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SMITHSONIAN CONTRIBUTIONS TO THE MARINE SCIENCES • NUMBER 38



Proceedings of the Smithsonian Marine Science Symposium

Edited by
Michael A. Lang,
Ian G. Macintyre, and Klaus Rützler

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ABSTRACT
Lang, Michael A., Ian G. Macintyre, and Klaus Rützler, editors. Proceedings of the Smithsonian Marine Science Symposium. *Smithsonian Contributions to the Marine Sciences,* number 38, 529 pages, 217 figures, 47 tables, 2009.—The Smithsonian Marine Science Symposium was held on 15–16 November 2007 in Washington, D.C. It represented the first major dissemination of marine research results since the establishment of the Smithsonian Marine Science Network (MSN). The 39 papers in this volume represent a wide range of marine research studies that demonstrate the breadth and diversity of science initiatives supported by the MSN. The first section contains an overview of the MSN along with papers describing the multidisciplinary investigations spanning more than 37 years for the four Smithsonian marine facilities that constitute the Network: the Smithsonian Environmental Research Center at the Chesapeake Bay, Maryland; the National Museum of Natural History's Smithsonian Marine Station at Fort Pierce, Florida; the Caribbean Coral Reef Ecosystems Program, with its Carrie Bow Marine Field Station in Belize; and the Smithsonian Tropical Research Institute in Panama. Subsequent papers represent findings by Smithsonian scholars and their collaborators on overarching topics of marine biodiversity, evolution, and speciation; biogeography, invasive species, and marine conservation; and forces of ecological change in marine systems.

Cover images: (left) *Aurelia aurita* sea jelly with juvenile carangid jacks in its bell, Carrie Bow Cay, Belize; (middle) *Dendronephthya* soft corals and *Anthias* school, The Brothers Islands, Red Sea, Egypt; (right) grey reef shark *Carcharhinus amblyrhynchos*, Kingman Reef, Northern Line Islands (all photos by Michael A. Lang).

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Contents

MARINE BIODIVERSITY, EVOLUTION, AND SPECIATION

Foreword

Nearly two-thirds of Earth's surface is covered by the ocean, a global system essential to all life. Impacts on one part of the ocean can have worldwide effects. The ocean moderates our climate, provides valuable resources, and produces at least half the oxygen we breathe: it makes our planet livable. We know little, however, about the physical, chemical, geological, and biological aspects of this crucial life support system.

The Smithsonian Institution, in efforts to increase knowledge about the ocean, has established a network of marine laboratories that monitors coastal habitats along a latitudinal gradient from the Chesapeake Bay through the Indian River Lagoon to the Mesoamerican barrier reef and on both sides of the Isthmus of Panama. The maintenance of long-term research projects and environmental monitoring is crucial to understanding changes that exceed in time the professional career of any given scientist. The information gained from such studies at stable sites enables scientists to differentiate between long-term changes and local or short-term environmental variations. Results contribute to our knowledge of systematics and ecology, physiology, behavioral sciences, geology, and paleoecology.

Our marine science universe comprises Smithsonian staff scientists and external collaborators and encourages the next generation of scientists, graduate students, and fellows. This symposium presents a Smithsonian-wide sample of marine science results.

Ira Rubinoff
Smithsonian Institution
Acting Under Secretary for Science, 2007–2008

Executive Summary

The results of the Smithsonian Marine Science Symposium, convened by the Marine Science Network on 15–16 November 2007 in Washington, D.C., are reported in 39 papers in this volume. These proceedings cover a wide range of marine research studies that demonstrate the breadth and diversity of science initiatives supported by the Smithsonian Marine Science Network. The first section treats an overview of the Smithsonian Marine Science Network established in 1998, and a brief background and history of multidisciplinary investigations spanning more than 37 years for each of the four marine facilities that constitute the Network: the Smithsonian Environmental Research Center at the Chesapeake Bay, Maryland; the National Museum of Natural History's Smithsonian Marine Station at Fort Pierce, Florida; the Caribbean Coral Reef Ecosystems Program, with its Carrie Bow Marine Field Station in Belize; and the Smithsonian Tropical Research Institute in Panama. Subsequent papers in this volume represent findings by Smithsonian scholars and their collaborators on overarching topics of marine biodiversity, evolution, and speciation; biogeography, invasive species, and marine conservation; and forces of ecological change in marine systems. The volume includes contributions on historical and geological aspects of coral reef and mangrove development; on biodiversity, developmental biology, and evolution (including molecular genetics) of sponges, cnidarians, sipunculan worms, crustaceans, and fishes; on ecology and population dynamics of algae, sponges, bryozoans, zooplankton, and miscellaneous invasive species; on environmental parameters, including pollutants, oceanographic factors, and hydrological regimes, and their effects on primary and secondary productivity, bleaching of symbiotic foraminiferans, benthic community structure, herbivory, development of toxic algal blooms, and land–sea connectivity in coastal habitats, that is, temperate bays and lagoons and tropical reefs and mangroves; and on conservation and education initiatives encompassing a range of organisms from sponges and corals to sea turtles, as well as communities such as tidal marshes, mangrove swamps, and coral reefs. As we prepare to face the challenges of rapidly accelerating biodiversity loss and global environmental stresses, particularly in highly vulnerable tropical shallow-water ecosystems such as reefs and mangroves, the focus of our scientific expertise on the member laboratories of the Marine Science Network has, during decades of documentation, established ecological standards that will help us monitor and evaluate future changes or trends and contribute to forthcoming education and conservation initiatives.

Michael A. Lang
Smithsonian Institution
Office of the Under Secretary for Science
April 2009

Introduction to the Smithsonian Marine Science Network

Michael A. Lang

ABSTRACT. The "Smithsonian Marine Science Symposium" contained more than 70 oral and poster presentations by Smithsonian scholars and collaborators and represented the first major dissemination of marine research results since the establishment of the Marine Science Network (MSN) in 1998. The MSN operates a unique array of laboratories and research vessels that spans the latitudinal gradient of the western Atlantic (Chesapeake Bay, Indian River Lagoon, Mesoamerican Barrier Reef, and Panamanian Coast) and crosses the isthmus of Panama. The Network is dedicated to understanding the rich biodiversity and complex ecosystem dynamics that sustain coastal processes and productivity. We study evolutionary, ecological, and environmental change in the ocean's coastal zones, increasing scientific knowledge of these environments and improving society's appreciation of the ocean's effect on our lives. Coastal environments are of immense economic and environmental importance and comprise 95% of the ocean's fisheries. Our coasts are the most densely populated and fastest growing communities in the USA. The MSN ensures integrated support of "Discovering and Understanding Life's Diversity," a core Smithsonian scientific mission. The MSN's goals are to ensure that the whole of the integrated Network is larger than the sum of its parts, leading to enhanced productivity through collaborative and comparative research, marine infrastructure development and support, professional training and outreach, and effective allocation of resources.

INTRODUCTION

The "Smithsonian Marine Science Symposium" was held 15–16 November 2007 to celebrate individual and long-term pan-institutional marine research, with a particular focus on highlights of the first ten years since the establishment of the Marine Science Network (MSN) in 1998. The symposium was convened by the Office of the Under Secretary for Science and represented the first gathering, of this magnitude, of Smithsonian marine scientists. The symposium presented marine research findings by Smithsonian scholars and their collaborators with emphasis on marine biodiversity, evolution, and speciation; biogeography, invasive species, and marine conservation, including life histories and microbial and behavioral ecology; and forces of ecological change in marine systems. The symposium carried on a tradition of Smithsonian marine science that began nearly 150 years ago and resulted in some of the world's foremost collections of marine specimens. More than 70 presentations

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and posters discussed results of marine research from the Chesapeake Bay, Indian River Lagoon and Florida Keys, the Mesoamerican Barrier Reef in Belize, the Atlantic and Pacific Coasts of the Isthmus of Panama, and other international research sites. Thirty-nine papers from this symposium are presented in this 38th volume of *Smithsonian Contributions to the Marine Sciences*, and additional marine education posters reside on http://www.si.edu/marinescience. Smithsonian speakers included marine research leaders, collaborators, and fellows from the Smithsonian Environmental Research Center, National Zoological Park, National Museum of Natural History, Smithsonian Marine Station at Fort Pierce, Caribbean Coral Reef Ecosystems Program, Smithsonian Tropical Research Institute, and the Office of the Under Secretary for Science.

The Smithsonian Institution operates a unique network of coastal laboratories and long-term research sites on the east coast of North and Central America that extends along the western Atlantic Ocean and bridges the Panamanian isthmus from the Caribbean Sea to the Pacific Ocean (Figure 1). Scientific diving supports a significant amount of Smithsonian marine research throughout the Network and internationally (Lang and Baldwin, 1996; Lang, 2007).

The Marine Science Network concept was developed in 1998 from the bottom up and has achieved the following important milestones:

1998: Formalization of a pan-institutional Smithsonian Marine Science Network initiated at two-day inaugural workshop at Smithsonian Environmental Research Center, with more than 50 Smithsonian Institution participants.
1999: Dedication of new Carrie Bow Cay Marine Field Station.
1999: Dedication of new Smithsonian Marine Station at Fort Pierce.
2000: MSN concept and infrastructure allocations approved by the Under Secretary for Science.
2001: Launch of the MSN website www.si.edu/marinescience.
2001: Annual MSN Calls for Proposals for infrastructure, marine research awards, and postdoctoral fellowships.
2003: Dedication of Bocas del Toro Marine Laboratory.
2006: Science Executive Committee review of Smithsonian marine science, including MSN.
2007: Formulation of Big Questions in Marine Science:
    1. What are the major spatial and temporal patterns in distribution of biodiversity?
    2. How does biodiversity, and the loss of biodiversity, affect the functioning of ecosystems?
    3. How are humans changing the magnitude and distribution of biodiversity and what are the patterns and consequences?
2007: Smithsonian Marine Science Symposium.

The MSN is administered as a pan-institutional program through the Office of the Under Secretary for Science. It is governed by a seven-member Steering Committee composed of Michael Lang (Office of the Under Secretaray for Science), Anson Hines (Smithsonian Environmental Research Center), Eldredge Bermingham (Smithsonian Tropical Research Institute), Klaus Ruetzler (National Museum of Natural History), Robert Fleischer (National Zoological Park), Valerie Paul (Smithsonian Marine Station at Fort Pierce), and Phillip Taylor (National Science Foundation). Additional Smithsonian scientists participate by invitation in MSN research proposals and postdoctoral fellowship review panels, MSN symposia and workshop committees, and special projects. Support for MSN infrastructure, research, and postdoctoral fellowships is provided by the Office of the Under Secretary for Science's Johnson and Hunterdon Oceanographic Research Endowments.

There are four main unifying disciplinary themes to Smithsonian marine research: systematics, evolutionary biology, ecology, and geology. Biogeography is a key research element, linking systematics, ecology, and evolutionary biology. Mechanisms of biogeographic isolation are central elements in evolutionary theory, population dynamics, conservation biology, and patterns of biodiversity. Biogeographic patterns are crucial data in the determination of introduced and native species. Site-specific, long-term measurements of environmental variables allow for analysis of change over multiple time scales, which is necessary to detect patterns in typically complex ecological data. The Smithsonian Marine Science Network is uniquely positioned to monitor long-term change at its component sites. It has an extensive array of programs that address many of the most pressing environmental issues in marine ecosystems, including biological invasions, eutrophication, harmful species and parasites, plankton blooms and red tides, linkages among coastal ecosystems, global warming including sea-level rise, El Niño/La Niña effects, UV radiation impacts, habitat destruction, fisheries impacts, ecology of key habitats (estuaries, coral reefs, mangroves, seagrasses, wetlands), and biodiversity inventories.

The Smithsonian's marine education programs consist of public outreach and professional training. A series

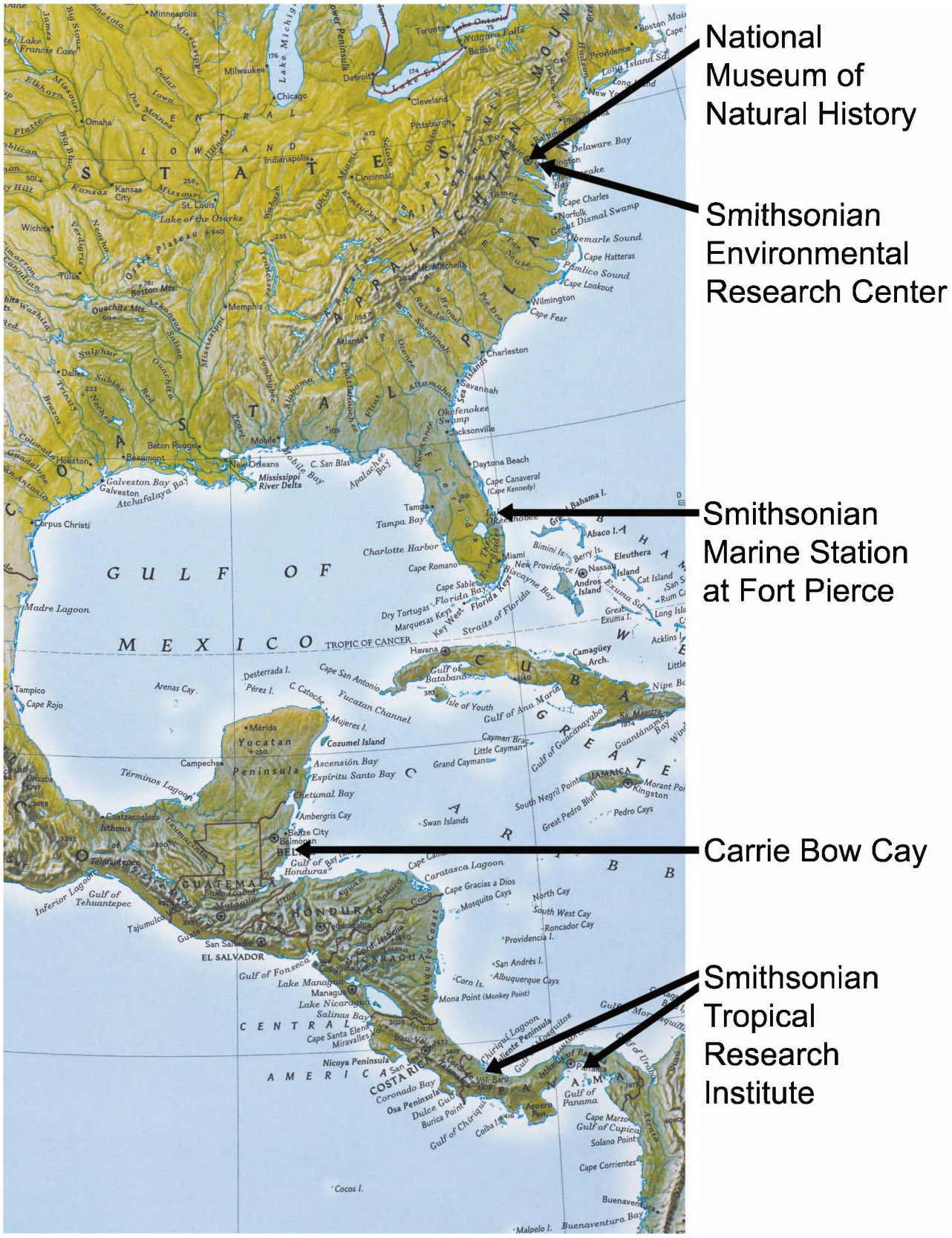

FIGURE 1. Map showing locations of the Smithsonian Marine Science Network members.

of these activities are aimed at promoting awareness and conservation of marine environments and at communicating the Smithsonian's research findings to the general public. By integrating research with education, the Smithsonian produces tomorrow's discoverers while pursuing today's discoveries. The public is engaged with interactive exhibits and scientists (e.g., the National Museum of Natural History's Sant Ocean Hall), symposia, popular books, lectures, and films about the marine environment. The Smithsonian Marine Science Network contributes to the public interest by disseminating novel environmental information around the globe. Its research helps build a solid foundation for informed decisions about environmental policy, natural resource management, and conservation.

Other recent coordinated Smithsonian marine science efforts of note are marine-terrestrial flora and fauna of Cayos Cochinos Archipelago, Honduras (Guzman, 1998), natural history of the Pelican Cays (Macintyre and Rützler, 2000), the Twin Cays mangrove ecosystem, Belize (Macintyre et al., 2004), and investigations of the marine fauna and environments of Bocas del Toro, Panama (Collin, 2005). Smithsonian taxonomic field guides and keys of algae, invertebrates, and fishes (Littler and Littler, 2000; Collin et al., 2005; Robertson, 2009) are valuable tools for biologists, divers, and fishermen alike.

Marine Science Network Mission and Vision

The MSN mission is dedicated to understanding the rich biodiversity and complex ecosystem dynamics that sustain coastal processes and productivity, and its vision is "to increase scientific knowledge of marine coastal environments and to improve society's appreciation of the ocean's effect on our lives."

Marine Science Network Goals

The MSN provides integrated support of "Discovering and Understanding Life's Diversity," a core scientific mission of the 2005–2010 Smithsonian Science Strategic Plan. The MSN ensures that the whole of the integrated Network is greater than the sum of its parts, leading to enhanced productivity through collaborative and comparative research facilitated by increased inter-unit coordination, marine infrastructure development and support, professional training and outreach, effective allocation of research funding, and transparent management, participation, and support for Smithsonian marine scientists through availability of shared resources and facility access.

SMITHSONIAN ENVIRONMENTAL RESEARCH CENTER (CHESAPEAKE BAY)

The Smithsonian Environmental Research Center (SERC) advances stewardship of the biosphere through interdisciplinary research and education. With a resident staff of more than 100 scientists, technicians, fellows, and students, SERC has experienced significant growth in the last few years. The SERC laboratories, educational facilities, and primary field sites are located 25 miles east of Washington, D.C., on the western shore of Chesapeake Bay. Its campus includes a growing complex of offices, laboratories, maintenance shops, a library, housing, and facilities for public programs. A dock, fleet of research vessels, dive locker, wet laboratory, aquarium room, and large fish-weir are used in support of estuarine research.

The greatest resource at SERC is its main research site on the Rhode River subestuary, which includes more than 1,072 ha of land and 26 km of undeveloped shoreline of the Chesapeake Bay. Since 1965, SERC's long-term studies have focused on the interactions among ecosystems in complex landscapes, tidal marshes, and estuaries. With the Rhode River site as its hub, SERC research radiates to sites around the world to address effects of global change, landscape ecology, coastal ecosystems, and population and community ecology. Much of SERC's comparative research across latitudes extends to the other sites of the MSN and includes studies of mangrove biocomplexity, invasive and native species biodiversity, estuarine food webs, land use impacts linked to water quality, carbon processing and global change, nutrient loading and low dissolved oxygen, ecosystem management of fisheries and crucial habitats, and life history patterns and evolution. Research at SERC focuses on five grand environmental challenges (Hines, 2009): (1) impacts of atmospheric change on climate, sea level, ultraviolet radiation, pollutant deposition, and carbon balance; (2) impacts of watershed nutrient discharges causing harmful algal blooms, depletion of oxygen, and destruction of submerged vegetation; (3) food web disruption by pollution and overfishing; (4) invasive species; and (5) landscape disturbance by agriculture and development.

Goals of SERC in marine education include professional training (interns, graduate students, postdoctoral fellows, and visiting scientists), teacher training, site visits and public programs, and distance learning.

NATIONAL ZOOLOGICAL PARK (WASHINGTON, D.C.)

The Smithsonian's National Zoological Park (NZP) was founded in 1889. Its mission is to provide leadership in animal care, science, education, and sustainability. Approximately 2,000 individuals of 400 different species constitute its animal collection. The NZP consists of a 163 acre urban park located in Rock Creek Park in northwest Washington, D.C., and the 3,200 acre Conservation and Research Center in Front Royal, Virginia, emphasizing reproductive physiology, analysis of habitat and species relationships, and the training of conservation scientists.

The National Zoological Park conducts international marine research on sea turtles and sea birds, ecology of bottlenose dolphins, Weddell seal lactation, life history and reproductive strategies of gray and harbor seals, nutritional ecology of sea otters, and cryopreservation of endangered coral species. Marine exhibits include the Seal and Sea Lion Pool and the Invertebrate Exhibit, which opened in 1987, where marine invertebrates comprise 75% of its live collections on display. The NZP's tools to inspire, train, and empower successive generations to care for the world's biological diversity are its exhibits, science, outreach, and education programs. Ultimately, efforts must be oriented toward protecting wildlife and other forms of biological diversity so that we, and future societies, continue to enjoy the incalculable benefits of our natural world.

NATIONAL MUSEUM OF NATURAL HISTORY (WASHINGTON, D.C.)

The National Museum of Natural History (NMNH) has a distinguished history of more than 150 years of sampling and collections-based research. Major collections represent algae and dinoflagellates, foraminifera, sponges, cnidarians, ctenophores, worms, crustaceans, mollusks, bryozoans, echinoderms, tunicates, fishes, marine reptiles, birds, and mammals), now numbering more than 33,000,000 specimens of plants and animals. Of approximately 2,415 families of marine invertebrates, nearly 67% are represented in the NMNH invertebrate collection, which is not limited solely to the diversity-rich tropics. The NMNH provides professional collection management services to the National Science Foundation United States Antarctic Program (USAP) and the international scientific community. A primary focus of this project is improving access to the collections through its cataloging (inventory) program (more than 900,000 USAP specimens) and loan program. More than 170,000 USAP specimens in 138 separate transactions were either lent or returned from loan between 1995 and the end of 2004, supporting the research efforts of scientists in 22 countries. Several hundred lots of archive samples from the Palmer Long-Term Ecological Research Program were also accessioned (Lemaitre et al., 2009).

The focus of marine science at NMNH addresses the diversity of marine life, where species occur, how they are related to each other, how marine diversity developed and how it is maintained, what are the human impacts on marine life, and how marine life-forms are used by people.

The Museum administers the Laboratories of Analytical Biology for state-of-the art molecular work and two marine field stations (Carrie Bow Cay, Belize, and Smithsonian Marine Station at Fort Pierce, Florida), member facilities of the MSN. Since 1966 the Museum has funded the *Atoll Research Bulletin*, which publishes research reports on the geology and ecology of islands and their adjacent coral reef and mangrove communities in tropical sites around the world.

The NMNH Ocean Initiative comprises the Sant Ocean Hall, the Ocean Web Portal, the Sant Chair in Marine Science, and interdisciplinary marine research at NMNH. Virtual access to the Museum's key marine collections is being created. The Initiative aims to train future generations of marine scientists and educate the public about, and raise awareness of, the importance of the ocean as a global system.

SMITHSONIAN MARINE STATION AT FORT PIERCE (FLORIDA)

The Smithsonian Institution has had a presence in Fort Pierce, Florida, since 1969 and was known then as the Fort Pierce Bureau. From 1969 to 1981, the Fort Pierce Bureau carried out studies including underwater oceanography with research submersibles, a survey of the Indian River Lagoon, coral reef research, and research on life histories of marine invertebrates, partly in collaboration with the newly formed Harbor Branch Foundation (now the Harbor Branch Oceanographic Institution at Florida Atlantic University). In 1981, the Fort Pierce Bureau was dissolved, and in its place the Smithsonian Marine Station at Link Port was formally recognized as an organizational unit under the auspices of the National Museum of Natural History. The Station took over the barge, acquired originally by the Smithsonian in 1973 from federal surplus, that

was docked at the Harbor Branch campus. In 1996, the Smithsonian purchased, from the MacArthur Foundation, 8 acres of property 7 miles south near the Fort Pierce Inlet with easement access to the Indian River Lagoon. St. Lucie County enacted a 25 year lease of a county dock and adjacent land at a site on the inlet across from the Station, whose main building was completed and dedicated in 1999.

The Smithsonian Marine Station at Fort Pierce (SMSFP) is a marine science research center located on the Indian River Lagoon along 156 miles of Florida's central Atlantic coast. The Indian River Lagoon is a long, narrow, and shallow estuary adjacent to the Atlantic Ocean, separated by a strip of barrier islands. Biologists at SMSFP have the advantage of working just 20 miles from the Florida current, a stream of warm water from the Caribbean that moves northward past Florida's coastline as part of the larger, complex system of currents known as the Gulf Stream. The current carries with it many tropical marine organisms, allowing researchers to work at the interface of the Northern Hemisphere's tropical and temperate regions. Situated in a biogeographic transitional zone between the temperate and subtropical provinces, the SMSFP facility provides access to an extraordinary diversity of marine and estuarine species and to a variety of habitats, which include mangroves, salt marshes and sandy beaches, rocky intertidal substrates, seagrass beds, mud and sand flats, coral reefs, worm reefs, *Coquina* hard bottoms, deep coral rubble zones, shallow- to deep-water sandy plains, and the blue waters of the Gulf Stream.

The Marine Station supports and conducts scholarly research in the marine sciences, emphasizing studies of biodiversity, life histories, and ecology of marine organisms (Paul et al., 2009). The results of this research enable policy makers to make informed environmental decisions in guiding conservation and sustainable management of marine resources, as well as providing the basis for innovative applications in medicine, aquaculture, and the effective balance between development and conservation. For Smithsonian scientists, the SMSFP provides an important link with other MSN facilities in the tropics at the Smithsonian Tropical Research Institute (STRI) in Panama and Carrie Bow Cay in Belize and in the temperate region, the Smithsonian Environmental Research Center on the Chesapeake Bay.

The facilities at the Smithsonian Marine Station at Fort Pierce include an 8,000 square foot facility that houses a histology laboratory, an electron microscopy lab, a confocal microscope, a combination electrophoresis/DNA/chemistry laboratory, a photographic darkroom, flow-through seawater tables and aquaria, an industrial shop, and offices and laboratories for visiting scientists and fellowship recipients. The 39-foot R/V *Sunburst* and two smaller vessels are used for scientific diving, dredging, and trawling in the Indian River Lagoon, Continental Shelf, and Gulf Stream.

The Marine Station's educational efforts include postdoctoral fellows and interns, public events and lectures, school programs and public tours, a web site, the Indian River Lagoon Species Inventory, and the Marine Ecosystems Exhibit, which was established in 2001 with the following ecosystems on display: coral reef, seagrass, mangrove, hardbottom and nearshore habitats, and *Oculina* reef).

CARIBBEAN CORAL REEF ECOSYSTEMS PROGRAM (CARRIE BOW CAY, BELIZE)

Coral reefs are unique biogeological structures that thrive in clear, nutrient-poor (oligotrophic) tropical oceans and support a rich and diverse biological community. Reef systems are driven by the symbiosis between scleractinian corals and microscopic dinoflagellate algae (zooxanthellae) as their chief energy source. The largest, best developed, least polluted, and least commercially exploited coral reef in the Atlantic region is the Mesoamerican Barrier Reef in Belize. It is a complex of reefs, atolls, islands, oceanic mangroves, and seagrass meadows that extends over 160 km. For its unique characteristics and unperturbed condition, the Belize barrier reef has been declared a World Heritage Site.

In the early 1970s, Rützler et al. (2009) discovered the formidable qualities of the Belize (then British Honduras) barrier reef. After careful comparison with other locations in the western Caribbean, it was chosen as the site of an interdisciplinary long-term study of systematics, ecology, behavior, and evolution of reef organisms and the dynamics and historical development of reef communities (Rützler and Macintyre, 1982). Carrie Bow Cay, only three hours by plane and boat from Miami, was found to be the ideal logistical base because of its location on top of the barrier reef, only meters away from a variety of habitat types (reef flat, spur and groove, deep fore-reef slope, patch reefs, seagrass meadows, and mangroves), and its undisputed ownership by a Belizean family able to cater to all Smithsonian needs for lodging, food, local transportation, and contacts with government.

In 1985, as part of the U.S. Congress Caribbean Basin Initiative, the National Museum of Natural History received an increase to its budget base to continue and intensify study of Caribbean coral reef ecosystems. These funds allowed for the expansion of research facilities on

Carrie Bow Cay and the update of CCRE equipment. In the years since, CCRE has accomplished the following: amassed thousands of specimens of marine plants, invertebrates, and fishes, which are organized in an enormous database; assisted the government of Belize in shaping and justifying its coastal conservation policy; participated continuously in the Caribbean-wide reef monitoring network (CARICOMP); established the first meteorological oceanographic monitoring station in coastal Belize; and, above all, published well over 850 scientific papers in reviewed journals, as well as several books, doctoral dissertations, popular articles, and photo and video documentaries. Between 60 and 80 scientists use Carrie Bow Cay each year as a part of ongoing CCRE research.

The Carrie Bow Cay Laboratory serves primarily in support of SI marine scientists' research projects and their external collaborators. Seasonal hurricanes during the past 35 years could not destroy Carrie Bow Cay facilities to the extent that a devastating fire did in December 1997. Improved facilities now include dry and wet labs, housing, generator, compressor, small boats and scuba cylinders, and essential facilities such as solar power, a running-seawater system, and weather station.

CCRE's educational and outreach programs include its Belize teachers' mangrove workshops, publications, symposia, advisory consults with Belizean Ministries, and fellows and interns.

SMITHSONIAN TROPICAL RESEARCH INSTITUTE (REPUBLIC OF PANAMA)

The Smithsonian Tropical Research Institute's (STRI) marine research program in the Republic of Panama dates to 1964 when small laboratories were established on the Pacific and Caribbean coasts within the former Canal Zone. Today, STRI operates marine stations at Bocas del Toro and Galeta Point in the Caribbean and the Naos marine laboratory complex in the Pacific. Until 2008, the R/V *Urraca*, a 96 foot nearshore coastal oceanographic vessel, was outfitted with remotely operated vehicle, scientific diving, and dredging capabilities, and was operated under University National Oceanographic Laboratory System (UNOLS) research fleet standards.

At the Panama Canal, the Isthmus of Panama narrows to less than 100 km, separating oceans that are very different tropical marine ecosystems. The Caribbean is a relatively stable ocean, with small fluctuations in temperature and relatively low tidal variation. Its transparent, nutrient-poor waters are ideal for the growth of reefs, and it ranks just behind the Indian Ocean and the Indo-West Pacific in terms of numbers of marine species. The tropical eastern Pacific, in contrast, exhibits much greater fluctuations in tides and temperature, with seasonal upwelling locally and longer-term variation resulting from the El Niño southern oscillation cycle. Its more nutrient-rich waters support commercial fisheries of major importance. The creation of these two distinct marine realms by the rise of the Isthmus of Panama during the past 10 million years also contributed to the formation of the modern biological and geological world. During this interval, the Gulf Stream was established, the mammals of North America conquered a newly connected South America, the Ice Ages began, and modern man arose. The Isthmus played a major role in this history, and set in motion a fascinating natural experiment, as the animals and plants of the two oceans went their separate evolutionary ways.

There are also major differences within each ocean. In the Pacific, seasonal upwelling of nutrient-rich waters is strong in the Gulf of Panama, where trade winds blow freely across the Isthmus, but absent in the Gulf of Chiriqui, where the high terrain blocks these winds. The more stable conditions in the Gulf of Chiriqui support the best developed coral reefs in the tropical eastern Pacific. On the Caribbean side, the San Blas Archipelago is bathed in clear oceanic waters, whereas the reefs and mangroves of the enormous Chiriqui Lagoon of Bocas del Toro are enriched by runoff from the land. Thus, Panama can be considered a nation of four ocean types, providing unique opportunities for understanding how and why marine ecosystems function as they do.

Understanding the history and ecology of Panama's diverse marine environments has been a major theme of STRI's research over the past four decades (Robertson et al., 2009). Major programs include between-ocean comparisons of physical and biological oceanography, geological reconstruction of events leading up to and following the rise of the Isthmus, studies of marine biodiversity, and analyses of the vulnerability of marine habitats to natural and anthropogenic change. In celebration of STRI's role in coral reef research, the Smithsonian's 150th anniversary, and the International Year of the Reef, the Smithsonian hosted the Eighth International Coral Reef Symposium in Panama in 1996. This meeting brought 1,500 reef scientists and managers to Panama from around the world and resulted in the publication of a two-volume proceedings (Lessios and Macintyre, 1997) and an international traveling exhibit that has already brought STRI's marine discoveries to Miami, the District of Columbia, Honduras, and Jamaica.

The Marine Environmental Sciences Program (MESP) at STRI collects and analyzes fundamental oceanographic information that provides critical information for studies such as El Niño and coral bleaching. The Panama Paleontology Project in Bocas del Toro seeks to record the history of the divergence between the two oceans over the past 10 million years and the evolutionary response of marine organisms to these changes. Results from this project are the geological reconstruction of the closure of the Isthmus of Panama 3 million years ago and the discovery of a major extinction event in the Caribbean about 2 million years ago. Through a combination of molecular and paleontological information, STRI's molecular evolution program has developed a model system for determining the rate at which organisms diverge genetically through time (Panama molecular clock). This achievement allows for the phylogenetic reconstruction of marine life elsewhere in the world.

Educational programs within STRI include a marine fellowship program, school group visits (Culebra Nature Center, Galeta Point Marine Lab, Bocas del Toro Lab), public seminars, advisory consults with the Panamanian government, and graduate courses.

CONCLUSION

The Smithsonian Institution is unique among federal agencies, research organizations, and universities with its investment in comprehensive, long-term marine studies of crucial ecosystems at a latitudinal gradient of stable sites. Thousands of marine science publications provide results and data for synthesis and a new baseline for modeling and forecasting. Continued opportunities remain for many important marine organisms to be identified by conventional and molecular techniques and described. Deep reefs are becoming increasingly important as a focal area to understand how the reef system in toto functions and to quantify their physical, chemical, and biological contributions to the shallow reefs that we have studied for more than three decades (Lang and Smith, 2006). Life histories require further analysis to aid ecological understanding and fisheries management. Thirty-five-year multidisciplinary databases allow for early detection and evaluation of community changes, invertebrate diseases, invasive species, and recruitment caused by environmental degradation and catastrophic events.

The MSN continues to provide support for individual and pan-institutional collaborative research, postdoctoral marine science fellows, marine science staff and infrastructure support, marine outreach and education, and workshops and symposia: for example, Bocas del Toro taxonomy; coral reef management; mangrove ecology of Twin Cays, Belize; marine genetics; sea turtle conservation and population management; seagrass and mangrove ecosystems; neogastropod evolution; marine invasives of the Gulf of Mexico; and marine invasive species across latitudinal gradients. Outcomes of the integration of the Smithsonian marine facilities and programs since 1998 are the facilitated freedom of movement of scientists between units and the increased collaborations and co-authored publications. The MSN was highlighted as a model for pan-institutional Smithsonian programs by the Smithsonian Science Commission in 2003. The most likely keys to its success were the bottom-up development of the Network concept, starting with the Institution's staff scientists, and the availability of research funding through the Office of the Under Secretary for Science to enable marine research and postdoctoral fellowships.

The Smithsonian Marine Science Network and the Smithsonian Scientific Diving Program provide the facilities and support for the efficient conduct of marine research. The primary objective of the marine research effort is the advancement of science. The deliverable is mainly in the form of peer-reviewed publications for dissemination throughout the scientific community and to the public.

The importance of the MSN is its contribution to the knowledge of complex ecosystems including seagrasses, mangrove islands, bays, estuaries, and coral reefs, and the preservation of these precious resources by learning about their rich biodiversity, function, and interconnectedness. Only a long-term commitment will allow us to understand the dynamics of coastal processes and organisms, obtain the cooperation of the public, and educate a new generation.

Acknowledgments

Support for the Smithsonian Marine Science Network is provided by the Hunterdon and Johnson Oceanographic Research Endowments in the Office of the Under Secretary for Science. I wish to extend appreciation to the Marine Science Symposium Committee: Biff Bermingham, Tuck Hines, Ian Macintyre, Olav Oftedal, Valerie Paul, Klaus Rützler, and Phil Taylor; and to Session Co-Chairs: Carole Baldwin, Allen Collins, Candy Feller, Ian Macintyre, Jon Norenburg, Valerie Paul, and Mark Torchin. I thank Ira Rubinoff for his long-term support of the Scientific Diving Program and MSN. Editorial collaboration with Ian Macintyre and Klaus Rützler on this volume was much appreciated, as was the assistance of Ginger Strader and

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Land–Sea Interactions and Human Impacts in the Coastal Zone

Anson H. Hines

ABSTRACT. The Smithsonian Environmental Research Center (SERC) conducts research on land–sea interactions to understand natural processes and human impacts in linked ecosystems of the coastal zone. Coastal ecosystems support great biological productivity and are of immense ecological and economic importance. In addition, more than two-thirds of the human population resides in the coastal zone, where human activities cause chronic and acute disturbance of every habitat and marked degradation of ecological balance and productivity. The Chesapeake Bay and its Rhode River subestuary are used by SERC as model study systems to conduct long-term, intensive monitoring and experiments. Research at SERC focuses on five grand environmental challenges: (I) impacts of atmospheric change on climate, sea level, ultraviolet radiation, pollutant deposition, and carbon balance; (II) impacts of watershed nutrient discharges causing harmful algal blooms, depletion of oxygen, and destruction of submerged vegetation; (III) food web disruption by pollution and overfishing; (IV) invasive species; and (V) landscape disturbance by agriculture and development. Research by SERC on these grand challenges serves to advise policy and management from improved stewardship of coastal resources.

INTRODUCTION

The coastal zone is of immense economic and environmental importance. More than 50% of the Earth's human population (3 billion people) resides in the coastal zone and relies on the goods and services of coastal ecosystems, and this number is expected to double by 2045 (Creel, 2003). Coastal communities are the most densely populated and fastest growing areas in the United States: 14 of the nation's largest 20 cities are in coastal locations; more than 50% of the U.S. population lives in 17% of the country's land, comprising coastal counties; this population concentration is expected increase to 70% within 25 years; and 23 of the 25 most densely populated counties encompass coastal cities and their surrounding sprawl (Crossett et al., 2004). The coastal environment includes the Earth's most biologically productive ecosystems, and this diverse environment includes unmeasured reserves of strategic minerals, oil and gas, and other nonliving resources. The coastal zone encompasses major hubs of global transportation and commerce and unparalleled opportunities for recreation and tourism, as well as the majority of fisheries and aquaculture industries. At the same time,

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these activities cause chronic and acute disturbance of every coastal habitat: overfishing has removed most large species at the top of the food web, and coastal waters receive most of the waste of urban centers and agricultural runoff of the coastal plain.

Research at the Smithsonian Environmental Research Center (SERC) focuses on land–sea interactions. Scientists at SERC study linked coastal ecosystems to understand natural processes and human impacts in the coastal zone. Ocean productivity is concentrated in the coastal fringe where nutrients run off the land and well up from the deep. The coastal environment includes the Earth's most biologically diverse ecosystems: estuaries, wetlands, mangroves, seagrasses, coral and oyster reefs, kelp forests, and pelagic upwelling areas. Bottom communities and water column processes of the photic zone are most tightly coupled in the nearshore shallows. Coastal waters comprise 95% of the oceans' fisheries. Thus, SERC research focuses on improved stewardship of these marine resources.

CHESAPEAKE BAY AND THE RHODE RIVER SUBESTUARY AS A MODEL SYSTEM

The Smithsonian Environmental Research Center utilizes the nation's largest estuary, Chesapeake Bay and its 177,000 km^2 watershed including six states and the District of Columbia (Figure 1), as its primary research landscape and main study site. In addition to SERC, this study area includes the Smithsonian's museum complex, zoological exhibits, and administrative offices. An area with a long American history of exploitation of coastal resources, the Chesapeake watershed is home to 17 million people, who are mostly concentrated in the urban centers and suburban sprawl of Baltimore, Washington, D.C., and Norfolk. Agriculture, particularly row crops, is the major land use of the Chesapeake watershed, and farming has been the major source of disturbance to the eastern deciduous forest for 400 years.

Established in 1965, SERC owns a unique 1,072 ha land holding for long-term descriptive and experimental studies of linked ecosystems in a model subestuary and subwatershed of Chesapeake Bay—the Rhode River, which is located 40 km east of Washington, D.C., and 10 km south of Annapolis, Maryland (Figure 2). The property at SERC includes cropland, forests in various successional stages, wetlands, and 26 km of undeveloped shoreline; this is the largest contiguous block of land dedicated to environmental research, science education, public access, and stewardship on the western shoreline of Chesapeake Bay. The 585 ha Rhode River subestuary is a shallow (maximum depth = 4 m), soft-bottom embayment in the lower mesohaline zone of the Bay. The facilities at SERC provide strategic support for research at the site and ready access to the rest of the Chesapeake watershed and estuary.

GRAND CHALLENGES OF COASTAL ENVIRONMENTAL RESEARCH

The purpose of this paper is to present examples that highlight SERC's coastal research on five grand environmental challenges. With data sets extending back to the 1970s and 1980s, SERC research monitors decadal-length changes to distinguish seasonal and annual fluctuations from long-term trends in the environment. Importantly, SERC research seeks to determine mechanistic understanding of the causes of change at multiple spatial scales ranging from global change to landscape, watershed, ecosystem, and community levels of organization. The land and long-term studies at SERC's Rhode River site afford multidisciplinary experimental analyses of mechanisms controlling ecological interactions. The research there addresses the grand challenges and advises environmental policy and management for improved stewardship of coastal resources.

Grand Challenge I: Impacts of Atmospheric Change

Human alterations of the atmosphere are causing rapid changes in climate, sea level, ultraviolet radiation, pollutant deposition, and ecosystem carbon balance. Research by SERC on the salt marshes of the Rhode River subestuary provides a good example of the ecological complexities of this challenge. B. G. Drake and colleagues have been conducting the world's longest running experimental manipulation of CO_2 on natural plant communities (1985 to present), which has been testing the effects of rising atmospheric CO_2 concentration in these salt marshes. The experiment measures response of the two dominant plant species at the site: *Spartina patens* and *Scirpus olneyi*. The experiment applied nine treatment combinations of three CO_2 levels in open-top chambers (ambient air at 340 ppm; elevated CO_2 at a twofold increase in concentration of 680 ppm; and a control treatment without chambers) crossed with types of patches (nearly monospecific *S. patens*; nearly monospecific *S. olneyi*; and patches with mixes of the two species) (Drake et al., 1989). Chambers were replaced exactly on replicate marked plots of the nine treatment

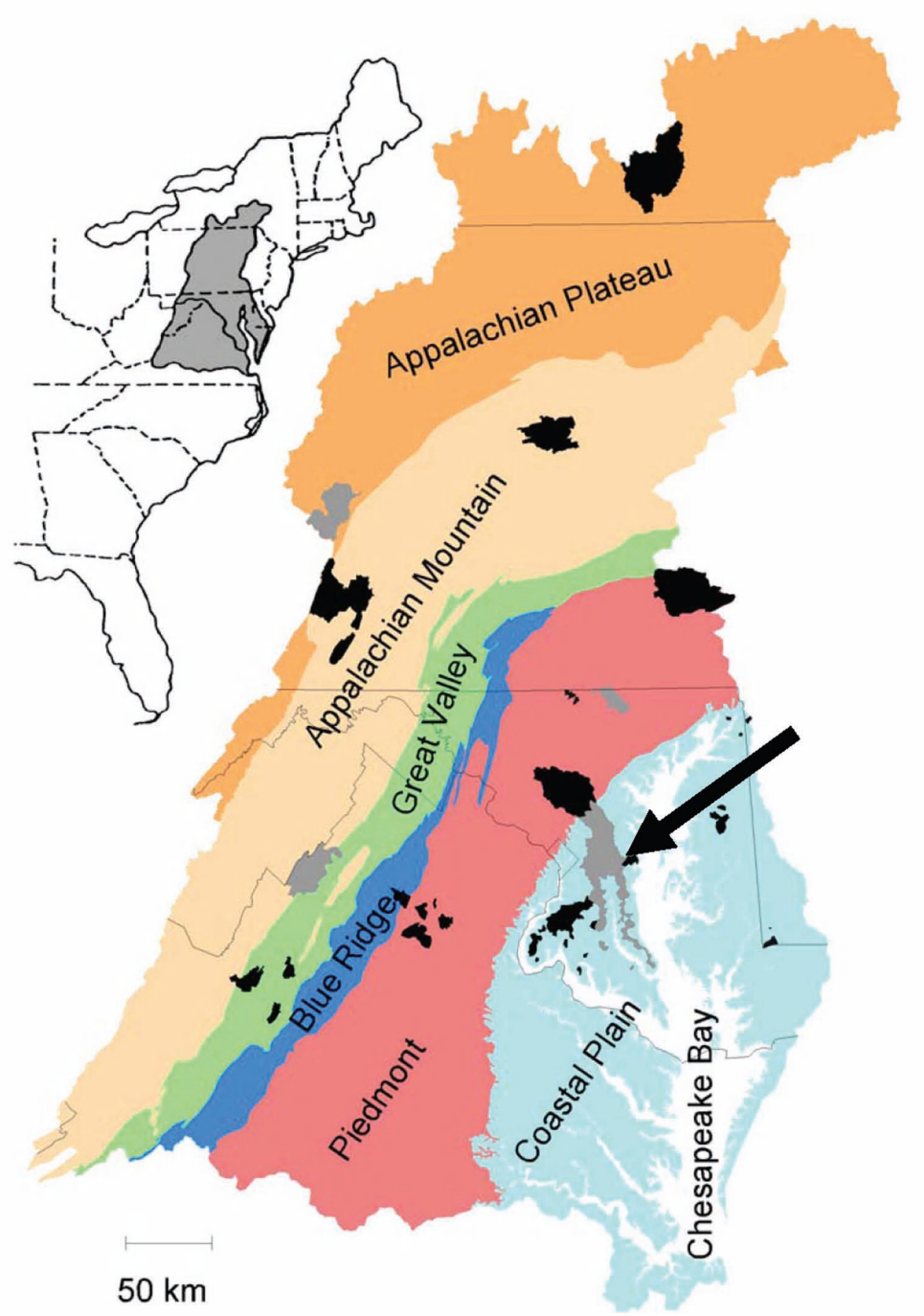

FIGURE 1. Map of Chesapeake Bay and its watershed with six physiographic provinces. Arrow indicates the location of the Smithsonian Environmental Research Center on the Rhode River subestuary and watershed. Darkened areas indicate 17 clusters of 500 subwatersheds that differed in land use and were monitored for stream discharges of nutrients.

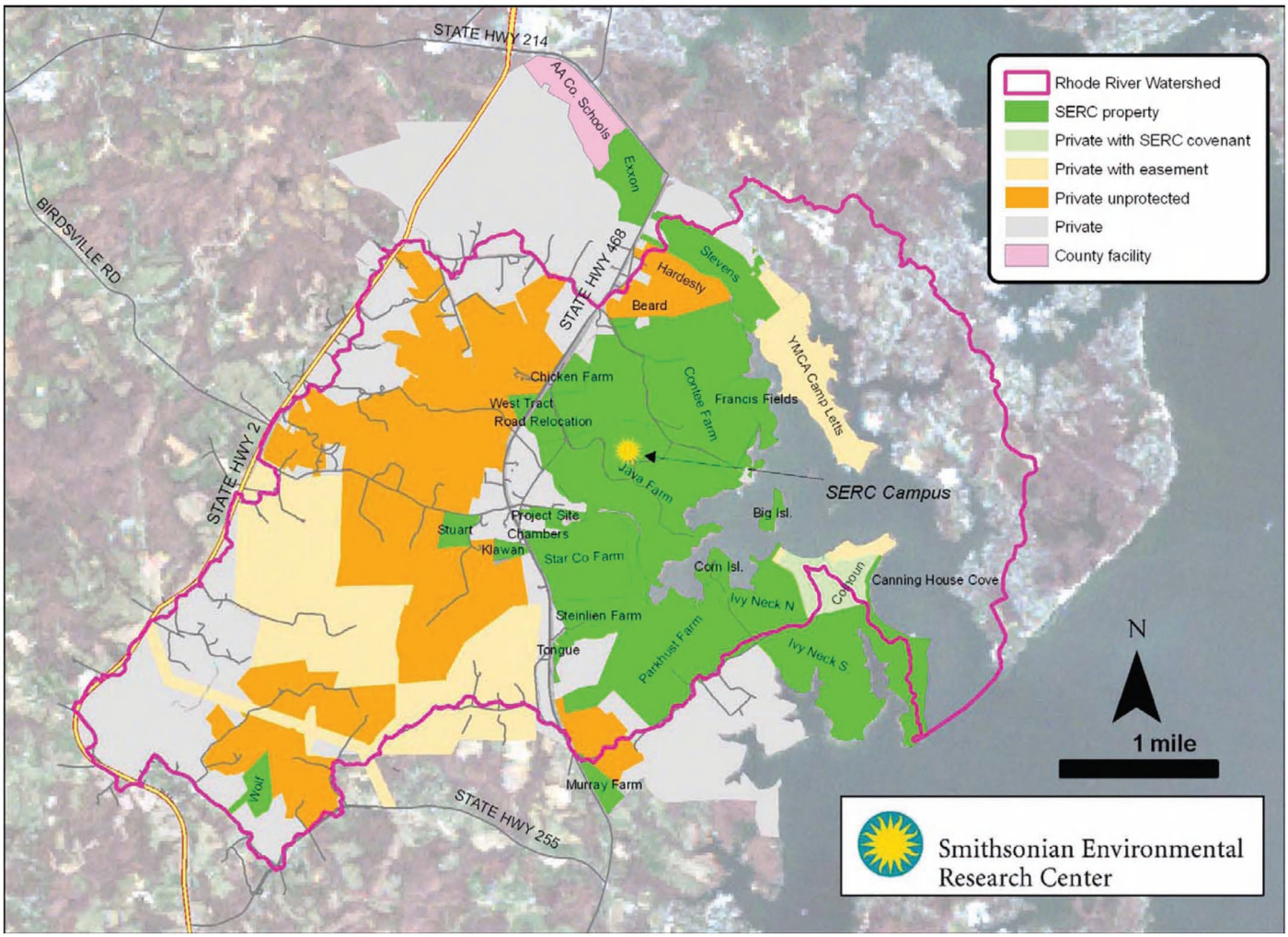

FIGURE 2. Map of land holdings (shaded green) of the Smithsonian Environmental Research Center (SERC) surrounding the Rhode River subestuary. Red outline shows the boundary of the watershed.

combinations for the duration of the growing season for the past 23 years (1995–2008). Photosynthesis and respiration were measured in each chamber during the growing season, and plant production was measured at the end of each season. As predicted, *Spartina patens* is a C_4 plant that responds weakly to rising CO_2, whereas growth and production were greatly stimulated in *Scirpus olneyi* as a C_3 plant (Drake and Rasse, 2003). However, the amount of stimulation of *S. olneyi* is significantly inversely dependent on salinity (i.e., water stress), with lower production in years of high salinities (i.e., low rainfall) (Rasse et al., 2005; and Figure 3).

Salt marsh research at SERC's Rhode River site also explores other ecosystem complexities. New research is tracking the fate of the carbon added by growth stimulation of the plants, which appears to be sequestered in the peat-forming roots of the salt marsh (Carney et al., 2007). Research conducted by J. P. Megonigal and colleagues at the same marsh study site compares effects of increased CO_2 interacting with nutrient additions to the marsh to determine whether peat accumulation is sufficient to keep up with rising sea level. Their initial results indicate that the peat accumulation is equivalent to the current rate of sea-level rise of approximately 3 mm year^{-2}, allowing the marsh to persist instead of becoming submerged. Additionally, a nonnative species, Phragmites australis, is rapidly invading the marsh site, similar to most others in the region (King et al., 2007); and its responses to the interaction of rising CO_2 and nutrients are unknown. The Chesapeake region has high levels of mercury deposition

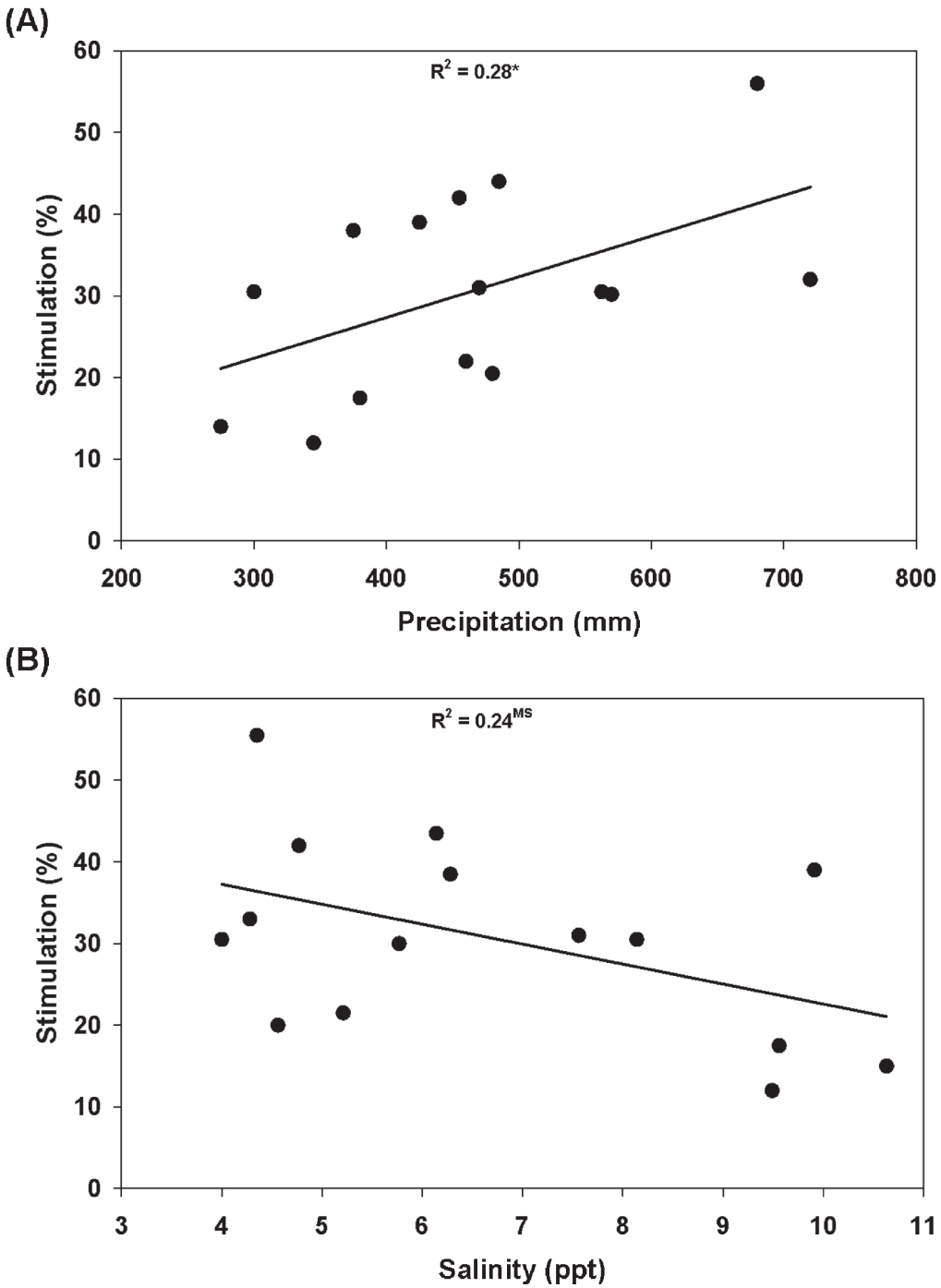

FIGURE 3. Effect of (A) precipitation and (B) salinity (ppt = parts per thousand) on the stimulation of photosynthesis by twofold increase in CO_2 concentration on the sedge *Scirpus olneyi* in open-top chambers placed on a salt marsh of the Rhode River subestuary during a 17-year period (1989–2003). (After Rasse et al., 2005.)

that is derived from coal-burning power plants. New work at the SERC salt marsh site shows that microbes rapidly activate the mercury (mercury-methylation) (Mitchell et al., 2008) deposited into marshes, thus feeding it into biological processes on the coastal food web (C. Mitchell and C. Gilmour, Smithsonian Environmental Research Center, 2008, personal communication).

Grand Challenge II: Impacts of Nutrient Loading

Over-enrichment of coastal waters with nutrients causes harmful algal blooms, depletion of oxygen, and destruction of submerged vegetation. Eutrophication in Chesapeake Bay and many other coastal systems is causing "dead zones" of anoxic and hypoxic waters along deeper bottom areas. A major focus of the restoration efforts of the Environmental Protection Agency's Chesapeake Bay Program has been to reduce nutrient loading by phosphorus and nitrogen runoff into the Bay. Long-term watershed and estuarine water quality monitoring by SERC at the Rhode River site and throughout Chesapeake Bay shows the dynamic interactions of stream discharge, nutrient inputs, and plankton responses affecting oxygen levels.

Watershed nutrient discharge occurs primarily in storm events and is related to both geologic position (e.g., Piedmont or Coastal Plain provinces of the Chesapeake watershed) and land use, especially development and agriculture (Figure 4). Plankton productivity is much higher in years with high runoff, which leads to plankton blooms (Figure 5). Long-term monitoring from 1986 to 2004 shows that water clarity (Secchi disc depth) and near-bottom oxygen levels have declined significantly in the Rhode River subestuary (Figure 6). Although oxygen levels at SERC's long-term monitoring station in the shallow edge of the Bay generally do not fall below alarming levels of approximately 6 ppm, oxygen levels in the deeper mainstem of the Bay drop to very low levels (Hagy et al., 2004) and occasionally spill into the mouth of the Rhode River, killing benthic organisms (A. Hines, personal observations).

With the decline in water clarity, light levels are not sufficient to support growth of seagrasses and other submerged aquatic vegetation, which had largely disappeared from the Rhode River subestuary and much of Chesapeake Bay by the early 1970s. These structured ecosystems are important nursery habitats for fish and crabs in coastal systems such as Chesapeake Bay. Recent SERC research

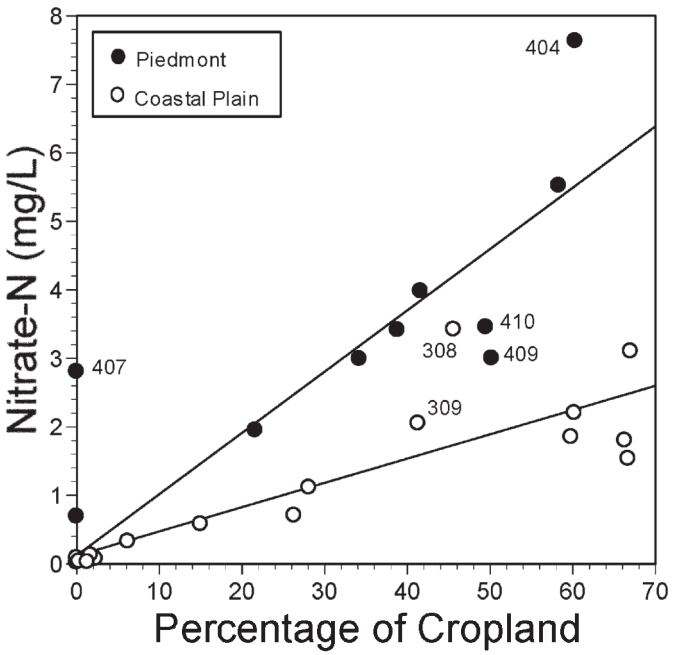

FIGURE 4. Effects of cropland on stream discharge of nitrogen for watersheds in the Piedmont and Coastal Plain physiographic provinces of Chesapeake Bay (see Figure 1). Nitrogen is shown as nitrate concentration on the *y*-axis; cropland is shown as a percentage of land use of the subwatershed area on the *x*-axis. (After Jordan et al., 1997.)

emphasizes the linkage of submerged aquatic vegetation to watershed characteristics (Li et al., 2007).

Grand Challenge III: Food Web Disruption by Pollution and Overfishing

Pollution and overfishing result in severe disruptions of coastal food webs (Jackson et al., 2001). The combined effects of low dissolved oxygen and loss of submerged aquatic vegetation comprise much of the major impact of pollution in coastal systems such as Chesapeake Bay. However, inputs of mercury and other toxic chemicals also markedly affect the food web as they become concentrated at its upper levels, often causing serious effects on seafood that affect human health (Krabbenhoft et al., 2007). Impacts of overfishing and habitat loss have resulted in the loss of sustainable stocks for nearly every fishery species in Chesapeake Bay and in nearly every coastal system worldwide. After a century of intense exploitation, disease, and ecosystem impacts, oysters, as the Bay's most productive

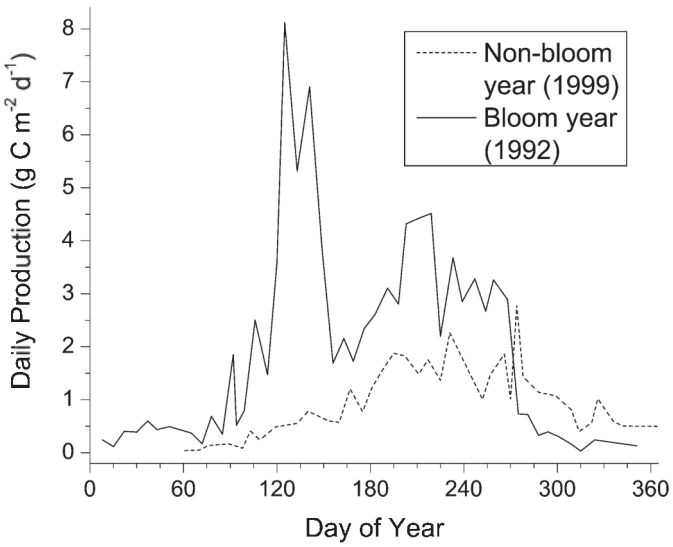

FIGURE 5. Comparison of carbon production in the Rhode River subestuary during two years, one with and one without a spring plankton bloom, which is mainly regulated by variation in spring precipitation and watershed discharge. (After Gallegos and Jordan, 1997.)

fishery historically, are now at only 1% of their biomass in 1900 (Rothschild et al., 1994). Eutrophication and overfishing act as multiple stressors on coastal food webs, and management's too narrow focus on single factors may have adverse consequences for restoring ecosystem health and fishery production (Breitburg et al., 2009).

Blue crabs are the remaining major lucrative fishery in the upper Bay, but the blue crab stock has also declined by 60% since 1991 (CBSAC, 2008). Research by SERC at the Rhode River subestuary provides the most detailed analysis of blue crab ecology available (Hines, 2007). Nearly 30 years of SERC experiments show that blue crabs are the dominant predator on benthic communities in the estuary, and their foraging limits abundance and species composition of infaunal invertebrates as well as causing major bioturbation of the upper 10 cm of sediments (Hines et al., 1990). Long-term monitoring of fish and blue crabs throughout the Rhode River subestuary shows the marked seasonal and annual variations in population abundance (Figure 7), as blue crabs migrate from the nursery habitat and become inactive below 9°C in winter. Annual variation in recruitment into the Rhode River causes more than a 10-fold fluctuation in abundance, with obvious variation in effects of predation on infaunal invertebrates. Many upper Chesapeake Bay nursery habitats now appear to be below carrying capacity for juvenile blue crabs (Hines et al., 2008). Recent SERC blue crab research has focused on de-

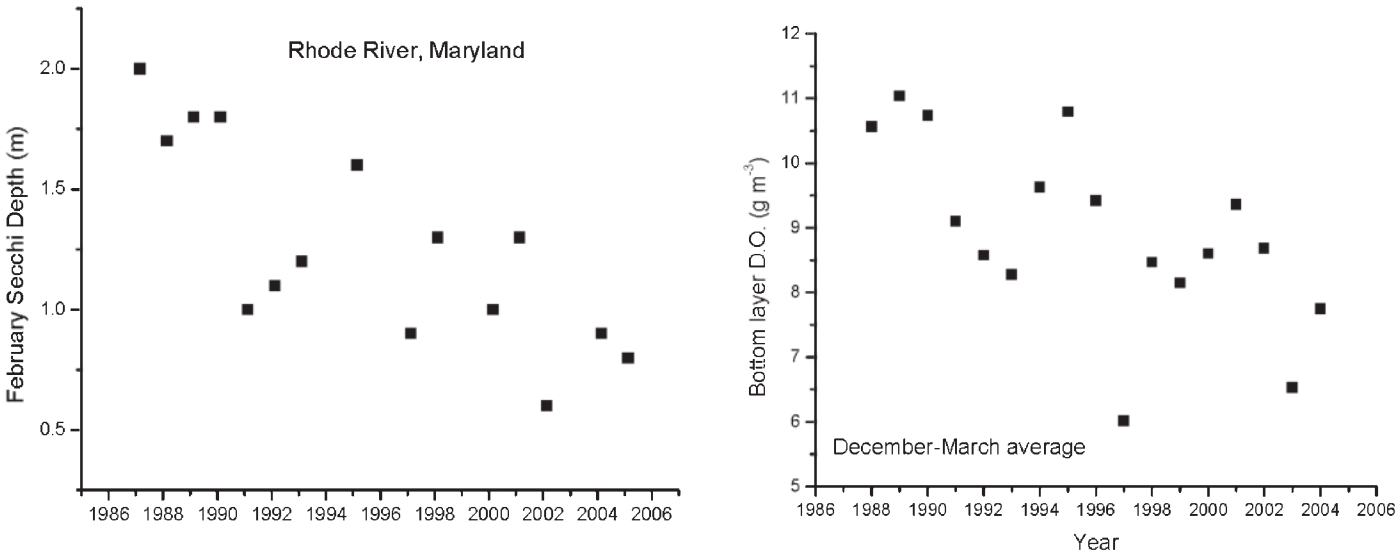

FIGURE 6. Long-term trends in water clarity as determined by Secchi (disk) depth (left) and in oxygen concentration (D.O. = dissolved oxygen; right) in the Rhode River subestuary. (Figure courtesy of C. Gallegos.)

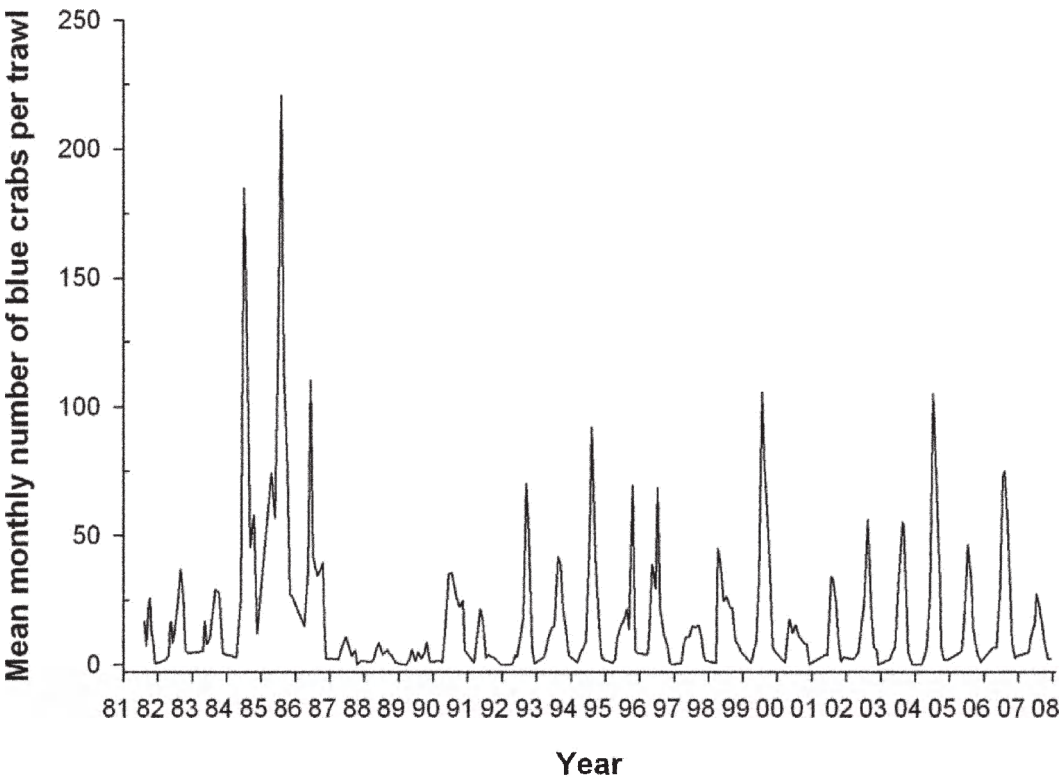

FIGURE 7. Seasonal and annual variation in abundance of blue crabs caught in 3 m otter trawls in the Rhode River subestuary. Abundance is the monthly mean of three trawls at each of three permanent stations within the estuary.

veloping innovative approaches to restoring the blue crab population in the Bay, especially by testing the feasibility of releasing hatchery-reared juvenile blue crabs into nursery areas such as the Rhode River (Hines et al., 2008).

Grand Challenge IV: Invasive Species

Invasions of nonindigenous species are drastically altering biodiversity, structure, and function of coastal ecosystems (Ruiz et al., 2000). The largest, most comprehensive research program on marine invasive species in the USA is conducted by SERC. Rates of invasion into coastal ecosystems are increasing markedly as a result of a wide range of human-mediated vectors, but most importantly as a result of shipping, both ballast water discharge and hull fouling (Ruiz et al., 2000). The SERC database for invasive species (NEMESIS) documents more than 500 invasive species of invertebrates, algae, and fish in North American coastal waters. For Chesapeake Bay approximately 176 species are documented as established invasions (Figure 8). Invasions are dynamic and ongoing in Chesapeake Bay, as indicated by recent records of Chinese mitten crabs (Ruiz et al., 2006). Many species are having large but poorly understood impacts in Chesapeake ecosystems, such as the salt marsh reed *Phragmites australis* (King et al., 2007).

Grand Challenge V: Landscape Disturbance by Agriculture and Development

Agriculture and urbanization are causing widespread modifications of landscape structure. Researchers at SERC recently analyzed various indicators of estuarine habitat quality for 31 Chesapeake subwatersheds that differed in five categories of land use composition: forest, agriculture, developed, mixed agriculture, and mixed developed (Figure 9). These land uses have profound effects on estuarine habitat quality because they increase stormwater runoff and loading of nutrients. Nitrogen discharge into subestuaries of

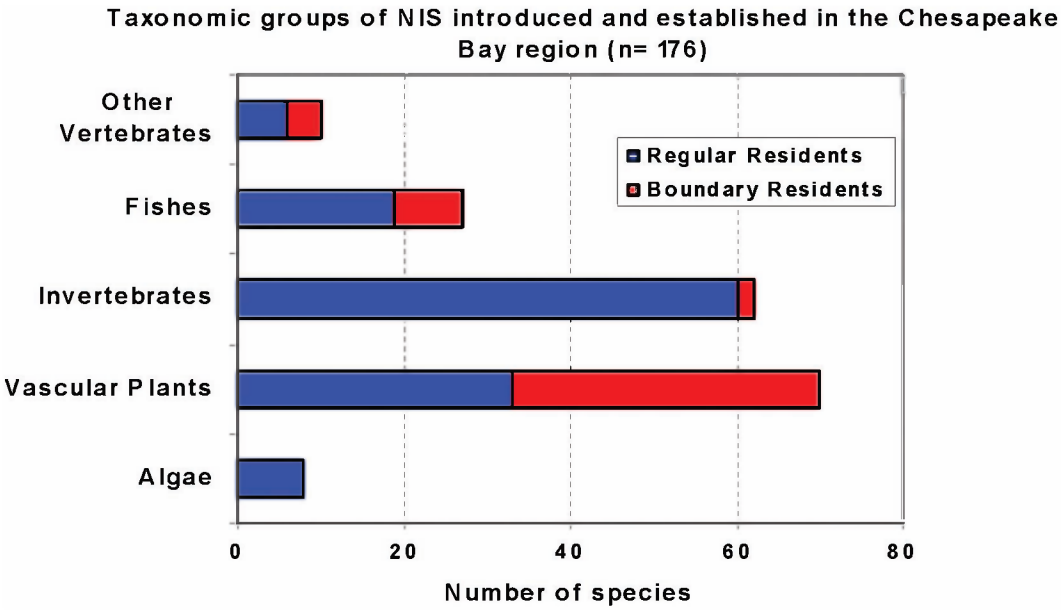

FIGURE 8. Numbers of invasive species documented for algae, vascular plants, invertebrates, fishes, and other vertebrates (total number = 176 species) in Chesapeake Bay. Regular residents are species living in habitats below tidal influence; boundary residents are species primarily living either above the intertidal zone or in non-tidal freshwater and that occasionally move into tidal portions of the Bay. (NIS = noninvasive species.)

agricultural and developed watersheds was high in both wet and dry years, but in dry years it was high only in developed watersheds, which continue to have high human water use regardless of rainfall (Figure 10) (Brooks et al., 2006). Land use also has marked effects on levels of toxic chemicals in the food webs of the subestuaries. Level of polychlorinated biphenyls (PCBs) was highly correlated with percentage of developed lands on the subwatershed (Figure 11).

In addition to effects on the watershed, development of the shoreline has large impacts on coastal ecosystems. Research by SERC in the Rhode River shows that the shallowest fringe of the subestuary serves as a critical refuge habitat for juvenile fishes and crabs to avoid larger predators, which are restricted to deeper water (Ruiz et al., 1993; Hines and Ruiz, 1995). Coarse woody debris from forested shores also plays a valuable role as structural habitat and refuge from predators (Everett and Ruiz, 1993). As development results in cutting down the riparian forest and hardening the shoreline with bulkheads and riprap to prevent erosion, water depth at the shoreline increases and the source of woody debris is lost. With the loss of functional refuge in the nearshore shallows, juvenile fish and crabs become increasingly accessible to predators.

CONCLUSION

The decadal data sets generated by SERC for the linked ecosystems of the Rhode River and Chesapeake Bay clearly show the importance of sustaining long-term, intensive studies to distinguish natural variation and trends of human impacts. The rate of change associated with human impacts is increasing markedly as the effects of global change become manifest and as the human population of the watershed continues to grow rapidly, with another 50% increase predicted in the next 25 to 50 years. The interactive effects of these multiple stressors require much more research to define improved management solutions to restore and sustain these resources. Scientists at SERC also extend studies of the large-scale systems of the Rhode River and Chesapeake Bay through comparative studies with other coastal areas, especially latitudinal comparisons of systems in the Smithsonian Marine Science Network along the western Atlantic. Although each site has its idiosyncratic traits, the common impacts of the grand challenges of atmospheric change, nutrient loading, food web disruption by pollution and overfishing, invasive species, and land development are all manifested pervasively in the linked ecosystems throughout the coastal zone.

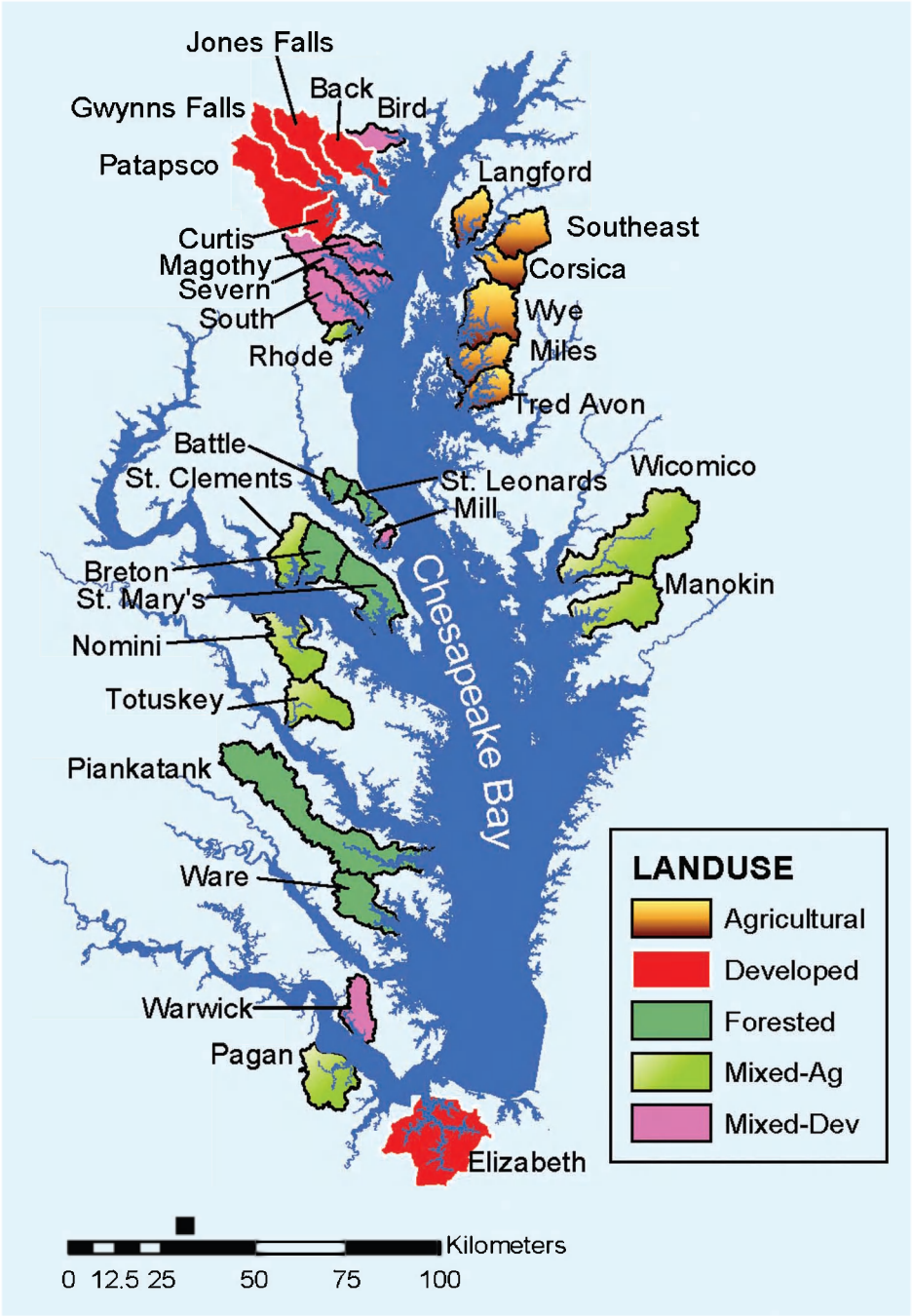

FIGURE 9. Map of 31 subwatersheds of Chesapeake Bay that were sampled for effects of land use on estuarine habitats. Watersheds were categorized in the five predominant categories shown: forest, agriculture, developed, mixed-agriculture, and mixed-developed.

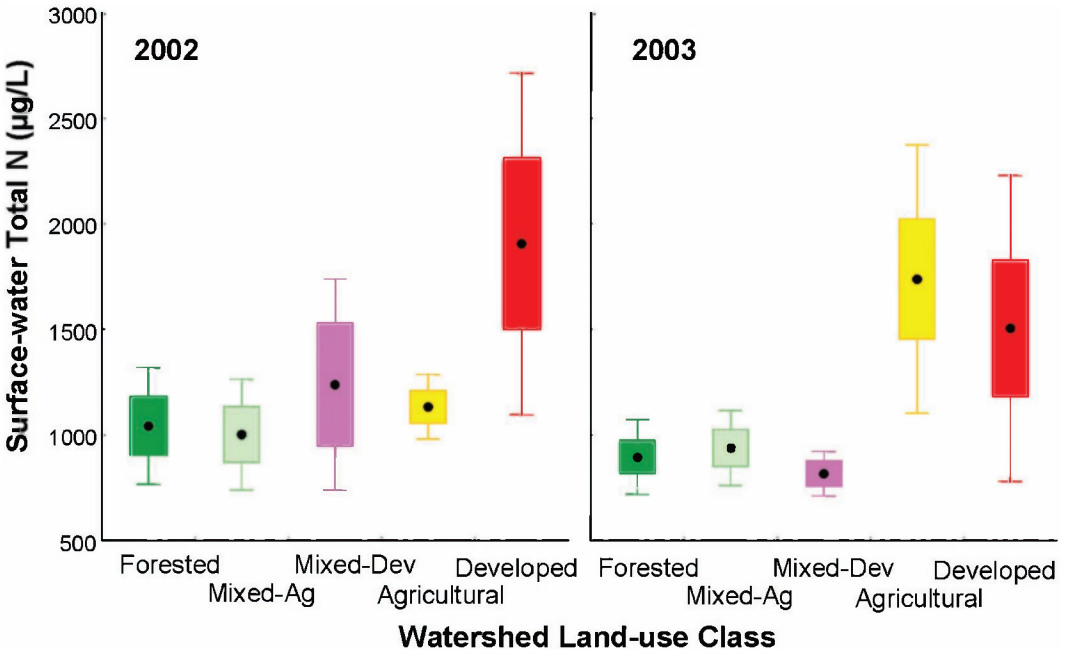

FIGURE 10. Effect of land use on nitrogen discharge from watersheds in the five land use categories shown in Figure 9. Stream surface discharges are compared among land use categories between a dry year with record low rainfall (2002, left) and a wet year (2003, right) with high rainfall. (After Brooks et al., 2006.)

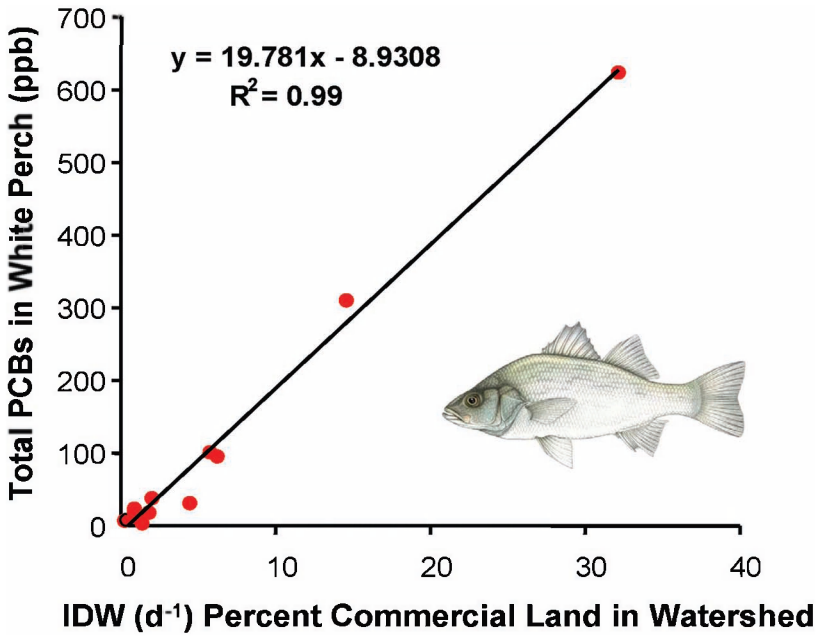

FIGURE 11. Concentration of toxic polychlorinated biphenyls (PCBs) in white perch (*Morone americana*) sampled from Chesapeake subestuaries with watersheds of varying percentages of commercially developed land use (IDW = inverse distance weighted). Watersheds sampled are shown in Figure 9. (After King et al., 2004.)

Acknowledgments

I thank my colleagues at SERC for use of their data and publications to illustrate this paper, especially Denise Breitburg, Bert Drake, Chuck Gallegos, Cindy Gilmour, Tom Jordan, Pat Megonigal, Rick Osman, and Greg Ruiz. I thank the Smithsonian Marine Science Network and the Smithsonian Environmental Sciences Program for long-term funding to SERC's Rhode River research program. Thanks go to Michael Lang for his steady assistance in managing the Marine Science Network and for organizing the symposium and editing these proceedings.

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Smithsonian Marine Station at Fort Pierce: Thirty-Eight Years of Research on the Marine Biodiversity of Florida

Valerie J. Paul, Julianne Piraino, and Laura Diederick

ABSTRACT. The Smithsonian Marine Station at Fort Pierce, located on South Hutchinson Island in Fort Pierce, Florida, has had an ongoing program in the marine sciences since the early 1970s. Funded by a private trust from J. Seward Johnson, Sr., to the Smithsonian, the marine program has supported the research of Smithsonian scientists and their associates, postdoctoral fellows, resident scientists, and the operations of the station, including a small support staff. The station is administered by the National Museum of Natural History as a facility for research dedicated to the marine sciences. The Smithsonian Marine Station at Fort Pierce has developed a strong, broadly based research program focusing on ecology, evolution, systematics, and life histories of marine organisms. Ongoing studies address important issues in biodiversity, including global climate change, invasive species, harmful algal blooms, larval ecology, and evolutionary developmental biology.

INTRODUCTION

The Smithsonian Marine Station at Fort Pierce (SMS) is dedicated to studying the rich diversity of marine life of the Indian River Lagoon and Florida coast. In sharing its findings with the scientific community, resource managers, and the general public, the Marine Station promotes the conservation and stewardship of Florida's vast marine resources. Research activities focus on the Smithsonian Institution's core scientific emphasis of discovering and understanding life's diversity. Although most research projects focus on biodiversity, life histories, and ecology of marine and estuarine organisms, complementary studies of physical and chemical processes related to the marine environment are also part of the Station's investigations. The insights gained by the research conducted at SMS are widely disseminated through scientific publications (more than 780 to date; see complete listing on the Station's website www.sms.si.edu), scientific and public presentations, popular articles, and the media, thus contributing to the broader mission of the Smithsonian Institution for the "increase and diffusion of knowledge."

The Smithsonian's presence in Fort Pierce, Florida, began in 1969 through an association with Edwin Link, an inventor and engineer who was involved at that time in the design of research submersibles, and J. Seward Johnson, Sr.,

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founder of the Harbor Branch Foundation, now known as the Harbor Branch Oceanographic Institute (HBOI) at Florida Atlantic University (Figure 1). In late 1969, the Smithsonian was given two trust funds through the generosity of J. Seward Johnson, Sr., for the development and maintenance of a submersible (then under construction) and for research in underwater oceanography. At its completion in 1971, the submersible, the Johnson Sea-Link I, was donated to the Smithsonian. In 1973, after a tragic accident in the Johnson Sea-Link in which two men died, the Smithsonian transferred ownership of the submersible to Harbor Branch. Following the transfer of the submersible, the Smithsonian's marine research program in Fort Pierce continued to be supported by income from both trust funds, then later, after certain legal resolutions, by one of the two funds, designated as the Hunterdon Fund. The Smithsonian carried out its activities in Fort Pierce on the grounds of the Harbor Branch Foundation (Link Port) under the auspices of the Fort Pierce Bureau, a unit administered directly by the Office of the Secretary and then later by the Assistant Secretary for Science. In March 1981 this Bureau was dissolved as an organizational entity, and the administrative responsibility for the Smithsonian research programs at Link Port was transferred to the Director of the National Museum of Natural History (NMNH). The organization was then retitled by the Secretary of the Smithsonian as the Smithsonian Marine Station at Link Port. At the time of the transfer of administrative responsibility, the directive from the Office of the Assistant Secretary for Science was that a strong research program in marine science should be established and that the program should be open to all marine scientists in the Smithsonian Institution. In response, Richard Fiske, then Director of NMNH, established an inter-unit advisory committee, appointing Catherine Kerby, his administrative assistant, as chair of the committee, and Mary Rice, Department of Invertebrate Zoology (on assignment to the Fort Pierce Bureau), as director of the facility and research programs at Link Port. Rice held this position until her retirement in 2002, at which time Valerie Paul was selected as her successor.

The Smithsonian Marine Station at Link Port was initially set up with a small on-site staff and well-equipped laboratories and field facilities to provide opportunities for Smithsonian scientists and their colleagues to conduct field research in a highly diverse subtropical marine environ-

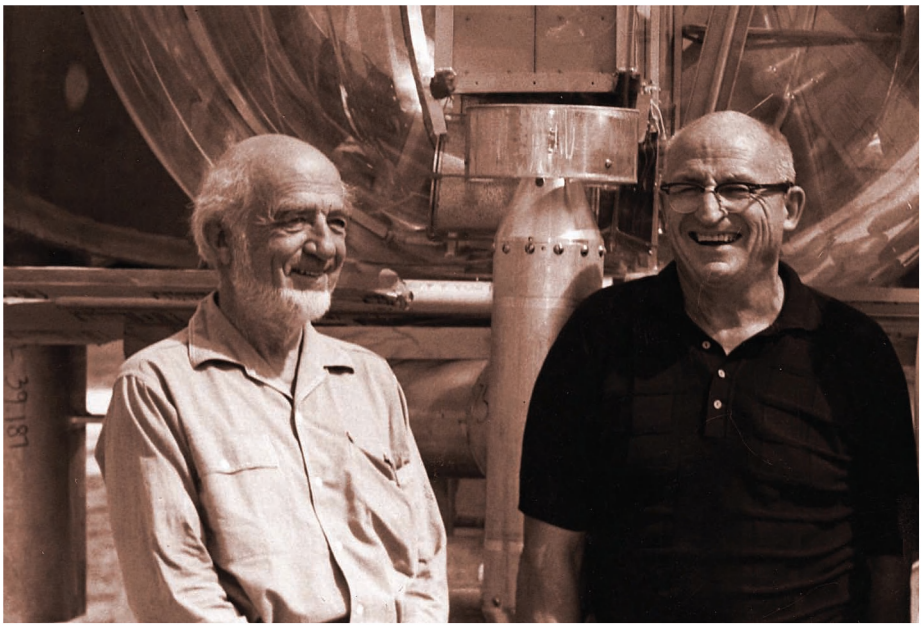


FIGURE 1. J. Seward Johnson, Sr. (left), and inventor Edwin Link were instrumental in providing funding and submersibles for the Smithsonian's marine research program in Fort Pierce, Florida.

ment. This plan gave Museum scientists the opportunity to extend and broaden their research from museum collections to field studies in such areas as behavior, ecology, physiology, and life histories. Moreover, it provided the opportunity for all Smithsonian marine scientists to carry out comparative studies of the diverse ecosystems and biota within the Fort Pierce vicinity and peninsular Florida and, most importantly, to establish long-term databases and to conduct long-term studies. An important component of the plan was to include postdoctoral fellows, both to complement the research of Smithsonian scientists and to contribute to training of future generations of marine scientists. In addition, by serving many Smithsonian scientists (as opposed to a few resident scientists), the program was conceived to yield maximum productivity of high-quality modern science and to be the most equitable and effective use of available funds.

For the first 18 years the Smithsonian Marine Station at Link Port used a vintage WW II barge as a floating laboratory docked at Harbor Branch (Figure 2) as the base of operations for its highly successful research program, which was carried out primarily by visiting scientists from the Smithsonian, their colleagues, and postdoctoral fellows. Restrictions imposed by the space and structural limitations of the barge for many research activities as well as its high maintenance requirements led the Smithsonian to pursue plans for a land-based laboratory.

In May 1999 these plans were realized when, with the approval of J. Seward Johnson, Jr., and a signed Memo of Understanding, the Smithsonian Marine Station relocated to an 8 acre site acquired from the MacArthur Foundation near the Fort Pierce Inlet, 7 miles south of Harbor Branch. At this time the official name of the station was changed to the Smithsonian Marine Station at Fort Pierce. The move was made into a newly constructed 8,000 square foot building with offices and laboratories for visiting scientists, resident staff and postdoctoral researchers, general-use laboratories for chemistry, microscopy, and molecular research (Figures 3, 4), and a wet laboratory supplied by a small seawater system. In March 2003, a 2,400 square foot storage building was completed. The building includes a workshop and storage for scientific supplies, scuba equipment, and other marine research equipment. In April 2004 a research dock was completed on the Indian River Lagoon, which is accessible by an easement on adjacent property. A flow-through seawater building was

FIGURE 2. A retired World War II Army barge was remodeled to include two levels of offices and laboratories for use by the Smithsonian Marine Station scientists from the early 1970s to 1999.

FIGURE 3. This aerial view shows the location of the Smithsonian Marine Station on the Fort Pierce Inlet of the Indian River Lagoon in Florida.

added to the campus in August 2005. The relocation to the new research building and campus provided the opportunity for the Smithsonian Marine Station to increase and strengthen the breadth and diversity of its research as well as to establish new collaborative interactions. The move also made it possible to expand the Station's educational mission, initiating new cooperative ventures in education and public outreach.

In the struggle to understand life, how its diversity has come about, and the current rapid loss of biodiversity on a global scale, the Smithsonian Marine Station is positioned as are few laboratories in the world to study this exceptional diversity from an array of environments. The Smithsonian Marine Station is located on the Fort Pierce Inlet of the Indian River Lagoon (IRL) (see Figure 3), an estuary extending along one-third the length of the east coast of Florida. The IRL is widely recognized as one of the most diverse estuaries in North America, and it has been designated an estuary of national significance by the Environmental Protection Agency. The Marine Station's unique location on the Fort Pierce Inlet puts it in a prime position to access oceanic waters and to sample organisms from the Florida Current and other offshore habitats. This region of Florida's coast, characterized as a transitional zone where temperate and tropical waters overlap, offers access to a great variety of habitats and an extraordinary diversity of species. To the south of Fort Pierce, within a few hours of travel, are Florida Bay and the Florida Keys, the only living tropical coral reefs in the continental United States.

Specialized equipment and instrumentation at the Smithsonian Marine Station include temperature-controlled aquaria and incubators, equipment for preparing tissues for light and electron microscopy, an ultracold freezer, equipment for electrophoresis, a thermocycler for DNA amplification, high performance liquid chromatographs, a gas chromatograph/mass spectrometer, and a UV-visual spectrophotometer. For microscopic studies, equipment is available for light, epi-fluorescent, and Nomarski microscopy, time-lapse and normal-speed cinematography, photomicrography, video recording and editing, inverted microscopy, scanning and transmission electron microscopy (Figure 5), and confocal laser scanning microscopy.

Confocal laser scanning microscopy (CLSM) has become an increasingly important tool in modern environmental microbiology, larval ecology, developmental biology, and biochemistry. CLSM involves the use of a light microscope, laser light sources, a computer, and special software to image a series of in-focus optical sections through thick specimens. The specimens, which can be live or fixed, are stained with fluorescent dyes that highlight specific structures when excited by the lasers. Once the stacks of two-dimensional (2-D) images are collected, the computer software constructs spectacular, information-rich, three-dimensional (3-D) images that yield a wealth of information. In June 2008, the Smithsonian Marine Station acquired a Zeiss LSM510 confocal system that is already providing data in the cutting-edge studies of Postdoctoral

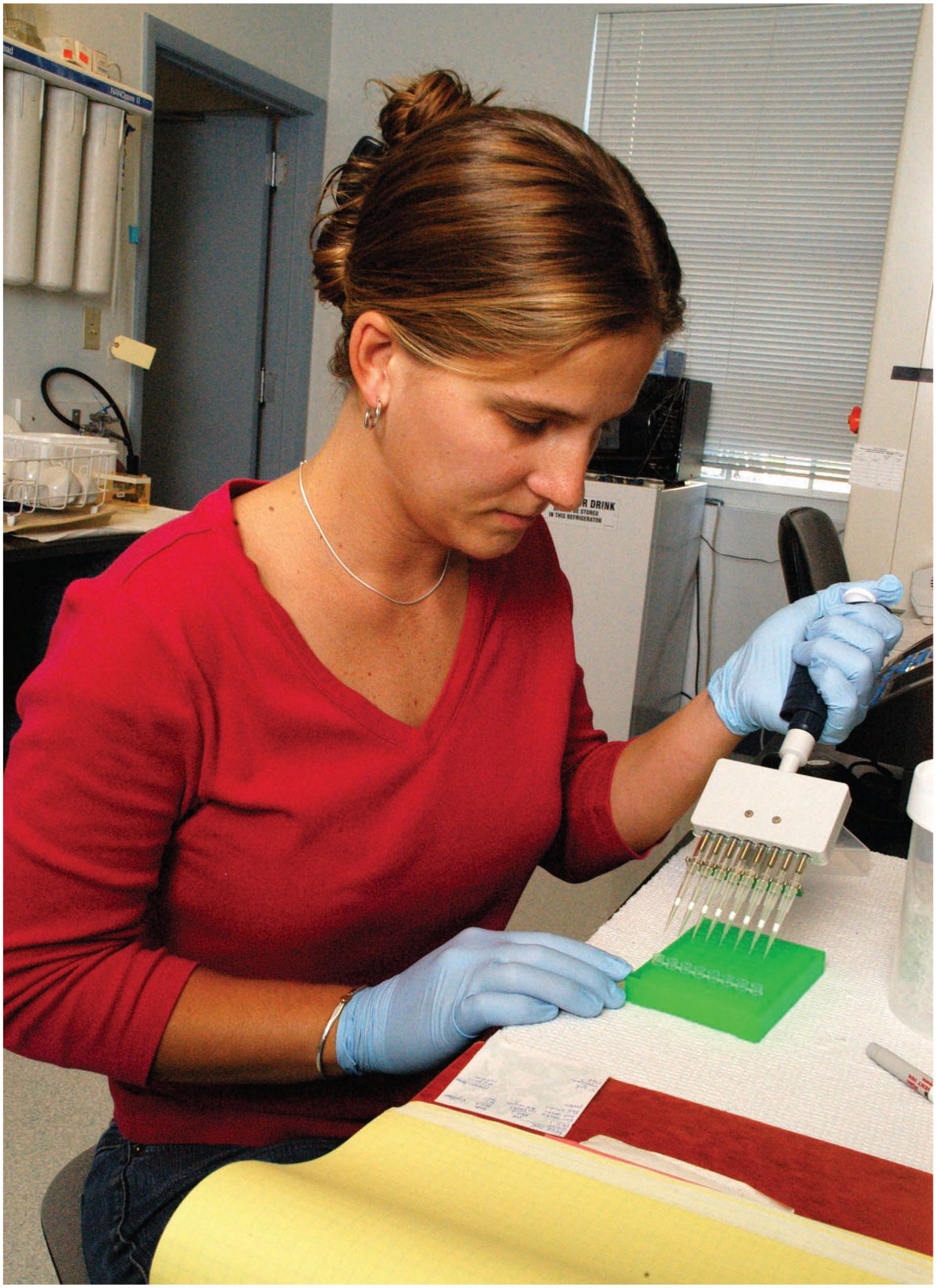


FIGURE 4. Smithsonian Marine Science Network Postdoctoral Fellow Koty Sharp uses molecular methods to determine the diversity of bacteria associated with corals.

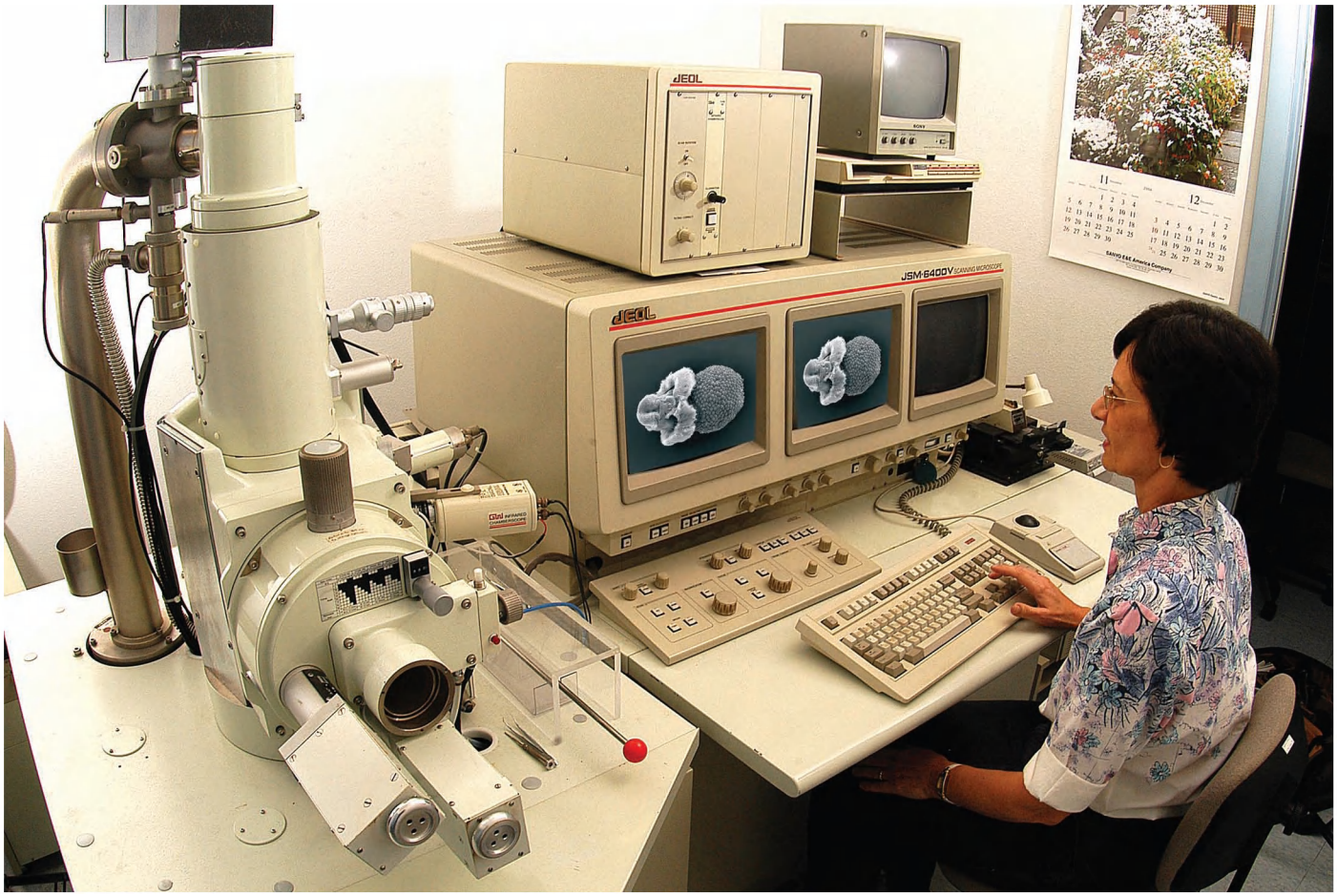


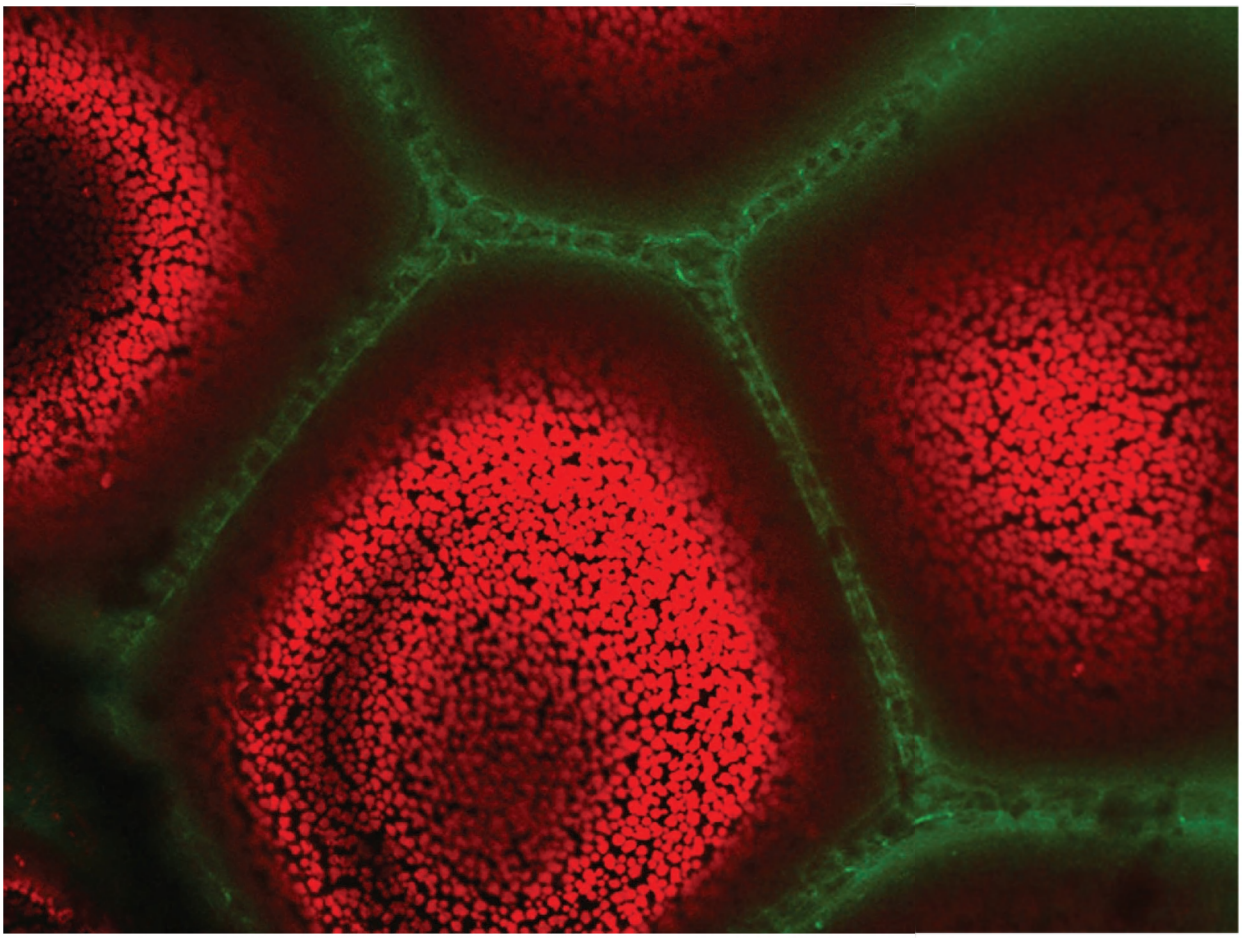
FIGURE 5. Julie Piraino, Laboratory Manager, examines the larva of a sipunculan worm on the scanning electron microscope.

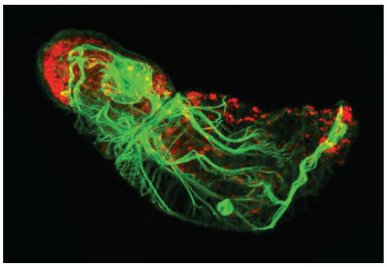
Fellow Koty Sharp on the presence and transmission of bacteria in corals, and in research conducted by Postdoctoral Fellow Kate Rawlinson on the fate of individual cells in the development of embryos of polyclad flatworms. This new microscope will greatly increase the capabilities of Smithsonian marine scientists to conduct probe-based subcellular studies in biochemistry, microbiology, and developmental biology (Figure 6).

The Marine Station owns four boats for use in field studies: a 17 foot Boston Whaler and a 21 foot Carolina Skiff for work in the shallow waters of the IRL, a 21 foot center-console boat to access nearshore waters, and a 39 foot vessel, the R/V *Sunburst*, for offshore research activities. These vessels provide access to the diverse marine and estuarine environments in the vicinity of SMS. The excellent location, facilities, instrumentation, and skilled staff of the Smithsonian Marine Station facilitate research on many diverse topics in marine biology and marine biodiversity.

RESEARCH ACTIVITIES

The Smithsonian Marine Station at Fort Pierce is an important contributor to the marine research and collections at the National Museum of Natural History. It provides a vital link between tropical and temperate ecosystems in a coastal network of marine research stations known as the Smithsonian Marine Science Network. The Marine Science Network (MSN) is an array of laboratories spanning the western Atlantic coastal zone and across the Isthmus of Panama, facilitating long-term interdisciplinary, comparative research among MSN sites, including the Smithsonian Environmental Research Center (SERC)





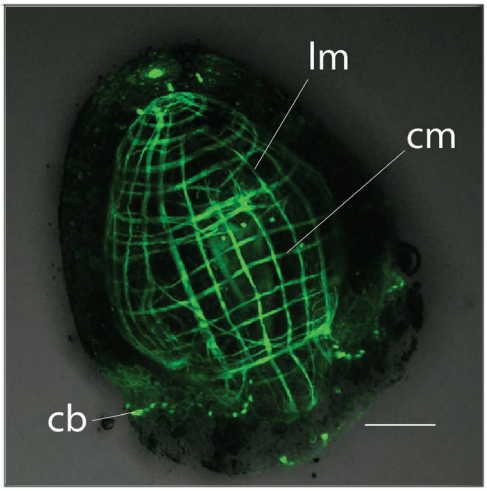


FIGURE 6. Left: Confocal microscopy captures an oxidative burst reaction by the green alga *Dictyosphaeria cavernosa* following exposure to the fungus *Lindra thalassiae*. An oxidative burst is an explosive production of reactive oxygen species (hydrogen peroxide is an example) intended to act as first defense against invading pathogens. Middle: Confocal image shows development of musculature and nervous system in a larva of a sipunculan worm. Right: Confocal laser scanning micrograph of the musculature of a Müller's larva of the flatworm *Cycloporus variegatus*. Phalloidin staining shows circular and longitudinal muscles (cm, lm, respectively) and the ciliary band (cb). Scale = 30 μm.

in Maryland, the Carrie Bow Cay Marine Field Station in Belize, and the Smithsonian Tropical Research Institute (STRI) in Panama.

Research at SMS continues to be carried out by Smithsonian scientists from various units within the Institution along with their colleagues from other national and international institutions, as well as by resident SMS scientists, postdoctoral fellows, and graduate students (Figure 7). Ongoing research programs by resident scientists at the Smithsonian Marine Station involve coral reef research,

FIGURE 7. Visiting Scientist Anastasia Mayorova (kneeling) collects sipunculan worms with the assistance of Mary Rice, Director Emeritus of SMS (left), and Research Technician Woody Lee.

monitoring that is supporting restoration of the Florida Everglades, harmful algal blooms, marine natural products, and invertebrate larval life histories, evolution, and development. The Smithsonian Marine Station promotes the education of emerging scientists by offering pre- and post-doctoral research fellowships and supporting the work of student interns. Examples of ongoing research activities are discussed below.

Marine Biodiversity

The Smithsonian Marine Station has long had a central focus on documenting biodiversity of marine life in the most diverse coastal waters of the continental United States. NMNH invertebrate zoologist David Pawson has discovered and documented echinoderms (sea urchins, sand dollars, sea cucumbers) in shallow and deep waters of Florida for more than 25 years (Hendler et al., 1995). He has found sand dollars that are probably hybrids between two species in the offshore waters of Fort Pierce. Other groups of organisms that have been well studied by NMNH researchers for many decades include the marine algae (Mark and Diane Littler), foraminifera (Marty Buzas), crustaceans (Rafael Lemaitre and colleagues), deep- and shallow-water mollusks (Jerry Harasewych and Ellen Strong), and meiofaunal organisms (animals less than 1 mm in size that live in sand and sediments) (Jon Norenburg and coworkers). Additionally, many SMS scientists, including former director Mary Rice, have focused on understanding the diversity and distribution of larval forms of different groups of marine invertebrates. These larval stages are morphologically and ecologically very different from adult life stages and are extremely important for the transport and propagation of marine species, sometimes over long distances (Figure 8). Tuck Hines and Richard Osman (SERC) have also studied recruitment patterns and larval ecology for a variety of invertebrate larval forms in the IRL. A few examples of the many biodiversity studies conducted at SMS are highlighted below.

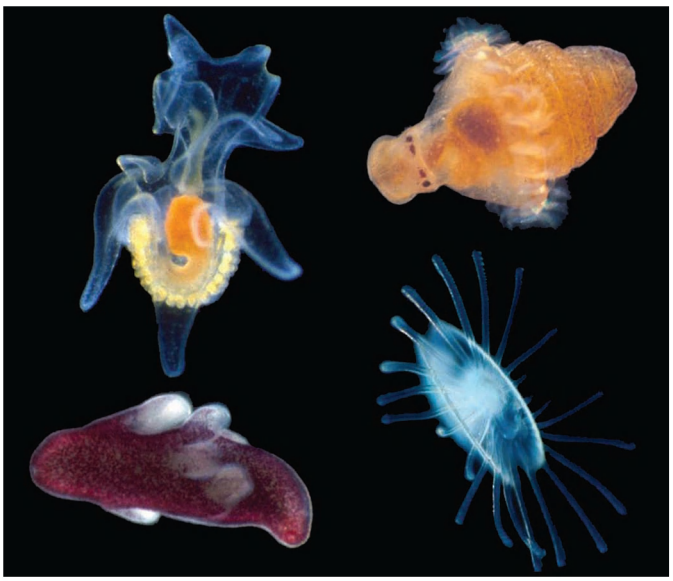


FIGURE 8. Examples of marine invertebrates, the larval development of which has been studied by Mary Rice and colleagues at SMS for more than 30 years. Organisms shown here, clockwise from upper left, are a starfish brachiolarian larva, a sipunculan pelagosphera larva, a brachiopod larva, and a flatworm Muller's larva.

With much of their research focused on ecology, physiology, and pollution-oriented work, Mark and Diane Littler observed a growing need for an easier means for field scientists and resource managers to identify the diverse and abundant marine algal species in the field. They have published user-friendly field guides, including the award-winning book *Caribbean Reef Plants* (Littler and Littler, 2000), with much of their laboratory and field research based at SMS. More recently, with co-author M. Dennis Hanisak (Harbor Branch Oceanographic Institute), they published *Submersed Plants of the Indian River Lagoon: A Floristic Inventory and Field Guide* (Littler et al., 2008), a book rich with photography and illustrations depicting the taxonomy and distributional patterns of more than 250 species of submersed plants in the Indian River Lagoon. The book was based on six years of field and laboratory work along the central east coast of Florida.

D. Wayne Coats, a protistan ecologist at the Smithsonian Environmental Research Center, has worked on the biology and ecology of free-living and symbiotic protists for 20 years. His work has enabled comparisons between the Chesapeake Bay and Indian River Lagoon estuaries and provided enhanced understanding of how eukaryotic microbes influence the structure of marine food webs (Snoeyenbos-West et al., 2004). Much of Coats' work at SMS has considered the biodiversity and trophic biology of protists living in coastal waters of Florida or associated with local marine fauna. He has shown parasitism of planktonic ciliates in the Indian River Lagoon to be a major pathway for recycling material within the microbial loop. Coats and his graduate students have also shown that many free-living photosynthetic dinoflagellates have the ability to feed on ciliate protozoa. Although ingestion rates are typically low, the high densities attained by red-tide dinoflagellates in the Indian River Lagoon and Chesapeake Bay make their ability to ingest ciliates an important microbial food web interaction. Feeding on ciliates and other protists may help sustain blooms when nutrient resources for photosynthesis are limited. Coats and his colleagues have also revealed a rich and poorly known ciliate fauna associated with the respiratory tract of bottle-nosed dolphins and other cetaceans (Ma et al., 2006). Previously reported to be parasitic, these ciliates appear not to directly impact the health of animals held in captivity. Through his work at SMS, Coats has helped define the significance of protists within the marine ecosystem. In some instances, these protists compete directly with zooplankton for food resources, thus limiting the upward movement of energy and matter in the food web. In other instances, they can recycle biomass not readily grazed by zooplankton, thus repackaging it in a form that can move more readily through the food web.

Mary Rice, former director of SMS, established a program of life history studies more than 30 years ago involving numerous postdoctoral fellows and visiting scientists who have worked on a variety of marine invertebrates. Her research has focused on an enigmatic group of marine worms known as sipunculans. Presumably a primitive group related to annelids and mollusks, sipunculans are unique in their complete lack of segmentation and single unpaired ventral nerve cord. One of several objectives of her studies has been the use of developmental studies to understand phylogenetic affinities both within the group and with other spiralian phyla (Schulze and Rice, 2009). Other objectives have been comparative studies of reproductive biology and ecology of shallow-water and deep-sea species, an investigation of the biology of oceanic larvae, including their metamorphosis and their role in species distribution, and a systematic survey of the Sipuncula of Florida and the Caribbean.

In studies of reproductive biology, comparative information was gathered for numerous species on gametogenesis, spawning, egg sizes, egg maturation, fertilization, and reproductive seasonality (Rice, 1989). Year-long observations of reproductive activity in *Phascolion cryptum*,

a small species inhabiting discarded gastropod shells in the subtidal waters of the Indian River Lagoon, revealed that—in contrast to temperate species—animals were reproductive throughout the year. A collaborative ultrastructural study of spermiogenesis was also conducted. The most abundant sipunculan of the Indian River Lagoon, this species was recorded in densities up to 2,000 to 3,000 per square meter. No longer found in these densities, the population has declined for reasons unknown (Rice et al., 1983).

Studies of larval biology by Rice and collaborators have concentrated on the oceanic pelagosphera larvae of sipunculans that occur in abundance in the Florida Current, a component of the Gulf Stream System that flows along the edge of the Continental Shelf offshore from Fort Pierce. Reported in warm water currents throughout the world's oceans, these larvae are known to be long lived, existing in the larval stage for 6 to 7 months, and hence to have the potential for widespread species dispersal (Rice, 1981). Continuing for more than three decades, the studies have included descriptions of the various larval morphotypes through light and scanning microscopy, as well as an investigation of factors inducing metamorphosis and the identification of species by rearing larvae to adulthood. Several of the larvae were identified by rearing, surviving in the laboratory for periods of 3 to 26 years as adults. In more recent collaborative studies (with Postdoctoral Fellow Anja Schulze and staff of the NMNH Laboratory of Analytical Biology), genomic analysis, comparing larval and known adult sequences, was utilized to identify additional larval types. These analyses suggested the presence of two cryptic species, characterized by morphologically similar adults but different larval types.

Jon Norenburg (NMNH), together with students, postdoctoral fellows, and collaborators, has been and is focused on discovering nemertean diversity in Florida and using that diversity to address broader questions. In short visits over the course of 20 years they have collected as many as 70 putative species, primarily from the shoreline and shallow coastal waters, from a region with 24 previously known species. Many of the additional species are potential range extensions that await confirmation with specimens from type locales, especially those in southern Brazil, which is the nearest subtropical nemertean fauna that also is well documented. There also are tantalizing preliminary data for close genetic links with European species (Maslakova and Norenburg, 2008). New species have been named, and another 10 to 15 potential new species await additional specimens or genetic work. Most nemerteans have few to no external diagnostics to characterize and discriminate species unambiguously. Almost all the species collected in Florida by Norenburg and coworkers in the past 15 years were processed with genetic work in mind, which will resolve some questions of identity and yield realistic estimates of true diversity and contribute important samples for studying diversification of the phylum (Thollesson and Norenburg, 2003). That effort in Florida is an important component of two global-scale nemertean projects headed by Norenburg: (1) diversity and coevolution of the specialized, ectosymbiotic carcinonemertid worms with their decapod crustacean hosts (mostly crabs), and (2) phylogeny and biogeography of *Ototyphlonemertes*, which are specialized and miniaturized worms occupying the aqueous pore space in coarse sediments, such as coarse sand beaches and in high-current subtidal habitats. Norenburg's study of nemerteans in Florida has contributed important original observations about developmental biology of nemerteans, and one species in particular has revolutionized our understanding of nemertean evolution (Maslakova et al., 2004).

Carole Baldwin and Lee Weigt from the National Museum of Natural History are studying fish diversity, including larval fishes, through DNA barcoding methods. Fish taxonomists have traditionally classified fishes based on morphological features that can be seen and described. However, many families of fishes, such as parrotfishes and gobies, have members that look so similar they are virtually indistinguishable without examining the genetic material. Baldwin and her research team have now cataloged more than 200 species (from more than 1,000 specimens) from the Indian River Lagoon. Processing involved identifying and measuring each fish, photographing its live coloration, taking a tissue sample for DNA analysis, and preserving the rest of the specimen as a voucher for NMNH archival collections. Tissue samples from each specimen were used to create a DNA barcode, which is unique to the individual fish species and can be used for identification purposes. Not only will this work be important for establishing a database of genetic information for fishes of the Indian River Lagoon, it will greatly increase our understanding of shorefish diversity. The overall goal of the work is to provide a new, more realistic estimate of species diversity in the Caribbean, Florida, and adjacent areas. Having amassed DNA extractions from fishes from a variety of taxa and from multiple localities in the tropical Atlantic, the investigators can now examine interspecific phylogenetic relationships to investigate patterns of speciation and potential patterns of morphological divergence accompanying speciation.

Important reasons often cited for understanding biological diversity are the possible benefits these species might yield as foods, medicines, or for other human uses. Valerie Paul, Director of SMS, and members of her research group investigate the chemical diversity of marine organisms by studying marine natural products, small molecules produced as chemical signals or as toxins or chemical defenses. Members of Paul's research team isolate and characterize natural products from Florida's marine life (seaweeds and invertebrates) and have discovered compounds that have never previously been found in nature. A current area of interest for her research group is the biodiversity and chemical diversity of benthic marine Cyanobacteria. Through collaborations with medicinal chemists, they are actively investigating the beneficial uses of these compounds for treatment of human diseases such as cancer and bacterial infections.

Marine Ecology

Valerie Paul studies marine plant–animal interactions in coral reef habitats. Coral reefs in Florida and throughout the world are declining, in part the consequence of shifts from coral- to algal-dominated communities. Paul and members of her research team study grazing by reef fishes and sea urchins and the effects of herbivory on coral reef community structure (Paul et al., 2007). They have found that chemical defenses of marine algae allow some well-defended marine plants to dominate on coral reefs despite grazing pressure. Key to the recovery of coral reefs is the successful recruitment of coral larvae to become juvenile and eventually adult corals (Ritson-Williams et al., 2009). Paul's research group and their collaborators examine positive and negative interactions between coral larvae and the marine algae that dominate coral reef habitats. Some of the same species of algae that are chemically protected from grazers can inhibit the settlement of coral larvae, thus preventing the successful recovery of coral reefs.

Algae are an essential part of marine ecosystems and when maintained in balance can provide food, shelter, oxygen, and more to millions of organisms, including people. But some algae can produce harmful toxins and, under certain conditions, can grow out of control. These so-called harmful algal blooms have been increasing in frequency and severity along the world's coastlines. NMNH scientist Maria Faust has been investigating the types of planktonic harmful algae, often called red tides, which occur along Florida's east coast (Faust and Tester, 2004). Valerie Paul, NMNH Statistician Lee-Ann Hayek, and Postdoctoral Fellows Karen Arthur and Kate Semon have been studying formation of blooms of marine cyanobacteria in Florida's estuaries and coral reefs and trying to elucidate environmental factors that contribute to bloom formation (Paul et al., 2005). Increased nutrients from land-based sources, such as runoff from fertilizers and sewage treatment plants, may help to fuel some of these algal blooms. The biological and biochemical diversity of harmful algae is the subject of ongoing research, which has led to the discovery of novel toxins produced by these cyanobacteria.

Estuaries and coasts around the world are approaching critical levels of degradation, and the southern Indian River Lagoon is no exception. Large-scale, collaborative efforts are underway to restore biodiversity and the vital ecological functions these ecosystems provide. Bjorn Tunberg and members of his benthic ecology research team at SMS are involved in one of the most ambitious of these projects, the Comprehensive Everglades Restoration Plan (CERP). Extensive modifications to the southern IRL watershed over the past 100 years have decreased the system's ability to store water and have increased nutrient-rich stormwater runoff. The CERP plan, under the direction of the South Florida Water Management District and the U.S. Army Corps of Engineers, aims to restore wetlands and build water storage basins to improve estuarine health. Tunberg and his team established a benthic monitoring program five years ago to provide a baseline data set of species distribution and abundance in the sediments of the Indian River Lagoon. This team is acquiring quarterly data that will allow them to detect and predict long-term changes in the benthic communities throughout the central and southern Indian River Lagoon.

The location of the Smithsonian Marine Station on the Indian River Lagoon for 37 years has allowed Smithsonian researchers to establish long-term and intensive research projects that are valuable in understanding and assessing marine biodiversity. Long-term biological monitoring is most effectively carried out on organisms with high densities, many species, short generation times, quick responses to changes in environmental variables, and a long history of extensive study on a worldwide basis. The benthic foraminifera fit these requirements, and their populations have been monitored in the Indian River Lagoon for more than 30 years by Marty Buzas (NMNH).

At one station near the Harbor Branch Oceanographic Institute, monthly replicate sampling of foraminifera living in the sediment has been carried out since 1977. These data indicate significant differences between seasons, as well as among years, but no overall increase or decrease

over a longer time span. The spatial distribution of the foraminifera forms an environmental mosaic of patches whose densities change with time. This newly discovered phenomenon was termed pulsating patches (Buzas et al., 2002; Buzas and Hayek, 2005). At the St. Lucie Inlet, observations were made in 1975–1976 and again 30 years later in 2005. Species richness had greatly declined over 30 years, and the community structure of the foraminifera in this area was completely destroyed (Hayek and Buzas, 2006). Monitoring at this Inlet during 2007–2008 has shown that species richness has increased; however, except for the abundant species, the fauna does not contain the same species as it did 30 years ago. Monitoring efforts are continuing, and Buzas and Hayek have also begun a coring program to determine the effects of both natural and anthropogenic effects on community changes during the past 150 years.

Candy Feller, Dennis Whigham, coworkers from the Smithsonian Environmental Research Center (SERC), and national and international collaborators have conducted long-term studies of the mangrove ecosystems of the Indian River Lagoon. The overall goal of this project is to collect hydrological, nutrient, microbial, and vegetation data in support of their long-term ecological studies of factors that control the structure and function of mangrove ecosystems (Figure 9, top). Feller has continued a study of how nutrient enrichment affects the mangrove communities along the Atlantic coast of Florida for the past 10 years. Fertilization experiments designed to enrich nitrogen (N) and phosphorus (P) in sediments have shown that black mangrove forests in Florida are nitrogen limited. When nitrogen was added in the IRL, the black mangroves grew out of their dwarf form (Feller et al., 2003). Addition of N also affected internal dynamics of N and P, caused increases in rates of photosynthesis, and altered patterns of herbivory (Lovelock and Feller, 2003). These findings contrast with results for mangrove forests in Belize and Panama where the seaward fringe was N-limited but the dwarf zone was P-limited. Their studies have demonstrated that patterns of nutrient limitation in mangrove ecosystems are complex, that not all processes respond similarly to the same nutrient, and that similar habitats are not limited by the same nutrient when different mangrove forests are compared (Lovelock et al., 2006; Feller et al., 2007).

Feller and her colleagues have also studied the effects of the 2004 hurricanes Frances and Jeanne on the mangrove communities (Figure 9, bottom). Over the past 4 years they have continued to monitor and quantify the recovery of the mangroves, documenting tree height, leaf area index, mangrove type, mangrove defoliation and recovery, hydrology, and salinity. Damage to the mangroves was higher in the fringe and transition zones than in the dwarf zone. The N-fertilized trees sustained significantly higher damage than controls in all zones and have been slower to recover. After 2.5 years, the leaf area index (LAI) of P-fertilized and control trees was equal to pre-storm levels, whereas +N trees were less than 90% recovered. LAI again decreased dramatically in January 2007, presumably as the result of an intense 2 year drought in Florida.

Dennis Whigham and colleagues from the University of Utrecht, The Netherlands Institute for Ecology–Centre for Limnology, and the University of South Florida are determining the relationships between the structure and productivity of different mangrove habitat types and hydrological processes and nitrogen cycling, including characteristics of the microbial community associated with nitrogen cycling. Their hydrological studies have shown that there is no evidence of freshwater input from groundwater into their study site and that the groundwater chemistry is primarily influenced by evapotranspiration. Subsequently, salt pans and dwarf mangrove communities develop in areas that are characterized by hypersaline conditions associated with evapotranspiraton. Growth rates are lower in the salt pan and dwarf mangrove habitats, and preliminary results indicate that the microbial community in those habitats differs from other habitats.

The studies described above document the morphological, genetic, and biochemical diversity of Florida's marine life. Collectively, these biodiversity and ecological studies and investigations of long-term changes in Florida's coastal waters, including the Indian River Lagoon, mangrove ecosystems in Florida, and coral reef habitats of southeast Florida and the Florida Keys, give us the background essential to document changes in biodiversity resulting from human and climatic impacts on Florida's coastal environments.

EDUCATION AND OUTREACH

As a resource for educators, students, researchers, and the public, the Marine Station maintains a species inventory of plants and animals in the Indian River Lagoon. The Indian River Lagoon Species Inventory website (www.sms.si.edu/IRLspec) is continually expanding and now includes more than 3,000 species with many photographs and scientific references. In addition to individual species

FIGURE 9. Top: From front to back, Smithsonian Marine Station graduate fellow Juliane Vogt (University of Dresden), postdoctoral fellow Cyril Piou, and volunteer Rainer Feller explore the mangrove at Hutchinson Island, Florida, looking for light gaps. Bottom: Sharon Ewe (on left) and Anne Chamberlain examine damage to the mangroves at Hutchinson Island, Florida, immediately after Hurricane Jeanne.

reports that give habitat, distribution, life history, population biology, physical tolerance, and community ecology information, the database includes information on nonnative and endangered, threatened, and special-status species. An electronic companion publication to the Species Inventory is the Field Guide to the Indian River Lagoon (www.sms.si.edu/IRLfieldguide). Both projects have been supported by the Indian River Lagoon National Estuary Program administered by the St. Johns River Water Management District. Features such as an interactive glossary, enhanced indexing, and links to other relevant websites add to the educational value of these websites.

The Smithsonian Marine Ecosystems Exhibit in the St. Lucie County Marine Center celebrated its seventh anniversary in August 2008. Administered by the Smithsonian Marine Station, the exhibit showcases the Caribbean coral reef ecosystem that was a popular exhibit at the National Museum of Natural History for more than 20 years and the first living model of an Atlantic coral reef ecosystem available for public viewing. Through an outpouring of local interest and support, the exhibit was transferred to Fort Pierce to a building constructed and maintained by St. Lucie County for the sole purpose of housing this educational attraction. At the Ecosystems Exhibit, visitors are invited to explore six Florida marine habitats and learn about the complexity and importance of these ecosystems (Figure 10). The largest aquarium houses a Caribbean coral reef display. Additional aquaria depict a seagrass bed, red mangrove coastline, estuarine and nearshore habitats, and a deep-water *Oculina* coral reef. Smaller aquarium displays highlight single species of interest, and a touch tank offers visitors personal interaction with various local invertebrates, such as horseshoe crabs, sea urchins, sea cucumbers, and peppermint shrimp.

This public aquarium is unlike any other, providing an accurate representation of the underwater worlds of the Indian River Lagoon and Atlantic Ocean. Although these waters are a common sight to many coastal Florida residents, few have experienced the unsurpassed diversity of life just below their surface. By highlighting this diversity and displaying local ecosystems as complex communities of organisms interacting in their environments, the Exhibit aims to provide the public with a better understanding of the fragile coastal ecosystems of the Indian River Lagoon and the surrounding area, including the impacts people have on them.

The Smithsonian Marine Ecosystems Exhibit is a field trip destination for thousands of school-aged children each year (Figure 11). Although some choose a self-guided visit, most participate in one of several structured programs facilitated by Education staff members. Program options are age- and grade-appropriate and are structured in compliance with Florida's Sunshine State Standards. Activities include scavenger hunts, water quality experiments, food web and energy transfer studies, simulated benthic sampling, and field experiences in the Indian River Lagoon.

In 2005, education staff at the Exhibit began offering community and visitor programs. Ranging from informative breakfast programs to sleepovers and summer camps, the new programs target traditional visitor groups in new ways, providing more focused and in-depth learning experiences for those interested in taking advantage of the many resources the Smithsonian has to offer. The enthusiastic response from the community has resulted in continual additions to the events calendar.

In addition to being a physical destination for the local community, the Ecosystems Exhibit has also established itself as a valuable resource for local schools and community organizations that do not have the means to travel. Education staff provides classroom outreach programs, bringing the wonders of the underwater world to hundreds of students each school year. Education staff members have also developed Resource Loan Kits for area teachers to borrow for in-classroom use for a two-week time period. The Exhibit website also hosts three webcams that provide live feeds to three of the Exhibit's displays. Online visitors have alternate, unparalleled views into the seagrass and coral reef model ecosystems, as well as through the lens of a laboratory microscope. Future plans include the development of online curricula and activities based on observations made via the webcams.

LOOKING TO THE FUTURE

During the past 37 years the Smithsonian Marine Station at Fort Pierce has developed a strong, broadly based program in marine biodiversity research focusing on systematics, ecology, and life histories of marine organisms. With nearly four decades of research along the IRL, the Smithsonian Marine Station has been able to establish long-term and intensive research projects that are valuable in understanding and assessing marine biodiversity as well as the changes in biodiversity occurring on a global scale. As a result of its excellent location, modern facilities, and experienced staff, the Marine Station is well positioned to continue to address important research topics including global climate change, invasive species, harmful algal blooms, systematics, larval ecology, and evolutionary developmental biology.

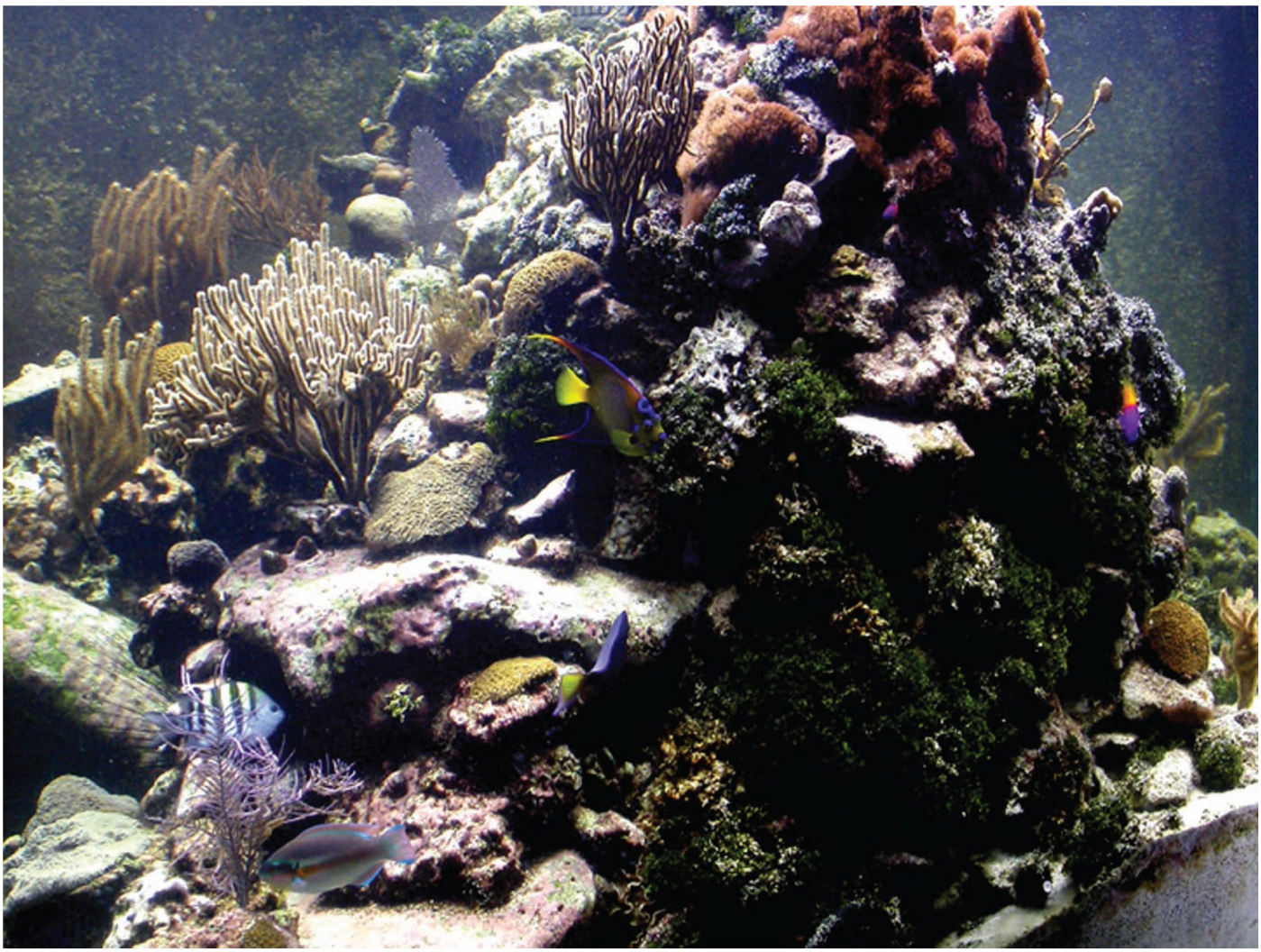


FIGURE 10. The coral reef at the Smithsonian Marine Ecosystems Exhibit.

The Smithsonian Marine Ecosystems Exhibit makes the work of Smithsonian marine researchers accessible to a broad, non-scientific audience. The living displays capture the dynamic quality of natural ecosystems, and the educational offerings are a reflection of the same. Programs, displays, and live exhibits are constantly changing, evolving, and taking on new life, providing a foundation to ensure the Exhibit's future in an ever-changing community.

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FIGURE 11. Top: A young girl takes a closer look at the inhabitants of the seagrass ecosystem. Bottom: Excited children view the nearshore reef ecosystem.

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Caribbean Coral Reef Ecosystems: Thirty-Five Years of Smithsonian Marine Science in Belize

Klaus Rützler

ABSTRACT. With foresight and tenacity, Smithsonian Institution marine scientists have devoted more than three decades to understanding and preserving one of the planet's vital natural resources: the coral-reef ecosystem. In the late 1960s marine scientists from the Smithsonian National Museum of Natural History, Washington, founded a long-term Caribbean coral-reef field program, now known as Caribbean Coral Reef Ecosystems (CCRE), to investigate the biodiversity, community structure and dynamics, and environmental processes that control this ecosystem. Its core group of botanists, zoologists, paleobiologists, and geologists found an ideal study site—with high biological diversity, significant geological features, and minimal anthropogenic disturbance—on the barrier reef off Southern Belize, and in 1972 established a field station on one of its tiny islands, Carrie Bow Cay. Within a radius of less than 2 km lie a great variety of richly populated habitats, from mangrove to fore-reef. The Belize mainland and three offshore atolls are within easy reach by small boat. Each year, up to 120 Smithsonian staff and associated scientists, with assisting students and technicians, study the area's reefs, nearby mangroves, and seagrass meadows. Their "whole-organism" expertise encompasses many fields of biology—systematics, evolution, paleobiology, ecology, and ecophysiology—supported by molecular techniques to expand upon traditional morphological taxonomic analyses. An oceanographic-meteorological monitoring station on Carrie Bow Cay records environmental data, now available on the World Wide Web, and monitors the productivity of selected reef, mangrove, and seagrass communities. Field research is complemented by the large resources of the Smithsonian home base. Today, the CCRE program is a member of the Smithsonian's Marine Science Network, which includes coastal laboratories in Panama, Florida, and Maryland. In these and other respects—CCRE now has more than 800 papers in print—the program's accomplishments are indeed impressive.

INTRODUCTION

How does one summarize in a few pages 35 years of research on a complex ecosystem by more than 200 investigators? Clearly, it cannot be done in a complete fashion. With apologies for any omissions, I present this review as a tribute to every single participant in the Caribbean Coral Reef Ecosystems program (CCRE) dating back to the late 1960s, when it was titled Investigations of Marine Shallow-Water Ecosystems (IMSWE). The founders' unifying objective was to apply a multidisciplinary, long-term team approach to studies of marine shallow-water animals and plants, and to examine their interactions in

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their environment—today as well as in the past—for information on the determinants of community structure and evolutionary change. A coral-reef ecosystem, we agreed, is the most extensive and biologically productive shallow-water community on Earth and thus would fully meet our purposes. After conducting literature reviews and several joint surveys throughout the Caribbean, we chose Belize (then British Honduras) as the program's locale because of its pristine environment and high diversity of organisms and reef types.

Program Founders and Objectives

Coral reefs are among the true wonders of the world: they cover 190 million km^2 of the world's ocean floors, are tremendously productive, protect tropical continental coasts and islands from the eroding forces of the oceans, supply humans with large quantities of high-quality protein, and are a unique recreational resource. For all their aesthetic and economic value, coral reefs remain invisible to most people unless they live close to tropical coasts or engage in skin or scuba diving. Without such contact, many are insensitive to the catastrophic effects of pollution and uncontrolled land development, which can rapidly decimate entire communities and thus their benefits, or are unaware of the effects of natural phenomena such as global warming and acid rain.

Fortunately, the unique composition of the Smithsonian Institution, with specialists in many disciplines of the life and earth sciences, provided a substantial number of researchers interested in reefs and willing to team up for the common good of an integrated study. Some experts from other institutions were expected to join for specific tasks. Our original team, all staff of the Natural Museum of Natural History (NMNH), consisted of Walter H. Adey, Department of Paleobiology, a specialist in fossil and modern coralline algae; Ian G. Macintyre, Paleobiology, a carbonate sedimentologist studying calcification, reef-building organisms, and reef evolution; Arthur L. Dahl, Botany, an algal ecologist; Mary E. Rice, Invertebrate Zoology, an expert in sipunculan worm systematics and developmental biology; Tom Waller, Paleobiology, a malacologist focusing on the systematics and distribution of scallops in time and space; Arnfried Antonius, a postdoctoral fellow in Invertebrate Zoology working on stony corals; and myself, Invertebrate Zoology, a sponge biologist with an interest in reef ecology and bioerosion. We were joined in our early search for the optimal research site by David R. Stoddart, a geographer at the University of Cambridge, England; Porter M. Kier, an actuopaleontologist (later a director of the Natural History Museum) looking for modern clues to interpreting fossil echinoderm assemblages; Richard S. (Father Joe) Houbrick, a former priest turned malacologist and working at the Smithsonian Marine Sorting Center; Ernst Kirsteuer, an invertebrate zoologist specializing in nemertine worms at the American Museum of Natural History, New York; and Fred Hotchkiss, a postdoctoral fellow in Invertebrate Zoology specializing in ophiuroid echinoderms. David Stoddart was a particular asset because he had a wealth of research experience with the distribution, geomorphology, terrestrial botany, and dynamics of Belizean islands (cays), having been a member of the 1959 Cambridge Expedition to British Honduras (Carr and Thorpe, 1961) and participant in numerous post-Hurricane Hattie (1961) surveys (Stoddart et al., 1982).

Our main objective was to study the historical and present conditions in a well-developed coral reef far removed from the stressful impacts of an industrial society with a view to compiling baseline data on how an established reef community adjusts to natural environmental parameters. These data would include information on diagenetic alteration of the reef structure, as revealed in drill cores. With the resulting information, we hoped to develop a predictive model of the impact of anthropogenic stress. As we quickly discovered, most previous reef studies consisted of short-term surveys during large-scale expeditions, with superficial sampling during a single season; moreover, many of the reports on reef fauna and flora had been prepared by specialists who had never observed the organisms and processes in the field. A complex ecosystem such as a coral reef obviously required a more rigorous, long-term, and multidisciplinary approach if we had any hope of determining the relative importance of diversity, biomass, energy flow, and environment to community function.

Carrie Bow Cay, Base of a New Marine Field Station

The team chose a Caribbean reef site for several reasons: most of us had already worked in that area, and it would be "close to home," would permit comparison with the already-stressed reefs of Florida, and would minimize travel time and cost. Equally important, to be sure, was the fact that all the characteristic reef types and zones were within workable distance, reef growth was vigorous with a good geological record of past development, and the locale was remote from terrestrial and human influences.

Moving ahead with small grant awards from Smithsonian Institution endowments, we purchased an inflatable boat with an outboard motor, dive tanks, a small compressor, and tents for reconnaissance trips across the Carib-

bean. In another step forward, the U.S. National Science Foundation provided support for a planning meeting on Glovers Reef atoll, Belize, attended by representatives of some 40 academic institutions. We envisioned starting up the program there and eventually conducting comparative studies on an Indo-Pacific atoll. As fate would have it, the proposal emanating from this meeting was not funded.

Returning to Glovers Reef in February 1972 to retrieve our IMSWE equipment from storage, Arnfried Antonius and I discovered a small unoccupied islet with three shuttered buildings on the southern Belize barrier reef. Its name, we learned, was Carrie Bow Cay (16°48′N, 88°05′W; originally spelled Caye), and it was owned by the family operating the Pelican Beach Motel in Dangriga (Stann Creek District), a small town on the mainland (Figures 1, 2). To our happy surprise, this reef tract met all our scientific requirements, studies there would garner generous cooperation from Belize's Fisheries Department, and excellent local logistical support would be available. The motel, now called Pelican Beach Resort, was owned and operated by Henry Bowman, Jr., and his wife Alice. After negotiating storage for our equipment, we initiated a contract to lease part of Carrie Bow Cay, including the two smaller cottages, for a three-month research period that spring and summer.

Carrie Bow Cay was owned by Henry Junior's father, Henry T. A. Bowman, a third-generation descendant of Scottish settlers. The enterprising Henry senior was a citrus grower, businessman, and one-time legislator who had bought the island from his father as a vacation retreat, changed its name (from Ellen or Bird Caye) to Carrie for his wife, and put up an old farmhouse that he had bought on the mainland and carried out to the cay in sections. With his love of fishing, "Sir Henry" (as I referred to him when we became friends) and some of his relatives (daughter Norma and daughter-in-law Alice, in particular) developed a keen interest in the sea and the reef's myriad animals and plants. This interest persuaded him to allow us unrestricted access to most of his island and provided many opportunities to share our observations over drinks during the sunset hour.

A great concern for both of us then, and for all of CCRE today, was the rate of coastal erosion, mainly the consequence of frequent hurricanes, which had reduced the size of the island from 2 acres (0.8 ha) in the 1940s to a little more than half that in the 1970s. In his delightful autobiography (Bowman, 1979), Henry took the blame himself, admitting that he had carelessly removed mangrove trees "that build and bind these cayes." At the same time, he did make a significant contribution to the island's morphology: in 1942 he built a 27 m long concrete boat dock on the leeward (lagoon) side. It has remained unchanged to this day and has served as a reference in our mapping of the geomorphology and communities nearby.

Since then, both Henrys have passed away, but their naturalist spirit lives on. Therese Rath, who is Junior and Alice's daughter (Sir Henry's granddaughter), runs Pelican Beach with her mother and continues to offer us logistical support on Carrie Bow. Therese's husband, Tony Rath—one of our early volunteer station managers who moved to Dangriga from Minnesota two decades ago—is a successful nature photographer and runs the premier web design business in Belize; he still helps us out as a naturalist adviser and provides documentary photography.

Facilities at Start-Up

Between 1972 and 1975, our team operated on a shoestring. The relatively small grants available to us (the Smithsonian has no direct access to National Science Foundation funding) kept the field station open for no more than four months a year and supported up to 25 scientists and assistants per season. Our facilities consisted of a small three-room building with a tin roof to the south of the main house (it contained our lab, living quarters for two, and a kitchen); a 4 m^2 shed that could house two; and a tent, when needed, that could accommodate up to six. The dive compressor and a small generator were installed in improvised shelters.

Our shower consisted of a spray-head on a pipe screwed into the bottom of a huge wooden vat that collected rainwater running off the roof of the main building. To preserve decency, there was an enclosure (its sign read: "Save Water, Shower with a Friend"). The toilets for all island occupants were two outhouses accessed from a wooden pier extending over the reef flat to the island's east. The cabin's seats were rough-cut planks with holes. However, its window opening allowed a spectacular view of the reef flat, barrier reef, and unobstructed horizon, with pelicans and 1 m long parrotfish jumping and feeding in the foreground.

After getting used to us, Sir Henry turned his children's "museum" in the main house into a station manager's quarters by adding some wood siding for walls and a door. It was a roofed-over corner of the house's wide, upper-level porch, where Norma and Alice had kept and displayed an assortment of shells, corals, quirky driftwood, and stranded and mummified algae, invertebrates, and fishes. Working for a museum ourselves, we found that a quaint step forward.

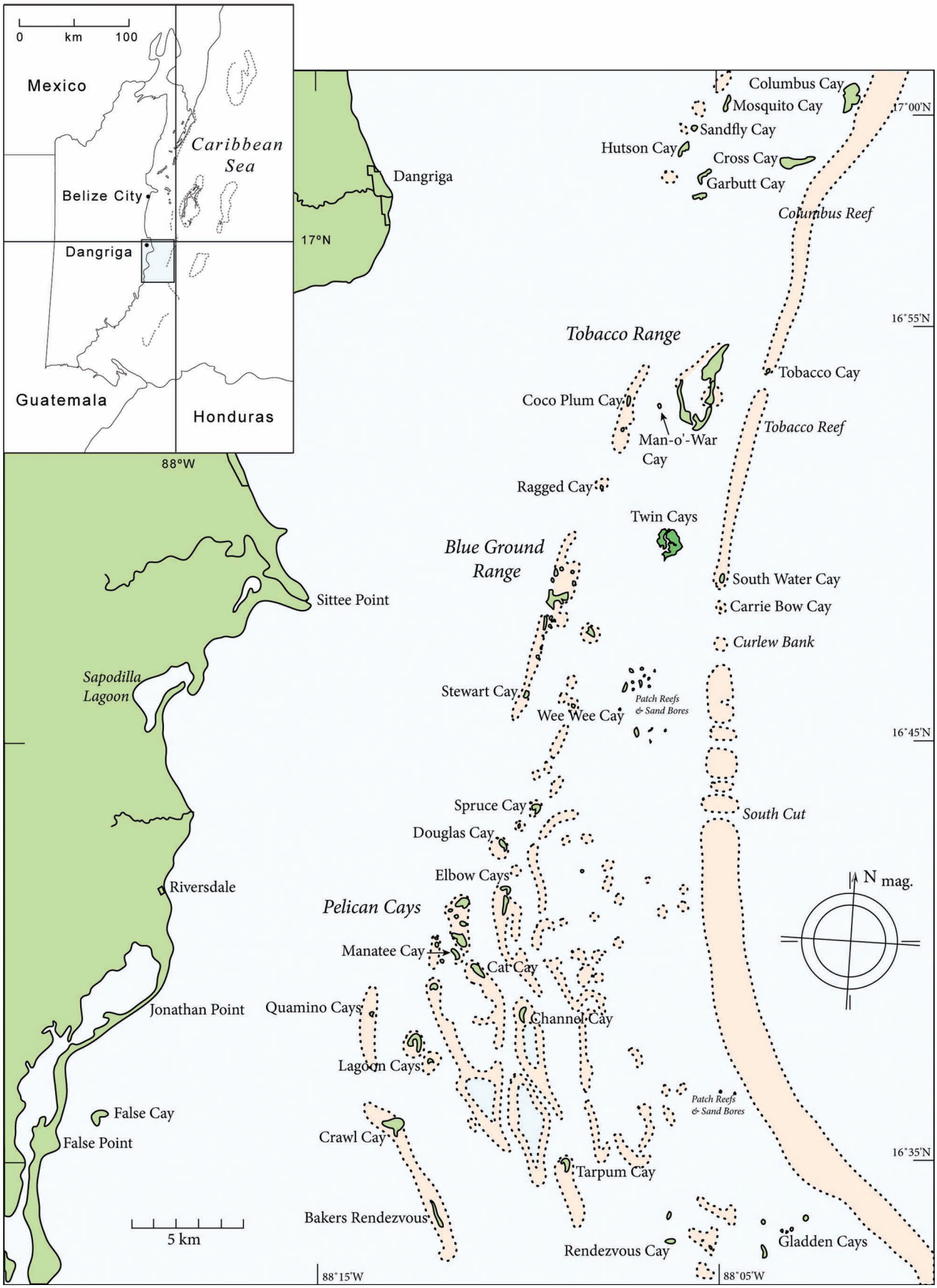


FIGURE 1. Map of research area in coastal Belize, Central America. The barrier reef and other reef tracts appear in pink.

FIGURE 2. The original Investigations of Marine Shallow-Water Ecosystems (IMSWE) survey team, the Belize barrier reef, and Carrie Bow facilities in the early 1970s. Upper left: The team included (left to right) Walter Adey, Arthur Dahl, Tom Waller, Klaus Ruetzler, and Arnfried Antonius (missing from the picture are Porter Kier, Ian Macintyre, and Mary Rice). Upper right: Belize barrier reef looking south, with South Water Cay in foreground and Carrie Bow Cay near center. Center left: Carrie Bow Cay looking southwest, with ocean-side reef flat in foreground. Center right: Carrie Bow facilities looking south, the Bowmans' "Big House" to the right and our lab building in the center. Lower left: Photographer Kjell Sandved working in the aquarium area. Lower right: Scientists in the lab are Anne Cohen (left) and Jim Thomas.

For the "lab," we removed some of the cottage partitions that originally defined the bedroom space for the parents and three children, built a long bench along the oceanfront wall with a supply cabinet and photo table opposite it, and weatherproofed windows by inserting acrylic panes in the lower half to allow the wooden shutters to remain open under most weather conditions and thus let in more light. The sun gave us light for microscopy and photography, with a couple of small gasoline-driven generators doing the job whenever needed. We brought microscopes, cameras, some portable instruments, labware, and boating and dive gear from home but improvised on most additional laboratory or field needs. Our original IMSWE inflatable boat and 25 horsepower outboard engine were still in working order, supplemented by a similar inflatable recently added. A shortwave radio provided contact with Pelican Beach in Dangriga for ordering supplies, brought out once a week. A local cook prepared our meals and lived in a room under the big house; next to her was the simple residence of a native fisherman who served as caretaker and watchman, particularly when the island was deserted during the off-season. Our station manager was usually one of us, or one of our enthusiastic young museum technicians, or some other volunteer with technical know-how. When the lab was closed, all valuables were stored in high places (in case of storm floods), the windows shuttered, and the door padlocked and nailed to its frame. ("This," the locals said, "does not keep the crooks out but keeps the honest people honest.") During hurricane season, all major equipment was taken to Dangriga and stored in the Maya Hut behind Pelican Beach.

ANALYZING A COMPLEX ECOSYSTEM

The Early Years

The program's first targets were to map the reefs and other habitats near the field station, including Carrie Bow Cay itself, and to identify the key organisms in the communities (Figures 3, 4). Because the north–south-oriented barrier reef is the dominant feature separating the lagoon from open ocean, we established a transect perpendicular to its trend, originating well inside the lagoon in a seagrass bed 2 m deep; it then crossed the barrier-reef crest some 150 m north of Carrie Bow and extended due east across the reef and down the fore-reef slope to a depth of 30 m. This transect would become the baseline reference for all our topographic studies and future observations and experiments.

We also tried to develop some standard methods of sampling, extracting interstitial organisms, and determining biomass (Dahl, 1973; Macintyre, 1975; Rützler, 1978a). Because of the complexity of the reef framework (with its three-dimensional structure) and the diversity and size range of its inhabitants (which varied by at least three orders of magnitude), we had to modify many of the commonly used ecological methods to ensure compatible results.

Unable to employ self-contained recording instruments to monitor important environmental parameters, we established a manual routine for taking tide and temperature readings, and for observing solar radiation, wind speed and direction, precipitation, humidity, cloud cover, wave action, and turbidity with simple handheld devices. For specific projects, we measured salinity, oxygen concentration, pH values, water current speed, and submarine daylight with off-the-shelf instruments for which we built waterproof housings when necessary. These data, along with the first reef maps and results from transect surveys, were summarized in our 1975 progress report and distributed to program participants and supporters.

Many colleagues helped identify key organisms and determine biomass and spatial and temporal distribution (Adey and Macintyre, 1973; Kier, 1975; Pawson, 1976). Early on, we discovered unexpectedly high numbers of new species in almost all taxa, which was surprising because the Caribbean Sea is generally considered among the best-studied oceanic regions of the world. Using in situ methods, we identified and quantified environmental parameters such as light, water flow, and sediments making up the "microclimate" of particular organisms (Graus and Macintyre, 1976). We also investigated important associations and interactions between organisms, such as symbioses and space competition, predation, diets, and behavioral patterns. In addition, we measured primary production of benthic macroalgae and symbiotic microalgae, and growth and reproduction rates of reef-forming organisms, the first steps toward determining key metabolic processes (Macintyre et al., 1974).

Some geologists and biologists collaborated in the study of geological processes such as the construction and destruction of the coral-reef framework and the calcification rates of corals, coralline algae, and other bioherms. Others studied physical and biological erosion and sediment production rates (Rützler, 1975), sediment sorting and colonization by meiofauna, and processes of recementation. Ian Macintyre initiated a drilling project with colleagues from the U.S. Geological Survey's Energy Resource Division to learn about the historical development of the

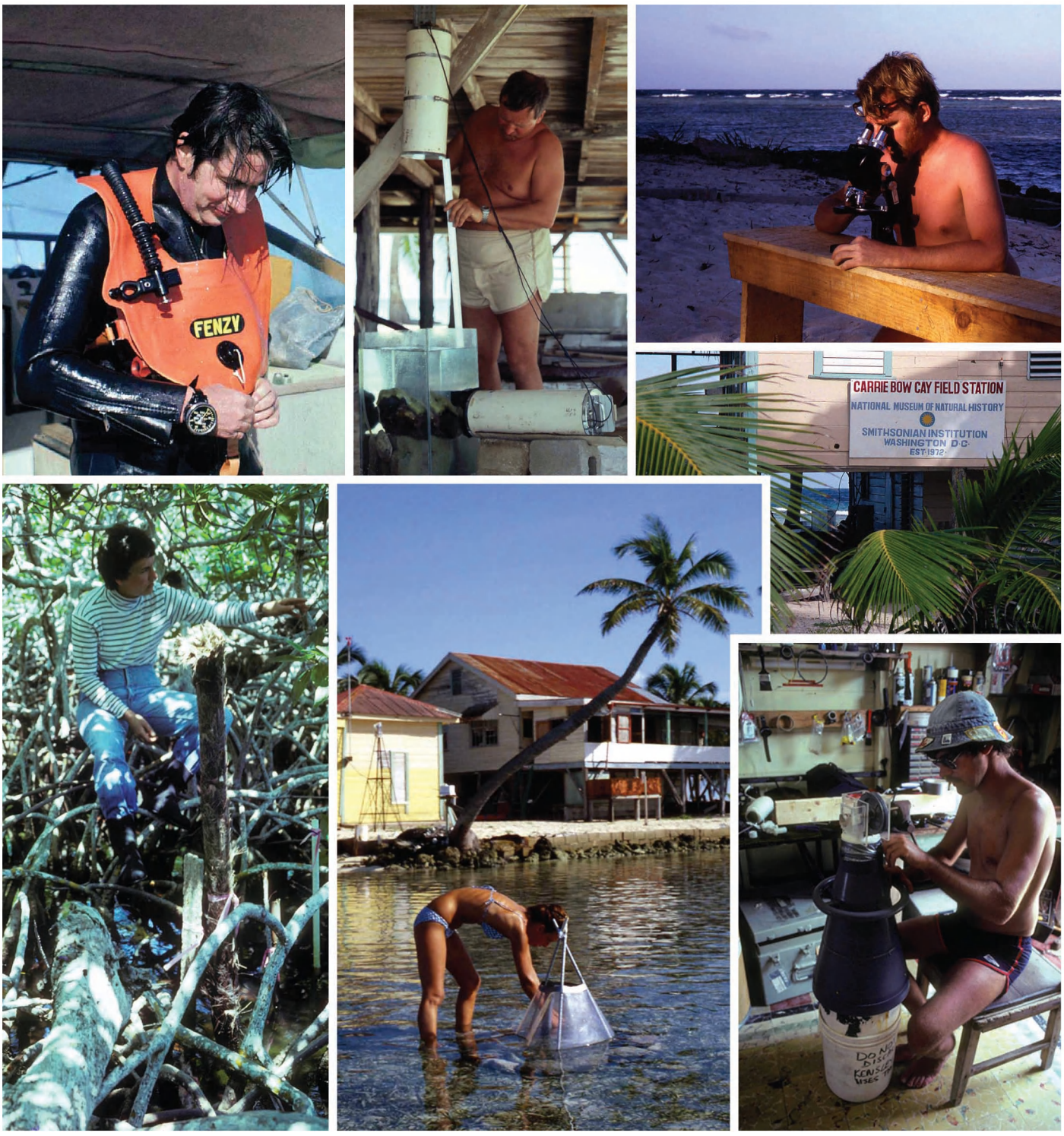


FIGURE 3. Some early program participants. Clockwise, from top left: Ian Macintyre about to enter the submarine Colombus Cay Cave; Arnfried Antonius setting up time-lapse camera for study of black band coral disease; Klaus Ruetzler catching the evening sun for a microscope examination; view of entrance of station; Mike Carpenter fixing an underwater viewer; Joan Ferraris measuring oxygen consumption of benthic community on the Carrie Bow reef flat; and Ilka Feller surveying red-mangrove insects at Twin Cays.

FIGURE 4. Principal marine habitats near Carrie Bow Cay. Top row: left, patch reef near the south tip of the island; right, barrier reef crest composed of elkhorn and fire coral (Acropora, Millepora). Middle row: left, outer fore-reef with corals, sponges, and gorgonians; center, small cave in the fore-reef framework; right, seagrass stand in the barrier-reef lagoon. Bottom row: left, red mangrove at Twin Cays, with Carrie Bow Cay in the background; center, gorgonian and barrel-sponge community on the outer fore-reef; right, diver on the fore-reef slope.

barrier reef. Cores from a series of holes yielded information on past community patterns and successions as well as the distribution of contemporaneous submarine cements within the reef structure (Macintyre et al., 1981).

Detailed maps and inventories of terrestrial plants on several Belizean cays, including Carrie Bow, from 1960, 1962, and 1972 (see Stoddart et al., 1982) aided in our observation of morphological and floral changes, particularly in relationship to the frequent hurricanes in the region. In 1974, only two years into our presence on Carrie Bow, Hurricane Fifi, which destroyed large coastal areas of Honduras, hit the barrier reef just south of our island. Although our reef habitats experienced only minor changes, primarily local breakdown of the framework and accumulation of rubble, most terrestrial life on Carrie Bow, particularly vascular plants, was killed by flooding—except for coconut trees, which suffered about a 20% loss (16 trees)—and there was severe coastal erosion. Upon remapping the island in the wake of this event, we noted some redepositing of beach sand and recolonization by plants through drift and windblown seeds.

Gaining Momentum

Our venture took a significant step forward with the award, in 1975, of an annual grant by the Exxon Corporation from the company's public relations budget for Central America. Although relatively small, the funds nearly doubled our support and had no strings attached, except they were to be dedicated to Caribbean coral reef research. Added to this welcome development was a new and beneficial relationship with Captain Graham Thomas of the Royal Signals Detachment in Belize, a helicopter pilot detailed to support the training of British forces in jungle environments. Graham was able to equip his helicopter with an aerial camera and take vertical pictures of the Carrie Bow reefs that provided excellent photo coverage for a detailed mapping of the area's reef structures at a scale of 1:800 and to a depth of 10 m. This information constituted enormous progress over available nautical charts (with a scale of 1:125,000) that dated back to British surveys in the 1830s and were only partly updated in the 1940s. Even greater resolution in aerial mapping (but at the expense of areal coverage) was achieved by introducing a helium weather balloon equipped with a remotely operated camera. This technique (Rützler, 1978b), like several others devised by our team, was documented in a volume on coral reef research methodology sponsored by the United Nations Educational, Scientific and Cultural Organization (UNESCO) (Stoddart and Johannes, 1978).

In 1977, in association with the Third International Symposium on Coral Reefs in Miami, several of our team organized a well-received field trip to Belize, highlighted by a detailed field guide based on maps, transect data, and aerial and underwater habitat photographs emanating from the program (Miller and Macintyre, 1977). In short order, the program launched several new projects (Figure 5) to investigate the fate of siliceous skeletons in the calcium carbonate environment of the reef (Rützler and Macintyre, 1978), the feeding behavior of scyphomedusae (Larson, 1979), and the systematics of the unexpectedly diverse ostracod crustaceans (Kornicker and Cohen, 1978). Other innovative and pioneering work focused on the fine structure of bivalve anatomy as revealed through scanning electron microscopy (Waller, 1980) and, with collaborators from the Scripps Institution of Oceanography, on the chemistry of marine plant and invertebrate secondary metabolites that showed promise as antibiotics or other therapeutical substances (Kokke et al., 1979). By the end of 1978, more than 50 papers had been published or were in press to document the biology, ecology, and geology of the Belize barrier reef in the vicinity of Carrie Bow Cay.

The program's success appeared to be short lived, however: in late September 1978, Hurricane Greta passed across the Belize barrier reef just 6 km north of Carrie Bow. Four lives were lost in Dangriga, the citrus harvest in the valley to the west was destroyed, and there was heavy beach erosion at Pelican Beach Motel. Although no members of our group were on location because we had closed down for the season, part of our equipment was damaged when ocean storm surge and rain flooded the storage area. Storm waves from the east and strong backlashing winds from the northwest caused severe erosion of beach sand on Carrie Bow and wiped out some 30 coconut trees, the small house, and the outhouses. The ocean-side wall of the laboratory building also caved in, exposing equipment and supplies to saltwater spray. Following the storm, visibility in the usually very clear ocean water remained at less than 3 m for two weeks, most elkhorn (*Acropora palmata*) and fire coral (*Millepora complanata*) near the reef crest was reduced to rubble, and the salinity in the lagoon dropped from the usual 35‰ to 25‰.

Despite this setback, we decided to press our team and collaborators to complete work in progress and prepare a state-of-the-art summary of our accomplishments. The resulting volume (Rützler and Macintyre, 1982), later known as the Blue Book (for the color of the hard cover), became a platform for the next phase of investigations, as well as for raising funds. The first section presented a

FIGURE 5. A few examples of research activities of the 1970s to 1980s. Clockwise, from top left: boat operator Frank (Pelican Beach Resort) helps Ron Larson to lower plankton net from the stern of the boat; Ian Macintyre's group drill-coring down a sand groove on the fore-reef; Joan Ferraris (left) tending incubation chambers to measure temperature-salinity tolerance of invertebrates and Sara Lewis preparing aquaria for fish herbivory experiments; Sara measuring algal abundance in a quadrat frame on the reef flat; Klaus Ruetzler retrieving trace paper from tide recorder on Carrie Bow dock; Mark and Diane Littler's team assessing effects of nutrients on algal growth.

detailed overview of the physical and biological environment of our study site—its habitats and community structure, geological history, tides, water currents, climate, and terrestrial conditions—compiled by Ian Macintyre and myself and various outside collaborators, including Björn Kjerve (University of South Carolina, Colombia), Joan Ferraris (Mount Desert Island Biological Laboratory, Maine), and Eugene Shinn (U.S. Geological Survey, Miami). The next section focused on the benthic and planktonic communities—the carbonate microborers, micro- and macrobenthos, zooplankton, and the populations of a large submarine cave at nearby Columbus Cay—and their productivity. The principal collaborators were Joan Ferraris, Paul Hargraves (University of Rhode Island, Kingston), Jeffrey May (Rice University, Houston), and David Young (Department of the Navy, Mississippi). A section on biodiversity included many important groups of reef organisms, notably the algae and seagrasses (James Norris, NMNH), hydroids (Barry Spracklin, University of New Hampshire, Durham), medusae (Ronald Larson, a former NMNH technician who moved on to the University of Victoria, Colombia), stony corals (Stephen Cairns, NMNH), octocorals (Katie Muzik, postdoctoral fellow, NMNH), sipunculan worms (Mary Rice, NMNH), crustaceans and pycnogonids (Brian Kensley, Allan Child, NMNH), and echinoderms (Frederick Hotchkiss, postdoctoral fellow, NMNH; Bradford Macurda, Jr., University of Michigan, Ann Arbor). The most unusual discovery was that chironomid insect larvae, caught in emergence traps, are part of the offshore benthic community and live in fore-reef sand bottoms to depths of 30 m (Gernot Bretschko, Biological Station Lunz, Austria). A section on species interactions and responses to the environment addressed chemical defense in algae (James Norris), the life history and ecology of cnidarians (Ronald Larsen), growth patterns of reef corals (Richard Graus, NMNH), sponge–zoanthid associations (Sara Lewis, Duke University, Durham), bivalve larval settlement (Thomas Waller, NMNH), and resource partitioning in chaenopsid, coral-associated fishes (David Greenfield, Field Museum of Natural History, Chicago). The concluding chapter puts Carrie Bow Cay and its reefs in the larger context of the Belize barrier reef complex (contributed by Randolf Burke, North Dakota Geological Survey, Grand Forks, and David Stoddart).

Having overcome many of the start-up problems, including setbacks caused by the hurricanes, we forged ahead in the new decade with increasing productivity and innovation. CCRE members authored a number of important monographs and other reports on reef biodiversity. We started a series of papers on the fungi (Kohlmeyer, 1984) and algae (Littler and Littler, 1985); prepared analyses of several large crustacean groups, including parasitic copepods on fishes (Cressey, 1981), decapods (Kensley and Gore, 1981), isopods (Kensley, 1984), and amphipods (Thomas and Barnard, 1983); and published the first survey of local moss animals, bryozoans, by a colleague then at the American Museum of Natural History in New York (Winston, 1984).

We also conducted a series of day and night plankton tows over the fore-reef and over lagoon seagrass bottoms, which were surprisingly devoid of larval stages of some of the area's common animals, such as an assortment of cnidarians and sponges. We speculated that the larvae might be swimming close to or within the reef framework, where our boat-towed nets could not be operated, and decided to tow or push the plankton nets by hand, while swimming close to the bottom. Although more successful, this technique took time and effort to obtain sufficient samples. Eventually, we hit on the idea of building a stationary net supported by a frame that could be placed close to or among the coral heads or branches. For locations without strong directional currents, we added a waterproof electric motor with propeller and a flow meter to measure the volume of water that passed through the net. This setup ultimately produced excellent samples of great diversity considerably beyond the composition of plankton tows by boat (Rützler et al., 1980).

Having a small budget and intent on disturbing our study environment as little as possible, we sought creative field and laboratory techniques that would not require sophisticated instrumentation or climate control. In keeping with these goals, our colleague Sara Lewis, for one, completed the experimental fieldwork for her entire dissertation on fish herbivory on the Carrie Bow reef flat, just a few meters east of the lab building (Lewis, 1986). Some of our Museum's phycologists experimented with the influence of algal growth forms on herbivores at the same location (Littler et al., 1983). Ecophysiological work on temperature and salinity tolerance of polychaetes and other reef invertebrates was accomplished in situ and with simple, specially designed acrylic incubation chambers (Ferraris, 1981; Ferraris et al., 1989). Submarine cementation processes were determined experimentally in the karst cave habitat of Columbus Cay (Macintyre, 1984). Benefits of algal symbionts to sponge hosts were explored by in situ trials on a nearby patch reef (Rützler, 1981). And, with an innovative underwater time-lapse camera with strobe light borrowed from its inventor, Harald Edgerton at the Massachusetts Institute of Technology, we recorded several unattended day–night activities on the reef, including the

nocturnal feeding behavior of basket stars (*Astrophyton*) (Hendler, 1982).

During reef surveys in the early phase of our program, we were already seeing a number of dead or damaged corals with no clear sign of the common physical impacts related to storms or boat anchors. Our postdoctoral fellow Arnfried Antonius pioneered these observations at Carrie Bow and elsewhere in the Caribbean, as well as in the Indo-Pacific (Antonius, 1982). One notable feature of many of these flagging corals was a black line between live coral tissue and recently dead (white) skeleton. During collaborative studies, we determined that the black band consisted of a mat of entangled filamentous cyanobacteria, with a number of associated microbes, and that the photosynthetic bacteria had an appetite for coral tissue, thereby causing what has been called "black band disease" (Rützler et al., 1983).

Oceanic Mangrove Swamps

Anyone looking through our 1982 "Blue Book" will notice that mangroves are barely mentioned, except for a few remarks about Twin Cays, a mangrove island in the lagoon just over 3 km northwest of Carrie Bow (Figure 6). This lack should not be taken as a sign of little interest. CCRE workers have in fact been highly impressed by the relatively clear (for a swamp) water in the tidal channels and the rich flora and fauna, particularly the sponges, covering the stilt roots of red mangrove *(Rhizophora mangle)*.

On an earlier visit to a very similar mangrove island, East Bimini in the Bahamas, I had been so struck by its subtidal diversity that it seemed an ideal community for multidisciplinary study. The "discovery" of Twin Cays during the early 1980s rekindled this interest, and coincidentally our Exxon supporters indicated that they wanted to diversify their generosity in Central America beyond coral reef research. We therefore submitted a new proposal to their open competition for the comprehensive study of a Caribbean mangrove ecosystem at Twin Cays. A factor in our favor was that oil pollution caused by tanker ballast-water discharge or wrecks was affecting Caribbean beaches and reefs at that time. Indeed, a colleague and I had studied the effect of such an oil spill at Galeta Island, Caribbean Panama, a decade earlier and found that the subtidal reef corals were barely affected by the spill but that the oil slick had caused severe damage to the nearby intertidal mangrove community (Rützler and Sterrer, 1971). Our proposal won another five years of research grants, and we named our initiative SWAMP (Smithsonian Western Atlantic Mangrove Program). This support was supplemented by internal grants for specific purposes, notably Fluid Research Funds travel awards, a Scholarly Studies grant for mangrove research, a Smithsonian Associates Women's Committee award for scientific illustration, W. R. Bacon Scholarships (for external collaborators), Seidell Funds for library enhancements, and National Science Foundation grants to outside collaborators (who were to some extent also supported by their home institutions).

Because mangroves are tidal communities with terrestrial, intertidal, and subtidal components, we could expand our fields of interest, adapt our methods to the new environment, and add a number of disciplines to our study that are not usually applicable in the subtidal reef environment (Rützler and Feller, 1988; Figure 7). With a wider biodiversity horizon, we could now conduct surveys of microbes, fungi, algae, sponges and their endofauna, polychaetes, crustaceans, echinoderms, and bryozoans. We were fortunate to have a rare expert on the quantitatively important ascidian tunicates join us at this time, Ivan Goodbody of the University of the West Indies, Jamaica. Our team also explored the geological history of the mangrove by coring through massive peat accumulations and dating the different horizons and also initiated terrestrial studies of the mangrove's lichens, insects, spiders, reptiles, and birds.

An important first step was to explore and map Twin Cays and name the many bays, ponds, creeks, mud flats, and lakes and give them coordinates (before Global Positioning System [GPS] devices were available) that would allow us to relocate research sites. We also wanted to garner more interest in the mangrove ecosystem, but because swamps tend to be viewed as undesirable environments, it took considerable effort to win over our sponsors, local hosts, and even many colleagues. Good photography was a decided help, but even the best pictures convey but a tiny segment of a process in nature, although they are absolutely necessary for documenting shapes, expressions, or colors, of course. To depict the entirety of, say, an animal–plant association, we needed to capture the obvious and the hidden, the large and the small (in proper detail and perspective), and the dynamics of day versus night—in a word, we needed to combine art with science. We did just that in a new project called Art in a SWAMP (Figure 8). The lead artist was Ilka ("Candy") Feller, a contract illustrator at the time with vast experience in the fields of botany and entomology, having worked with numerous colleagues in those departments in our Museum over many years. Candy not only employed her artistic talent in the illustration of mangrove communities, but she was so captivated by the entire ecosystem that she resumed academic studies (after

FIGURE 6. Twin Cays mangrove habitats. Clockwise, from top left: the island viewed southwest toward Carrie Bow Cay and the barrier reef; mangrove fringe lining the Main Channel; sponge clusters in one of the tidal channels supported by red-mangrove stilt roots; diverse community of sponges and ascidians on a root substrate; a newly discovered and described sponge (genus *Haliclona*) anchored on and in the mangrove-peat bank that lines many channels; juvenile barracuda hiding among mangrove roots; a snorkeler exploring Hidden Creek, which connects a shallow mangrove lake with the open Main Channel.

FIGURE 7. (*facing page*) Examples of projects initiated at Twin Cays. Clockwise, from top left: mapping and exploring team landing on the western shore; our weather station erected in a large tidal mud flat; scientific illustrator Mary Parrish sketching mangrove communities; Molly Ryan photographing community samples returned to the lab at Carrie Bow for one of her scientific illustrations; student volunteer helping to collect specimens; ichthyologists Will Davis and C. Lavett Smith comparing catches; Ilka Feller measuring salinity in Hidden Lake, the location of one of her mangrove fertilization and growth experiments; Ian Macintyre, with Ilka, dissecting termite nest during an exploration of the mangrove's interior.

having raised two daughters), completed a dissertation on the Twin Cays mangrove, and became one of the foremost mangrove ecologists working on communities between Florida and Brazil and as far away as Australia and New Zealand. Other artists on the project were Mary Parrish, first a staff member in my department and now illustrator in the Department of Paleobiology; Molly Kelly Ryan, Invertebrate Zoology staff illustrator; and Jennifer Biggers, then my contract research assistant and illustrator. This team, along with Paleobiology's Ian Macintyre, Natural History Museum photographer Chip Clark, Invertebrate Zoology technician Mike Carpenter, and several more associates and volunteers engaged in detailed surveys and mapping of Twin Cays geomorphology and in analysis and graphic reconstruction of habitats and communities, ranging from epiphytic sponges to intertidal algae–invertebrate associations to red mangrove–insect interactions, and an entire mudflat population (Rützler and Feller, 1996).

Our growing familiarity with mangrove organisms raised a number of significant ecological and behavioral questions concerning the composition and ecology of floating cyanobacterial mats (addressed by Maria Faust in the Department of Botany; Faust and Gulledge, 1996) and herbivory in macroalgal communities (investigated by Mark and Diane Littler and colleagues; e.g., Littler et al., 1983). A rare immune disease was discovered in a sponge (Rützler, 1988) in which the usually beneficial microbial symbionts turn against their host. The dynamics and behavioral patterns of swarming copepod crustaceans among mangrove roots were investigated by Frank Ferrari, Invertebrate Zoology, and colleagues (Buskey, 1998; Ferrari et al., 2003). Also, new work was done on invertebrates living in complex burrows in the sediment substrata of mangrove channels (Dworschak and Ott, 1993), and on the importance of mangroves for the recruitment and protection of commercial species such as spiny lobster (Acosta and Butler, 1997). In research on another puzzling question—the role of sponge cyanobacterial symbionts in both mangrove and reef nutrient cycling—it was shown that nitrogen fixing by bacteriosponges is indeed an important input to the community (Diaz and Ward, 1997). With the new emphasis on the biology of the Twin Cays mangrove, Ian Macintyre applied geological techniques over several years to reveal its biological history: using a portable vibracore, he mapped and carbon-dated the subsurface layering of peat, sand, and rubble, all the way to Pleistocene level (Macintyre et al., 2004b).

As our financial situation improved, we were able to address the bothersome problem of coping without automated instrumentation for continuous monitoring of basic meteorological and oceanographic parameters. Up to then we had documented the tidal regime at Carrie Bow Cay with a pressure sensor (Kjerfve et al., 1982) and relied on various borrowed or leased instruments to meet the needs of specific projects for monitoring water currents, temperature, solar radiation precipitation, or wind (Greer and Kjerfve, 1982; Rützler and Ferraris, 1982). None of these improvised methods provided long-term, reliable records even if we were on site. With the help of contract engineer George Hagerman, we adapted a leased Anderaa (Bergen, Norway) automatic weather station for our purposes and installed it on a massive, elevated wood platform in an extended tidal mud flat to the north of Twin Cays. This setup provided us with continuous data for several years until it became outdated and fell victim to vandalism.

CARIBBEAN CORAL REEF ECOSYSTEMS: A BREAKTHROUGH

In the early 1980s, Richard Fiske, then Director of the National Museum of Natural History, asked for proposals that would interest our sponsors in the U.S. Congress, who at that point appeared to favor the expansion of promising research already in progress. To our surprise, the Museum received an increase to its budget base for the study of Caribbean Coral Reef Ecosystems beginning in 1985. Modeled on the IMSWE-SWAMP initiatives, the CCRE program encompassed reef, mangrove, seagrass, and plankton communities, with a primary focus on the Carrie Bow Cay region.

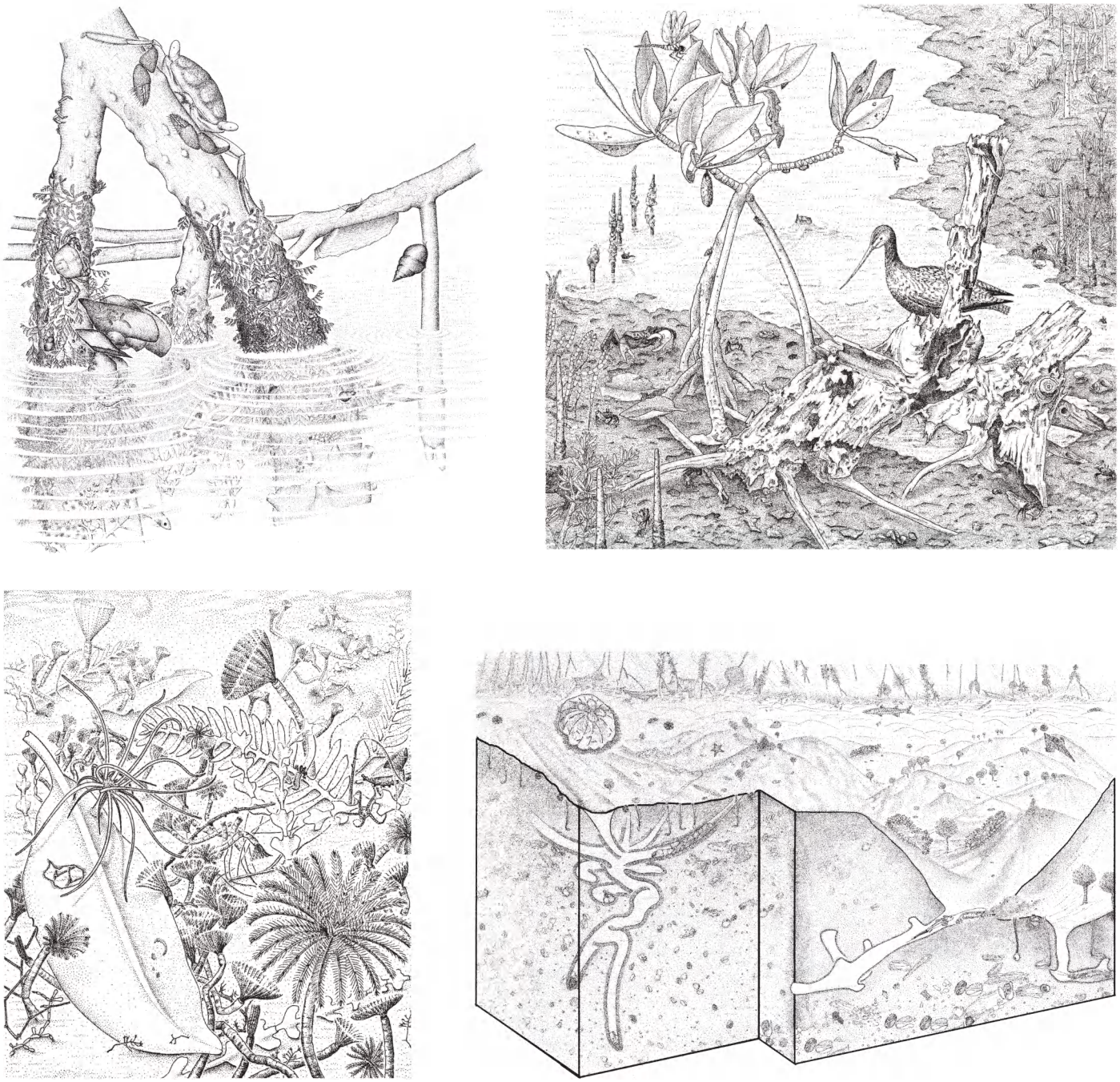


FIGURE 8. Examples of illustrations of mangrove swamp communities. Clockwise, from top left: characteristic intertidal red algal cover (*Bostrychietum*) on red-mangrove prop root, with mangrove oyster, mangrove crab, and periwinkle; tidal mudflat showing developed red-mangrove seedlings, black-mangrove pneumatophores, driftwood, and a marbled godwit; cut-away view of mangrove channel bottom showing characteristic benthic organisms including decapod burrowers; close-up of peat-bottom community with algae, fallen mangrove leaves, sea anemone, and sabellid tube worms.

Under a new administrative structure approved by Director Fiske, CCRE would be governed by a steering committee chaired by me and composed of representatives of different departments and disciplines, including outside advisers. Marsha Sitnik, who worked with all the Museum's interdepartmental biodiversity initiatives, became program administrator. Now we could afford the important position of operations manager, filled by Mike Carpenter, who serves as field station logistics director and has trained and supervised our volunteer station managers, each of whom typically spends three to six weeks at Carrie Bow Cay.

The new funding allowed us to lease all of Carrie Bow Cay year round and remodel the big house for much-needed dry-lab space for instruments, a library, computer, and additional living accommodations. We built a separate, sound-insulated compressor-generator shed, added propane gas refrigerators to kitchen and labs, and improved radio-communication and other safety features for boating and diving. New equipment included microscopes, balances, centrifuge, and other analytical equipment. We also upgraded our weather station with real-time data access and connected it with the Belize Meteorological Office, which had no offshore monitoring facility. Even more important, we had a modest budget for travel stipends to attract outside collaborators to work on organisms or disciplines not covered by Smithsonian staff scientists.

At the end of the first CCRE program year, our publication list exceeded 200 entries. Several of the projects mentioned earlier were continued or completed and new ones begun with like-minded colleagues whose expertise filled the gaps in our experience. To name a few of these projects, some focused on the control of reef zonation by light and wave energy (Graus and Macintyre, 1989), the taxonomy and ecology of hydroids (Calder, 1991), oligochaete worms (Erséus, 1988), mysid crustaceans (Modlin, 1987), and ascidians (Goodbody, 1995); the predation and feeding ecology of sponges (Wilkinson, 1987), echinoderms (Aronson, 1987), and fishes (Wainwright, 1988); the ecophysiology of invertebrate–bacterial symbiosis supporting life in hydrogen sulfide environments (Ott and Novak, 1989; Ott et al., 1991) and mangrove-tree metabolism (McKee et al., 1988); and island groundwater hydrology (Urish, 1988).

In 1988 we held a workshop at the Calvert Marine Museum in Solomons, Maryland, to review the accomplishments and gaps in our research on the Twin Cays mangrove ecosystem. Close to 40 program participants summarized the progress of their work on internal structure, development over time, sedimentology, meteorology, hydrology, vegetation, productivity, nutrient cycling, temperature-salinity tolerance, and biodiversity of fauna and flora from microbes to amphibious fishes. The most obvious deficiencies were in oceanography, a number of important organism groups such as mollusks and fishes, and marine benthic and terrestrial ecology. One of the highlights was a report on a complementary team study of the Holocene geological history, peat composition, and terrestrial and marine vegetation of Tobacco Range, another large mangrove island about 3.5 km north of Twin Cays (Littler et al., 1995). This atoll-like range drew CCRE's attention when a large area of fractured and slumped fossil peat was discovered off its west shore.

At a subsequent planning workshop in Jamaica, CCRE established a protocol for studies at Twin Cays and Carrie Bow Cay. This initiative, called Caribbean Coastal Marine Productivity (CARICOMP), calls for simple but universally applicable methodologies in the monitoring of major oceanographic parameters and health of the Caribbean's principal communities. To this end, we established representative plots and transects in mangrove, seagrass meadow, and fore-reef, which are being evaluated yearly for changes in structure and productivity, while climatic factors are determined on a weekly basis (Koltes et al., 1998).

As our scientific drawing and photography of swamp communities gained scientific importance and aesthetic value, we were invited display some of this work to the public at the Smithsonian's S. Dillon Ripley Center in an exhibition titled "Science as Art." It included a video documentary on mangrove swamp biology, produced in collaboration with colleagues from the University of Vienna (Joerg Ott and Alexander Bochdansky). The video also served as a teaching aid in an annual educational workshop for Belize high school teachers, conducted by Candy Feller and Marsha Sitnik in collaboration with the Belize Fisheries and Forestry departments and titled "Mangrove Conservation through Education." This was a timely workshop, indeed: our research area at Twin Cays was showing the first signs of anthropogenic stress in response to tourist visitation, garbage dumping, vandalism (of our weather station and boat dock), and the clear-cutting of mangrove trees to gain land for development. These developments had a particularly adverse impact on Candy and her colleagues, whose work on mangrove plant ecology required extended undisturbed natural conditions to single out parameters (nutrients, in particular) that enhance or impede growth (Feller, 1995; Feller et al., 1999). Fortunately, at our urging, Belize's Forestry and Natural Resources Departments helped slow the disturbances and started work on a conservation plan for the South Water

Cay Marine Protected Area (MPA) that would include Carrie Bow and Twin Cays.

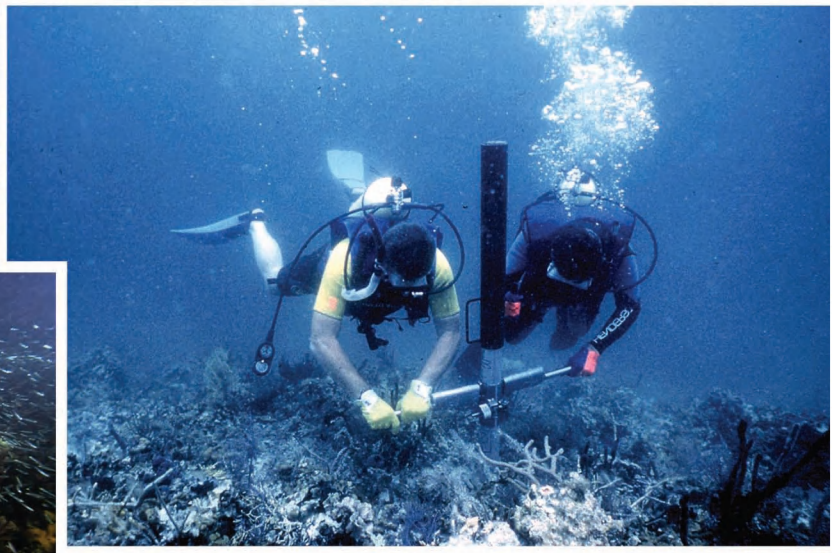
Side-tracked by the discovery of the Pelican Cays biodiversity hotspot (see the next section), the impact of a hurricane, and coral bleaching events, we were unable to convene another Twin Cays symposium until 2003. Meeting at the Smithsonian Marine Station in Fort Pierce, Florida, we found our mangrove program had accumulated enough scientific results not only to fill a volume of multidisciplinary papers but also to demonstrate changes in the structure of the ecosystem over a span of two decades (Macintyre et al., 2004a). Articles on geological history and sedimentary conditions were spearheaded by Ian Macintyre and those on aquatic ecology by Rützler and colleagues. Other contributions covered a wide range of topics: changes in the mangrove landscape, documented through aerial and satellite imagery by Wilfrid Rodriguez and Ilka Feller at the Smithsonian Environmental Research Center; marine botany, investigated by Maria Faust and the Littler team; Foraminifera, by Susan Richardson of the Smithsonian Marine Science Network (MSN); symbiotic ciliates, by Joerg Ott; sponge ecology, by Cristina Diaz (then an MSN Fellow with me) and Janie Wulff (now at the Florida State University, Tallahassee); and two very different worm groups, the interstitial gnathostomulids by Wolfgang Sterrer (Natural History Museum, Bermuda), and the burrowing sipunculans, by Anja Schulze, postdoctoral fellow, and Mary Rice, emeritus scientist, both at SMS, Ft. Pierce, Florida (Mary is also the founding director of that laboratory). William Browne (University of Hawaii, Honolulu) summarized years of genetic and developmental research on mangrove crustaceans, Judith Winston her work on bryozoans, and Ivan Goodbody his observations on ascidian diversity. Years of genetic research at Twin Cays on a highly unusual amphibious fish, the mangrove rivulus, was summarized by Scott Taylor and his collaborators, and terrestrial biology received a welcome boost from observations by Seabird McKeon (then at SERC) and Stephen Mitten (University of Missouri, St. Louis, now based in Belize). Energy flow was also examined in a paper on nutrients derived from microbial mats by Samantha Joye (University of Georgia, Athens), and another on the planktonic food web by Edward Buskey (University of Texas at Austin). To round out the reports, Mary Parrish explained the important role of scientific field illustration—a collaboration between scientist and artist—in analyzing and explaining mangrove communities.

CCRE's recent accomplishments also include two far-reaching initiatives. The first, begun by collaborator Emmett Duffy from the Virginia Institute of Marine Science (Gloucester Point), is a study of the systematics and ecology of snapping shrimp (Alpheidae) that live in reef sponges with a large interior cavity system, such as the genus *Agelas*. As the work progressed with various specialists and graduate students coming on board, alpheids were found to have much more genetic diversity and ecological complexity than previously thought. Another discovery, a first among marine life, was that these crustaceans have the same advanced social structure (eusociality) as some well-studied terrestrial animals, such as termites (Duffy, 1996). Second, a logistical breakthrough, made possible through our collaboration with colleagues at the University of Rhode Island, was the development of a new integrated environmental sensing system with a radio-telemetry link to the Internet (Opishinski et al., 2001).

Pelican Cays, Biodiversity Hotspot

In the early 1990s, our neighbors on Wee Wee Cay, Paul and Mary Shave, alerted us to another amazing ecosystem: the Pelican Cays (16°59.8′N, 88°11.5′W), a biologically rich mangrove island group less than 20 m south-southwest of our Carrie Bow station (Figure 9). We now had an 8 m boat, more substantial than any before, that could take us there in about an hour. Ivan Goodbody, the first to visit the Pelicans on the rumor of an

FIGURE 9. (*facing page*) The Pelican Cays "mangreef." Clockwise, from top left: aerial photograph of Cat Cay showing mangrove, reef ridges, and deep lagoons (Manatee Cay, left, and Fisherman's Cay are in the background); diver swimming over coral (*Agaricia*) below the canopy of a red-mangrove tree; Rich Aronson and Ian Macintyre operating a hand corer to retrieve subbottom coral and other deposits; close-up of ascidian (*Clavellina*)–sponge (*Monanchora*, *Spirastrella*) community, enveloped by brittle star arms, on Manatee Cay mangrove root; Klaus Ruetzler sampling sponges (*Aplysina*) in a marine pond on Fisherman's Cay; Coral Ridge (*Agaricia*, *Palythoa*) with sponges (*Chondrilla*) at Cat Cay lagoon entrance (sponge-covered mangrove roots in background).



ascidian paradise there, found the area teeming with ascidians—and much more. Many of us followed in short order, eager to investigate the Pelicans' atoll-like reef, an elongate north–south-oriented structure measuring almost 10 × 3 km and studded with about a dozen mangrove cays on its northern rim. Most of the islands enclose deep circular ponds that support and protect a diverse community of marine plants and sessile filter feeders—particularly sponges and ascidians—flourishing on red-mangrove stilt roots and peat banks. A wealth of other invertebrates and fishes live just below the tide line, a mix of reef and mangrove organisms, some species previously seen only in much deeper water on the barrier fore-reef, although the Pelican Cays are situated deep inside the barrier-reef lagoon, halfway between the reef and the mainland.

We were so impressed by the unusual diversity and ecological complexity of the Pelican Cays ecosystem that we asked the Belize Coastal Zone Management and Fisheries units to include the region in the South Water Cay MPA. Tony Rath (NaturaLight, Dangriga) and Jimmie Smith (Islands from the Sky, Houston, Texas) helped with aerial photography, Molly Ryan with mapping, and our research team along with outside collaborators addressed the new scientific perspectives (Macintyre and Rützler, 2000). Macintyre and his team, and Karen McKee (U.S. Geological Survey, Lafayette, Louisiana), spearheaded the study of geological underpinnings and vegetation history, Dan Urish (University of Rhode Island) and Tracy Villareal (University of Texas at Austin) the hydrography of the ponds, Thomas Shyka (National Oceanographic and Atmospheric Agency, Silver Spring, Maryland) the nutrient cycle and water flow patterns in the ponds, Steve Morton (Bigelow Laboratory, West Boothbay Harbor, Maine) and Maria Faust the phytoplankton, Mark and Diane Littler the marine algae and seagrasses, Susan Richardson (then at Yale University, New Haven, Connecticut) the epiphytic foraminiferans, Rützler and colleagues the sponges, Janie Wulff (then at Middlebury College, Vermont) sponge predation, Wolfgang Sterrer the gnathostomulids, Gordon Hendler (Natural History Museum, Los Angeles, California) the echinoderms, and Ivan Goodbody the tunicates. At the height of these investigations, we were able to introduce the spectacular coral communities of the cays to participants of the 8th International Coral Reef Symposium, along with other points of interests, such as community changes in the reef zones of the Carrie Bow reference transect over the past two decades (Macintyre and Aronson, 1997). The fishes of the Pelicans were investigated (just after the edited volume was published) by a team of ichthyologists led by our Museum's James Tyler and included former American Museum of Natural History curator (now retired) C. Lavett Smith (Smith et al., 2003). More recently, important suspension feeders that had not been covered by the earlier surveys, the bryozoans, were studied by our long-time collaborator Judith Winston of the Virginia Museum of Natural History (Winston, 2007).

Although still uncertain of the causes of this archipelago's unusually high biodiversity, CCRE researchers saw ample evidence of its fragility and warned of the irreparable harm that could come to its delicate communities from careless visitors or water warming during long periods of calm (as observed in course of some hurricanes). Little did we know that our concerns would soon prove to be well founded. In the course of a number of routine survey flights over the reef, we noted disturbing signs of land "development" on several of the Pelican islands, subsequently confirmed by ground-truthing: we found large areas of mangrove clear-cut and bottom sediments near the cays dredged to obtain fill material on which homes and resorts could be built. We reported our observations to the authorities because by that time the cays were already part of the South Water Cay MPA and mangrove cutting was illegal without a special permit. At the time of this writing, the activities have stopped and are under investigation by the government of Belize. Unfortunately, a great natural treasure has been severely damaged, without any clear sense of whether and how soon a recovery will be possible.

A Memorable Year, 1997

In CCRE's 35-year history, 1997 stands out for its remarkable highs—and lows. Scientifically, many significant field projects were launched or carried to completion: investigations of coral bleaching, a new and unsettling phenomenon on the barrier reef (Aronson et al., 2000); ecophysiological analysis of periodic crustacean swarming among red-mangrove stilt roots (Buskey, 1998); a pioneering initiative to match poorly known fish larvae to the adults of the species, first by morphological means after rearing in the laboratory, later by DNA analyses (Baldwin and Smith, 2003); and a workshop on Caribbean sponge systematics with experts from five nations that led to a better understanding of the barrier-reef and mangrove poriferan fauna (Rützler et al., 2000). This was also the International Year of the Reef, and to celebrate the occasion we made every effort to share our enthusiasm for this unique environment with students and the general public through numerous lectures, poster sessions, and demon-

strations, on site in Belize and back home at the National Museum of Natural History. To add to the festivities, our Carrie Bow field station, the logistical base and catalyst of our program, had reached the respectable age of 25 (1972–1997).

But the Gods of the Sea must have had other plans for this venerable facility. On 6 December 1997 an accidental fire broke out, aided by old, termite-riddled lumber and fanned by a strong northerly wind. Most of the station was reduced to ashes—laboratory, kitchen, living quarters, even wooden vats filled with water, all except a small cottage and the generator hut, which were isolated on the south end of the island. The blaze destroyed much valuable equipment, including microscopes, balances, solar system, weather station, and the contents of the library. As a result, little fieldwork could be done in the following year, although we did investigate the fire's damage to the island (20 or more coconut trees were lost) and the impact of recent complete flooding (which caused substantial coastal erosion). Two other points of interest were the impact on the reef after being subjected to stormy seas with waves up to 6 m and to an extensive calm period with shallow-water warming that appeared to precipitate the bleaching and death of large numbers of corals.

At this juncture, we gave serious consideration to terminating the CCRE program at this location—but not for long. Buoyed by the positive spirit of the Bowman family (and some insurance payback) and the talent of a young Cuban-trained architect, Hedel Gongora, we designed a new field laboratory to take the place of the old main house. It was built by local carpenters with lumber from pine forest in the west of the country, complete with wet lab, dry lab, library, running seawater system, workshop, and kitchen (Figures 10, 11). The facility was rededicated as the Carrie Bow Marine Field Station in August 1999, with more than 100 visitors in attendance to celebrate the occasion, including local fishermen, cooks, the Minister of Environment, the U.S. ambassador to Belize, scientists, and representatives of all major conservation societies. Over the next two years, we added one cottage for living quarters and rebuilt the one spared by the fire. With generous donations from a number of U.S. companies and individuals, we replaced and improved most laboratory equipment and instrumentation, and Tom Opishinski installed a new meteorological-oceanographic monitoring station enhanced by COASTMAP software (donated by the University of Rhode Island). By the beginning of 2000, CCRE was back on its feet, functioning as a year-round scientific program.

Resurgence and Biocomplexity

With a renovated field station, CCRE's scientific momentum took off once again, with new scientific opportunities as well as challenges. Nearly 80 scientific staff resumed field research disrupted by the fire or initiated new projects. A number centered on the sad effects of environmental stress or degradation on delicate but essential reef-building corals. To aid in the understanding of possible coral revival, Ken Sebens (then at the University of Maryland) and colleagues evaluated the benefit of water currents for the growth of the reef-building shallow-water coral *Agaricia*, which has been adversely affected by extended calm periods during hurricanes (Sebens et al., 2003). A parallel ecophysiological study found different tolerances to elevated temperature among species of *Agaricia* and speculated that their abundance may therefore vary with environmental disturbance (Robbart et al., 2004). However, corals that have survived such events may have their recovery impeded by the grazing of parrotfish, which are otherwise considered beneficial to the health of reefs (Rotjan et al., 2006). According to a series of investigations on predators and competitors of reef and mangrove sponges, these aggressors help defense mechanisms in sponges evolve (Wulff, 2005). In another sponge study, we concluded that encrusting excavating sponges have a competitive edge over reef corals weakened by elevated temperatures: the sponges can undermine the weakened opponent as well as displace its living tissue (Rützler, 2002). Assessments by Rich Aronson (Dauphin Island Sea Lab, Alabama), with collaborators, showed alarming recent changes in the composition of reef-building coral species as a result of stress and disease (Aronson et al., 2002, 2005), while John Pandolfi (then, Paleobiology) identified trends responsible for the decline of coral-reef ecosystems worldwide (Pandolfi et al., 2003). A discovery with harmful implications for human consumption of seafood (ciguatera poisoning) was the increase in toxic dinoflagellate algal blooms in our area (Faust and Tester, 2004), a phenomenon attributed to increased nutrient levels in lagoon waters, earlier considered a potential threat (Morton and Faust, 1997). On a more positive note, studies by Ana Signorovitch (then a graduate student at Yale University) applying innovative molecular methods to the enigmatic *Trichoplax* in the one-species phylum Placozoa found considerable genetic diversity there as well as signs of sexual reproduction (Signorovitch et al., 2005). Using a new approach to sponge systematics from the cytochrome oxidase subunit 1 gene tree, Sandra Duran (postdoctoral

FIGURE 10. New Carrie Bow Marine Field Station (2000) and Biocomplexity Program. Top row: left, aerial view of Carrie Bow Cay looking north (see details on map, Figure 11); right upper, the island with laboratory and living facilities as seen from the barrier reef (open-ocean side) and, image immediately below, view from the dock (lagoon side). Center row: left, flow-through seawater system photographed from the storage tank above; center, dock-mounted oceanographic sensors; right, view of upper-level dry lab. Bottom row: Starting the Twin Cays Biocomplexity Program on mangrove nutrient cycle: left, Ilka Feller with experimental enclosure in a tidal mudflat surrounded by black mangrove; right, subtidal bacterial mat with decaying mangrove leaves, an early stage in the cycle.

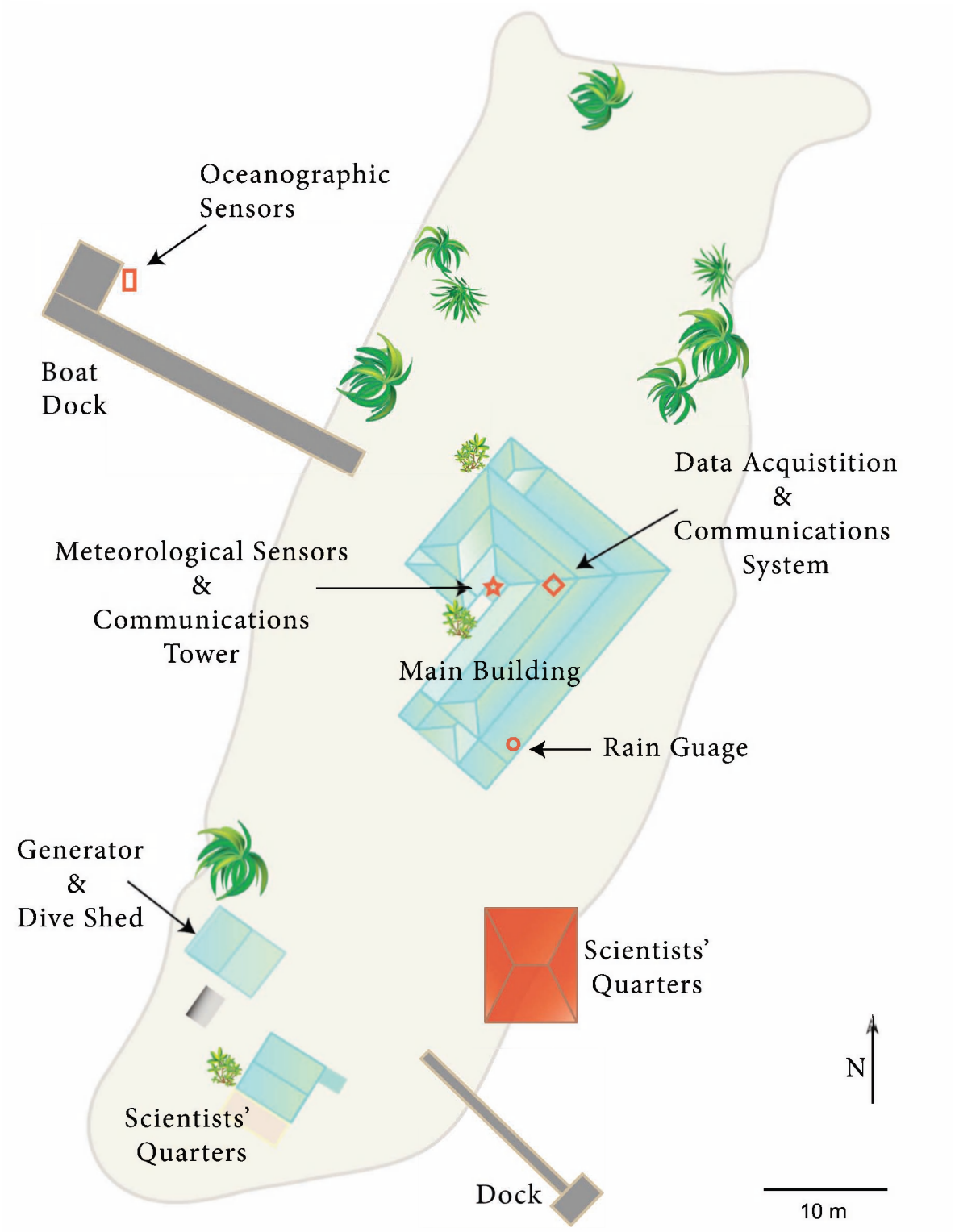


FIGURE 11. Map of Carrie Bow Cay (in 2000) identifying principal structures (number of coconut palms reduced for clarity).

fellow with Valerie Paul at SMS, Ft. Pierce) uncovered important information on rDNA phylogenies and an interesting case of ecological speciation wherein populations from reefs and mangroves in close vicinity were genetically more distant than those from similar habitats separated by thousands of kilometers (Duran and Rützler, 2006; Erpenbeck et al., 2007).

One breakthrough during this period was the five-year multidisciplinary "Mangrove Biocomplexity Study at Twin Cays: Microbial and Nutrient Controls," funded by the National Science Foundation and headed by Ilka Feller, with nine collaborators from outside institutions (see Figure 10). Several important contributions to an understanding of nutrient production, cycling, limitation, effects on organisms, and related ecophysiological phenomena have already been published (Feller et al., 2002, 2007; Lovelock et al., 2006; Cheeseman and Lovelock, 2004), but many more are expected in the near future now that the fieldwork phase of the Biocomplexity Program has come to a conclusion. One surprising discovery, in a complementary project by CCRE postdoctoral fellow Amy Erickson, was that the intertidal tree crab *Aratus*, long thought to be a mangrove leaf eater, actually prefers an animal diet if given a choice (Erickson et al., 2008). Another important finding, by a group from the University of Vienna, related to the microenvironment of sessile ciliates growing on mangrove peat banks and associated with chemoautotrophic bacteria coating their surface. By analyzing motion behavior of the ciliate and measuring microelectrodes in situ, scientists could show that the hydrogen sulfide required by the symbionts seeped into the boundary layer between the peat surface and oxygenated water column (Vopel et al., 2002).

CONCLUSION AND OUTLOOK

In 1972 a group of enthusiastic, like-minded young scientists embarked on a comprehensive, long-term field investigation of unprecedented dimensions for the Museum of Natural History. The team was unified in its belief that organisms had to be studied in their natural settings for a clear understanding of their features and role in their community (Figures 12, 13). Only then could a preserved museum collection aid in documenting the building blocks of an ecosystem. This approach was particularly true for coral reefs, which were all but inaccessible to scientists until scuba diving allowed in situ observations and experimental study. Similarly, it was essential to probe and sample substrata to better understand the structure of communities, present and past. The team directed its attention to the Caribbean because it is the Americas' tropical sea, to which our own nation is connected by weather, ocean currents, and marine resources, as well as by cultural and economic exchange.

In the beginning, we were convinced that together, and with the cooperation of selected specialized collaborators, we could pierce most of the secrets of a functioning coral reef in little more than a decade and generate models for predicting future trends. It did not take long for the restrictions of space, limits of available talents, and chronic shortage of funds to show us how naïve we were. Besides, as all scientists know, every resolved problem opens up new questions. Nevertheless, we can look back on over a third of a century of substantial progress, with more than 800 research papers in print and many more under way, all focused on a particular reef ecosystem and covering a significant time span. Our investigators addressed a vast array of subjects: biodiversity, from microbe to mammal; the geological and sedimentological setting and its developmental history; the physical and chemical factor regime; developmental biology, genetics, food chains, nutrient production, and cycling; behavior, competition, predation, and disease; and fisheries and conservation. We produced an impressive database that a new generation of motivated researchers can build upon with the benefit of technical advancements such as molecular analysis, which should shed further light on eutrophication, climate change, and other stress factors responsible for the increasing occurrence of algal blooms and devastating invertebrate diseases. These topics and more will need our full attention to help guide resource management and conservation efforts—and, above all, to preserve the aesthetic and economic value of the world's reefs.

Over the past few years our program has once again come up against a number of hurdles. Financial shortages in the Natural History Museum's budget have eroded our funding, while staff has been reduced and not replaced, leaving our scientific and management capabilities somewhat shaky. However, some of the slack was picked up by endowed funds, and our field station became part of the Smithsonian-wide Marine Science Network, joining the ranks of our "big brothers," the Environmental Research Center at Chesapeake Bay, the Marine Station in Florida, and the Tropical Research Institute in Panama.

It is gratifying to find that half the papers in this volume of the *Smithsonian Contributions to Marine Science* series emanated from our CCRE program and the Carrie Bow Marine Field Station. The scientific advances

FIGURE 12. Examples of recent projects conducted at Carrie Bow. Top row: left, diver sampling fish larvae in situ, a project on larval rearing and molecular identification headed by Carole Baldwin and Lee Weigt; right, Juan Sanchez setting up in situ experiment for study of gorgonian ecology and growth. Middle row: left, Randi Rotjan recording fish bites on coral (*Porites*) on the reef shallows (inset below: larval fish [*Rypticus*] reared by the Baldwin team in the Carrie Bow seawater system to develop characteristics used in identification of adults); center, colonial ciliates (*Zoothamnnium*), barely 15 mm tall, with bacterial ectobiont, dwell on mangrove peat and are studied by Joerg Ott's group; right, collaborator Kay Vopel measuring the microclimate surrounding *Zoothamnium*, primarily the oxygen versus hydrogen sulfide balance. Bottom row: left, Klaus Ruetzler recording progress of an excavating encrusting sponge (*Cliona*) that competes with temperature-stressed coral (*Diploria*) (center); right, new records of sponge disease are exemplified by this specimen (*Callyspongia*).

FIGURE 13. Research in progress and unanticipated new opportunities. Top row: left, Klaus Ruetzler initiated (with Carla Piantoni) a study of cryptic and cave-dwelling reef communities in shallow-water (center, upper photo) and deep-water (center, lower photo) habitats with little or no light exposure; right, Laurie Penland assists recording cave fauna using a digital HD video camera. Middle row: left, water warming during hurricanes killed many shallow-water corals in the Pelican Cays, which became overgrown by sponges (*Chondrilla*) that benefit starfish (*Oreaster*) as a source of food, thus starting a new ecological cycle; right, clear-cutting of mangrove and filling in resulting tidal flats with coral sand for "land development" started here at Twin Cays in the 1990s and continued at the Pelican Cays. Bottom row: left, Manatee Cay shown in 2008; right, this environmental disaster, recently stopped by the government of Belize, offers opportunities to study parameters of recovery of stressed and depleted marine and terrestrial communities.

achieved through CCRE research indicate that our decisions and actions over the years have blazed a fertile trail for the future of our science.

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The Smithsonian Tropical Research Institute: Marine Research, Education, and Conservation in Panama

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ABSTRACT. A grand geological experiment with a global reach to its biological impact, the formation of the isthmus of Panama between 15 and 3 million years ago split the tropical Interamerican Seaway into two and substantially changed the physical oceanography of each part. That event subjected the now-divided halves of the neotropical marine biota to new environmental conditions that forced each along a different evolutionary trajectory. For the past 45 years the Smithsonian Tropical Research Institute (STRI) marine sciences program has taken full advantage of this event by sponsoring research on a great diversity of topics relating to the evolutionary effects of the formation of the isthmus. That research, which has been supported by multiple laboratories on each coast and a series of research vessels, has produced more than 1,800 publications. Here we provide an overview of the environmental setting for marine research in Panama and an historical perspective to research by STRI's scientific staff at the different marine facilities.

INTRODUCTION

The unique geological history of Panama encourages a wider variety of research on tropical marine organisms than can be accomplished anywhere else in the world. The Central American Isthmus narrows in Panama to approximately 70 km, separating oceans that have very different oceanographic regimes and marine ecosystems. The formation of the central American isthmus, starting approximately 15 million years ago (Ma) and finishing in Panama about 3 Ma, had wide ramifications not only for the nature of the modern biological and geological world of the Americas but also for the entire global oceanic circulation. With the completion of this process the Gulf Stream strengthened, changing the Atlantic circulation. That change was soon followed by Northern Hemisphere glaciation, which in turn brought about a period of climate change in Africa that may have stimulated the origins of modern man. From a more local perspective, the completion of the isthmus set in motion a vast natural experiment: single populations of marine animals and plants were split and isolated in different and changing environments that forced their evolutionary divergence and fundamental changes in their biology.

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The Smithsonian Tropical Research Institute (STRI) marine research program in the Republic of Panama has taken full advantage of this globally significant geological event. In 1964 STRI established its first laboratories on the Pacific and Caribbean coasts within what then constituted the Panama Canal Zone. Since that time, marine research at STRI has expanded greatly and made major contributions to understanding of tropical marine biodiversity: the geological history of the isthmus and the development of environmental differences in the Caribbean and eastern Pacific, patterns of biodiversity in neotropical marine habitats, coral reef development, coral symbioses and diseases, the modes and tempo of species formation and diversification, evolutionary change within many groups of organisms relating to environmental differences on the two sides of the isthmus of Panama, and invasions by marine organisms facilitated by the Panama Canal and its shipping activity. To date marine research at STRI has resulted in more than 1,800 scientific publications; half of these have been produced by staff scientists and more than 200 published in high-profile journals such as *Science, Nature, Proceedings of the National Academy of Sciences of the United States of America, Proceedings of the Royal Society, American Naturalist, Evolution, Ecology,* and *Annual Review of Systematics and Ecology*.

In celebration of its role in coral reef research, the Smithsonian's 150th anniversary, and the International Year of the Reef, STRI hosted the Eighth International Coral Reef Symposium in Panama in 1996. This meeting brought 1,500 reef scientists and managers to Panama from around the world, resulting in the publication of a two-volume proceedings (Lessios and Macintyre, 1997), and an international traveling exhibit of coral reefs that is now resident at the Bocas del Toro Research Station.

Here we present an overview of the marine setting of Panama that clearly indicates its potential for research, and a historical summary of the diversity of marine studies conducted at the different STRI marine facilities. We then briefly outline STRI's marine education and outreach activities. Although this review focuses on the research activities of STRI's marine staff scientists, a strong fellowship program and a larger suite of visiting students and scientific collaborators have acted as substantial multipliers of STRI scientists' activities.

THE COASTAL OCEANOGRAPHIC SETTING OF THE ISTHMUS OF PANAMA

The emergence of the Isthmus of Panamá likely was the most crucial event for tropical marine ecosystems in the last 15 million years of earth's history. In Central America the marine environment experienced drastic changes in the two seas formed by the isthmus. As the isthmus approached closure, the Caribbean gradually became cut off from the eastward flow of Pacific water and became warmer, saltier (westward winds carried away evaporated moisture), and more oligotrophic. The Caribbean now is a relatively stable sea, with small fluctuations in temperature, relatively low tidal variation, and a relatively high salinity. Its relatively clear and nutrient-poor waters (D'Croz and Robertson, 1997; D'Croz et al., 2005; Collin et al., 2009) are ideal for the growth of coral reefs, and the wider Caribbean area ranks third behind the Indian Ocean and the Indo-West Pacific in terms of numbers of marine species. Annual rainfall is high on the Caribbean coast of Panama and follows the same basic seasonal pattern as on the lower-rainfall Pacific side of the isthmus (Kaufmann and Thompson, 2005). Relative to the Caribbean, the Tropical Eastern Pacific (TEP) exhibits much greater fluctuations in tides and temperature and has substantially lower salinity as a consquence of an area of very high rainfall along the Intertropical Convergence Zone. The TEP also has more and much larger areas of seasonal upwelling than the Caribbean. In addition, and in contrast to the Caribbean, the TEP also experiences strong longer-term variation in temperature and productivity from the influence of El Niño–Southern Oscillation Cycle (ENSO) events (D'Croz and O'Dea, 2009). Sea warming related to ENSO (which occurs at intervals of four to nine years) has drastic affects on coral reef development in the TEP. The direct marine effects of ENSO events in the tropical and warm temperate parts of the eastern Pacific are stronger than anywhere else in the world. Although coastal biological productivity is strongly related to benthic communities in the Caribbean, pelagic productivity and high availability of ocean-derived dissolved nutrients dominate the TEP, with high variability in those nutrient levels producing matching variability in the abundance of pelagic organisms (Miglietta et al., 2008). In Panama the nutrient-rich waters of its Pacific coast support commercial fisheries of major importance, fisheries that have no counterpart on the Caribbean coast.

The coastal marine communities of Panama are affected not only by inter-ocean differences in oceanography but also by marked local variation in shoreline environmental conditions along each coast. The Pacific shelf of Panama is wide and is divided, by the southward-projecting Azuero Peninsula, into two large areas, the (eastern) Gulf of Panama and the (western) Gulf of Chiriquí. The Gulf of Panama is subject to strong seasonal wind-driven upwelling, but the Gulf of Chiriquí is

not (Figure 1; and see D'Croz and Robertson, 1997). In the latter Gulf, high mountains block the wind and prevent wind-induced upwelling (D'Croz and O'Dea, 2007). In contrast, the Caribbean coast of Panama is relatively straight and has a narrow continental shelf, except in the (western) Bocas del Toro Archipelago. Hydrological conditions vary substantially along the Caribbean coast, ranging from the nutrient- and plankton-poor waters in the (eastern) San Blas Archipelago, where river discharge is low and the influence of open ocean water is high (D'Croz et al., 1999), to the more turbid environments of the Bocas del Toro Archipelago, where rainfall and river discharge are higher as a result of the blockage of westward moisture flow by the highest mountains in Panama (D'Croz et al., 2005; Collin et al., 2009). Thus, Panama lays claim to having "four oceans," providing unique opportunities for

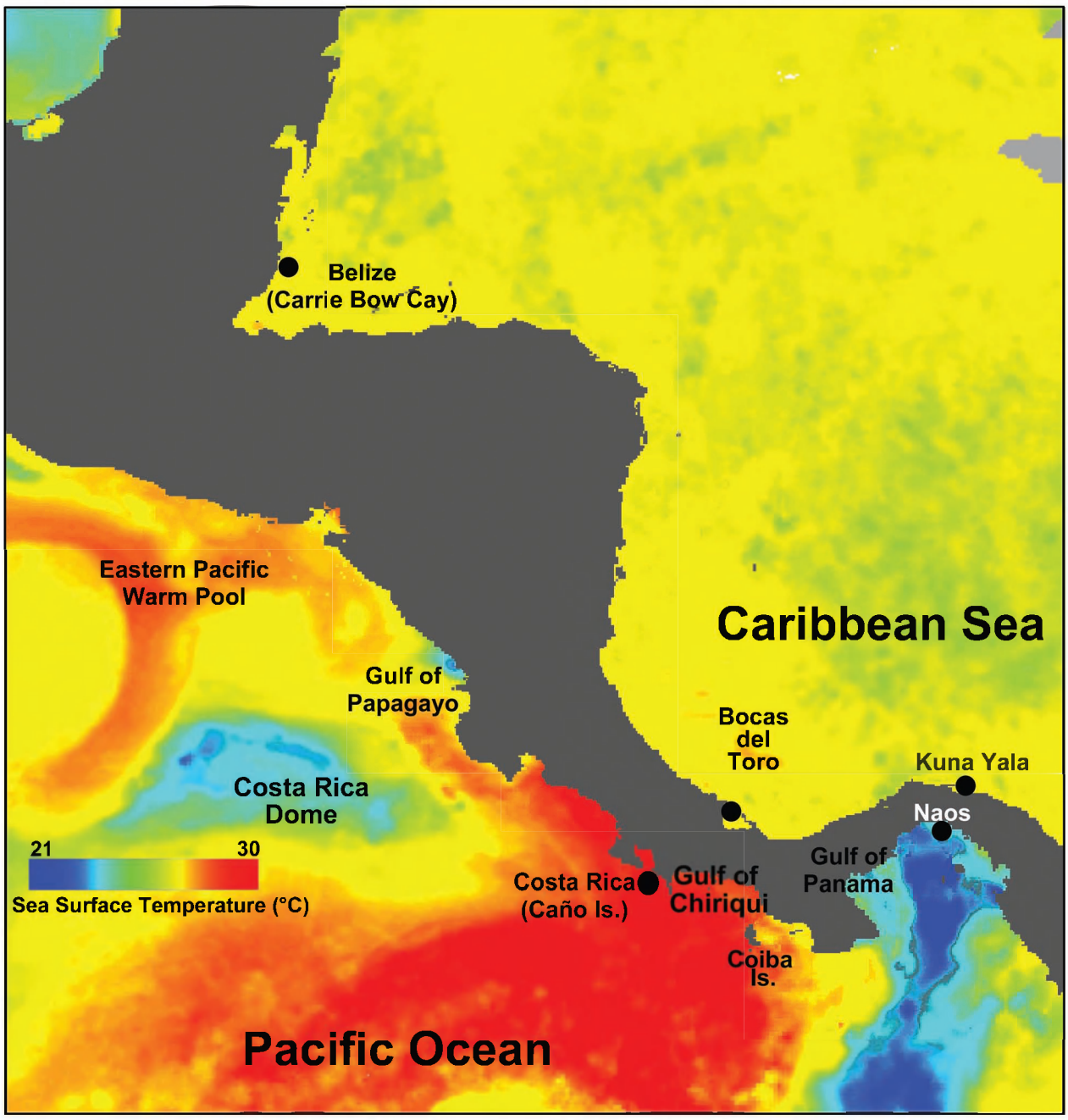

FIGURE 1. Temperature regimes on the Atlantic and Pacific coasts during the seasonal upwelling in the Gulf of Panama.

understanding how and why marine ecosystems vary and function as they do.

A HISTORICAL RESUME OF RESEARCH AT STRI MARINE FACILITIES

Marine research began at STRI in 1961with the doctoral work of STRI Director (Emeritus) Ira Rubinoff on trans-isthmian sister species of fishes (Rubinoff and Rubinoff, 1971), which led to STRI's first marine publication, based on work done near Punta Galeta (Rubinoff and Rubinoff, 1962). Since then the marine program has undergone exponential growth in its productivity. STRI currently operates two land facilities on the Caribbean coast of Panama, Punta Galeta Laboratory and Bocas del Toro Research Station, and two on the Pacific coast, Naos Island Laboratory complex and Rancheria Island field station. Between 1977 and 1997 STRI also operated a small, highly productive field station in the San Blas islands (Figure 2). In addition, STRI has maintained a series of small coastal research vessels that greatly expanded the geographic reach of its activities well beyond STRI's land facilities and, in fact, far beyond Panama.

Marine Environmental Sciences Program (MESP)

Monitoring the Physical Environment

In 1974, the Smithsonian Institution Tropical Environmental Sciences Program began monitoring physical environmental variables at Galeta, recording rainfall, wind speed and direction, solar radiation, reef flat water level, and air and water temperature hourly with automated equipment. Today, such physical data are recorded more frequently, automatically sent to a central processing facility via radio and internet, and added to a database that is available online at http://striweb.si.edu/esp/physical_monitoring/index_phy_mon.htm. Organization of physical data collection from Galeta has now been combined with that from Barro Colorado Island, Bocas del Toro, and several other sites, under the management of Karl Kaufmann. Recording of sea-surface temperature started at Galeta in 1988, and this monitoring now covers 33 shallow-water stations throughout the coastal waters on both coasts of Panama. Published summaries of the marine physical data include Meyer et al. (1974), Cubit et al. (1988), and Kaufmann and Thompson (2005). Physical environmental monitoring was conducted at the San Blas station from 1991 until its closure in 1998 and has been in progress at Bocas del Toro since 1999.

Monitoring the Biological Environment

The first phase of biological monitoring consisted of work done at Galeta that was stimulated by the two oil spills and formed part of their resultant studies. At San Blas, biological (plankton) monitoring co-occurred with the physical monitoring. At Bocas del Toro, biological monitoring that started in 1999 includes minor activity directed at seagrasses and mangroves in connection with CARICOMP. The major activity however, has been an expanding set of monitoring surveys of coral reefs by Hector Guzman, which now cover reefs scattered along both coasts of Panama (see http://striweb.si.edu/esp/mesp/reef_monitoring_intro.htm). This program developed from a survey of coral reefs in the general vicinity of Galeta made in 1985 (Guzman et al., 1991; and see also Guzman et al., 2008b).

Galeta Point Marine Laboratory

The Galeta Point installation became a STRI laboratory in 1964 when a military building constructed in 1942 on a fringing reef flat was turned over to STRI, thanks to the efforts of Ira Rubinoff. From his research on inshore fishes in that area (Rubinoff and Rubinoff, 1962) Rubinoff recognized its value as an easily accessible Caribbean study site. By 1971 Galeta Point was STRI's primary marine research site, providing access to a fringing reef flat, seagrass beds, and mangroves within a few meters of a laboratory building, with housing in nearby Coco Solo. Early work emphasized the comparison of reefs on both sides of the isthmus (Glynn, 1972) and the geological structure and history of the reefs (Macintyre and Glynn, 1976). Fundamental insights into differences between the Caribbean and eastern Pacific at Panama also were developed by Chuck Birkeland (Birkeland, 1977) when, during his long-term residence at Galeta, he analyzed patterns of biomass accumulation on settling plates deployed on both sides of the isthmus.

Permanent monitoring of the biota at Galeta Point was started in 1970 by Chuck Birkeland, David Meyer, and Gordon Hendler to provide baseline data on a tropical Caribbean reef flat; this was done to determine the effect of the Witwater oil spill, which occurred in December 1968 about 5 km east of Galeta. Because no baseline data were available to determine effects of that spill on reef communities, the US Environmental Protection Agency provided funds to set up the study and to perform experiments testing susceptibility of corals to oil. Transects were established at both Galeta Point and Punta Paitilla on the

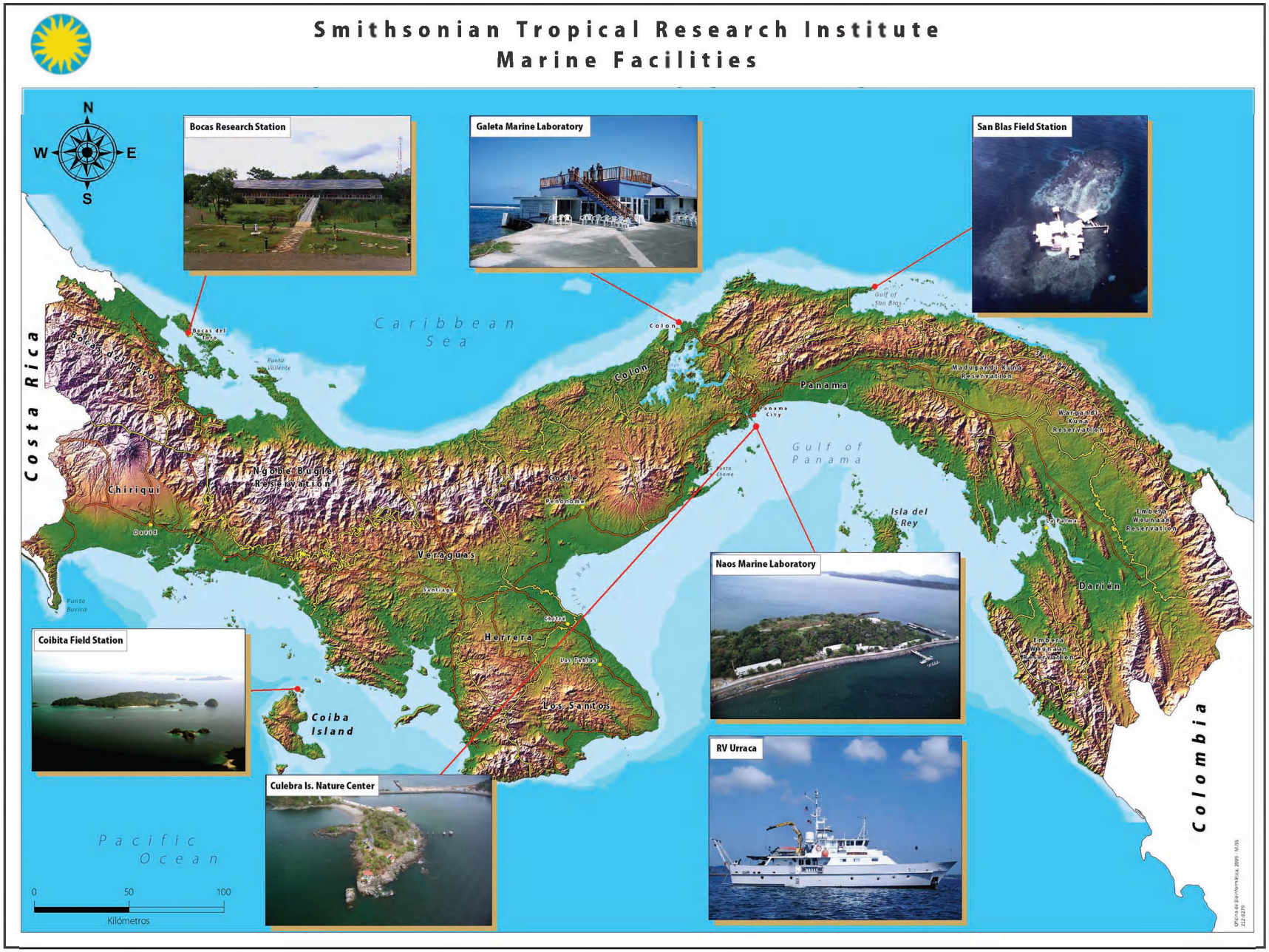

FIGURE 2. Map of Panama showing the distribution of Smithsonian Tropical Research Institute (STRI) marine facilities.

Pacific side to compare community structure, recruitment patterns, and the effect of oil on both communities (Birkeland et al., 1973).

In April 1986 a storage tank ruptured at an oil refinery about 4 km east of the laboratory, spilling 60,000–100,000 barrels of oil into the sea. The reef flat, grass beds, and mangroves around the Galeta laboratory were heavily oiled. This time a substantial amount of baseline data was available, thanks to the original transects set up by Birkeland, Meyer, and Hendler, whose monitoring had ended in 1982, and to the wider reef surveys in that area by Ernesto Weil. The Minerals Management Service of the U.S. Department of the Interior bestowed a 5 year grant to STRI to study the effects of the second oil spill in tropical areas (Keller and Jackson, 1993). That effort involved a considerable expansion of the types of data gathered, organisms studied, and habitats monitored over those in the first oil spill study. Subsequent to the second oil spill study the center of STRI research on coral reefs shifted first to San Blas then to Bocas del Toro. A long-term study (since 1988) of mangrove forest dynamics by Wayne Sousa (Sousa, 2007), occasional short-term projects, and physical environmental monitoring by MESP (see below) have continued at Galeta. The site also supports public education and outreach programs organized by Stanley Heckadon (see below). To date 315 publications include data obtained at Galeta laboratory, and the lab itself has produced 288 marine publications.

San Blas Field Station

The sparsely populated San Blas archipelago, in the autonomous Kuna Yala comarca, consists of several hundred sand cays scattered along the relatively sparsely populated eastern third of the Caribbean coast of Panama. The archipelago has the richest and most extensive development of coral reefs and associated fauna (including reef fishes) and flora in Caribbean Panama. Marine research sponsored by STRI began in San Blas in 1970, and research activity increased greatly in the late 1970s following the gradual construction by STRI from 1977 onward of a small field station that provided basic living accommodations and so allowed year-round research.

The San Blas field station, with its year-round access to a 15 km^2 area of rich reefs in calm, clear water, was the Caribbean base for many of STRI's comparative studies of the biology of closely related organisms on the Atlantic and Pacific sides of the Isthmus of Panama. Early research by STRI staff in San Blas included studies by STRI's founding director, Martin Moynihan, on the behavior of cephalopods (Moynihan, 1975; Moynihan and Rodaniche, 1982) and by Peter Glynn in the 1970s on coral reef development (Glynn, 1973). These investigations were followed by others on a broad range of organisms: Ross Robertson on the sexual patterns of labroid fishes, with Robert Warner (Warner et al., 1975; Robertson and Warner, 1978; Warner and Robertson 1978), and the recruitment dynamics and demography of reef-fishes (Robertson et al., 1999, 2005); Haris Lessios on the evolution and biology of echinoderms on the two coasts of the isthmus of Panama (Lessios, 1979, 1981, 2005); deputy director Eric Fischer on the sexual biology of simultaneously hermaphroditic groupers (Fischer, 1980, 1981; Fischer and Petersen, 1987); Nancy Knowlton on the biology and evolution of snapping shrimps and the reproductive biology, coral–algal symbioses, and evolution of corals (Rowan et al., 1997; Knowlton et al., 1977, 1992; Knowlton and Weigt, 1998); Jeremy Jackson on the comparative population and reproductive biology and evolutionary history of bryozoans on both sides of the Isthmus of Panama (O'Dea and Jackson, 2002; Dick et al., 2003; O'Dea et al., 2004); Luis D'Croz on comparative oceanographic conditions on the Caribbean and Pacific coasts of Panama (D'Croz and Robertson, 1997); and Hector Guzman on coral reef distribution and conservation (Andrefouet and Guzmán, 2005). During this period STRI also sponsored several anthropological projects on traditional Kuna society, acted as a conduit for international funding of Kuna marine management and conservation activities, and provide fellowships to Kuna University students.

The San Blas station provided essential support for projects on long-term ecological change on surrounding coral reefs. The combination of ease of access to shallow reefs, access as good as anywhere in the world, and the ability to do much work while on snorkel rather than scuba meant that it was possible to accumulate enormous data sets involving daily or shorter time period observations over months or years. These kinds of data are all too rarely available for tropical marine systems.

In early 1983 a Caribbean-wide mass die-off of an ecologically key organism on Caribbean reefs, the black sea urchin *Diadema antillarum*, began near San Blas and spread within the year throughout the entire Greater Caribbean. The year-round presence of biologists conducting long term studies of reef organisms at STRI's field station enabled the documentation of the start and spread of that event, which produced large, long-term effects on algal and coral growth on Caribbean coral reefs. Haris Lessios has followed the population and evolutionary consequences of that event for the urchin since it started (Lessios et al.,

1984; Lessios, 2005). Year-round monitoring of reef-fish populations on reefs around that station over a 20 year period contributed key information to a meta-population study that documents a gradual Caribbean-wide decline in the overall abundance of reef fishes since the *Diadema* dieoff (Paddack et al., 2009). Long-term monitoring of climatic and oceanographic conditions by MESP enabled detailed examination of linkages between environmental dynamics and the dynamics of recruitment of pelagic larvae of reef-fishes (Robertson et al., 1999). In addition regular station visitors accumulated the world's only long-term data sets on gorgonians and sponges. The former work includes data on a combination of population dynamics and genetics of clone structure obtained by Howie Lasker and Mary-Alice Coffroth (Coffroth et al., 1992; Lasker, 1991; Lasker et al., 1996). The latter work includes data on the dynamics of sponge communities collected by Janie Wulff (Wulff, 1991, 1997).

Over the 20 years of its existence, research supported by the San Blas field station produced 363 publications on the biology of plants and animals living on the coral reefs around the station, at a peak annual operating cost of about US$100,000. The cheapness of this operation provides a startling example of how effective a small station can be for very little expense, so long as the necessary tools for field research are supplied: grass huts for living, rainwater for drinking and washing, communal kitchens, small boats, a scuba compressor, and, above all, field sites in calm clear water at the station's doorstep.

Local political events in this autonomously governed indigenous reserve led to the closure of the San Blas station in 1998. Although this closure terminated the activities of STRI staff scientists in that area for some time, several external researchers were able to make private arrangements to continue their work there. After the closure of the San Blas station the center of STRI's Caribbean research efforts moved to Bocas del Toro Province, at the opposite end of the Caribbean coast of Panama.

Bocas del Toro Research Station (BRS)

The Smithsonian Institution (SI) has a long history of terrestrial and geological research in Bocas del Toro Province. In the 1970s and 1980s Charles Handley of the Natural History Museum mounted a number of expeditions to the province to survey the mammal and bird fauna (Handley, 1993; Anderson and Handley, 2002). This phase was followed with ground-breaking geological work by STRI's deputy director, Anthony Coates. He used the rock outcrops in the province, which contain the most complete record of marine environments of the last 10 million years in the southern Caribbean, to help clarify events associated with the rise of the Isthmus of Panama (Collins et al., 1995; Collins and Coates, 1999; Coates et al., 2005).

In 1998 STRI purchased 6 hectares (ha) just outside the town of Bocas del Toro on Isla Colon. A dormitory was built on the site in 2001 and a modern, well-equipped laboratory in 2003. The BRS now houses 28 resident scientists and will soon add accommodation for 16 more. BRS can now host approximately 325 scientific visitors from more than 30 countries each year: 40% undergraduates, 25% graduate students, 10% postdoctoral fellows, and 25% researchers. About half the postdoctoral fellows and researchers are SI scientists. Research at the station has resulted in 201 scientific publications in the five years since its inauguration in 2003, with Rachel Collin as its director.

The BRS is now among the preeminent research stations in the Caribbean. It is better equipped and provides access to a larger diversity of habitats than almost any other research facility in that region. The wealth of natural diversity available near BRS combined with technical support facilities is reflected in the wide range of research projects that are conducted at the station. Significant research has focused on the coral bleaching response to stress and disease. These studies have shown that sugars are one of the most damaging components in pollution from rain runoff (Kline et al., 2006) and that coral disease is related to temperature stress. An SI fellow identified candidate genes that participate in coral's bleaching response to elevated temperature (DeSalvo et al., 2008). Research at the laboratory also has shown that some coral disease and death in the Caribbean results from a protozoan infection. Another strong line of research at the BRS is the investigation of the factors that lead to speciation in the marine environment. Groundbreaking work on hamlet fishes has shown that mate choice based on color pattern may drive divergence and that color patterns may evolve via aggressive mimicry, a previously undemonstrated mechanism of diversification (Puebla et al., 2007, 2008).

The BRS is also a local focus of taxonomic work and studies aimed at documenting marine biodiversity that were published in a special issue of the *Caribbean Journal of Science* dedicated to the marine environment and fauna of Bocas del Toro (Collin, 2005a, 2005b). Extensive work has been done there on the taxonomy of marine shrimps (Anker et al., 2008a, 2008b, 2008c). Bocas del Toro is a global hotspot of shrimp diversity and ranks within the top 10 sites in the world. More than 20 new shrimp species from Bocas del Toro have been described in the past five

years. New species of other marine organisms, including snails, tunicates, sponges, flatworms, and meiofauna, have also been described on the basis of work at the BRS. As a result of these taxonomic and faunal studies, Bocas del Toro has the highest recorded tunicate diversity anywhere in the Caribbean and the third highest sponge diversity.

Other long-term projects based at BRS include studies focused on breeding success at major Caribbean turtle nesting beaches, the effects of noise polluction and tour boat operations on dolphin vocalization and behavior, effects of anthropogenic substrates, such as docks, on invasive tunicate abundance, effect of nutrient limitation on mangrove forest structure and diversity, emerging sponge diseases, and Caribbean-wide speciation in *Montastraea* corals caused by temporal shifts in spawning cycles.

Naos Island Laboratories

Naos is one of a cluster of four islands at the end of a 2 km long causeway at the Pacific entrance to the Panama Canal. STRI's first marine laboratory was established there in 1964 in an old military bunker and has since expanded to four buildings, three of them ex-US Navy buildings refurbished by STRI. This laboratory provides ready access to the upper bay of Panama with its extensive mangroves and a scattering of inshore islands, plus the coral reefs of the Perlas Archipelago, 50 km away. The laboratory complex, with a flow-through aquarium system, diving locker, small boat support, research vessel, and molecular laboratories, supports a wide range of research by all the marine scientific staff. Organismal studies based primarily at Naos cover or have covered the following topics: the Panama Canal as a hub for marine invasions, rocky intertidal community ecology, physiological ecology, behavioral ecology of intertidal organisms, coral reef development in the TEP, molecular evolution of marine organisms, life history evolution and evolution of mode of development, and marine zooarchaeology.

Panama as a Hub for Marine Invasions

Biological invasions are a potent force for change across the globe. Once established, introduced species can become numerically or functionally dominant, threatening native biodiversity and altering ecosystem processes. The flip side to the damage they cause is that introduced species can provide opportunities for insight as large-scale experiments to understand ecological and evolutionary processes. In marine and coastal environments, shipping is a major pathway for biological invasions and appears largely responsible for the recent dramatic increase in invasions.

Beginning with the studies of Hildebrand (1939) in the 1930s, followed by several investigations surrounding the potential problems associated with the construction of a sea-level Canal in Central America (Rubinoff, 1965, 1968; Rubinoff and Rubinoff, 1969), STRI has been central in evaluating the role of the Canal as a passageway for shorefishes. Interestingly, despite the Canal's 100 years of existence and the occurrence of approximately 1,500 species of marine and brackish-water fishes on the two coasts of Panama, only a handful of such species have successfully passed through the Panama canal and established populations in the "other" ocean. For example, only 8 species of such successful immigrants are known in the tropical eastern Pacific and only 3 have spread beyond the immediate confines of the Pacific entrance to the canal. Why are there so few successful invasions through a short, suitable corridor? Why do some invasions fail and others succeed? Panama and its canal have much to offer studies aimed at determining success or failure of invasions.

STRI is ideally situated to study marine and coastal invasions. Panama is one of the world's largest hubs for shipping. The Canal serves as an aquatic corridor connecting the Atlantic and Pacific Ocean basins, and ports on either side serve as hubs for international trade. Since its opening in 1914, approximately 800,000 ocean-going commercial vessels have passed through the Canal. Today, approximately 12,000 to 14,000 commercial ships transit the Canal annually (Ruiz et al., 2009). Moreover, Panama is initiating a major effort to expand shipping in the Canal by constructing additional locks on the Pacific and Atlantic coasts. Although the freshwaters of Lake Gatun, a large lake that constitutes the bulk of the canal, have strongly limited the inter-oceanic invasions of purely marine species, the new locks being added to the canal have the potential to increase the salinity of Gatun Lake and increase such interchange. With the Naos and Galeta marine laboratories strategically located at the Pacific and Atlantic entrances to the Canal, STRI is well positioned to continue to conduct a broad range of basic research on marine invasions.

In contrast to the limited exchange of fishes across the Isthmus, various introduced invertebrate species have been documented recently in the Canal, underscoring the fact that invasions are occurring. Some examples include a North American mud crab that has established a population in the Panama Canal expansion area (Roche and Torchin, 2007) and an invasive Japanese clam that reaches densities greater than 100 m^{-2} in the Canal, as well as an

invasive snail that is known to host medically important trematode parasites. Although there are likely other such species, with few exceptions (Abele and Kim, 1989) invertebrate diversity of the Canal remains largely unexplored. Recently, STRI and SERC scientists teamed up to evaluate the role of the Canal in biological invasions and determine how patterns and processes driving invasions in tropical and temperate regions may differ.

Although the potential for invasions in Panama is likely to be high, with the exception of studies on fishes that have passed through the Canal in the past 40 to 50 years, we know surprisingly little about other coastal invasions that have resulted, and many established invasions probably have been overlooked (Miglietta and Lessios, 2009). With the current expansion of the Panama Canal, evaluating the importance of the Canal in regional and global invasions is arguably an imperative goal for the conservation of our coastal resources and an ideal opportunity to illuminate our understanding of biological invasions.

Rocky Intertidal Community Ecology

The rocky intertidal zone of the TEP appears to be largely bare rock, with very little macroalgal cover and few sessile invertebrates, which are not distributed in clear zones according to tidal height or wave exposure. The striking contrast between this system and those of temperate North America and Europe, which are well vegetated and have an abundance of invertebrates in regularly arranged zones, drew researchers such as Jane Lubchenco (currently director of the NOAA) and Bruce Menge to STRI in the late 1970s to seek an explanation. Their field exclusion experiments indicated that year-round predation and herbivory by a diverse community of highly mobile fishes, crabs, and mollusks forces their prey into refugia in cracks and under boulders and regulates directly, or indirectly, species interactions such that species capable of dominating space are kept in check (Menge and Lubchenco, 1981; Menge et al., 1986).

Physiological Ecology

The marine environment of the eastern Pacific is much more variable than that of the Caribbean, especially so during upwelling and in shallow-water and intertidal habitats. Temperatures in tidal pools at Naos range between approximately 18°C and more than 50°C. Jeffery Graham made contributions to basic understanding of how fishes and sea snakes contend with this and other physiological challenges in the TEP (Graham, 1970, 1971) and later investigated heat regulation in tunas (Graham, 1975). Ira Rubinoff, together with Graham and Panamanian cardiologist Jorge Motta, performed pioneering work on the temperature physiology and diving behavior and respiratory physiology of the neotropics' only sea snake species (Graham et al., 1971; Rubinoff et al., 1986).

Behavioral Ecology of Intertidal Animals

Marine behavioral and estuarine (soft-bottom) ecology has been the focus of long-term research programs by John Christy and his students on the reproductive ecology (larval release cycles in relation to predation risks; Morgan and Christy, 1995) and behavior (burrow ornaments as sexual signals; Christy et al., 2002) of intertidal crabs, particularly fiddler crabs. The latter reach their highest species diversity in the world on the Pacific coast of Central America (Sturmbauer et al., 1996). Christy recently completed five years of daily observations of the reproductive timing of a fiddler crab on Culebra beach, the results of which demonstrate that these crabs have a remarkable ability to track, on several time scales, complex variation in environmental conditions suitable for larval release. Research by Christy's lab on mechanisms of mate choice in fiddler crabs has shown that male courtship signals elicit responses in females that have been selected by predation, not because the signals lead to choice of the best male as a mate. This research has provided the best empirical support to date for the "sensory trap" mechanism of sexual signal evolution (Christy, 1995; Backwell et al., 2000, Kim et al., 2009). Together with work by terrestrial biologists at STRI, this research has made STRI a global center for the study of the evolution of sexual signals.

Coral Reef Development in the Tropical Eastern Pacific (TEP)

Following the closure of the isthmus, different components of the tropical biota of the TEP reacted in different ways to resultant dramatic changes in the local marine environment. Most of the coral fauna was wiped out (~85% of the current, depauperate fauna is derived from Indo-Central Pacific immigrants), probably largely by extreme environmental fluctuations during ENSO events. Documentation of effects of environmental changes on coral reef development in that area has been the focus of 35 years of studies by Peter Glynn and his collaborators, not only in Panama but also further afield in the TEP in places such as the Galapagos (Glynn et al., 1979; Glynn and Wellington, 1983). STRI research on Panama's Pacific coral reefs began in the earlier 1970s, when, contrary to

previous ideas, fully developed coral reefs were found in the Gulfs of Panama and Chiriquí (Glynn, 1972; Glynn et al., 1972; Glynn and Stewart, 1973). It also became evident that differences in reef growth in those gulfs were related to their different temperature regimes (Glynn and Stewart, 1973). Coral reefs in the Gulf of Panama are mainly confined to the warmer sides of the Pearl islands and grow at lower rates than reefs in the year-round warmth of the Gulf of Chiriquí (Glynn and Macintyre, 1977). The latter reefs grow at rates comparable to those on the Caribbean coast of Panama (Macintyre and Glynn, 1976), and corals in each gulf differ in their responses to temperature (D'Croz et al., 2001; Schloeder and D'Croz, 2004). A major thrust of work on TEP reefs has been to understand the effects of ENSO warming events on the survival and dynamics of reef ecosystems. Observations linked coral bleaching in Panama to high-temperature anomalies of the severe 1982–1983 ENSO (Glynn et al., 1988; Glynn and D'Croz, 1990; Glynn et al., 2001). Such bleaching led to region-wide mass coral mortality during the intense 1982–1983 and 1997–1998 ENSO events (Glynn, 1984; Glynn et al., 2001). Microcosm experiments at STRI confirmed that temperature stress produced bleaching and mass mortality of corals (Glynn and D'Croz, 1990) and that slow-growing massive species are more resistant than branching types to temperature-induced beaching (Huerkamp et al., 2001). There has been continuous monitoring of reef recovery since the mass coral mortality produced by the 1982–1983 ENSO, providing one of the longest term databases of this type in the world (Glynn, 1984, 1990; Glynn and Colgan, 1992; Glynn et al., 2001). Major efforts have also been made to investigate the reproductive ecology of corals, relating fecundity, spawning activity, and recruitment of surviving species to community recovery and reef resilience in Pacific Panama (Glynn et al., 1991, 1994, 1996, 2008; Colley et al., 2006; Manzello et al., 2008). Bleaching patterns have been related not only to the diversity of zooxanthellae symbionts of corals (Glynn et al., 2001) but also to coral genotypes (D'Croz and Maté, 2004), with both factors likely playing an important role in adaptive responses by corals to climate change. Research on corals in Pacific Panamá additionally involves the taxonomy and biogeography of gorgonian soft corals (Vargas et al., 2008; Guzman and Breedy, 2008).

Molecular Evolution of Marine Organisms

STRI has played a leading role in development of molecular techniques for studies of marine organisms, not only in relationship to trans-isthmian biology of neotropical taxa (reviewed by Lessios, 2008) but also in studying the global biogeography of pantropical groups. A 30 year history of such work, the longest in SI, began with studies of sea urchins by Haris Lessios (Lessios, 1979). That work, although centered at the molecular laboraties at Naos Laboratory, has relied on all other STRI marine facilities for collections and maintenance of live organisms. Since that start, molecular evolution studies at STRI have undergone explosive growth. Such studies include assessments of effects of the rise of the isthmus on the ecology and biology of neotropical organisms (Collin, 2003a) and patterns and processes involved in the evolutionary divergence of such taxa (Knowlton and Weigt, 1998; Hurt et al., 2009). Molecular studies also have led to the delineation of species boundaries in marine organisms (Knowlton, 2000) and understanding of global historical biogeography of pantropical groups (Lessios et al., 1999, 2001; Collin, 2003a, 2003b, 2005a; Quenoiville et al., 2004), invasions of the tropical Atlantic by Indo-Pacific taxa around southern Africa (Bowen et al., 2001; Rocha et al., 2005a), patterns of dispersal among different tropical biogeographic regions within the Atlantic (Lessios et al., 1999; Rocha et al., 2002, 2005b), physiological mechanisms involved in species formation (McCartney and Lessios, 2002; Zigler and Lessios, 2004), non-allopatric speciation within biogeographic regions (Rocha et al., 2005a; Puebla et al., 2007, 2008), patterns and processes involved in speciation of corals (Fukami et al., 2004), and the history of two-way transfers of species across the 4,000–7,000 km wide Eastern Pacific Barrier, the world's widest stretch of deep open ocean (Lessios and Robertson, 2006). Molecular evolution studies at STRI have produced 163 marine-themed publications to date.

Marine Archaeology: Historical Human Reliance on Marine Resources in Panama

Zooarchaeology has played an important role in STRI's anthropology program for the past 40 years (Linares and Ranere, 1980) through studies originated by Richard Cooke of pre-Columbian usage of marine resources in Panama, primarily in Panama Bay (Cooke, 1981). The expanding reference collection of 1,540 skeletons of 340 species of fishes and other organisms used in this research has also enhanced knowledge of the zoogeography of these organisms (Cooke and Jiménez, 2008b). This work has charted the time course of geographic changes in patterns of marine resource usage in Panama Bay. By 7,000–4,500 bp, humans on the shores of that bay exploited a wide variety of inshore marine resources, including more than

80 species of marine fishes (bony fishes, sharks, sawfish, sting rays) taken in a variety of different habitats (beaches, mangroves, estuaries, reefs, open water) using various methods (hook-and-line, nets, stationary wood-and-stone traps) (Cooke, 1992; Cooke and Jiménez, 2004, 2008a; Cooke et al., 2008). Other marine resources used include sea turtles, dolphins, manatees, and seabirds. The ritual importance of marine animals in pre-Columbian Panama is underlined by frequent images of sea turtles, fish, and marine invertebrates on pottery and goldwork (Linares, 1977; Cooke, 2004a, 2004b). Although currently there is no convincing zooarchaeological evidence for overfishing in pre-Columbian times in Panama, ongoing research in the Pearl Islands seems likely to identify pressures that produced changes to populations of mollusks and reef fish around individual islands. Intensive collection of colorful marine shells and marine birds for making ornaments likely led to local impacts on populations of these taxa.

Rancheria Island Field Station

Rancheria Island is situated in the center of the largest and best managed marine reserve in Panama: the Coiba National Park (and World Heritage Site) in the Gulf of Chiriqui. The park area has a long history of environmental protection (Coiba acted as a "free-range" prison island for almost 85 years) and hosts the largest area of coral reefs and richest [number of species] accumulation of corals on the entire continental shore of the TEP. A tiny, relatively undeveloped field station at Rancheria has supported research on coral reefs in the surrounding area by Peter Glynn and his collaborators (see above).

The Research Vessels

Four vessels were operated by STRI between 1970 and 2008: the 65-foot *Tethys* (1970–1972), the 45-foot RV *Stenella* (1972–1978), the 63-foot RV *Benjamin* (1978–1994), and the 96-foot RV *Urraca* from 1994 to 2008. None of those vessels was purpose built. The equipping of the *Urraca*, after its purchase, with an A-frame and oceanographic winch allowed intensive trawling and dredging activities (to depths of 250 m) and thus greatly extended the range of studies that could be supported beyond the previous emphasis on scuba-based research. These research vessels, and particularly the *Urraca*, enabled fieldwork in remote locations that lacked land bases for marine research and thus vastly extended the geographic reach of STRI biologists. The *Urraca* acted as such a base not only throughout Panama's territorial waters but also in localities as far afield as Clipperton Island (1,000 km west of Acapulco) in the Pacific (Robertson and Allen, 2008) and Honduras in the Caribbean (Guzman, 1998).

To date, 14 years service by the *Urraca* has produced 350 scientific publications. Research supported by the *Urraca* proved vital to the declaration of two large Marine Protected Areas (MPAs) on the Pacific coast of Panama, principally through the research activities of H. Guzman on coral diversity and conservation (see below). *Urraca* support of collecting along the entire Pacific coast of Panama, as well as Costa Rica, Clipperton and Cocos Islands (remote oceanic islands in the eastern Pacific), and El Salvador was essential for the development of the world's first online information system on a regional shorefish fauna (www.stri.org/sftep). In addition the *Urraca* provided extensive and extended support to the Panama Paleontology Program (see below) and for collecting fishes (Bermingham, Robertson), echinoderms (Lessios), soft corals (Guzman), and mollusks (Collin) for taxonomic and evolutionary studies, and hydrologic surveys (D'Croz).

HISTORICAL MARINE ECOLOGY: THE PANAMA PALEONTOLOGY PROJECT

STRI is unique in having an institutional marine program that includes both biology and geology, as well as a series of strong programs in various aspects of tropical terrestrial biology. Intellectual cross-fertilizations between scientists steeped in terrestrial and marine systems have maintained STRI as a place known for creative research.

The striking differences in environmental conditions and ecology from opposite sides of the Isthmus of Panama today, and their changes over time during Isthmus closure, provides marine paleontologists with a "natural experiment" with which to address, on an evolutionary and ecologically large scale, the impact of environmental change and genetic isolation on marine invertebrate faunas. In 1986 the Panama Paleontology Project (PPP) was initiated by Jeremy Jackson and Anthony Coates. Their aim was to survey coastal sediments of the isthmian area to establish if the fossil record were sufficiently complete to explore the evolutionary responses of marine communities to the gradual emergence of the Isthmus of Panama.

Stratigraphically complete Neogene deposits were soon discovered in the Panama Canal basin and Bocas del Toro, and excavations were subsequently extended to several other richly fossiliferous regions of Panama and Costa Rica, Venezuela, Ecuador, Jamaica, and the Dominican Republic. In addition, large-scale benthic

surveys of modern shallow-water communities across the Caribbean and Tropical Eastern Pacific serve as a baseline for understanding biotic changes through geological time.

The PPP has so far involved more than 50 scientists from 20 institutions in seven countries and undertaken almost 40 expeditions to eight countries. The resultant collections comprise thousands of replicated samples and many millions of individual, quantitatively collected fossil specimens. The rigorous paleontological framework of the PPP presents evolutionary biologists with a unique view of 15 million years of life and environments in a tropical region. Using these samples and framework, the PPP has documented the environmental, lithologic, and biological changes in Isthmian nearshore marine habitats from 15 Ma to the present day, producing almost 200 publications to date (see http://www.fiu.edu/~collinsl/pppcon.html).

Placing igneous and sedimentary rock formations in sequence established a high-resolution stratigraphic system that was critical to effectively reconstruct patterns of biological change (Coates et al., 1992, 2005; Collins et al., 1996b; Collins and Coates, 1999). Aligned with taxonomic and paleoenvironmental analyses, these geological studies also permit reconstructions of land and water masses as the isthmus shoaled, providing dates of final closure that are essential for estimates of the timing of divergence of modern marine organisms (Collins et al., 1995; Coates and Obando, 1996) (Figure 3).

Data from PPP studies have revealed the following. (i) Faunal composition of Caribbean and Pacific fossil assemblages and the timing of paleoenvironmental change demonstrate that major cross-isthmian marine connections ceased approximately 3 Ma (Collins et al., 1995, 1996a; Coates et al., 2003, 2005; O'Dea et al., 2007a), consistent with dates from previous (non-PPP) oceanographic studies. (ii) Seasonal upwelling was strong in what is now the southwestern Caribbean (SWC) before isthmian closure, and constriction of the forming isthmus led to a rapid decline in upwelling intensity, resulting in a collapse in primary productivity from around 5 to 3 Ma (Collins, 1996). The increasing oligotrophy allowed reefal habitats to expand in the SWC while reducing the amount of filter-feeding molluscan habitat, and the cessation of upwelling also stabilized environments to modern-

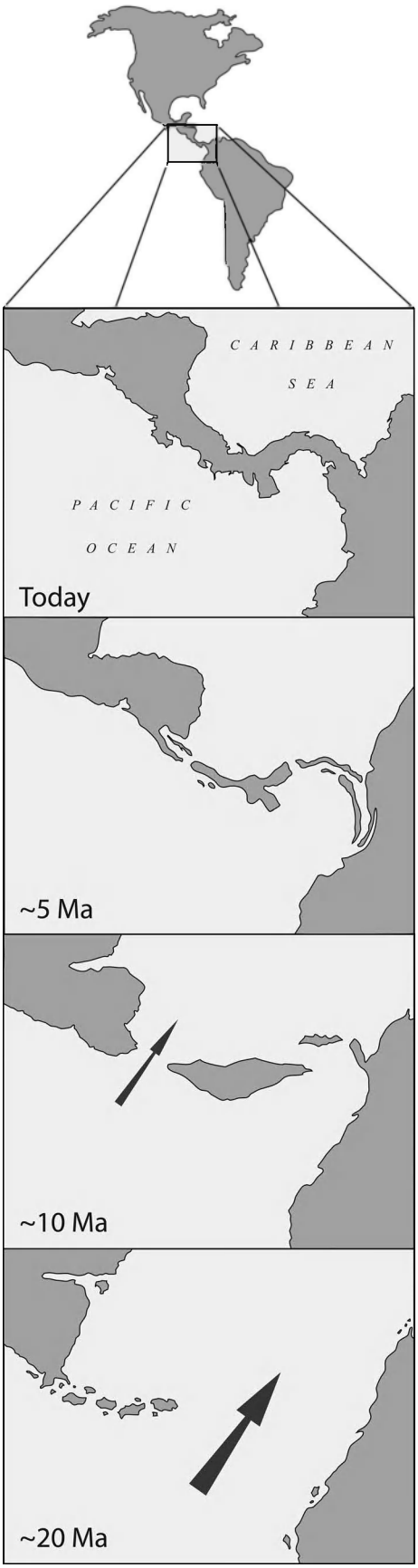

FIGURE 3. Formation of the Isthmus of Panama during the last 20 million years (Ma = million years ago). Arrows indicate direction of principal water flow through the Central American Seaway. (From O'Dea et al., 2007b.)

day conditions (O'Dea et al., 2007a; Jackson et al., 1999). Meanwhile, upwelling continued in what is now the TEP to the present day. (iii) A wide assortment of marine taxa experienced a major turnover in the now-SWC during the last 10 million years (Jackson et al., 1993; Jackson and Johnson, 2000; O'Dea et al., 2007a; Smith and Jackson, 2009). Origination of new species in all major groups of macroinvertebrates peaked at about 5–3 Ma, coincident with the formation of new habitat along the SWC coast of the Isthmus. (iv) From approximately 5–3 Ma the SWC remained connected to the TEP but coastal conditions became instable. This transition period saw most SWC faunas reach their peaks in diversity (Jackson and Johnson, 2000; Todd et al., 2002; Smith and Jackson, 2009). As old and new species coexisted in time, richness of most groups was around 30% to 60% higher than in the modern SWC. (v) Following isthmus closure and the birth of the modern-day Caribbean, a widespread extinction reduced numbers of gastropod, bivalve, coral, and bryozoan taxa by 30% to 95%. (vi) This massive extinction was strongly selective against nutriphilic taxa, indicating that the collapse in primary productivity was the causal mechanism. However, fine-scale environmental and community composition data reveal that extinction in most groups lagged well behind the shift to more oligotrophic conditions as the Isthmus closed (O'Dea et al., 2007a) (Figure 4). Time lags of this scale challenge the conventional wisdom that cause and effect have to be contemporaneous in macroevolution. (vii) Other ecological characteristics of organisms also shifted dramatically. Average coral colony and snail egg-size increased, larval durations of scallops decreased, and rates of clonality in free-living bryozoans declined dramatically. Ongoing field and laboratory work aims to analyze the fates and trajectories of clades that preserve modes of life, life histories, and feeding strategies in fossils within the rigorous framework provided by the PPP. This approach will help tease apart the drivers of macroevolutionary change in the neotropical seas (Jackson and Erwin, 2006).

MARINE EDUCATION AND OUTREACH

At the level of both the institution and the individual scientist, STRI, along with other SI bureaus, has become deeply involved in two global efforts connected with marine biodiversity: the Census of Marine Life (COML) and the Consortium for the Barcode of Life (CBOL). The COML aims to provide rapid and full documentation of marine biodiversity, while CBOL provides easy

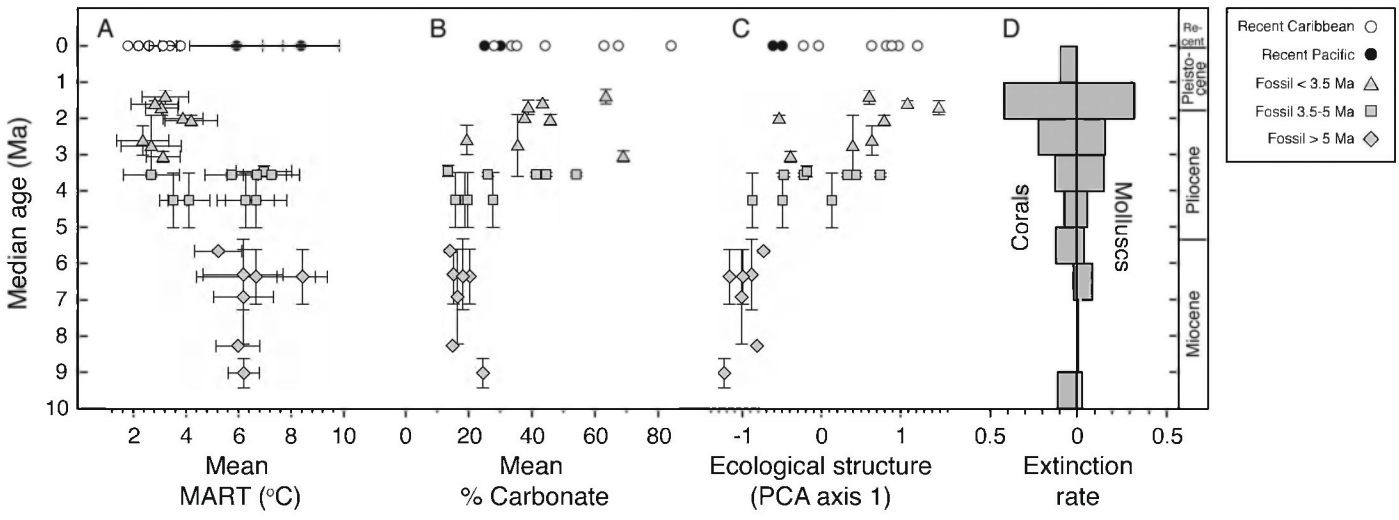

FIGURE 4. The sequence of environmental and ecological changes in the southwest Caribbean in response to the closure of the isthmus of Panama (Ma = million years ago). A. Upwelling intensity, as estimated by the mean annual range of temperature (MART), shifted rapidly from high values similar to the modern-day tropical eastern Pacific values to modern Caribbean values. B. Carbonate levels in sediments followed suit, with an increase in the Caribbean. C. Biotic assemblages shifted from mollusk-dominated to a mix of coral-, algae-, and mollusk-dominated communities (PCA = principal components analysis). D. Extinction rates of corals and mollusks peaked 1–2 million years after the environmental and ecological changes. (From O'Dea et al., 2007a.)

molecular means to confirm the identities of a broad array of species in both marine and terrestrial ecosystems. Substantial contributions of information on neotropical marine organisms have been made by STRI to both those efforts. Recently, the Encyclopedia of Life (EOL) began to make use of information generated by STRI scientists, and STRI also recently became part of the United States Geological Survey's Caribbean tsunami monitoring network.

Educational and outreach programs at STRI include a marine fellowship program for graduate students (worldwide, plus targeted to Latin America), hosting of K–12 school groups and teacher training (at Galeta Point Marine Laboratory and Bocas del Toro Research Station), conducting public seminars, responding to requests for information from Panamanian government entities, and supporting graduate student courses. The public marine education program at STRI consists of a series of activities aimed at promoting awareness and conservation of marine environments and communicating its research to the general public. Since 1992 the program has consisted of docent-led educational visits, seminars for teachers, and the development of educational materials (posters, newspapers and supplements, exhibits), and curricular materials for the classroom.

Culebra Island Education Center

The Punta Culebra Nature Center (PCNC) of STRI lies at the Pacific entrance to the Panama Canal immediately adjacent to the Naos Laboratory. For nearly a century, access to Culebra was restricted to U.S. military personnel, a practice that protected Culebra's shoreline organisms, which now exist in abundances not seen elsewhere in Panama Bay. The general health of the intertidal and shallow-water marine communities at Culebra makes the area especially attractive for research. Culebra has been a major research site for John Christy (since 1983), Mark Torchin (since 2004), and their students.

The PCNC relies on the support of the Smithsonian Foundation of Panama and international entities. The academic and public programs at Culebra encourage direct experiences with organisms in the local habitats and in touch pools. Exhibits promote environmental awareness, understanding, and conservation, emphasizing marine systems. Since it opened in 1996, 750,000 people have visited PCNC, with about 25,000 schoolchildren annually taking part in its educational program. The PCNC also fosters research on site, which allows visitors to see STRI scientists and students "in action."

Galeta Point Marine Laboratory

The education and outreach program at Galeta Laboratory was initiated by Stanley Hecakdon in 2000 to build bridges between research at Galeta on coral reefs, seagrass beds, and mangrove forests and the schools of Colon and wider Panama. The program seeks to motivate public interest on the importance of the sciences and the value of coastal tropical habitats, currently under severe threat because of a destructive style of economic development. Private donors have been vital to the success of this program, funding the construction of enhancements to Galeta buildings, a 300 m long mangrove boardwalk, and science equipment used by the program.

Attendance in the student education program climbed from 200 from an orphanage in nearby Colon in 2000 to a current 10,000 per year from all over Panama. These programs are hosted by 12 nature guides and 19 volunteers. Recently, the first live Internet broadcast was made from Galeta to elementary schools in New Jersey. The next step will be an online program to schools in Colon and, eventually, the rest of Panama. Galeta's public outreach program began in 2003 with the support of students from McGill University's "Panama Field Semester Studies." The first project was a socioeconomic study of a local fishing community, with fishermen then being trained in nature tourism to provide an alternative source of income. In 2006 Galeta began the Smithsonian Talk of the Month, at which STRI researchers share their work with the people of Colon. Teacher training aimed at raising the quality of science education in Colon started in 2007. To date 120 local elementary and high school teachers have been trained. Galeta laboratory also participates in a variety of community events: the yearly community beach cleanup; scientific and environmental fairs; and events such as Bio Diversity Day, World Mangrove Day, and Earth Day.

Bocas del Toro Research Station

The BRS has had active public programs, almost entirely funded by income from station fees, since the completion of the main laboratory building in 2003. Activities organized by the BRS for the general public include bimonthly public seminars given by researchers working at the station as well as weekly open houses and an annual Earth Day beach clean-up. In addition the Station opens its doors to the public during the annual Feria Ambiental weekend, at which environmental non-governmental organizations (NGOs) and governmental organizations from the region present information to the public, debate

local conservation issues in a round-table format, and give public lectures on their projects. This Feria has proven to be highly successful, with representatives form organizations such as IUCN (International Union for Conservation of Nature) and The Nature Conservancy attending from Costa Rica and Panama City.

The BRS also has an active program working with local schools. School groups visit the station three days a week during the school year, and a biodiversity summer program is available for interested children on Isla Colon and Bastimentos. More than 1,000 children per year participate in these programs or, in more remote areas, receive visits from presenters of the public programs. Finally, the Station presents an annual teacher training workshop, which offers teachers development credit for learning about environmental issues and conservation.

The BRS is also active in undergraduate and graduate teaching. The station hosts undergraduate courses from 12 institutions from the USA, Colombia, Canada, and Germany and trains graduate students in the advanced Training in Tropical Taxonomy Program. This program aims to bring taxonomic experts and experts in training together in the field to provide hands-on training in taxonomy. This program focuses on groups for which taxonomic expertise is in immediate danger of disappearing. This program, the only one of its kind in the Neotropics, has so far trained 100 students from 30 countries and receives some funding from the National Science Foundation Pan-American Advanced Studies Institutes (NSF PASI) program as well as individual Assembling-the-Tree-of-Life grants.

The Online BRS Bilingual Biodiversity Database

The public face of the Bocas del Toro Research Station extends into cyberspace. The Online BRS Bilingual Biodiversity Database, available at http://biogeodb.stri.si.edu/bocas_database/?&lang=eng, has resulted from work at the BRS and now includes 6,000 species and 8,000 photos of organisms from Bocas del Toro province. This website is supplemented by printed identification guides to local organisms (Collin et al., 2005).

Marine Zooarchaeology

The zooarchaeology reference collection at STRI is frequently used by students and researchers to identify archaeofaunal materials. Specimens are often loaned or donated to outside institutions. Panamanians have strong interests in their cultural heritage, and STRI zooarcheologists frequently give public lectures in Panama on the history of human–animal interactions in Panama and the relevance of zooarchaeology to tropical zoogeography and biodiversity. STRI's Bioinformatics office recently started work on an online database that will provide photographic, geographic, and biometric information on all identified zooarchaeological materials and specimens from Panamanian sites.

Online Information System on Tropical Eastern Pacific Shorefishes

This Shorefishes of the Tropical Eastern Pacific Online Information System (www.stri.org/sftep) exemplifies the Smithsonian's commitment to carrying information that its research generates to the widest possible audience. It provides free, public access to comprehensive information on the biology of almost 1,300 shorefish species. Systems such as these are useful for managers, biologists, students, and fishers wanting to identify fishes and obtain information about their biology. The information that systems such as this bring together allows comprehensive assessments of our level of knowledge about biodiversity (Zapata and Robertson, 2006) and regional geographic distribution of that diversity (Mora and Robertson, 2005; Robertson and Cramer, 2009).

MARINE CONSERVATION ACTIVITIES

The work that STRI biologists, notably Hector Guzmán, have done on organisms as diverse as corals, sea cucumbers (Guzman et al., 2003), conchs (Tewfik and Guzman, 2003), lobsters, and crabs (Guzman and Tewfik, 2004) has been instrumental in the establishment not only of management regulations for specific organisms but also of a large marine reserve on the Pacific coast of Panama: the Pearl Islands Special Managment Area in the Gulf of Panama (Guzman et al., 2008a). In addition, efforts by Todd Capson and research on corals by Hector Guzman (see Guzman et al., 2004) were instrumental in the declaration of Coiba National Park (where Rancheria Island is situated) as a World Heritage Site in 2005. In 2009 Panama's government established the Matumbal Reserve, a STRI-managed marine reserve that protects 34 ha of reefs, seagrass beds, and mangroves immediately adjacent to BRS. This reserve will ensure maintenance of the research potential of the station in an area of explosive tourism and developmental growth. During 2008–2009 STRI (primarily through the efforts of Juan Maté) has been involved with the recently completed development

of a comprehensive zoning and management plan for Coiba Park and workshops aimed at informing government resource managers about the utility, methods, and needs of STRI's marine research activities.

The online information system on TEP shorefishes (see above) provided the primary database used in the first comprehensive IUCN Redlist Assessment of an entire regional shorefish fauna through workshops held in Costa Rica (2008) and Panama (2007). An equivalent information system encompassing more than 1,500 species of Greater Caribbean shorefishes, currently in production, will facilitate an equivalent Redlist assessment planned for the Greater Caribbean regional shorefish fauna.

Marine conservation activities by STRI staff also have a global and historical reach through the work of J. B. C. Jackson and colleagues on historical declines of coral reef growth and organisms induced by human activities, and the depletion of their marine resources, in the Caribbean area and throughout the rest of the tropics (Jackson, 1997, 2001; Jackson et al., 2001; Pandolfi et al., 2003, 2005; Pandolfi and Jackson, 2006).

BRS has been a member of CARICOMP (the Caribbean Coastal Marine Productivity Program) since 1997, contributing data to Caribbean-wide monitoring of seagrasses, corals, and mangroves (Collin, 2005a; Collin et al., 2009; Guzman et al., 2005). BRS also recently became part of a global IUCN program to assess the resilience of coral reefs worldwide. As part of this program, rapid assessments of the state of coral reefs at each site are linked to long-term monitoring of physical environmental data to predict the local response to future bleaching stress from elevated temperatures. Since 2000 STRI has also been involved with Conservation International, the United Nations Environmental Program, the International Union for the Conservation of Nature, and the governments of Panama, Costa Rica, Colombia, and Ecuador in an effort to develop the Eastern Tropical Pacific Seascape. This 2.1 million km^2 marine conservation area, in the equatorial part of the TEP, is based on a cluster of Marine Protected Areas, among them the Coiba National Park (see also Guzman et al., 2008a).

2008—A TIME OF TRANSITION

After 48 years and 1,800 publications the marine program, which remains an integral part of research at STRI, is undergoing rapid change. The year 2008 marked the end of an era, with the retirement of Ira Rubinoff and the succession of Eldredge Bermingham as STRI director. It also marked the start of a hiatus in the research vessel program, with the retirement of the RV *Urraca*, as its absence leaves a significant gap in research capability that STRI seeks to rapidly fill. The continuing development of the laboratory at Bocas del Toro will open up new opportunities for research. The development of a facility at Rancheria Island, and, perhaps, the Pearl Islands would greatly enhance accessibility of coral reefs and other marine habitats in the two largest nearshore archipelagos in the equatorial part of the eastern Pacific, archipelagos that to date have experienced relatively low impacts from economic development. STRI geologist Carlos Jaramillo is currently taking advantage of a unique event—major excavations to widen the Panama Canal—to clarify the history of the formation of the isthmus and thus help shed light on the history of changes in the neotropical marine ecosystems and the evolution of their organisms. In future STRI also will emphasize the development of tools that exploit the World Wide Web to enhance the diffustion of knowledge derived from its marine research, both through its own Bioinformatics office and through participation in global enterprises such as the Census of Marine Life, the Consortium for the Barcode of Life, and the Encyclopedia of Life. STRI's marine program will play an increasingly important role in efforts to understand the role of the oceans in global climate variability, interactions between terrestrial and marine ecosystems, and the response of marine ecosystems to climate change and more direct human-induced stresses.

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STRI's marine program thanks Panama for its cooperation in hosting STRI, and for the long-term support of the Panamanian government entities that manage marine resources and marine reserves and cooperate with STRI's research activities (the Autoridad Maritima de Panamá, the Autoridad Nacional del Ambiente, and the Autoridad de los Recursos Acuaticos de Panamá). Many local and international donors have contributed generously to the development of STRI facilities and to its education and research programs, notably D. Cofrin, F. Hoch, P. Peck, K. and E. Himmelfarb, the Upton Trust, and the Fundación Smithsonian de Panamá. Marine science at STRI also has benefitted immensely over the years from grants made by the National Science Foundation, the Secretaría Nacional de Ciencia Tecnología de Panamá (SENACYT), Conservation International, the Darwin Initiative, the Nature Conservancy, the National Geographic Society, and various Smithsonian entities: the

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Protandric Simultaneous Hermaphroditism Is a Conserved Trait in *Lysmata* (Caridea: Lysmatidae): Implications for the Evolution of Hermaphroditism in the Genus

J. Antonio Baeza

ABSTRACT. Shrimps from the genus *Lysmata* are unusual because of their peculiar sexual system. Individuals in a population first reproduce as males, to change later in life to functional simultaneous hermaphrodites. The evolutionary origin of this sexual system, called protandric simultaneous hermaphroditism (PSH), is a longstanding question overdue for consideration. A previously proposed "historical contingency" hypothesis suggested that PSH evolved in the tropics from an ancestral protandric species of *Lysmata* that became socially monogamous and symbiotic with sea anemones. The restricted probability of encountering mating partners by shrimps because of their association with their hosts would have favored PSH. Here, I first provide evidence that PSH is a fixed trait within the genus. Second, I examine whether the historical contingency hypothesis appropriately explains the origin of PSH in the genus. Using anatomical observations and laboratory experiments combined, I demonstrate that two shrimps from the genus *Lysmata*, *L. galapagensis* and *L. boggessi*, feature PSH. Study of museum specimens suggests that nine other species of *Lysmata* are protandric simultaneous hermaphrodites. The foregoing information indicates that PSH represents a fixed trait in the genus *Lysmata*. Ancestral character state reconstruction using Bayesian inference allowed testing whether the ancestral *Lysmata* featured a symbiotic lifestyle and a socially monogamous mating system, as proposed by the historical contingency hypothesis. In agreement with this hypothesis, analysis indicated that the most common recent ancestor of *Lysmata* was most likely socially monogamous. However, the ancestral lifestyle was equally likely to be free-living or symbiotic. Thus, the present study provides partial support for the historical contingency hypothesis. Studies on the sexual system and lifestyle of more species and development of a more robust phylogeny are needed to reveal the evolutionary origin of PSH in the genus *Lysmata*.

INTRODUCTION

In decapod crustaceans, the greatest diversity of sexual systems is found in the infraorder Caridea. Most caridean shrimps are gonochoric, with individuals in a population producing only male or female gametes during their entire life. Well-studied examples include *Rhynchocinetes typus* (Correa et al., 2000), *Hippolyte obliquimanus* (Terossi et al., 2008), *Pontonia margarita* (Baeza, 2008a), and *Hippolyte williamsi* (Espinoza-Fuenzalida et al., 2008). The second most common sexual system is protandry. In at least 31 species of shrimps, individuals in a population reproduce first as males and change to females later in life

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(Bauer, 2000). Although several variants of protandry have been reported (e.g., protandry with primary females in *Crangon crangon*; Schatte and Saborowski, 2006), no study has reported protogyny (changing sex from female to male) among shrimps. Most recently, a particular variant of simultaneous hermaphroditism, that is, adolescent protandry sensu Ghiselin (1974), protandric cosexuality sensu Policansky (1982), or protandric simultaneous hermaphroditism (PSH) sensu Bauer (2000), has been described for shrimps from the genera *Lysmata* (Baeza et al., 2008) and *Exhippolysmata* (Kagwade, 1982; Braga et al., 2009). It must be noticed that a recently developed molecular phylogeny for *Lysmata* and other related genera demonstrated that the genus *Exhippolysmata* represents a derived group of shrimps within the genus *Lysmata* (Baeza et al., 2009). Thus, species of *Exhippolysmata* are treated here as members of the genus *Lysmata*.

In protandric simultaneous hermaphroditic shrimps, juveniles consistently mature as functional male individuals (also called male-phase [MP] shrimps; Bauer and Holt, 1998) bearing typical caridean male characters (i.e., coupling hoods and appendix masculina on the first and second pleopods, respectively) (Bauer and Holt, 1998; Baeza, 2008b; Baeza and Anker, 2008; Baeza et al., 2008). These functional males later attain female sexual function and develop into functional simultaneous hermaphrodites (hereafter, hermaphrodites; but also called female-phase [FP] shrimps; Bauer and Holt, 1998). Resembling females of caridean gonochoric species, hermaphrodites mate as females shortly after molting, spawn oocytes to an abdominal chamber where fertilization takes place, and brood their embryos for relatively long periods of time (e.g., 10–15 days in *Lysmata wurdemanni*; Baeza, 2006). These hermaphrodites retain testicular tissue, male ducts, and gonopores and thus have the ability to reproduce as both male and female (Bauer and Holt, 1998). After becoming hermaphrodites, individuals do not revert to males (Baeza, 2007a), and no self-fertilization has been demonstrated (Bauer and Holt, 1998; Baeza, 2008b; Baeza and Anker, 2008; Baeza et al., 2008).

So far, the various studies on the sexual biology of shrimps from the genus *Lysmata* suggest that all species exhibit PSH. Protandric simultaneous hermaphroditism is suspected to be a fixed trait in the genus. Nonetheless, additional information from more species is needed to confirm this notion. In turn, other life history traits differ within these two genera. Shrimps have been reported to inhabit the shallow subtidal and intertidal of subtropical and tropical rocky and coral reefs around the world. Some species of *Lysmata* live in groups, others are solitary, while some species are socially monogamous (pair-living; e.g., *L. grabhami* (Gordon, 1935)) (Wirtz, 1997). Several species with an inconspicuous coloration dwell freely among rocks in temperate zones, while other more colorful species inhabit tropical sponges (*L. pederseni* Rhyne and Lin, 2006) (Rhyne and Lin, 2006). Other strikingly brilliant species clean fishes (*L. amboinensis* (De Man, 1888)) (Limbaugh et al., 1961). Species from this genus represent ideal candidates to explore the role of ecological conditions in explaining evolutionary innovations in the marine environment (see Baeza and Thiel, 2007).

Recent studies have examined various aspects of the biology of various *Lysmata* and *Exhippolysmata* shrimps (Baeza, 2008b; Baeza and Anker, 2008; Baeza et al., 2008; Lopez-Greco et al., 2009). Furthermore, shrimps from the genera *Lysmata* and *Exhippolysmata* are currently being used as models in evolutionary biology and behavioral ecology because of their peculiar sexual system (Baeza and Bauer, 2004; Baeza, 2006, 2007a, 2007b, 2007c). In spite of the increasing knowledge regarding the behavior and ecology of several species of *Lysmata*, the evolutionary origins of PSH in the genus remain uncertain. Although recent studies have shown that the variety of lifestyles of *Lysmata* is greater than originally recognized (Baeza, 2008b; Baeza and Anker, 2008; Baeza et al., 2008), an emerging dichotomy in social organization and ecology was noted in initial studies. One group of species (named "Crowd" species by Bauer, 2000) was described as inhabiting warm subtropical environments, occurring as dense aggregations in their refuges, and exhibiting no specialized fish-cleaning behavior (i.e., *L. californica*: Bauer and Newman, 2004; *L. wurdemanni*: Baeza, 2006). A second group (named "Pair" species by Bauer, 2000) was described as mostly tropical, occurring at low densities in the subtidal, and dwelling as socially monogamous pairs on sea anemones used as spots for fish-cleaning activities (i.e., *L. grabhami*: Wirtz, 1997; *L. amboinensis*: Fiedler, 1998). Based on this initial dichotomy, Bauer (2000) proposed that PSH evolved in the tropics from an ancestral symbiotic protandric species of *Lysmata* that became a specialized fish cleaner. Restricted mobility of individuals resulting from their association with the host and, hence, reduced probability of encountering mating partners would have favored PSH (also see Bauer, 2006). Under such a scenario, the "Crowd" warm temperate species that do not exhibit specialized cleaning behaviors would have evolved from tropical species with specialized cleaning behaviors and more complex mating systems (Bauer, 2006). A recent phylogeny developed for the genus found no support for Bauer's hypothesis because socially monogamous species

presented a more derived position than gregarious species (Baeza et al., 2009). However, no formal testing of Bauer's ideas was conducted. Current advances in ancestral character state reconstruction using Bayesian inference (Pagel et al., 2004) make it possible to test whether the ancestral *Lysmata* featured a symbiotic lifestyle and a socially monogamous mating system, as proposed by Bauer (2000).

Here, I provide evidence that PSH is a fixed trait within the genus *Lysmata* (including *Exhippolysmata*), as suspected by previous studies (see Bauer, 2000; Baeza, 2008b; Baeza and Anker, 2008; Baeza et al., 2008). For this purpose, I examined the sexual system of two shrimps from the genus, *L. galapagensis* Schmitt, 1924 and *L. boggessi* Rhyne and Lin, 2006, using anatomical observations and laboratory experiments. I also examined specimens from another nine species deposited at the National Museum of Natural History (NMNH), Washington, D.C. The information altogether strongly suggests that PSH is a conserved trait within the genus *Lysmata*. My second goal was to examine Bauer's (2000) hypothesis regarding the evolution of PSH in *Lysmata*. I tested whether the ancestral *Lysmata* was socially monogamous (1) and strictly symbiotic with, for example, sea anemones (2), as proposed by this author. To accomplish this second goal, a review of the literature on the socioecology of *Lysmata* was conducted. Next, the lifestyle of shrimps was mapped onto the phylogeny of the genus, and the likelihood of specific traits to occur at particular ancestral nodes in the phylogeny was tested.

METHODS

Collection and Maintenance of Shrimps

Individuals from the two studied species were collected between February and August, 2006, at different localities in Panama and Florida, USA. Individuals from *L. boggessi* were collected at night during low tides from seagrass beds at Madelaine Key (27°38′51.87″N, 82°42′56.50″W), Fort De Soto National Park, Florida. Specimens from *L. galapagensis* were collected from Islas Secas (7°58′37.54″N, 82°02′18.02″W), Gulf of Chiriqui, Panama. Immediately after collection, specimens were transported to the R/V Urraca and then to the Naos Marine Laboratories, Panama (*L. galapaguensis*) or directly to the Smithsonian Marine Research Station at Fort Pierce, Florida (*L. boggessi*). Individuals were maintained in 15–70 L aquaria at a water temperature of 22°–33°C and 34–36 ppt salinity and were fed every other day with shrimp pellets before being selected for dissections or experiments.

Dissections

Observations on reproductive anatomy were conducted as in Baeza (2008b) in a total of six specimens of each species, three presumptive males (3.6–3.8 and 4.0–4.6 mm carapace length [CL] in *Lysmata galapagensis* and *L. boggessi*, respectively) and three presumptive hermaphrodites that were brooding embryos (4.4–5.1 and 6.5–5.6 mm CL in *Lysmata galapagensis* and *L. boggessi*, respectively). First, the presence or absence of male gonopores on the coxae of the fifth pereiopods was recorded for each individual. Individuals with male gonopores (all) had sperm collected from the ejaculatory ducts using short electric shocks that results in the ejection of a spermatophore (as noted in Baeza, 2006, 2007c). Each individual was then dissected to extract the gonad for examination under the stereomicroscope. Finally, the first and second pleopods were dissected and the presence or absence of appendices internae and masculinae, respectively, were recorded. Specimens were defined as males or hermaphrodites by the presence (males) or absence (hermaphrodites) of coupling hooks (cincinnuli) and appendices masculinae on the endopods of the first and second pleopods, respectively (see Baeza, 2007c, 2008b).

Experiments

Three experiments, as described in Baeza et al. (2008) and Baeza (2008b), were conducted to determine the sexual system of the three species under study. In summary, the different experiments determined whether (1) brooding shrimps (reproducing as females) were capable of mating as males, (2) brooding shrimps were capable of self-fertilization, and (3) males were capable of becoming hermaphrodites with time (see Results). In the first experiment ($n = 5$), pairs of brooding shrimps were maintained in 21 L aquaria. In the second experiment, five brooding shrimp were each maintained alone. In the third experiment ($n = 5$), pairs of males (small nonbrooding shrimp with no externally visible female gonads and visible cincinnuli and appendices masculinae) were maintained separately in 21 L aquaria for at least 50 days. Individuals were examined daily for hatching of the embryos, the presence of exuvia from molting, development of mature oocytes in the gonad (visible through the carapace), and spawning of a new batch of eggs. The development of any newly spawned embryos was examined in detail after four days of spawning.

Following the rationale developed by Baeza et al. (2008), if in the first experiment ovigerous shrimps that

paired together produced normally developing broods, then it was inferred that either the other ovigerous shrimp in the aquarium acted as a male to inseminate its partner, or that the shrimp was capable of self-fertilization. If in the second experiment shrimps in isolation failed to successfully produce and brood developing eggs, then the possibility of self-fertilization was eliminated. If in the third experiment individuals identified as males at the beginning of the experiment developed the ovarian portion of the ovotestis and produced eggs, then I inferred that male shrimps mature as hermaphrodites (see Baeza et al., 2008).

Population Structure, Sex Ratio, and Abundance

Information on the abundance, population structure, and sex ratio (males to hermaphrodites) of each species was collected from the field. The carapace length (CL) and number of shrimps of each sexual phase and each species captured during the different samplings were recorded. The sampling effort (total number of hours spent collecting shrimps) was calculated for each sampling event. Relative abundance of shrimps was estimated by dividing the sample abundance (number of shrimps captured) by the sampling effort.

Museum Specimens

Specimens from nine different species of *Lysmata* deposited at the Collection of Crustaceans, National Museum of Natural History (NMNH; Smithsonian Institution, Washington, D.C.) were examined. Dissection of specimens pertaining to the collection was not possible because only a few individuals were available from several of the examined species and many of the specimens were part of the type series used to describe the species. Therefore, the identification of males and hermaphrodites was mostly based on external morphological characters (see foregoing). When identifying sexual phases, particular attention was given to the presence of male gonopores at the base of the coxae of the fifth pair of pereiopods in brooding shrimps as a likely indicator of simultaneous hermaphroditism (see Results).

Testing the Historical Contingency Hypothesis

To examine whether the historical contingency hypothesis proposed by Bauer (2000) appropriately explains the origins of PSH in shrimps from the genus *Lysmata*, the lifestyle (in terms of the propensity to develop symbiotic partnerships and natural group size) was reconstructed using BayesTraits (Pagel and Meade, 2006; available at www.evolution.rdg.ac.uk).

A pruned set of sequences (from the 16S mitochondrial gene) recently published by Baeza et al. (2009) was used to generate a phylogenetic hypothesis for the group on which to reconstruct the evolution of lifestyles in shrimps. The sequences pertained to 20 species of *Lysmata* and *Exhippolysmata* plus 3 other species (*Merguia rhizophorae*, *Hippolyte williamsi*, and *H. inermis*) used to root the trees during the initial phylogenetic analysis. The set of aligned sequences was first imported to BayesPhylogenies (Pagel et al., 2004) to obtain a Bayesian posterior distribution of phylogenetic trees. Metropolis coupled–Markov chain–Monte Carlo analyses were conducted using a GTR + I (invariant) + G (gamma) model of nucleotide substitution. The analysis was run on two different simultaneous chains. A total of 6,000,000 iterations were conducted, and sampling was performed every 100th tree. The last 1,000 posterior probability trees generated by BayesPhylogenies were then imported to BayesTraits. The submodule MultiState in BayesTraits uses Markov chain Monte Carlo (MCMC) methods to infer values of traits (that adopt a finite number of discrete states) at ancestral nodes of phylogenies. Additionally, this method permits testing for particular ancestral characters at specific nodes taking phylogenetic uncertainty into account (Pagel et al., 2004).

The two traits here analyzed have three states each. For group size, the states were (1) aggregations (including swarms), (2) small groups, and (3) pair-living (social monogamy). The three character states used for describing the symbiotic propensity of different shrimp species were (1) free-living, (2) facultative associate (with different moray eel species, such as *L. californica* and *L. seticaudata*; with sea anemones, such as *L. ankeri*), and (3) strictly symbiotic with either sponges (e.g., *L. pederseni*) or sea anemones (*L. amboinensis, L. grabhami*). Information on the lifestyle of each species was obtained by direct observation of shrimps in nature (personal observations), from the literature (see literature review), or from both sources.

During the analysis, a reversible-jump MCMC search was used with two independent chains that were run for 6,000,000 iterations with a burn-in of 50,000. I choose the prior distribution of the parameters in the model with the option Hyperprior (see Pagel et al., 2004), seeding an exponential distribution from uniform on the interval 0.0 to 30 and a rate deviation of 18. These values were selected considering preliminary runs and were used to keep the acceptance rate at approximately 0.3, as recommended by Pagel et al. (2004). Character states at internal nodes

were reconstructed using the most recent common ancestor method. I tested hypotheses about particular character states at specific nodes when comparing the MCMC run-in which the node was "fossilized" (constrained) to one state versus an alternative. The command *Fossil* allows testing whether a particular state is "significantly" more likely at a specific node than an alternative state. For each tested character, the same set of conditions (prior distribution, burn-in) as used in the ancestral character state reconstructions already described were used. However, the MCMC was run 5 times for each trait state tested, and a total of 100,000,000 iterations were conducted. Bayes factors were calculated as the difference between the highest harmonic mean of the marginal likelihood from the five MCMC runs for each state (Pagel et al., 2004). The strength of support for one model over another was measured using the scale from Kass and Raftery (1995).

RESULTS

Dissections

Dissections demonstrated that all shrimps (brooding or nonbrooding) from the two species had male gonopores at the coxae of the fifth pair of pereiopods (Figure 1A). Female gonopores at the coxae of the third pair of pereiopods were more difficult to reliably observe. From all shrimps (brooding or nonbrooding), sperm cells shaped in the form of an inverted umbrella were retrieved from the male gonopores by electroshocks (Figure 1A,B). Dissections of the gonads from small shrimps not brooding embryos (presumptive males) demonstrated the presence of an ovotestes (Figure 1C) with an undeveloped anterior female portion full of immature oocytes (lacking coloration) (Figure 1D) and a posterior male gonad containing sperm cells with the same morphology as the sperm retrieved from the gonopores (see Figure 1B). Gonads dissected from brooding (presumptive hermaphrodites) shrimps also had ovotestes, but with a large ovarian portion full of mature oocytes and a relatively small posterior testicular portion with sperm (Figure 1E). In both brooding and nonbrooding shrimps, vas deferentia and oviducts extended laterally from the testicular and ovarian portions, respectively (Figure 1C,E).

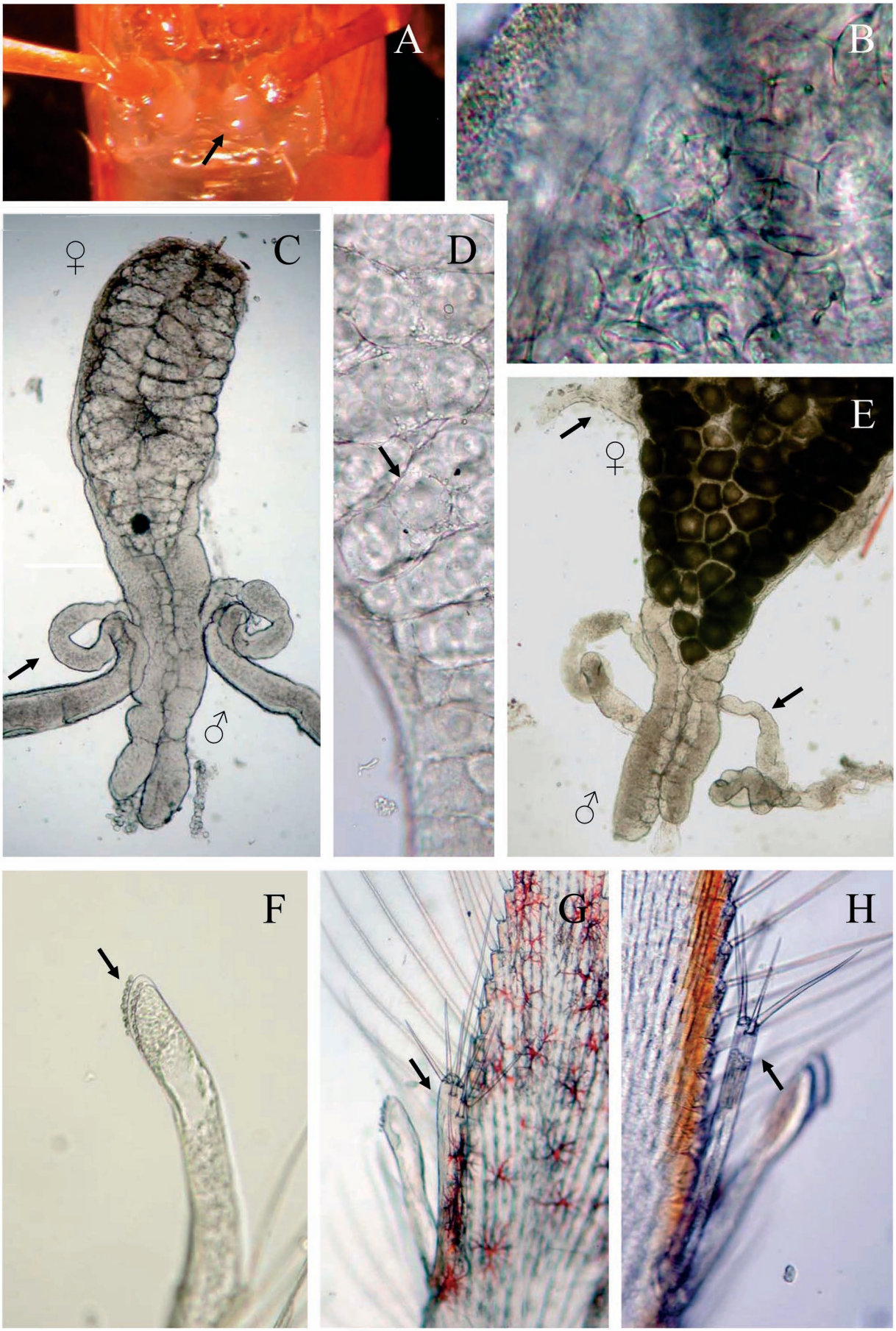
Shrimps brooding embryos invariably lacked cincinnuli and appendices masculinae in the endopod of the first and second pereiopods, respectively. In contrast, appendices masculinae bearing relatively long spines and numerous cincinnuli were observed in the second and first pleopods, respectively, of nonbrooding shrimps (Figure 1F–H). Some minor differences between the two species were noticed regarding the relative length and number of spines borne by the appendix masculinae; in *L. boggessi*, the spines were more numerous and longer than those of *L. galapagensis* (Figure 1G,H). Overall, all the anatomical differences observed between brooding and nonbrooding shrimps indicate that the populations of all the *Lysmata* species studied herein are indeed composed of males and hermaphrodites.

Experiments

When two brooding individuals (presumed hermaphrodites) were paired, all individuals in the two species examined successfully hatched their embryos as larvae, molted, and spawned a new batch of oocytes below the abdomen. The oocytes remained attached to the pleopods and showed embryonic development as embryos (i.e., early blastulae formation) after three days. This embryological development suggests the ability of the other hermaphrodites in the same aquarium to reproduce as males or, alternatively, the possibility of selfing by the hermaphrodites acting as females. However, none of the 10 hermaphrodites (5 of each species) maintained in isolation from conspecifics successfully reared their embryos to larvae. These solitary shrimps molted and spawned oocytes to beneath the abdomen. However, the oocytes invariably disappeared from the pleopods within a few days after spawning. Overall, the observations from these first two experiments strongly suggest that brooding hermaphrodites do not have the capability of self-fertilization. Therefore, brooding shrimps (hermaphrodites) maintained in pairs indeed acted as males and fertilized eggs when their partners molted and reproduced as females.

In the experiment conducted to determine whether males mature as hermaphrodites later in life, all six males of *L. galapagensis* turned into simultaneous hermaphrodites within four months. Males showed signs of ovarian maturation during intermolt periods. When the gonad was full of large green (vitellogenic) oocytes, the male shrimps molted into hermaphrodites. Most probably, these shrimps mated as females shortly after molting for the first time in their lifetime because the spawned embryos beneath the abdomen were observed developing normally several days after spawning.

In contrast to *L. galapagensis*, all six male shrimps from *L. boggessi* died of unknown reasons within the first month of the experiment. However, observations on three males of *L. boggessi* in the maintenance aquaria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indicated that they turn into hermaphrodites before four months. This change of sexual phase was accomplished after a single month, as observed in *L galapagensis*. Thus, it may be concluded that *L. galapagensis* and *L. boggessi* are protandric simultaneous hermaphrodites, incapable of self-fertilization.

Population Structure, Sex Ratio, and Abundance

Abundances of *L. galapagensis* and *L. boggessi* at the different sampling locations were high and low, with a mean of 2.79 and 0.317 individuals collected per minute per sampling period, respectively. In the two species, population was biased toward males. The ratio of males to total shrimps collected during the sampling period was 0.024 and 0.16 for *L. galapagensis* and *L. boggessi*, respectively. The range of body size registered for males varied from 1.9 to 3.8 and from 3.13 to 5.75 mm CL in *L. galapagensis* and *L. boggessi*, respectively. Hermaphrodites ranged in size between 4.1 and 5.1 and 5.63 and 6.5 mm CL in *L. galapagensis* and *L. boggessi*, respectively (Figure 2).

Museum Specimens

A variable number of specimens from *L. anchisteus*, *L. argentopunctata*, *L. chica*, *L. kuekenthali*, *L. moorei*, *L. philippinensis*, *L. rathbunae*, *L. trisetacea*, and *L. vittata* were available at the NMNH. Small shrimps in each species appear to be males as they have cincinnuli and appendices masculinae in the second and first pleopod, respectively. In turn, shrimps brooding embryos (the great majority of them above average size) invariably lacked cincinnuli and appendices masculinae in the endopod of the first and second pereiopods, respectively. This last observation suggests they were hermaphrodites. It was not possible to detect transitional individuals in these species because no dissections were possible and gonad condition was not easily observed. The carapace of formaldehyde- and alcohol-fixed specimens is not translucent as it is in living or recently preserved specimens. Also, shrimps less than 3.0 mm CL were not sexed because of the risk of inflicting damage. For all species examined except *L. anchisteus*, *L. argentopunctata*, and *L. philippinensis*, a relatively large sample of specimens was available. The size–frequency distribution of the different species strongly resembled that of the two species studied above, with small shrimps resembling males and large shrimps resembling hermaphrodites (Figure 3). Observations of the coxae of the fifth pair of pereiopods of the largest brooding shrimps in each species demonstrated the presence of male gonopores. Overall, the distribution of the sexes across size classes and the limited observations on the external male and female anatomy suggest that all these other *Lysmata* shrimps are protandric simultaneous hermaphrodites.

FIGURE 1. (*facing page*) *Lysmata galapagensis* and *Lysmata boggessi*: anatomical and morphological differences between males and hermaphrodites. A, spermatophore (arrow) retrieved from gonopores of hermaphrodite (*L. boggessi*); B, sperm from male (*L. galapagensis*); C, ovotestes from male (anterior female and male portions on top and bottom, respectively; arrow points at left vas deferentia) (*L. galapagensis*); D, close-up of female gonad portion in male (arrow points at immature oocyte) (*L. boggessi*); E, ovotestes from dissected hermaphrodite (anterior female and male portions on the top and bottom, respectively; top and bottom arrows point at right oviduct and left vas deferentia, respectively) (*L. galapagensis*); F, endopod of first pleopod in male (arrow points at cincinulli) (*L. galapagensis*); G, endopod of second pleopod in male (arrow points at appendix masculina) (*L. galapagensis*); H, endopod of second pleopod in male (arrow points at appendix masculina) (*L. boggessi*).

Literature Review

The literature review of the 41 species of *Lysmata* (including *Exhippolysmata*) described to date revealed that the geographic and bathymetric distribution, coloration, and habitat of these species are relatively well known. Shrimps from the genus *Lysmata* occur in tropical, subtropical, and temperate waters around the world and can be found among rocks or fossilized coral, live coral, seagrass blades, on muddy and shell bottoms, or associated with sponges or sea anemones in the intertidal or subtidal to 360 m depth. Most species have an inconspicuous coloration (red striped, translucent reddish with reddish flagella on both pairs of antenna). Only 4 species are reported as featuring a striking color pattern (contrasting body colors, bright white flagella on both antenna). This dichotomy in coloration was previously noticed by Bauer (2000). *Lysmata splendida*, one of the 4 species with a brilliant coloration, most probably is a cleaner shrimp. However, nothing is known about its reaction to fish and its propensity to clean them. Similarly, information regarding the degree of specialization of the cleaning behavior is unknown for most of the species (Table 1).

Information on the socioecology and sexual system is, in general, poorly known. Information on lifestyle

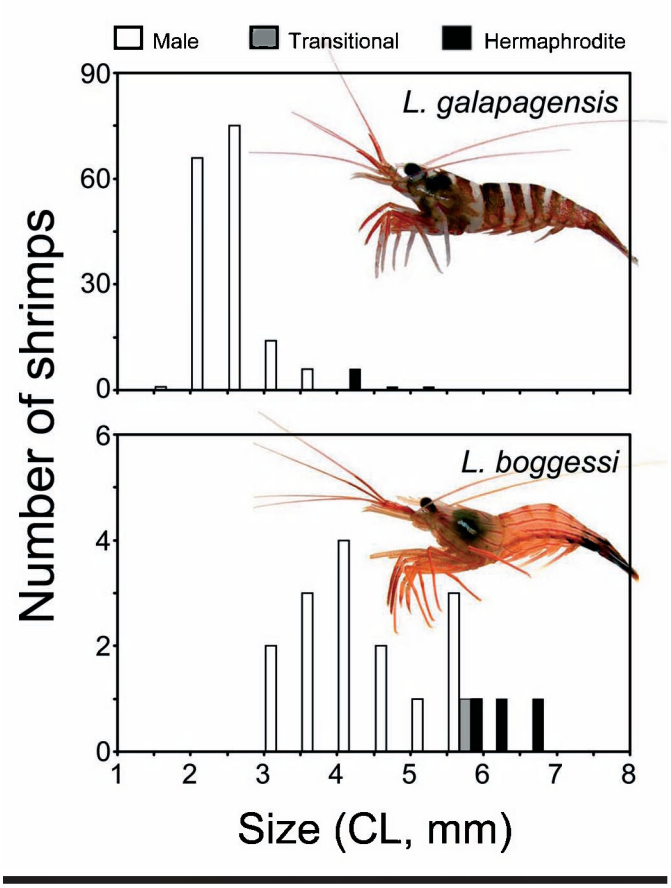

FIGURE 2. *Lysmata galapagensis* and *L. boggessi* population structure (n = 178 and 22 shrimps, respectively, from *L. galapagensis* and *L. boggessi*; CL = carapace length).

(socioecological attributes) is available only for 18 of the 41 described species. Of these, 7 species live in crowds (aggregations), 7 species live in small groups, 3 species live in pairs (i.e., they are socially monogamous), and 1 species is reported as living in extremely large aggregations (in swarms; *Exhippolysmata oplophoroides*). Demonstration of PSH using a combination of experimental, morphological, and anatomical findings and population structure is available for 12 species. A strong indication of PSH exists for another 10 species. Although the information is incomplete (PSH has been reported for a total of 22 species, or 54% of the described species), this review clearly demonstrates that the lifestyle and socioecology of shrimps from this genus are more complex than originally thought and further confirms the idea that PSH is a fixed trait in the genus *Lysmata* (including *Exhippolysmata*).

Testing the Historical Contingency Hypothesis

The 50% majority-rule consensus tree obtained during the initial phylogenetic analysis confirms the existence of the three natural clades (tropical-American, cosmopolitan, and cleaner) noticed previously by Baeza et al. (2009). However, one important difference between the present consensus tree and that previously published is that *L. olavoi* is not supported as the most basal species within the genus. This difference between trees might (1) be an effect of the different set of species used for the phylogenetic analysis or (2) perhaps have occurred because the different software programs used for phylogenetic inference function with different algorithms. On the other hand, the monophyly of *Lysmata* is well supported in this new tree, with a 100% posterior probability (Figure 4; Baeza et al., 2009: fig. 1).

The lifestyle of shrimps mapped onto the consensus tree indicated that the most recent common ancestor of the species pertaining to the neotropical and cosmopolitan clades was gregarious. In contrast, the ancestor of the species comprising the cleaner clade most probably was socially monogamous (see Figure 4). On average, the node of the most common recent ancestor of all *Lysmata* species is reconstructed to be in state 2 (social monogamy) with 80% of certainty. The degree of certainty varied from tree to tree but was generally high, as indicated by the low standard deviation of this value (SD = 0.03, calculated from 2,000,000 iterations using 1 of 1,000 randomly sampled posterior probability distribution trees at each iteration). The largest harmonic log-likelihood obtained from five independent runs when the node was fossilized to state 0 and 2 was –22.309507 and –20.865237, respectively. The almost three log-unit improvement in likelihood (Bayes factor = 2.89) of the model when the node was fossilized to state 2 represents evidence that the ancestral lifestyle of Lysmata was social monogamy.

With regard to the propensity for developing symbiotic interrelationships, the reconstructions suggest that the ancestor of the neotropical and cosmopolitan clades most probably had a free-living lifestyle and did not develop any symbiotic partnership with other macroinvertebrates. It should be noticed that the degree of certainty of these two inferences is relatively low, as indicated by the large standard deviations of the distribution of the character (see Figure 4). Also, the reconstructions indicate that, with a probability of 0.46 ± 0.20 or 0.41 ± 0.18, either facultative partnerships or strict symbiosis,

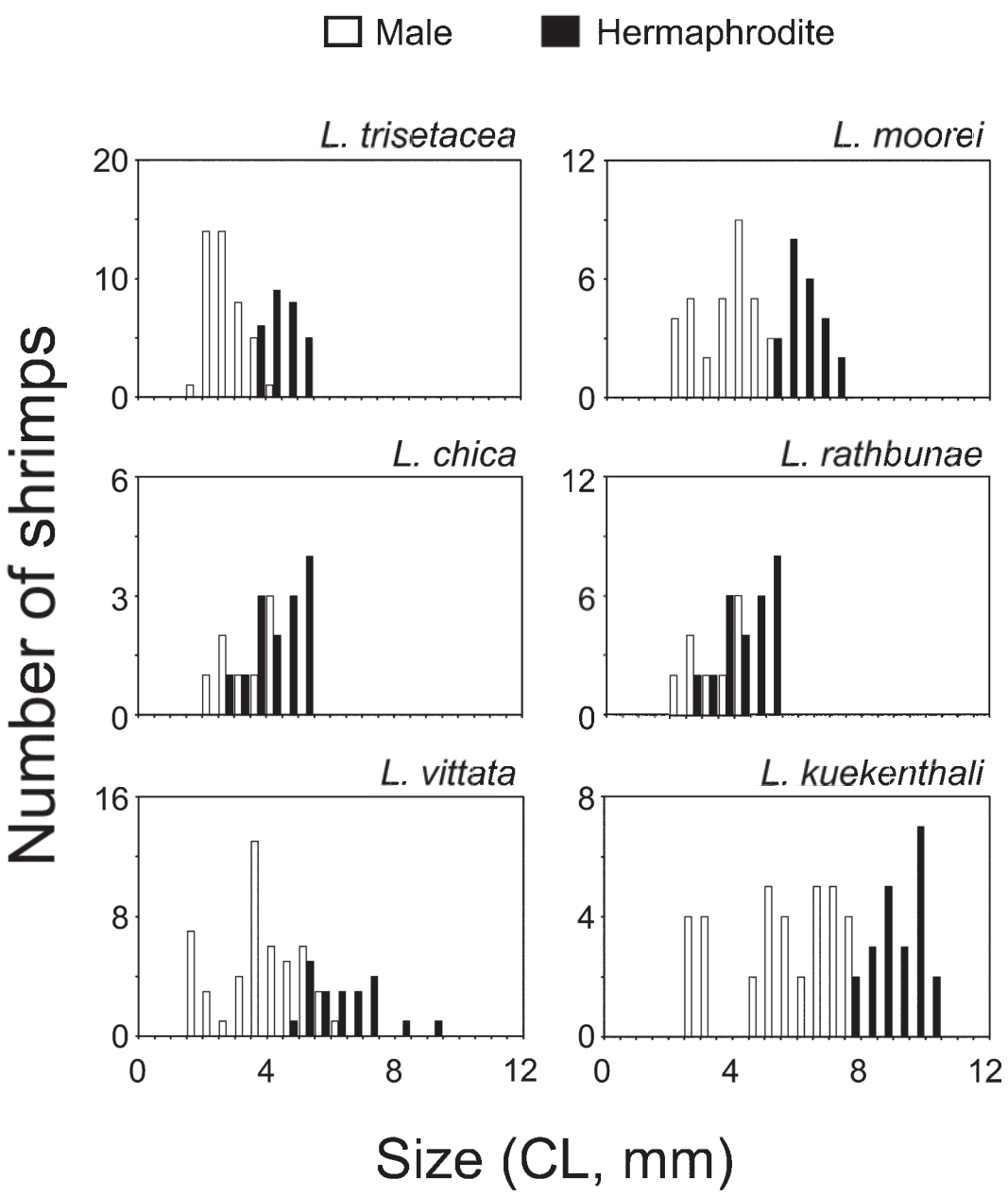

FIGURE 3. *Lysmata* spp. Population structure of selected species from the National Museum of Natural History (n = 71, 56, 22, 31, 70, and 57 shrimps from *L. trisetacea*, *L. moorei*, *L. chica*, *L. rathbunae*, *L. vittata*, and *L. kuekenthali*, respectively).

respectively, was the ancestral state of the genus *Lysmata*. The improvement in the likelihood of the model (Bayes factor = 1.51) when the node was fossilized to state 2 (strict symbiosis) was low compared to when the node was fossilized to state 0 (free-living). Therefore, there is no evidence indicating that symbiosis is significantly more likely than a free-living lifestyle in the ancestral *Lysmata*.

Overall, the present ancestral character state reconstruction provides partial support for Bauer's (2000) hypothesis about the evolution of PSH in shrimps from the genus *Lysmata*.

TABLE 1. Diversity of lifestyles and sociobiology in shrimps from the genera *Exhippolysmata* and *Lysmata*. A question mark (?) indicates data not available.

Species	Geographic distribution	Bathymetric distribution (m) [a]	Habitat [b]	Latitude	Lifestyle	Coloration	Cleaning behavior [c]	Sexual system (SS) [d]	Evidence used forSS [e]	References [f]
E. ensirostris	Indo-Pacific	I & S	?	Tropical	?	?	?	PSH?	E,M	1,10,13
E. hastatoides	W. Africa	12–48	?	Tropical	?	?	?	?	?	1
E. oplophoroides	W. Atlantic	7–27	R	Subtropical	Swarms	?	?	PSH	D,M,P	1,4,14
E. tugelae	South Africa	22–48	?	Subtropical	?	?	?	?	?	1
L. amboinensis	Red Sea, Indo-Pacific	S	S	Tropical	Pairs	Brilliant	S	PSH	E	1,15,26
L. anchisteus	Caribbean	0–3	R,M	Tropical	?	?	?	?	?	1,3
L. ankeri	Caribbean	0–2	S,C	Tropical	Groups	Dull	U	PSH	D,E,M,P	27
L. argentopunctata	E. Pacific	0–35	R,C	Tropical	?	Dull	?	?	?	2
L. bahia	W. Atlantic	I	F	Subtropical	Groups	Dull	U	PSH	D,E,M,P	16
L. boggessi	Caribbean	0–2	S,R	Tropical	Crowd	Dull	U	PSH	D,E,M,P	29
L. californica	E. Pacific	I & S	R,S	Tropical	Crowd	Dull	U	PSH	D,E,M,P	1,2,17
L. chica	Galapagos	I & S	R,C	Tropical	?	Dull	?	?	?	2
L. debelius	Indo-Pacific	10–28	R,S	Tropical	Pairs	Brilliant	S	PSH	?	1,11,26
L. galapagensis	E. Tropical Pacific	0–2	R	Tropical	Crowd	Dull	U	PSH	D,E,M,P	1,2,29
L. grabhami	Caribbean, Atlantic	Subtidal <55	S	Tropical	Pairs	Brilliant	S	PSH	D,M	1,3,18,26
L. gracilirostris	E. Tropical Pacific	0–138	R	Tropical	?	Dull	U	PSH?	M	2,27
L. hochi	Caribbean	I	F	Tropical	Groups	Dull	U	PSH	D,E,M,P	19
L. intermedia	Caribbean, Atlantic	0–22	A,R,C	Tropical	Groups	Dull	U	PSH	D,E,M,P	1,3,16
L. kempi	Burma	37	?	Tropical	?	?	?	?	?	1
L. kuekenthali	Red Sea, Indo-Pacific, South Africa	0–11	?	Tropical	?	Dull	?	PSH	?	1,10,27, 29
L. moorei	Caribbean, W. Atlantic	I	R,F	Tropical	Groups	Dull	U	PSH?	M	1,9,27
L. morelandi	New Zealand	I & S	R	Temperate	?	?	?	?	?	1
L. multiscissa	Djibouti, Africa	?	?	Tropical	?	?	?	?	?	1
L. nayaritensis	E. Pacific	I & S	R	Tropical	Crowd	Dull	U	PSH	D,E,M,P	2,20
L. nilita	Mediterranean, E. Atlantic	?	?	Subtropical	?	Dull	?	PSH	E,M	1,21
L. olavoi	Azores and Savage Is.	135–360	?	Tropical	?	Dull	?	PSH?	M	1,6,28
L. pederseni	Caribbean	S	S	Tropical	Groups	Dull	U	PSH	D,E,M,P	22,27
L. philippinensis	Philippines	267	?	Tropical	?	?	?	?	?	1
L. porteri	Juan Fernandez, E. Pacific	0–12	R	Subtropical, temperate	?	?	?	?	?	1
L. rafa	Caribbean	I & S	R	Tropical	Groups	Dull	U	PSH	D,E,M	22,27
L. rathbunae	Caribbean	13–119	?	Tropical	?	Dull	?	PSH?	M,P	1,3,23

continued

L. seticaudata	Mediterranean, E. Atlantic, Black Sea	I,S	R,S	Subtropical	Crowd	Dull	U	PSH	D,E,M,P	1,21,24
L. stenolepsis	Cape Verde	150–275	?	Tropical	?	?	?	?	?	1,7
L. striata	Caribbean	I	R,M	Tropical	Crowd	Dull	U	PSH	D,E,M,P	1,25,27
L. splendida	Indo-Pacific	6–35	W,C	Tropical	?	Brilliant	?	?	?	12
L. ternatensis	Djibouti, Indonesia	<62	Sa	Tropical	?	Dull	?	?	?	1
L. trisetacea	Red Sea, New Zealand	0–150	R,C	Tropical	?	?	?	?	?	1,2
L. uncicornis	NW Africa	4–5	R	Subtropical	?	Dull	?	?	?	1,8
L. vittata	Indo-Pacific, Australia, E. Africa	0–54	A	Tropical	?	?	?	?	?	1,5
L. wurdemanni	Caribbean, E. Atlantic, Gulf of Mexico	0–30	R,J	Subtropical	Crowd	Dull	U	PSH	D,E,M,P	1,3,26,27
L. zacae	Indo-Pacific	?	?	?	?	?	?	?	?	1

[a] I = intertidal; S = subtidal; numbers refer to depth in meters.

[b] A = associated with algae; S = symbiont (with moray eels in the case of *L. californica* and *L. seticaudata*); Sa = coarse sand; R = rocky bottoms and/or rubble; C = among corals; F = fossilized coral terraces; J = jetties; M = mud; W = caverns, on reef roof walls.

[c] S = specialized; U = unspecialized.

[d] PSH = protandric simultaneous hermaphroditism.

[e] D = dissections; E = experiments; M = morphology; P = population structure.

[f] References: 1, Chace, 1997; 2, Wicksten, 2000; 3, Chace, 1972; 4, Williams, 1984; 5, Kemp, 1914; 6, Fransen, 1991; 7, Crosnier and Forest, 1973; 8, Holthuis and Maurin, 1952; 9, Rathbun, 1906; 10, Holthuis, 1948; 11, Bruce, 1983; 12, Burukovsky, 2000; 13, Kagwade, 1982; 14, Braga et al., 2009; 15, Fiedler, 1998; 16, Baeza, 2008b; 17, Bauer and Newman, 2004; 18, Wirtz, 1997; 19, Baeza and Anker, 2008; 20, Baeza et al., 2008; 21, d'Udekem d'Acoz, 2002; 22, Rhyne and Anker, 2007; 23, Rhyne and Lin, 2006; 24, Dohrn and Holthuis, 1950; 25, Anker et al., unpublished; 26, Bauer, 2000; 27, unpublished observations; 28, Fransen, personal communication; 29, this study.

DISCUSSION

The present study suggests that the sexual system in shrimps from the genus *Lysmata* (including *Exhippolysmata*) represents a fixed trait. Anatomical observations, behavioral experiments, and field samples demonstrated that the 2 species studied here are protandric simultaneous hermaphrodites, as reported for all other *Lysmata* species (Table 1). Size–frequency distributions and additional but limited anatomical observations of museum specimens further suggest that at least 9 other species are protandric simultaneous hermaphrodites. Including the information generated in the present study, PSH has been reported for a total of 22 species, or 54% of the 41 species described worldwide.

The well-conserved sexual system in *Lysmata* contrasts with that reported for other genera from the closely related family Hippolytidae. For instance, two different genera of Hippolytidae shrimps, *Thor* and *Hippolyte*, are known to contain both gonochoric and strict sequentially hermaphroditic species (Espinosa-Fuenzalida et al., 2008, and references therein). The reasons for PSH to be fixed in *Lysmata* are not clear, especially when considering the diversity of environments inhabited by these species (see Table 1). Different habitats with varying degrees of structural complexity, seasonality, and predation regimes should favor different sexual systems. For instance, the rather heterogeneous environment (i.e., seagrass beds, seaweed meadows) in which the gregarious *L. boggessi* and *L. wurdemanni* occur is expected to favor sequential hermaphroditism over PSH. In these complex environments, male mating success most likely decreases with increasing body size because small body size is expected to increase searching ability and, ultimately, male mating success when encounter rate among conspecifics is high (Baeza and Thiel, 2007). This small-male advantage together with the well-reported exponential relationship between fecundity and body size in female shrimps is expected to favor strict protandry over simultaneous hermaphroditism in these species (Charnov, 1982).

On the other hand, hermaphroditic shrimps are known to experience brooding constraints (e.g., *L. wurdemanni*; Baeza, 2007c), a condition that theoretically favors simultaneous hermaphroditism (see Charnov, 1982, and references therein). Similarly, in socially monogamous *Lysmata* (e.g., *L. grabhami*; Wirtz, 1997), infrequent encounter rates among conspecifics should be favoring strict simultaneous hermaphroditism over PSH. It should pay (in term of fitness) for each individual in a pair to reproduce both as male and female as soon as possible during their lifetime because this strategy increases reproductive success through both sperm donation to the partner and female reproduction. Thus, an early male phase in these socially monogamous species should not be adaptive. On the other hand, differing costs between the sex functions might explain the existence of an early male phase before the simultaneously hermaphroditic phase in these monogamous species. The relatively large energetic and temporal costs of producing ova might delay maturation of the female function, resulting in a functional adolescent male phase previous to the simultaneously hermaphroditic phase (see Baeza, 2006). Additional studies in gregarious and socially monogamous cleaner shrimp species should improve our understanding about the conditions favoring PSH under a social monogamous mating system in *Lysmata*.

The literature review conducted herein indicates that the diversity of lifestyles in the genus is greater than previously recognized. Initial studies reported a distribution for the genus restricted to tropical-subtropical waters. The present review suggests that shrimps also inhabit cold temperate environments. *Lysmata porteri* is reported from southern Chile, and *L. morelandi* inhabits New Zealand (see Table 1). Because *Exhippolysmata* spp. represents a derived group of *Lysmata*, the deep water environment represents another environment colonized by the species in this group (see Baeza et al., 2009). Also, the dichotomy in social organization ("Crowd" versus "Pair" species) noted in initial studies (Bauer, 2000) is not supported. In addition to tropical pair-living and temperate gregarious species, the present review indicates other species forming swarms (extremely large aggregations) in temperate deep water soft-bottom environments (i.e., *E. oplophoroides*) or living in small groups in the tropical or subtropical intertidal that might or not associate with sea anemones (*L. ankeri*; Table 1). The possibility of an adaptive radiation in this group of shrimps is currently being explored. The rather unusual sex allocation pattern of this shrimps might represent the key innovation allowing species in these two genera to colonize and persist in environments where species with conventional sexual systems might fail.

The ancestral character state reconstruction analysis conducted in this study provides partial support for Bauer's (2000) hypothesis about the evolution of PSH in *Lysmata*. The analysis suggested that the ancestral *Lysmata* shrimp lived as socially monogamous pairs either facultatively associated to other macroinvertebrates or featuring a strictly symbiotic lifestyle (with sea anemones, for example). The free-living condition of several species pertaining to the cosmopolitan and neotropical clades is likely to be derived according to the present analysis. PSH might have

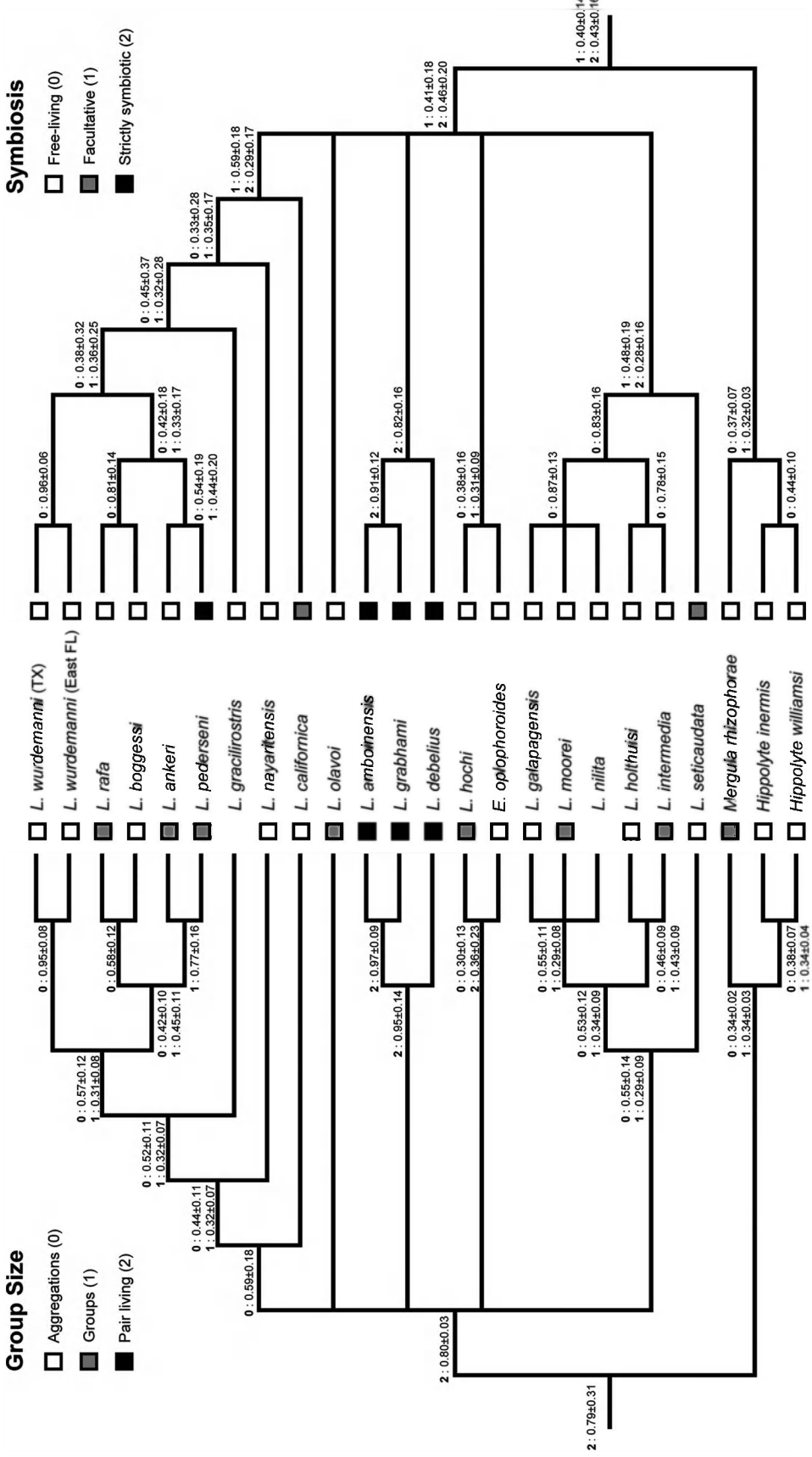

FIGURE 4. A 50% majority-rule consensus tree obtained using Bayesian inference phylogenetic methods showing the posterior densities of the reconstructed ancestral states (symbiosis and group size) at different nodes. For each node, the posterior probability for group size (left tree) and symbiosis (right tree) obtained when reconstructing the ancestral state of the most recent common ancestor to different clades in each tree is shown.

evolved in the tropics from an ancestral protandric species of *Lysmata* that became a specialized fish cleaner, as suggested by Bauer (2000). Restricted mobility of individuals resulting from their association with the host, and, hence, the reduced probability of encountering mating partners, would have favored PSH (Bauer, 2000) (see foregoing for further details about this hypothesis). Nonetheless, the inferences about ancestral character states from the present analysis need to be considered with caution. Indeed, the present analysis did not support an ancestral symbiotic condition as significantly more likely than a free-living condition. Also, several internal nodes in the phylogenetic tree were not well supported by the Bayesian analysis of phylogenetic inference (see Baeza et al., 2009). This low support for internal nodes, together with the breadth of the posterior distributions of the character inferred for these nodes, means that other alternative routes to the evolution and maintenance of this peculiar sexual system in *Lysmata* cannot be ruled out.

Among alternative historical scenarios (to that proposed by Bauer, 2000), PSH might well have evolved from a strict simultaneous hermaphrodite or even from a strict gonochoric free-living ancestor inhabiting tropical environments. The evolution of PSH from an ancestral strictly simultaneous hermaphroditic condition has been reported previously for the worm *Ophryotrocha diadema*, one of the few other marine invertebrates in which PSH has been demonstrated (Dahlgren et al., 2001). Acting together with the conditions favoring simultaneous hermaphroditism (i.e., low abundance), sex-dependent energetic costs might have favored an early maturation of the male reproductive function compared to that of the female function in the ancestral free-living shrimp (regardless of its sexual system), ultimately resulting in the evolution of PSH as we observe it today in *Lysmata* (and *Exhippolysmata*). Similarly, brooding constraints experienced by hermaphroditic shrimps might have favored the retention of the male function later in life. If the space for brooding embryos in the abdomen becomes saturated, allocation of energy to sperm production is expected to maximize fitness. This argument is similar to that of Ghiselin (1987) to explain apparent protogynous simultaneous hermaphroditism in chitons. In some species of polyplacophorans, individuals brood eggs along the side of the body. Early in life, they reproduce strictly as females until they reach a size at which the space in which they brood is saturated. At that point, the same individuals start producing sperm while they are brooding. Brooding constraints have been previously reported for at least one species of *Lysmata* (*L. wurdemanni*; Baeza, 2006). New studies are needed to confirm whether brood constraints are common in the genus.

In the scenarios depicted here, we should expect that, in a phylogeny of the group, "tropical–low abundance" species would have a more basal position than the "Pair" and "Crowd" species ("Pair" and "Crowd" sensu Bauer, 2000). The rather complex mating system (social monogamy) and specialized fish-cleaning behavior of the "Pair" species most probably evolved from "tropical–low abundance" species without complex cleaning behavior and with rather simple mating systems (i.e., without long-lasting associations between mating partners), as appears to be the case for most shrimps from the closely related family Hippolytidae. The unresolved position of the different natural clades with respect to each other in the current phylogeny (see also Baeza et al., 2009) constrain testing this last hypothesis against Bauer's (2000) ideas. Future studies attempting to resolve the natural relationships among species of *Lysmata*, *Exhippolysmata*, and other related taxa together with the detailed examination of their sexual system should allow explaining the origin of simultaneous hermaphroditism in shrimps from the genus *Lysmata*.

Last, it is worth mentioning one of the main assumptions of the present analysis. PSH was treated as a singular innovation only originating in the genus *Lysmata* (which contains *Exhippolysmata*), as initially suggested by Bauer (2000). To the best of my knowledge, shrimps from the genus *Merguia*, apparently the sister group to *Lysmata*, seem to have a gonochoric sexual system. However, this observation needs experimental confirmation. Most importantly, future studies need to test for the existence of protandric simultaneous hermaphroditism in members from other closely related genera (i.e., *Mimocaris*, *Parahippolyte*, *Merguia*, *Merhippolyte*). These studies might reveal that PSH is not a singularity. Indeed, PSH has independently evolved in the past at least four other times outside the Caridea. In addition to *Lysmata* shrimps, PSH has been confirmed in the polychaete worm *Ophryotrocha diadema* (Premoli and Sella, 1995), the land snail *Achatina fulica* (Tomiyama, 1996), the tunicate *Pyura chilensis* (Manríquez and Castilla, 2005), and the symbiotic barnacle *Chelonibia patula* (Crisp, 1983). If simultaneous hermaphroditism turns out not to be a singularity in shrimps from the families Hippolytidae and Lysmatidae, then it should be possible to explore the environmental conditions that favor this unique sexual system in shrimps.

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Reconciling Genetic Lineages with Species in Western Atlantic *Coryphopterus* (Teleostei: Gobiidae)

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ABSTRACT. Species identification of western Atlantic *Coryphopterus* can be problematic because some of the species are morphologically similar, there is confusing morphological variation within some species, no taxonomic key includes all currently recognized species, and the validity of some species is questionable. The most recently published keys do not include *Coryphopterus tortugae* or *C. venezuelae*, the validity of which as distinct from *C. glaucofraenum* has been questioned. Neighbor-joining trees derived from mitochondrial cytochrome *c* oxidase I (COI) sequences (DNA barcoding) were used to determine the number of genetically distinct lineages of *Coryphopterus* from collections made off Belize, Curacao, and Florida. Additional specimens for genetic and morphological analysis were obtained from Panama, Venezuela, and the Bahamas. Subsequent comparative analysis of preserved voucher specimens from which DNA was extracted and digital color photographs of those specimens taken before preservation yielded, in most cases, sufficient morphological information to separate the genetic lineages. Species identification of the lineages was then determined based on review of original and subsequent descriptions of *Coryphopterus* species and examination of museum specimens, including some type material. Many museum specimens are misidentified. Twelve species of *Coryphopterus* are herein recognized in the western Atlantic and Caribbean: *C. alloides*, *C. dicrus*, *C. eidolon*, *C. glaucofraenum*, *C. hyalinus*, *C. kuna*, *C. lipernes*, *C. personatus*, *C. punctipectophorus*, *C. thrix*, *C. tortugae*, and *C. venezuelae*. *Coryphopterus bol* Victor, 2008 is a synonym of *C. venezuelae* (Cervigón, 1966). Although genetically distinct, *C. glaucofraenum* and some specimens of *C. venezuelae* are extremely similar and cannot be separated on the basis of morphology 100% of the time. Comments on the identification of each *Coryphopterus* species and a revised key to western Atlantic species are provided.

INTRODUCTION

To provide specific identifications of larvae of Caribbean reef fishes at Carrie Bow Cay, Belize, a small coral-fringed island on the Belizean Barrier Reef (16°48.5′N, 88°05′W), we have been matching larvae to adults through DNA barcoding (mitochondrial cytochrome c oxidase I [COI] sequences). In addition to greatly increasing our success rate of identifying larvae, DNA barcoding is also providing a method of checking existing species-level classifications by revealing the numbers of distinct genetic lineages within genera.

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Attempts to identify Belizean *Coryphopterus* species using the most recently published keys (Böhlke and Robins, 1960, 1962; Böhlke and Chaplin, 1968; Murdy, 2002) proved problematic for certain species. None of those keys includes *C. tortugae* (Jordan) or *C. venezuelae* Cervigón, presumably because the validity of both species as distinct from *C. glaucofraenum* Gill has been questioned (e.g., Böhlke and Robins, 1960; Cervigón, 1966; Thacker and Cole, 2002). Longley and Hildebrand (1941) and Böhlke and Robins (1960) considered *C. tortugae* (Jordan; type locality, Dry Tortugas, Florida) a synonym of *C. glaucofraenum* Gill. Garzón-Ferreira and Acero (1990) redescribed *C. tortugae* as distinct based on new collections from the Colombian Caribbean. Thacker and Cole (2002) acknowledged the latter work but did not recognize *C. tortugae* in their phylogenetic analysis of *Coryphopterus* species. Victor (2008) recognized *C. tortugae* as distinct from *C. glaucofraenum* and identified what he considered a cryptic new species within Garzón-Ferreira and Acero's (1990) *C. tortugae*, which he named *Coryphopterus bol.* Cervigón (1994) elevated *C. venezuelae* from a subspecies of *C. glaucofraenum* to a distinct species, but it was not included in Murdy's (2002) key or Thacker and Cole's (2002) and Victor's (2008) molecular phylogenies of *Coryphopterus* species.

Another problem with identification of western Caribbean *Coryphopterus* is that stated distributions of many species are conflicting, and some do not include the western Caribbean. Greenfield and Johnson (1999) identified nine species of *Coryphopterus* from Belize (all of the 12 recognized herein except for *C. venezuelae*, *C. punctipectophorus*, and the recently described *C. kuna* (Victor, 2007)). Murdy (2002) listed only *C. alloides*, *C. dicrus*, *C. glaucofraenum*, *C. hyalinus*, *C. lipernes*, and *C. personatus* as having ranges that include Central America, western Caribbean, or Caribbean. A search for reef-associated species in Belize in FishBase (www.fishbase.org) returned only *C. alloides*, *C. eidolon*, *C. glaucofraenum*, and *C. personatus*.

The purposes of this paper are to assess the number of valid *Coryphopterus* species known from the western Atlantic and to provide comments on the identification of, and a revised key to, those species based on results of DNA barcoding, subsequent examination of voucher specimens and color photographs of them, examination of museum specimens, and reference to original and other descriptions of the species. A neotype for *C. glaucofraenum* is designated because the location of Gill's (1863) holotype is unknown.

METHODS

Depending on the locality, fish specimens were collected using the fish anesthetic quinaldine sulfate or rotenone. Specimens were measured to the nearest 0.5 mm, photographed with a Fujifilm FinePix 3 digital camera to record color patterns, sampled for genetic analysis, and then preserved as vouchers. Tissue sampling for molecular work involved removing a muscle biopsy, eye, or caudal body portion (depending on size) and storage in saturated salt buffer (Seutin et al., 1990). Genomic DNA was extracted from up to approximately 20 mg minced preserved tissue via an automated phenol:chloroform extraction on the Autogenprep965 (Autogen, Holliston, MA) using the mouse tail tissue protocol with a final elution volume of 50 μL. For polymerase chain reaction (PCR), 1 μL of this genomic DNA is used in a 10 μL reaction with 0.5 U Bioline (BioLine USA, Boston, MA) Taq polymerase, 0.4 μL 50 mM $MgCl_2$, 1 μL 10× buffer, 0.5 μL 10 mM deoxyribonucleotide triphosphate (dNTP), and 0.3 μL 10 μM each primer FISH-BCL (5′-TCAACYAATCAYAAAGATATYGGCAC) and FISH-BCH (5′-TAAACTTCAGGGTGACCAAAAAATCA). The thermal cycler program for PCR was 1 cycle of 5 min at 95°C; 35 cycles of 30 s at 95°C, 30 s at 52°C, and 45 s at 72°C; 1 cycle of 5 min at 72°C; and a hold at 10°C. The PCR products were purified with Exosap-IT (USB, Cleveland, OH) using 2 μL 0.2× enzyme and incubated for 30 min at 37°C. The reaction was then inactivated for 20 min at 80°C. Sequencing reactions were performed using 1 μL of this purified PCR product in a 10 μL reaction containing 0.5 μL primer, 1.75 μL BigDye buffer, and 0.5 μL BigDye (ABI, Foster City, CA) and run in the thermal cycler for 30 cycles of 30 s at 95°C, 30 s at 50°C, 4 min at 60°C, and then held at 10°C. These sequencing reactions were purified using Millipore Sephadex plates (MAHVN-4550; Millipore, Billerica, MA) per manufacturer's instructions and stored dry until analyzed. Sequencing reactions were analyzed on an ABI 3730XL automated DNA sequencer, and sequence trace files were exported into Sequencher 4.7 (GeneCodes, Ann Arbor, MI). Using the Sequencher program, ends were trimmed from the raw sequences until the first and last 10 bases contained fewer than 5 base calls with a confidence score (phred score) lower than 30. After trimming, forward and reverse sequences for each specimen were assembled, each assembled contig was examined and edited by hand, and each sequence was checked for stop codons. Finally the consensus sequence from each contig was aligned and exported in a nexus format. Neighbor-joining trees (Saitou and Nei, 1987) and

distance matrices were generated using Paup*4.1 (Swofford, 2002) on an analysis of Kimura 2-parameter (K2P) distances (Kimura, 1980).

MATERIAL

The *Coryphopterus* material examined is listed in the Appendix (Table A.1). This table includes the voucher specimens represented in the neighbor-joining tree (Figure 1), as well as non-voucher specimens collected as part of this or other projects. Most specimens examined genetically for this chapter are juveniles or adults, except those of *C. kuna*; that species is represented in our samples only by larvae. For most specimens analyzed genetically, a digital color photograph of the specimen taken before dissection and preservation is housed at the Smithsonian Institution. Cytochrome *c* oxidase I (COI) sequences for specimens analyzed genetically are deposited in GenBank (accession numbers GQ367306–GQ367475). Genetic information for several specimens collected in the Bahamas was not available in time for inclusion in the neighbor-joining tree, but identifications of those specimens based on that information are discussed in the text.

RESULTS

Twelve distinct genetic lineages of *Coryphopterus* are present in our material (see Figure 1). One of those lineages, a single specimen identified as *C. alloides* from Curacao is under additional investigation and is not discussed further here. Tissue samples of *C. punctipectophorus* were not available for genetic analysis. The other lineages, from top to bottom in Figure 1, are *C. lipernes*, *C. hyalinus*, *C. personatus*, *C. tortugae*, *C. glaucofraenum*, *C. venezuelae*, *C. dicrus*, *C. thrix*, *C. eidolon*, *C. alloides*, and *C. kuna*. Comments on the identification of each lineage, as well as *C. punctipectophorus*, are provided below. The COI sequence of *Coryphopterus bol* Victor, 2008 (PR SIO0869, fig. 1 [SIO = Scripps Institution of Oceanography]) is part of the *C. venezuelae clade*, and the synonymy of that species is discussed below. Intra- and interspecific differences in percent sequence divergence for COI for all species are provided in Table 1. We have not plotted distribution maps of *Coryphopterus* species because our samples are from a limited number of locations, and historical confusion about the identification of some species precluded our relying on geographic information based on museum catalogues. Based on extensive recent collecting throughout the Caribbean, Ross Robertson (Smithsonian Tropical Research Institute, personal communication, 8 June 2009) and James Van Tassell are providing distribution maps of *Coryphopterus* species in their *Shorefishes of the Greater Caribbean* CD, expected to be released in 2009.

Coryphopterus lipernes Böhlke and Robins, 1962

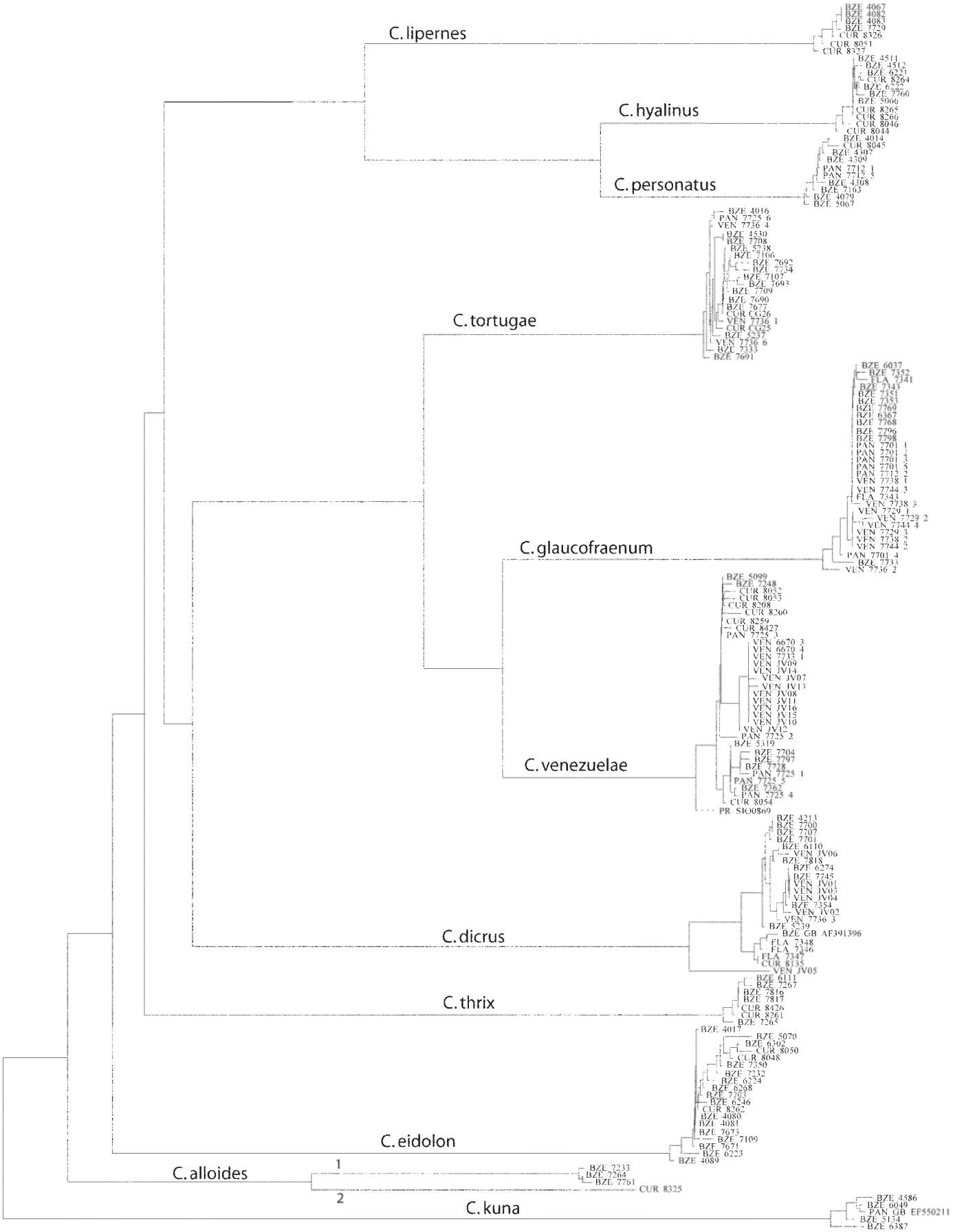
FIGURE 2

Our specimens of *C. lipernes* from Belize and Curacao form a close genetic clade. Identification of *C. lipernes* presents no problems: It is distinguished from all *Coryphopterus* species except *C. hyalinus* and *C. personatus* by the presence of black pigment surrounding the anus; from *C. hyalinus* by the presence of a single (vs. two) anterior interorbital pore; and from *C. personatus* by color pattern (see Figure 2). We did not make fin-ray counts for *C. lipernes*, but according to Böhlke and Robins (1962), *C. lipernes* also differs from *C. personatus* in having 10 (vs. 11) second dorsal- and anal-fin elements. Murdy (2002) distinguished *C. lipernes* and *C. personatus* from *C. hyalinus* by the presence of two pores between the eyes (vs. three), but as noted by Böhlke and Robins (1962), there is one *anterior* interorbital pore in *C. lipernes* and *C. personatus* and two in *C. hyalinus*.

Coryphopterus hyalinus Böhlke and Robins, 1962

FIGURE 2

The validity of *C. hyalinus* as distinct from *C. personatus* has been questioned (e.g., Smith et al., 2003), but the two are genetically distinct (see Figure 1, Table 1). Of the *Coryphopterus* gobies with a black ring of pigment around the anus (*C. hyalinus*, *C. personatus*, and *C. lipernes*), *C. hyalinus* is the only one with two anterior interorbital pores (Böhlke and Robins, 1962; Böhlke and Chaplin, 1968). Because head pores can be difficult to see in fresh material (considerably easier to see in preserved specimens), separation of *C. hyalinus* and *C. personatus* in the field can be difficult. We have observed no consistent differences in pigmentation in fresh or preserved specimens of the two species, but we often collect *C. hyalinus* in deeper water than *C. personatus*.


C. lipernes
C. hyalinus
C. personatus
C. tortugae
C. glaucofraenum
C. venezuelae
C. dicrus
C. thrix
C. eidolon
C. alloides
C. kuna
1
2
0.005 substitutions/site

Coryphopterus personatus (Jordan and Thompson, 1905)

FIGURE 2

Identification of *C. personatus* also presents no problems using published keys. It can be distinguished from *C. hyalinus* by the presence of a single interorbital pore and from *C. lipernes* by pigment pattern (see Figure 2). According to Böhlke and Robins (1962), *C. personatus* also can be separated from *C. lipernes* by having 11 (vs. 10) total elements in the second dorsal and anal fins.

Coryphopterus tortugae (Jordan, 1904)

FIGURE 3

Longley and Hildebrand (1941) and Böhlke and Robins (1960) considered *C. tortugae* (Jordan: type locality, Dry Tortugas, Florida) to be a synonym of *C. glaucofraenum* Gill. Garzón-Ferreira and Acero (1990) redescribed *C. tortugae* as distinct based on new collections from the Colombian Caribbean. Victor (2008) concurred with Garzón-Ferreira and Acero's (1990) recognition of *C. tortugae* but noted that their Santa Marta specimens constitute a distinct species, which he described as *C. bol*. As noted below (see "Synonymy of *Coryphopterus bol*"), *C. bol* appears to be a synonym of *C. venezuelae*.

We had initially identified all specimens of the *C. tortugae*, *C. glaucofraenum*, and *C. venezuelae* clades as *C. glaucofraenum* using published keys (Böhlke and Robins, 1960; Böhlke and Chaplin, 1968; Murdy, 2002). However, those specimens separate into three well-defined lineages based on COI sequences. Specimens in one of those lineages are usually paler than those of the other two and almost always have a central bar of basicaudal pigment (vs. usually two spots or a dumbbell- or C-shaped marking), characters described by Garzón-Ferreira and Acero (1990) as diagnostic for *C. tortugae*. Böhlke and Robins (1960), who considered *C. tortugae* to be a pallid form of *C. glaucofraenum*, noted that the pigment markings along the side of the body are round (upper row) or vertically elongate (lower row) versus X-shaped as in *C. glaucofraenum*, usually a consistent feature in our specimens of *C. tortugae*. The pigment spots in the lower row of markings along the side of the body in *C. tortugae* are usually vertically elongate (crescents or some part of an X), but they are rarely distinct X-shaped markings. If some of the anterior markings do resemble X's (Figure 3D), the height of each X is considerably smaller than the height of the X's in *C. glaucofraenum* and, when present, in *C. venezuelae* (half or less of eye diameter in *C. tortugae*, approximately three-quarters of or equal to eye diameter in the other two species). The pigment spots in the lower row also are not rounded, as they are in pale specimens of *C. venezuelae*.

We have not found the basicaudal pigment to be a reliable character for separating *C. tortugae* from *C. glaucofraenum* and *C. venezuelae*, as all three species may have a central bar of pigment; however, *C. tortugae* does not have two distinct spots in any of our material, so if that feature is present in a specimen, it is not *C. tortugae*. *Coryphopterus tortugae* shares with *C. glaucofraenum* and *C. venezuelae* the presence of a distinct dark blotch or triangle behind the eye above the opercle and with *C. glaucofraenum* the absence of a pigment spot on the lower portion of the pectoral-fin base. Garzón-Ferreira and Acero's (1990) redescription of *C. tortugae* did not mention the absence of this spot, presumably because the Santa Marta specimens included in their description do have the spot and appear to be *C. venezuelae* (see "Synonymy of *Coryphopterus bol*," below). Our investigations indicate that the absence of this pigment spot on the pectoral-fin base, along with the presence of vertically elongate versus round pigment spots in the lower row of markings on the body, is significant in separating *C. tortugae* from pale specimens of *C. venezuelae*. Examination of photographs of the holotype of *Ctenogobius tortugae* (SU 8363) confirms that there is no pigment on the lower portion of the pectoral-fin base.

Coryphopterus tortugae is most easily separated from all other *Coryphopterus* by the following combination of characters: a dark blotch or triangle of pigment above the opercle is present; large X-shape markings on the side of the body and a spot on the lower pectoral-fin base are absent; at least some of the pigment markings in the lower row along the side of the body are vertically elongate or crescent shaped; and the overall coloring is pale.

Coryphopterus glaucofraenum Gill, 1864

FIGURE 4

The location of the single type specimen upon which Gill described *C. glaucofraenum* is unknown (Eschmeyer, 2008). Böhlke and Robins (1960:108–109) described

FIGURE 1. (*facing page*) Neighbor-joining tree derived from cytochrome *c* oxidase I sequences showing genetically distinct lineages of western Atlantic *Coryphopterus*.

TABLE 1. Average (and range) Kimura two-parameter distance summary for *Coryphopterus* species based on cytochrome *c* oxidase I (COI) sequences of individuals represented in the neighbor-joining tree in Figure 1. Intraspecific averages are shown in bold; n/a = data not available.

Coryphopterus sp.	*lipernes* (*n* = 7)	*hyalinus* (*n* = 11)	*personatus* (*n* = 10)	*tortugae* (*n* = 21)	*glaucofraenum* (*n* = 29)	*venezuelae* (*n* = 33)	*dicrus* (*n* = 22)	*thrix* (*n* = 7)	*eidolon* (*n* = 19)	*alloides* 1 (*n* = 3)	*alloides* 2 (*n* = 1)	*kuna* (*n* = 5)
lipernes	**0.13%** (0.00–0.35)	–	–	–	–	–	–	–	–	–	–	–
hyalinus	14.88% (14.21–15.40)	**0.06%** (0.00–0.31)	–	–	–	–	–	–	–	–	–	–
personatus	15.66% (15.10–16.03)	7.14% (6.79–7.65)	**0.14%** (0.00–0.46)	–	–	–	–	–	–	–	–	–
tortugae	19.60% (18.65–20.23)	21.14% (20.64–21.82)	20.08% (19.46–21.02)	**0.20%** (0.00–0.61)	–	–	–	–	–	–	–	–
glaucofraenum	20.71% (19.49–21.20)	21.68% (20.61–22.44)	21.50% (20.48–22.30)	12.07% (11.07–12.99)	**0.19%** (0.00–0.92)	–	–	–	–	–	–	–
venezuelae	21.37% (19.27–22.13)	20.86% (19.59–21.69)	20.12% (18.58–21.18)	9.84% (8.10–10.77)	9.51% (8.59–10.21)	**0.53%** (0.00–1.24)	–	–	–	–	–	–
dicrus	21.72% (20.10–22.56)	19.68% (18.61–20.37)	19.03% (18.30–19.94)	17.53% (16.59–18.07)	20.65% (19.82–21.32)	18.28% (16.46–19.00)	**0.61%** (0.00–2.82)	–	–	–	–	–
thrix	21.86% (20.85–22.69)	21.10% (20.11–22.44)	19.70% (19.19–20.48)	19.00% (18.43–19.63)	21.03% (20.30–21.54)	19.30% (17.61–20.12)	21.30% (20.74–21.84)	**0.11%** (0.00–0.48)	–	–	–	–
eidolon	25.16% (24.45–26.19)	19.19% (18.19–20.11)	17.92% (17.12–18.82)	19.74% (18.34–21.42)	23.17% (21.98–25.04)	18.72% (16.75–20.36)	19.39% (18.69–20.87)	19.54% (18.96–20.41)	**0.24%** (0.00–0.99)	–	–	–
alloides 1	22.13% (21.53–22.53)	17.90% (17.09–18.64)	18.16% (17.69–18.93)	19.44% (18.79–20.14)	21.69% (20.98–22.48)	18.62% (17.67–19.70)	18.15% (17.52–19.03)	20.39% (19.91–21.18)	18.06% (17.28–19.08)	**0.21%** (0.16–0.31)	–	–
alloides 2	21.15 (21.02–21.68)	17.75% (17.62–17.96)	19.27% (19.04–19.94)	21.73% (21.38–22.18)	21.30% (21.04–21.81)	19.68% (18.58–20.23)	17.11% (16.70–17.80)	19.16% (19.10–19.38)	19.34% (18.82–19.90)	9.68% (9.48–9.88)	**n/a** n/a	–
kuna	26.41 (25.59–27.00)	23.22% (22.62–23.65)	25.70% (24.86–26.37)	25.51% (24.58–26.27)	27.87% (26.79–28.53)	25.78% (24.48–26.64)	24.91% (23.36–25.57)	25.63% (24.96–26.27)	25.54% (24.61–26.20)	23.30% (22.77–23.65)	23.97% (23.59–24.30)	**0.57%** (0.15–1.24)

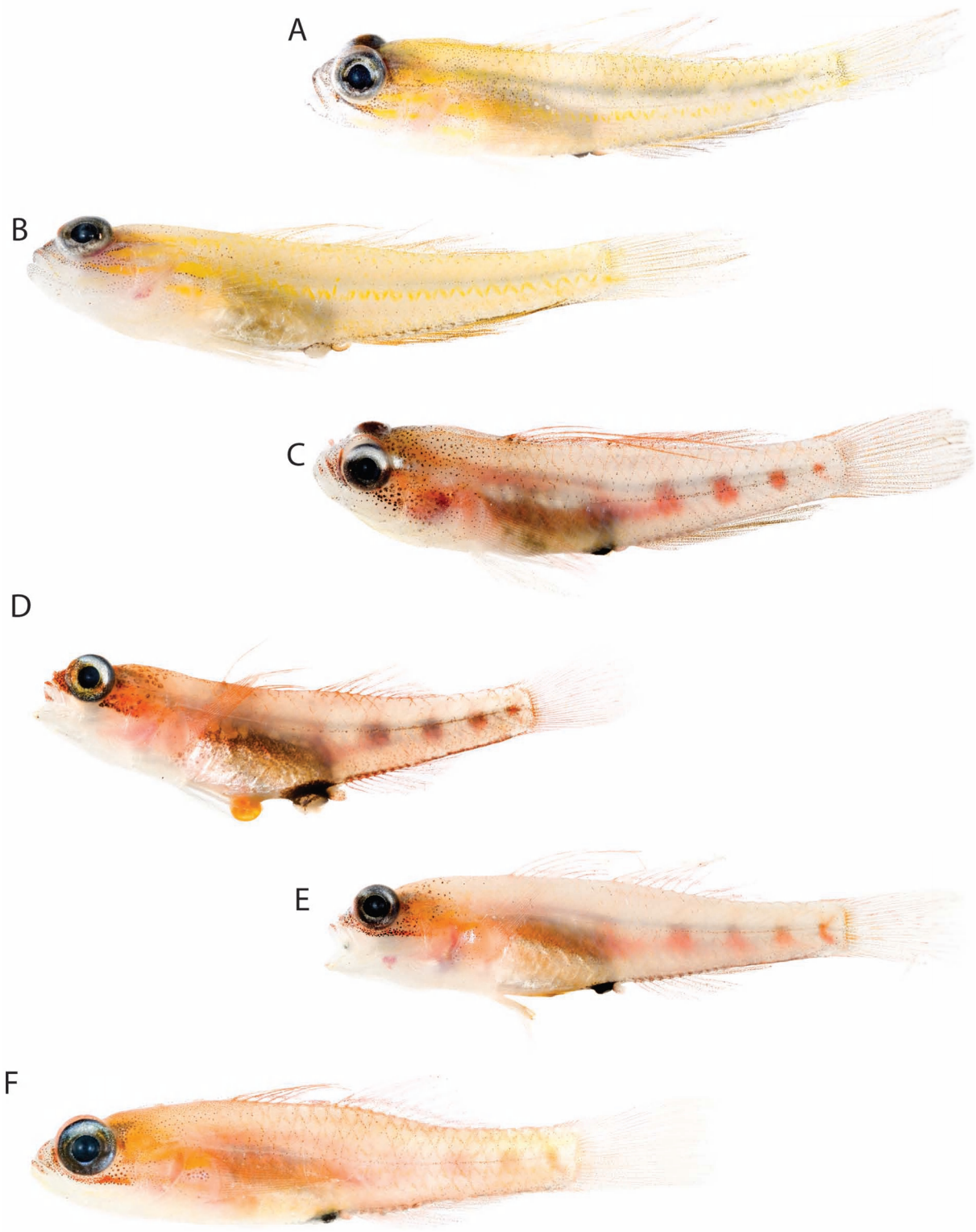


FIGURE 2. *Coryphopterus lipernes*: A, Curacao, 20 mm SL, DNA 8326, USNM 394896; B, Curacao, 21 mm SL, DNA 8051, USNM 394895. *Coryphopterus hyalinus*: C, Curacao, 20 mm SL, DNA 8044, USNM 394890; D, Curacao, 17 mm SL, DNA 8265, USNM 294889. *Coryphopterus personatus*: E, Curacao, 21 mm SL, DNA 8045, USNM 294897; F, Belize, 15 mm SL, DNA 7163, USNM 394742.

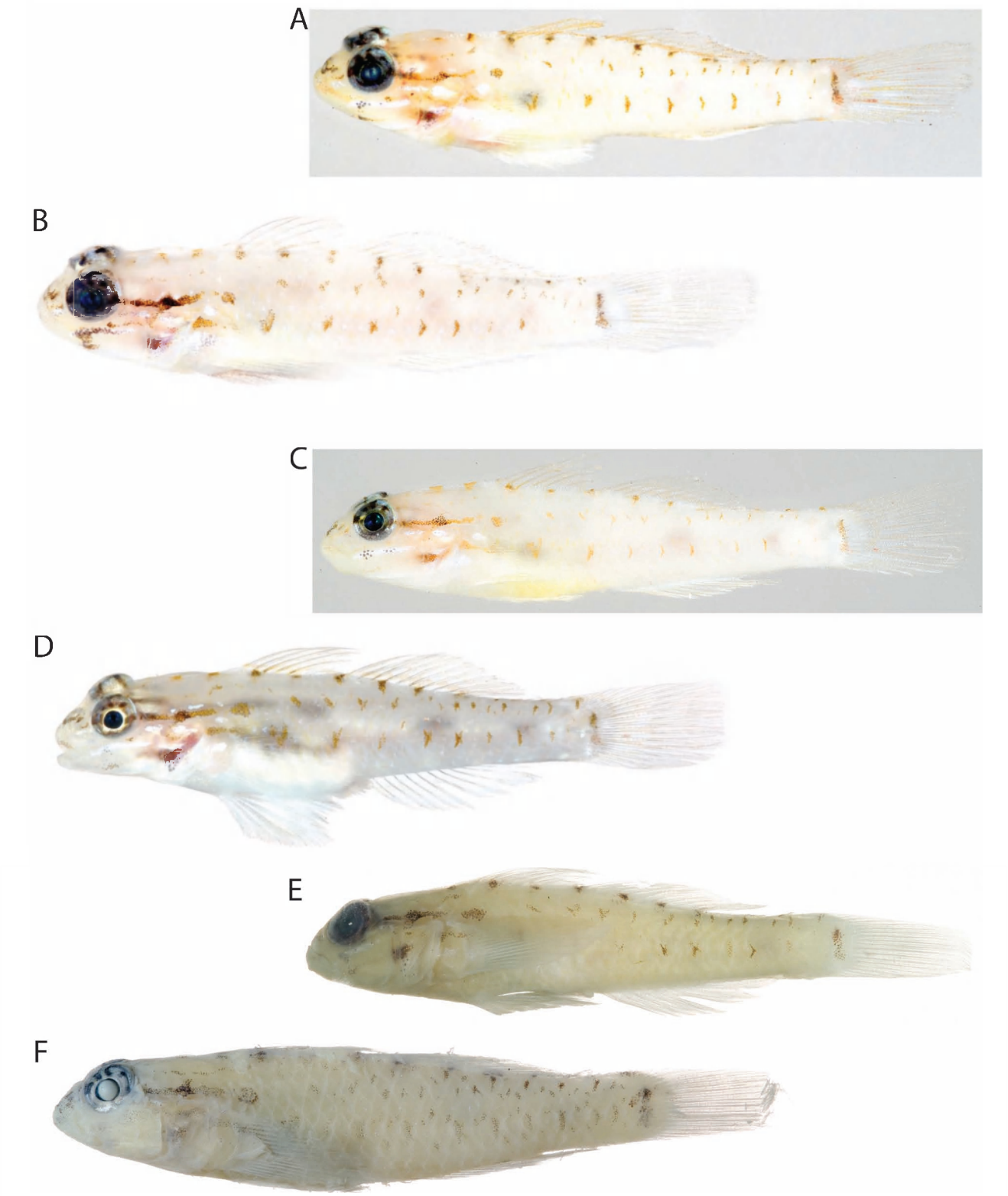


FIGURE 3. *Coryphopterus tortugae*: A, Belize, 25 mm SL, DNA 7333, USNM 394744; B, Belize, 34 mm SL, DNA 5237, USNM 394743; C, Belize, 36 mm SL, DNA 7107, USNM 394733; D, Belize, 40 mm SL, DNA 4530, USNM 394730; E, Belize, 40 mm SL, DNA 4530, USNM 394730, preserved; F, Venezuela, 37 mm SL, DNA 7736 4, AMNH 247340, alcohol preserved.

two forms of *C. glaucofraenum*: "[D]ark inshore form (typical *glaucofraenum*)" and"[P]allid white-sand form." Specimens in our genetic clade identified as *C. glaucofraenum* match the Böhlke and Robins (1960) "typical *glaucofraenum*," an identification supported by the fact that the pallid form is now recognized as *C. tortugae*. Below (see "Designation of Neotype for *Coryphopterus glaucofraenum*") we select a neotype for *C. glaucofraenum* Gill.

In our material, adult *C. glaucofraenum* can always be separated from *C. tortugae* by having at least some large, well-formed X-shaped markings along the side of the body. It can almost always be separated from *C. venezuelae* by lacking a prominent dark marking on the lower portion of the pectoral-fin base and sometimes by having 10 total anal-fin elements. Rarely, *C. glaucofraenum* has a dark pectoral-fin base that includes pigment on the lower portion (Figure 4G), and *C. venezuelae* may have 9–11 anal-fin elements, 10 being the typical count in our material (Table 2). *Coryphopterus glaucofraenum* usually can be separated from both *C. tortugae* and *C. venezuelae* by the shape of the pigment marking above the opercle: a two-peaked blotch in *C. glaucofraenum*, and a triangular or rounded blotch in *C. tortugae* and *C. venezuelae*.

If a specimen has a two-peaked blotch of pigment above the opercle, has at least some large (height approximately three-quarters of or equal to diameter of eye) X-shaped markings along the side of the body, has 10 anal-fin elements, and lacks pigment on the lower portion of the pectoral-fin base, it is unquestionably *C. glaucofraenum*.

Coryphopterus venezuelae (Cervigón, 1966)

FIGURE 5

The most recent keys to western Atlantic *Coryphopterus* (Böhlke and Robins, 1960, 1962; Böhlke and Chaplin, 1968; Murdy 2002) do not include *C. venezuelae*, originally described as a subspecies of *C. glaucofraenum* by Cervigón (1966), but recognized as a separate species by Cervigón (1994) and known at the time only from Venezuela. In the *Coryphopterus* material from the northeast coast of Venezuela that we examined are specimens that are clearly *C. venezuelae* based on Cervigón's (1966, 1994) descriptions: most notably the presence of 11 second dorsal- and anal-fin elements, a dark blotch of pigment on the lower portion of the pectoral-fin base, and two dark spots on the base of the caudal fin (e.g., Figure 5D herein). However, those Venezuelan specimens are part of a clade based on COI analysis (see Figure 1) that includes specimens from Venezuela, Curacao, Panama, Belize, Puerto Rico, and the Bahamas (the last not shown on the tree) that usually have 10 second dorsal- and anal-fin elements and various patterns of pigment on the base of the caudal fin, including a central bar, two spots joined by a bar, and a C-shaped blotch (Figure 5A–C,E). The Venezuelan specimens on the tree (Figure 1), including two that have 10 second dorsal- and anal-fin elements (VEN 7733 1 and VEN JV12), cluster within the *C. venezuelae* clade, but the genetic distance between the Venezuelan specimens and other members of the clade is only 0.41% to 0.85%. This distance is extremely small relative to the genetic distance between the *C. venezuelae* clade and other species on the tree (9.51%–20.86%; see Table 1), suggesting that the individuals in this clade represent a single species. Corroborating the identification of the clade as Cervigón's *C. venezuelae* is the presence in all individuals in the clade of a dark spot on the lower portion of the pectoral-fin base. Among western Atlantic *Coryphopterus*, only *C. punctipectophorus* and *C. dicrus* have a prominent pigment spot on the lower portion of the pectoral-fin base: *C. punctipectophorus* is not known from the Caribbean, and it differs morphologically from *C. venezuelae* in, among other features, lacking a dark blotch of pigment behind the eye above the opercle; in *C. dicrus*, there is also a prominent spot of equal size on the dorsal portion of the pectoral base that is lacking in *C. venezuelae* (which may have a slash of pigment but never a well-defined dorsal spot equal in size and intensity to the lower spot); *C. dicrus* also lacks the dark pigment behind the eye above the opercle and lacks a pelvic frenum (both present in *C. venezuelae*).

Our data thus suggest that *C. venezuelae* is a much more widespread species than previously recognized, and fin-ray counts alone are not sufficient in diagnosing the species. Cervigón (1994) believed that the presence of 10 second dorsal- and anal-fin elements in *C. glaucofraenum* distinguished it from *C. venezuelae*. In his material of the latter, all specimens had 11 second dorsal-fin elements and most had 11 anal-fin elements (two had 10). Most of our specimens of *C. glaucofraenum* have 10 second dorsal- and anal-fin elements, but two specimens have 11 second dorsal-fin elements, and two have 9 anal-fin elements (see Table 2). Both 10 and 11 second dorsal- and anal-fin elements are common in specimens in our *C. venezuelae* clade (Table 3), although we found 11 in both fins only in some of our material from Venezuela. It is significant that one of the *C. venezuelae* specimens from Venezuela that has 10 elements in both fins was caught in the same sample as

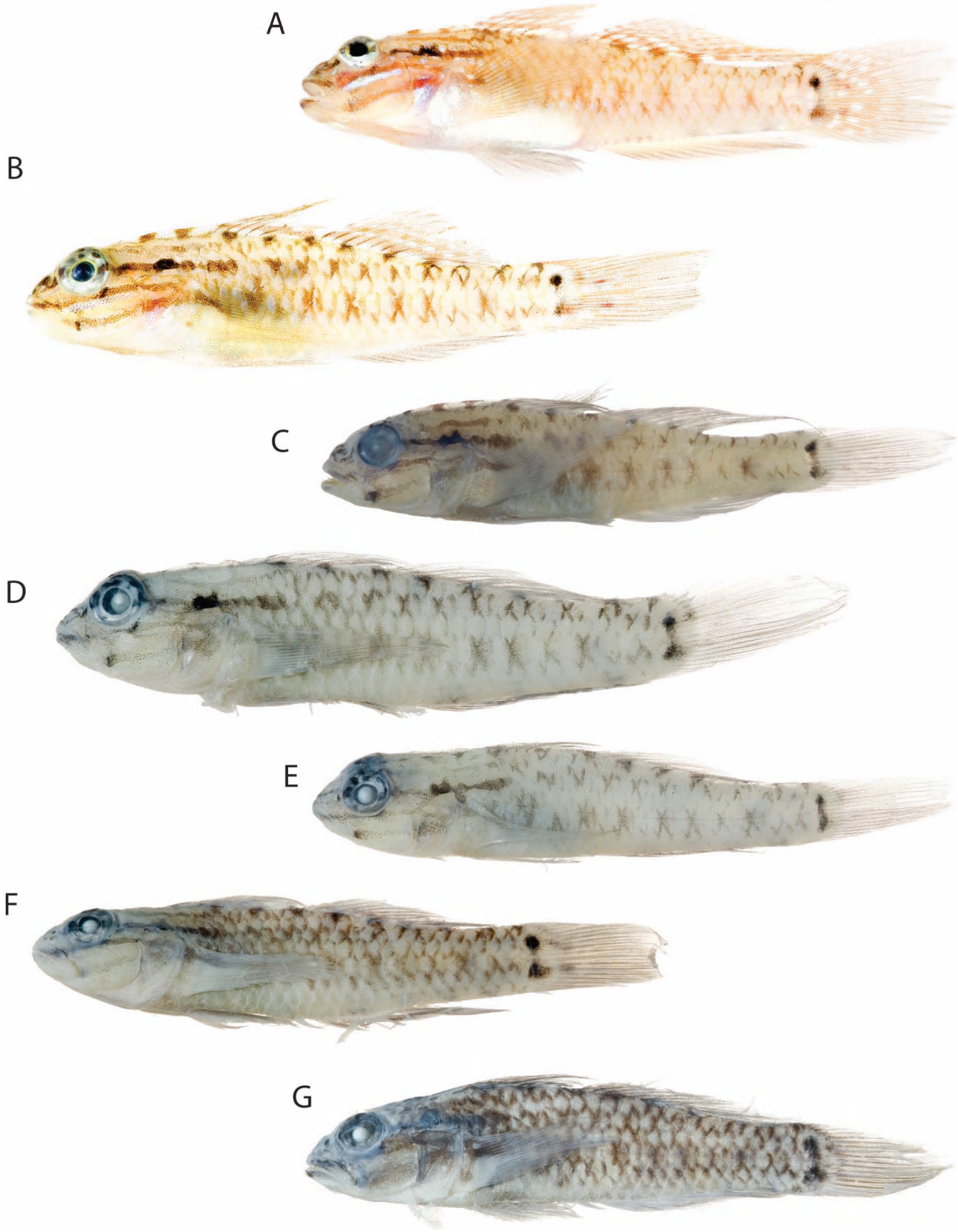


FIGURE 4. *Coryphopterus glaucofraenum*: A, Belize, 44 mm SL, DNA 6367; B, Belize, 25 mm SL, DNA 7352, USNM 394354; C, Belize, 35 mm SL, DNA 7351, USNM 394353, preserved; D, Venezuela, 31 mm SL, DNA 7744 2, AMNH 247339, alcohol preserved; E, Venezuela, 27 mm SL, DNA 7744 3, AMNH 247339, alcohol preserved; F, Panama, 34 mm SL, DNA 7712 2, AMNH 247335, alcohol preserved; G, Panama, 37 mm SL, DNA 7701 1, AMNH 247334, alcohol preserved.

several with 11 in both fins. There is thus more variability in numbers of second dorsal- and anal-fin elements than Cervigón indicated, and those fin-ray counts are of value in separating *C. glaucofraenum* and *C. venezuelae* only when 11 elements are present in both fins—a condition we have not observed in *C. glaucofraenum*, which may have 11 second dorsal-fin elements but no more than 10 anal-fin elements (see Table 2).

If a specimen has a dark blotch or triangle of pigment above the opercle, 11 second dorsal-fin *and* 11 anal-fin elements, and a prominent pigment spot on the lower portion of the pectoral-fin base, it is *C. venezuelae*.

If a specimen has those features and has 10 second dorsal- and anal-fin elements, it is usually *C. venezuelae* but could be *C. glaucofraenum*: as noted under "*Coryphopterus glaucofraenum*," rarely specimens of that species may have pigment on the ventral portion of the pectoral-fin base. The shape of the pigment marking above the opercle (with two peaks in *C. glaucofraenum*, a single triangular or rounded blotch in *C. venezuelae*; see "*Coryphopterus glaucofraenum*") will frequently resolve the species identification.

There are two distinct forms of *C. venezuelae* in terms of body pigment: one has at least some large X-shaped markings in the ventral row of markings similar to those of *C. glaucofraenum* (Figure 5B,D,E); the other is a much paler form, and the ventral pigment markings along the side of the body are usually fairly small, somewhat circular blotches (Figure 5A,C). Both forms, including the palest specimens, have a pigment spot on the lower pectoral-fin base, but this spot may be composed primarily of yellow chromatophores versus melanophores in pale specimens. The less-pigmented form is most easily confused with *C. tortugae*, but some of the pigment spots in the ventral row of *C. venezuelae* are usually more circular than the vertically elongate ones of *C. tortugae*. Additionally, none of our specimens of *C. tortugae* has a spot of pigment (yellow or black) on the ventral portion of the pectoral-fin base. Although unusually divergent intraspecifically in patterns of pigmentation (see Figure 5) relative to, for example, the very similar patterns between species such as *C. personatus* and *C. hyalinus*, the two forms of *C. venezuelae* form a tight genetic clade (intraspecific variation, 0.53%; see Figure 1, Table 1). The different pigment patterns do not correspond to different fin-ray counts, as we have observed 10 and 11 second dorsal- and anal-fin elements in both forms. For example, note the similar patterns of pigmentation in a specimen of *C. venezuelae* from Venezuela (Figure 5D) that has 11 second dorsal- and anal-fin elements and a specimen of *C. venezuelae* from Panama (Figure 5E) that has 10 second dorsal- and anal-fin elements. Furthermore, the differences are not attributable to sexual dimorphism or geography, but they could reflect differences in local habitat. Some specimens of *C. venezuelae* collected in mangrove areas tend to be dark, and those collected in reef areas pale, although we note that a dark form was collected on a reef off Panama (Figure 5E).

TABLE 2. Frequency distributions of numbers of second dorsal-fin and anal-fin elements in two species of *Coryphopterus*.

	No. of second dorsal-fin elements		No. of anal-fin elements		
Species	10	11	9	10	11
C. glaucofraenum	22	2	2	20	—
C. venezuelae	20	13	1	22	11

There is some correlation with size: the pale form of *C. venezuelae* is more common among small specimens (<30 mm standard length [SL]), and the form with prominent X-shaped markings is more common among larger specimens (>40 mm SL). Adults of the pale form of *C. venezuelae* (e.g., Figure 5A) look remarkably similar to juveniles (e.g., see Figure 7C). There is also a trend toward increasing depth of the head and anterior body in larger specimens. Similar differences in body shape and pigment with increasing size are evident in *C. glaucofraenum* (compare the juvenile in Figure 7B with adults in Figure 4). Possibly in *C. venezuelae*, growth is not always accompanied by a transformation in pigment and body depth, and adults retain more of the juvenile features. More investigation is needed to determine the relationships in *C. venezuelae* among pigment pattern, body shape, size, maturity, and local habitat. Cervigón (1966, 1994) did not illustrate any of his type specimens of *C. venezuelae*, but we obtained digital photographs of two of his paratypes (MOBR-P-0867 [Museo Oceanológico Hermano Benigno Román, Venezuela]; one is shown in Figure 6). The holotype is not in good condition (J. C. Capelo, MOBR, personal communication, 4 July 2008). The pigment of the paratypes most closely resembles that in Figure 5D herein: a triangular to rounded mark above the opercle, a roughly circular dark spot on the ventral pectoral-fin base, and two basicaudal spots joined by a light dusky bar. There is some evidence of X-shaped markings on the side of the body, but the body pigment is mostly faded. Cervigón (1966, 1994) did not mention X-shaped markings in his descriptions; rather, he noted that there are three longitudinal rows of dark spots.

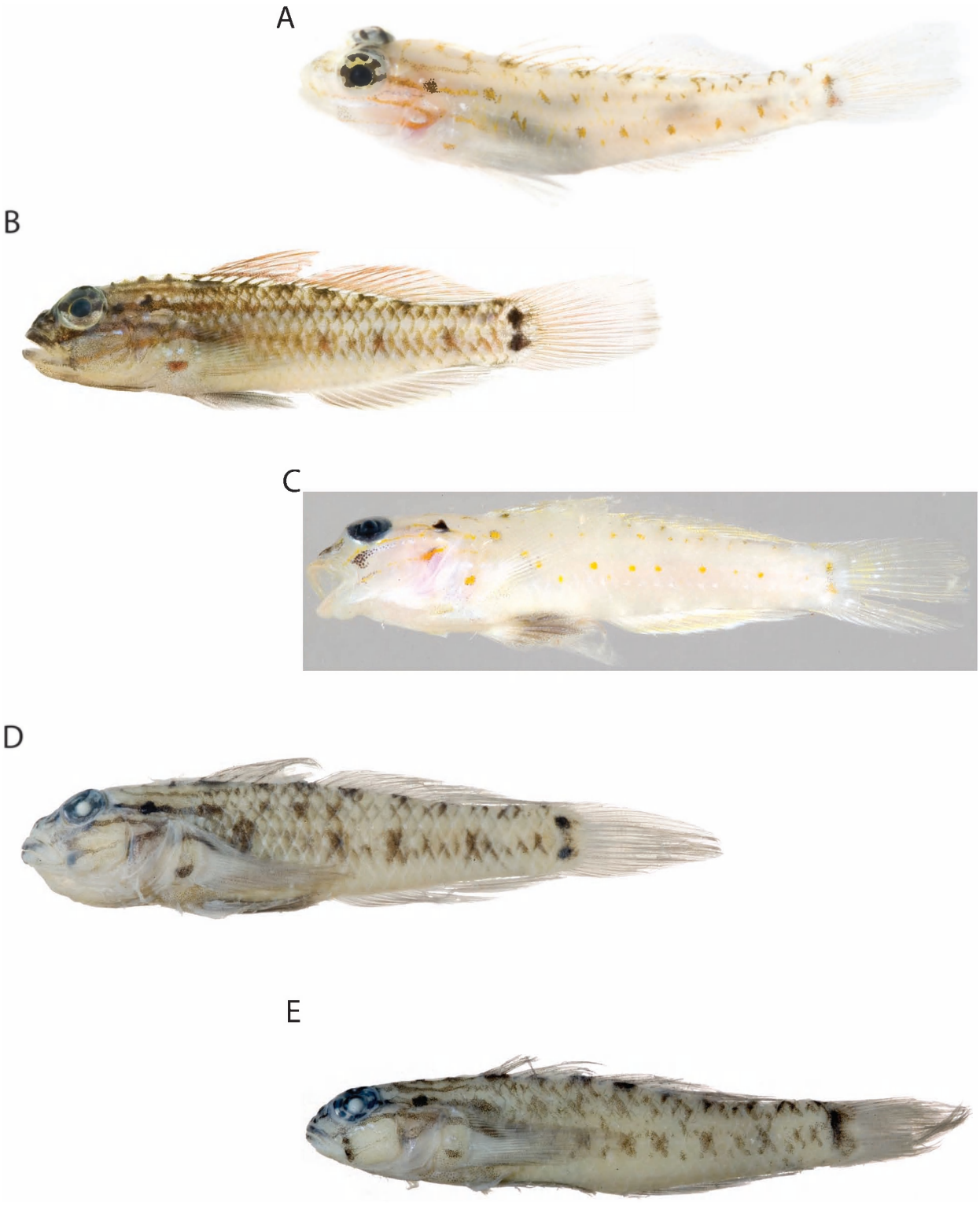


FIGURE 5. *Coryphopterus venezuelae*: A, Curacao, 29 mm SL, DNA 8260, USNM 394740; B, Venezuela, 54.4 mm SL, no DNA, Photo No. 1907 VT-05-530, photo by J. V. Tassell and D. R. Robertson; C, Belize, 35 mm SL, DNA 7248, USNM 394736; D, Venezuela, 50 mm SL, DNA JV15, AMNH 247345, alcohol preserved; E, Panama, 42.5 mm SL, DNA 7725-1, AMNH 247341, alcohol preserved.

Synonymy of *Coryphopterus bol*

Victor (2008) described *Coryphopterus bol* as a species that heretofore had been masquerading under *C. tortugae* (e.g., Garzón-Ferreira and Acero 1990:107, fig. 1A, Santa Marta specimens). We believe that Victor (2008) was correct in recognizing that the Santa Marta specimens are not *C. tortugae*, but our investigation indicates that they are *C. venezuelae*. The COI sequence that Victor (2008) provided for the new species (from the holotype from Puerto Rico) places it solidly with our *C. venezuelae* clade (PR SIO 0869, fig. 1). The average genetic distance between *C. bol* and individuals of *C. venezuelae* is 0.38% (range, 0.00%–0.85%) and, for comparison, the average genetic distance between the holotype of *C. bol* and the next most closely related clade (*C. tortugae*) is more than 20-fold greater, or 8.47% (range, 8.10%–9.21%). Diagnostic features of Victor's (2008:4) *C. bol* include 10 second dorsal- and anal-fin elements; 19 pectoral-fin rays; pelvic fins fully joined and with a distinct frenum; a prominent, dark, upward-pointed triangular marking on the stripe behind the eye; a discrete blotch of small melanophores on the lower third of the pectoral fin base; and a basicaudal marking that resembles a thick "C." The combination of the triangular marking on the stripe behind the eye above the opercle, the pigment blotch on the lower portion of the pectoral-fin base, and 10 second dorsal- and anal-fin elements matches most of our *C. venezuelae* specimens. Victor (2008) distinguished his new species from *C. venezuelae* based on the presence of 11 second dorsal- and anal-fin elements in *C. venezuelae*, but, as noted above (also see Table 2), specimens matching Cervigón's *C. venezuelae* based on the pre-pectoral pigment may have 10 or 11 second dorsal- and anal-fin elements.

Coryphopterus bol also matches *C. venezuelae* in number of pectoral-fin rays (19 in *C. bol*, 61% of specimens with 19 in Cervigón's [1994] *C. venezuelae* material), pelvic-fin morphology, and other pigment. For example, the basicaudal mark in *C. venezuelae* may be C-shaped, but it ranges in our material from two separate spots to a central bar of pigment (some examples are shown in Figure 5). The basicaudal pigment is also somewhat variable in the type material of *C. bol* (Victor, 2008:fig. 1). Two of the type specimens of *C. bol* most closely resemble the pale form of *C. venezuelae*; that is, the form with round spots on a relatively slender body (holotype and a 32.1-mm SL paratype). Two paratypes (24.5 and 29 mm SL) are darker and have at least some X-shaped markings. None of Victor's type material is larger than 32 mm SL, and, as noted under *C. venezuelae*, above, most of our dark, deeper-bodied specimens of *C. venezuelae* are >40 mm SL.

TABLE 3. Frequency distributions of the combinations of second dorsal-fin and anal-fin elements in *Coryphopterus venezuelae* by country.

	No. of second dorsal-fin elements / anal-fin elements				
Country	10/9	10/10	10/11	11/10	11/11
Belize	—	2	—	—	—
Curacao	1	11	1	1	—
Panama	—	6	1	—	—
Venezuela	—	2	—	1	9
Puerto Rico	—	1[a]	—	—	—

[a] Holotype of *Coryphopterus bol*.

In summary, one cannot distinguish *C. bol* and *C. venezuelae* on the basis of numbers of second dorsal- and anal-fin elements because there is more variation in those counts than previously reported. One might argue that specimens from Venezuela that have 11 elements in both the second dorsal and anal fins and heavy pigment with X-shape markings are *C. venezuelae* and that everything else in our *C. venezuelae* clade is *C. bol*. However, some specimens with those features, except with 10 elements in the second dorsal and anal fins, were taken in the same station off Venezuela as those with 11 elements (AMNH 247345 [American Museum of Natural History]), so would one identify the former as *C. venezuelae* or *C. bol*? Species identification of specimens with 11/10 or 10/11 second dorsal-/anal-fin elements also would be nebulous, as would species identification of dark forms with 10/10 but otherwise virtually identical to those with 11/11 (e.g., Figure 5D,E). Variation in COI among all specimens in the *C. venezuelae* clade is well within typical intraspecific levels for the genus. However, even if COI is masking recent divergence within the clade, there is a diagnostic morphological feature for the clade: a conspicuous spot or blotch of pigment on the lower pectoral-fin base; in combination with a triangular or circular blotch of pigment behind the eye above the opercle, this character is unique to *C. venezuelae*. The more common presence of 11 second dorsal- and anal-fin elements in some Venezuelan specimens may best be interpreted as regional variation. Known currently from Belize, Panama, Curacao, Venezuela, the Bahamas, the U.S. Virgin Islands, Puerto Rico, Saba, and Brazil, *C. venezuelae* appears to be a widespread species. It is misidentified in the USNM (U.S. National Museum;

FIGURE 6. Paratype of *Coryphopterus venezuelae*, MOBR-P-0867, 42 mm SL (length estimated from ruler included with original photograph; this is likely Cervigón's 41.2 mm SL paratype).

i.e., National Museum of Natural History, Smithsonian Institution)—and likely other museum collections—as *C. glaucofraenum* or *C. tortugae*.

Key Notes for *C. tortugae*, *C. glaucofraenum*, and *C. venezuelae*

Juveniles (Figure 7), and occasionally adults, of *C. tortugae*, *C. glaucofraenum*, and *C. venezuelae* may lack the black marking or triangle above the opercle, or it is not as dark as other pigment in the stripe posterior to the eye. As we have used this feature in the "Revised Key to Western Atlantic *Coryphopterus*" (see below) to separate *C. tortugae*, *C. glaucofraenum*, and *C. venezuelae* from other species, absence of this feature in specimens of any of those species could present identification problems. If there are well-defined X's of pigment along the sides of the body (*C. glaucofraenum* and some *C. venezuelae*) or the basicaudal pigment comprises two spots or a dumbbell-shaped marking (most *C. glaucofraenum* and some *C. venezuelae*), users of the key should follow the option in the couplet that indicates the dark marking is present above the opercle (4b). If a specimen lacks the dark pigment spot above the opercle, has 11 second dorsal- and anal-fin rays, and has a prominent dark blotch on the lower portion of the pectoral-fin base, it can only be *C. venezuelae*. *Coryphopterus punctipectophorus* is similar in lacking the pigment spot above the opercle and having 11 second dorsal-fin elements, but it has 10 anal-fin elements (Springer, 1960). Furthermore, geography will currently separate those two species: *C. venezuelae* occurs in the Caribbean, and *C. punctipectophorus* is known only from the Gulf of Mexico and off the southeastern USA.

Coryphopterus dicrus Böhlke and Robins, 1960

FIGURE 8

Numerous features, in combination, separate *C. dicrus* from other western Atlantic *Coryphopterus*, including the following: no black ring of pigment around anus; no distinct dark spot behind eye above opercle; anal-fin elements 10; pelvic frenum absent; pectoral-fin base with two prominent dark spots of equal intensity, one above the other; and sides of body freckled with scattered large and smaller pigment blotches. The last two characters are the quickest way to make the identification. The only other species that usually have pigment dorsally *and* ventrally on the pectoral-fin base are *C. venezuelae* and *C. thrix*, but the dorsal pigment on the pectoral-fin base in *C. venezuelae*, when present, is a slash versus a spot, and the dorsal pigment on the pectoral-fin base in *C. thrix* is usually much more pronounced than the ventral marking. Additionally, both species have a pelvic frenum, which is lacking in *C. dicrus*.

Coryphopterus thrix Böhlke and Robins, 1960

FIGURE 8

Coryphopterus thrix is the only western Atlantic species of *Coryphopterus* that lacks black pigment around the anus *and* has the second dorsal-fin spine elongated into a filament. If the spine is broken, however, the species is still identifiable by the combination of features presented in the key, most notably the absence of a distinctive pigment blotch above the opercle, presence of a conspicuous dark blotch on the dorsal portion of the pectoral-fin base, and presence of a pelvic frenum.

Coryphopterus alloides Böhlke and Robins, 1960

FIGURE 9

Distinguishing features of *C. alloides* include having a low anal-fin count (8–9 total elements), a dark blotch of pigment on the lower portion of the membrane between the second and third dorsal spines, and the pelvic fins almost completely separate. Only *C. kuna*, among the *Coryphopterus* species lacking a black ring of pigment around the anus, has as few as 9 anal-fin elements, but that species has 9 second dorsal-fin elements and 15 pectoral rays (vs. usually 10 and 16–17, respectively, in *C. alloides*). *Coryphopterus kuna* may have a stripe and distal flag of pigment on the first dorsal fin, but it never has the pigment blotch on the lower portion of the first dorsal fin characteristic of *C. alloides*. The living color pattern of *C. alloides* is also distinctive: the head and anterior portion of the body bear a considerable amount of orange pigment, whereas the posterior portion of the body is yellow. An apparently cryptic species related to but genetically distinct from *C. alloides* and known only from Curacao is currently under investigation.

Key Note

In some preserved specimens of *C. alloides*, there are melanophores above the opercle that may lead the user of the key to select "4b. Distinct black blotch or triangle behind eye above opercle . . ." However, this pigment is never as consolidated and prominent in *C. alloides* as in

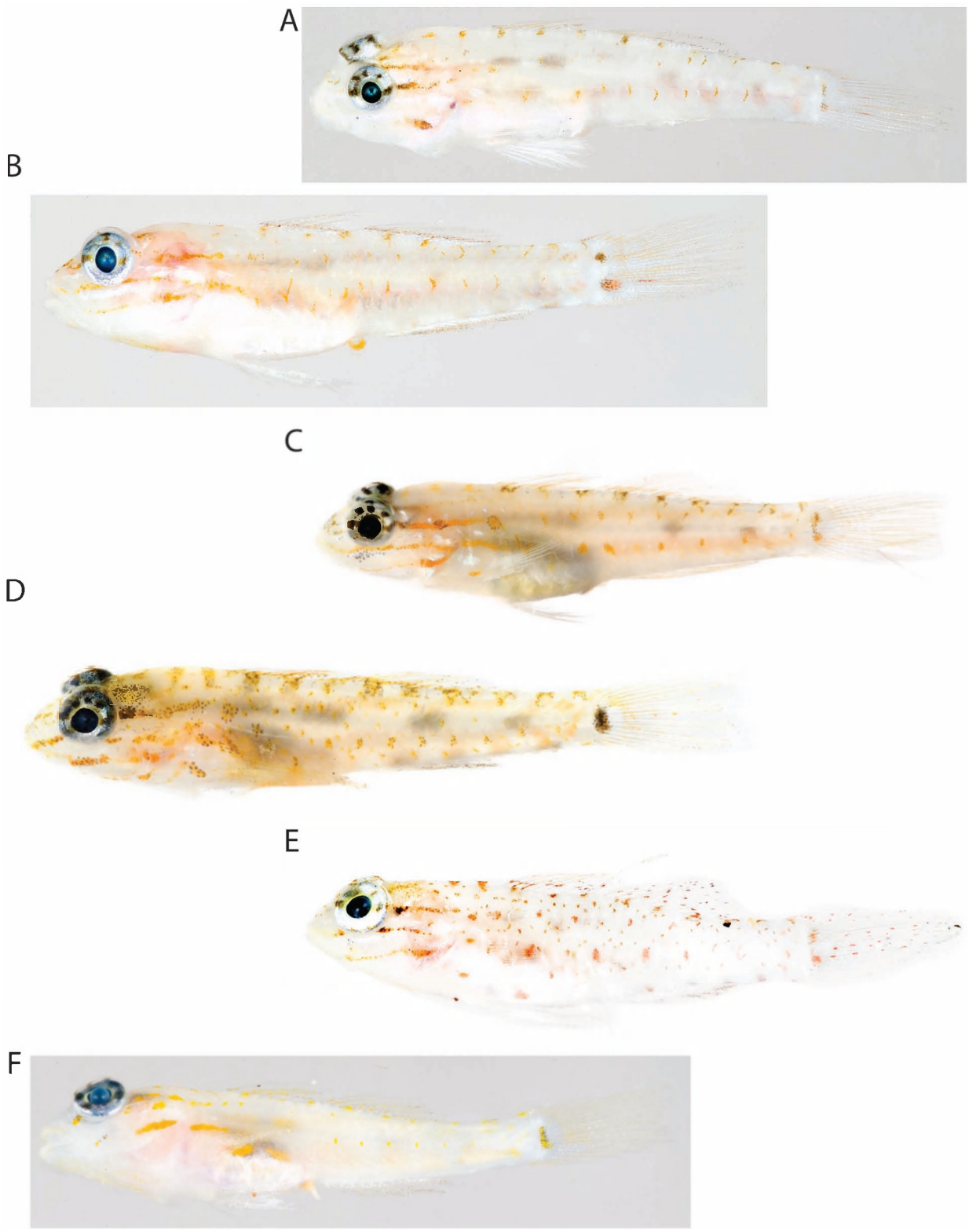


FIGURE 7. *Coryphopterus* juveniles: A, *C. tortugae*, Belize, 20 mm SL, DNA 7693, USNM 394800; B, *C. glaucofraenum*, Belize, 17 mm SL, DNA 7769, USNM 394793; C, *Coryphopterus venezuelae*, Belize, 17 mm SL, DNA 7728, USNM 394881, D, *Coryphopterus thrix*, Curacao, 16 mm SL, DNA 8261, USNM 394760; E, *Coryphopterus dicrus*, Belize, 13 mm SL, DNA 6110, USNM 394779. F, *Coryphopterus eidolon*, Belize, 18 mm SL, DNA 6223, USNM 394788.

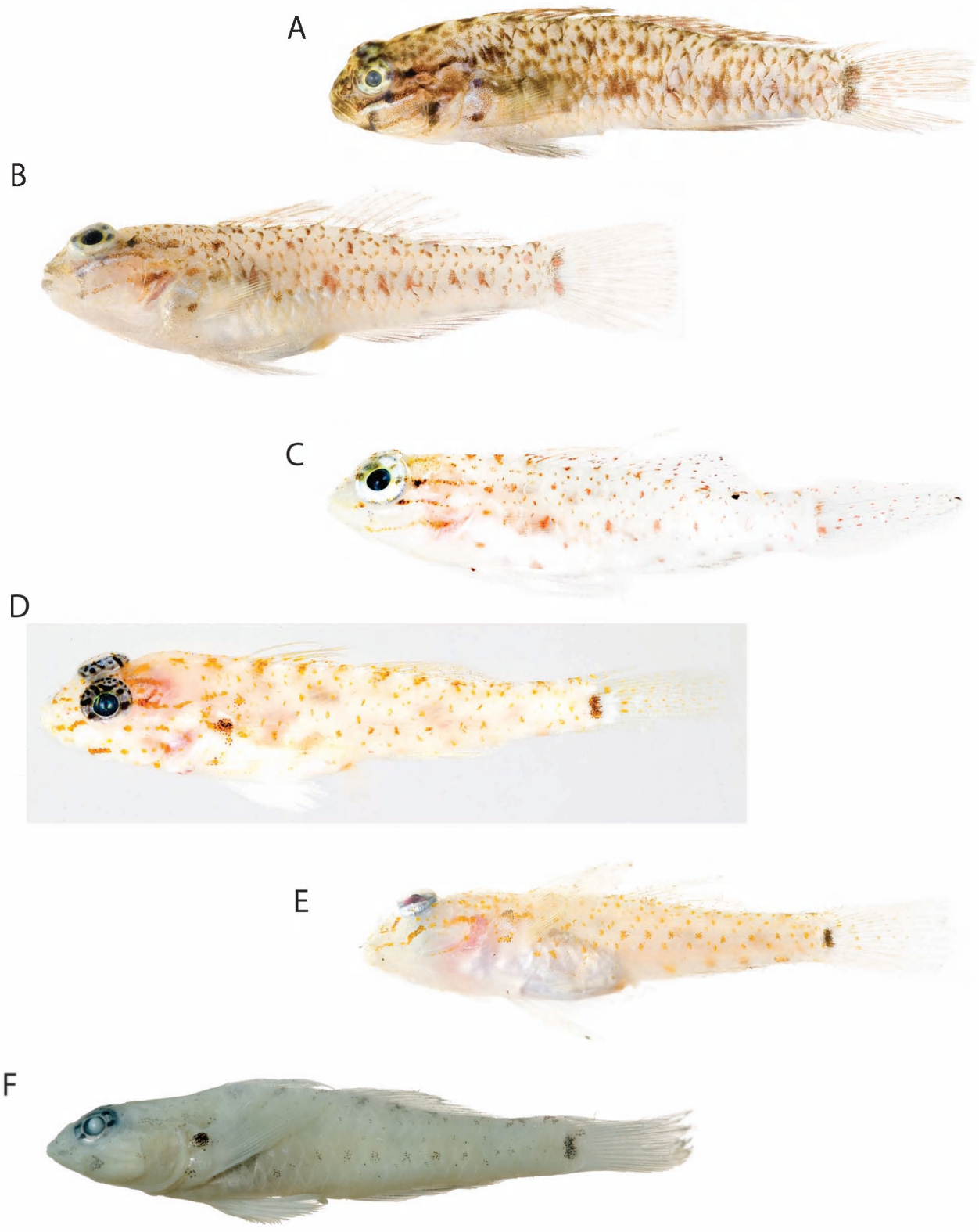


FIGURE 8. *Coryphopterus dicrus*: A, Florida, 38 mm SL, DNA 7348, USNM 394345; B, Curacao, 30 mm SL, DNA 8135, USNM 394747; C, Belize, 13 mm SL, DNA 6110, USNM 394779. *Coryphopterus thrix*: D, Belize, 23.5 mm SL, DNA 7816, USNM 394914; E, Curacao, 23 mm SL, DNA 8426, USNM 394761; F, Venezuela, AMNH 244983, 26 mm SL, alcohol preserved, no DNA.

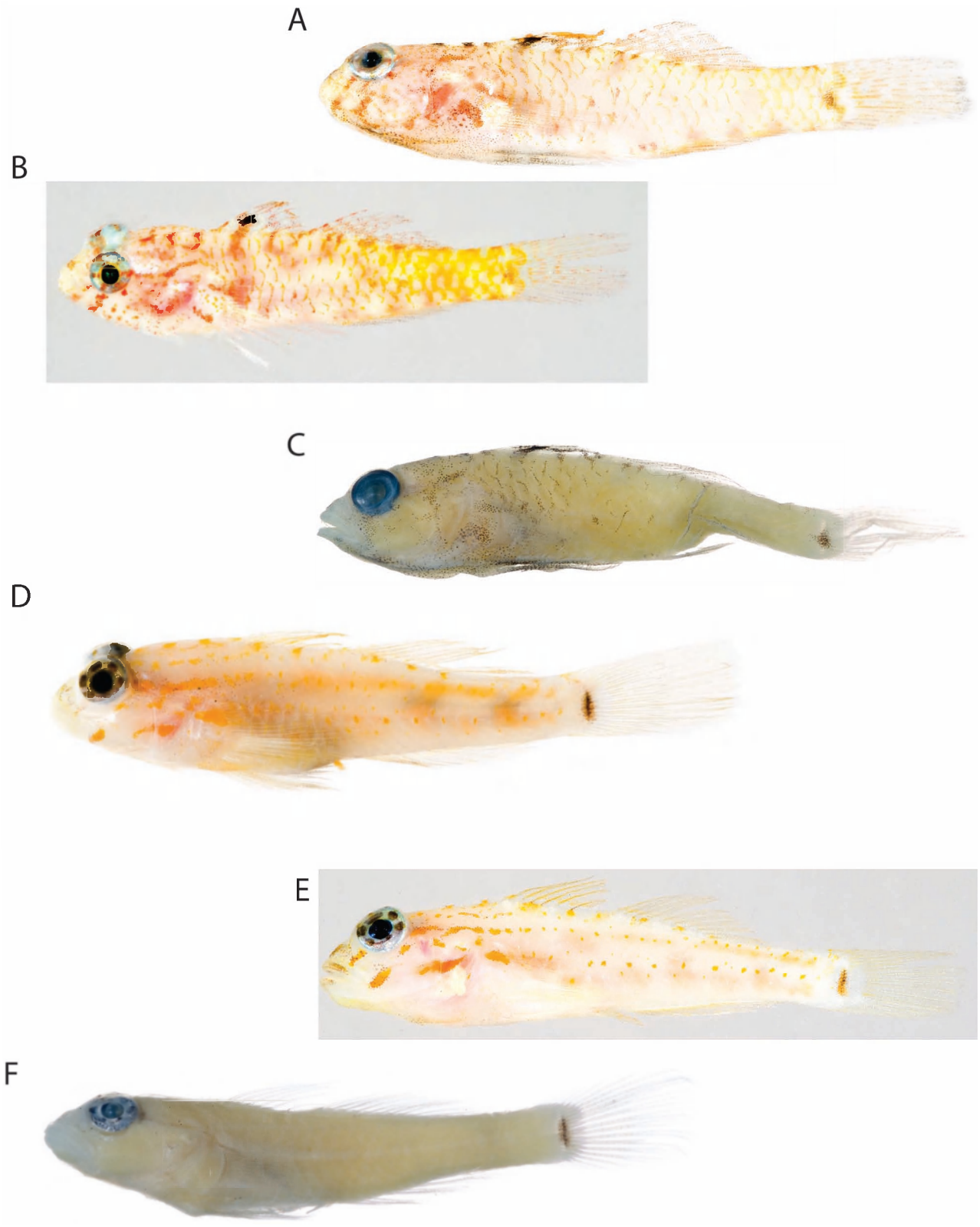


FIGURE 9. *Coryphopterus alloides*: A, Belize, 24 mm SL, DNA 7233, USNM 394754; B, Belize, 19 mm SL, DNA 7264, USNM 394755; C, Belize, 24 mm SL, preserved, DNA 7233, USNM 394754. *C. eidolon*: D, Curacao, 38 mm SL, DNA 8050, USNM 394885; E, Belize, 34 mm SL, DNA 7109, USNM 394752; F, Belize, 33 mm SL, preserved, DNA 5070, USNM 394750.

C. tortugae, *C. glaucofraenum*, and *C. venezuelae*; furthermore, *C. alloides* lacks a pelvic frenum, a conspicuous feature in the other three species.

Coryphopterus eidolon Böhlke and Robins, 1960

FIGURE 9

Pigment, except for basicaudal and scattered small body melanophores, is yellow, which disappears during preservation, typically rendering this a very pale goby. In life there is a yellow stripe behind the eye bordered by small melanophores that remain in preserved specimens after the color fades. There are no dark markings above the opercle, on the pectoral-fin base, or on the first dorsal fin. The absence of distinctive markings (other than the basicaudal mark) is the easiest way to recognize *C. eidolon*, a very abundant species in many of our samples, particularly from Belize and Curacao.

Coryphopterus kuna Victor, 2007

FIGURE 10

Baldwin and Smith (2003) described *Coryphopterus* B larvae from Belize as likely belonging to an unidentified species based on the low second dorsal- and anal-fin counts (9 in both fins) and low pectoral-fin count (15). Victor (2007) described *C. kuna*, which has the low fin-ray counts of the *Coryphopterus* B larvae, as a new species from off Panama. Incorporation of the COI sequence published in the original description of *C. kuna* into our analysis revealed that *Coryphopterus* B larvae are *C. kuna*. This species is distinctive in typically having 9 second dorsal- and anal-fin elements, as well as a low pectoral-ray count of 15 (found elsewhere only in *C. personatus* and *C. hyalinus*). Apparently a small fish—the adult male holotype is 17.1 mm SL—*C. kuna* has little dark pigment: numerous small spots on the pelvic fin of the holotype, a few scattered small spots on the sides of the body, no markings on the pectoral-fin base, and no basicaudal spot. It lacks a pelvic frenum.

Coryphopterus punctipectophorus Springer, 1960

FIGURE 10

Coryphopterus punctipectophorus is similar to *C. tortugae*, *C. glaucofraenum*, and *C. venezuelae* in having three rows of pigment spots along the side of the body, but it differs from those species in lacking a dark blotch or triangle behind the head above the opercle. It is most similar to *C. venezuelae* in having a prominent dark spot on the lower portion of the pectoral-fin base, and juvenile (and occasionally adult) specimens of *C. venezuelae* that lack the pigment blotch above the opercle will typically key to *C. punctipectophorus* based on the ventral pigment spot on the pectoral-fin base. Like *C. venezuelae*, *C. punctipectophorus* was originally described as having 11 second dorsal-fin elements, but as noted above (see *C. venezuelae*), the former has 10 or 11 second dorsal elements. The "dusky light buff" pigment spots along the dorsal contour and "coral pink" spots along the sides of the body in fresh material of *C. punctipectophorus* (Springer, 1960:240; see Figure 10B,E herein) apparently fade in preserved material (see Figure 10D). The known distribution of *C. punctipectophorus* includes both coasts of Florida, the Gulf of Mexico (including the southern Gulf where it meets the Caribbean), and South Carolina. It apparently inhabits deeper water than some *Coryphopterus* species: the type material was collected at 62 and 120 feet. It has not been reported from the Caribbean. We have not collected *C. punctipectophorus*, and fresh material of the species was not available for inclusion in our genetic analysis. Thacker and Cole's (2002) *C. punctipectophorus* from Belize (GenBank Accession No. AF391396) is *C. dicrus*, based on incorporation of their COI sequence into our data set.

REVISED KEY TO THE WESTERN ATLANTIC SPECIES OF *CORYPHOPTERUS*

1a. Black ring of pigment surrounding anus 2
1b. Black ring around anus absent 4
2a. One interorbital pore anteriorly 3
2b. Two interorbital pores anteriorly . . . . . ***Coryphopterus hyalinus***
3a. Second dorsal and anal fins each typically with 11 total elements; head with some orange pigment in life; body translucent, with several squares or rectangles of pale orange pigment internally; preserved specimens lacking conspicuous postorbital stripes of melanophores but with "mask" of pigment around eye . . . . . ***Coryphopterus personatus***

(continued on p. 130)

REVISED KEY TO THE WESTERN ATLANTIC SPECIES OF *CORYPHOPTERUS* *(continued)*

3b. Second dorsal and anal fins typically with 10 total elements; head and body predominantly yellow in life; a dusky internal stripe along posterior section of vertebral column; preserved specimens with postorbital stripes of melanophores and scattered spots over entire body . . . . . ***Coryphopterus lipernes***

4a. No distinct black blotch behind eye above opercle in adults; pigment above opercle, if present, no larger or darker than other markings behind eye; pelvic frenum present or absent (see "Key Note" for *C. alloides* in text) *5*

4b. Distinct black blotch or triangle behind eye above opercle in adults, blotch usually larger and darker than other pigment in stripe behind eye; pelvic frenum present (see "Key Notes for *C. tortugae*, *C. glaucofraenum*, and *C. venezuelae*" in text) 10

5a. Anal-fin elements 8–9 (usually 9), pectoral-fin rays 15–17, pelvic frenum absent *6*

5b. Anal-fin elements 10–11, pectoral-fin rays 17–20, pelvic frenum present or absent *7*

6a. Second dorsal and anal fins each with 9 elements; pectoral-fin rays 15; pelvic fins fully joined; first dorsal fin with stripe of black pigment; in life, head and body with orange spots and blotches and sometimes with flag of dark pigment on 1st–3rd dorsal spines . . . . . ***Coryphopterus kuna***

6b. Second dorsal fin with 10 elements, anal fin with 9 (rarely 8); pectoral-fin rays 16–17; pelvic fins almost completely separate; black blotch or bar between 2nd and 3rd dorsal spines; head and anterior body mottled orange in freshly caught specimens, posterior body mottled yellow . . . . . ***Coryphopterus alloides***

7a. Pectoral-fin base with two prominent dark spots of equal intensity, one *dorsally* and one *ventrally*; upper spot usually with swath of melanophores extending posteriorly onto pectoral-fin rays; sides of body freckled with scattered large and smaller blotches of melanophores (blotches associated with coral, tan, yellow pigment in life); pelvic frenum absent . . . . . ***Coryphopterus dicrus***

7b. Pectoral-fin base not with two prominent dark spots (or, if two spots present, upper spot more intense); sides of body with few dark markings (with few to many yellow spots in life) or with three rows of light markings (coral pink/orange in life); pelvic frenum present *8*

8a. Pectoral-fin base without prominent dark markings but sometimes with a few to many scattered melanophores; sides of body with few if any dark markings (with yellow spots in life) except for basicaudal spot . . . . . ***Coryphopterus eidolon***

8b. Pectoral-fin base with prominent markings; sides of body with or without numerous dark markings *9*

9a. Pectoral-fin base with distinct pigment spot *dorsally*, spot usually dark above, diffuse below, often with dots trailing ventrally; ventral dots coalescing into a separate spot in some specimens (ventral spot, if present, less intense than dorsal spot); second dorsal-fin elements 9–10; second dorsal spine filamentous . . . . . ***Coryphopterus thrix***

9b. Pectoral-fin base with prominent dark spot or blotch only on *ventral* portion; second dorsal-fin elements 11; second dorsal spine not filamentous . . . . . ***Coryphopterus punctipectophorus***

10a. Body usually pale, pigment primarily comprising three rows of markings on side of body; lower row comprising small, mostly vertically elongate markings, some of which may be crescent shaped or some part of an X-shape but rarely well-defined X's; if X-shaped markings present, their height is considerably shorter than eye diameter; pigment marking above opercle usually a triangle, and basicaudal pigment usually a central bar . . . . . ***Coryphopterus tortugae***

10b. Body heavily pigmented or pale but without vertically elongate or crescent-shaped markings in ventral row of pigment on side of body; height of X-shaped markings, if present, three-quarters of or equal to diameter of eye; pigment marking above opercle triangular, rounded, or with two peaks; basicaudal pigment comprising two separate spots, two spots connected by a line of pigment and resembling a dumbbell, a central bar, or a C-shaped marking. ***11***

11a. Pigment on pectoral-fin base variable but always with dark spot or rectangular-shaped blotch ventrally (may be associated with bright yellow pigment in life); one or two additional bars, blotches, or concentrations of pigment sometimes present dorsally; three rows of dark markings on side of body, some in lower row large, X-shaped markings in heavily pigmented specimens, small, circular blotches in paler specimens; pigment marking above the opercle triangular or round . . . . . ***Coryphopterus venezuelae***

11b. Pectoral-fin base rarely with prominent dark marking ventrally, although melanophores may form one to three light to moderate concentrations on base; body with three rows of dark markings, most of those in the lower row large, distinctive X-shaped markings; pigment marking above opercle usually with two well-defined peaks . . . . . ***Coryphopterus glaucofraenum***

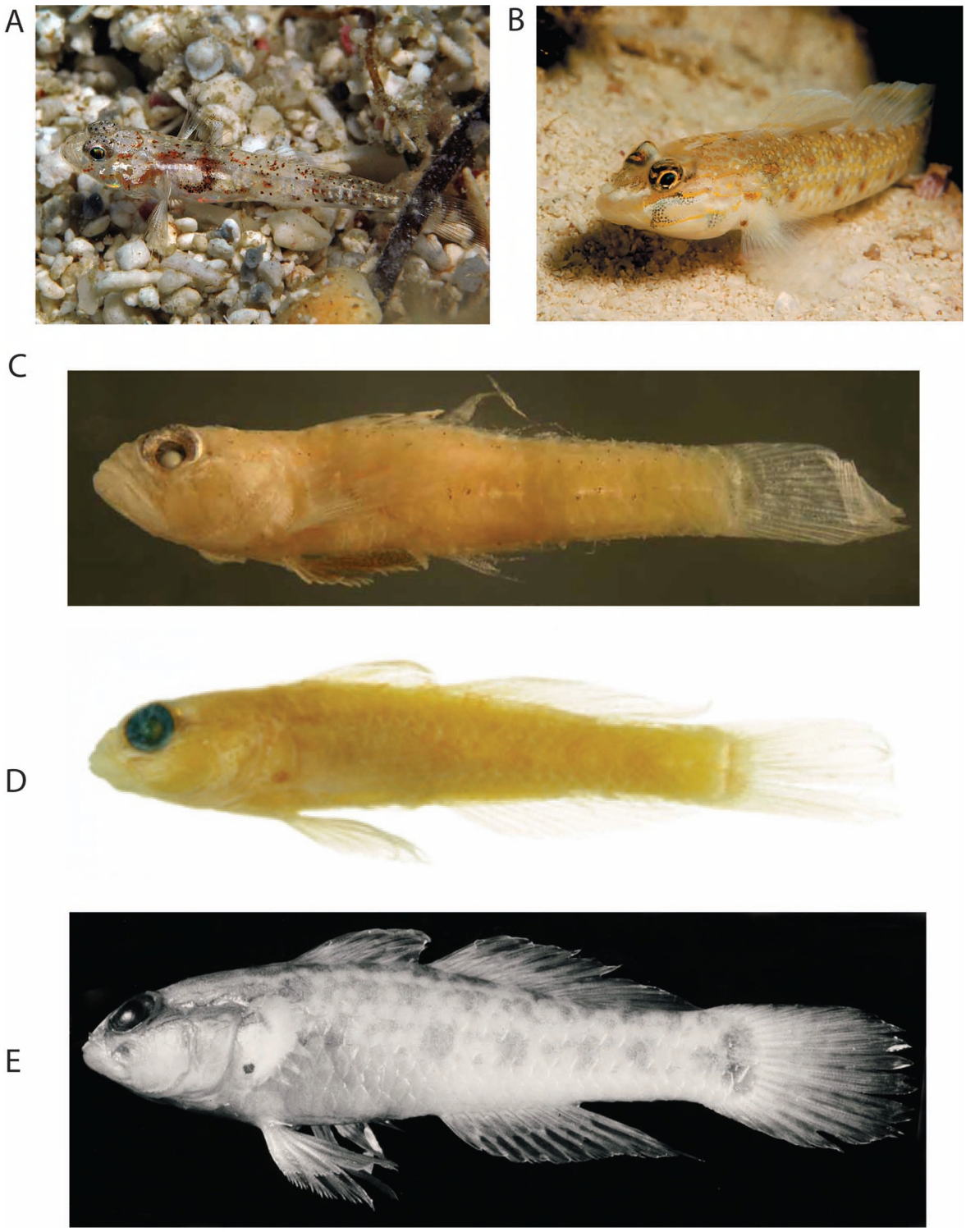


FIGURE 10. A, *Coryphopterus kuna*, San Andres, Colombian Caribbean (photo by Keri Wilk, ReefNet Inc.); B, *Coryphopterus punctipectophorus*, Holbox Island, Mexico (photo by Hilario Itriago); C, *Coryphopterus kuna*, Panama, 17.1 mm SL, holotype, SIO-07-5, preserved, DNA GB EF55021 (reproduced from B. Victor, 2007, fig. 1A, *Zootaxa* 1526:53); D, *Coryphopterus punctipectophorus*, South Carolina, 28 mm SL, USNM 315530, preserved, no DNA; E, *Coryphopterus punctipectophorus*, Florida, Gulf of Mexico, 42 mm SL, holotype, ANSP 90103, preserved, no DNA.

FIGURE 11. *Coryphopterus glaucofraenum*, neotype, USNM 393907, Belize, 44 mm SL, DNA 6367: A, fresh; B, preserved.

Designation of Neotype for *Coryphopterus glaucofraenum*

FIGURE 11

Eschmeyer (2008) noted the need for designating a neotype for *Coryphopterus glaucofraenum* Gill, because the whereabouts of the holotype are unknown. He also noted that four MCZ specimens assumed to be syntypes do not constitute type material because Gill's (1863) description was clearly based on a single specimen. Because of the historical confusion regarding the validity of *C. tortugae* and *C. venezuelae* as distinct from *C. glaucofraenum*, and because the three species can be difficult to separate, we have elected to designate a neotype for *C. glaucofraenum* from which we have successfully obtained a COI sequence that places the specimen in the *C. glaucofraenum* clade. We hereby make the following type designation:

Neotype

Coryphopterus glaucofraenum Gill, USNM 393907, 44 mm SL, DNA 6367, Twin Cays, Belize, mangrove edge on interior channel, 0–6 ft. (GenBank accession no. GQ367355.)

SUMMARY AND FUTURE WORK

Cytochrome *c* oxidase I sequences (DNA barcoding) were useful in determining the number of distinct genetic lineages within Caribbean *Coryphopterus*. We used the neighbor-joining tree (see Figure 1) derived from those sequences to assemble voucher specimens (and color photographs of them taken before preservation) into clades and then compared the morphology of specimens among those clades. Assigning clades to species was relatively easy based

on review of original literature and examination of some type specimens (or photographs of them). Resolving the identities of many Caribbean *Coryphopterus* in the absence of the DNA data would have been extremely difficult.

We are continuing to expand our geographic coverage of *Coryphopterus* sampling and will continue sequencing COI, and ultimately other genes, from specimens from a diversity of locations. The precise geographic distributions of most western Atlantic *Coryphopterus* are not known, and our genetic analyses have revealed the presence of one or more additional cryptic species. Additionally, the existence of two morphological forms within the genetic clade identified as *C. venezuelae* warrants further investigation. Ultimately, our multi-locus data set will enable us to reanalyze phylogenetic relationships among *Coryphopterus* species, from which we can investigate patterns of speciation and morphological divergence. Finally, testing of the species identifications of *Coryphopterus* larvae proposed by Baldwin and Smith (2003) based on morphology is currently in progress based on COI sequences of larvae collected as part of this study.

Acknowledgments

Cody Payne contributed to the organization of our Belizean *Coryphopterus* material, made radiographs and counts of numerous specimens, helped distinguish *C. tortugae* from *C. glaucofraenum*, and provided data helpful in developing the revised species key. James Van Tassell contributed numerous specimens, tissue samples, and photographs of Venezuelan and Panamanian *Coryphopterus* and engaged in many helpful discussions about *Coryphopterus* with the first author. Amy Driskell and Andrea Ormos provided laboratory and logistical assistance. Jon Fong provided images of the holotype of *Ctenogobius tortugae*. Victor Springer, Lisa Palmer, and Hilario Itriago provided images of *Coryphopterus punctipectophorus*. Benjamin Victor provided the photograph of the holotype of *C. kuna*, and Zhi-Qiang Zhang, Chief Editor of *Zootaxa*, allowed us to reproduce this image. Keri Wilk provided the in situ image of *C. kuna*. Annemarie Kramer allowed us to include her sequences of *C. tortugae* from Curacao in our analysis. Oscar M. Lasso-Alcala, Juan C. Capelo, and Ramon Varela provided digital images of two paratypes of *C. venezuelae*. Michael Carpenter, Zachary Foltz, Amy Driskell, and Justin Bagley provided field assistance in Belize and Florida. Research in Florida was conducted pursuant to Special Activity License 07SR-1024. Amos Gazit, Kate Wilson, and Maureen Kunen made it possible for us to collect fish samples through the CARMABI laboratory in Curacao. Fieldwork in the Bahamas was conducted under the auspices of the Perry Institute of Marine Science, with logistical assistance from Brenda Gadd. Fourteen members of the first author's family contributed to the fieldwork in the Bahamas, and a portion of that work was funded by a generous donation from Christine B. Lang in memory of David E. Baldwin and Richard A. Lang. The Smithsonian Marine Science Network provided most of the funding for fieldwork, and the Smithsonian DNA Barcoding Initiative provided funding for molecular analyses. This is contribution number 837 of the Caribbean Coral Reef Ecosystems Program (CCRE), Smithsonian Institution, supported in part by the Hunterdon Oceanographic Research Fund, and Smithsonian Marine Station at Fort Pierce (SMSFP) Contribution No. 756.

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APPENDIX

TABLE A.1. *Coryphopterus* material. A number in the DNA column indicates that the specimen was analyzed for cytochrome *c* oxidase 1. An asterisk beside this number indicates the entry appears in the neighbor-joining tree in Figure 1; because of space constraints, not all specimens for which DNA was successfully sequenced are included in Figure 1. Extracting DNA was not attempted on formalin-fixed specimens. If the specimen was not sampled for DNA, "no DNA" is recorded in this column; BZE, Belize; FLA, Florida; CUR, Curacao; BAH, Bahamas; PAN, Panama; VEN, Venezuela.

Species	DNA	Standard length (mm)	Specimen voucher [a]	Photo voucher at NMNH
C. lipernes	BZE 4067*	—	No voucher	No
	BZE 4082*	23	No voucher	No
	BZE 4083*	21	No voucher	No
	BZE 7729*	18	USNM 394796	Yes
	CUR 8051*	21	USNM 394895	Yes
	CUR 8326*	20	USNM 394896	Yes
	CUR 8327*	17	USNM 394894	Yes
C. hyalinus	BZE 4511*	15	No voucher	No
	BZE 4512*	15	No voucher	No
	BZE 5066*	13	No voucher	Yes
	BZE 6221*	13.5	USNM 394795	Yes
	BZE 6222*	14.5	USNM 394794	Yes
	BZE 7760*	7	No voucher	Yes
	CUR 8044*	20	USNM 394890	Yes
	CUR 8046*	19.5	USNM 394891	Yes
	CUR 8264*	19	USNM 394893	Yes
	CUR 8265*	17	USNM 394889	Yes
	CUR 8266*	16.5	USNM 394892	Yes
C. personatus	BZE 4014*	—	No voucher	No
	BZE 4079*	19	No voucher	Yes
	BZE 4307*	24	USNM 394756	Yes
	BZE 4308*	21	USNM 394757	Yes
	BZE 4309*	18	USNM 394758	Yes
	BZE 5067*	19	USNM 394913	Yes
	BZE 7163*	15	USNM 394742	Yes
	CUR 8045*	19.5	USNM 394897	Yes

Species	DNA	Standard length (mm)	Specimen voucher [a]	Photo voucher at NMNH
	BAH 8263	23	USNM 394904	Yes
	BAH 8264*	22	USNM 394905	Yes
	PAN 7712-1*	22	AMNH 247346	No
	PAN 7712-5*	22	AMNH 247346	No
C. tortugae	BZE 4016*	28	No voucher	Yes
	BZE 4530*	40	USNM 394730	Yes
	BZE 5237*	34	USNM 394743	Yes
	BZE 5238*	30	USNM 394731	Yes
	BZE 7106*	20	USNM 394732	Yes
	BZE 7107*	36	USNM 394733	Yes
	BZE 7333*	25	USNM 394744	Yes
	BZE 7677*	31	USNM 394801	Yes
	BZE 7690	37	USNM 394878	Yes
	BZE 7691*	29	USNM 394802	Yes
	BZE 7692*	36	USNM 394879	Yes
	BZE 7693*	20	USNM 394800	Yes
	BZE 7708*	33	USNM 394877	Yes
	BZE 7709*	29	USNM 394798	Yes
	BZE 7734*	26	USNM 394799	Yes
	BZE (no DNA)	40	USNM 329834	No
	BZE (no DNA)	33	USNM 334838	No
	CUR CG25*	—	No voucher	No
	CUR CG26*	—	No voucher	No
	PAN 7725-6*	36	AMNH 247347	No
	VEN (no DNA)	45	USNM 194103	No
	VEN 7736-1*	33	AMNH 247340	No
	VEN 7736-4*	37	AMNH 247340	No
	VEN 7736-6*	46	AMNH 247340	No
	Bermuda (no DNA)	9 (15–31)	USNM 330023	No
	FLA (no DNA, photo of holotype)	—	SU 08363	No
C. glaucofraenum	BZE 6037*	35	USNM 394347	Yes
	BZE 6367*	44	USNM 393907	Yes
	BZE 7343*	6	No voucher	Yes
	BZE 7351*	35	USNM 394353	Yes
	BZE 7352*	25	USNM 394354	Yes
	BZE 7353*	17.5	USNM 394355	Yes
	BZE 7733*	25	USNM 394748	Yes
	BZE 7768*	22	USNM 394792	Yes
	BZE 7769*	17	USNM 394793	Yes
	BZE 7796*	8.5	No voucher	Yes
	BZE 7798*	8.5	No voucher	Yes
	FLA 7341	49	USNM 394348	Yes
	FLA 7342	42	USNM 394349	Yes
	FLA 7343*	35	USNM 394350	Yes
	FLA 7344	36	USNM 394351	Yes
	FLA 7345	30	USNM 394352	Yes
	FLA 7674	49	USNM 394356	Yes
	FLA 7675	44	USNM 394357	Yes
	FLA 7676	38	USNM 394358	Yes
	FLA 7677	32	USNM 394729	Yes
	PAN 7701-1*	39	AMNH 247334	No
	PAN 7701-2*	40.5	AMNH 247334	No
	PAN 7701-3*	32	AMNH 247334	No
	PAN 7701-4*	26.5	AMNH 247334	No
	PAN 7701-5*	33	AMNH 247334	No
	PAN 7712-2*	35	AMNH 247335	No

continued

TABLE A.1. *continued*

Species	DNA	Standard length (mm)	Specimen voucher [a]	Photo voucher at NMNH
	VEN 7729-1*	31	AMNH 247336	No
	VEN 7729-2*	30	AMNH 247336	No
	VEN 7729-3*	31	AMNH 247336	No
	VEN 7736-2*	37.5	AMNH 247337	No
	VEN 7738-1*	38	AMNH 247338	No
	VEN 7738-2*	36	AMNH 247338	No
	VEN 7738-3*	39	AMNH 247338	No
	VEN 7744-2*	32	AMNH 247339	No
	VEN 7744-3*	27	AMNH 247339	No
	VEN 7744-4*	28.5	AMNH 247339	No
	Bahamas (no DNA)	31	USNM 386863	No
	Bahamas (no DNA)	2 (30–32)	USNM 386955	No
	Bermuda (no DNA)	4 (27–35)	USNM 178908	No
	Bermuda (no DNA)	2 (45–46)	USNM 178555	No
C. venezuelae	BZE 5099*	16	USNM 394735	Yes
	BZE 5319*	8.5	No voucher	Yes
	BZE 7248*	35	USNM 394736	Yes
	BZE 7362*	7.5	No voucher	Yes
	BZE 7704*	20	USNM 394880	Yes
	BZE 7728*	17	USNM 394881	Yes
	BZE 7797*	8.5	No voucher	Yes
	CUR 8052*	30.5	USNM 394737	Yes
	CUR 8053*	30	USNM 394764	Yes
	CUR 8054*	26.5	USNM 39475	Yes
	CUR 8055	28	USNM 394766	Yes
	CUR 8208*	31.5	USNM 394738	Yes
	CUR 8259*	29	USNM 394739	Yes
	CUR 8260*	29	USNM 394740	Yes
	CUR 8427*	35	USNM 394741	Yes
	BAH 8048*	43	USNM 394908	Yes
	BAH 8049*	42	USNM 394906	Yes
	BAH 8262*	39	USNM 394909	Yes
	PAN 7725-1*	42.5	AMNH 247341	No
	PAN 7725-2*	38	AMNH 247341	No
	PAN 7725-3*	33	AMNH 247341	No
	PAN 7725-4*	39	AMNH 247341	No
	PAN 7725-5*	42.5	AMNH 247341	No
	VEN 6670-3*	41	AMNH 247342	No
	VEN 6670-4*	45	AMNH 247342	No
	VEN 7733-1*	29	AMNH 247343	No
	VEN JV07*	20	AMNH 247344	No
	VEN JV08*	29.5	AMNH 247344	No
	VEN JV09*	36	AMNH 247345	No
	VEN JV10*	29	AMNH 247345	No
	VEN JV11*	29	AMNH 247345	No
	VEN JV12*	52	AMNH 247345	No
	VEN JV13*	50	AMNH 247345	No
	VEN JV14*	50	AMNH 247345	No
	VEN JV15*	50	AMNH 247345	No
	VEN JV16*	29	AMNH 247345	No
	VEN (no DNA; photo of paratype)	~42	MOBR-P-0867	No
	Puerto Rico; holotype of *C. bol** (DNA from Victor, 2008)	26.8	SIO 0869	No
	Saba (no DNA)	15	USNM 387726	No

Species	DNA	Standard length (mm)	Specimen voucher [a]	Photo voucher at NMNH
	Brazil	4 (2–39)	USNM 357709	No
C. dicrus	BZE 4213*	22	USNM 394337	Yes
	BZE 5239*	27	USNM 394763	Yes
	BZE 6274*	25	USNM 394774	Yes
	BZE 6110*	13	USNM 394779	Yes
	BZE 7238	29	USNM 294338	Yes
	BZE 7266	24	USNM 294339	Yes
	BZE 7354*	22	USNM 394745	Yes
	BZE 7410	27	USNM 394746	Yes
	BZE 7700*	19	USNM 394778	Yes
	BZE 7701*	17	USNM 394776	Yes
	BZE 7707*	21	USNM 394777	Yes
	BZE 7745*	23	USNM 394780	Yes
	BZE 7818*	22	USNM 394775	Yes
	FLA 7346*	43	USNM 394343	Yes
	FLA 7347*	41	USNM 394344	Yes
	FLA 7348*	38	USNM 394345	Yes
	FLA 7680	39	USNM 394340	Yes
	FLA 7681	42	USNM 394341	Yes
	FLA 7682	44	USNM 394342	Yes
	CUR 8135*	30	USNM 394747	Yes
	BAH 8134*	43	USNM 394900	Yes
	BAH 8135*	38	USNM 394898	Yes
	BAH 8232	36	USNM 394899	Yes
	VEN 7736-3*	35	AMNH 247332	No
	VEN JV01*	33	AMNH 247333	No
	VEN JV02*	35	AMNH 247333	No
	VEN JV03*	36	AMNH 247333	No
	VEN JV04*	20.5	AMNH 247333	No
	VEN JV05*	21	AMNH 247333	No
	VEN JV06*	20	AMNH 247333	No
	Saba (no DNA)	4 (25–28)	USNM 388525	No
	Tobago (no DNA)	35	USNM 318808	No
	Tobago (no DNA)	3 (23–25)	USNM 318818	No
	Dominica (no DNA)	11 (13–27)	USNM 325165	No
C. thrix	BZE 6111*	15	USNM 394797	Yes
	BZE 7265*	10	USNM 394734	Yes
	BZE 7267*	30	USNM 394759	Yes
	BZE 7816*	23	USNM 394914	Yes
	BZE 7817*	22	USNM 394915	Yes
	BZE (no DNA)	3 (20–28.5)	USNM 328240	No
	CUR 8261*	16	USNM 394760	Yes
	CUR 8426*	23	USNM 394761	Yes
	Venezuela (no DNA)	26	AMNH 244983	No
	Navassa (no DNA)	31	USNM 359403	No
	Tobago (no DNA)	32	USNM 318811	No
	Tobago (no DNA)	2 (23–24)	USNM 317133	No
C. eidolon	BZE 4017*	31	USNM 394749	Yes
	BZE 4080*	20	USNM	Yes
	BZE 4081*	29	No voucher	No
	BZE 4089*	–	No voucher	No
	BZE 5070*	33	USNM 394750	Yes
	BZE 5099	16	No voucher	Yes
	BZE 6223*	18	USNM 394788	Yes
	BZE 6224*	24	USNM 394789	Yes
	BZE 6246*	25	USNM 394787	Yes
	BZE 6268*	23.5	USNM 394790	Yes
	BZE 6302*	33	USNM 394751	Yes

continued

TABLE A.1. *continued*

Species	DNA	Standard length (mm)	Specimen voucher [a]	Photo voucher at NMNH
	BZE 7108	21	USNM 394785	Yes
	BZE 7109*	34	USNM 394752	Yes
	BZE 7152	19	USNM 394346	Yes
	BZE 7232*	31	USNM 394762	Yes
	BZE 7350*	36	USNM 394753	Yes
	BZE 7671*	28	USNM 394786	Yes
	BZE 7672	24	USNM 394784	Yes
	BZE 7673*	22	USNM 394781	Yes
	BZE 7702	31	USNM 394783	Yes
	BZE 7703*	26	USNM 394782	Yes
	BZE 7726	24	USNM 394912	Yes
	BZE 7727	17	USNM 394911	Yes
	BZE 7735	23	USNM 394791	Yes
	CUR 8047	37	USNM 394886	Yes
	CUR 8048*	39	USNM 394884	Yes
	CUR 8049	33	USNM 394883	Yes
	CUR 8050*	38	USNM 394885	Yes
	CUR 8262*	24	USNM 394887	Yes
	CUR 8263	33	USNM 394888	Yes
	BAH 8046*	41	USNM 394903	Yes
	BAH 8047*	37	USNM 394902	Yes
	Navassa (no DNA)	3 (32–33)	USNM 360458	No
C. alloides	BZE 7233*	24	USNM 394754	Yes
	BZE 7264*	19	USNM 394755	Yes
	BZE 7761*	12	USNM 394910	Yes
	BZE (no DNA)	21	USNM 267843	No
	CUR 8325*	18	USNM 394882	Yes
C. kuna	BZE 4586*	6	No voucher	No
	BZE 5134*	7.5	No voucher	Yes
	BZE 6049*	7	No voucher	Yes
	BZE 6387*	7.5	No voucher	Yes
	PAN; holotype* DNA from GenBank	17.1	SIO-07-5	No
C. punctipectophorus	FLA; paratype (no DNA)	28	USNM 179307	No
	South Carolina (no DNA)	28	USNM 315530	No

[a] USNM = U.S. National Museum (National Museum of Natural History), Smithsonian Institution; AMNH = American Museum of Natural History; MOBR = Museo Oceanológico Hermano Benigno Román, Venezuela; SIO = Scripps Institution of Oceanography.

Recent Insights into Cnidarian Phylogeny

Allen G. Collins

ABSTRACT. With representatives of more than 10,000 species from diverse clades scattered throughout the world's oceans, Cnidaria is a moderately diverse phylum of Metazoa. As such, various taxa within Cnidaria have been the subjects of recent phylogenetic analyses. Because of its diversity, it has not yet been possible to conduct any extensive phylum-level phylogenetic analyses. In addition, new information suggests that the large group of parasites known as Myxozoa is part of Cnidaria. The present contribution summarizes recent findings to create a picture of a current working hypothesis of cnidarian phylogeny. This summary, which treats the relationships among taxa down to the approximate level of order, likely provides a suboptimal estimation of cnidarian phylogeny as compared to a detailed phylogenetic analysis of data sampled densely from all the Cnidaria component clades. Nevertheless, it should provide points of comparison for upcoming efforts to more comprehensively assess cnidarian phylogeny. Even at the basic level of order, many taxa are thought to be polyphyletic. Understandably, current classifications are not fully reflective of recent phylogenetic advances.

INTRODUCTION

Early in the history of Metazoa, the nematocyst evolved. This capsular organelle encloses venom and a tightly coiled, hollow, dart-like thread that is discharged at incredibly rapid accelerations of up to 5 million *g* (Nüchter et al., 2006). This explosive discharge can be achieved because of extreme osmotic pressures (Holstein and Tardent, 1984; Weber, 1989) within the highly stable nematocyst wall, the molecular structure and function of which are becoming ever clearer (e.g., Meier et al., 2007). Cnida is the more general term for this organelle, the nematocyst being just one type. However, it is reasonably clear, based on the distribution across cnidarian taxa, that the ancestral form of the cnida was as a nematocyst (Marques and Collins, 2004). The lineage in which the nematocyst originated gave rise to the moderately diverse phylum Cnidaria, most likely during the Ediacaran period (Peterson and Butterfield, 2005; Cartwright and Collins, 2007). Since this time, cnidarians have evolved an enormous variety of forms and a great diversity of life history strategies. Representative cnidarians build reefs, fish the depths, and parasitize other species. Extant valid species number a bit more than 11,000 (Daly et al., 2007),

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or more than 13,000 when roughly 2,200 myxozoan species (Lom and Dyková, 2006) are included (see following), and can be found living in all marine environments. Many myxozoans infect freshwater taxa, but just a small number of other cnidarian species live in freshwater (Jankowski et al., 2008).

Daly et al. (2007), in honor of the 300th anniversary of the birth of Linnaeus, recently provided a summary of phylogenetic knowledge about currently recognized cnidarian taxa (exclusive of Myxozoa) typically ranked at ordinal and family levels in current classifications. I aim to provide a summary of recent insights into cnidarian phylogeny focusing on relationships among the different cnidarian orders. No phylum-level analyses of the evolutionary relationships among these taxa have been carried out, although attempts have been made to assess phylogenetic hypotheses for large subclades of Cnidaria; i.e., Anthozoa (Berntson et al., 1999; Won et al., 2001), Hexacorallia (Daly et al., 2003; Brugler and France, 2007), Octocorallia (Bertnson et al., 2001; McFadden et al., 2006); Medusozoa (Collins, 2002; Marques and Collins, 2004; Collins et al., 2006a; Van Iten et al., 2006), and Myxozoa (Kent et al., 2001; Fiala, 2006). In addition, several recent studies have assessed the phylogenetic affinities of taxa that have been problematic (Collins et al., 2006b; Van Iten et al., 2006; Dyková et al., 2007; Jiménez-Guri et al., 2007). The approach taken here is to cobble together results from these various analyses to provide a reasonable picture of our present understanding of cnidarian relationships (Figure 1). Representative cnidarians are illustrated in Figures 2 and 3.

As it concentrates on recent insights, the present paper does not provide a thorough review of the history of ideas about relationships among cnidarian orders, nor does it attempt to summarize what recent phylogenetic results tell us about cnidarian character evolution. For that type of information, one should consult the studies referenced herein. The working hypothesis of cnidarian relationships (see Figure 1), as well as the summary provided by Daly et al. (2007), should provide points of comparison for phylum-wide analyses of cnidarian phylogeny, which will soon be attempted by researchers engaged in the cnidarian tree of life project (http://CnidToL.com). Because it is a representation of a hypothetical history of Cnidaria, every node in Figure 1 is uncertain and is subject to change in light of new information. In a couple of instances, question marks are inserted on the working hypothesis to indicate relationships that are particularly tentative at present.

A WORKING HYPOTHESIS OF CNIDARIAN PHYLOGENY

Cnidaria is one of the earliest diverging clades within Metazoa, and surprisingly its precise position within the early diverging animal lineages—Porifera, Placozoa, Bilateria, Ctenophora, and Cnidaria—has remained elusive (Collins et al., 2005b; Dunn et al., 2008). That said, it has become ever clearer that Cnidaria is more closely related to Bilateria than is Ctenophora, a finding based on a synthetic consideration of morphology (Salvini-Plawen, 1978), later supported by 18S rDNA data (Wainright et al., 1993; Collins, 1998), and most recently confirmed by a large analysis of many sequences of data from expressed gene transcripts (derived from large-scale sequencing of messenger RNA; known as expressed sequence tags, or ESTs) (Dunn et al., 2008; although note that the analyses published therein suggest that Ctenophora is the earliest diverging extant metazoan lineage, which is either a radical new finding or an indication of bias in the results). Ribosomal data, both 18S (e.g., Collins, 1998) and combined 18S and 28S (Medina et al., 2001; Cartwright and Collins, 2007), strongly suggest that Cnidaria forms a clade with Bilateria and the little-known phylum Placozoa, and that Cnidaria may be the sister group of either taxon or both together. More recently, phylogenetic analyses using entire genomes (unfortunately without any representatives of Ctenophora) found Placozoa to be the sister group of a clade composed of Cnidaria plus Bilateria (Srivastava et al., 2008).

Myxozoa is an interesting group of parasites that very well may be part of Cnidaria. Although some early workers suggested that they are cnidarians, based on the similarity between nematocysts and myxozoan polar capsules (Weill, 1938), they were mainly considered as protists throughout the twentieth century. In 1995, an analysis of 18S and morphological data suggesting that myxozoans were derived from within Cnidaria was published (Siddall et al., 1995). However, this conclusion was doubted by many because the 18S gene of myxozoans appears to have evolved very quickly relative to that of most other metazoans, and different analyses involving different sets of taxa came to conflicting conclusions about the precise position of Myxozoa within Metazoa (Smothers et al., 1994; Siddall et al., 1995; Hanelt et al., 1996; Siddall and Whiting, 1999; Kim et al., 1999; Zrzavý and Hypsa, 2003). This uncertainty was claimed to have been resolved when it was discovered that an unusual worm-shaped animal known as *Buddenbrockia* was a myxozoan (Okamura

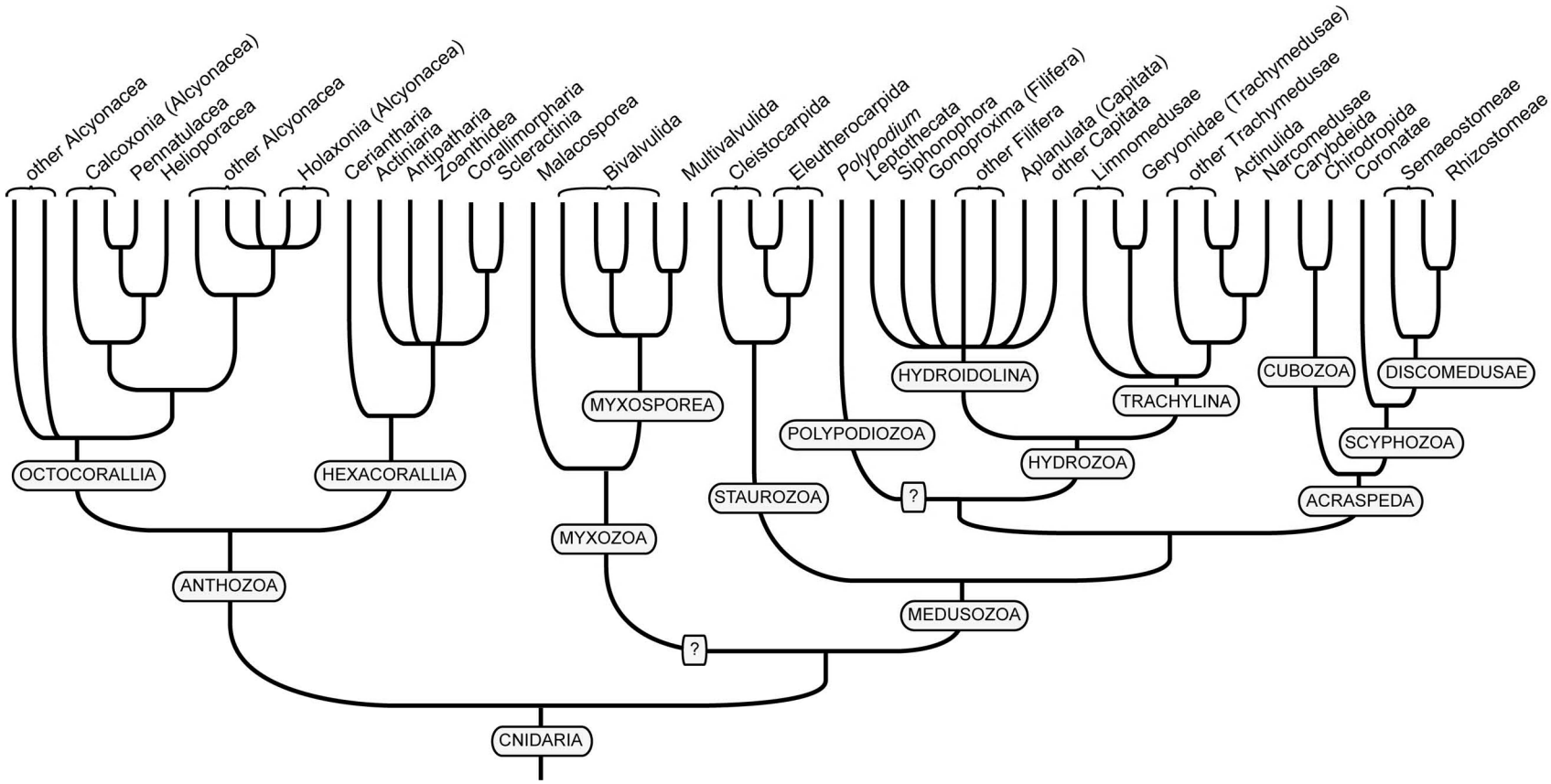

FIGURE 1. Working hypothesis of evolutionary relationships among extant cnidarian taxa usually classified at or near the rank of order.

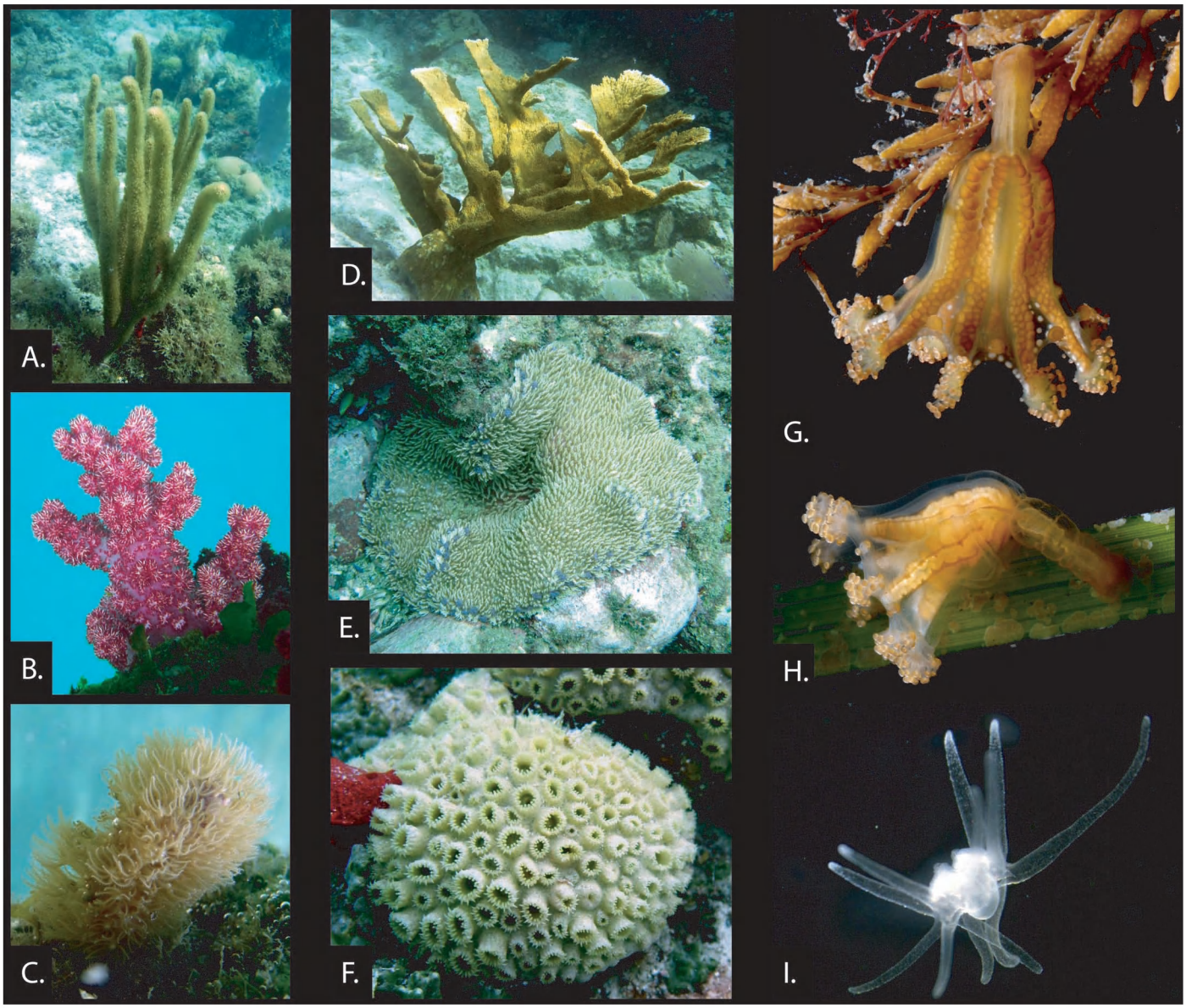

FIGURE 2. Representative cnidarians: Anthozoa, Staurozoa, and Polypodiozoa. A, Octocorallia, Holaxonia, *Plaxaura* from St. John, U.S. Virgin Islands; B, Octocorallia, Alcyonacea (part of the unnamed clade including Holaxonia), *Dendronephthya* from Shirahama, Japan; C, Octocorallia, Alcyonacea, *Briareum* from St. John, U.S. Virgin Islands; D, Hexacorallia, Scleractinia, *Acropora* from St. John, U.S. Virgin Islands; E, Hexacorallia, Actiniaria, *Thalassianthus* from Shirahama, Japan; F, Hexacorallia, Zoanthidea, *Palythoa* from St. John, U.S. Virgin Islands; G, Staurozoa, Eleutherocarpida, *Haliclystus* from Hokkaido, Japan; H, Staurozoa, Cleistocarpida, *Manania* from Hokkaido, Japan. I, Polypodiozoa, *Polypodium* (photographed by N. Evans).

et al., 2002; Okamura and Canning, 2003). Okamura and colleagues showed that the morphology and DNA of *Buddenbrockia* firmly placed it within Myxozoa. However, they also argued that the presence of four muscles located between the endoderm and ectoderm of *Buddenbrockia* indicated that it, and by extension Myxozoa as a whole, was a close relative of nematodes and firmly derived from within Bilateria. Most recently, this hypothesis was falsified by analyses of EST data taken from *Buddenbrockia* and other metazoans indicating that Myxozoa is part of Cnidaria (Jiménez-Guri et al., 2007), as suggested by earlier workers (Weill, 1938; Siddall et al., 1995).

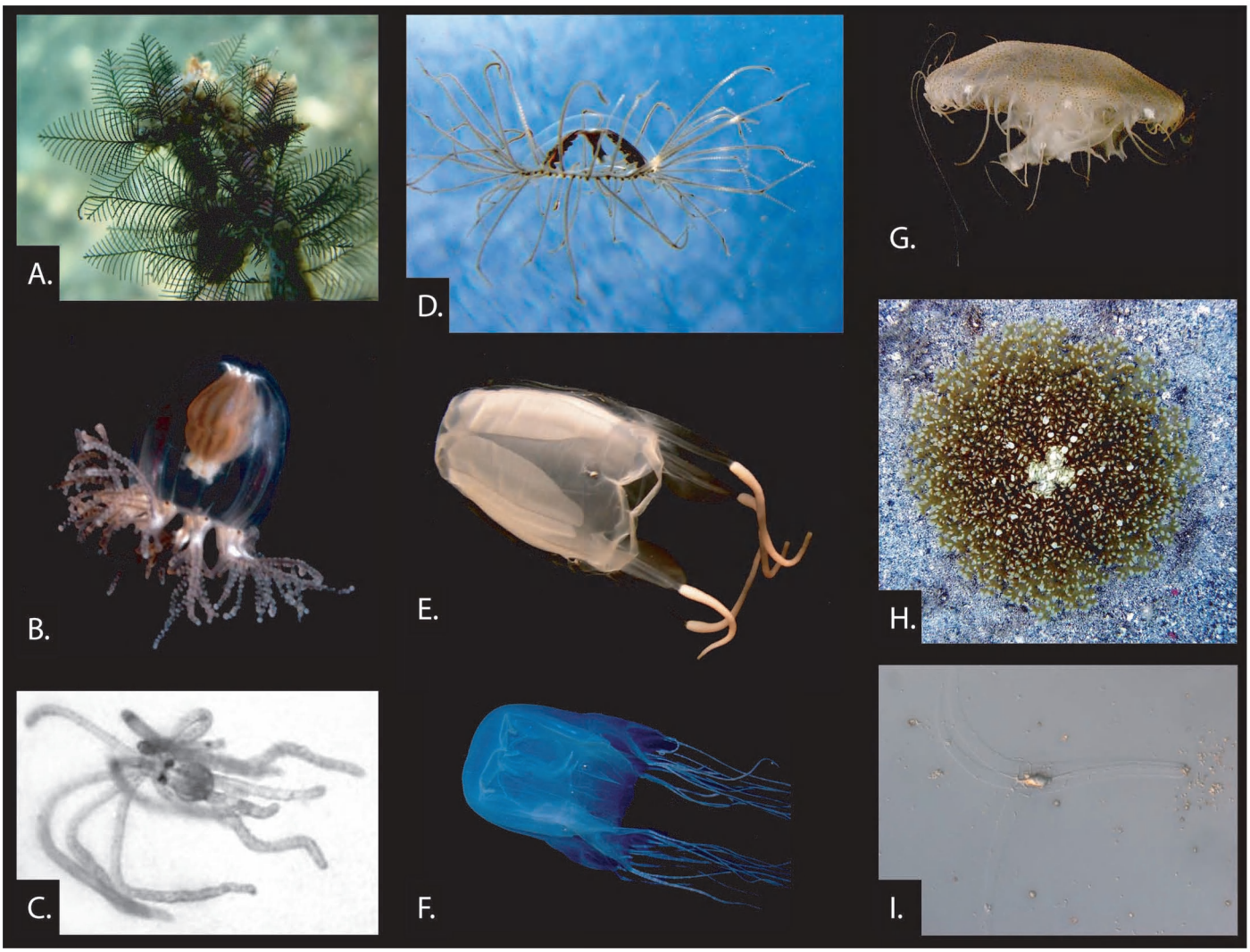

FIGURE 3. Representative cnidarians: Hydrozoa, Cubozoa, Scyphozoa, and Myxozoa. A, Hydroidolina, Leptothecata, *Lytocarpia* from Shirahama, Japan; B, Hydroidolina, other Capitata, *Cladonema* from Hokkaido, Japan; C, Trachylina, Actinulida, *Halammohydra* from Bocas del Toro, Panama (still taken from video by J. Norenburg); D, Trachylina, Limnomedusae, *Gonionemus* from Hokkaido, Japan; E, Cubozoa, Carybdeida, *Carybdea* from Bocas del Toro, Panama; F, Cubozoa, Chirodropida, *Chironex* from Southern Japan (photographed in Enoshima Aquarium); G, Scyphozoa, Semaeostomeae, *Chrysaora* from Bocas del Toro, Panama; H, Scyphozoa, Rhizostomeae, *Cassiopea* from St. John, U.S. Virgin Islands; I, Myxozoa, Myxosporea, Bivalvulida, actinospore stage of *Myxobolus* (photographed by A. Nawrocki and N. Evans).

Not surprisingly, given the type of data analyzed, taxon sampling for the EST analysis was rather limited, with just two anthozoans, two hydrozoans, and one scyphozoan included. The myxozoan was shown to fall as the earliest diverging lineage in a clade including the two hydrozoans and the scyphozoan (Jiménez-Guri et al., 2007). In contrast, even more recent analyses of rDNA data with excellent taxon sampling resulted in best trees in which Myxozoa was the sister group of Bilateria, a result thought to be biased by long-branch attraction (Evans et al., 2008). Figure 1 shows Myxozoa as the sister lineage of Medusozoa, as indicated by the EST results, but the branch also includes a question mark because of the small number of taxa included in the analysis by Jiménez-Guri et al. (2007). Knowing whether myxozoans possess linear or the typical circular mitochondrial genomes could help place Myxozoa within Cnidaria, as this is a major distinction between Anthozoa and Medusozoa (Bridge et al., 1992, 1995; see following).

The inclusion of Myxozoa's 2,200 species (Lom and Dyková, 2006) within Cnidaria increases the richness of

the phylum significantly. As parasites with complex life cycles involving multiple hosts, the diversity of Cnidaria is substantially enriched as well. In recent years, relationships within Myxozoa have mainly been addressed using 18S rDNA data. These data give a strong signal that species parasitic of freshwater bryozoans in the class Malacosporea (=order Malacovalvulida) form a small clade (just three species are known) that is sister to the remaining myxozoans classified in the class Myxosporea (Canning et al., 2000; Kent et al., 2001). Recent classifications of Myxosporea break the class into two orders, Bivalvulida and Multivalvulida. While neither taxon as traditionally recognized is monophyletic, including one aberrant bivalvulidid member in Multivalvulida renders Bivalvulida paraphyletic and Multivalvulida monophyletic (as shown in Figure 1; Kent et al., 2001; Whipps et al., 2004; Fiala, 2006; Lom and Dyková, 2006). The great majority of myxosporeans appear to fall into two large clades, one dominated by species inhabiting freshwater hosts, and the other including Multivalvulida and other species that primarily infect marine hosts (Kent et al., 2001; Holzer et al., 2004). Examples of reversals in freshwater and marine habits continue to accumulate, and a third smaller clade has been identified (Fiala, 2006). Considerable diversity of Myxozoa remains to be sampled, but existing studies indicate that many myxozoan taxa, even genera, are polyphyletic. Continued efforts to identify morphological features reflecting shared ancestry, which could be used to improve the existing classification, are necessary (Fiala, 2006).

In analyses of Cnidaria (exclusive of Myxozoa), it has generally become accepted that Anthozoa is the sister clade of Medusozoa, a hypothesis that is buttressed by morphology (Salvini-Plawen, 1978; Bridge et al., 1995), mitochondrial genome structure (Bridge et al., 1992), and rDNA sequences (e.g., Berntson et al., 1999; Kim et al., 1999; Medina et al., 2001; Won et al., 2001; Collins, 2002). One recent exception is a study by Kayal and Lavrov (2008) based on complete mitochondrial genome sequences, which found Medusozoa (just two representatives) derived from within Anthozoa as the sister group of three sampled representatives of Octocorallia. Although certainly worthy of consideration and future testing, limitations in taxon sampling in the Kayal and Lavrov (2008) analysis cast some doubt on the veracity of this finding. Similar arrangements were also presented in early rDNA analyses that similarly suffered from poor taxon sampling, as shown by pioneering work of Bridge et al. (1995). As indicated in Figure 1, Anthozoa is hypothesized to consist of two well-supported sister clades with diverse representatives, Octocorallia and Hexacorallia. Anthozoa is usually considered to be a class within the phylum (e.g., Daly et al., 2007), but making it a subphylum, with Hexacorallia and Octocorallia as its classes, would go some way toward balancing the classification of Anthozoa with that of Medusozoa.

The phylogeny of Octocorallia has posed some of the most troublesome questions in recent cnidarian systematics because of a relatively dramatic incongruence between traditional taxonomy and molecular-based hypotheses of relationships (Berntson et al., 2001). Nevertheless, consistent progress has been made; many of the alliances first suggested by the rDNA analyses of Berntson et al. (2001) have been confirmed, and some morphological synapomorphies of recently recognized clades have been identified (McFadden et al., 2006). It has been premature, given the great diversity of Octocorallia remaining to be sampled, to erect a new classification for the group, but some patterns are emerging. There appear to be two major clades and a minor clade or grade that branched early in the history of Octocorallia (McFadden et al., 2006). One of the three octocoral orders, Alcyonacea (soft corals and sea fans), which is by far the most diverse and least distinctive, is clearly paraphyletic. The other two orders, Pennatulacea (sea pens) and Helioporacea (blue corals), are monophyletic, and each appears to be independently descended from a paraphyletic Calcoxonia (one group of sea fans), a suborder of Alcyonacea (McFadden et al., 2006). Another group of sea fans known as Holaxonia all appear in one of the major clades, along with other alcyonaceans, but there is no strong evidence for holaxonian monophyly.

In contrast to Octocorallia, the overall picture of the phylogeny of Hexacorallia has been relatively clear. Of the six hexacorallian orders, several lines of evidence indicate that Ceriantharia (tube anemones) is the earliest diverging lineage (Berntson et al., 2001; Won et al., 2001; Daly et al., 2002, 2003; Brugler and France, 2007). Similarly, this same set of studies all concur in finding a close relationship between Scleractinia (stony corals) and Corallimorpharia. However, there has been some confusion about whether Corallimorpharia might be derived from within Scleractinia, that is, one version of the "naked coral hypothesis," which posits that one or more hexacorallian groups without skeletons are derived from stony coral ancestors. Abundant evidence refutes the idea that Actiniaria (true anemones) or Zonanthidea are derived from scleractinian ancestors (Berntson et al., 2001; Won et al., 2001; Daly et al., 2002, 2003; Brugler and France, 2007), but several analyses of mitochondrial genes, including whole mitochondrial genomes, have found corallimorpharians to be derived from within Scleractinia (France et al., 1996; Romano and Cairns, 2000; Medina et al., 2006). In contrast,

however, better taxon sampling of mitochondrial genomes (Brugler and France, 2007) and analyses of other genes with better taxon sampling (Fukami et al., 2008) favor the hypothesis that Corallimorpharia and Scleractinia are monophyletic sister groups. No clear picture of the relationships between this clade, Actiniaria, Antipatharia (black corals), and Zoanthidea has emerged from recent work, as different data sets or analytical approaches have yielded conflicting results (Berntson et al., 2001; Won et al., 2001; Daly et al., 2002, 2003; Brugler and France, 2007).

In present classifications, Medusozoa is usually presented as including four classes: Cubozoa, Hydrozoa, Scyphozoa, and Staurozoa. As mentioned earlier, a recent analysis of EST data suggested that Myxozoa is derived from within Cnidaria, as the sister group of the three medusozoans included in the analysis (Jiménez-Guri et al., 2007). Another taxon, Polypodiozoa, which is sometimes considered a class because of the unusual nature of the parasitic species within its single genus, *Polypodium* (Raikova, 1988), has also recently been hypothesized to fall within Medusozoa, most likely as a close relative of Hydrozoa (Evans et al., 2008). Thus, Medusozoa may have as many as six classes representing rather distinct, evolutionarily independent lineages. Evidence for the monophyly of Medusozoa, albeit with the exclusion of Myxozoa and Polypodoizoa, comes from rDNA data (Collins, 1998, 2002; Medina et al., 2001; Collins et al., 2006a) and observations of morphology (Werner, 1973; Salvini-Plawen, 1978; Schuchert, 1993; Bridge et al., 1995).

Attempts to incorporate data from Myxozoa and Polypodiozoa in analyses of cnidarian phylogeny have been complicated by the relatively rapid rate of molecular evolution in these two taxa. In many analyses of rDNA, representatives of these two groups appear to be artificially attracted to bilaterian examplars and end up forming sister group relationships with Bilateria (Siddall et al., 1995; Kim et al., 1999; Zrzavý and Hypsa, 2003). In a recent study of 18S and 28S data, dense taxon sampling of medusozoans appears to have overcome some of this long-branch attraction problem, at least so far as Polypodiozoa is concerned (Evans et al., 2008). Although the optimal trees of Evans et al. (2008) had Myxozoa branching as the sister group of Bilateria, perhaps because the 28S marker was only partially sampled for the myxozoans, Polypodiozoa consistently fell within Medusozoa, as one would expect based on its morphology (Raikova, 1980, 1994). Unfortunately, however, the exact position of Polypodiozoa within Medusozoa was shown to be dependent upon method of analysis and the inclusion or exclusion of myxozoan representatives (Evans et al., 2008), prompting the question mark shown in Figure 1.

Among the taxa more traditionally considered as part of Medusozoa, Staurozoa (or Stauromedusae) may possibly be the earliest diverging lineage, a result obtained through the analysis of both molecular and morphological data (Collins, 2002; Dawson, 2004; Collins and Daly, 2005; Collins et al., 2006a; Van Iten et al., 2006). As benthic, so-called stalked medusae, the finding that Staurozoa might branch early in the history of Medusozoa was of some interest because it very clearly implied that the pelagic medusa stage was a feature derived within this clade. However, Collins et al. (2006a) noted that some methodological choices in their phylogenetic analyses impacted the position of Staurozoa. Further, although not specifically addressed, the position of Staurozoa was not stable in the analyses of Evans et al. (2008). Thus far, no strong evidence has been published suggesting that Staurozoa is not an early diverging lineage of Medusozoa. Within Staurozoa, there are two main taxa, Cleistocarpida and Eleutherocarpida, neither of which appears to be monophyletic despite the fact that taxon sampling was relatively limited (Collins and Daly, 2005).

Another small class of Medusozoa is Cubozoa (box jellyfishes). Although 18S data provide no clear signal about the precise position of Cubozoa within Cnidaria (Collins, 2002; Evans et al., 2008), 28S data strongly suggest that Cubozoa is the sister group of Scyphozoa (true jellyfishes), together forming the clade Acraspeda (Collins et al., 2006a; Evans et al., 2008). Both 18S and 28S strongly support cubozoan monophyly, as well as that of its two main subtaxa, Carydeida and Chirodropida (Collins, 2002; Collins et al., 2006a). Similarly, there is relatively strong and stable evidence concerning the evolutionary relationships among the scyphozoan orders Coronatae, Rhizostomeae, and Semaeostomeae, although it should be noted that taxon sampling has been sparse. The earliest diverging lineage is Coronatae, and Rhizostomeae is a well-supported clade that is derived from within Semaeostomeae (Collins, 2002; Collins et al., 2006a).

The largest and most diverse class within Medusozoa is Hydrozoa. As indicated in Figure 1, an ancient divergence within Hydrozoa divides the group into two clades, Trachylina and Hydroidolina (Collins, 2002; Marques and Collins, 2004; Collins et al., 2006a). Each clade has been the subject of recent papers aimed at bringing increased taxon and genetic marker sampling to bear on the evolutionary relationships among their respective component groups (Cartwright et al., 2008; Collins et al., 2008). As Figure 1 shows, relationships among the major lineages of

Hydroidolina are uncertain (Collins et al., 2006a; Cartwright et al., 2008). In terms of taxonomy, the clade includes Leptothecata (thecate hydroids and leptomedusae) and Siphonophora (colonial siphonophores including the Portuguese man o' war), two groups with ample evidence for monophyly (Collins, 2002; Collins et al., 2006a; Cartwright et al., 2008). Hydroidolina also includes the large and diverse taxon Anthoathecata (athecate hydroids and anthomedusae), which is typically subdivided into Capitata and Filifera. There is no evidence supporting the monophyly of Capitata, Filfera, or Anthoathecata (Collins, 2002; Collins et al., 2006a; Cartwright et al., 2008). Despite the difficulty in working out the relationships among hydroidolinan clades, some advances have been made in identifying large clades that had not been previously recognized. For instance, Capitata appears to be composed of two well-supported clades, one dubbed Aplanulata (includes the well-known model organisms of *Hydra*) in reference to the group's lack of a ciliated planula stage (Collins et al., 2005a, 2006a) and the other consisting of all the other capitate groups (Cartwright et al., 2008). The name Capitata has recently been applied to this more restrictive clade (Cartwright et al., 2008). Similarly, within Filifera, a previously unrecognized alliance of species that bear gonophores, but not on their hydranth bodies, has been given the name Gonoproxima. There is no support for the monophyly of the remaining filiferans.

Trachylina is composed of four orders: Actinulida, Limnomedusae, Narcomedusae, and Trachymedusae. The monophyly of Narcomedusae seems to be relatively certain (Collins, 2002; Collins et al., 2006a, 2006b, 2008), whereas the monophyly of Actinulida has yet to be tested because just a single representative has been included in any phylogenetic analysis (Collins et al., 2008). Trachymedusae, a group of pelagic species that lack polyp stages, appears to be polyphyletic. One family (Geryonidae) has a close relationship with a subgroup of Limnomedusae (Collins et al., 2006a, 2008), whereas another (Rhopalonematidae) may have given rise to the interstitial Actinulida (Collins et al., 2008). Limnomedusae appears to represent a grade at the base of Trachylina (Collins, 2002; Collins et al., 2006a, 2006b, 2008). As with many cnidarian groups, the classification of Trachylina requires refinement to better reflect our phylogenetic knowledge.

CONCLUSION AND CLASSIFICATION

The working hypothesis of cnidarian phylogeny presented here (see Figure 1), as do all others, requires continued testing and refinement. Many of the studies behind it have limitations, especially in taxon sampling, and the original papers should be consulted for more detailed assessments of strengths and weaknesses of the analyses that they report. As the working hypothesis results from no single analysis and was instead put together from numerous sources, some of my biases, in the form of judgments, have had an impact on the final form of the working hypothesis. This effect is certainly a weakness in such an exercise and demonstrates why it is less preferable than an analysis that relies on data sampled from diverse representatives across Cnidaria. When such an analysis is conducted, the working hypothesis presented here may provide a helpful reference point for comparison.

Figure 1 makes it clear that the current classification of Cnidaria, even at the basic level of order, has not kept up with phylogenetic advances. A new classification using taxa hypothesized to be monophyletic is not feasible until more thorough and robust phylogenetic analyses are conducted. Conflicting results from different phylogenetic studies create one hindrance to advances in classification, but this is not really new, as different taxonomists have always offered different classifications to reflect their changing perceptions of taxa. More detrimental to progress in classification is the lack of completeness in existing phylogenetic analyses. With molecular data, individuals are sampled, and assessments of the phyletic status (monophyletic, paraphyletic, or polyphyletic) of larger taxa are not very strong until large numbers of component species are included in an analysis. Moreover, the relevant morphological features that distinguish any particular clade (especially if not corresponding to a traditional taxon) are not easily discerned without thorough sampling and examination of its members.

Nevertheless, classifications are made to enhance communication. Therefore, it may be prudent to attempt classifications that better reflect ongoing advances in phylogenetics. Below I present one such attempt for Cnidaria. It is not meant to be adopted, as this author has little expertise in non-medusozoan cnidarians. Instead, it is presented to illustrate one possible system for classifying traditional taxa in light of ongoing phylogenetic advances. It relies on annotation indicating whether a given taxon is likely to be monophyletic, paraphyletic, or polyphyletic. Taxa for which reasonable evidence suggests monophyly are followed by a superscript M. Taxa thought to be paraphyletic are followed by superscript P and a list of taxa [in brackets] hypothesized to be derived from it. Taxa that are likely polyphyletic are placed in quotation marks. Finally,

taxa whose phyletic status is essentially unknown are left with no annotation.

Phylum Cnidaria[M]
- Subphylum Anthozoa[M]
  - Class Hexacorallia[M]
    - Order Actiniaria[M]
    - Order Antipatharia[M]
    - Order Ceriantharia[M]
    - Order Corallimorpharia[M]
    - Order Scleractinia[M]
    - Order Zoanthida[M]
  - Class Octocorallia[M]
    - Oder "Alcyonacea"[P] [Calcoxonia, Helioporacea, Holaxonia, Pennatulacea]
    - Order Calcoxonia[P] [Helioporacea, Penatulacea]
    - Order Helioporacea[M]
    - Order Holaxonia
    - Order Pennatulacea[M]
- Subphylum Medusozoa[M]
  - Class Cubozoa[M]
    - Order Carybdeida[M]
    - Order Chirodropida[M]
  - Class Hydrozoa[M]
    - Subclass Hydroidolina[M]
      - Order Aplanulata[M]
      - Order Capitata[M] (excluding Aplanulata)
      - Order Filifera (excluding Gonoproxima)
      - Order Gonoproxima[M]
      - Order Leptothecata[M]
      - Order Siphonophora[M]
    - Subclass Trachylina[M]
      - Order Actinulida
      - Order Limnomedusae (including Geryonidae)
      - Order Narcomedusae[M]
      - Order Trachymedusae[P] [Actinulida, Narcomedusae]
  - Class Polypodiozoa[M]
    - Genus *Polypodium*
  - Class Scyphozoa[M]
    - Order Coronatae[M]
    - Subclass Discomedusae[M]
      - Order Rhizostomeae[M]
      - Order Semaeostomeae[P] [Rhizostomeae]
  - Class Staurozoa[M]
    - Order "Cleistocarpida"
    - Order "Eleutherocarpida"
- Subphylum Myxozoa[M]
  - Class Malacosporea[M]
    - Order Malacovalvulida[M]
  - Class Myxosporea[M]
    - Order Bivalvulida[P], [Multivalvulida]
    - Order Multivalvulida[M]

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Biodiversity and Abundance of Sponges in Caribbean Mangrove: Indicators of Environmental Quality

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ABSTRACT. We have long been fascinated by the lush biological diversity seen on subtidal substrates in Caribbean saltwater mangroves. Several groups of plants and sessile invertebrates flourish on the submerged prop roots of red mangrove (*Rhizophora mangle*), competing for space and tolerating a stressful range of ecological variables (temperature, salinity, nutrients, sedimentation) that is quite different from the more stable climate on nearby coral reefs. To test the limits of tolerance, we monitored populations of these organisms, the abundant sponges in particular, at environmentally and geographically dissimilar locations in Panama and Belize. We used relative abundance estimates and frequency counts of major ecologically functional groups and common sponge species to establish baselines, and we repeated our surveys over long time spans (months to years) to find correlations between community and environmental changes. Both study locations demonstrated environmental quality decline during the time of observation, mainly through mangrove clear-cutting, followed by increase of suspended fine sediments from dredging reef sands and filling in intertidal land, and elevation of nutrient levels from terrestrial inputs. Although our methods are still in a stage of refinement, our data are leading the way to responsible monitoring of our most precious coastal resources in the tropics. We find that photosynthetic organisms (cyanobacteria, algae) and filter-feeding invertebrates (sponges, ascidians, bivalves, bryozoans) count among the "canaries in the coal mine" as effective indicators of environmental change.

INTRODUCTION

Red mangrove trees, *Rhizophora mangle*, grow along thousands of kilometers of Caribbean shorelines, protecting them from storm erosion and offering habitat to many organisms (Rützler and Feller, 1988, 1996; de Lacerda et al., 2002). Caribbean mangroves harbor from a handful to more than 100 sponge species at any one particular site (Table 1). Available data indicate that sponges may make up 10% to 70% of epiphytic species diversity on submerged mangrove roots. The best studied mangrove sponge faunas are described from islands off southern Belize, with species richness reported between 50 and 147 species (Rützler et al., 2000; Wulff, 2000; Diaz et al., 2004), followed by faunas from a few islands in the Bocas del Toro Archipelago, Panama, with 65 species (Diaz, 2005), from various mainland and island sites off Venezuela with 62 species (Sutherland, 1980; Diaz et al. 1985; Orihuela et al., 1991; Pauls,

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TABLE 1. Number of species of Porifera and other epifaunal taxa reported from Caribbean mangroves (n.a. = no data available).

Country	Locality	Porifera	Other taxa	Author
Antilles	Guadalupe, Trinidad, Puerto Rico	4–10	32–70	Toffart (1983)
Bahamas	Bimini	13	n.a.	Rützler (1969)
Belize	Four cays	24	59	Farnsworth and Ellison (1996)
	Twin Cays	54	n.a.	Rützler et al. (2000)
	Pelican Cays	147	217	See Macintyre and Rützler (2000)
Cuba	n.a.	48	n.a.	Alcolado (unpublished data)
Panama	Bocas del Toro	60	n.a.	Diaz (2005)
USA	Indian River, Florida	3	25	Bingham and Young (1995)
Venezuela	Buche Bay	16	32	Sutherland (1980)
	Morrocoy National Park	23	n.a.	Diaz et al. (1985)
	Turiamo Bay	10	n.a.	Pauls (2003)
	Cienaga Bay	26	n.a.	Pauls (1998)
	La Restinga National Park	18	35	Orihuela et al. (1991)
	La Restinga National Park	40	n.a.	Diaz et al. (2003)

1998, 2003; Ramirez, 2002; Diaz et al., 2003; Pérez, 2007), and various mangrove sites in Cuba, with 41 species (Alcolado, unpublished data). Other reports are from Colombia, with 26 species (Zea, 1987; S. Zea, National University of Colombia, personal communication, 2006); Jamaica, with 18 species (Hechtel, 1965; Lehnert and van Soest, 1998); and Trinidad and Guadalupe, with 6 species (Toffart, 1983) (clearly representing only a portion of the mangrove sponge diversity there).

Most of the mangrove systems in the Caribbean remain unexplored, leaving a large void in biodiversity information. Most studies just cited show that the more closely these communities are investigated, the more new species are being discovered. An example is the research by the Caribbean Coral Reef Ecosystems Program in Belize during the past 25 years (Rützler et al., 2000, 2004). In particular, specialists on certain sponge taxa discovered and described numerous species in the families Suberitidae (order Hadromerida) (Rützler and Smith, 1993), Chalinidae (order Haplosclerida) (de Weerdt et al., 1991) and Mycalidae (order Poecilosclerida) (Hadju and Rützler, 1998). A recent revision of Caribbean *Lissodendoryx* allowed the reinterpretation of *L. isodyctyalis* (Carter, 1882) and seven other species, four of them new to science (Rützler et al., 2007). Similarly, two unique haplosclerids were found in Belizean and Panamanian mangroves: a thin, erect, and fragile undescribed species of *Haliclona* from Twin Cays, and *Xestospongia bocatorensis*, a thin crust occurring in Bocas del Toro mangroves and reefs. Both are in an endosymbiotic relationship with filamentous Cyanobacteria, a very unusual occurrence in this order of sponges (Diaz et al., 2007, Thacker at al., 2007).

Besides the importance of sponges species richness, they may be one of the most abundant animal groups in mangrove habitats. In Belize, for instance, on the leeward sides of islands, sponges cover 10% to 50% of the root surfaces, followed in importance by sea anemones, ascidians, and algae (Farnsworth and Ellison, 1996; Diaz et al., 2004). In the Caribbean, epibiont mangrove communities have been interpreted as highly heterogeneous (Rützler, 1969; Sutherland, 1980; Alcolado, 1985; Alvarez, 1989; Calder, 1991a; Bingham, 1992; Diaz et al., 2004) as a result of low recruitment rates (Zea, 1993; Maldonado and Young, 1996), low and fragmented available space (Jackson and Buss, 1975; Sutherland, 1980), and stochastic processes in the long term (Bingham and Young, 1995; Ellison et al., 1996). Abundance and distribution for sponges and algae in these communities have been related to environmental factors, such as light intensity, tides, wave impact, air exposure, and sedimentation (Rützler, 1995), and to biological factors, such as larval supply (Farnsworth and Ellison, 1996), root abundance, competition, and predation (Calder, 1991b; Littler et al., 1985; Taylor et al., 1986; Ellison and Farnsworth, 1992; Rützler, 1995; Rützler et al., 2000; Wulff, 2000). Algae abound on the shallow, well-lit parts of stilt roots, and their abundance and species composition are highly susceptible to the presence of grazers. Sponges are most abundant on the lower subtidal portions of the stilt roots and dominate peat bank walls and undercuts.

The major physical and biological processes are modulated by competitive abilities, such as growth rates and chemical defenses against predation (Wulff, 2000, 2004, 2005; Engel and Pawlik, 2005). Short-term epibiont abundances are likely to be determined by interspecific competitive interactions and predation, while long-term abundances are limited by seasonal environmental changes, such as freshwater inputs during periods of rain, strong tidal currents, waves, and stochastic processes that make these communities unstable (Bingham and Young, 1995; Ellison et al., 1996). Despite important generalizations about mangrove benthos ecology, we lack understanding of the temporal or spatial variation within most epibiont groups and knowledge about species occurrence, abundance, dominance, and interactions. For example, we do not know which species are generally abundant in these communities, how the hierarchy changes with the year's seasons, and if there are predictable succession patterns. Our current lack of knowledge prevents us from discerning between natural variations, for instance, seasonal or yearly dynamics, and artificial disturbances caused by humans.

The present work pursues the overall goal of a better understanding of diversity, biogeography, and ecological dynamics and their causes among the sponges in Caribbean mangroves. It encompasses two major aspects: evaluation of our current knowledge of epiphytic sponge taxa and the contribution of new data on causes for species richness, distribution, abundance, and dynamics, particularly from the examples of mangrove in Panama and Belize. The survey carried out in Bocas del Toro (Panama) intends to follow short-term changes (over one year) in the epiphytic fauna of mangrove roots, whereas the study in Belize will clarify shifts in distribution of taxa over a longer period (four years).

METHODS

Sponge Species Distribution in Caribbean Mangroves

The distribution of species in Caribbean mangroves was determined from currently published data or unpublished data provided to the authors. Faunas from different regions were compared by using a cluster analysis with the Bray–Curtis dissimilarity coefficient, which is part of the Multivariate Statistical Package (MVSP 3.1) (van Soest, 1993).

Sponge Identification

Specimens were identified in situ or, when necessary, briefly characterized and photographed, with a sample preserved in ethanol. In the laboratory, routine microscope preparations were made by cleaning spicules in household bleach and hand-cutting perpendicular and tangential sections, which were dehydrated and mounted in Permount and examined under the light microscope.

Physicochemical Variables

Temperature and salinity were measured at 0 and 50 cm depth at the Belize sites (January and August 2004), and in Bocas del Toro (February, June, and September 2004). Sedimentation rates were estimated from accumulations in buried sediment traps (plastic pipes, 10 cm diameter, 50 cm length) left in place for 210 days in Belize and 150 days in Panama. The trapped sediment was oven-dried (50°C), and its composition was determined as percentage of mud (including the very fine clay fraction) (<0.002–0.05 mm) and sand (0.05–2 mm). Approximate values of calcium carbonate content were determined from weight loss after exposure to changes of dilute hydrochloric acid, and sediment deposition rates were calculated (g/m^2/day). Seawater samples (500 mL each) were taken in Belize (September 2003) and at Bocas del Toro (September 2004) at low and high tide, filtered (0.2 μm, GF/F filter) and frozen for nutrient analysis (Astor, 1996). Nutrient values were determined by spectrophotometric technique using the procedure described earlier (Diaz and Ward, 1997). Qualitative observations about habitat types surrounding the mangrove fringe were recorded, as well as an estimate of the level of human disturbance.

Survey sites, Bocas del Toro, Panama

Four sites within a perimeter of 10 km were selected and surveyed during 2004 (Figures 1, 2): (1) STRI Point: location of the Smithsonian Tropical Research Institute's laboratory, several mangrove stands close to reef patches in the southwest of Colon Island, and near a well-developed area with a housing complex that is part of the station; (2) Solarte In: a protected lagoon in the east of Solarte Island, site of a modest housing development; (3) Solarte Out: a pristine mangrove island close to reef patches to the west side of Solarte Island; and (4) Big Bight: a pristine, mangrove-lined lagoon surrounded by a well-developed terrestrial forest on Colon Island, less than 5 km northwest of STRI Point. General physicochemical characterization and geographic location of the sites are presented in Table 2 and the nutrient regime in Table 3.

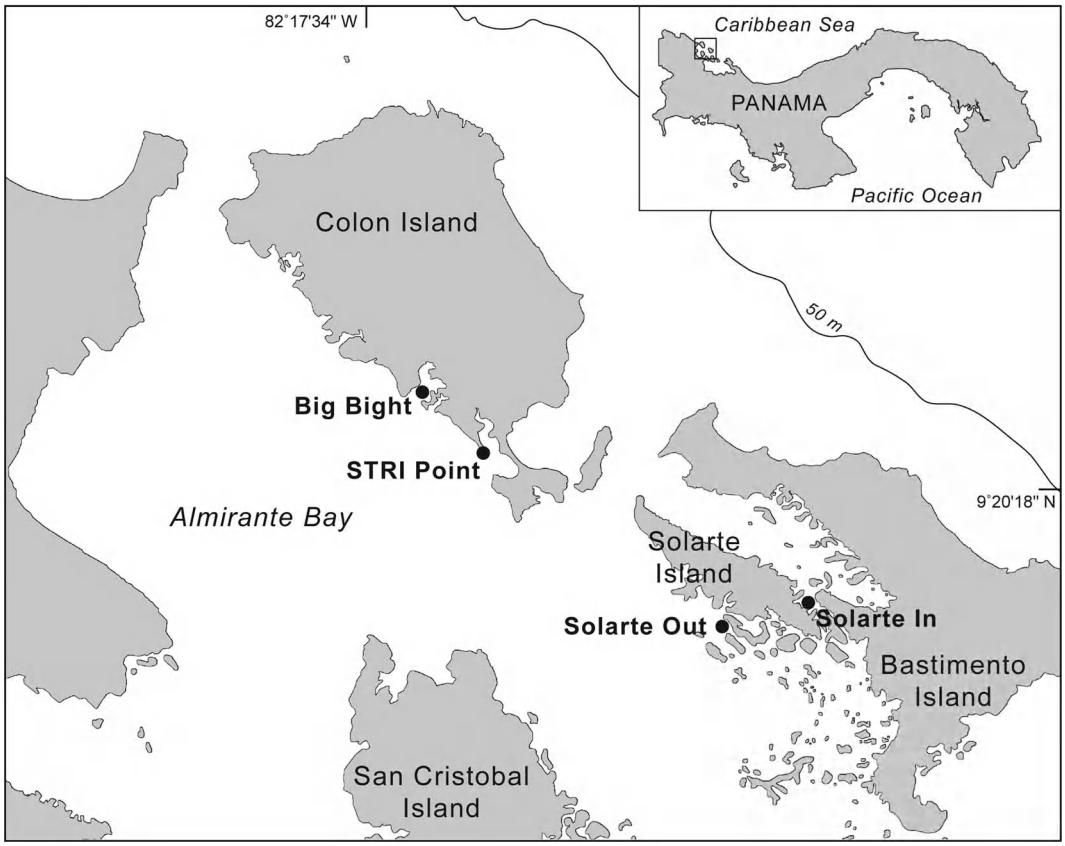

FIGURE 1. Map of research area at Bocas del Toro, Panama (STRI = Smithsonian Tropical Research Institute).

Mangrove prop roots (25 per site) were haphazardly selected within a 30 m length of the mangrove fringe. The front side (facing the channel) of each selected root was photographed along its entire length and set to scale by tying a transparent measuring tape to the high-tide water mark. The tape was prevented from floating by attaching a metal weight to its lower end. Three to seven photographs were taken, depending on the root length. Roots were rephotographed four times during the year (February 2004, June 2004, September 2004, and February 2005). From these images, abundance values of epifauna were estimated by measuring the projected area of each species using the CPCe program. The area (cm^2) covered by each taxon was divided by the root length (m), so that the relative abundance values are related to a measure of available substrate. Cover of each taxon is reported as the sum of its abundances (cm^2/m root) on all roots at a particular site. Eight categories of epiphytes were distinguished: cyanobacterial mats (monospecific stands of cyanobacteria), green algae, red algae (including both crustose calcareous and fleshy species), turf (mixture of densely packed red, green, and cyanobacterial filaments), sponges, hydroids, bivalves, and ascidians; the ninth category was "empty" (spaces not occupied by macrofauna or macroalgae). When small organisms were found overgrowing a large one (such as *Spongia tubulifera*, *Hyrtios proteus*) the projected area of both species was included. The number of roots finally analyzed per studied site was reduced to 14–22 because there was some accidental loss of photographic data.

Survey Sites, Twin Cays and Pelican Cays, Belize

Three sites at Twin Cays and one in the Pelican Cays were surveyed in August 2003 and four years later in August 2007 (Figures 3, 4; see Tables 2, 3). Two of the Twin Cays sites, the Lair Channel and Hidden Creek, are deep creeks that branch off the Main Channel; Sponge Haven

FIGURE 2. Views of research locations at Bocas del Toro, Panama. Top row from left: STRI Point looking south, where the transect was located in the right foreground; Solarte Island, with transect location Solarte In near the center. Bottom row from left: mangrove fringe at Solarte In; underwater view of mangrove prop roots showing a specimen of sponge, *Chalinula molitba*; mangrove roots covered by the encrusting sponge *Halisarca* sp. (undescribed; note scale in centimeters [cm] to the left), along with bivalves, algae, bryozoans, ascidians, and other fouling invertebrates.

is a bay in the southwest of the Main Channel. The Pelican Cays site was in the northern part of the lagoon of Manatee Cay. Transects (30 m long) were placed along the red mangrove fringe at each site, with number of roots ranging between 52 and 143. In all, the presence of the six most conspicuous epiphyte categories was recorded on each root within each transect: cyanobacterial mats, macroalgae, sponges, sea anemones (*Aiptasia pallida*), bivalves, and ascidians.

RESULTS

Caribbean Mangrove Sponge Species Richness and Distribution

The distribution of 177 sponge species currently reported from Caribbean mangroves is presented in Table 4. A cluster analysis (Figure 5) of the best studied sites (Belize, Cuba, Panama, Venezuela) shows the highest similarity between Venezuela (62 species) and Panama (65 species).

TABLE 2. Characterization of study sites in Panama and Belize.

Country and locality	Habitat [a] (depth, m)	Human impact [b]	Temperature range (°C)	Salinity range (ppm)	Sedimentation			Turbidity [b]	Coordinates
					Type	$CaCO_3$ (% dry wt)	Rate (g/m²/day)		
Panama									
STRI Point	PR (1.5–2)	+	26–29	29–34	Mud	14–23	34–41	±/+	09°21′29.1″N, 82°16′28.9″W
Solarte In	SG (2–2.5)	−/±	26–29	27–32	Sand	4–24	28–58	−	09°17′05.0″N, 82°10′03.3″W
Solarte Out	PR (1)	−	27–29	29–33	Sand	80–98	88–248	−	09°17′35.6″N, 82°12′08.3″W
Big Bight	SG (1.5–2)	−/±	27–29	27–34	Mud/ sand	25	40	−/±	09°22′31.1″N, 82°17′38.3″W
Belize									
Sponge Haven	SG (1–1.8)	±	26–32	33–35	Mud	48.75	25	±	16°49′40.5″N, 88°06′16.5″W
Hidden Creek	TC (2–2.5)	±/+	25.5–33	32–36	n.a.	n.a.	n.a.	±	16°49′40.5″N, 88°06′16.5″W
Lair Channel	TC (1.5–1.8)	−	25.3–33	32–36	Mud	25.9	44	−	16°49′33.7″N, 88°06′11.6″W
Manatee Lagoon	PR/SG (1–2)	− [c]/+ [d]	25.5–32	35–36	Mud	38.9	45	−	16°40′03.3″N, 88°11′32.4″W

[a] Habitat abbreviations: PR = mangrove prop roots; SG = seagrass (Thalassia); TC = tidal creek with peat walls and undercuts.
[b] Human impact and turbidity designations: +, high; ±, medium; −, low.
[c] Survey of 2003.
[d] Survey of 2007.

TABLE 3. Ranges of nutrient concentrations (low tide to high tide) at the study sites: Panama samples taken in September 2004 and Belize samples taken in September 2003.

Country and locality	Phosphate (μmol/L)	Ammonium (μmol/L)	Nitrate (μmol/L)
Panama			
STRI Point	0–0.048	1.32–0.988	0.26–0.253
Solarte In	0.02–0.85	0.845–0.88	0.264–0.23
Solarte Out	0.024–0.048	0.096–0.071	0.345–0.276
Big Bight	0.048–0.048	1.55–1.100	0.345–0.230
Belize			
Sponge Heaven	0.528–0.624	4.79–1.88	0.5–0.41
Hidden Creek	0.336–0.786	3.19–2.35	1.1–0.39
Lair Channel	0.384–0.672	2.72–1.59	1.06–1.24
Manatee Lagoon [a]	0.576	1.41	0.32

[a] Only one sample taken, at intermediate tide.

These faunas were paired with Twin Cays (54 species) and Cuba (48 species). The most dissimilar fauna in the analyses resulted from comparison with the Pelican Cays mangroves (147 species).

Mangrove Surveys at Bocas del Toro, Panama

Changes in Abundance of Major Epifaunal Taxa

The relative abundance of major taxa at each of the four localities studied between February 2004 and February 2005 is shown in Figure 6. In terms of the hierarchy of major taxa, sponges were first or second in abundance on mangrove roots at all sites, followed by algal turfs. An exception to this pattern was found in Solarte In (see Figure 1), where large mats of green algae, mostly *Caulerpa verticillata* and *Halimeda* spp., dominated over the sponges in February 2004 and 2005. Bivalves were the third most abundant group, followed closely by unoccupied (empty) spaces.

The abundance of the two most dominant groups, sponges and algae/cyanobacteria, showed a considerable decrease at STRI Point and Solarte In by the end of the

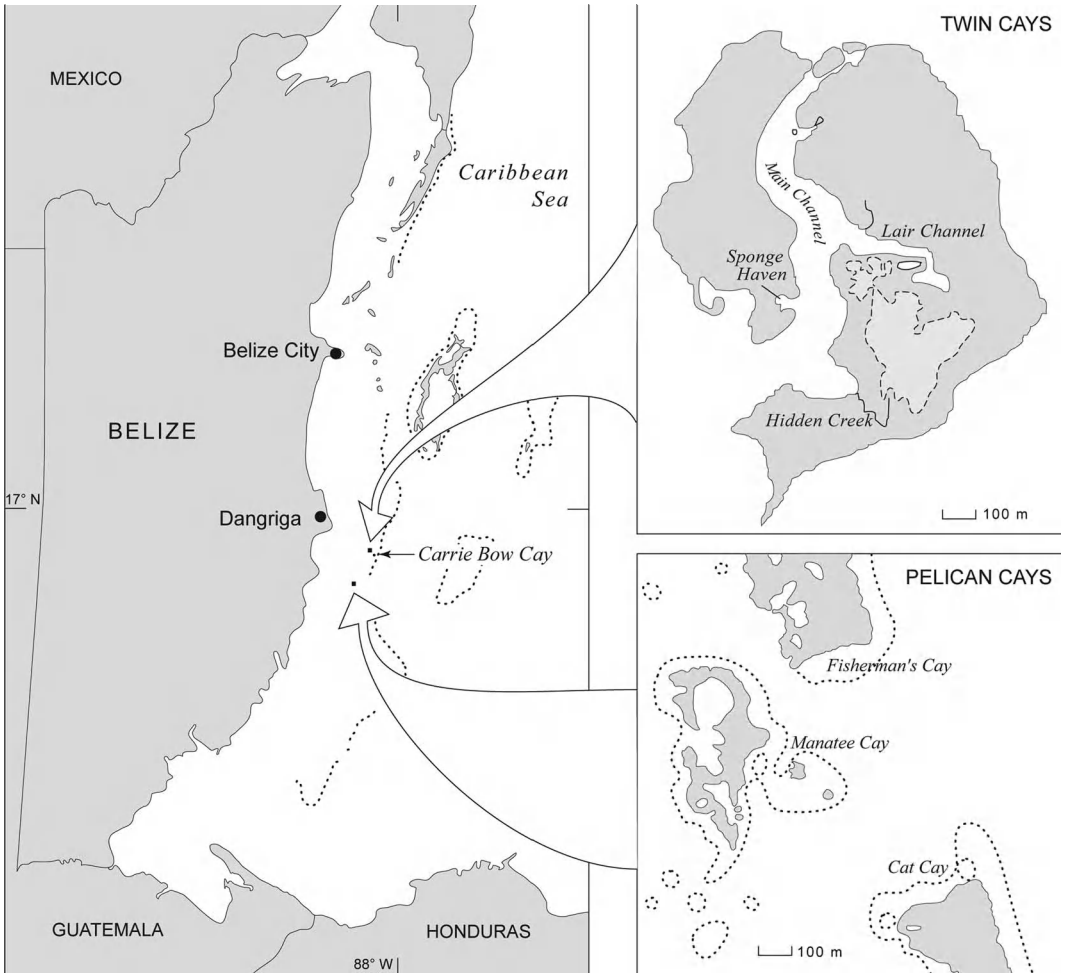

FIGURE 3. Map of research areas at Twin Cays and Pelican Cays, Belize.

study (February 2005), whereas abundance of both groups increased or stayed at similar levels at the other two sites.

Sponge Species Abundances per Site

Because of the high level of heterogeneity in sponge composition and dominance among sites, the relative abundance of the most conspicuous epiphytic sponge species is presented separately, by sites.

STRI Point

Sixteen of a total of 23 species found at this site comprise 99% of total sponge abundance. Most of them (13 species) belong to the order Haplosclerida, specifically the family Chalinidae, and to the order Poecilosclerida. *Tedania ignis* was the most abundant, followed by *Clathria schoenus*, *Spongia tubulifera*, *Mycale microsigmatosa*, *Chalinula molitba*, *Haliclona manglaris*, and *H. tubifera*. Figure 7a shows the relative abundance of the six most common species at this site, which added up to 87% of the total sponge abundance. It is interesting to note that the presence of both *T. ignis* and *Clathria schoenus* had decreased considerably by February 2005, whereas *S. tubulifera* remained with similar abundance throughout the year. *Chalinula molitba* shows a considerable increase (>200%) for June 2004 and a decrease to its initial values by February 2005.

Solarte In

Eight of 14 species found at this site constituted 99% of the total sponge abundance. Figure 7b demonstrates

FIGURE 4. (*facing page*) Views of research locations at Twin Cays and Pelican Cays, Belize. Top left, aerial view of Twin Cays looking south, where transect locations were in the Lair channel (branching from the Main Channel center toward the left), in Sponge Haven (the small bay at the top right), and Hidden Creek (a narrow, deep tidal channel hidden by mangrove canopy, connecting the Main Channel in the far right background with Hidden Lake in the center background); top right, aerial view of Manatee Cay where a transect was placed in the large lagoon to the left (Cat Cay is in the background); middle left, mangrove fringe at Sponge Haven; middle right, red mangrove prop roots hanging free near the Pelican Cays site and covered mainly by the ropy sponge *Iotrochota birotulata*; bottom left, close-up of *Tedania ignis* and *Tedania* sp. (probably *T. klausi* Wulff, a species described after this survey was made), both red, attached to exposed roots in the main channel of Twin Cays; bottom right, close-up of purple ascidian (*Clavelina puertosecensis*) with sponges (turquoise *Haliclona curacaoensis*, primarily) on root at Manatee Cay lagoon.

TABLE 4. Distribution of sponge species reported from Caribbean mangrove localities by various researchers (X = presence). Localities are abbreviated as follows: BEL, Belize; TC, Twin Cays; PC, Pelican Cays; PAN, Panama; COL, Colombia; VEN, Venezuela; TRI, Trinidad; GUA, Guadalupe; JAM, Jamaica; and CUB, Cuba. Data sources are given in table footnotes.

	BEL [b]								
Species [a]	TC	PC	PAN [c]	COL [d]	VEN [e]	TRI [f]	GUA [f]	JAM [g]	CUB [h]
Plakina jamaicensis	—	X	—	—	—	—	—	—	—
Plakinastrella onkodes	—	X	—	—	—	—	—	—	—
Plakortis halichondriodes	X	X	—	—	—	—	—	—	—
Plakortis angulospiculatus	—	—	X	—	—	—	—	—	X
Oscarella sp. 1 (purple)	X	X	X	—	—	—	—	—	—
Oscarella sp. 2 (drab)	—	X	X	—	—	—	—	—	—
Cinachyrella apion	X	X	X	—	X	—	—	—	—
Ecionemia dominicana	—	X	—	—	—	—	—	—	—
Myriastra kallitetilla	X	—	—	—	—	—	—	X	X
Erylus formosus	—	X	—	—	—	—	—	—	—
Geodia gibberosa	—	X	—	—	X	—	X	X	X
Geodia papyracea	X	X	X	—	X	—	—	X	—
Dercitus sp.	—	X	—	—	—	—	—	—	—
Chondrilla caribensis	X	X	X	—	X	—	—	—	X
Chondrosia collectrix	—	X	X	—	—	—	—	—	X
Cervicornia cuspidifera	—	X	—	—	—	—	—	—	—
Cliona caribbaea	—	X	—	—	—	—	—	—	—
Cliona raphida	—	—	—	—	X	—	—	—	—
Cliona varians	X	X	—	—	—	—	—	—	X
Cliona sp.	—	X	—	—	—	—	—	—	—
Placospongia intermedia	—	X	X	—	X	—	—	—	—
Diplastrella megastellata	—	X	—	—	—	—	—	—	—
Spirastrella coccinea	—	X	—	—	—	—	—	—	—
Spirastrella hartmani	—	X	—	—	—	—	—	—	—
Spirastrella mollis	X	X	X	—	—	—	—	—	—
Aaptos duchassaingi	—	X	—	—	—	—	—	—	—
Aaptos lithophaga	—	—	—	—	—	—	—	—	X
Terpios fugax	—	X	—	—	—	—	—	—	X
Terpios manglaris	X	X	X	—	X	—	—	—	—
Prosuberites laughlini	—	—	X	—	X	—	—	X	—
Suberites aurantiaca	—	X	—	—	X	—	—	X	—
Tethya actinia	X	X	X	—	X	—	—	—	X
Tethya aff. *seychellensis*	—	—	X	—	X	—	—	X	—
Discodermia dissoluta	—	—	X	—	—	—	—	—	—
Paratimea ? sp.	X	—	—	—	—	—	—	—	—
Timea unistellata	—	X	—	—	—	—	—	—	—
Agela conifera	—	X	—	—	—	—	—	—	—

continued

TABLE 4. *continued*

Species [a]	BEL [b]		PAN [c]	COL [d]	VEN [e]	TRI [f]	GUA [f]	JAM [g]	CUB [h]
	TC	PC							
Phorbas amaranthus	—	X	—	—	—	—	—	—	—
Coelosphaera raphidifera	—	X	—	—	—	—	—	—	—
Lissodendoryx colombiensis	—	X	X	—	—	—	—	—	—
Lissodendoryx isodictyalis	X	X	X	—	X	—	X	—	X
Lissodendoryx sigmata	X	—	—	—	—	—	—	—	—
Monanchora arbuscula	—	X	—	—	X	—	—	—	—
Desmapsamma anchorata	—	X	X	—	X	—	—	—	—
Biemna caribea	X	—	X	—	X	—	—	—	X
Desmacella janiae	—	X	—	—	—	—	—	—	—
Desmacella meliorata	—	X	—	—	X	—	—	—	—
Neofibularia nolitangere	—	X	—	—	—	—	—	—	—
Hymedesmia sp.	—	X	—	—	—	—	—	—	—
Acarnus sp.	—	X	—	—	—	—	—	—	—
Artemisina melana	—	X	X	—	X	—	—	—	—
Clathria affinis	—	X	—	—	—	—	—	—	—
Clathria cf. *ferrea*	—	—	X	—	X	—	—	—	—
Clathria microchela	—	X	—	—	X	—	—	—	—
Clathria schoenus	X	—	X	—	X	—	—	—	X
Clathria aff. *schoenus*	X	—	—	—	—	—	—	—	—
Clathria spinosa	—	X	—	—	—	—	—	—	—
Clathria venosa	X	X	X	—	X	—	—	—	—
Clathria virgultosa	X	—	—	—	—	—	—	—	—
Mycale cf. *americana*	—	—	—	—	X	—	—	—	—
Mycale angulosa	—	—	—	—	X	—	—	—	—
Mycale arenaria	—	—	—	—	—	—	—	—	—
Mycale arndti	—	X	—	—	—	—	—	—	—
Mycale carmigropila	X	X	X	—	X	—	—	—	—
Mycale citrina	X	—	—	—	X	—	—	—	—
Mycale escarlatei	—	—	—	—	—	—	—		—
Mycale laevis	X	—	—	—	—	—	—	X	—
Mycale laxissima	X	X	—	X	X	—	—		—
Mycale magniraphidifera	X	X	X	—	X	—	—		X
Mycale aff. *magniraphidifera*	X	X	X	—	—	—	—		—
Mycale microsigmatosa	X	X	X	X	X	X	—	X	X
Mycale aff. *microsigmatosa*	—	X	—	—	—	—	—	—	—
Mycale paresperella	—	X	X	—	—	—	—	—	—
Iotrochota birotulata	—	X	X	—	X	—	—	—	—
Strongylacidon sp.	—	X	—	—	—	—	—	—	—
Ectyoplasia ferox	—	X	—	—	X	—	—	—	—
Eurypon laughlini	—	X	X	—	X	—	—	—	—
Tedania ignis	X	X	X	—	X	—	—	X	X
Tedania aff. *ignis*	—	X	—	—	—	—	—	—	—
Dragmacidon reticulata	—	X	—	—	—	—	—	—	—
Pseudaxinella ? sp.	—	X	—	—	—	—	—	—	—
Ptilocaulis walpersi	—	X	—	—	—	—	—	—	—
Dictyonella sp.	X	X	—	—	—	—	—	—	—
Scopalina hispida	—	X	—	—	X	—	—	X	X
Scopalina ruetzleri	X	X	X	X	X	—	—	—	X
Scopalina ? sp.	—	X	—	—	—	—	—	—	—
Ulosa funicularis	—	X	—	—	—	—	—	—	—
Amorphinopsis sp. 1	—	X	—	—	X	—	—	—	—
Amorphinopsis sp. 2	—	X	—	—	—	—	—	—	—
Ciocalypta ? sp.	—	X	—	—	—	—	—	—	—
Halichondria corrugata	—	—	—	—	—	—	—	—	X
Halichondria magniconulosa ?	X	X	X	—	X	X	X	—	X
Halichondria melanadocia	X	X	X	X	X	—	—	X	X
Halichondria poa ?	X	X	—	—	—	—	—	—	—

Species [a]	BEL [b]		PAN [c]	COL [d]	VEN [e]	TRI [f]	GUA [f]	JAM [g]	CUB [h]
	TC	PC							
Hymeniacidon caerulea	—	X	—	—	—	—	—	—	—
Myrmekioderma rea	—	X	—	—	—	—	—	—	—
Topsentia ophiraphidites	—	X	—	—	—	—	—	—	—
Callyspongia arcesiosa	—	—	—	—	X	—	—	—	—
Callyspongia fallax	—	X	X	—	—	—	—	—	X
Callyspongia pallida	—	X	X	—	—	—	—	—	—
Callyspongia vaginalis	—	X	—	—	—	—	—	—	—
Haliclona caerulea	—	X	X	—	X	—	X	X	—
Haliclona curacaoensis	X	X	X	—	X	—	—	—	X
Haliclona aff. *curacaoensis*	—	X	—	—	—	—	—	—	—
Haliclona implexiformis	X	X	X	X	X	—	—	—	X
Haliclona aff. *implexiformis*	—	X	—	—	—	—	—	—	X
Haliclona magnifica	X	X	X	—	X	—	—	—	—
Haliclona manglaris	X	X	X	—	X	—	—	—	X
Haliclona mucifibrosa	X	X	X	—	—	—	—	—	—
Haliclona picadaerensis	X	X	X	—	X	—	—	—	—
Haliclona tubifera	X	X	X	X	X	—	—	—	X
Haliclona aff. *tubifera*	—	X	—	—	—	—	—	—	—
Haliclona twincayensis	X	X	X	—	X	—	—	—	—
Haliclona vermeuleni	X	X	X	—	—	—	—	—	—
Chalinula molitba	X	X	X	—	X	—	—	X	X
Chalinula zeae	—	—	X	—	—	—	—	—	—
Amphimedon compressa	—	X	—	—	X	—	—	X	—
Amphimedon erina	X	X	—	X	X	—	—	—	—
Amphimedon aff. *erina*	—	X	—	—	—	—	—	—	—
Amphimedon viridis	—	—	—	—	—	—	—	—	X
Niphates caicedoi	—	X	X	—	—	—	—	—	—
Niphates digitalis	—	X	—	—	—	—	—	—	—
Niphates erecta	—	X	X	—	X	—	—	—	—
Niphates sp.	—	X	—	—	—	—	—	—	—
Petrosia pellasarca	—	X	—	—	—	—	—	—	—
Petrosia weinbergi	—	X	—	—	—	—	—	—	—
Strongylophora davilai	—	X	—	—	—	—	—	—	—
Xestospongia carbonaria	—	X	—	—	—	—	—	—	—
Xestospongia muta	—	X	—	—	—	—	—	—	—
Xestospongia proxima	—	X	—	—	—	—	—	—	—
Xestospongia subtriangularis	—	X	—	—	X	—	—	—	—
Aka coralliphaga	—	X	—	—	—	—	—	—	—
Aka siphona	—	X	—	—	—	—	—	—	—
Aka sp.	—	X	—	—	—	—	—	—	—
Calyx podatypa	X	X	X	—	—	—	—	—	—
Oceanapia nodosa	—	—	X	—	X	—	—	—	—
Oceanapia oleracea	—	—	X	—	—	—	—	—	—
Cacospongia sp.	—	X	—	—	—	—	—	—	X
Fasciospongia? sp.	—	X	—	—	—	—	—	—	—
Hyrtios proteus	X	X	X	—	X	—	—	—	X
Hyrtios sp.	X	X	—	—	—	—	—	—	—
Smenospongia aurea	—	X	—	—	—	—	—	—	—
Ircinia campana	—	X	X	—	—	—	—	—	—
Ircinia felix	X	X	X	X	X	—	—	X	X
Ircinia strobilina	X	X	—	—	X	—	—	—	X
Spongia pertusa	X	X	X	—	X	—	X	—	X
Spongia tubulifera	X	X	X	X	X	—	—	—	X
Dysidea etheria	X	X	X	X	X	—	X	—	X
Dysidea fragilis	—	—	—	—	—	—	—	X	—
Dysidea janiae	—	X	—	—	—	—	—	—	X
Aplysilla glacialis	X	X	X	—	X	—	—	—	—
Chelonaplysilla aff. *erecta*	—	X	X	X	X	—	—	—	X
Darwinella rosacea	—	X	—	—	X	—	—	X	X

continued

TABLE 4. *continued*

Species [a]	BEL [b] TC	BEL [b] PC	PAN [c]	COL [d]	VEN [e]	TRI [f]	GUA [f]	JAM [g]	CUB [h]
Halisarca caerulea	—	X	—	—	—	—	—	—	—
Halisarca sp. (white)	X	X	X	—	X	—	—	—	—
Aiolochroia crassa	—	X	—	—	—	—	—	—	—
Aplysina archeri	—	X	—	—	—	—	—	—	—
Aplysina fistularis	—	X	—	—	X	—	—	—	X
Aplysina insularis	—	X	—	—	—	—	—	—	—
Aplysina fulva	—	X	—	—	—	—	—	—	X
Aplysina lacunosa	—	X	—	—	—	—	—	—	—
Verongula rigida	—	X	—	—	—	—	—	—	—
Clathrina primordialis	X	X	—	—	—	—	—	—	X
Sycon sp.	X	—	X	—	X	—	—	—	—
Leucandra aspera	—	—	X	—	X	—	—	—	—

[a] Species are listed in taxonomic order according to class, order, and family.
[b] Rützler et al., 2000.
[c] Diaz, 2005; Lehnert and van Soest, 1998.
[d] Zea, 1987; unpublished data.
[e] Sutherland, 1980; Diaz et al., 1985; Orihuela et al., 1991; Pauls, 1998, 2003; Ramirez, 2002; Diaz et al., 2003; Perez, 2007.
[f] Toffart, 1983.
[g] Hechtel, 1965.
[h] Alcolado, unpublished data.

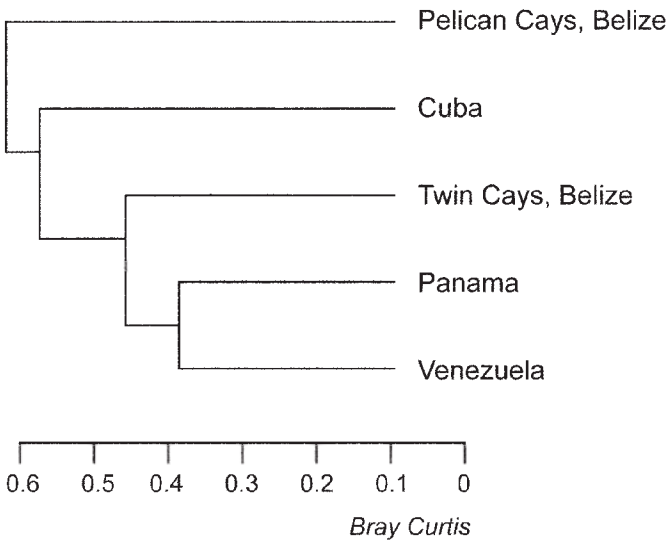

FIGURE 5. Similarities of mangrove sponge faunas from Belize, Panama, Venezuela, and Cuba. The dendrogram is built from a binary matrix (presence or absence) of species distribution using an unweighted pair-group method with arithmetic mean (UPGMA) clustal analysis program, with the Bray–Curtis distance index.

the relative abundance of the six most common species, which comprised 96% of all sponges. *Tedania ignis* and *Mycale microsigmatosa* were among the top species; *Halisarca* sp. (a species so far undescribed) and *Mycale carmigropila* appeared to be among the major components. Similar to STRI Point, most of the dominant species decreased in abundance or disappeared by the end of the study, while *Halisarca* remained steady in abundance throughout the study period. Three of the common species at this site (*Dysidea etheria*, *Haliclona curacaoensis*, and *Mycale carmigropila*) show an increase of sponge growth in the warmer periods (either June or September 2004), followed by a decrease in size during cooler periods (February 2005).

Solarte Out

Six of nine species found at this site made up 99% of total sponge abundance (Figure 7c). *Tedania ignis* continues to dominate, followed by three species not seen in the previously discussed sites: *Spirastrella mollis*, *Haliclona vermeuleni*, and *H. caerulea*. It is notable that the (projected) area coverage of the dominant species is much lower here than that at the other sites (most values are less than 500 cm^2/m).

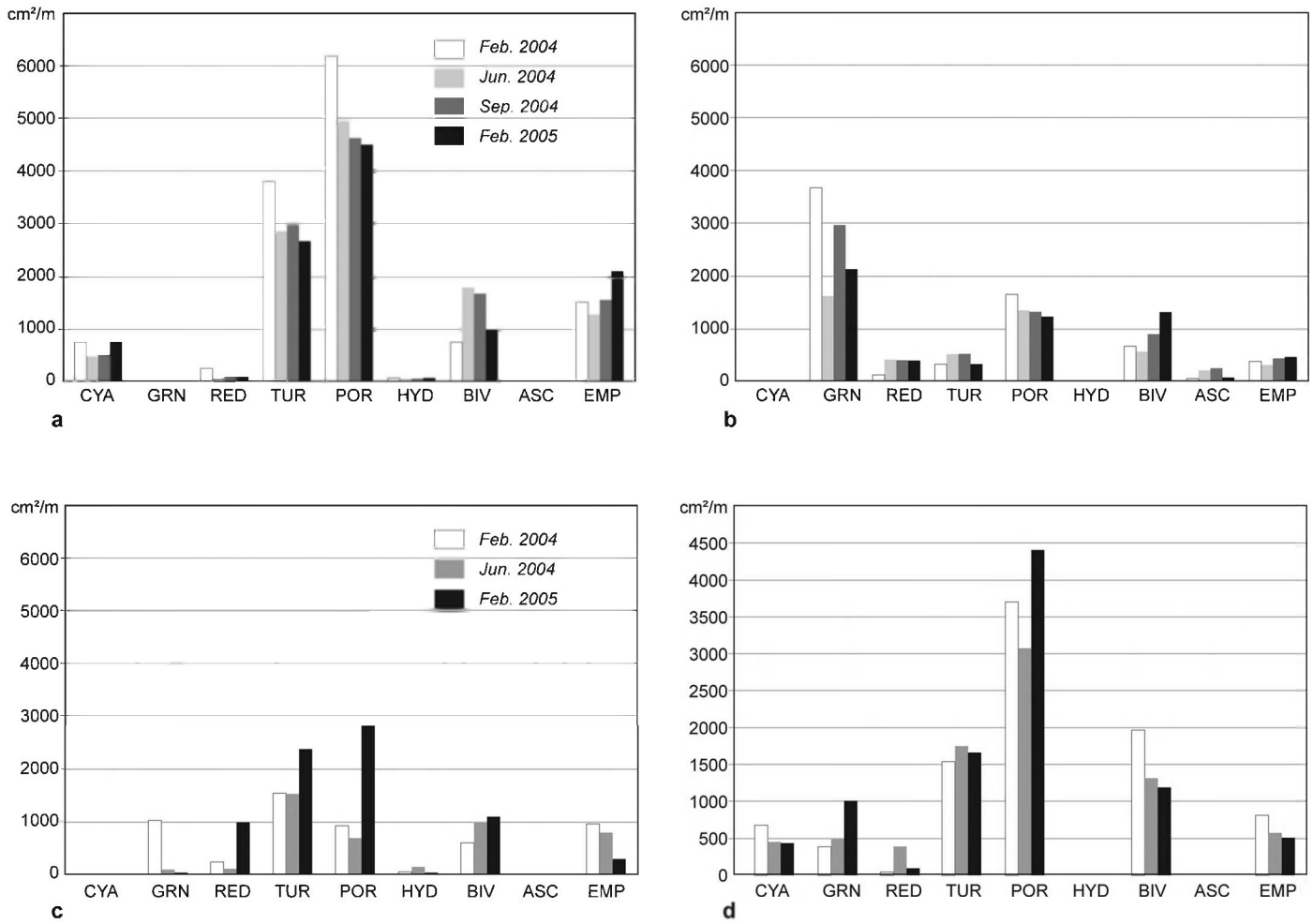

FIGURE 6. Relative abundance of major functional groups growing on mangrove roots (expressed as projected area [cm²] per length [m] of root) at four Bocas del Toro sites, between February 2004 and 2005: a, STRI Point; b, Solarte In; c, Solarte Out; d, Big Bight. (ASC = ascidians; BIV = bivalves; CYA = Cyanobacteria; EMP = empty space; GRN = green algae; HYD = hydroids; POR = sponges [Porifera]; RED = red algae; TUR = algal–cyanobacterial turf.)

Big Bight

Twelve of 17 species found at Big Bight comprised 99% of the total sponge abundance; 6 of these amounted to 90% (Figure 7d). The most abundant species—*Tedania ignis*, *Mycale microsigmatosa*, and *Haliclona manglaris*—increased in size throughout the year, whereas *Lissodendoryx colombiensis* and *Dysidea etheria* gained in size up to September 2004 but disappeared altogether in February 2005. It is worth pointing out the large values for area coverage, as compared to the other locations. The September 2004 data from this site were accidentally lost.

Sponge Species Ranks

The most common sponges at each site amount to 21 species, from a total of 40 distinguished in the studied areas. Abundance ranks from each site are listed in Table 5. Only one species, *Tedania ignis*, maintained the same rank at all sites, as the most abundant species. The second and third most abundant species were *Clathria schoenus* and *Spongia tubulifera* at STRI Point, *Mycale microsigmatosa* and *Halisarca* sp. at Solarte In, *Spirastrella mollis* and *Haliclona manglaris* at Solarte Out, and *M. microsigmatosa* and *H. manglaris* in Big Bight. Seven of these 21 common sponges were only found at one site.

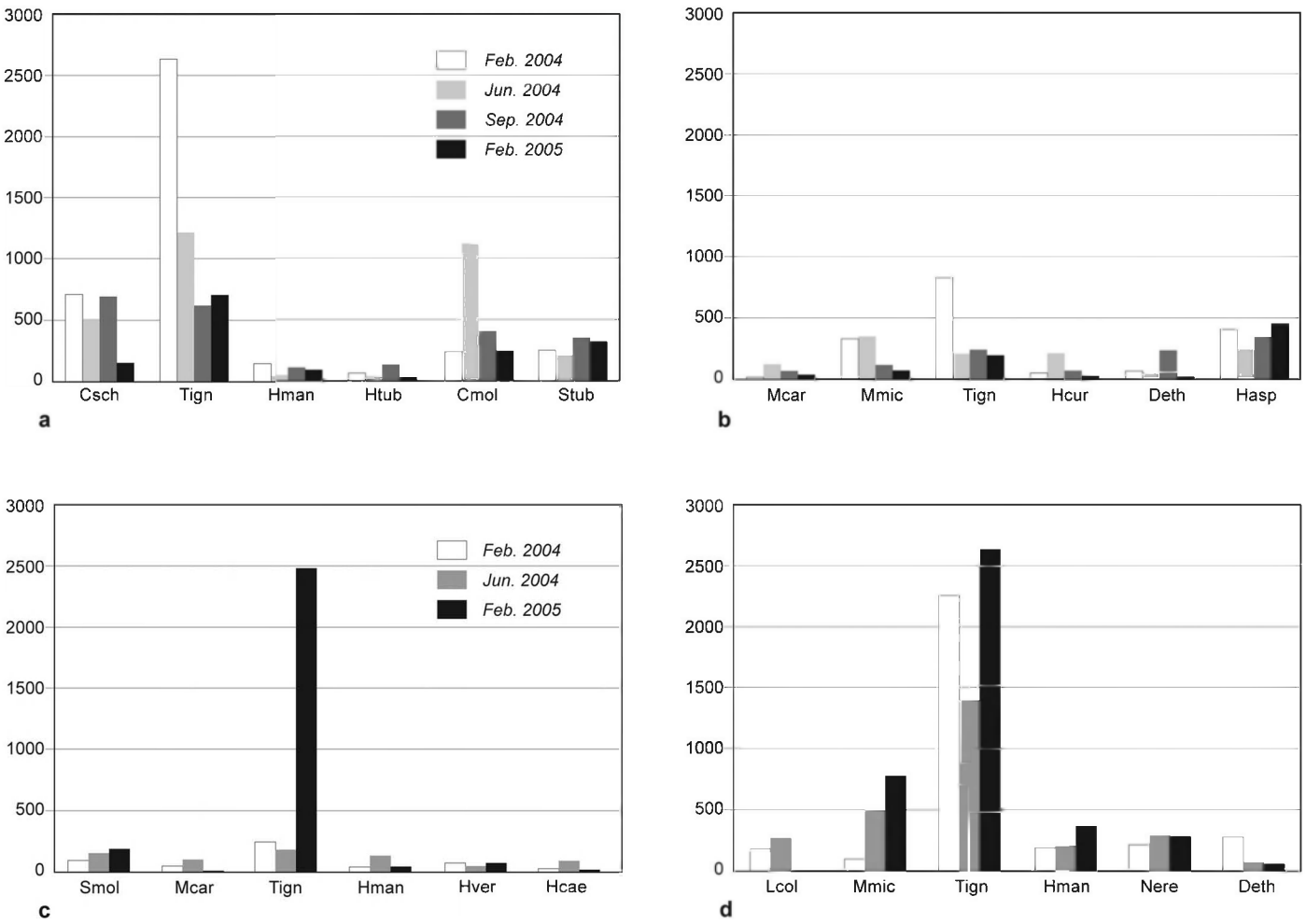

FIGURE 7. Relative abundance of most common sponge species growing on mangrove roots (expressed as projected area [cm^2] per length [m] of root) at four Bocas del Toro sites between February 2004 and 2005: a, STRI Point; b, Solarte In; c, Solarte Out; d, Big Bight. (Cmol = *Chalinula molitba*; Csch = *Clathria schoenus*; Deth = *Dysidea etheria*; Hasp = *Halicarca* sp.; Hcae = *Haliclona caerulea*; Hcur = *H. curacaoensis*; Hman = *H. manglaris*; Hpro = *Hyrtios proteus*; Htub = *Haliclona tubifera*; Hver = *H. vermeulei*; Lcol = *Lissodendoryx colombiensis*; Mcar = *Mycale carmigropila*; Mmic = *M. microsigmatosa*; Nere = *Niphates erecta*; Smol = *Spirastrella mollis*; Stub = *Spongia tubulifera*; Tign = *Tedania ignis*).

Mangrove Surveys in Belize

Changes in Frequency of Occurrence of Major Functional Groups

We determined the frequency of occurrence of important functional groups growing on mangrove roots to be able to assess changes over time (Figure 8). The six compound groups recorded in our surveys were cyanobacteria, algae, sponges, sea anemones, bivalves, and ascidians. In terms of the hierarchy, sponges were first or second at all sites, followed by colonial ascidians, macroalgae, and cyanobacteria. Only at Manatee Cay had sponge occurrence on roots decreased since 2003, whereas at the other three sites it either increased or stayed nearly the same. Ascidian occurrence decreased considerably (10%–26%) at all sites between 2003 and 2007. These changes in sponge and ascidian populations were accompanied by cyanobacterial blooms at three sites (Lair Channel, Hidden Creek, and Manatee Cay), where increases of 10% to 57% of these organisms were recorded. One of the less abundant

TABLE 5. Ranking of the most common sponge species according to their abundance at each studied site in the Bocas del Toro region, 2004–2005.

Species	Rank in abundance			
	STRI Point	Solarte In	Solarte Out	Big Bight
Spirastrella mollis	0	0	2	0
Lissodendoryx colombiensis	0	0	0	5
Lissodendoryx isodictyialis	11	0	0	11
Clathria schoenus	2	0	0	12
Mycale carmigrophila	16	4	6	16
Mycale microsigmatosa	5	2	0	2
Iotrochota birotulata	7	0	0	0
Tedania ignis	1	1	1	1
Haliclona caerulea	0	0	5	0
Haliclona curacaoensis	13	5	0	0
Haliclona implexiformis	10	0	0	0
Haliclona manglaris	6	7	3	3
Haliclona tubifera	8	0	0	0
Haliclona vermeuleni	0	0	4	0
Chalinula molitba	4	0	0	10
Amphimedon sp.	0	0	0	8
Niphates erecta	0	0	0	4
Hyrtios proteus	15	0	0	6
Spongia tubulifera	3	8	0	13
Dysidea etheria	0	6	0	7
Halisarca sp.	0	3	0	18

groups, the sea anemone *Aiptasia pallida* (Cnidaria), is worth mentioning for its striking change in occurrence at the Twin Cays sites. Although the population remained steady at Hidden Creek (8%), it doubled in Lair Channel (10%–24% of roots occupied), but it apparently disappeared from Sponge Haven where it had been present on 20% of the roots in 2003. The number of roots available for settlement per site increased considerably at Sponge Haven and Manatee Cay lagoon, although it decreased in Hidden Creek and Lair Channel.

Sponge Species Frequencies per Site

The distinctive species composition and richness at each site warrant separate presentations.

Sponge Haven

Most mangrove-specific species, such as *Halichondria magniconulosa*, *Haliclona curacaoensis*, *H. manglaris*, *H. implexiformis*, *Hyrtios proteus*, *Lissodendoryx isodyctialis*, and *Spongia tubulifera*, remained the most common among sponges, and some even increased in frequency between 2003 and 2007 (Figure 9a).

Lair Channel

In this mangrove channel most species remained in place between survey periods; some increased in root occurrence (*Tedania ignis*, *Haliclona manglaris*, *H. tubifera*, *Dysidea etheria*) and a few decreased (*Haliclona curacaoensis*, *H. implexiformis*, *Hyrtios proteus*) (Figure 9b). Overall, this change was accompanied by a slight decrease in root numbers (from 105 to 91) and an increase in all non-sponge groups except ascidians.

Hidden Creek

This tidal channel site is opposed to Sponge Haven in its changes between 2003 and 2007 (Figure 9c). Ten of the 12 sponge species found on the transect decreased considerably in occurrence on roots; only the opportunistic

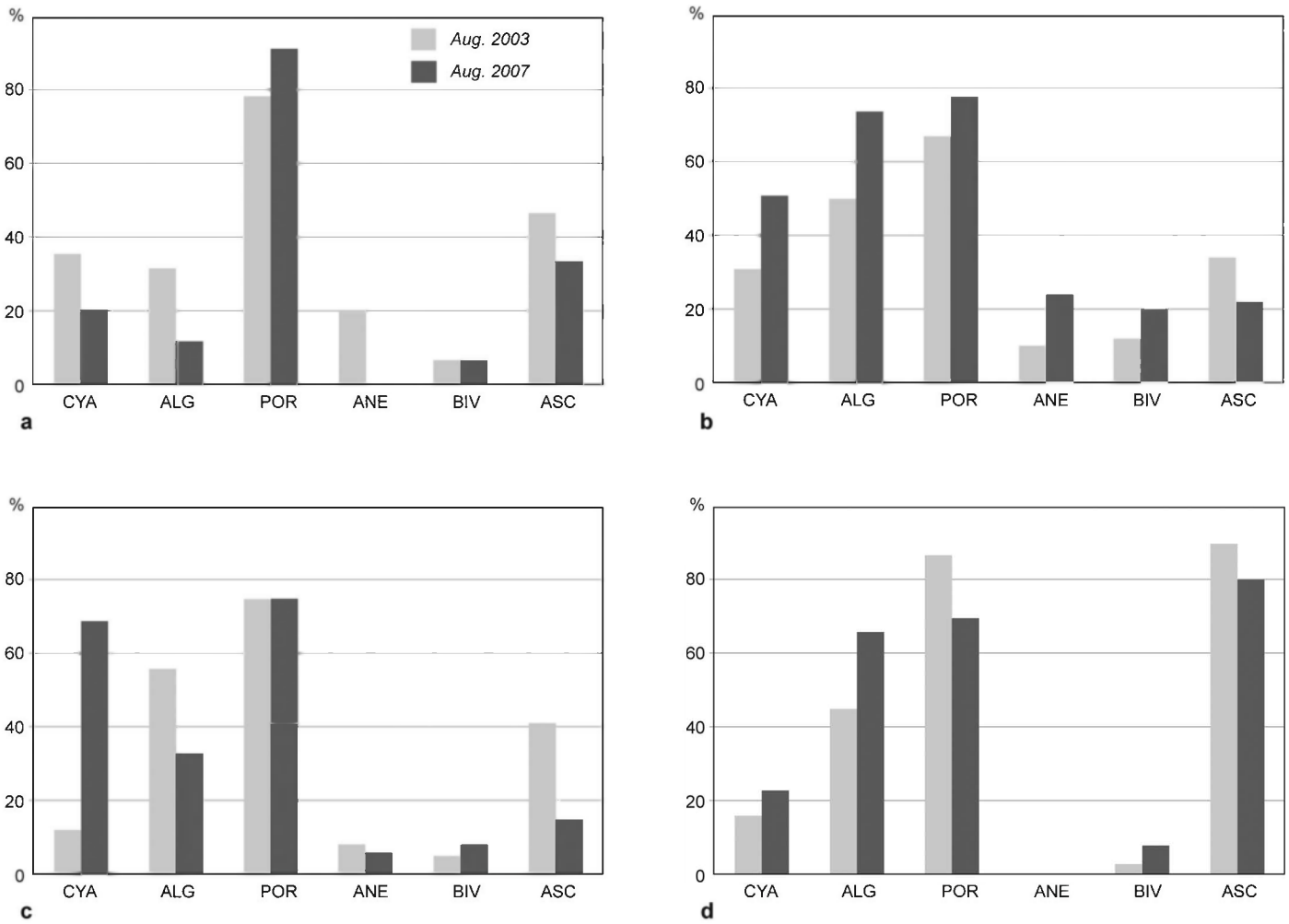

FIGURE 8. Frequency of occurrence (% of roots occupied) of major functional groups growing on mangrove roots at four sites in Belize: a, Sponge Heaven, Twin Cays; b, Lair Channel, Twin Cays; c, Hidden Creek, Twin Cays; d, Manatee Lagoon, Pelican Cays. (ALG = algae; ANE = sea anemones [*Aiptasia pallida*]; ASC = ascidians; BIV = bivalves; CYA = cyanobacteria; POR = sponges [Porifera]).

generalist *Tedania ignis* and *Lissodendoryx isodictyalis* increased slightly.

Manatee Cay

At this lagoon site, abundance of most typical mangrove sponge species decreased considerably during the survey period while two common opportunistic species (*Tedania ignis*, *Clathria schoenus*) experienced a considerable boost in their populations (Figure 9d). This trend coincided with a major increase in root numbers (from 89 to 123), similar to that which took place at Sponge Haven during the same time span.

DISCUSSION

Biogeography of Caribbean Mangrove Sponges

Available reports describing sponge species distribution in Caribbean mangroves suggest the importance of geographic vicinity, with high similarities between the faunas of Panama and Venezuela. On the other hand, this geographic concept is upset by the incongruence of faunas encountered at two nearby sites in Belize (Twin Cays and the Pelican Cays). This dissimilarity is caused mostly by the presence of several unique or usually coral reef-associated species in the mangroves of Manatee Lagoon, an environment of particular geomorphological structure and prevailing ecologi-

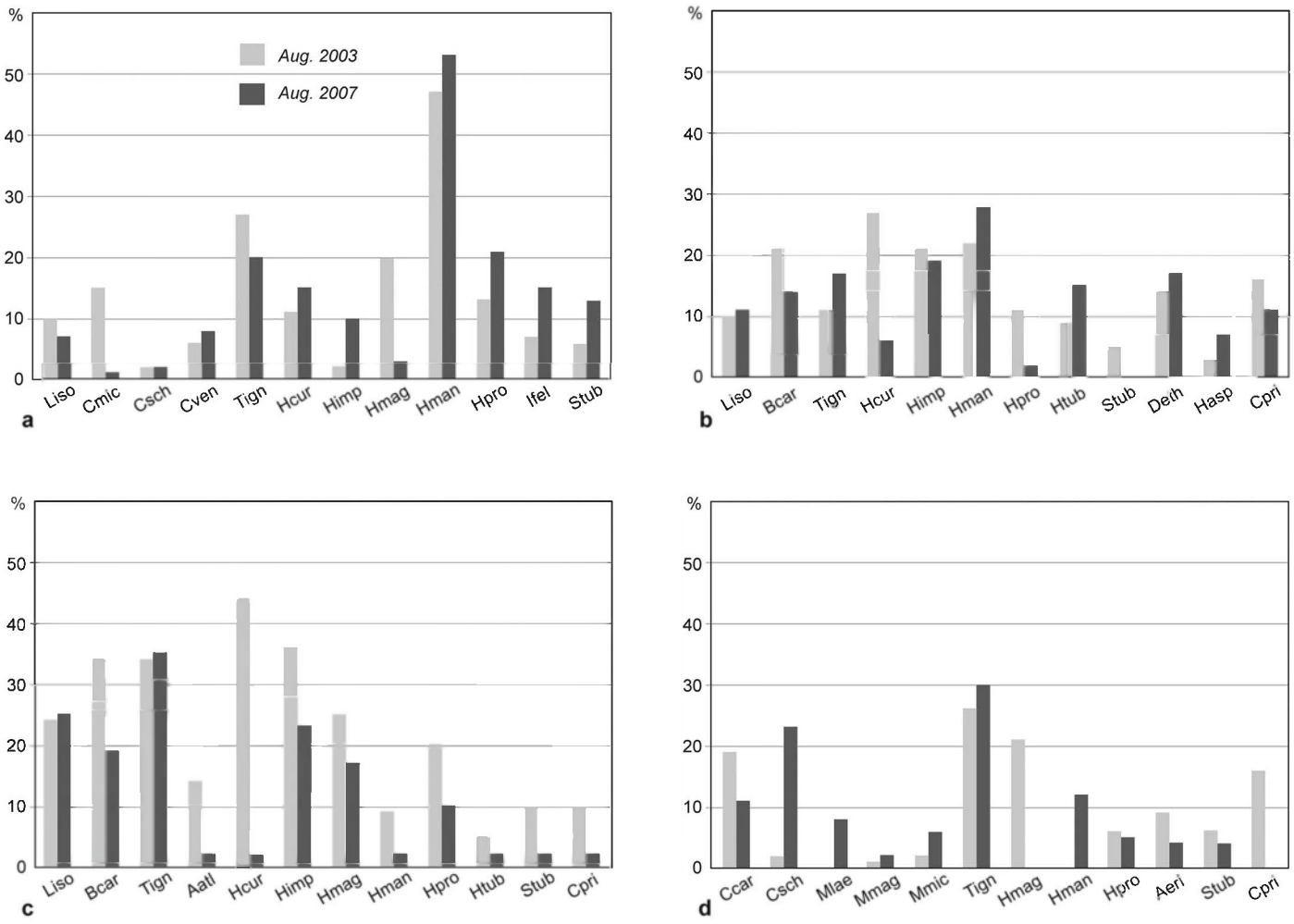

FIGURE 9. Frequency of occurrence (% of roots occupied) of sponge species growing on mangrove roots at four sites in Belize: a, Sponge Heaven, Twin Cays; b, Lair Channel, Twin Cays; c, Hidden Creek, Twin Cays, d, Manatee Lagoon, Pelican Cays. (Aatl = *Amorphinopsis atlantica*; Aeri = *Amphimedon erina*; Bcar = *Biemna caribbea*; Ccar = *Chondrilla caribensis*; Cmic = *Clathria microchela*; Cpri = *Clathrina primigenia*; Csch = *Clathria schoenus*; Cven = *Clathria venosa*; Deth = *Dysidea etheria*; Hasp = *Halisarca* sp.; Hcur = *Haliclona curacaoensis*; Himp = *H. implexiformis*; Hmag = *Halichondria magniconulosa*; Hman = *Haliclona manglaris*; Hpro = *Hyrtios proteus*; Htub = *Haliclona tubifera*; Ifel = *Ircinia felix*; Liso = *Lissodendoryx isodictyalis*; Mlae = *Mycale laevis*; Mmag = *M. magniraphidifera*; Mmic = *M. microsigmatosa*; Stub = *Spongia tubulifera*; Tign = *Tedania ignis*.)

cal conditions in the Pelican Archipelago (Macintyre and Rützler, 2000; Rützler et al., 2000; Wulff, 2000).

Short-Term Dynamics of Mangrove Epifauna in Panama

Major Functional Groups

As previously reported, mangrove-root epiphytic communities in Bocas del Toro are dominated either by sponges or by algae/cyanobacteria (Farnsworth and Ellison, 1996; Diaz et al., 2004; Peréz, 2007). Elsewhere in the Caribbean, other groups, such as bivalves, anemones, or ascidians, may rival these taxa in abundance (Sutherland, 1980; Toffart, 1983; Bingham, 1992). The dominance of macroalgae at the protected lagoon of "Solarte In" might be a consequence of the eastward orientation of this site (as opposed to the westward orientation of the other three sites), which would expose the mangrove fringe to sunlight

for longer periods, thus promoting the growth of typical shallow-water algal species. However, further studies are required to sustain this hypothesis. There were no observations of seasonal changes in the composition of epiphytic taxa from one sampling period to the other at any site. The decrease in abundance (20%–35%) found for the most dominant groups at two sites (STRI Point and Solarte In) coincides with housing developments that occurred since the study started in 2004. Increases in suspended sediments and incidences of sponges covered by silt, which were observed at STRI Point during September and February 2005, may have impacted the community. In contrast, at the more pristine sites (Solarte Out and Big Bight), these same organisms demonstrated considerable quantitative increases.

Sponge Species

The six most common species at each site constitute from 87% to 99% of the total root area covered by sponges. These dominant species differed between sites, bringing the number of the most abundant sponges to 21, of a total diversity of 40 species. Only *Tedania ignis* was the most common species at all sites. At Solarte In the second most common species was a thin crust of the genus *Halisarca*, whereas at STRI Point it was *Clathria schoenus*, a species with a highly variable growth form (thick crusts to branching), supporting the common observation that mangrove fauna can be highly heterogeneous within one biogeographic region. It is interesting to note that 5 species that ranked near the top at the four sites were encrusting sponges (*Mycale microsigmatosa*, *Dysidea etheria*, *Haliclona manglaris*, *Halisarca* sp., *Clathria schoenus*, and *Spirastrella mollis*). This result suggests that, at least in Bocas del Toro, encrusting species are highly successful competitors. The dominance of *Tedania ignis* was also reported from other Caribbean locations (Toffart, 1983; Sutherland, 1980; Wulff, 2004; Diaz et al., 2004) and is probably related to its high and nearly year-around production of larvae (Ruetzler, unpublished data) and rapid growth rate (Wulff, 2005).

Dominant species were not always consistent in abundance at all sites. For example, during the observation time *Tedania ignis* decreased considerably at STRI Point and Solarte In but increased at Solarte Out and Big Bight. Furthermore, increase or decrease in abundance was not necessarily restricted to certain species or localities. Certain locality trends, however, were observed. At STRI Point, where *T. ignis* and *Clathria schoenus* decreased or disappeared entirely from the roots, the few large specimens of *Spongia tubulifera* remained with only slight size changes throughout the year. At least at one location, Solarte In, deterioration of sponges appeared to be coinciding with the aforementioned housing development, which caused an increase in suspended and deposited sediment.

An interesting trend is the predominance of large sponges at Big Bight versus the much smaller sizes at Solarte Out. Solarte Out is a shallow habitat in an exposed position and subjected to strong wave action and scouring by predominantly sandy sediments (see Table 2). These parameters must impede the growth of large individuals, with the result that small and better adapted forms, such as *Haliclona vermeuleni*, *H. caerulea*, and *Spirastrella mollis*, become very abundant. Even an opportunistic species such as *Tedania ignis*, common and large-growing elsewhere, tends to be considerably restricted in size there. On the other hand, Big Bight sponges were found to have rapid growth rates that can be attributed to high nutrient concentrations measured at this site, possibly related to runoff from the dense forest that surrounds this lagoon (see Table 3). The high variability of sponge species composition between contiguous sites corroborates previous reports that the mangrove sponge fauna is rather heterogeneous in species distribution and dominance within relatively small geographic areas (Farnsworth and Ellison, 1996; Rützler et al., 2000; Diaz et al., 2004). This characteristic is probably the result of low recruitment rate in most species studied and, in some cases, selective physicochemical variables, such as those described for Solarte Out. A third aspect that became evident in this study is the intrinsic growth dynamics of species over time, high in species such as *Tedania ignis* and *Chalinula molitba*, and low or barely noticeable in *Hyrtios proteus* and *Spongia* spp. It must be recognized that species have different lifespans, growth rates, growth periods, and frequency of reproduction. Understanding these processes is essential to the interpretation of community dynamics.

Long-Term Dynamics of Mangrove Epifauna in Belize

Major Functional Groups

The distribution of the four primary components of mangrove-root ephiphytic communities in Belize—cyanobacteria, macroalgae, sponges, and ascidians—varied differently at each of the four studied sites between August 2003 and 2007. Sponges were the most frequent occupants at all four locations in 2003; by 2007, the population had either increased (Sponge Heaven, The Lair), decreased (Manatee Lagoon), or remained steady. The decrease at Manatee Cay seemed to be related to macroalgal blooms

that coincided with the recent clear-cutting of the mangrove adjacent to this lagoon and to dredging for land-fill that released large quantities of fine sediments. Ascidian occurrence followed a similar pattern, indicating that all filter feeders are impacted by environmental events such as increase of sedimentation and blockage of vents by cyanobacterial blooms. The effect of changing root numbers seems to be obscured by the environmental factors, because there was no obvious relationship between changes in root number and frequency of any of the major taxa in the community.

Sponge Species

Comparing species composition and frequency at the four study sites in Belize, we found that they varied considerably during the four years between observation periods. The most obvious parameters affecting sponge populations were space competitors (cyanobacteria, macroalgae), number of roots available for settlement, and anthropogenic destructive events. The considerable decrease in cyanobacteria and macroalgae and increase in root numbers (from 99 to 143) in Sponge Haven may be related to the strong increase of mangrove-specific sponges because important competitors were no longer present and new substrata became available. In contrast, at Hidden Creek, the increase of filamentous cyanobacteria (to 57% of substrate area) and decrease in root numbers (from 59 to 52) must have caused the dramatic reduction of most mangrove-specific sponge species. In Manatee Cay Lagoon, mangrove-specific species lost in frequency while opportunistic species (*Tedania ignis*, *Clathria schoenus*) gained. Overall, however, there was a reduction of sponge populations despite an addition in root numbers. This trend can be explained by increased algal competition and an artificial incursion, the clear-cutting of mangrove trees and dredging of fill material for a housing development sometime before the 2007 survey. The dredge operation in particular can be blamed in the short term as it causes suspension of fine sediment, affecting the delicate filtration system of the sponges. A shift of species toward more robust opportunists rather than typical mangrove forms is therefore not surprising.

Comments on Methods for Evaluating Mangrove Prop-Root Communities

Two criteria were used in the present study to evaluate epiphytic communities on mangrove roots. To determine short-term dynamics (within one year; Bocas del Toro), it was expected that specimen size rather than numbers would change. Therefore, a photographic record was made of a specific number of roots (25) along their entire lengths (the side facing the open water), and planimetry was used to measure projected area cover of the fouling organisms. From these values and the record of root length, an index of species abundance could be calculated. Area cover has been extensively used to compare the abundances of plant and sessile animal communities, and it has been proven a most practical and reliable method for reef surveys (Weinberg, 1981). Considering that in mangroves substrate availability is quite low, measuring area cover gives a good indication of how important an organism is in this community. The limitation of this method applies mostly to stoloniferous organisms for which cover may underestimate their importance. The photo-transect method proved to be most useful in areas where visibility was very good, but it was problematic in locations with high freshwater or sediment input. Such conditions caused whole sets of photographs to be impossible to interpret. This method is also time consuming, both the work underwater and that during photo analysis in the lab. For this reason there was a limit to the root numbers that could be included in each survey. Usually, to complete a survey of 25 roots in one site it was necessary to visit twice, and evaluation of all (3–8) photos for one root took from 30 to 60 min. In the end, after excluding useless images, the data set was reduced to only 14 to 22 recorded roots, depending on the site.

Alternatively, in Belize we used data on the presence or absence of taxa on each root and thus were able to survey a much larger number of roots (50–150), from which we determined the frequency of occurrence of major taxonomic groups and species of sponges. These data allowed monitoring the presence of each group or species and change in distributions over time. This type of survey follows the fate of the community rather than fluctuations in biomass. The method also aids detection of a species or community reaction to particular environmental disturbances. In terms of time investment, it takes only 2 to 5 h to obtain frequency data from a 30 m transect along the mangrove fringe. The data were in hard copy once the fieldwork was completed and were independent of visibility conditions and other variables that may ruin photographic data.

CONCLUSIONS

Many more Caribbean mangroves must be studied before we can expect a full understanding of the biodiversity and the biogeographic relationships of their unique and

fascinating prop-root fouling communities, particularly the sponges. The rather disjunct pattern of sponge species distribution found in the Panama and Belize study sites suggests that biodiversity is better evaluated by surveying extended stretches of mangrove fringe at numerous sites in any region rather than short lengths of transects. Interpretation of species composition and interactions can be based on smaller-scale levels of inquiry. The most abundant organisms in the studied sites were sponges, macroalgae, cyanobacteria, ascidians, and bivalves. The hierarchical ranking of these groups showed great variability on spatial and temporal scales, making generalization and prediction of structure and dynamics of communities very difficult.

The one-year study of four sites in the Bocas del Toro region, Panama, showed various important aspects of abundance changes in these fouling communities. First, a few sponge species contribute most of the abundance; second, the identity of major community components varies within a small geographic scale; third, species have adopted distinct life strategies (in growth potential, recruitment rates, and asexual reproduction capabilities) that allow for adaptations to resist stressful environmental variables; and fourth, the combination of the factors of large sediment grain size and energy from wave or current action limits species habitat access, survival, and growth, as demonstrated by the increase in turbidity from landfilling and development in some areas.

The four-year observations in Belize made it evident that the frequency of occurrence of sponge species and other taxa, such as cyanobacterial and macroalgal blooms, is a relatively simple and fast measure to detect major environmental changes. Even if sponge frequency on the roots is not much affected by algal blooms, the presence of mangrove-specific species certainly shows a decline; only a couple of generalist species seem to profit from such stressful events. The degree of disappearance of ascidians at all four sites in Belize suggests that these organisms may be even more sensitive to algal and cyanobacterial competition, as well as suspended fine sediments, than sponges. We find, both in Belize and in Panama, that two sponge growth forms are highly successful among sponge root occupiers: encrusting and irregularly massive. This observation is in contrast to open reef environments where tubular and ramose forms predominate.

Close monitoring of the abundance and frequencies of key mangrove benthos at specific sites and their correlation with short-term or long-lasting environmental impacts and stress will be a useful tool for assessing mangrove health throughout the Caribbean region in the future.

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Internal Transcribed Spacer 2 (ITS2) Variation in the Gorgonian Coral *Pseudopterogorgia bipinnata* in Belize and Panama

Daniel Dorado and Juan A. Sánchez

ABSTRACT. One of the most intriguing aspects of molecular evolution is the concerted evolution of ribosomal genes, yet the presence of intragenomic rDNA variants is still not well understood. We studied the intragenomic variation of the internal transcribed spacer 2 (ITS2, rDNA) in the gorgonian coral *Pseudopterogorgia bipinnata* (Gorgoniidae: Octocorallia) using a combined approach of denaturing gradient gel electrophoresis (DGGE), DNA sequencing, and RNA secondary structure prediction. We examined intragenomic variants of colonies from Carrie Bow Cay (Belize) and Bocas del Toro (Panama). Despite frequent intragenomic ITS2 variation in *P. bipinnata*, predicted RNA secondary structures exhibited no signs of including pseudogenes and comprised functional copies. Given the low divergence among the ITS2 sequences recovered from DGGE gels, intragenomic variation was restricted to a few mutations that did not compromise the functionality of the ITS2 secondary structure. The presence of common ITS2 intragenomic variants at two distant populations raises new questions such as whether sharing similar copies can be the product of gene flow. Regardless of the limited number of individuals analyzed in this study, the method used here, excising bands from DGGE gels for further amplification and sequencing, examined the reliability of the technique to separate intragenomic variants with up to one nucleotide difference. Studying the intragenomic variation of ITS2 has potential to provide us with information on recent population events such as introgressive hybridization.

INTRODUCTION

Ribosomal DNA (rDNA) intragenomic variation has puzzled molecular systematists and ecologists during the past few years. The rDNA is a multigene family arranged in tandem repeats, frequently achieving several hundreds of repetitions per chromosome. Each repetition is composed of three ribosomal subunits (18s, 5.8s, and 28s), separated by two internal transcribed spacers (ITS1 and ITS2, or ITSs), an external transcribed spacer (ETS), and the non-transcribed intergenic spacers, IGS. The ITS1 and ITS2 spacers form secondary structures that are crucial for ribosomal maturation as well as important for the maturation of the rRNA (Coté and Peculis, 2001). The ITSs are known to have conserved core structures throughout the metazoans (see reviews in Coleman, 2003; Schultz et al., 2005). Changes in the ITS secondary structure are known to produce inhibition of the maturation of rRNA as a consequence of

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coevolution between RNA secondary structures and the processing molecular machinery responsible for its removal (Van Nues et al., 1995). As multicopy genes, the rDNA is assumed to evolve via concerted evolution, resulting in the homogenization of the sequences throughout the genome (Harris and Crandall, 2000; Hillis and Davis, 1988), that is, homogenization of copies through unequal crossing-over and gene conversion processes (Liao, 2000). However, variations within individuals have been reported primarily as a result of slow concerted evolution (Harris and Crandall, 2000; Coté and Peculis, 2001), hybridization, or the presence of pseudogenes (Marquez et al., 2003; Harpke and Peterson, 2006). The latter can appear because of the presence of highly divergent rDNA types in different chromosomes (Arnheim et al., 1980), which retain ancestral rDNA polymorphisms for long periods of time (Marquez et al., 2003). Hybridization phenomena between species per se could increase the rDNA diversity in an individual, but as an additional consequence could result in silencing some rDNA loci by chromatin modifications in a nucleolar dominance process (Chen et al., 1998; Frieman et al., 1999; Muir et al., 2001), which can drive some rDNA loci by neutral selection toward pseudogenes (Muir et al., 2001). However, the presence of ITS2 intragenomic variants is a phenomenon that we do not clearly understand.

Pseudopterogorgia bipinnata Pallas is one of the most abundant shallow-water gorgonian corals in the Caribbean Sea (Bayer, 1961; Sánchez et al., 1997). This species has two particular characteristics: it exhibits large phenotypic plasticity along the depth–wave exposure gradient, and it presents clear intragenomic variation in the ITS2 sequence (Sánchez et al., 2007). Consequently, *P. bipinnata* constitutes an appropriate model species to study the nature and genetics of ribosomal intragenomic variation. In this study we had two main objectives: (1) to isolate sequences of intragenomic ITS2 variants in *P. bipinnata* from populations at Belize and Panama and (2) to examine if intragenomic ITS2 variants were functional copies using predicted RNA secondary structures.

MATERIALS AND METHODS

Samples from *Pseudopterogorgia bipinnata* colonies were obtained by scuba diving at Carrie Bow Cay ($n = 27$), Belize, and Cristobal Island ($n = 11$), Bocas del Toro, Panama. A few *P. bipinnata* from the Bahamas (San Salvador) and Colombia (Bancos de Salmedina, Cartagena), as well as a sequence of *Gorgonia mariae*, were chosen as outgroups. However, there was no a priori information on the genetic distance between western and eastern Caribbean populations. Total DNA was extracted using a cetyltrimethylammonium bromide (CTAB), proteinase K, phenol–chloroform–isoamyl alcohol extraction method (Coffroth et al., 1992); DNA was resuspended and conserved in TE buffer at −70°C; DNA quality was checked in agarose (1%) electrophoresis at 80 V for 30 min. Using the best DNA extraction quality, primers 5.8s 5′-AGCATGTCTGTCTGAGTGTTGG-3′ and 28s 5′-GGGTAATCTTGCCTGATCTGAG-3′, designed by Aguilar and Sánchez (2007), were used for the ITS2 amplification. Conditions for polymerase chain reaction (PCR) were as follows: an initial denaturizing step of 2 min at 94°C; followed by 35 cycles of 30 s at 94°C, 30 s at 56.8°C, and 1 min at 72°C; and a final extension step of 2 min at 72°C; using 1 unit Taq polymerase (Invitrogen), 3.5 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphates (dNTPs; Biorad Mix), 0.15 μM primers (each), and 4 μL DNA (dilution 1/50) in 20 μL as the final volume. The amplification was standardized with an efficiency of 95%. PCR reactions were screened in denaturing gradient gel electrophoresis (DGGE) containing 8% polyacrylamide, 1 × TAE buffer, and a linear urea-formamide denaturing gradient from 45% to 80%. The gels were pre-run at 60°C and 90 V for 30 min, followed by electrophoresis at 60°C and 90 V for 13 h. Gels were stained with ethidium bromide during 15 min and visualized using a BIORAD Chemidoc system. DGGE separates DNA fragments not only by the fragment size but also by the DNA sequence, where GC-richer sequences migrate further independently of small differences in size (Figure 1). All reactions were conducted without a CG-clamp in the primers, which is a 40 bp GC-rich sequence added before the 5′-primer that adds an additional denaturing domain allowing further migration of the DNA before denaturing (LaJeunesse and Pinzon, 2007). In the case of gorgonian corals, there was no need for the GC-clamp owing to the great migration in the DGGE of gorgonian ITS2 sequences, which avoided the problems involved with PCR reaction tailed primers. Bands visualized in the DGGE gel were excised using sterilized micropipette tips in the Bio-Rad Chemidoc system and placed in 0.5 mL tubes with 100 μL sterilized double distilled water. The tubes were incubated in a shaker at room temperature for 24 h at 150 rpm. Each band extract was collected in a 0.5 mL tube and the DNA was precipitated with 300 μL cold absolute ethanol; tubes were placed at −20°C for 24 h and then centrifuged at 13,000 rpm for 30 min; the supernatant was discarded,

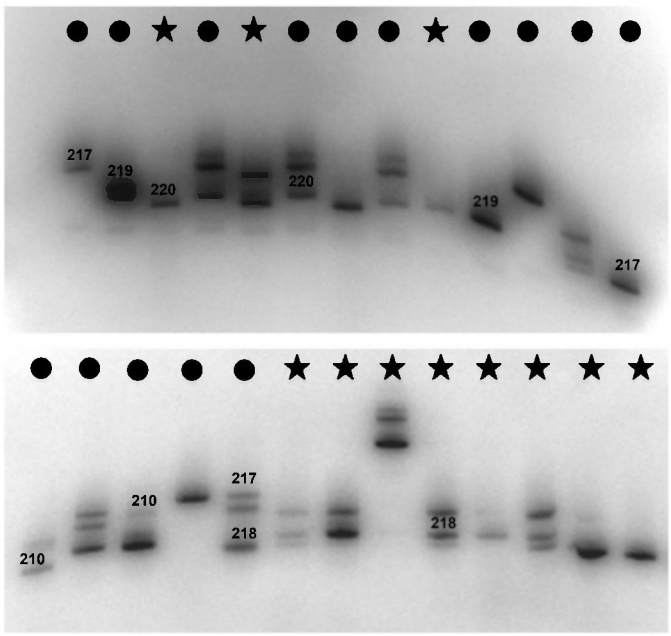

FIGURE 1. Runs (2) of internal transcribed spacer 2 (ITS2) denaturing gradient gel electrophoresis (DGGE) banding patterns from *Pseudopterogorgia bipinnata* colonies from Panama (Bocas del Toro; stars) and Belize (Carrie Bow Cay; circles). Numbers correspond to the sequence size when available. The gels have a common artifact in the form of a "smile" (more accentuated in the upper gel), where lateral wells tend to migrate slightly further because of the pressure acting on the gel edges.

and the pellet was dried and resuspended in 15 μL sterilized double distilled water.

Reamplification of bands was conducted using PCR as just described, using the same set of primers, except that DNA was used without dilution and the annealing temperature was raised a few degrees to increase specificity. Purification of PCR products for sequencing was performed by the Exo-SAP (Exonuclease 1 and shrimp alkaline phosphatase) method using 1 unit Exonuclease, 0.2 units shrimp alkaline phosphatase, and 2 μL SAP buffer 10× per 20 μL in a 0.2 mL tube. Reactions were held at 37°C for 1 h and at 80°C for 15 min. Sequencing reactions were performed with the BigDye 3.1 system according to the manufacturer's instructions (Applied Biosystems) and sequenced in a capillary electrophoresis automated sequencer (ABI310). Each sample was sequenced with forward and reverse primers. The consensus sequences were obtained by assembling the two complementary electrophenograms in Sequencer 4.7 software.

Secondary structures of all sequences were obtained by reconstructing by comparison via Pairwise Alignment (Bioedit) with previously reported structures in octocorals (Aguilar and Sánchez, 2007). The sequences were then submitted with a few constraints and restrictions in MFOLD at a default temperature of 37°C (Zuker, 2003). Constraints force bases to be double stranded whereas restrictions cause them to be single stranded, which are chosen depending on the sequence homology between the sequences with known structure against each problem sequence without known structure. A good example for a constraint are the two complementary sequences that make a stem; an example of a restriction is a string of free nucleotides between helices or any kind of loop. The structure chosen was the one with the greater negative free energy but conserving the ring model known for ITS2. The obtained secondary structures were used to construct a matrix for cladistic analysis as described by Aguilar and Sánchez (2007). Phylogenetic analyses included maximum parsimony and maximum likelihood as well a Bayesian inference for a combined sequence-molecular morphometric analysis (see details in Grajales et al., 2007).

RESULTS

Denaturing gradient gel electrophoresis (DGGE) analysis revealed that most individuals from Belize and Panama contained intragenomic variants of ITS2 (see Figure 1). There were as many as three different bands per individual that were similar or nearly equal in length because of their closeness in the DGGE gel (Figure 2). Some banding patterns were identical for individuals from both Belize and Panama, which indicated exact ITS2 copies, although some patterns unique to each population were also observed (see Figure 1). Great effort was made to obtain sequences from most bands, but not all of them were successfully recovered. The sequences had an average GC content of 55.6%, which afforded the great migration of intragenomic ITS2 variants in the DGGE. The sequences from *P. bipinnata* had more than 85.6% of sequence similarity, contrasting with just 48% with respect to *G. mariae*.

Predicted secondary structures from all excised bands exhibited functional structures with the conserved six helicoidal ring model previously reported for octocorals (Aguilar and Sánchez, 2007), but great variability was observed in the length and complexity of each stem and spacer (Figures 2, 3). Intragenomic differences were frequently discrete changes that did not affect the predicted secondary

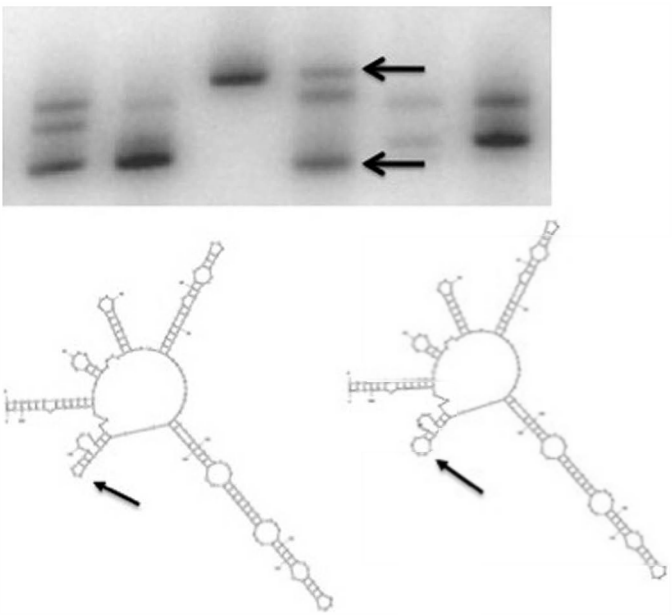

FIGURE 2. Two different intragenomic ITS2 variants from an individual colony of *Pseudopterogorgia bipinnata* from Belize. The variants were excised from the two bands indicated by arrows in the DGGE gel above, reamplified, and sequenced. The arrows below show the differences between the two predicted RNA secondary structures corresponding to one INDEL (insertion or deletion) only.

structures (see Figure 2). The ITS2 in *P. bipinnata* from Panama and Belize varied from 212 to 224 nucleotides (Figure 3). In general, the stems 2, 3, and 6 were shorter than the stems 4 and 5, with stem 5 being the longest. Multiple internal loops were frequent in stems 3, 4, and 5, with more nucleotides (nt) on stem 5, where up to six internal loops were observed (Figure 3). The spacers were often short, ranging from 1 to 4 nt. Spacer '1i' showed a conserved sequence, UG, with little variation across individuals, while spacer '4i' was the longest, with 4 to 12 nt and a conserved core sequence (AGUNCAGC) observed in most of individuals (Figure 3). Phylogenetic results from sequence and alignments or combined data sets, including 11 excised bands from individuals from Belize and 3 from Panama, showed little divergence between Panama and Belize despite the long distance with respect to a few individuals from Bahamas and Colombia (Figure 4). Very modest support was found within individuals from Panama or Belize, and no particular grouping could be discerned (data not shown). In addition, no particular features of the ITS2 secondary structure as seen with helix 5, which showed the largest number of characters, were supporting any particular clade or group of individuals (Figure 4).

DISCUSSION

The intragenomic ITS2 variation in *Pseudopterogorgia bipinnata* individuals involved functional copies, as corroborated by reconstructing their predicted RNA secondary structures. Given the low divergence among the ITS2 sequences recovered from DGGE gels, intragenomic variation was restricted to a few mutations that did not compromise the functionality of the ITS2 secondary structure. Despite frequent intragenomic ITS2 variation in *P. bipinnata*, predicted RNA secondary structures exhibited no signs of including pseudogenes or structural degeneration. Having in mind that the ITS2 secondary structure has a major role in the maturation of the ribosomal RNA (Coté and Peculis, 2001), little tolerance of changes is expected as a result of the restrictions imposed by the ITS2 splicing machinery (Van Nues et al., 1995; Coleman, 2003); this means purifying selection is acting on secondary structural constraints (Coté and Peculis, 2001) or concerted evolution mechanisms are acting similarly (Liao, 2000; but see Nei and Rooney, 2005; Harpke and Peterson, 2006). Similarly, compensatory base changes (CBC), occurring at the stem regions, are very unlikely to occur at the intraspecific level (Müller et al., 2007). Thus, it is expected that only variants or alleles carrying only minor changes occur, which was evident with the functionality of co-occurring secondary structures found at the intraspecific level.

ITS intragenomic variation has been also observed in scleractinian corals. Van Oppen et al. (2001) examined diverse nuclear and mitochondrial DNA sequences, concluding that paraphyly from intragenomic ITS copies could be explained by extensive introgressive hybridization and reticulate evolution. Similarly, Marquez et al. (2003) found the presence of ribosomal pseudogenes as a possible consequence of multiple hybridization events. However, Vollmer and Palumbi (2004) examined the multiple copies of the Caribbean *Acropora* species and concluded that there is no proper way to evaluate if the intragenomic shared variation of genes such as ITS1 and ITS2 was the result of incomplete lineage sorting or recent hybridization processes. Nonetheless, all the studies mentioned studied the intragenomic variation of ITS using the DGGE technique, and it is clear that traditional cloning methods overestimate the intragenomic diversity (LaJeunesse and Pinzon, 2007).

Regardless of the limited number of individuals analyzed in this study, the method used here, excising bands from DGGE gels for further amplification and sequencing, probed its reliability to separate intragenomic variants up to one nucleotide difference (see Figure 2). DGGE is a

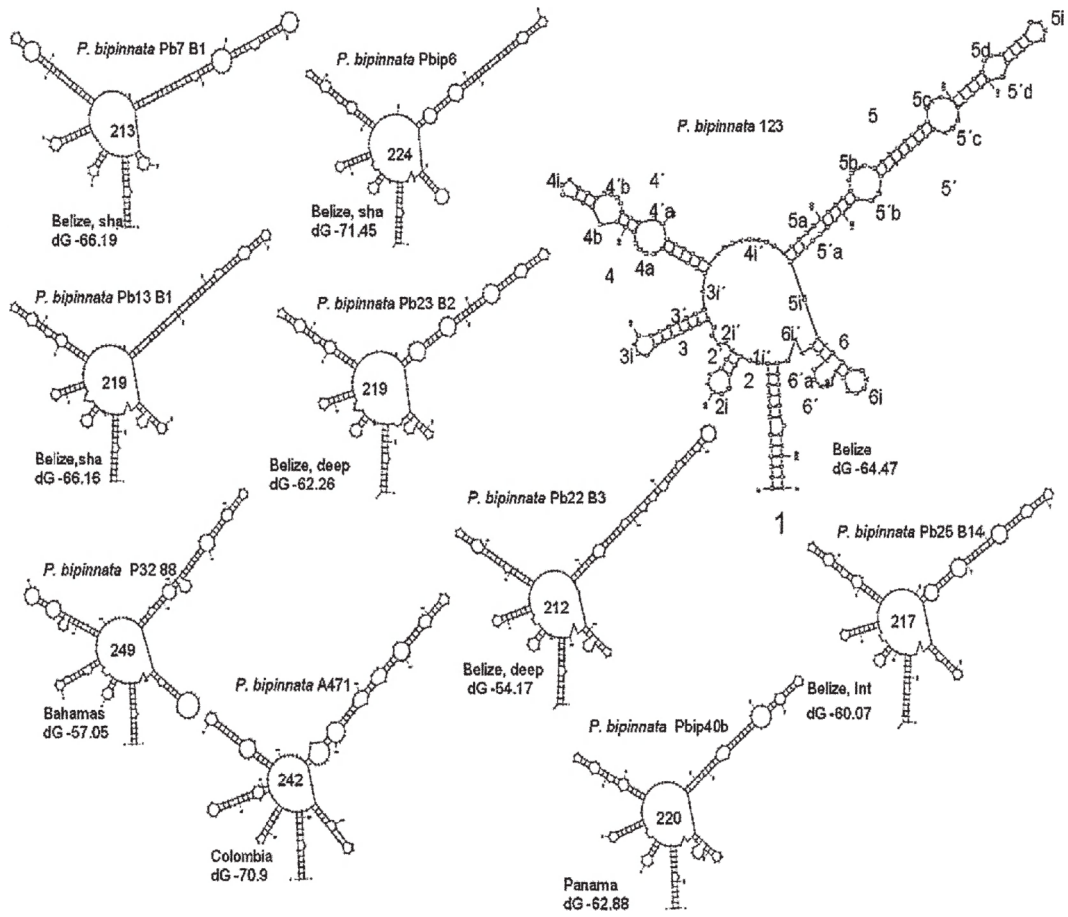

FIGURE 3. Predicted ITS2 RNA secondary structures in *Pseudopterogorgia (P.) bipinnata*. The upper right structure shows the characters for the molecular morphometrics analysis used in the combined Bayesian inference analysis. The number within the ring structure refers to the total number of nucleotides at each structure.

method useful to detect the most prevalent intragenomic variants of ribosomal genes, whereas traditional methods to screen intragenomic variation such as cloning miscalculate the codominance of the different copies (LaJeunesse and Pinzon, 2007; Thornhill et al., 2007). There are two main approaches for depicting the nature of intragenomic ITS2 variants in octocorals. One method is to study in detail the genetics of the different ITS2 variants by crossing individuals with different intragenomic patterns, which can provide inheritance information and linkage disequilibrium configurations. An alternative method includes techniques such as reverse transcription (RT)-PCR and quantitative real-time PCR, which can offer more accurate information on the functionality of the different intragenomic ITS2 copies. The RT-PCR technique can filter copies that are not expressed in the cell, and quantitative PCR can quantify the amount of ITS transcripts from each particular copy. These methods could also test if the intensity of bands in DGGE actually corresponds to the number of copies of a particular intragenomic variant.

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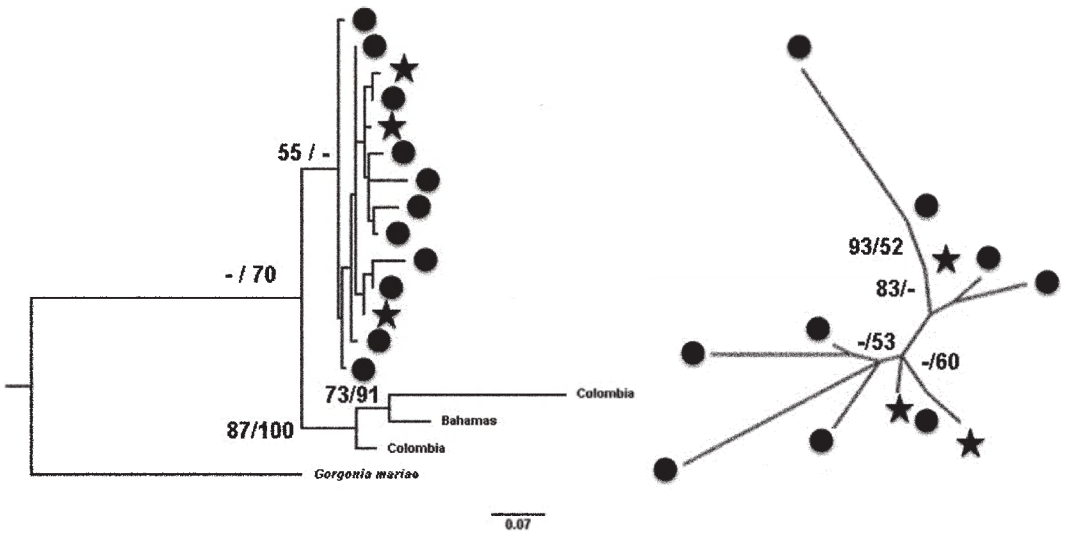

FIGURE 4. Maximum-likelihood phylograms show above-node support from the combined sequence-molecular morphometrics Bayesian analysis (left) and maximum-parsimony bootstrapping (1000 replicates, right): *Pseudopterogorgia bipinnata* colonies from Panama (Bocas del Toro; stars) and Belize (Carrie Bow Cay; circles). The tree at the right is a radial representation of a set of terminal branches corresponding to Panama and Belize sequences pruned from the left tree.

Cairns, BIOMMAR colleagues, and the Smithsonian Station at Carrie Bow Cay, Belize. The Minister of Environment, Household and Territorial Development of Colombia granted access to genetic resources to JAS for the DNA analyses included in this paper (Contract 007, resolution 634; 14 March 2007). This work is contribution number 841 of the Caribbean Coral Reef Ecosystems Program (CCRE), Smithsonian Institution, supported in part by the Hunterdon Oceanographic Research Fund.

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Obvious Invaders and Overlooked Infauna: Unexpected Constituents of the Decapod Crustacean Fauna at Twin Cays, Belize

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ABSTRACT. Decapod crustaceans in the vicinity of Carrie Bow Cay and Twin Cays, Belize, have been under study for more than 25 years. Large collections have been assembled, and new species have been discovered. The effort has included photographic documentation of coloration, yielding characters of value in identification of problematic tropical taxa. Measurements of diversity have been markedly enhanced by extraction corer (yabby pump) sampling in shallow subtidal sediments, especially at Twin Cays. This technique revealed species, genera, and families of thalassinidean decapods not previously known from the region. Studies continue on the ecological roles of these burrowers, dominant bioturbators in seagrass beds where they produce conspicuous mounds of sediment and constitute a major infaunal biomass at Twin Cays. By contrast, familiar large reptant decapods typically dominate shallow rocky substrates. Within the past four years, however, the nonindigenous portunid crab *Charybdis hellerii* has extensively invaded large portions of hard substrates at Twin Cays. In 2007, it was found to dominate cavities under coral heads in survey areas along the northeastern and southwestern shorelines, possibly displacing populations of large *Mithrax*, *Menippe*, *Callinectes*, and *Panulirus* previously found there in abundance.

INTRODUCTION

Fieldwork centered on Carrie Bow Cay and surrounding habitats, including a variety of settings at Twin Cays. The effort continues work by the first author in collaboration with the late Ray Manning in 1983, as well as work by the late Brian Kensley during the 1980s and early 1990s (Kensley, 1981, 1996). Early efforts produced abundant grass-bed and reef-crest species generally identifiable with known Caribbean taxa, along with small cryptic forms obtained by cutting open sponges, breaking rubble, poisoning in situ, or using several narcotants to drive out small decapods from rubble isolated in containers. Rich collections that have accumulated in the the Smithsonian Institution's National Museum of Natural History were fixed in formalin, limiting their value in genetic analyses. Efforts in 2002 and 2007 shifted emphasis to varied intertidal and subtidal habitats of Twin Cays and to resampling the regional fauna to obtain alcohol-fixed materials for molecular genetic analyses.

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Concerted effort has been made to photographically document coloration of fresh specimens, given the value of color in the identifications of tropical species and the long-term goal of producing a guidebook for the regional decapod fauna (DLF and RL, in progress). More than 260 decapod species have been enumerated in our collections from the Carrie Bow Cay region, some yet to be named. Under U.S. National Science Foundation AToL "Decapod Tree of Life" support, molecular and morphological systematic studies are under way concerning alpheid and other caridean shrimps, paguroid hermit crabs, thalassinidean shrimps, and panopeid, portunid, grapsoid, pinnotherid, and majoid crabs, as well as family-level relationships among all major decapod groups. Work incorporating porcelain crab collections from the region has been published (Rodríguez et al., 2005, 2006) as has work by other investigators on some alpheid shrimp groups (Duffy, 1996; Duffy and Macdonald, 1999; Duffy et al., 2000, 2002; Macdonald et al., 2006; Ríos and Duffy, 2007). Previous collections of upogebiid thalassinidean shrimp from Belize were included in Williams (1993). Several descriptions of new species from our Belize collections have also appeared (Goy and Felder, 1988; Manning and Felder, 1996; Felder and Manning, 1997), but many species remain to be described. The second author has been involved in several ecological studies of the infaunal decapods of the region (Dworschak and Ott, 1993; Abed-Navandi and Dworschak, 2005; Dworschak et al., 2006).

Our protracted field sampling program has in some cases allowed us to observe apparent changes in community composition. In a striking example, shallow subtidal habitats at Twin Cays have been recently invaded by the nonindigenous swimming crab *Charybdis hellerii* (A. Milne-Edwards, 1867), previously unreported from Belize. Recurrent trips have also provided opportunities for shallow subtidal sampling and burrow-casting of fossorial infauna in turtle grass (*Thalassia*) beds along shorelines of Twin Cays, revealing unexpected thalassinidean diversity. A brief account of these latest efforts is our present focus, preliminary to more comprehensive treatment of the full decapod assemblage.

MATERIALS AND METHODS

Sampling included the breaking of dead coral and conch shell rubble, netting, extraction of sediments, and sorting through hard-surface fouling organisms, but sampling of large crabs such as *Charybdis hellerii* (Brachyura) and its macrocrustacean associates was a targeted effort. These decapods were captured from under pieces of dead subtidal coral and debris that were lifted while snorkeling over and adjacent to seagrass (*Thalassia*) beds in water 1–2 m deep. Sampling of most thalassinideans and related decapod burrowers was accomplished with a suction extractor (yabby pump) and bag-sieve while wading, snorkeling, or SCUBA diving in water 0.5–4 m deep. In addition to collections of *Glypturus acanthochirus* (Callianassidae) by suction extractor, some specimens of this species were obtained with "weighted line" traps (de Vaugelas, 1985). Specimens of *Axiopsis serratifrons* (Axiidae) were obtained by baiting animals to the apertures of their burrows, where they were captured by cutting off the burrow or by spearing the specimens. Casts of burrows were made as described by Dworschak and Ott (1993). Specimens were immobilized by immersion in chilled seawater or by narcotization with clove oil before photography. Photographs of specimens immersed in a pan of seawater were made with a Fuji Fine Pix S1Pro digital camera equipped with a 60 mm macrolens while the subject was lighted by a combination of direct and reflected sunlight or high-intensity 5000°K fluorescent photographic lamps. All specimens were subsequently preserved in several exchanges of 95% nondenatured ethanol and then stored in 75% nondenatured ethanol. Photographic voucher specimens were archived in the Zoological Collections of the University of Louisiana at Lafayette (ULLZ), and most other materials were deposited in the Smithsonian Institution–National Museum of Natural History (USNM). Some collections by the second author (especially thalassinideans) were deposited in the Naturhistorisches Museum in Wien, Austria (NHMW) and the Muséum National d'Histoire Naturelle, Paris, France (MNHN). For figured specimens, size is indicated as carapace width (cw) or carapace length (cl).

RESULTS AND DISCUSSION

Invasion by *Charybdis hellerii*

Large bottom debris (waterlogged wood, discarded building materials, dead coral heads) typically provides cover for large reptants such as spiny lobsters (*Panulirus* spp.), swimming crabs (*Callinectes* spp.), stone crabs (*Menippe* spp.), and large spider crabs (*Mithrax* spp.), especially in shallow well-lighted waters. Sampling of these environments at both Carrie Bow Cay and Twin Cays in October 2002 revealed no large decapods other than these genera. That same year, however, a small specimen of the

nonindigenous portunid crab *Charybdis hellerii* was found in an empty conch shell on the inshore side of Carrie Bow Cay, the first such occurrence recorded in our sampling program.

In April 2007, sampling under large pieces of cover at Twin Cays was undertaken to obtain fresh materials of the aforementioned resident genera for genetic analyses. Initial sampling centered in the vicinity of the "Fisheries Camp" on the southeastern end of Twin Cays, where a storm had scattered sheets of metal building siding from the shoreline to depths of nearly 2 m. Inspections beneath 20 such sheets across this entire range of depths revealed none of the target species but at least seven variously sized individuals of the nonindigenous swimming crab *C. hellerii*.

Sampling was thereafter shifted to dead coral heads scattered among turtle grass beds on the northeast side of Twin Cays. A crude survey was there undertaken for coral heads in 1–1.5 m depths, each head roughly 0.5–0.7 m in diameter and separated from one another by roughly 6–15 m. Of the 25 coral heads inspected, 13 were uninhabited by large reptant decapods, 8 harbored large specimens of *C. hellerii* (Figure 1b), and four harbored only *Menippe nodifrons* Stimpson, 1859 (Figure 1a). Large single individuals of *C. hellerii* were found under 6 of the 25 heads that were lifted, a mating pair of *C. hellerii* was found under a single head, and a specimen of *C. hellerii* together with a large specimen of *M. nodifrons* was found under another head. No specimens of *Mithrax* spp., *Callinectes* spp.,

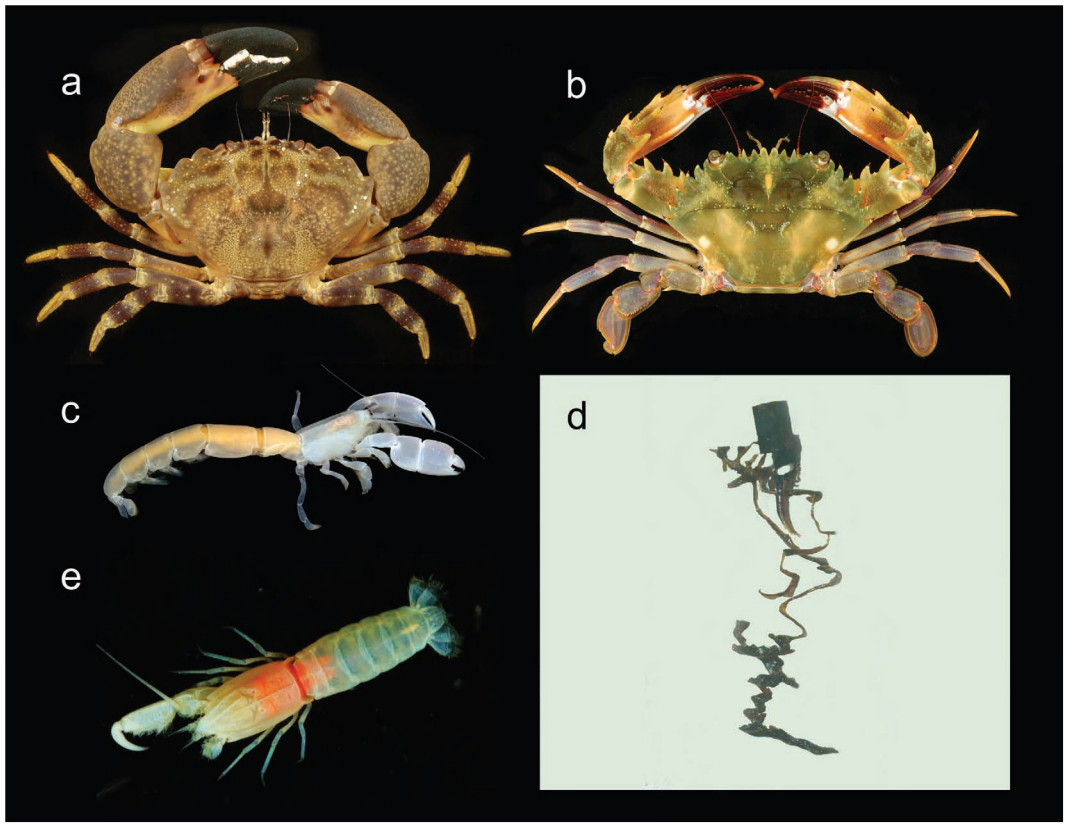

FIGURE 1. a, Stone crab *Menippe nodifrons*, male, 69.7 mm carapace width (cw), Twin Cays, Belize 10 April 2007, ULLZ 8991. b, Invasive Indo-Pacific swimming crab *Charybdis hellerii*, male, 75.3 mm cw, Twin Cays, Belize, 10 April 2007, ULLZ 8990. c, Callianassid *Eucalliax* sp., female, 8.3 mm carapace length (cl), South Water Cay, 22 October 2002, ULLZ 9230. d, Polyester resin burrow cast from Twin Cays, probably assignable to *Axianassa australis*, cast length 85 cm, made by PCD, Twin Cays, Belize, August 1989, NHMW 24001. e, Laomediid *Naushonia* sp. female, 5.8 mm cl, Carrie Bow Cay, Belize, 3 April 2007, ULLZ 8895. ULLZ, University of Louisiana at Lafayette; NHMW, Naturhistorisches Museum Wien. Photographs a–c and e by DLF; photograph d by PCD.

or *Panulirus* spp. were observed, despite these taxa being commonly found in such settings during 1983 and 2002.

Small or immature specimens of *Charybdis hellerii* are easily confused with *Cronius ruber* (Lamarck, 1818) and to a lesser extent with *Achelous tumidulus* Stimpson, 1871, both of which also occur in Belize and adjacent waters of the Caribbean, Gulf of Mexico, and other areas of the warm temperate Atlantic. This similarity led us to initially question the identity of the single small specimen collected in 2002, but it was confirmed to be *Charybdis hellerii* by 16S mtDNA sequence analysis by comparing to other sequence data for the species (Robles et al., 2007; Mantelatto et al., 2009). Widely used diagnostic morphological characters that apply well to full-sized adults do not readily facilitate identification of juveniles among these three species, and records of subadults could easily be in error if based on presently limited descriptions. At the very least, *A. tumidulus* differs from both *C. ruber* and *Charybdis hellerii* by lacking a striking posterior or posterodistal meral spine on the fifth pereiopod (swimming leg) in all crab stages. *Cronius ruber* and *Charybdis hellerii*, however, share a strongly spined fifth pereiopod, albeit with the spine usually occupying a relatively more distal position and being less posteriorly directed on the merus of *Cronius ruber*. The relative position of the spine is, however, difficult to distinguish in small juveniles. These two species also share the presence of small spinules bordering the posterior margin of the fifth pereiopod propodus, although these spinules are of relatively larger size in *Charybdis hellerii*. This characteristic is readily evident in adults, where setation obscures small acute granules along the margins of the propodus in *Cronius ruber*, which are unlikely to be confused with the well-formed adult spinules of *Charybdis hellerii* (see Figure 1b). In juveniles of *Cronius ruber*, microspination of this propodal margin is relatively stronger than in adults, and distinction from juveniles of *Charybdis hellerii* is somewhat subjective, especially if one lacks comparative specimens of similar size. No feature in the carapace of early crab stages (Dineen et al., 2001: fig. 24) appears to separate small individuals of these species.

Recent observations have revealed an ongoing invasion of *C. hellerii* into coastal western Atlantic locations, and its documented distribution must now include Belize along with Brazil, Venezuela, Colombia, Cuba, the Yucatán shelf of Mexico, both coasts of Florida, and other northern Atlantic U.S. coastal habitats through at least the Carolinas (Campos and Türkay, 1989; Gómez and Martínez-Iglesias, 1990; Hernández and Bolaños, 1995; Lemaitre, 1995; Calado, 1996; Mantelatto and Dias, 1999; Dineen et al., 2001; Mantelatto and Garcia, 2001; Mantelatto et al., 2007; Robles et al., 2007; McMillen-Jackson, 2008; Felder et al., 2009). Clearly, the foregoing chronology of reports reveals continued western Atlantic range expansion for *C. hellerii*, although the potential trophic impacts of this invader remain poorly documented. The first author has on two occasions observed individuals of *C. hellerii* in the Indian River Lagoon, Florida, feeding (inside shallow cavities of hard substrates that they occupied) on soft-shelled, postmolt individuals of native species of large decapods (one *Callinectes*, one *Panulirus*), and in another instance feeding on small mussels. As in the present report, all such observations and inferences of this invader's potential competitive and predatory impacts in the western Atlantic remain very limited and anecdotal, but they serve to justify a call for controlled experimental studies.

Thalassinideans

Our collections of cryptic burrowing thalassinideans from various habitats in the vicinity of Carrie Bow and Twin Cays, along with the few previously reported records, include at least 17 species representing the families Callianassidae, Laomediidae, Thomassiniidae, Axianassidae, Axiidae, and Upogebiidae. The species of these often overlooked groups are presented in the following list, with collection sites indicated as TC (Twin Cays), CB (Carrie Bow Cay), SW (South Water Cay), and SL (shorelines near Dangriga); catalogue numbers are shown for archived specimens.

Infraorder Thalassinidea *sensu lato*

Callianassidae (Ghost Shrimps)

Corallianassa longiventris (A. Milne-Edwards, 1870)—TC, CB: NHMW 6774, 6775, 15352–15355; ULLZ 4228–4230, 6083, 8997.

Eucalliax sp.—TC, SW: ULLZ 9230.

Glypturus acanthochirus Stimpson, 1866—TC, CB: NHMW 6765–6770, 15338–15342; MNHN Th 1181, Th 1185; ULLZ 8993–8995, 9233; USNM 266241–266244.

Lepidophthalmus richardi Felder and Manning, 1997—SL [near river mouths]: NHMW 15343–15349: ULLZ 3577, 5186–5188, 8992; USNM 277777–277779.

Neocallichirus grandimana (Gibbes, 1850)—TC, CB, SW: NHMW 6796–6799, 15356–15367; MNHN Th

1182–1184; ULLZ 8998, 9235–9237, 9239–9241, 9243, 9244.

Neocallichirus maryae Karasawa, 2004—TC: ULLZ 9234, 9238.

Laomediidae

Naushonia sp.—CB: ULLZ 8895, 8915.

Axianassidae

Axianassa australis Rodrigues and Shimizu, 1992—TC [identified by burrow cast]: NHMW 24001.

Thomassiniidae

Mictaxius thalassicola Kensley and Heard, 1991—TC: ULLZ 9246.

Upogebiidae (Mud Shrimps)

Pomatogebia operculata (Schmitt, 1924—CB: ULLZ 9231.

Upogebia acanthura (Coêlho, 1973)—?CB: USNM 251246.

Upogebia omissa Gomes Corrêa, 1968—TC, SL: ULLZ 5165.

Upogebia sp.—CB: ULLZ 9232.

Axiidae (Lobster Shrimps)

Axiopsis serratifrons (A. Milne-Edwards, 1873)—CB: NHMW 6771–6773, 15350–15351: ULLZ 4232, 4233, 5827, 8996; USNM 18905, 18907, 18908.

Coralaxius nodulosus (Meinert, 1877)—CB: USNM 170856, 171764–171766, 243431–243434.

Paraxiopsis hispida Kensley, 1996—CB: USNM 211462.

Paraxiopsis spinipleura Kensley, 1996—CB: USNM 211451.

Sediments in lower intertidal to subtidal seagrass beds at Twin Cays are densely populated by *Neocallichirus grandimana*, *Glypturus acanthochirus*, *Corallianassa longiventris*, *Neocallichirus maryae*, *Mictaxius thalassicola*, and *Eucalliax* sp., often burrowing more than 1 m into sediments. Dworschak and Ott (1993) previously analyzed burrow morphologies and distributions for three of these species, as well as for *Axiopsis serratifrons* and two species of pistol shrimp. Their food sources were investigated by stable isotope studies (Abed-Navandi and Dworschak, 2005). Among the species from Twin Cays, *M. thalassicola* has not previously been reported from the northern Caribbean, and *Eucalliax* sp. (Figure 1c) represents an undescribed taxon presently known only from Belize.

The newly reported *Neocallichirus maryae* is a replacement name for the more familiar *N. rathbunae* (Schmitt, 1935), which proved to be a junior primary homonym of a different fossil species (Karasawa, 2004). Although Sakai (2005) placed *N. raymanningi* Blanco Rambla and Lemaitre, 1999, in synonymy with *N. rathbunae* (Schmitt, 1935), and *N. raymanningi* would thus predate recent establishment of *N. maryae*, we do not accept the presently limited evidence for this synonymy.

Ejecta from burrows of these thalassinideans dominates bottom topography in intertidal to shallow subtidal seagrass beds of this area, but along intertidal muddy shorelines at Twin Cays, especially those immediately adjacent to mangroves, it appears that the axianassid *Axianassa australis* also occurs. As no specimens have been captured, this can be deduced only from highly characteristic ejecta patterns and spiraled burrow casts (see Dworschak and Rodrigues, 1997; Felder, 2001), the latter obtained by the second author in 1989 (Figure 1d).

Neocallichirus grandimana appears to be the most widely distributed callianassid among sites sampled in the vicinity, inhabiting both vegetated and nonvegetated sediments. Together with *Eucalliax* sp., it densely populates sparsely vegetated calcareous sands of shallow shoals bordering South Water Cay in addition to sites at Twin Cays. At South Water Cay, upper reaches of its burrows are commonly inhabited by *Processa* sp. and early juvenile stages of *Callinectes* sp., the latter being uniquely pigmented an opaque bluish-black. *Glypturus acanthochirus* and *Corallianassa longiventris* range into deeper grass beds, where they appear to draw grass blades into their burrows. Distributions of all the collected thalassinideans depend on sediment characteristics, depths, vegetation, and water quality, whereas characteristic burrow architectures are both diagnostic of species and suggestive of ecological adaptations (Dworschak and Ott, 1993, Abed-Navandi and Dworschak, 2005; Dworschak et al., 2006). Less conspicuous evidence of sediment ejecta characterizes areas among seagrasses that are burrowed primarily by nonthalassinidean decapods such as the *Alpheus* spp. reported by Dworschak and Ott (1993). Surface features of these burrows can be all

but indistinguishable from those made by what appear to be several species of *Upogebia*, including *U. omissa*.

The assemblage of upogebiids in the Carrie Bow Cay region remains poorly understood. It appears that *Upogebia omissa* ranges widely here, from the shoreline along the mainland to offshore cays, and the first author has identified specimens taken as "pests" from commercial penaeid shrimp farms on the mainland. General treatment of western Atlantic upogebiids by Williams (1993) included records of *U. acanthura* from a patch reef southwest of Carrie Bow Cay and *U. brasiliensis* Holthuis, 1956 from more distant shoreline areas of Belize, although our collections have produced no additional specimens. Two other species listed by Williams (1993) from nearby coastal environments of Quintana Roo (*U. corallifora* Williams and Scott, 1989 and *U. vasquezi* Ngoc-Ho, 1989) could also be expected in Belize, although we have yet to find them. Specimens of this genus from coralline rubble just off the reef crest at Carrie Bow Cay (ULLZ 9232) and other uncatalogued specimens from Twin Cays (in areas also burrowed by alpheid shrimp) cannot confidently be assigned to known species and warrant further study. Generally found in deeper subtidal habitats (Felder et al., in press), the upogebiid *Pomatogebia operculata* ranges into waters as shallow as 2 m depth off Carrie Bow Cay and likely occurs elsewhere between cays in appropriate deeper calcareous rubble habitats; these have been collected by breaking open highly eroded pieces of coralline rubble to expose the muddy interstices and cavities occupied by this upogebiid.

Axiids are also found in association with rubble and reef structures of outer cays, as, for example, at Carrie Bow. The widely distributed *Coralaxius nodulosus*, a small-sized species inhabiting cavities in subtidal coralline rubble from the fore-reef (see also Kensley, 1994), is routinely found along with the upogebiid *Pomatogebia operculata* in interstices of broken rubble retrieved from depths greater than 2 m. By contrast, the large and strongly armed *Axiopsis serratifrons* is widely distributed between pieces of coarse coral rubble in back-reef flats of Carrie Bow (0.5–2 m depths), there positioned to ambush prey from its somewhat concealed burrow aperture. In addition, two new species of *Paraxiopsis* described by Kensley (1996) both range into reef habitats of Carrie Bow Cay. Although *P. spinipleura* was originally found there in shallow (1.5 m) back-reef rubble, we have not encountered additional specimens. We have also not found additional materials of *P. hispidus*, previously collected at the reef drop-off in depths greater than 20 m.

A remarkable thalassinidean find at Carrie Bow was the April 2007 discovery of a laomediid assignable to *Naushonia* sp. (Figure 1e). Two specimens were captured, both from cavities of empty conch shells in shallow (<1.5 m) subtidal waters. These individuals appear to also represent an undescribed species of a rarely encountered genus in the northern Caribbean region. To date known only from Carrie Bow Cay, they are currently being described.

The thalassinidean fauna of the general region also includes an abundant nearshore species, *Lepidophthalmus richardi*, adapted to euryhaline waters and muddy sand shorelines of the Stann Creek District (Felder and Manning, 1997). This species has not been found in habitats immediately associated with Twin Cays or Carrie Bow Cay, despite intensive search.

These collections have allowed us to update and expand the burrow distribution schemes for Belize given by Dworschak and Ott (1993). We herewith add additional taxa and habitat distributions (Figure 2) to underscore the overlooked diversity of infaunal macrocrustaceans, some of which are dominant bioturbators.

Note Added in Press

Additional sampling in Belize was conducted in February 2009. Observations in shallow waters at Twin Cays confirmed that populations of *Charybdis helleri* remained as seen in 2007. Further sampling for thalassinideans supported accounts on the preceding pages, with noteworthy additions. Sampling among shoreline mangrove roots at Twin Cays produced the first specimens of the Axianassidae, representing new records for *Axianassa intermedia* Schmitt, 1924. Five such specimens were extracted by yabby pump from beneath a surface area of no more than 0.25 m^2 at low tide, but less productive adjacent sampling suggested heterogeneous patterning. Given the small size of these specimens, we question whether this species accounts for burrows provisionally attributed to *A. australis* on the basis of castings mentioned on the preceding pages. From these same habitats at Twin Cays, the first specimen of the callianassid *Biffarius fragilis* (Biffar, 1970) was captured, along with a specimen of the same *Naushonia* sp. reported from Carrie Bow Cay on preceding pages. Finally, the first specimen of the family Callianideidae, *Callianidea laevicauda* Grill, 1959, was taken from intertidal rubble of the exposed reef crest at Carrie Bow Cay. These latest efforts confirm presence of at least one species of the family Axianassidae, add a seventh thalassinidean family to our report, and bring the documented number of thalassinidean species in our survey to at least 19.

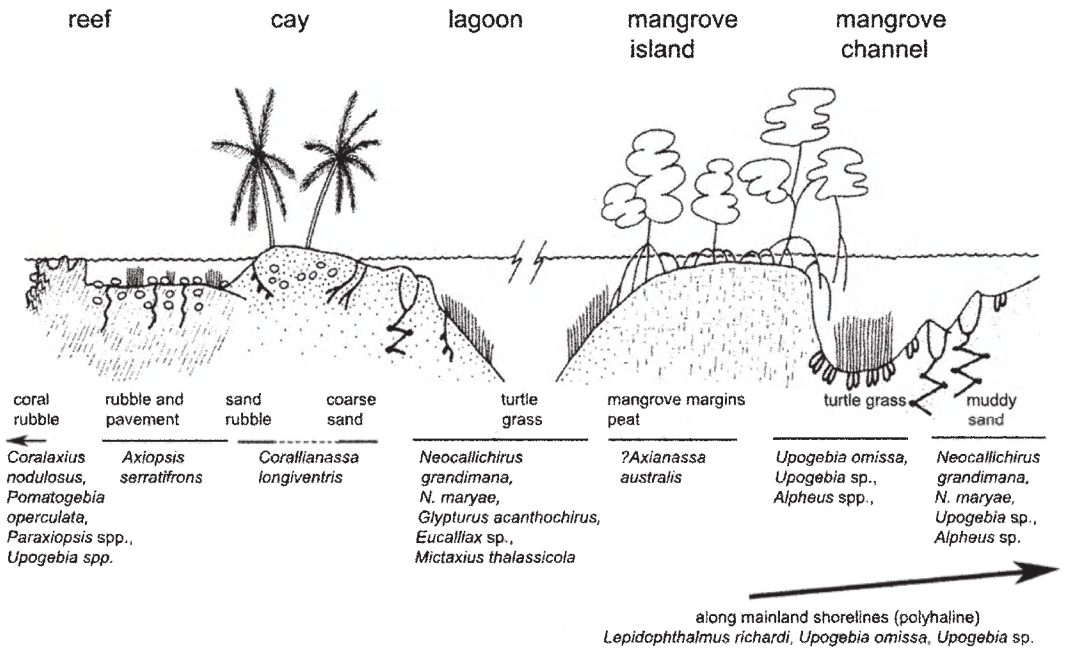

FIGURE 2. Schematic of thalassinidean distributions in channel and back-reef environments near Carrie Bow Cay and Twin Cays, Belize. Modified from Dworschak and Ott (1993:fig. 9).

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Imposex in One of the World's Busiest Shipping Zones

Carter Li and Rachel Collin

ABSTRACT. Tributyltin pollution from antifouling paint is well known to disrupt the endocrine system in female marine gastropods. The masculinization of females, including the aberrant growth of a penis and vas deferens and occlusion of the capsule gland, has been reported primarily in neogastropods and is particularly well documented in muricids. Compared to temperate areas, few studies of imposex have been undertaken in the tropics, and there are few studies in general on non-neogastropods. Here we report a high frequency of imposex near the Pacific mouth of the Panama Canal in two species of muricids and two species of calyptraeids. The frequency of imposex declined rapidly with distance away from the canal, and several species appeared to be mostly normal less than 10 km from the entrance. This is the first report of imposex in *Acanthais brevidentata*, *Thaisella kiosquiformis*, *Bostrycapulus calyptraeformis*, *Crepidula* cf. *nivea*, and *Anachis fluctuata*. Because imposex has not previously been reported for the Calyptraeidae, a family of protandrous gastropods, a laboratory study was conducted to verify that imposex was not simply retention of the penis after sex change. The 2007 ratification of the International Maritime Organization's convention on antifouling systems should reduce the levels of TBT worldwide, but the persistence of this compound in sediments suggests that imposex may continue to be a problem at the mouth of the canal as routine dredging and large tides frequently resuspend sediment.

INTRODUCTION

Tributyltin (TBT) is well known to be a highly effective antifouling agent, used primarily on ship hulls, but it has numerous detrimental effects on a wide variety of non-target taxa. Despite having demonstrable effects on molluscan shell growth (Alzieu et al., 1981), embryological development of fish and marine invertebrates (Hano et al., 2007; Inoue et al., 2006), neurulation in ascidians (Dolcemascolo et al., 2005), and testosterone metabolism in mysids (Verslycke et al., 2003), the most well studied and widespread effect is the disruption of the endocrine system in marine gastropods. Exposure to very low levels (as little as 0.5 ng/L) of TBT causes the masculinization of females, including the aberrant growth of a penis and occlusion of the capsule gland (Gibbs and Bryan, 1996). This condition is referred to as imposex, and severe cases can lead to reproductive failure. For example, an extreme case of population decline as a result of imposex has been demonstrated for *Nucella lapillus* in southwest England (Bryan et al., 1986; Gibbs and Bryan, 1986).

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In a recent review, Shi et al. (2005) reported that imposex has been recorded in 170 species of gastropods from 28 families. The vast majority, 134 species, are neogastropods. Among the non-neogastropod, caenogastropod families, ampullarids, rissioids, cypreaids, cymatids, and tonnids all contain several species for which imposex has been reported (Shi et al., 2005). Although the taxonomic coverage is wide, much of the basic information on imposex in relation to TBT pollution is centered on muricids, buccinids, and conids (Fioroni et al., 1991; Shi et al., 2005). On a worldwide scale, it is necessary to extend the scope of studies to include more tropical forms and locations (Ellis and Pattisina, 1990) to get a global picture of the effects of TBT pollution on gastropods.

The Panama Canal is one of the world's busiest shipping zones, and commercial transport through the canal represents about 5% of world trade. About 14,000 vessels pass through the Canal annually (statistics available from the Autoridad del Canal de Panama web site http://www.pancanal.com/), and the most common shipping route is between the east coast of North America and Asia. Most of the shipping traffic is composed of large, oceangoing vessels, which have not previously been subject to restrictions on the use of tributyltin antifouling paint. The entrance to the Canal, on the Pacific coast adjacent to Panama City, was the site of Rodman Naval Base (1943–1999), and is currently the site of the container port of Balboa and a shipyard. The anchorage for the canal commonly has more than 30 vessels waiting to transit the Canal. The substrate in this area is primarily a mix of rocky debris and sandy mud in the intertidal and fine mud in the subtidal. With the consistently high levels of shipping traffic, frequent dredging, and muddy substrate (which is known to retain TBT for years, as reviewed in de Mora, 1996), the local levels of TBT and, therefore, imposex are expected to be higher around the entrance to the Canal than they are along the open coast. We conducted a survey of four common intertidal gastropod species around the mouth of the canal to document the levels and geographic extent of imposex in this area.

MATERIALS AND METHODS

Gastropods were collected between February and April 2005 from four sites along the Pacific coast of Panama at varying distances from the mouth of the Panama Canal (Figure 1). The site closest to the mouth of the canal consisted of rocky outcrops near Farfan beach (8.93°N, 79.58°W) and the Bridge of the Americas. Progressively further away to the west were Isla Venado (8.91°N, 79.63°W), Chumical (8.5°N, 79.66°W), and Bique (8.90°N, 79.66°W). In November 2007 additional samples were collected from Punta Culebra (8.91°N, 79.53°W), which faces the entrance to the Canal and is at the edge of the Canal anchorage.

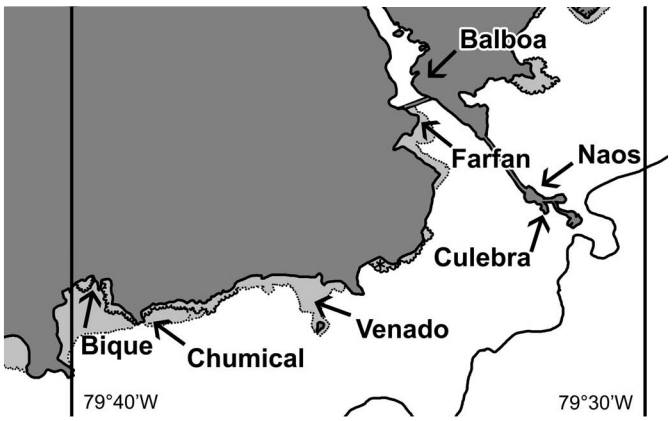

FIGURE 1. Map of the study area at the entrance to the Panama Canal. Arrows indicate the sites of sample collection and locations mentioned in the text.

We collected four species, which were clearly identifiable and abundant at two or more of the sites. Efforts were made to collect the same species from all sites, but because of the habitat heterogeneity in the area, we were not able to collect sufficient numbers of females for statistical analyses for several sites. Adequate samples were collected for the muricids *Acanthais brevidentata* (Wood, 1828) from Farfan and Chumical and *Thaisella kiosquiformis* (Duclos, 1832) from Farfan and Bique, and the calyptraeids *Bostrycapulus calyptraeformis* (Deshayes, 1830) and *Crepidula* cf. *nivea* from Farfan, Venado, and Chumical (Table 1).

Shell length was measured with vernier calipers, and live snails were extracted from their shells. The reproductive system was immediately examined under a stereomicroscope, and the sex was determined based on characteristics of the gonad and presence or absence of seminal receptacles and seminar vesicles. If the sex was not easily identified, sex was verified by examining gametes from a smear of gonad. The length of the penis (if present) was measured using an ocular micrometer on a stereomicroscope.

TABLE 1. Frequency of imposex in four gastropod species at sites arranged here from nearest to furthest from the Panama Canal entrance. Frequency at each site was compared to that at Farfan (the site at the entrance to the Canal) using a Fisher's exact test. A one-tailed test was used, but two-tailed results did not differ; * $P = 0.001$; ** $P = 0.0001$; *** $P = < 0.0001$.

	Site				
Species	Farfan	Culebra	Venado	Chumical	Bique
Muricids					
Thaisella kiosquiformis	29/53	–	–	–	13/52*
Acanthais brevidentata	8/32	–	–	0/57**	–
Calyptraeids					
Bostrycapulus calyptraeformis	60/63	22/43[a]***	2/79***	1/122***	–
Crepidula cf. *nivea*	87/90	19/22	–	0/99***	–

[a] Significantly different from Venado and Chumical <0.0001.

Significant differences in the frequency of imposex between the entrance to the Canal and more distant sites were tested for using Fisher's exact test. Analysis of covariance (ANCOVA) was used to examine the relationship between penis length in male and imposex females, with shell length as a covariate for samples collected from Venado and Farfan. Because samples from Culebra were preserved in ethanol before examination, the penis length from these samples could not be directly compared to the others that were measured fresh.

Experiments to determine if imposex develops in adult snails after exposure to ambient water levels of TBT were conducted at STRI's Naos Marine Laboratories, only a few hundred meters from the Culebra site. *Anachis fluctuata* (Sowerby, 1832) and *Bostrycapulus calyptraeformis* were both collected from Isla Venado, an area with low levels of imposex, and maintained in the laboratory. Sixty adult *Anachis fluctuata* were kept in a 100 L fiberglass tank in the outside seawater system and fed frozen commercial clams once a week. After five months the animals were killed and levels of imposex were determined as already described. *Bostrycapulus calpytraeformis* were collected as small males. They were maintained in pairs in the laboratory in 350 mL plastic cups. The water was changed every other day and the animals were fed 10 mL *Isochrysis galbana* culture every day. Animals were measured every four weeks, and their sexual state was recorded on the basis of external features. The experiment was terminated after 400 days. Both species were cultured using the same source of seawater (from the side of Isla Naos away from the Canal entrance), and neither was exposed to local sediment other than that which settled out of the seawater.

RESULTS

Field Collections

Imposex was detected in all four species. In the two muricids, the imposex was almost always in the early stages with limited penis development and no indication of any occlusion of the capsule gland. We never observed imposex that was so far advanced that the females were found to retain eggs or that an obvious vas deferens had developed. Imposex in the calyptraeids was more developed; penes were large in many specimens and could easily be confused with a normal male penis. Several imposex females of *Bostrycapulus calyptreaformis* and *Crepidula* cf. *nivea* were observed brooding egg capsules, showing that imposex females were not sterile. Near the entrance of the Canal the frequency of imposex ranged from 25% to 50% in muricids and was greater than 80% in calyptraeids. The number of females collected for each species at each site and the frequency of imposex are given in Table 1. In all cases the frequency of imposex was significantly higher near the entrance to the Canal than at farther sites (Table 1).

Acanthais brevidentata: Because there were no imposex individuals in Bique and because animals from that site were significantly larger (mean = 28.9 mm) than from Farfan (mean = 26.9 mm; $P < 0.001$), comparisons of imposex females with normal males and females were conducted for data collected from Farfan only. Imposex females were significantly larger (length = 30.1 mm) than non-imposex females (length = 26.4 mm; $P < 0.02$). ANCOVA showed that there were significant effects of shell length ($P = 0.01$) and imposex ($P < 0.001$) on

penis length as well as a significant interaction effect ($P < 0.04$). Penes of imposex females were smaller than those of normal males, and male penis length increased with shell length, although imposex penis length was not associated with shell length (Figure 2).

Thaisella kiosquiformis: Animals from Bique and Farfan did not differ in size, nor did the sexes differ in size. Imposex females were also the same size as non-imposex females. The average size for all categories was 26–27 mm. Data from Bique and Farfan were combined for the analysis. ANCOVA showed that there were significant effects of shell length ($P < 0.0001$) and imposex ($P < 0.0001$) on penis length as well as a significant interaction effect ($P = 0.003$). Penes of imposex females were smaller than those of normal males, and penis length increased with shell length in both sexes (Figure 2). There was a significant incidence of imposex at Bique, despite it being the site furthest from the Canal. We attribute this relatively high frequency of imposex to this site's prox-

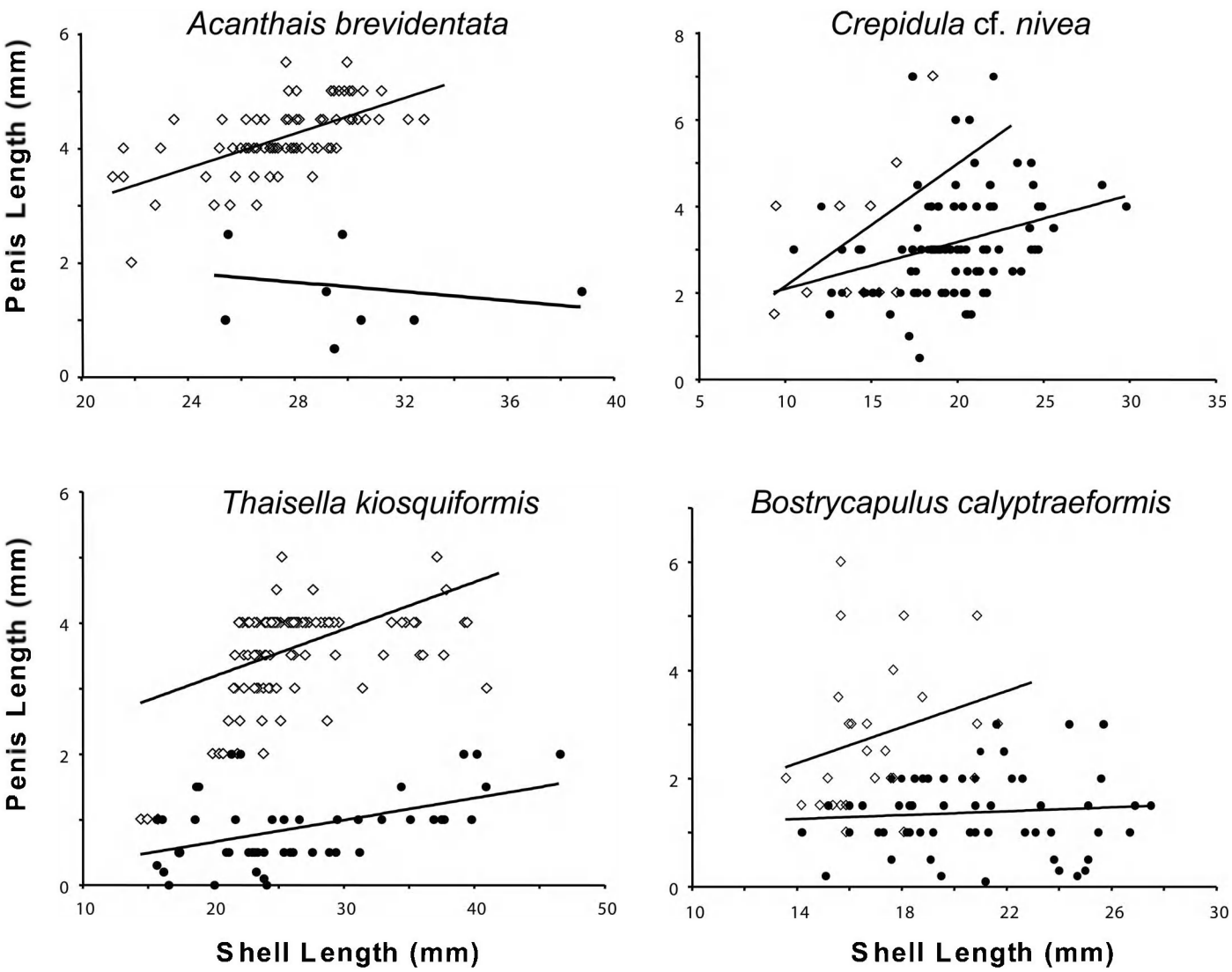

FIGURE 2. Relationship between shell length and penis length for males (white diamonds) and imposex females (black dots) of *Acanthais brevidentata*, *Thaisella kiosquiformis*, *Crepidula* cf. *nivea*, and *Bostrycapulus calyptraeformis*.

imity to a dry dock facility slightly further to the west in Vacamonte.

Crepidula cf. *nivea*: Calyptraeids are protandrous hermaphrodites (Collin, 2006) and the small animals are almost always males; therefore, we did not make as much effort to collect the smallest animals as we did in the other species. The size of females differed significantly between the sites (16.1 mm for Chumical vs. 19.6 mm for Farfan; $P < 0.0001$). Imposex females were larger than non-imposex females. Because all females at Chumical were normal and virtually all in Farfan had imposex, this size difference cannot be distinguished from a site effect on size. An ANCOVA showed that shell length had a significant effect on penis length ($P = 0.03$), that imposex females and males did not differ in penis length as there was considerable penis growth in the imposex females, and that there was no significant interaction between imposex status and shell length (see Figure 2). Although there were significant levels of imposex in samples from Culebra, in all these cases the penis was very small; they were not much more developed than a small bump at the base of the tentacle, whereas those from Farfan were often as long as or longer than the tentacles.

Bostrycapulus calyptraeformis: The average size of females differed significantly among the three sites (17.5 mm at Chumical; 16.5 mm at Venado; 20.6 at Farfan; $P < 0.01$). Again, because nearly all the females in Farfan had imposex but no imposex was detected in the other locations, the larger size of imposex females may have been a site effect. ANCOVA analysis of animals from Farfan showed that there was a significant effect of imposex on penis length ($P < 0.001$), and imposex females had smaller penes than males. Shell length and the interaction between shell length and imposex had no significant effect on penis length. The level of imposex in animals from Culebra was again very rudimentary, with penes little more than a nub at the base of the tentacle.

In summary, all four species showed significant higher rates of imposex near the entrance of the canal. By 20 km away, rates were generally of the order of 1%–2%. In the two muricids and one of the calyptraeids, the penes of imposex females were smaller than those of similar-sized males. In the two muricids and the other calyptraeid, shell length was a significant covariate of penis length, and in two species the penis length of imposex females increased with shell length.

Laboratory Experiments

After five months in the laboratory, 2 of 29 female *Anachis fluctuata* had developed penes, indicating that this species can develop imposex. However, this was not statistically significantly different from the frequency of imposex in the field in Venado ($P = 0.09$; Table 2). No comparisons to the entrance to the Canal could be made because this species could not be found there.

Of the 60 *Bostrycapulus calyptraeformis* that were raised in the laboratory, the largest animals in 6 of the 30 cups retained penes throughout the experiment and did not change to become female. In the remaining 24 cups, the larger of the 2 animals lost the penis, indicating sex change from male to female. Of these 24 animals, 10 lost the penis and then subsequently regained it 1 to 3 months after sex change. In many cases the penis was not as long or thick as a normal male penis, but they were fairly large, and casual observers would be likely to categorize such animals as males (Figure 3). The largest animals in the remaining 14 cups underwent transition to normal females and did not develop imposex before the end of the experiment. The smaller of the 2 animals in each cup was not examined, as they usually remain male in the presence of the larger animals (Collin et al., 2005).

TABLE 2. Frequency of imposex from field-collected and laboratory-reared snails.

Species	Laboratory	Venado	Chumical	Fisher's exact test, *P* value
Anachis fluctuata	2/29	1/133	–	$P = 0.091$
Bostrycapulus calyptraeformis	10/24	2/79	1/122	$P < 0.0001, < 0.0001$

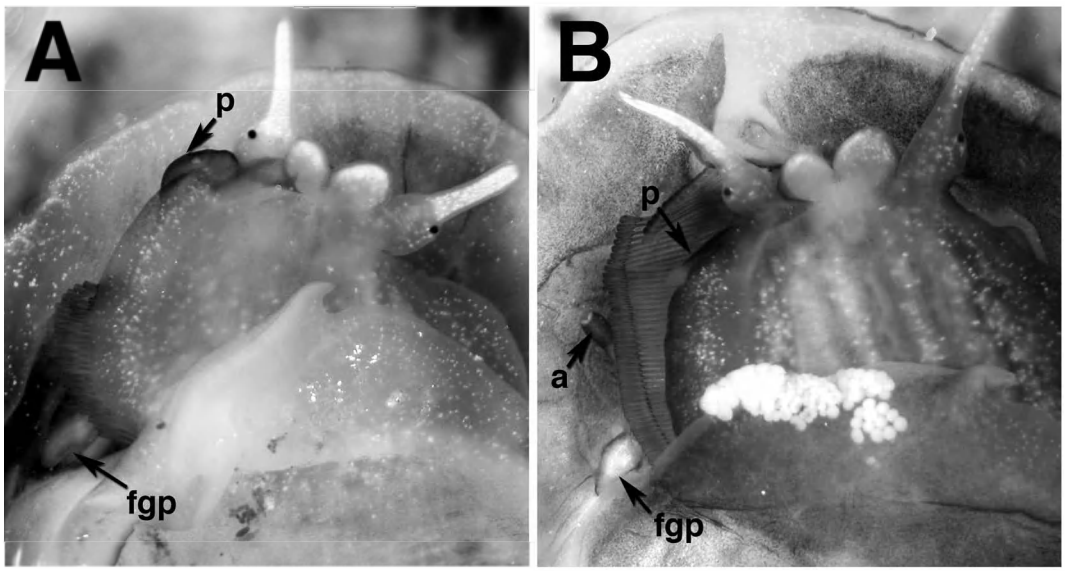

FIGURE 3. A, B. Photographs of two individuals with well-developed imposex in *Crepidula* cf. *nivea* from Farfan, with penis (p) and female genital papilla (fgp) indicated in each image. B. The female genital papilla can be confused with the anus (a), which is usually obscured by the gills; brooded eggs are visible as the light mass in this image.

DISCUSSION

Imposex was found in all snail species examined from the mouth of the Panama Canal, and in all cases the frequency and severity of imposex declined with distance away from the Canal. The frequency of imposex differed among the species examined, with calyptraeids more likely to display imposex than the muricids. Calyptraeids showed higher frequencies of imposex at the mouth of the Canal, and the penes of imposex females were much more fully developed then we ever observed in muricids. Species differences in sensitivity both to TBT (Wilson et al., 1993; Tan, 1999) and to its bioaccumulation (Liu et al., 1997) have been demonstrated in other surveys. Liu et al. (1997) found that imposex was much more severe in *Thais* species than *Morula*, despite similar organotin burdens, and suggested a genus-specific susceptibility to organotin pollution with the ranking order of *Nucella*, *Thais*, and *Morula*. The differences in habitat (high on the intertidal versus low on the intertidal), diet, and physiology have been suggested as causes of interspecific differences in imposex (Tan, 1999). If TBT were primarily waterborne either in solution as *bis*(tributyltin) oxide or adsorbed by suspended solids (de Mora, 1996) at our study sites, it is possible that filter-feeding calyptraeids would be exposed to more TBT, by filtering large volumes of water, than would other gastropods. Suspended particles may have TBT adhered to them and may be captured in the mucous net and ingested during filter feeding, thus increasing the exposure of calyptraeids relative to the muricids. These scenarios are not in agreement with a number of laboratory studies (Bryan et al., 1989; see Gibbs and Bryan, 1996, for review) that show that TBT accumulates more rapidly from the diet than from the ambient water and which suggest that carnivores could accumulate more TBT from their diet than would herbivores. However, controlled experimental comparisons of bioaccumulation between carnivorous and suspension-feeding gastropods have not been made, and the effects of suspended solids have not been examined.

Another factor that can influence the expression of imposex is age. Because extended exposure to TBT is necessary to elicit imposex, those species that are longer lived or slower growing may be more likely to have high levels of TBT and thus exhibit imposex. Studies have also shown that juvenile snails are more sensitive to TBT than are adults (Gibbs and Bryan, 1996). Our data for *Acanthais brevidentata*, showing that females with imposex are larger than normal females, are consistent with either increase in imposex development with long-term exposure or recent reductions in TBT levels. However, *Thaisella kiosquiformis* did not show this pattern. Few data on the

age or lifespans of tropical gastropods are available and so this possibility is difficult to evaluate. However, Panamanian calyptraeids grown in the laboratory generally reach maturity at sizes similar to animals that matured in the field, in less than a year (Collin et al., 2005, and personal observation), and it seems unlikely that TBT in the sediment, which has a half-life of years, would have changed drastically in such a short interval.

Imposex has not been previously reported in calyptraeid gastropods. Because animals normally change from males to females and transitional animals may sometimes retain a penis while also showing well-developed female reproductive structures, it is possible that imposex individuals have previously been misidentified as undergoing the normal transition between the male and females phases. Here we found, in sites with low expected TBT exposure, that there are virtually no individuals that display both male and female characteristics at the same time. In addition, our laboratory studies show that during sex change the penis can be reabsorbed and that the penes of imposex individuals can grow following this reabsorption. These results show that the abundant large females with penes collected at the entrance to the Canal are indeed imposex females and not transitional individuals that have yet to lose the penis.

Numerous studies have shown a tight relationship between levels of TBT in the environment, levels of TBT in gastropod tissues, and the frequency of imposex within species (Gibbs et al., 1987; Horiguchi et al., 1994; Minchin et al., 1997; Ruiz et al., 1998). However, the relationships between sites, species, and the different types of triorganotins are not always simple (Ide et al., 1997). Imposex has also been shown to be a more sensitive way to detect TBT than many chemical detection methods, and imposex has been used as a bio-indicator when TBT levels are too low for easy analytical detection (Gibbs and Bryan, 1996). Despite an extensive literature on the relationship between TBT and imposex, one study (Nias et al., 1993) indicates imposex could result from exposure to paint matrix or copper. However, this result has not been pursued or elaborated. Although we could not measure levels of TBT directly at the sites around the Canal, it can, in the light of this literature, be inferred with some level of confidence that the exposure of animals to TBT is higher at the entrance to the Canal than it is in the surrounding areas. Despite the high levels of shipping and presumably high levels of TBT leaching into the surrounding water, the development of imposex was not so severe as has been reported for areas with high shipping traffic in Europe and Asia, and TBT does not have an extreme impact on reproduction by occluding the pallial oviduct or splitting the bursa copulatrix and capsule gland, as has been reported from these regions (Oehlmann et al., 1996; Shi et al., 2005). Less obvious effects on reproduction were not directly evaluated in this study. The high amount of flushing in the area, from large volumes of discharge from the Canal and the 6 m tides, may help to prevent local buildup of high concentrations of TBT in this partially enclosed area.

In 2002 the International Maritime Organization adopted a Convention on Antifouling Systems (AFS) that called for a global prohibition of the application of organotin compounds as biocides in antifouling systems on ships by 1 January 2003 and a complete prohibition by 1 January 2008. However, the prohibition was only to be implemented 12 months after 25 states representing 25% of the world's merchant shipping tonnage ratified it. In September 2007 this quota was met when Panama ratified the convention, and therefore these regulations went into effect in September 2008. As the AFS convention applies to ships flagged in, operated by, or docking in states that have ratified it, the convention should significantly reduce the exposure of Panama's marine habitats to TBT pollution in the coming years. This regulation is especially important because the planned expansion of the Canal in 2014 will significantly increase shipping traffic along both the Pacific and Caribbean coasts of Panama.

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We are grateful to L. Weintraub and M. Salazar for assisting with field collections of animals, M. deMaintenon for verifying the identification of the columbellid samples, and M. Torchin for sharing his knowledge of gastropod reproductive systems. We thank the Autoridad Maritima de Panama for providing collecting permits. This project was conducted during a McGill University Field Semester in Panama.

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Shorefishes of the Tropical Eastern Pacific Online Information System

D. Ross Robertson

ABSTRACT. Shorefishes of the Tropical Eastern Pacific Online Information System (SFTEP) version 1, 2008, provides an online electronic identification guide and information system for the known fauna of shorefishes found in the Tropical Eastern Pacific. SFTEP allows users (i) to identify all shorefishes known from the Tropical Eastern Pacific (TEP) (1,287 species in version 1) and (ii) to analyze and conduct biogeographic research on the composition of that fish fauna at varying spatial scales. Tools for identification emphasize the use of color photographs, along with descriptive text that highlights key morphological features; allow comparison of similar species; facilitate identification of unfamiliar species using information on location and fish morphology (shape, color pattern, and color); and incorporate interactive keys to members of two species-rich families (Gobiidae, Sciaenidae) that have many similar-looking species. To accommodate nonspecialist users, scientific jargon is minimized; the interface is intuitive and user-friendly, and searches for species can be made using common names. The Research Engine, which provides information about the composition of local faunas and the regional fauna, allows users to compare geographic ranges of multiple taxa, to construct faunal lists of taxonomic and functional groups of species for single and paired sites, and, at varying spatial scales, to determine local endemism and to display region-wide patterns of species richness of different taxa and functional groups of fishes. The system is accessible online at www.stri.org/sftep.

INTRODUCTION

Shorefishes of the Tropical Eastern Pacific Online Information System (SFTEP), version 1, 2008, provides an online electronic identification guide and information system for the known fauna of shorefishes found in the Tropical Eastern Pacific (TEP). This version represents the latest iteration of a series that began with the 1994 English-language printed identification guide of the same name (Allen and Robertson, 1994). That book was followed by a Spanish-language printed edition in 1998 (Allen and Robertson, 1998). Both these works were succeeded by a dual-language CD-based information system in 2002 (Robertson and Allen, 2002), which was revised and expanded in 2006 (Robertson and Allen, 2006).

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SYSTEM FEATURES

Dual Language Interfaces

The system incorporates separate, full-capability English- and Spanish-language interfaces.

Aids to Visual Impairment

The system incorporates two types of aids:

1. Variable map-color formats are available. Users can select various color schemes designed to accommodate different patterns of color blindness, including monochrome or color with the ability to select colors.
2. Page layout structure accommodates variation in font size. Two page layouts are possible—landscape and portrait. Page structure is stable over a threefold range in font size.

SYSTEM MODULES

Home

The home page provides an overview of the capabilities of the system and access to all major modules through buttons and/or tabs that act as shortcuts (Figure 1). In addition there are links to several modules not accessible from other parts of the system: to the **Copyright notice**, a switch to change between **English** and **Spanish** interfaces, and to the websites of the **Smithsonian Tropical Research Institute** and **Coeus,** the company that programmed the system.

Each of the authors and major contributors of information directly related to the construction of SFTEP has an individual contributor page, accessible from the **Contributors** button and from a link at the top of any screen. In addition, the major contributors of information presented on each family are noted on each family page.

General Information

General information about Shorefishes of the Tropical Eastern Pacific Online Information System (SFTEP) is shown in Figure 2; this module includes three sections.

Introduction

The "Introduction" to the TEP and its shorefish fauna provides background information on the oceanography of the region and its marine habitats (geographic and temporal variation in climate, rainfall and salinity, primary production and coastal upwelling systems, ocean current systems, influences of the El Niño cycle, shoreline habitats and rocky and coral reefs in the region); a history of taxonomic fish guides, major modern guides, global online resources, systematic ordering of the fishes, and the scientific and common names of fishes); the ecology of TEP shorefishes (species that occur in the upper 100 m of the water column over the continental shelf or within ~50 km of the shore), their use of different environments and habitats, their depth-distribution patterns, their dietary groupings, and their modes of reproduction; and the zoogeography of the fauna—studies of the region's zoogeography, resident versus vagrant species, relationships of the fauna to the faunas of other areas, distribution of the fauna in different climate zones, the geography of variation in species richness and local endemism throughout the region, and biogeographic subdivisions of the TEP.

Features & User Guide

The "Features & User Guide" section describes system features, providing information available on taxon pages, databases on biological and zoogeographic characteristics, information used to identify fishes, an interactive glossary of ichthyological terms, the functioning of the zoogeographic research engine (comparison of taxon ranges, assembly of faunal lists, determination of local endemism, assembly of maps of species richness and sampling intensity, assembly of lists of species from predefined parts of the TEP), the functioning of the interactive library, the database of images, and credits to contributors.

Acknowledgments

The "Acknowledgments" section recognizes support from STRI, funding, government permissions, logistical support, assistance collecting fishes, identification of specimens and reviews of section, databases, Spanish translations, images and illustrations, database management, and digital image preparation.

The Fishes

A Page for Each Species, Genus, and Family

Information on the members of the fauna is provided through interlinked species, genus and family pages. Genera and species are ordered alphabetically within each family, with families being arranged in "phylogenetic" order. "The Fishes" module provides access to **Species**, **Genera**, and **Families** pages by browsing within each taxo-

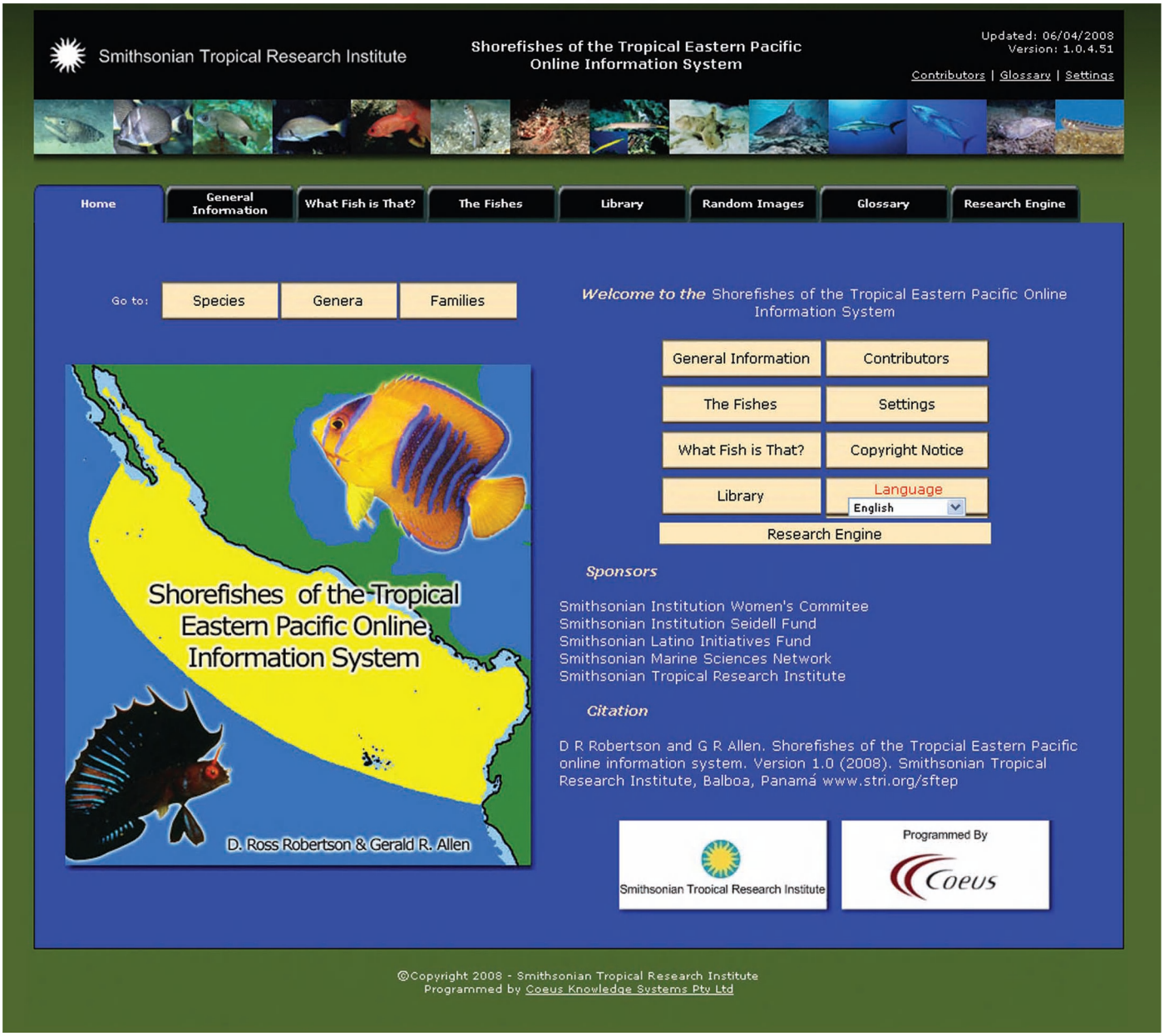

FIGURE 1. Opening screen and "Home" module.

nomic level, browsing from within a **Systematic Tree** (with optional alphabetic or systematic ordering, and optional use of common or scientific names), browsing from within a **Book Mode** (species within genera within families), or user-selection of level and taxon from pull-down lists.

Family and genus pages include a brief introduction to systematics, biology, global geographic distribution, and an estimate of the number of genera and species worldwide and present within the TEP; a text description of distinguishing morphological features—*black text* indicates the least distinctive features for identification purposes, *red text* indicates important features, and *red text with yellow high-lighting* shows the most important features (see Figure 3); a database map of the taxon's range limits distribution in the TEP (assembled from the distributional maps of component species) and a list of component genera and species with links to their pages; an image of a representative species that has a key feature

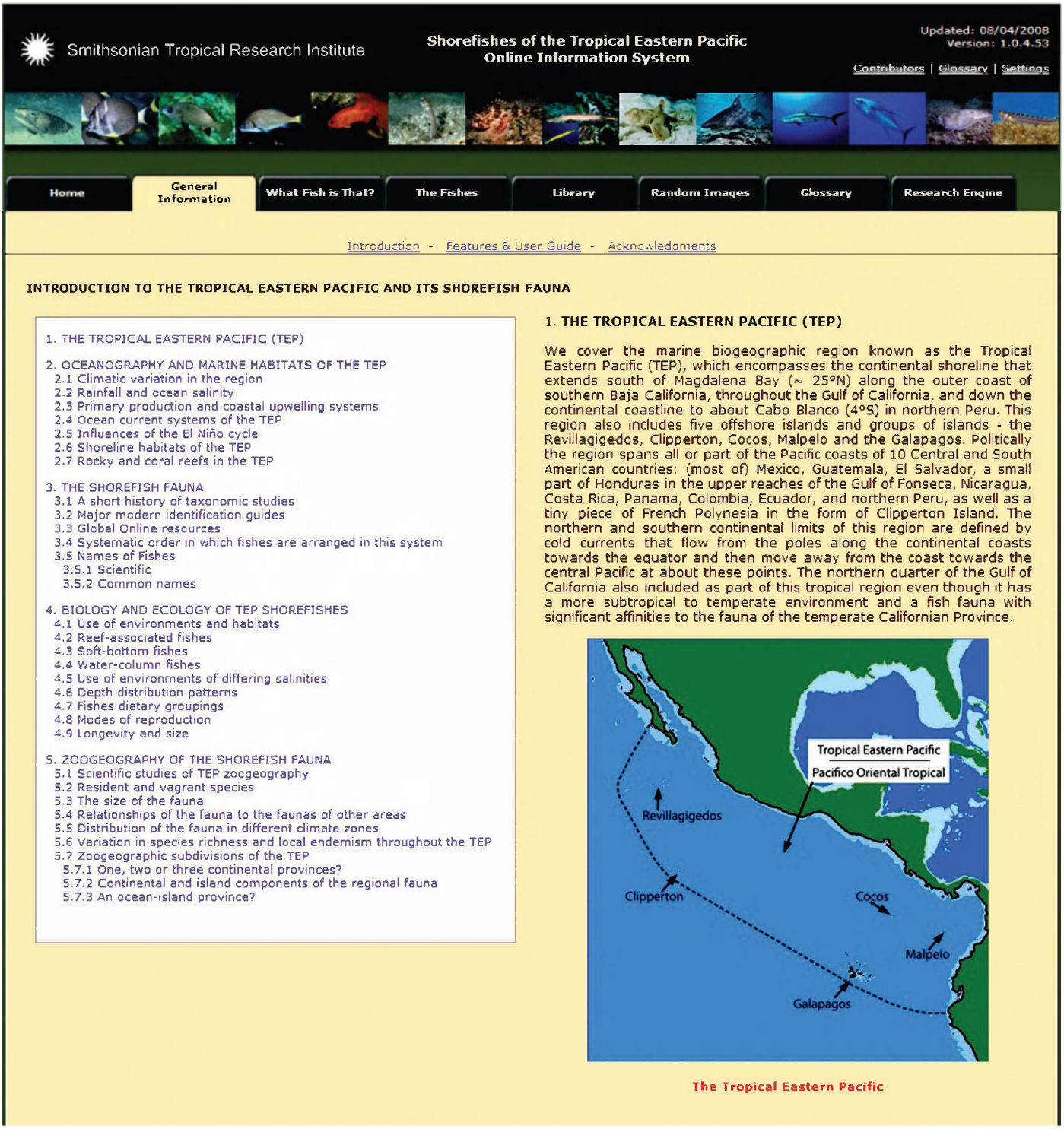

FIGURE 2. Opening screen from the "General Information" module.

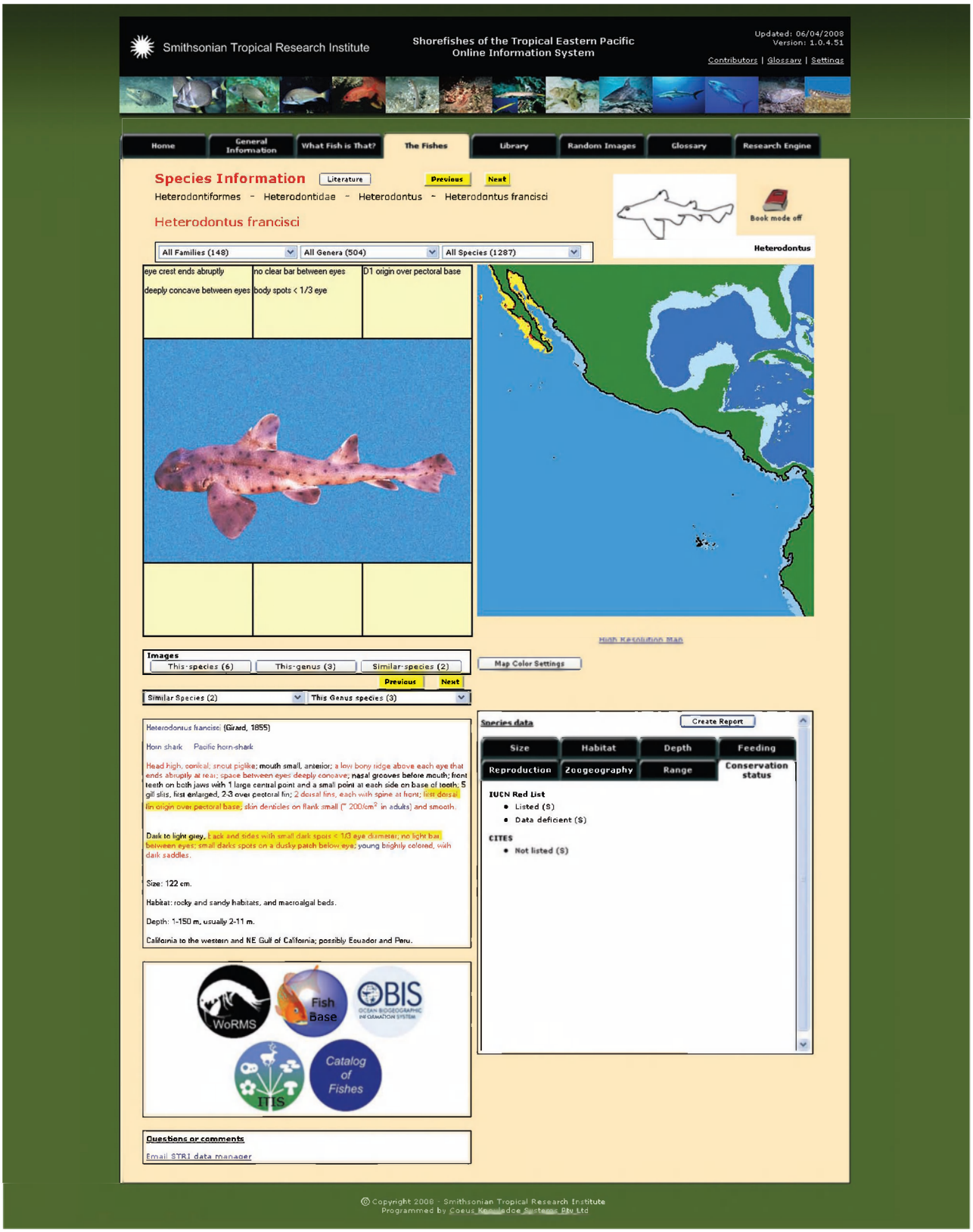

FIGURE 3. Example of a species page.

overlay indicating diagnostic features of the taxon that distinguish it from similar taxa; and comparisons with similar taxa. To assist in distinguishing look-alike fishes, each taxon page includes a button-link that allows the user to compare images (with key feature overlays) of such taxa. Each page also includes a list of designated similar fishes (at the same taxonomic level), with links to their taxon pages.

Species data pages are similar to genus and family pages but also include multiple images (e.g., juvenile, female, male, color morphs, specific morphological characteristics) and access to a downloadable list of species zoogeographic and ecological attribute data. For example, the **Zoogeography** tab includes **Global endemism**, a species global-scale distribution and its occurrence outside the TEP; **Regional endemism**, distributions of species within the TEP, including TEP endemics (species that occur only in the region or have the great bulk of their distributions within it), temperate eastern Pacific endemics (whose distributions are primarily to the north and south of the TEP, in the Californian and Peruvian provinces), eastern Pacific non-endemics (species that have populations outside the eastern Pacific, for instance circumtropical species). Categories relating to the distributions of species within the TEP include the occurrence of endemic and non-endemic species at offshore islands and/or the continental shore, whether TEP endemics are endemic to the offshore islands (and which islands) or to the mainland, and to which of the three mainland provinces (or combinations thereof) each continental TEP endemic is restricted. Attributes for **Climate zone** and **Residency** (whether the species appears to be a resident or a vagrant in the region) are also included.

Other species ecological attributes that are presented include the following:

- the known maximum total length of each species;
- a species' maximum and minimum depth of occurrence;
- the salinity of environment(s) in which a species occurs;
- the specific habitat(s) a species uses (as well as habitat categories as defined by FishBase, see www.FishBase.org);
- whether a species is restricted to inshore waters or occurs in offshore, oceanic conditions;
- the position in the water column at which a species lives (e.g., bottom, surface);
- a species' feeding group (e.g., carnivore);
- items in a species' diet (e.g., fishes, pelagic crustaceans, microalgae);
- a species' reproductive mode (e.g., different types of eggs, live birth); and
- a species' CITES and IUCN REDLIST status.

When information is available (e.g., for diet) for a species itself, an "S" is given after the value in the database. In cases for which such species-level information is lacking, the page displays information for the genus (indicated by "G"), or for the family ("F") if there is no information for the genus.

Taxon pages includes direct links to external websites concerning the same taxon in the following external online sources: William Eschmeyer's Catalog of Fishes (www.calacademy.org/research/icthyology/catalog), which provides comprehensive up-to-date data on the systematics of fishes; FishBase (www.fishbase.org), which covers a variety of aspects of the biology of fishes; ITIS, the International Taxonomic Information System (http://www.itis.gov) and WoRMS, the World Register of Marine Species (http://www.marinespecies.org), both of which focus on scientific names of fishes; and OBIS, the International Biogeographic Information System (http://www.iobis.org), which aggregates geo-referenced databases of collection records of fishes.

What Fish Is That?

This module facilitates identification of unknown fishes using four distinct tools (Figure 4).

Find a Fish

This tool allows users who are not scientifically trained to identify an unfamiliar fish by choosing among the following in any order or combination, with the ability to back-up steps: **Where was it?**—select location and size of area in question on a database map—and combinations of **Body Shape**, **Color Pattern**, and **Colors**. Each step narrows the list of possibilities, with each species on the possibilities list linked to its image, and hence to its species page.

Identification Keys Search

Illustrated dichotomous keys are provided for the genera and species in the two families with the largest number of species: Gobiidae (88 species in 27 genera) and Sciaenidae (82 species in 26 genera). Search results link to species pages.

Compare Images of Fishes

This function allows simultaneous comparison of images of any two to six families, genera, or species selected. The feature enables users to compare "apples" with "oranges," whereas the comparison of designated similar taxa on taxon pages limits users to comparing only "apples." Resultant images are linked to taxon pages.

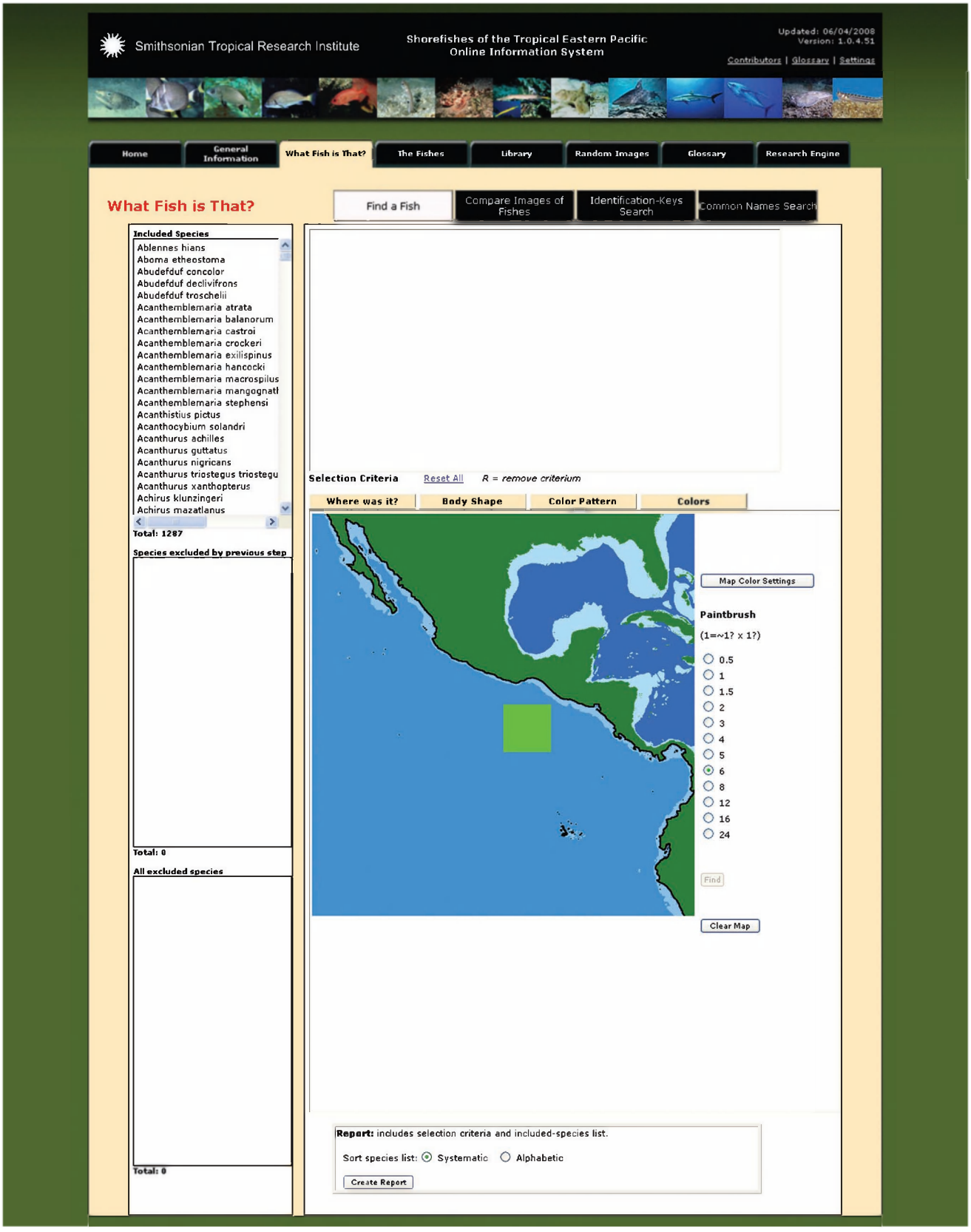

FIGURE 4. Screen capture from the "What Fish Is That?" module.

Common Names Search

Searches can be made for families, genera, and species from pull-down lists of common names, with results linked to taxon pages. The systematic tree or taxonomic hierarchy (see "The Fishes" module) also functions with the use of either common names or scientific names. The use of common names in this hierarchy helps users who are not scientifically trained to appreciate the relationships among fishes.

Glossary

An interactive glossary of taxonomic terms is provided that uses a combination of images and text to explain basic terms relating primarily to morphological characteristics that are used in the identification of fishes. In addition the usage of scientific jargon has been reduced as much as possible throughout the taxon pages by using simple descriptive phrases from everyday English to replace technical terms.

Research Engine

This module provides a variety of types of zoogeographical data and the ability to generate maps and site-specific species-lists based on complex queries constructed by the user (Figure 5).

Taxon Range Maps

This feature provides overlaid displays of the regional ranges of up to three taxa (species, genera, families, or a mixture thereof). In addition, maps can be generated of the geographic distribution of all species-range centroids (both paint and point data) and of all geo-referenced sampling points in the system's database.

Species Richness Displays

This feature provides maps with color-coded overlays of patterns of variation in species richness throughout the region. Those patterns include richness of individual families and richness of species in specified "functional groups" (e.g., species sharing one or more biogeographic and ecological attribute). Richness displays can indicate either absolute richness (number of species) or relative richness (number of species as a percentage of the local fauna). A display of relative sampling intensity indicates the number of species recorded at minimally one site within each unit of area (1° of latitude × 1° of longitude) as a percentage of the number of species whose ranges encompass that unit.

Species – List Assembly

Family and genus lists can be constructed for single locations. Species lists for "functional groups" of fishes for a particular location can be constructed using any combination of biogeographic and ecological attributes. Species lists include both single-location lists and lists of species found or not found at two locations. The spatial scale of a location in such a search varies from a single island to an area of variable shape and size, to the entire TEP or map. Locations are defined by the user employing a library of approximately 300 preformed templates that include geographic entities (e.g., shoreline, continental shelf, named gulfs), habitat features (e.g., mangroves, rocky shores, upwelling areas), islands (individual and archipelagos), biogeographic entities (provinces of the TEP), political areas (Exclusive Economic Zones and parts thereof), and marine reserves (individual reserves, combine country reserves). In addition, quadrants of varying sizes (12 groups ranging from 0.5° latitude × 0.5° longitude to 24° × 24°) used by the map of the **Find a Fish** tool in the "What Fish Is That?" module provide approximately 5,000 additional [square] templates.

Unconfirmed/Confirmed Occurrences

Single-area species lists indicate both likely occurrences (species whose ranges include the selected area) and confirmed occurrences (species with at least one collection record in the same area).

Local Endemism Indicator

This feature provides a list of species found only within one or two template areas, and nowhere else on the system's map.

List and Map Exports

Lists and Maps produced by searches are exportable/printable. Lists may be arranged alphabetically or systematically (genera and species arranged alphabetically within families arranged in systematic order).

Species Range Maps and Range Data

A database map on each taxon page incorporates two types of data: a two-dimensional painted representation of the geographic range based on museum and literature records of occurrence and range maps, and our own field surveys in Mexico, El Salvador, Costa Rica, Panama, Colombia, Ecuador, the Revillagigedos, Clipperton, Cocos,

FIGURE 5. Screen capture from the "Research Engine" module.

Malpelo, and the Galapagos; and points indicating site records from museum collections, the scientific literature, and our own field surveys. Geographic-range statistics derived from these two offsets of data and presented on species pages include: latitudinal and longitudinal limits, ranges and midpoints based on paint data, and, separately, site records; data on range characteristics derived from paint data have been adjusted in species that occur in the eastern Pacific beyond the limits of the system's base map; habitat area, based on number of painted pixels in the species range map; and separate range-area polygons with centroids based on painted data and site record data.

Continental Ranges

Comprehensive faunal lists exist for few locations, and large sections of the coastline and continental platform of the TEP remain poorly sampled, a situation that will not be rectified in the near future. Hence the range of most species on the continental shelf is derived from data on the northern and southern limits of occurrence. Thus painted areas on taxon page maps represent the potential range and potential habitat area, and a species is assumed to be present *in appropriate habitat* anywhere between those limits. Exceptions include species that are known to have wide gaps in their distributions, such as some well-known anti-tropical species. Those gaps are represented in the range maps of such species.

Habitat Area Calculations

Maps constructed for the determination of habitat areas incorporate information on habitat usage and depth range as well as the extent of the geographic range. Continental areas of range maps were modified to exclude large areas of habitat that was inappropriate for the particular species; for example, shorelines composed primarily of sand and mud were excluded from ranges of reef-fishes and rocky shores were excluded from ranges of fishes living on beaches or in lagoons and mangroves. The depth ranges of individual species were also taken into account: ranges of demersal species restricted to very shallow water (less than ~20 m depth) are indicated by lines that follow coastlines. For habitat area calculations of such species, the coastal strip of habitat was taken to be 1 km wide. Ranges of coastal species found in deeper water on the continental and insular shelves are divided into three groups: those occurring down to ~60 m have maps that span the inner continental shelf; species that are limited to depths below ~60 m occur on the outer shelf; and the third group has depth ranges (and maps) that span the entire shelf. Maps for pelagic species variously include parts of the shelf and/or open ocean, depending on the biology of the species.

Mercator Projection Distortions and Adjustments to Habitat Area Calculations

Mercator projection maps, such as that used in this system, incorporate distortions of both latitude and longitude that affect estimation of habitat area. In such projections lines of longitude are shown as parallel rather than converging with increasing latitude, and lines of latitude diverge with increasing latitude instead of remaining a fixed distance apart. When calculating the habitat area for each species those two distortions were taken into account by making appropriate adjustments to the areas of individual pixels in different latitudinal bands. Range polygon areas were calculated independently using the GIS (Geographic Information System) ArcInfo system.

Cleanup of the Geo-Referenced Records Database

Both the scientific literature and databases from museums inevitably include erroneous records as a consequence of misidentification of fishes and sites, as well as bookkeeping errors. In addition museum specimens of demersal species include not only individuals collected in demersal habitats but also larvae collected in the open sea far from adult habitat, and, in some cases, far from the known adult geographic range (Robertson, 2008). Records from the multisource database of ~67,000 collection site records included here that were adjacent to the currently known limits of the geographic range were used to adjust (by expanding) those ranges. However, we removed from the systems database those "suspect" records that were well outside the known habitat and geographic ranges of the "adult" phase of each species, based on extensive records from other sources, the biology of the species, and expert determinations of ranges. This cleanup process reduced the size of the database by approximately 6%. Points outside the current range were retained for some species that, because of overfishing, have had their historical ranges reduced. For example, historically the mackerel *Scomberomus concolor*, which currently occurs only in the northern Gulf of California, occurred throughout that gulf and also off California, USA (B. B. Collette, National Marine Fisheries Service Systematics Laboratory, personal communication, 2008).

Library

The library database (Figure 6) includes 1,143 citations. The citation for its original description is included for each species, along with citations of revisions of genera and families. Other citations include local and larger scale

FIGURE 6. Screen capture from "Library" module.

lists of species; identification guides to species; and publications about the biology and zoogeography of species inside and outside the TEP. Each citation is linked to the species it discusses (and hence to appropriate genera and families).

Exportable lists can be generated for:

- Citations linked to individual families, genera, and species.
- Species linked to a particular citation.
- Citations linked to a particular author, date, or source journal.
- The entire bibliography arranged alphabetically by author name.

Random Images

The image database incorporates 2,927 images. These include 2,346 color photographs that cover 83% of the fauna (1,068 of 1,237 species). In comparison, the 1994 book from which this system was developed included color images of 683 species and treated only 768 species.

This module presents color images in a randomized order.

Digital Manipulation of Images

The user should assume that **all** illustrations used in this system have been digitally manipulated to some extent, to increase their utility as identification aids. Such manipulation includes cropping, image sharpening, changes in lighting and contrast relationships of different parts of individual subjects, changes of subject-to-background contrast, changes of background to enhance subject visibility, the (occasional) combination of multiple images of fishes in a montage to provide examples of variation in color patterns within the same image, and minor repairs to fin membranes and removal of body blemishes (scratches, minor cuts, blood spots) that resulted from capture handling.

Image Credits

All images are accompanied by a relevant ownership credit, copyright notice, and usage notice. Each image is accompanied by a link to either the owner's e-mail contact or website.

Acknowledgments

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Nephasoma pellucidum: A Model Species for Sipunculan Development?

Anja Schulze and Mary E. Rice

ABSTRACT. Recent developments in metazoan phylogeny, especially with regard to the position of the Sipuncula in the annelid clade, have sparked a renewed interest in sipunculan development. If Sipuncula are annelids, they must have secondarily lost segmentation. By comparison with segmented annelids, they could provide important clues for the evolution of segmentation. A sipunculan model species is needed to examine fundamental developmental processes. Here we describe the development of *Nephasoma pellucidum* and explore its potential as a model species for sipunculan development. Like other sipunculans, *N. pellucidum* produces eggs with a thick, porous, multilayered egg envelope. Cleavage in *N. pellucidum* is spiral, holoblastic, and unequal. The species shows the most common, and likely ancestral, developmental mode in the group. Its life cycle includes a lecithotrophic trochophore and a planktotrophic pelagosphera larva. The trochophore is enclosed in the egg envelope, with cilia growing through the envelope's pores. The trochophore larva metamorphoses into the pelagosphera larva at approximately 60 h. Pelagosphera larvae reached metamorphic competence at about five weeks. Metamorphosis to the juvenile was induced by supplying sediment that had been inhabited previously by conspecific adults. Juveniles were observed for several weeks. We conclude that *N. pellucidum* is a good model species for sipunculan development, although rearing conditions in the laboratory still need to be optimized.

INTRODUCTION

During the past two decades, our understanding of metazoan relationships has changed radically, starting with the first use of ribosomal RNA sequences for phylogenetic analysis (Field et al., 1988). Many taxa for which evolutionary origins have long been mysterious or controversial can now be placed with more certainty into the metazoan tree of life (Dunn et al., 2008; Halanych, 2004). Among those, two groups that were long regarded as distinct phyla have been absorbed into the Annelida: the Echiura, or spoon worms, and the Siboglinidae, previously called Pogonophora and Vestimentifera (McHugh, 1997; Rouse and Fauchald, 1997).

The Sipuncula, commonly known as peanut worms or star worms, have had a complex taxonomic history but now appear to be following the same route as the echiurans and siboglinids. Nearly 50 years after Hyman (1959) affirmed phylum status for the group, recent authors place them into the annelid clade, based on

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phylogenetic analyses of mitochondrial gene order (Bleidorn et al., 2005; Boore and Staton, 2002), sequence data from several genes (Struck et al., 2007), and expressed sequence tags (Dunn et al., 2008). Although there is a growing consensus on the annelid affinities of sipunculans, it remains to be determined which of the incredibly diverse annelids is the sister group to the Sipuncula. With a simple body, consisting of a trunk and a retractable introvert with an array of tentacles at the anterior end, they show little similarity to any other polychaete group. In the molecular analyses, support for a sister group relationship with any other polychaete taxon is low. The monophyly of the Sipuncula is uncontested, and solid hypotheses of within-group relationships have been published (Maxmen et al., 2003; Schulze et al., 2005, 2007; Staton, 2003).

Sipuncula are an interesting case in the field of "EvoDevo," or the interface of evolution and development. Embryonic and larval characters have often been cited as support for phylogenetic hypotheses. Rice (1985) listed several similarities between sipunculan and annelid development, such as the larval prototroch and metatroch and the retention of the egg envelope to form the larval cuticle. She also noted that in some sipunculan larvae the ventral nervous system develops in paired cords, similar to most polychaetes. On the other hand, Scheltema (1993), comparing embryos and larvae of annelids, mollusks, and sipunculans, argued that sipunculan development shows more similarity with that of mollusks. The development of all three taxa includes spirally cleaving embryos and a trochophore larva. A long-held view is that annelid and mollusk embryos can be distinguished at the 64-cell stage by the arrangement of the micromeres around the animal pole: they form either an "annelid cross" or a "molluskan cross." Reproducing Gerould's (1906) drawing of the embryo of *Golfingia vulgaris* with a molluskan cross, Scheltema concluded that sipunculans and mollusks were sister groups. However, Maslakova et al. (2004) showed that the annelid and molluskan crosses are far from universal within the respective taxa and probably hold no phylogenetic significance.

The primary reason why few past researchers have recognized sipunculans and echiurans as annelids is that adults of both taxa show no sign of segmentation, either externally or internally. It took advanced techniques in immunohistochemistry and confocal laser scanning microscopy to demonstrate segmentation in the nervous system of echiuran larvae (Hessling and Westheide, 2002). Similar techniques initially failed to show segmentation in sipunculan larvae (Wanninger et al., 2005) but a recent study showed a segmental mode of neural patterning in the early pelagosphera stage (Kristof et al., 2008).

If the Sipuncula fall into the annelids, they must have secondarily lost segmentation in the later larval stages and the adult. If no morphological segmentation is evident, what happened with the molecular pathways responsible for segment formation in other annelids? By comparison with other species, sipunculans are valuable for the identification of the genetic and cellular basis of segment formation in annelids.

The recent developments in metazoan phylogeny have thus sparked a renewed interest in sipunculan development. A model species is needed to study fundamental developmental processes. A good model species has to be readily available, be easy and inexpensive to maintain in the laboratory, lend itself to a variety of examination techniques, and be representative for its taxonomic group. Here we describe the development of *Nephasoma pellucidum* and explore its potential as a model species. *N. pellucidum* is a relatively common species that inhabits cracks and crevices in hard substrates in shallow warm waters. The species exhibits the most common developmental mode within the Sipuncula, which includes a lecithotrophic trochophore stage and a planktotrophic pelagosphera larva (Rice 1967, 1975a, 1975b, 1976, 1989). We have accumulated these data between 1972 and 1984 and, more recently, between 2003 and 2006.

MATERIALS AND METHODS

Specimens of *Nephasoma pellucidum* were collected from numerous localities offshore from Fort Pierce, Florida, extending from Capron Shoal and Pierce Shoal 4 and 6 miles, respectively, southeast of the Fort Pierce Inlet to the Sebastian Pinnacles approximately 32 miles north of the Inlet. At the Pinnacles, worms inhabited rubble of oculinid coral at depths of 70 to 100 m, whereas on the more southern shoals they occurred in depths of 9 to 15 m in rubble composed of mollusk shells, sand dollar tests, and rocks. Occasionally specimens were found in the Fort Pierce Inlet in intertidal clumps of oyster shells. The worms were carefully removed from the rubble with hammer and chisel. Multiple adults from each collection were kept in glass dishes in approximately 200 mL seawater at room temperature. Spawning occurred in the lab, generally after changing the water. Whenever eggs were observed in the culture dishes, they were pipetted into a clean dish and observed for development. Larval cultures were kept until the larvae either died or metamorphosed. Water was changed at least every two days. The larvae were periodically fed with unicellular algae or diatoms (*Isochrysis*,

Dunaliella, or *Nanochloropsis*). To induce metamorphosis, larvae were pipetted into dishes with muddy sediment previously inhabited by conspecific adults.

For scanning electron microscopy (SEM) and transmission electron microscopy (TEM), specimens were fixed in 2.5% glutaraldehyde in Millonig's phosphate buffer (Millonig, 1964) for at least 1 h and up to several days at 4°C. Fixation was followed by three washes in a 1:1 mixture of Millonig's phosphate buffer and 0.6 M sodium chloride and postfixation in 1% osmium tetroxide (1:1:2 mix of 4% OsO_4 : Millonig's buffer : 0.75 M NaCl). Samples were then dehydrated in an ethanol series up to 100%. For SEM, they were critical point dried and mounted on SEM stubs using double-sticky tape and viewed in either a Nova Scan or a JEOL 6400 Visions scanning electron microscope. Images were either scanned from negatives or stored digitally. For TEM, the dehydrated specimens were transferred to propylene oxide and subsequently embedded in Epon resin and sectioned. Thin sections were stained with uranyl acetate and lead citrate and viewed in a JEOL 100CX transmission electron microscope.

RESULTS

Gametes

The spermatozoan of *Nephasoma pellucidum* is of the primitive type according to Franzén's classification (Franzén, 1958). The nuclear region is rounded and capped by a doughnut-shaped acrosome with a central nipple-like protuberance. The head, including nucleus and acrosome, measures 1.5 × 1.7 μm. Posterior to the nucleus, four mitochondrial spheres are arranged in a circle, from the center of which extends the flagellum (Figure 1A).

The egg at the time of spawning is spherical, measuring 105 μm in diameter (Figures 1B, 2A). In direct light the surface appears opalescent, and the color is pale gray. The egg envelope, up to 6 μm in thickness, is multilayered and perforated by numerous pores (Figure 3).

Spawning

As in most sipunculans, sexes are separate; eggs and sperm are spawned freely via the nephridipores into the surrounding water where fertilization occurs. From data accumulated on spawning in the laboratory, two spawning peaks are evident: one in the spring (April–May) and the other in the fall (September–November). Observations of spawning were carried out on animals in the laboratory, usually for a period of one month after collection from the field: 139 spawnings were recorded over a period of 8 years (1972–1980), and spawning occurred every month of the year except January. Although a few animals were observed to spawn after maintenance in the laboratory for as long as 18 months, 88% of the spawnings were recorded within 30 days of collection.

Cleavage

The eggs at spawning may be arrested in the first meiotic metaphase, or they may possess an intact germinal vesicle. In the latter case, the germinal vesicle breaks down soon (within at least 30 min) after spawning, regardless of whether the egg is fertilized. Within 40 min after fertilization (23°C), the first polar body is formed (Figure 2B), and at 55 min the second polar body makes its appearance. The first cleavage, occurring at 90 min, is unequal, the CD cell exceeding the AB cell in size (Figure 2C). The next three cleavages occur at approximately half-hour intervals, and the 16 cell stage is attained within 3 h after fertilization. The third cleavage, from 4 to 8 cells, is spiral and unequal. The A, B, and C cells, all approximately the same size, divide simultaneously, preceding the initiation of division of the larger D cell by about 1 min and completing their divisions 5 min before that of the D cell. In the 8 cell stage, the micromeres and macromeres of the A, B, and C quadrants are approximately the same size, the C sometimes being slightly larger, but all are smaller than the d cell which, in turn, is smaller than the D cell.

After the first few cleavages, the divisions are more frequent, and by 7 h after fertilization the egg has developed into an early blastula; cilia from the prototrochal cells protrude through the pores of the egg envelope and the embryo begins to rotate slowly on the bottom of the container. By 16 h the embryos are swimming throughout the dish, no longer confined to the bottom. At this time the stomodaeal invagination is evident, and the embryos show the first signs of positive phototropism (Figure 2D).

Trochophore: Morphology and Metamorphosis to the Pelagosphera

By 48 h the embryo has reached the stage of trochophore. The shape has changed from spherical to oval, as a result of a slight posttrochal elongation (see Figure 1C). A pair of dorsolateral red eyespots is present in the pretrochal hemisphere. Prototrochal cavities are evident to the inner side of the prototroch cells, and the gut is differentiated into three regions: esophagus, stomach, and intestine.

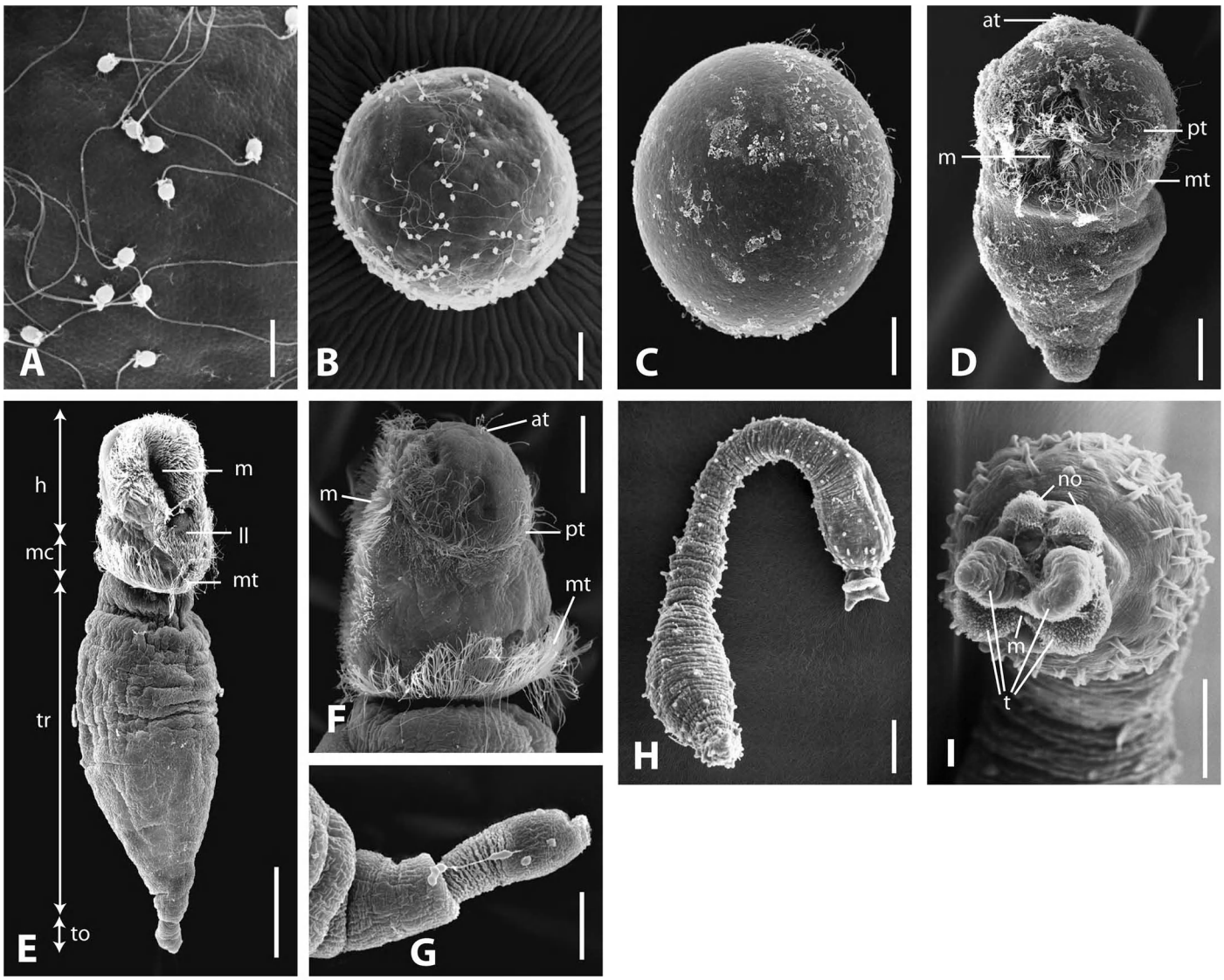
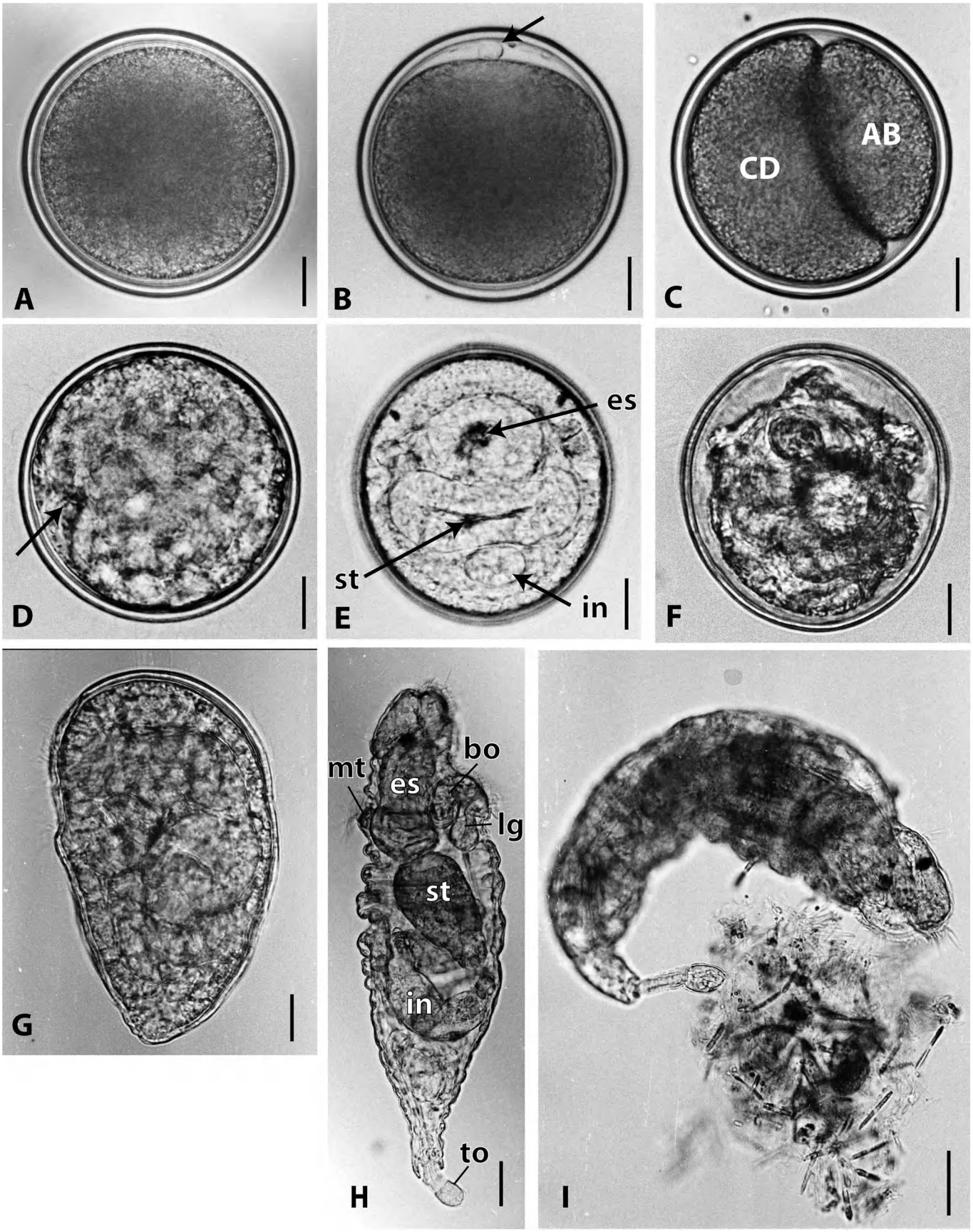


FIGURE 1. Scanning electron micrographs showing development of *Nephasoma pellucidum* (scale bar lengths here in parentheses). A. Sperm on the surface of the egg (5 μm). B. Egg with sperm on surface (20 μm). C. Trochophore larva; note cilia extending through egg envelope (20 μm). D. Early pelagosphera larva (20 μm). E. Fully formed pelagosphera larva, ventral view (50 μm). F. Head of a pelagosphera larva, lateral view (20 μm). G. Terminal organ of the pelagosphera larva (10 μm). H. Metamorphosed juvenile (100 μm). I. Tip of juvenile introvert with tentacle buds and lobes of nuchal organ (50 μm). Abbreviations: at = apical tuft; h = head; ll = lower lip; m = mouth; mc = metatrochal collar; mt = metatroch; no = nuchal organ; pt = prototroch; t = tentacles; to = terminal organ; tr = trunk. (Images A, B from Rice, 1989: fig. 4E,F; used with permission)

FIGURE 2. (*facing page*) Light micrographs showing development of *Nephasoma pellucidum* (scale bar lengths here in parentheses). A. Unfertilized egg (20 μm). B. Egg with polar body (arrow) (20 μm). C. Two-cell stage; note size difference between CD and AB blastomeres (20 μm). D. Blastula stage; beginning invagination of stomodaeum (arrow) (20 μm). E. Early trochophore; note eyespots at anterior end (top) (20 μm). F. Trochophore shortly before metamorphosis to pelagosphera (20 μm). G. Early pelagosphera in the process of elongation (20 μm). H. Fully metamorphosed pelagosphera larva (20 μm). I. Feeding pelagosphera, 10 d old (50 μm). Abbreviations: bo = buccal organ; es = esophagus; in = intestine; lg = lip gland; mt = metatroch; st = stomach; to = terminal organ.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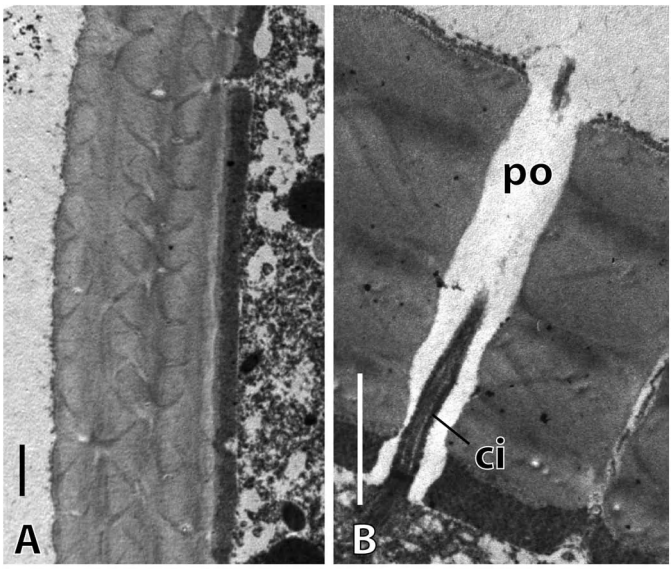

FIGURE 3. Transmission electron micrographs showing egg envelopes of *Nephasoma pellucidum* (scale bars = 1 μm). A. Section through multilayered egg envelope. B. Cilium growing through pore in egg envelope of trochophore larva. Abbreviations: ci = cilium; po = pore.

The trochophore is lecithotrophic, being completely enclosed by the egg envelope (Figure 2E,F).

Metamorphosis of the trochophore to the pelagosphera larva occurs at 60 to 65 h (23°C) and extends over a period of 6 to 8 h. The body elongates from 140 μm to 250 μm, by an increase in the length of the posttrochal hemisphere. The lumen of the gut is completed, and mouth and anus break through the overlying egg envelope (Figures 1D, 2G). The ventral ciliated surfaces of the head and the lower lip are formed apparently by an evagination and expansion of the anterior stomodaeum. Larval organs of the lower lip become functional: the buccal organ is protrusible, and the pore of the lip glands opens (Figure 2H). The metatrochal cilia project from a prominent metatrochal collar posterior to the mouth and lower lip. As the retractor muscles become functional, the entire pretrochal body is retractable into the posterior or posttrochal region of the larva. The coelom is considerably expanded, and the posttrochal body is capable of great extension and contraction. Whereas the posttrochal egg envelope is transformed into the larval cuticle, the pretrochal egg envelope is gradually sloughed off, leaving a thin cuticle covering the head. The terminal organ appears first as an evagination of the posterior extremity of the trunk and within a few hours differentiates into a more discrete elongate structure (40 μm) that is retractable into the trunk and provides a temporary attachment for the larva to the substratum (Figure 1G).

Pelagosphera: Morphology, Behavior, and Metamorphosis to the Juvenile

Four regions of the body can be distinguished: head, metatrochal collar, trunk, and terminal organ (see Figures 1E,F, 2H). The terminal organ is well developed with an unusually long neck, terminated by a bulbous posterior expansion (Figure 1G). The terminal organ of a 10-day larva may be extended to a length one-third that of the entire larva.

For approximately two weeks after metamorphosis, the majority of larvae are attached by their terminal organs; some continue to swim, or else attach and swim intermittently. At two weeks there is a high rate of mortality and, in the absence of substratum, most larvae die within two months; the maximum survival time of larvae reared in culture dishes is 103 days. Surviving larvae of one month of age attain a maximum size of 1.2 mm. At this age the body proportions have changed, the head being relatively smaller than in the younger stages. The external body wall is smooth, glistening in reflected light, and through the relatively transparent body wall the gut is apparent as an elongate dark yellow stomach and a lighter yellow recurved intestine, ending at the dorsal anus in the anterior trunk. Usually larvae are still attached by the terminal organ at these later stages, although some may lie on the bottom, relatively quiescent. Swimming occurs only rarely, although metatrochal cilia are still present.

Attached larvae are observed to feed on the substratum surrounding their points of attachment (Figure 2I). The body may be bent downward so that the ventral surface of the head is applied to the bottom of the dish, or the body may be stretched out from the point of attachment parallel to the substratum. In culture dishes in which there is an algal growth covering the bottom, the area surrounding the attached larva is often bare, indicative of larval grazing activity. The area of attachment is often marked by clumps of feces on which the larva may graze and ingest. Occasionally larvae release themselves from the attachment and swim or move along the bottom to a new site. Free larvae sometimes move with head applied to the substratum and posterior end directed upward, either exploring or feeding on the bottom. Frequently the terminal organ is placed in or near the mouth. Older larvae detach and move to new locations less often than younger larvae. A larval behavior, common to all sipunculans but of unknown function, is placement of the terminal organ in or near the mouth.

Metamorphosis of larvae reared in culture dishes could be induced at the age of 5 to 6 weeks by exposure to a fine, muddy sediment that had been occupied previously by adults. Attempts to induce metamorphosis before this age were not successful. Before metamorphosis, larvae buried themselves in the sediment and in 3 d underwent metamorphic changes to the juvenile stage.

The process of metamorphosis is initiated by the loss of the metatrochal cilia, reduction in the size of the lower lip, narrowing of the head, and elongation of the pretrochal body. At the end of 3 d, both posttrochal and pretrochal regions of the body are narrowed and elongated, the metatrochal collar is reduced, the terminal organ and lip are partly regressed, the mouth moves to a terminal position, and dorsal to the mouth a pair of developing tentacular lobes is apparent (Figure 1H,I). These morphological modifications, along with the behavioral changes of initiation of burrowing and cessation of swimming, mark the beginning of the juvenile stage. Regions of the body of the juvenile are reduced from the four found in the larva to two: (1) the broader and longer posterior trunk, formed from the posttrochal larva, and (2) the more narrow anterior introvert, which is terminated by mouth and developing tentacles and formed from the pretrochal larva. Similar to the pretrochal larval body, the introvert of the juvenile is retractable into the trunk.

The most immediate modifications are found in the head and metatrochal regions. As the mouth becomes terminal, the dorsal surface of the head is foreshortened. The ventral lip is lost, but ciliation of the ventral surface of the head persists to surround the mouth and the ventral surface of the developing dorsal lobes. On the dorsal head two heavily ciliated patches that will give rise to the paired nuchal organ have moved further anteriorly as the head foreshortens. In the 7- to 9-day-old juveniles the buccal organ is no longer apparent. One to four rings of simple hooks appear in the region of the former metatrochal band. Papillae, already apparent in older larvae, are more prominent and numerous. Scattered among the hooks, the papillae are dome shaped and, as seen in scanning electron micrographs, have central pores from which several cilia protrude. Papillae of similar structure, but somewhat larger, cover the entire trunk (Figure 1H). A vestigial terminal organ may persist for one or two weeks. Within two to four weeks a second pair of rudimentary tentacles appear ventral to the mouth.

The body wall of the juvenile thickens, losing its transparency. Externally circular constrictions, also seen in late larval stages, are more prominent. Juveniles of one week also show longitudinal "folds" of the body wall, resulting in a checkered appearance of the integument in some regions.

DISCUSSION

Nephasoma pellucidum is one of the few sipunculan species in which the life cycle has been observed from spawning to juvenile stage. Other species are *Siphonosoma cumanense* (Rice, 1988), *Thysanocardia nigra* (Rice, 1967), *Themiste pyroides* (Rice, 1967), *Themiste lageniformis* (Pilger, 1987), *Themiste alutacea* (Rice, 1975c), *Phascolion strombus* (Åkesson, 1958; Wanninger et al., 2005), and *Phascolion cryptum* (Rice, 1975c). Most of these species show abbreviated development, either omitting both the trochophore and pelagosphera stage, or omitting the pelagosphera stage, or having a lecithotrophic pelagosphera (Rice, 1976). The oceanic, planktotrophic pelagosphera larvae of many aspidosiphonid and phascolosomatid larvae have been recovered in plankton tows; however, their complete life cycles are unknown (Rice, 1981; Hall and Scheltema, 1975).

We argue that a model species should show the ancestral developmental mode for the taxon. Cutler (1994) concluded that indirect development with a planktotrophic pelagosphera was ancestral in Sipuncula. The most recent phylogenetic analyses (Schulze et al., 2007; Schulze and Rice, 2009) seem to confirm this view. The genera *Sipunculus* and *Siphonosoma*, which to our present knowledge only contain species with planktotrophic pelagosphera larvae, represent the two basal clades in both analyses. The remaining three major clades have species of *Phascolosoma* and *Apionsoma* as their basal branches, two additional genera in which abbreviated development is unknown.

Of the species listed above, only the life cycle of *Siphonosoma cumanense* includes a planktotrophic pelagosphera as *N. pellucidum* does. *Siphonosoma cumanense* is a large, sand-burrowing species. Like other sipunculans, it survives well under laboratory conditions, when supplied with sediment and adequate aeration. However, its potential for use as a model species is limited by two factors. First, even though the species has a wide geographic distribution, it is rarely found in significant numbers, and the establishment of a viable population would require major efforts. Second, larvae do not seem to be competent to metamorphose until about 8 weeks old (Rice, 1988).

Nephasoma pellucidum is geographically widespread, mostly in shallow warm waters, although it does not seem to be as abundant in most places as at our collecting station

near Fort Pierce, Florida. Collection of a significant number of individuals can be time consuming because they have to be carefully removed from the cracks and crevices of rubble; the removal process can damage the animals, often causing their death. After successful retrieval, however, adults are easy to maintain in laboratory conditions. Removed from their shelter, they survive in simple glass bowls without aeration or food supplement for at least a year, feeding only on the biofilm at the bottom of the dish. We assume that, left in their shelter or in sediment, with proper aeration and occasional food supply, they would survive for years. This assumption is based on the longevity of other sipunculans: individuals of *Apionsoma misakianum* have been kept in holding tanks at the Smithsonian Marine Station for nearly 30 years.

Nephasoma pellucidum spawns frequently during the warmer months of the year. Spawning can be induced by changing the seawater in the dish, although this procedure does not reliably yield the desired results, leaving some uncertainty as to when the spawning occurs. Embryos and larvae are easy to observe with different microscopic techniques. Mortality before the first metamorphosis, from trochophore to pelagosphera, is minor. The pelagosphera larvae are more transparent than other sipunculan larvae, facilitating observation by light and confocal laser scanning microscopy. In contrast to the pelagosphera larvae of some other sipunculan species, they relax relatively well when temporarily cooled to 4°C and treated with menthol, magnesium chloride, or 10% ethanol, leaving their head and terminal organ exposed.

The increase in mortality during the prolonged pelagosphera phase presents some difficulties. By the time metamorphic competence is reached, the percentage of surviving larvae is low. A further reduction in numbers occurs at metamorphosis, because not all larvae respond to the settlement cue, that is, adult-conditioned sediment. Therefore, postmetamorphic juveniles are only rarely observed. Common metamorphosis-inducing agents such as potassium chloride, cesium chloride, gamma-aminobutyric acid, 3,4-dihydroxy-L-phenylalanine (L-dopa), and isobutylmethylxanthine (Bryan et al., 1997; Morse et al., 1979; Yool et al., 1986) seem to have no effect on metamorphosis in *N. pellucidum*.

As a conclusion, among the sipunculans for which development has been studied, *N. pellucidum* is a good candidate for a model species. Rearing larvae through metamorphosis still presents some difficulties, and future work should focus on optimizing the conditions. Recently the cold-water species *Phascolosoma agassizii* from the Sea of Japan, which also has a planktotrophic pelagosphera, has been reared through metamorphosis (A. S. Maiorova and A. V. Adrianov, Institute of Marine Biology, Far East Branch of the Russian Academy of Sciences, Russia, personal communication) and might be another appropriate candidate for a model species, even though metamorphosis could never be observed in individuals of the same species collected in the Pacific Northwest and reared at the University of Washington Friday Harbor Laboratories.

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Mitochondrial Phylogeography of the Intertidal Isopod *Excirolana braziliensis* on the Two Sides of the Isthmus of Panama

Renate Sponer and Harilaos A. Lessios

ABSTRACT. The intertidal isopod *Excirolana braziliensis* Richardson possesses limited means of dispersal; there is no larval stage, and adults remain sedentary under the sand. It is represented on the two coasts of Panama by three morphs, two in the Pacific (P and C′) and one in the Atlantic (C). Previous work has quantified morphometric differences between the morphs, found that there are multiple allozyme differences between them, and produced indirect evidence that they are reproductively isolated from each other. Here we report comparisons of 345 bp of 12S and 678 bp of cytochrome oxidase I (COI) mitochondrial DNA (mtDNA) from three populations of each morph. The mtDNA sequences from the three morphs are reciprocally monophyletic, strengthening the case for recognizing them as separate species. As in morphology and isozymes, the C morph and the C′ morph are sister clades, and the P morph is an outgroup. In contrast to what was previously supposed, the C and C′ morphs neither are the result of a recent introduction from one ocean to the other, nor were separated at the final stages of the completion of the Isthmus of Panama three million years ago, but rather are anciently separated sister clades that now exist on separate shores. Patterns of mitochondrial gene flow between populations of the same morph vary. The C and C′ morphs show large genetic differences between local populations, as would be expected from an organism with such limited vagility. In the P morph, on the other hand, populations from localities 5 km apart are identical in mitochondrial DNA, even though they differ in one allozyme locus, suggesting the possibility of sex-biased migration.

INTRODUCTION

Many marine organisms are capable of dispersing over large distances at some point of their life cycle. The population genetics of such organisms usually reveal a genetic neighborhood size in the order of thousands or tens of thousands of kilometers. Some marine species, however, provide a contrast to this picture of wide dispersal in that they lack any means of transferring their genes by either vagile adults or free-swimming larvae, yet have wide geographic ranges. How much gene flow may occur between noncontiguous populations of such species and whether species cohesion is maintained in the face of limited vagility is of special interest to population genetics. The tropical isopod *Excirolana braziliensis* is an example of a species apparently spread over the tropical seas of Americas, even though it lacks the means of maintaining genetic contact between distant populations.

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Excirolana braziliensis Richardson is a common isopod of intertidal beaches on both sides of the Americas, from the Pacific coast of Mexico (30°N) to S. Chile (40°S) and from the northern Caribbean (31°N) to Uruguay (25°S) (Cardoso and Defeo, 2003). It is a small (approximately 3–4 mm in length), dioecious species, which reaches its highest abundance just above the high-tide mark, where it lives buried in the sand during low tide and rises to the water column at high tide to feed on live and dead fishes and invertebrates (Brusca and Iverson, 1985). *E. braziliensis* has very limited means of dispersal. The female carries broods of 4 to 17 offspring per reproductive event. Young are released directly into the adult habitat (Klapow, 1970). The frequency of reproduction is such that a population may turn over every four months (Brusca and Iverson, 1985). In Panama, recruitment occurs throughout the year (Dexter, 1977). Dispersal in *E. braziliensis* may occur as a result of feeding events, during which individuals of this genus have been observed attached to fish or other prey items for several minutes (Brusca, 1980). This behavior may represent the only means of transport of this organism between beaches because free-swimming individuals are likely to be eaten by fish.

Weinberg and Starczak (1988, 1989) reported the existence of three morphological variants of *E. braziliensis* from Panama. Two similar and presumably closely related types, termed C and C′, are found on the Caribbean and Pacific coasts, respectively. The third type (P) is morphologically distinct from C and C′; its distribution overlaps with that of C′ throughout most of its range (Weinberg and Starczak, 1989). In general, Pacific beaches contain only one of the two morphotypes. Nevertheless, 2 of 43 beaches sampled by Weinberg and Starczack (1989) were found to contain C′ and P morphs in approximately equal numbers. The geographic patterns of morphotype distribution and genetic composition remain stable over time, but there are occasional complete replacements of entire beaches by a different morph, presumably as the result of extirpation and subsequent recolonization (Lessios et al., 1994). Morphological and genetic divergence (based on allozyme data) between morphs are highly correlated and large enough to suggest that the P morph constitutes a distinct species (Lessios and Weinberg, 1994). The allozyme data are also consistent with the hypothesis that the C and C′ morphotypes are geminate species that resulted from the rise of the Panamanian Isthmus three million years ago (Lessios and Weinberg, 1994).

Allozyme analyses indicate that the three morphotypes of *E. braziliensis* are probably reproductively isolated, because they form few hybrids even when they co-occur at the same beach. Even within morphotypes, gene flow between populations from different beaches is low, as deduced from the predominance of distinct alleles in one or more loci, even among beaches situated less than 5 km apart. However, dispersal (as measured by individuals homozygous for alleles that otherwise occur on a different beach) is rather high, suggesting that some form of reproductive isolation prevents them from mating with individuals from the local population (Lessios and Weinberg, 1993).

The purpose of the present study is to investigate the phylogenetic and phylogeographic relationships within and between the three morphotypes of *Excirolana braziliensis* using sequences of mitochondrial DNA (mtDNA). Specifically, we are addressing the following questions: (1) Does mtDNA show patterns of genetic divergence, phylogeny, and geographic distribution congruent with those suggested by isozymes and morphology? (2) When did the three lineages diverge? (3) What are the patterns of population genetic structure? Do mtDNA data show similar levels of gene flow as isozymes within and between morphotypes? (4) What processes can explain mtDNA discrepancies between patterns from mtDNA and allozyme markers?

MATERIALS AND METHODS

Sample Collection

Excirolana braziliensis were obtained from nine locations along the Pacific and Caribbean coasts of Panama (Figure 1). Each of the three morphotypes was represented in our collections by three populations. Isopods were collected on beaches during low tide. The top 10 cm of sand at haphazard locations above the high-tide mark were sifted through a 500 μm sieve, and isopods were placed in plastic bags with wet sand. The collected isopods were brought alive to the laboratory and frozen at −80°C. The majority of samples used in this study were from collections made in 1988, the same collections used to assay isozymes (Lessios and Weinberg, 1993, 1994). Additional individuals were collected in 1998 from Isla Culebra. Specimens of *Excirolana mayana* were also collected at Isla Culebra to be used as outgroups.

DNA Extraction, Polymerase Chain Reaction, and mtDNA Sequencing

Genomic DNA was extracted using a standard phenol/chloroform protocol (Sambrook et al., 1989) with ethanol precipitation. For amplification and sequencing

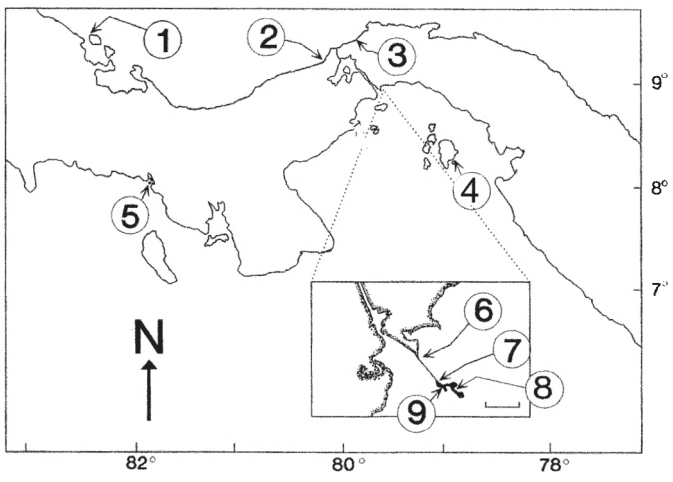

FIGURE 1. Map of Panama, indicating localities in which *Excirolana braziliensis* was sampled (sample size in parentheses). 1, Bocas del Toro (14); 2, Shimmey Beach (10); 3, Maria Chiquita (9); 4, Isla Santelmo (18); 5, Isla Adentro (15); 6, Causeway (6); 7, Lab (8); 8, Perico (10); 9, Isla Culebra (14).

of 345 base pairs (bp) of the 12S mtDNA gene, we used the universal primers 12Sa and 12Sb (Simon et al., 1994). A 678 bp fragment of cytochrome oxidase I (COI) was amplified and sequenced with combinations of the forward primers BWBK (5′-GAG CTC CAG ATA TAG CAT TCC-3′) and ISO-F1 (5′-CYC TTT TAT TAG GRA GGG GG-3′) and the reverse primers BWBJ (5′-CAA TAC CTG TGA GTC CTC CTA-3′) and ISO-R2 (5′-ACR GCA ATA ATT ATG GTA GC-3′). The following conditions were used for polymerase chain reaction (PCR): initial denaturation for 2 min at 94°C, then 30 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 50°–53°C, extension for 1 min at 72°C, and final extension for 10 min at 72°C. PCR products were cleaned for sequencing using silica gel purification columns. Cycle sequencing was carried out in both directions, with the ABI PRISM d-Rhodamine Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystematics). Sequences were obtained on an ABI 377 automated sequencer and verified and aligned by eye in the program Sequencher (Gene Codes Corporation). 12S was sequenced in 104 individuals; a subset of 22 individuals was also successfully sampled for COI, whereas the rest failed to amplify for this locus.

Phylogenetic Analysis

For phylogenetic analysis, identical haplotypes from multiple individuals were collapsed. We applied the program Modeltest 3.0 (Posada and Crandall, 1998) to calculate the goodness of fit of various models of DNA evolution. The selected model for the 12S data was that of Tamura and Nei (1993), with equal base frequencies, a gamma distribution with a shape parameter of 0.443, and the following substitution rates: [A—C] = 1.00; [A—G] = 6.00; [A—T] = 1.00; [C—G] = 1.00; [C—T] = 11.71; and [G—T] = 1.00. The selected model for the COI sequence was a transversional model (TVM + I; Posada and Crandall, 2001), with a proportion of 0.69 of invariable sites and the following substitution rates: [A—C] = 8908.76; [A—G] = 258211.81; [A—T] = 26092.37; [C—G] = 0.0001; [C—T] = 258211.82, and [G—T] = 1.00. A partition homogeneity test, executed in version 4.0b10 of PAUP* (Swofford, 2000), indicated that phylogenetic signals in the COI and 12S data were not significantly different ($P = 0.256$). The best fitting model for the combined 12S and COI data was HKY (Hasegawa et al., 1985) with a transition/transversion ratio of 11.46 and a gamma distribution shape parameter of 0.782. Employing these parameters, we ran phylogenetic analyses for 12S and COI separately and with the two DNA regions concatenated. We used the BioNJ algorithm (Gascuel, 1997) and heuristic searches in maximum parsimony and maximum likelihood with PAUP* (Swofford, 2000). Bootstrap confidence values for distance and likelihood trees were calculated in 5,000 and 500 iterations, respectively. Bayesian phylogeny inference was carried out in the program MrBayes v.3.04b (Huelsenbeck and Ronquist, 2001). Bayesian analyses on the COI and the combined data sets were run for 800,000 generations, of which the first 20,000 (2,000 trees) were discarded. For 12S, 2,760,000 generations were run, and 67,500 (6,750 trees) were discarded. Convergence of chains was determined by average standard deviations of split frequencies less than 0.01 and by potential scale reduction factors approximately equal to 1.0. The trees were rooted on sequences of *Excirolana mayana*. Clock-like evolution of sequences was tested with likelihood ratio tests. The tests were carried out in PAUP* 4.0b10 by calculating the difference in log-likelihood of the neighbor-joining trees (see above) with and without the enforcement of a molecular clock and comparing the likelihood ratios to the χ^2 distribution.

Geographic Distribution of Genetic Variation within Morphs

Genealogical relationships of haplotypes within species may be better represented by networks than trees, as

ancestral haplotypes may still be present in the population (Crandall and Templeton, 1993; Posada and Crandall, 2001). We calculated unrooted parsimony haplotype networks based on 12S for each of the three morphotypes separately, using the computer program TCS (Clement et al., 2000). In this method the parsimony limit (the maximum number of differences among haplotypes as a result of single substitutions) is calculated with 95% statistical confidence, and haplotypes are connected in order of increasing number of substitutions. To investigate the population genetic structure within each morphotype, we applied analysis of molecular variance (AMOVA; Excoffier et al., 1992) to the 12S data. Genetic variation for this analysis was assessed based on the Kimura (1980) two-parameter distance between haplotypes. The significance of fixation indices was tested by 10,000 rearrangements of haplotypes between populations. Calculations were carried out in version 2000 of the computer program ARLEQUIN (Schneider et al., 2000).

RESULTS

Descriptive Statistics and Phylogenetic Analysis

Although we sampled many more individuals of *Excirolana braziliensis* for 12S than for COI, trees based on the former DNA region (Figure 2) displayed less resolution than the combined analysis of both genes together. Despite this, all analyses of the 12S segment alone resulted in three distinct lineages, which correspond to the previously described C, C′, and P morphs. The 12S sequences of each morph were monophyletic in all analyses. The node joining C and C′ was well supported by maximum-parsimony analysis but fairly weakly supported by neighbor-joining, maximum-likelihood, and Bayesian analysis. The three main lineages were present in more than one beach, but each beach contained representatives of only one lineage. Although Santelmo had previously been found to contain a mixture of C′ and P morphotypes and the allozymes corresponding to these morphs (Weinberg and Starczak, 1989; Lessios and Weinberg, 1994), all nineteen 12S sequences from Santelmo, differing from each other by a maximum of three substitutions, belonged to the C′ morph. The tree based on fewer sequences of COI (not shown) and the tree based on the combined data (Figure 3) were well resolved and gave strong support to the expected grouping of the C and C′ lineages as sister groups, irrespective of the type of phylogenetic algorithm used.

The 12S Tamura and Nei average distances ranged between 11% and 18% between morphs (Table 1). Within morphs, distances varied between 0% and 2.3%. For COI, average distances (TMV) among lineages were 17.4%–26.1% and within lineages 0%–1.5%. There were five amino acid changes in the COI fragment, of which four were substitutions of nonpolar for nonpolar residues (Met/Ileu; Val/Ileu) and one was a nonpolar for a polar residue (Ala/Thr). Three of the changes differentiate the C/C′ and P lineage; one groups C and P, versus C′, and one is shared between C′ and P, compared to C. Likelihood ratio tests failed to reject the hypothesis of clock-like evolution of either the 12S or the COI sequences ($P > 0.05$).

Genetic Variation within Morphs

Parsimony haplotype networks showed that populations of the C and C′ morphs, but not the P morph, were genetically structured (Figure 4). The most common and (presumably) ancestral haplotype of the P morph was shared by all three populations. Two derived haplotypes were also shared, one between all populations and the other between two populations. In the C′ morph two haplotypes, including the ancestral one, were shared between Santelmo and Isla Culebra. Although Isla Adentro contained three haplotypes not found in any other population, the majority of specimens from this island were of a single haplotype, leading to a low haplotype diversity compared to other populations ($H = 0.14$). The population at Bocas del Toro (C morph), was characterized by high nucleotide diversity compared to other populations ($\pi = 0.0077 \pm 0.0045$). Haplotypes from Bocas del Toro were differentiated from Maria Chiquita and Shimmey Beach by one to eight substitutions whereas the latter two populations shared the ancestral haplotype.

In the C morph, AMOVA (Table 2) found that 67.44% of genetic variance was partitioned among populations; population pairwise F_{ST} comparisons (Table 3) showed that all populations of this morph were significantly differentiated from each other. In the C′ morph, 35.62% of the variance was the result of differences between populations. The population at Adentro had significantly high F_{ST} values when compared to both Santelmo and Culebra, whereas the latter two were not significantly different from each other (Table 3). In the P morph, all the genetic variance was contained within populations, a result in stark contrast with high levels of population subdivision seen in the other two morphs.

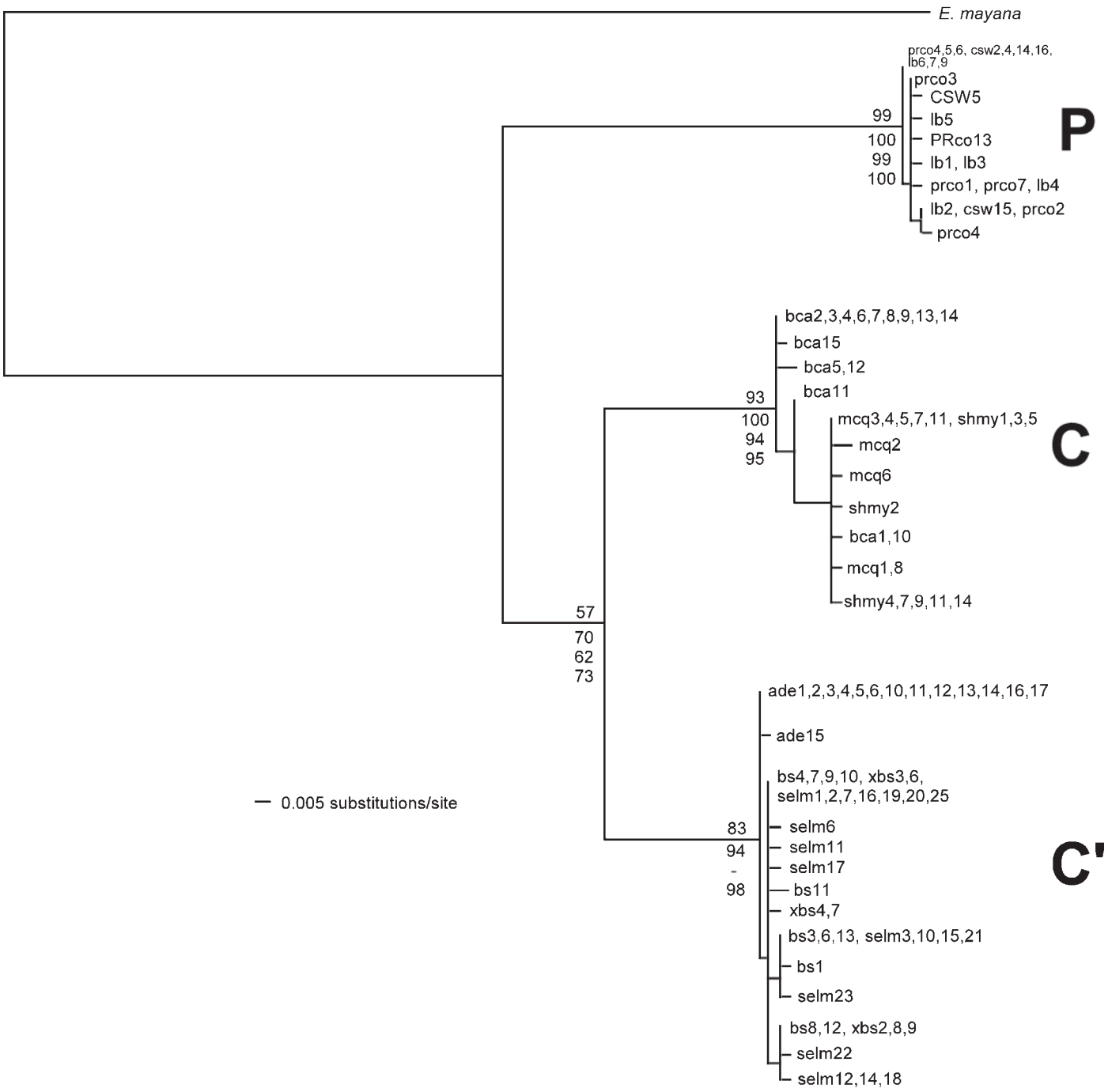

FIGURE 2. 12S mitochondrial DNA (mtDNA) maximum-likelihood bootstrapped consensus tree relating three morphotypes (C, C′, and P) of *Excirolana braziliensis*. Numbers above branches indicate maximum-likelihood bootstrap confidence values; numbers below branches refer to posterior probabilities (Bayesian analysis), neighbor-joining bootstrap support, and maximum-parsimony bootstrap support, respectively, from top to bottom. Support values <50% are not shown. Locality codes of specimens: prco = Perico; csw = Causeway; lb = Lab; bca = Bocas del Toro; mcq = Maria Chiquita; shmy = Shimmey Beach; ade = Isla Adentro; bs = Isla Culebra; selm = Santelmo; xbs = Isla Culebra (xbs specimens were collected in 1998; all other samples were collected in 1988). See Figure 1 for the position of each locality.

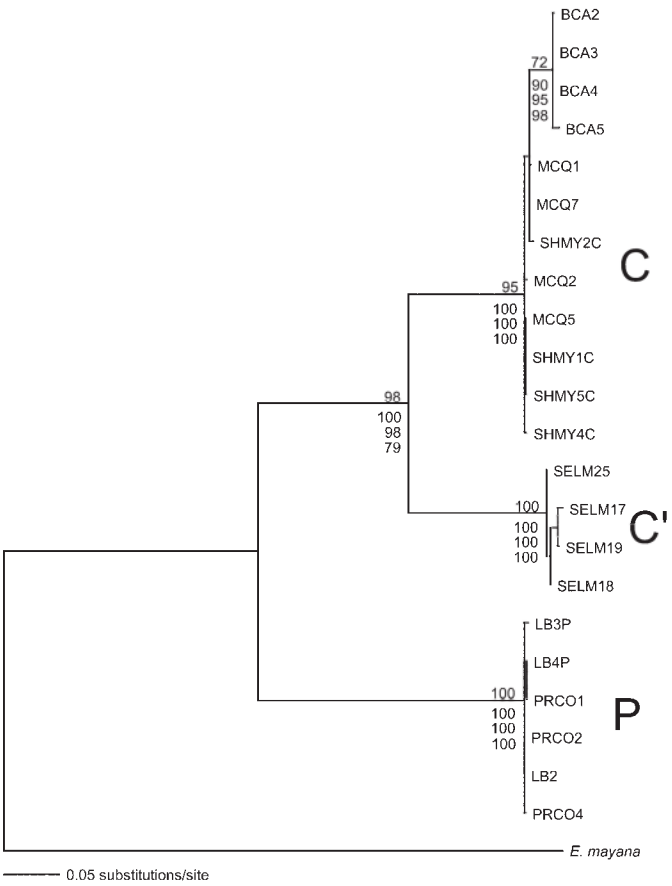

FIGURE 3. Combined 12S/cytochrome oxidase I (COI) mtDNA maximum-likelihood bootstrapped consensus tree relating three major lineages (C, C′, and P) of *Excirolana braziliensis* in Panama. Numbers above branches indicate maximum-likelihood bootstrap confidence values, numbers below branches refer to posterior probabilities (Bayesian analysis), neighbor-joining bootstrap support, and maximum-parsimony bootstrap support, respectively, from top to bottom. Localities: BCA = Bocas del Toro; MCQ = Maria Chiquita; SHMY = Shimmey Beach; SELM = Santelmo; LB = Lab; PRCO = Perico.

TABLE 1. Genetic distances within (along diagonal) and between (below diagonal) morphs of *Excirolana braziliensis*, for 12S (Tamura and Nei, 1993) and cytochrome oxidase I (COI) mitochondrial DNA (mtDNA) (in parentheses; TVM + I [Posada and Crandall, 2001]).

Morph	C	C′	P
C	2.25 (1.52)	–	–
C′	11.03 (17.38)	1.36 (0.49)	–
P	16.21 (24.32)	17.84 (26.12)	1.19 (0.84)

DISCUSSION

The mtDNA data presented here confirm the results from analysis of both morphology (Weinberg and Starczak, 1988, 1989; Lessios and Weinberg, 1994) and allozymes (Lessios and Weinberg, 1994) that *Excirolana braziliensis* populations from the Pacific and Caribbean coasts of Panama consist of three distinct lineages. Allozymes suggest that these lineages are reproductively isolated (Lessios and Weinberg, 1993) and should, therefore, be considered separate species. Although mtDNA data agree with morphological and allozyme data on the grouping of the C and C′ lineages as sister groups with respect to P, the relative magnitude of the measures of differentiation in the three sets of characters is different. Mahalanobis generalized distance from morphometric characters and Nei's D from allozymes indicate that the P morphotype is three times more distant from C and C′ than the latter are from each other (Lessios and Weinberg, 1994). Mitochondrial DNA, on the other hand, gives a P/(C, C′) distance that is only 1.2 to 1.3 times higher than that between C/C′. It is clear that each type of character evolves at a different rate.

A review of molecular divergence across the Isthmus of Panama in 34 lineages likely to have been separated by the final closure of the Isthmus of Panama (Lessios, 2008) has shown that during 3 million years of independent evolution (Coates and Obando, 1996; Coates et al., 2005), crustacean COI has accumulated genetic distances ranging from 4.1% to 8.7% (Knowlton and Weigt, 1998; Schubart et al., 1998; Williams et al., 2001; Morrison et al., 2004) and 12S ranging from 2% to 3% (Robles et al., 2007). Based on these calibrations, and given the differences we determined in COI and 12S, the divergence of the P morph from the two C morphs occurred between 9 and 25 million years ago and that of C from the C′ morph between 6 and 17 million years ago. Thus, in contrast to what was surmised by Lessios and Weinberg (1994) on the basis of isozymes, mtDNA data do not support the idea that the C and C′ morphotypes were isolated at the final stages of the closure of the Panamanian Isthmus, 3 million years ago, but rather that their populations were separated well before the final closure. On the basis of molecular divergence, this appears to be also the case in 73 other amphi-isthmian sister lineages of crustaceans, sea urchins, fishes, and mollusks (Lessios, 2008).

The combination of large mitochondrial differences and evidence for reproductive isolation from allozyme data (Lessios and Weinberg, 1993, 1994; Lessios, 1998) rules out the hypothesis that C′ merely represents a recently

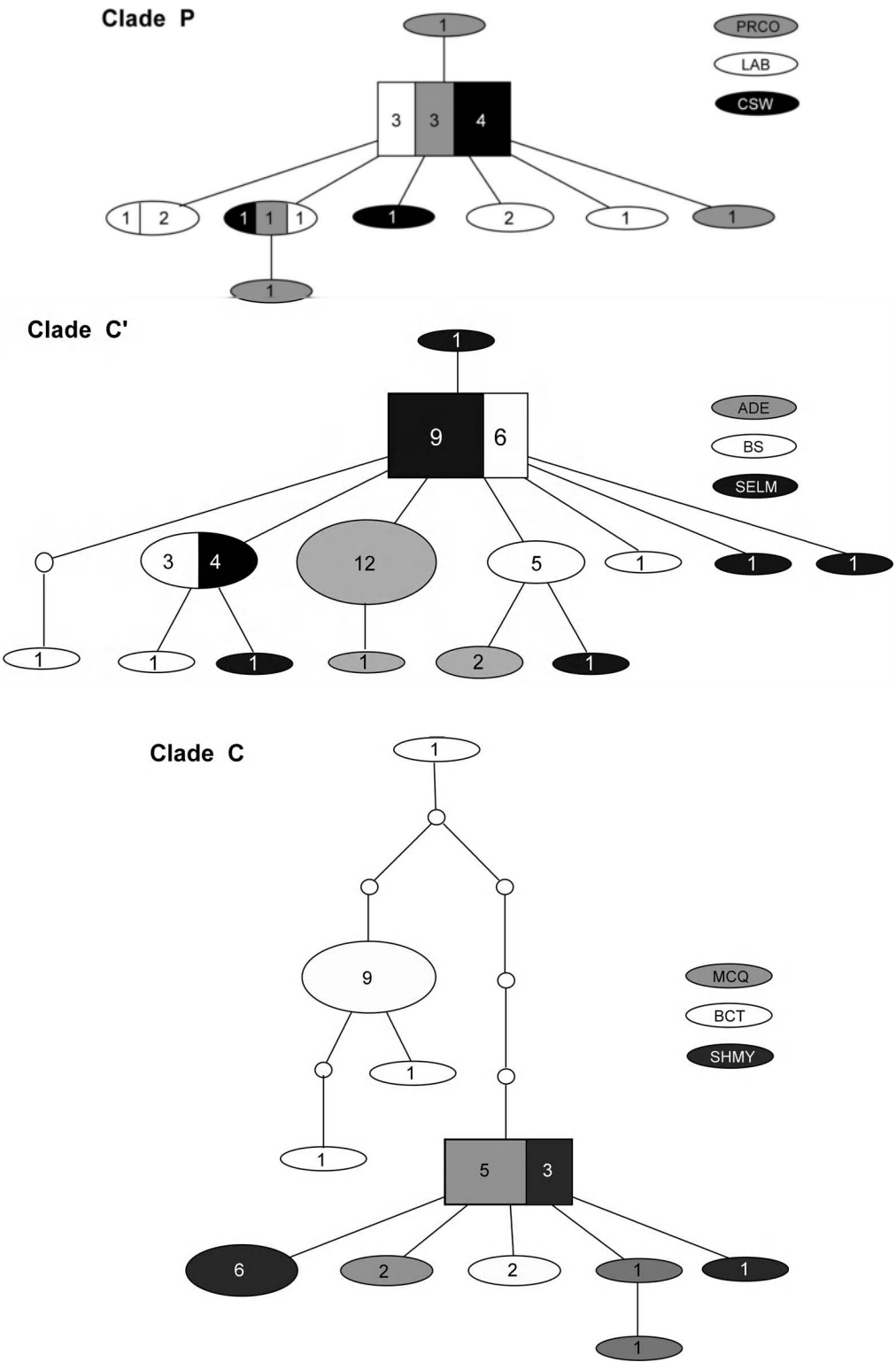

FIGURE 4. Parsimony network of 12S mtDNA haplotypes of the three morphs (clades) of *Excirolana braziliensis*. Each large oval represents a unique haplotype, boxes represent ancestral haplotypes, and small ovals indicate hypothetical, intermediate haplotypes not observed in the populations. The size of each shape represents the frequency of each haplotype. Numbers within each symbol indicate the number of individuals bearing each haplotype. Localities: Prco = Perico; Lab = Lab; CSW = Causeway; ADE = Isla Adentro; BS = Isla Culebra; SELM = Santelmo; MCQ = Maria Chiquita; BCT = Bocas del Toro; SHMY = Shimmy Beach.

TABLE 2. Analysis of molecular variance (AMOVA) of Panamanian populations of *Excirolana braziliensis* based on 12S mtDNA sequences. Partitioning of genetic variance within and between populations (beaches) was estimated for each morph (clade) separately. The significance of fixation indices was tested by 10,000 permutations.

	Variance (%)			
Morph	Between populations	Within populations	Φ_{CT}	*P*
C	67.44	32.56	0.67	<0.001
C′	35.62	64.38	0.36	<0.001
P	−2.39	102.39	−0.02	>0.05

TABLE 3. *Excirolana braziliensis* population pairwise F_{ST} values from 12S mtDNA sequences. Bold values are significant at the $P < 0.01$ level.

P Morph		
Locality	Lab	Perico
Perico	−0.02	–
Causeway	−0.02	−0.04
C Morph		
Locality	Maria Chiquita	Bocas del Toro
Bocas del Toro	**0.69**	–
Shimmey Beach	**0.27**	**0.71**
C′ Morph		
Locality	Isla Adentro	Isla Culebra
Isla Culebra	**0.58**	–
San Telmo	**0.50**	−0.02

introduced population of C from the Caribbean into the Pacific, as had been suggested by Weinberg and Starczak (1988, 1989) and strengthens the case that each of the three lineages represents a distinct species.

Population Structure and Dispersal

Populations of the C and C′ morphs were characterized by population subdivision, as illustrated by high F_{ST} estimates (overall values of 0.67 and 0.36, respectively), whereas samples from different localities of the P morph can be considered as belonging to the same genetic population ($F_{ST} = -0.02$). Populations from Isla Adentro (C′ morph) and from Bocas del Toro (C morph) stand out for their lack of alleles shared with individuals from other localities. Maria Chiquita and Shimmey Beach (C morph) also have significantly different allele frequencies, whereas the populations at Santelmo and Isla Culebra, as well as at Perico, Lab and Causeway, are not significantly differentiated. The two populations most divergent from others in the same morph, Adentro and Bocas del Toro, are also the most geographically distant from other localities containing individuals of their respective morphs, raising the possibility that dispersal to and from these localities is restricted as a result of physical distance. With only three populations per morph, statistical verification of a correlation between geographic and genetic distances is not meaningful.

We observed several differences in the degree of population subdivision when comparing mtDNA and allozyme markers (Lessios and Weinberg, 1994). Based upon mtDNA sequence, the populations at Bocas del Toro and Shimmey Beach were the most different of all ($F_{ST} = 0.71$), whereas their allozyme allele frequencies were rather similar ($F_{ST} = 0.097$, as calculated from data in Lessios and Weinberg, 1993). On the whole, mtDNA data suggest a higher divergence between the morphs, but a lesser degree of subdivision between populations of the same morph, compared to data on allozymes. These results support Lessios and Weinberg's (1993) findings that dispersal among populations is much higher compared to gene flow, because even individuals of the same morph show some sort of reproductive isolation. According to their estimates, up to 2.5% of individuals in a locality consist of new immigrants that do not inject their genes into the host population, indicating that some form of reproductive isolation exists between populations of the same morph, even at the scale of a few kilometers. The data from Santelmo are interesting in this connection: This is the only locality in which two morphs, P and C′, coexist (Lessios and Weinberg, 1993, 1994). The number of hybrids between them, as judged by allozymes, is lower than would be expected from random mating (Lessios and Weinberg, 1993), but hybrids do exist. However, all 19 mitochondrial haplotypes from this locality belong to the mtDNA clade that corresponds to the C′ morph, despite having been sampled from the same collections as the allozymes. Barring the unlikely possibility of a sampling accident, this finding indicates that some individuals with a P nuclear genotype, as manifested in morphology and isozymes, actually carry

a C′ mitochondrial DNA. This, in turn, suggests that hybridization between the morphs, when it occurs, is successful in only in one direction, that is, only if the mother belongs to the C′ clade.

In conclusion, *E. braziliensis* in Panama consists of at least three lineages (C, C′, and P), which diverged well before the final closure of the Isthmus and warrant separate species status. Populations that are more than 30 km distant from each other (C, C′) are genetically divergent, whereas those at less than 5 km (P) are panmictic in mtDNA, even though they are different in at least one allozyme locus (Lessios and Weinberg, 1994). It remains to be seen whether population structure is a result of isolation by physical factors or whether the three species have inherently different dispersal potential, and whether the higher degree of gene flow in mtDNA relative to isozymes is the result of sex-biased migration.

Acknowledgments

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Stability and Change in the Indian River Area Bryozoan Fauna over a Twenty-Four Year Period

Judith E. Winston

ABSTRACT. Two surveys describe changes and stability in bryozoan assemblages at sites in the temperate to tropical transition zone of the Florida Atlantic coast over a 24-year interval in which seawater temperatures increased. Results of a monthly survey of the Indian River Area bryozoan fauna carried out in 1974–1975 as part of a postdoctoral fellowship at the Smithsonian Marine Station were published in 1982. The existence of this baseline work made it possible to resurvey some of the same areas during 1998–1999 to determine whether the bryozoan communities at three of the sites in the original study had changed or remained stable. Results showed that most of the species that had been abundant at a site still occurred at that site 24 years later, indicating a high degree of stability. However, there were some important changes. Temperate species such as *Hippoporina verrilli*, *Cryptosula pallasiana*, and *Bugula stolonifera*, which had been abundant in 1974, were rare or absent in 1998. Those species were replaced by Caribbean species, such as *Exechonella antillea* and *Caulibugula armata*. Although local seawater temperatures during the time period were not available, the Fort Pierce air temperature records indicated that despite the year-to-year variability in both minimum and maximum temperatures over the seasons, mean winter air temperatures maintained a slow increase from 1974 to 1999.

INTRODUCTION

Most ecological research projects are carried out over a very short time period, the length of a research grant or dissertation project, a few years at most, and once the researcher moves on to new studies these research efforts are seldom repeated. Long-term studies are essential to document effects of climate change in communities over time, but the number of such publications for marine communities is extremely low compared with the number documenting the effects of climate change in terrestrial systems (e.g., Richardson and Poloczanska, 2008). This paper describes a repeated survey of coastal and lagoon sites conducted 24 years after the original survey was completed.

In 1974 and 1975, as part of my research as a postdoctoral fellow at the Smithsonian Marine Station, I carried out monthly surveys of the bryozoan fauna at five intertidal sites in the Indian River Lagoon region, both in the lagoon itself and on the coasts of North and South Hutchinson Islands. Descriptions of the species found at these sites, together with descriptions of species taken in one-time

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collections at 18 additional localities in the region and notes on their distribution and ecology, were published in a taxonomic paper, "Marine Bryozoans (Ectoprocta) of the Indian River Area (Florida)" (Winston, 1982). Over the years I returned to the area many times to study various aspects of the biology and ecology of the bryozoans of the region. The apparent persistence of species at particular sites year after year led me to believe that bryozoan communities in the area might be very stable. Yet, the patchiness and limited extent of the hard substrata available for settlement, combined with the fact that certain species were found consistently at only a single site, made me wonder about the potential effect of a man-made or natural disturbance. If a site were to be destroyed, would that mean the regional extinction of the bryozoan species uniquely found there, or did they, in fact, have additional refuges at other sites in the area? To begin to answer these questions, 24 years after the first study, I resurveyed three of the original sites over a one year period in 1998–1999 to learn how stable was the species composition and to look for additions or losses of species at each site.

STUDY AREA

The Indian River Lagoon system, including Mosquito Lagoon, extends along about a third of the Atlantic coast of Florida, from Ponce de Leon Inlet to Jupiter Inlet, a distance of 295 km. Its western boundary is the Florida mainland, while a barrier island complex broached by several inlets forms its eastern boundary. The Indian River Lagoon proper is a shallow microtidal lagoon 195 km in length. It is believed to have the highest biodiversity of any estuarine system in North America, perhaps in part because of its location at the transition between two biogeographic provinces, the warm temperate Carolinean and the tropical Caribbean (Swain et al., 1995).

METHODS

The samples taken in the original survey had been gathered at first only to acquire living colonies of as many species as possible for behavioral and morphological studies (Winston, 1978). As I became interested in the life histories of the species involved, I began collecting at the most convenient and interesting sites in the south central part of the Indian River Lagoon area on an approximately monthly schedule from the fall of 1974 through the summer of 1975. The sites studied were the inner breakwater at Sebastian Inlet, the Johnson House seagrass bed at Harbor Branch Oceanographic Institution at Link Port, the North Beach breakwater at Fort Pierce Inlet, Walton Rocks, South Hutchinson Island, and Seminole Shores, South Hutchinson Island. Collections from those sites were taken in all seasons, an important consideration in the seasonal environment of the Indian River Lagoon region. For bryozoans, as for many organisms inhabiting the area, the highest diversity is achieved and the greatest amount of reproduction, recruitment, and growth of colonies of most species take place during the cooler months (Winston, 1982, 1995). However, tropical species are more apt to be present or active in summer. It was not possible to return to Florida monthly in 1998–1999, but for the best comparison to 1974–1975, the sampling dates were selected to span the seasons and thus reflect the known seasonality of the bryozoan fauna.

Collections were made quarterly (in November 1998, and February, April, and July 1999) at four sites: two within the lagoon and two on the open coast.

Sites Sampled

It was not possible to resurvey all the sites sampled in the original study, for reasons of time and because changes such as the development of some sites into official county or state parks had increased restrictions on scientific collecting. The coastal sites sampled in the restudy were the North Beach breakwater, Fort Pierce Inlet State Park (by special permit), and the Walton Rocks area, South Hutchinson Island, plus one site in the Indian River Lagoon, the Johnson House seagrass bed. One additional site was chosen for the 1998–1999 survey: the intertidal bridge pilings on the east side of the Route A1A causeway to the North Beach in Fort Pierce. This site was added because it was within the Lagoon, yet was close enough to the Fort Pierce Inlet, local marinas, and the commercial port in Fort Pierce to be a likely settlement spot for any newly arrived bryozoan species.

Collecting Methods

Some bryozoan species have colonies several centimeters or more in size and are recognizable in the field, but in many other species the colonies are microscopic and cryptic. Therefore collections were made by scraping hard surfaces and by gathering encrusted substrata: algae, hydroid stems, rocks, shells, or trash. As in the original study, sampling was not quantitative but was thorough. At each locality all microhabitats available—crevices of break-

waters, surfaces of rocks, shells, wood, algae, hydroids, octocorals, sabellariid worm tubes, etc.—were examined carefully for bryozoans. In addition, encrusted examples of each kind of substratum available were taken back to the laboratory and examined alive in seawater; attached bryozoans were identified under a dissecting microscope at 12–100×. Careful microscopic examination made it possible to identify the many tiny and/or uncalcified specimens that could not be identified or even detected in the field. The condition of the colonies and the presence of reproductive structures and/or embryos were also noted, as was the relative abundance of each species at a site. Voucher samples for the project are deposited at the Virginia Museum of Natural History.

Temperature and Salinity Data

Seawater temperatures and salinities were recorded at each census in this study. Temperature ranges are given in the results for each site. Salinity varied little. All readings were in the normal ocean range of the area (35–37‰). The salinity range in the Indian River Lagoon can be more variable than that recorded at any of the sites during the resurvey, but low salinities are connected with periods of heavy rainfall, and 1998–1999 was a drought year. No temperature or salinity data were collected in the original study, and no seawater temperature data were available for the area that covered the entire time period. Air temperatures for Fort Pierce were available (Figure 1) and are summarized in the Discussion section.

RESULTS

North Beach Breakwater, North Hutchinson Island, Fort Pierce Inlet

This site was located at the southern tip of North Hutchinson Island. Specimens were collected from the intertidal rocks on the north side of the north breakwater that protects Fort Pierce Inlet. Habitats sampled included the rocks of the breakwater; sabellariid tubes, hydroids, octocorals, and algae attached to the rocks; and driftwood and other debris wedged among the rocks. The bryozoan diversity at the breakwater is largely dependent on the presence of the hydroid *Thyroscyphus ramosus* Allman, 1877 and the soft coral *Carijoa riisei* (Duchassaing and Michelloti, 1860), whose colonies provide habitat for most of the epifaunal invertebrates at the site. The large

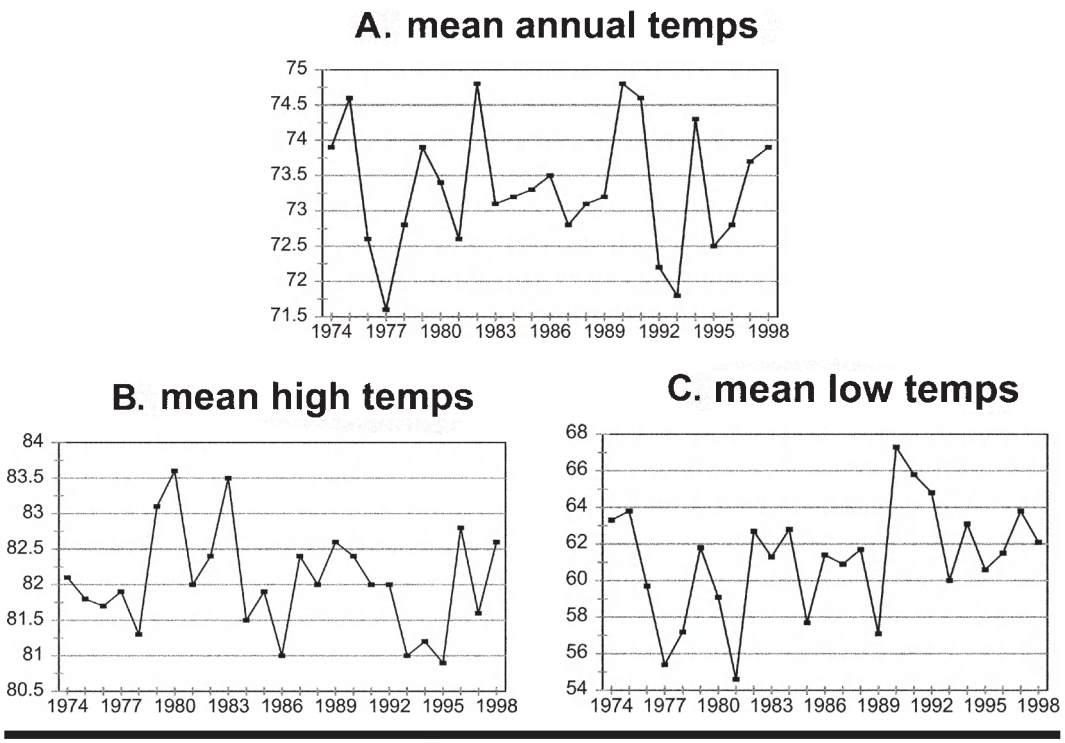

FIGURE 1. Mean annual (A), high (B), and low (C) air temperatures (in degrees Fahrenheit) for Fort Pierce, Florida, from 1974 to 1998. Note: lowest, 54°F = 12.2°C; highest, 83.6°F = 28.7°C.

mounds produced by the sabellariid worm *Phragmatopoma lapidosa* Kinberg, 1867 stay clean and unfouled when the worms are growing actively but break down as they age, the mounds dissolving or becoming riddled with holes and channels in which other organisms settle.

At the November census, water temperature was 23.3°C. New sabellariid tubes were covering the old eroded sabellariid mounds on many of the rocks. The hydroids *Thyroscyphus ramosus* and *Eudendrium carneum* Clarke, 1882 were in an active phase of growth. The cyclostome *Crisia elongata* Milne-Edwards, 1838 was the most common bryozoan found; masses of short young *Crisia* colonies were attached to hydroid roots and branches. Colonies of the encrusting cheilostome *Watersipora subtorquata* d'Orbigny, 1852 were also common, attached directly to the rocks near the low water mark.

At the February census the water temperature was 23.1°C. Hydroids had proliferated. *Thyroscyphus* and *Eudendrium* colonies were thriving, and *Tubularia* sp. and *Halocordyle disticha* (Goldfuss, 1820) were also present along with colonies of the octocoral *Carijoa riisei*. The worm reef was extensive and in healthy condition. *Watersipora* was abundant, with some small, recently recruited colonies present along with large mature colonies. *Crisia* was still a dominant, with large mature colonies producing gonozooids containing yellow embryos.

At the April census water temperature was 25.3°C. Old fouled colonies of *Watersipora* were still present, but the most abundant encrusting cheilostome was *Thalamoporella floridana* Osburn, 1940, which formed thin whitish crusts and bilaminate expansions around the stems of *Thyroscyphus*. *Crisia* colonies with gonozooids were still abundant.

At the July census the water temperature was 29.2°C. The worm reef mounds were crumbling in places but still showed areas of active growth. *Carijoa* was abundant, and there were large, well-grown colonies of *Thyroscyphus*, still active, with functional polyps and characteristic garlicky smell. The most abundant bryozoan at this census was the primitive cheilostome *Aetea sica* (Couch, 1844). This bryozoan has a runner-like growth form, producing uniserial rows of semierect zooids, and an ephemeral life history. Species of *Aetea* occasionally appear in an area in large numbers, encrusting almost every substratum. At other times they may be rare or absent at the same locality. At this census *Aetea* colonies were attached to sabellariid worm tubes, sponges, and *Codium* species of algae, as well as to hydroid stems. *Crisia* was still abundant, but colonies were short and there were very few gonozooids. The other species common at this census was the branching cteno-stome *Amathia distans* Busk, 1886, whose colonies form limp yellow-speckled clumps. They were attached to various substrata, including the senescent worm reef mounds.

Overall, the North Beach Breakwater site was remarkably stable in its bryozoan fauna over the 24-year interval. Thirty species were recorded at the site during this study, including 20 of the 31 species originally found there (Table 1). The species that were dominant in the original survey—*Amathia vidovici* (Heller, 1867), *Beania hirtissima* (Heller, 1967), *Beania klugei* Cook, 1968, *Celleporina hassalli* (Johnston, 1848), *Crisia elongata*, *Pasythea tulipifera* (Ellis and Solander, 1786), *Savignyella lafontii* (Audouin, 1826), *Synnotum aegyptiacum* (Audouin, 1826), *Thalamoporella floridana*, and *Watersipora subtorquata*—were still abundant and were present during at least three of the four censuses. In addition, two new species were common at this site. *Amathia alternata* Lamouroux, 1816 was present at other Indian River sites in the past and still occurs at those sites, but it had not previously been recorded at the North Beach Breakwater. *Caulibugula armata* Verrill, 1900 is new to the region since the original study was carried out.

Walton Rocks

This site is located about 13.7 km south of Fort Pierce Inlet on South Hutchinson Island, on the beach just south of the Hutchinson Island Nuclear Power Plant (which was not yet constructed at the time of the original survey). The habitat consists of exposed sandy beach, with intertidal beach rock ledges; their upper surfaces are covered year-round by a macroalgal turf and seasonally by mounds of sabellariid worm reef. Numerous loose slabs of beach rock are present in a sandy trough in the surf zone between the ledges and the low water line. The exposed location makes collecting at this site difficult or impossible under high surf or wind conditions, and the full extent of the ledges is revealed only during the lowest tides of the year, and then only under calm sea conditions. Encrusting bryozoan species and branching species such as *Scrupocellaria regularis* Osburn, 1940 occur on the undersides of both the beach rock ledges and loose beach rock slabs and stones. Other branching and encrusting species grow on the algae and hydroids attached to the ledges.

At the November census the water temperature was 23.3°C. Most common were the spiny mats of the cheilostome *Beania hirtissima*, which were found on the underside of almost every piece of beach rock. Also common were the beach rock-encrusting species *Exechonella antillea* (Osburn, 1927), *Schizoporella "unicornis,"* and

TABLE 1. Bryozoans found (+) in 1998–1999 four-season resurvey in comparison with those found in original survey at the North Beach Breakwater, North Hutchinson Island, Fort Pierce, Florida. Dominant species are shown in **bold** type; a dash (–) indicates species not found during the resurvey.

Species	November 1998	February 1999	April 1999	July 1999
Species found in 1974–1975 survey				
Aetea sica	–	–	–	+
Aeverrillia armata	+	+	–	–
Amathia distans	–	–	–	–
Amathia vidovici	+	+	+	–
Anguinella palmata	–	–	–	–
Antropora leucocypha	–	–	+	–
Beania hirtissima	+	+	+	–
Beania klugei	–	+	+	+
Beania mirabilis	–	–	–	–
Bowerbankia imbricata	+	–	–	+
Bowerbankia maxima	+	+	–	–
Bugula minima	–	–	–	–
Bugula turrita	–	–	–	–
Celleporina hassalli	+	+	+	+
Crisia elongata	+	+	+	+
Cryptosula pallasiana	–	–	–	–
Exechonella antillea	–	–	–	–
Hippoporina verrilli	–	–	–	–
Jellyella tuberculata [a]	+	–	–	–
Nolella stipata	+	–	–	–
Pasythea tulipifera	+	+	+	+
Pourtalesella incrassata [a]	–	–	–	–
Savignyella lafontii	+	+	–	+
Scrupocellaria regularis	–	–	–	+
Synnotum aegyptiacum	+	+	+	+
Thalamoporella floridana	+	+	+	+
Valkeria atlantica	–	–	–	–
Vittaticella contei	+	+	–	–
Vittacella uberrima	–	–	–	+
Watersipora subtorquata [a]	+	+	+	+
Zoobotryon verticillatum	–	–	–	–
Additional species found, 1998–1999				
Amathia alternata	+	+	+	–
Caulibugula armata	+	+	+	+
Caulibugula pearsei	–	–	+	–
Biflustra arborescens [a]	–	–	–	+
Biflustra denticulata [a]	–	+	+	–
Bugula neritina	–	+	–	–
Bugula stolonifera	–	+	+	–
Parasmittina sp. 3	–	–	–	+
Rhynchozoon sp.	–	–	–	+
Schizoporella "unicornis"	–	+	–	–

[a] Species for which nomenclature has been revised since Winston (1982).

Pourtalesella incrassata (Canu and Bassler, 1928), actively growing peach or pink colonies with red embryos present in ovicells, along with the ctenostome *Nolella stipata* Gosse, 1855. *Nolella* zooids are straight mud-covered tubes resembling miniature polychaete tubes. They are connected by a thin stolon, but at this site zooids were so thickly aggregated that the stolons were invisible and the colony appeared as a fuzzy mat of tubes.

At the February census the water temperature was 23.0°C. The wind was strong because of a cold front, and the surf was high, making collection difficult. The macroalgal turf was thriving and mostly unfouled except

by epiphytic hydroids. There were few branching bryozoans. Loose rock in the trough was almost all buried under sand. The colonies of beach rock-encrusting bryozoans collected were abraded and bleached in color.

At the April census the water temperature was 23.7°C. The algal turf was growing luxuriantly. Many more beach rock stones, some freshly broken off the ledges, were uncovered. The undersides of most rocks were completely covered by a cryptic community that included zooanthids, didemnid ascidians, sponges, anemones, and branching and encrusting bryozoans. *Beania hirtissima* was again dominant, but other colonies of encrusting bryozoans, including *Schizoporella "unicornis," Exechonella antillea*, *Watersipora subtorquata*, and *Cryptosula pallasiana* (Moll, 1803), were brightly colored and healthy. *Nolella stipata* zooids were clean and translucent, less mud-coated than in February. Colonies were sexually reproductive, as well; many zooids brooded two or three yellow-ochre eggs near their distal ends.

At the July census water temperature was 30.9°C. Surf was moderate, sand had filled in around ledges again, and a considerable amount of detached beach rock ledge algae was washed up on the beach. The undersides of large beach rock slabs still had a healthy cryptic fauna consisting of zooanthids, ascidians, sponges, and bryozoans on their undersides, despite being buried in sand. Dominant bryozoans were *Exechonella antillea*, *Biflustra denticulata* (Busk, 1856), and *Beania hirtissima*, as well as *Nolella stipata* (which was still reproducing), plus two erect branching species, the ctenostome *Amathia vidovici* and the cyclostome *Crisia elongata*, both present as large, old, fouled colonies.

This site, Walton Rocks, had been the most diverse intertidal site in the original study, with 36 species recorded at that time. Twenty-five of the same species were found in 1998–1999 (Table 2). Of the dominant species in the original survey, all were still present in at least two of the four censuses, and all but one, *Parasmittina betamorphaea* Winston, 2005, was present at three of the four.

The biggest change at this site was a decline in abundance of *Cryptosula pallasiana* and its apparent replacement in beach rock undersurface habitats by *Exechonella antillea*, which in 1974 had been found only once, at the North Beach Breakwater, and which had not been collected at Walton Rocks.

Johnson House Seagrass Bed, Indian River Lagoon

This seagrass bed is located about 9.7 km north of Fort Pierce Inlet. It lies in a shallow cove just north of the Harbor Branch Canal, behind the Johnson residence on the campus of Harbor Branch Oceanographic Institution. The grass bed has been the site of several studies of seagrass and soft substratum communities (e.g., Mook, 1976; Kulczycki et al., 1981; Virnstein and Carbonara, 1985; Virnstein and Howard, 1987) and was one of the bryozoan sites studied monthly in 1974–1975. The turtle grass, *Thalassia testudinum* Banks and Soland. ex Koenig, is the most abundant seagrass at this site, but manatee grass, *Syringodium filiforme* Kuetz., is also common. Drift algae float among the grass blades.

At the November census water temperature was 24.4°C. Collections were made of all substrata: *Thalassia*, *Syringodium*, and drift algae. Most drift algae were fouled by a colonial ascidian, *Lissoclinum fragile* (Van Name, 1902). The stoloniferous ctenostome *Bowerbankia maxima* Winston, 1982, and the encrusting cheilostome *Conopeum tenuissimum* (Canu, 1908) were the dominant bryozoans.

At the February census, the water temperature was 15.2°C, with a strong north wind. Masses of drift algae had been cast up on shore. *Bowerbankia maxima*, *Conopeum tenuissimum*, and the branching cheilostome *Bugula neritina* (Linnaeus, 1758) were the dominant bryozoans on seagrass and drift algae, respectively.

At the April census the water temperature was 23.5°C. Drift red algae appeared bleached in color compared with their February condition; other algal species appeared to be thriving. There had been a new settlement of spirorbid polychaetes onto the seagrass since February, and *Conopeum* had decreased in abundance on *Thalassia*. However, there were larger numbers and larger sexually reproductive colonies of *Bugula neritina* on the *Syringodium*, along with small recent recruits.

At the July census the water temperature was 29.7°C. *Thalassia* and *Syringodium* blades were heavily fouled by filamentous algae and hydroids. Large colonies of *Bowerbankia maxima*, clean and healthy in appearance, with long free-trailing masses of stolons and zooids, occurred on the drift algae. *Conopeum tenuissimum* and *Schizoporella floridana* Osburn, 1914, with recently settled recruits and with embryos in mature colonies, were found on the *Thalassia*.

In the original study nine species of bryozoans were recorded from this site. Six of these were collected at least once in the re-study (Table 3). The dominant species, *Conopeum tenuissimum*, *Schizoporella floridana*, and *Bowerbankia maxima*, remained unchanged. Four additional species, *Aetea sica*, *Aeverrillia armata* (Verrill, 1873), *Hippoporina verrilli* Maturo and Schopf, 1968, and *Scrupocellaria "bertholletii,"* not recorded here in

TABLE 2. Bryozoans found (+) in 1998–1999 four-season resurvey in comparison with those found in original survey at Walton Rocks, South Hutchinson Island, St. Lucie County, Florida. Dominant species are shown in **bold** type; a dash (–) indicates species not found at this location during the resurvey.

Species	November 1998	February 1999	April 1999	July 1999
Species found in 1974–1975 survey				
Aetea sica	–	–	–	+
Alcyonidium polypylum	+	+	+	–
Amathia alternata	–	–	+	+
Amathia distans	–	–	+	+
Anguinella palmata	–	–	–	–
Antropora leucocypha	–	+	–	–
Beania hirtissima	+	+	+	+
Beania klugei	–	+	+	+
Biflustra denticulata [a]	–	–	+	+
Bowerbankia gracilis	–	–	–	–
Bowerbankia imbricata	–	–	–	–
Bowerbankia maxima	–	+	–	–
Bugula neritina	–	+	–	–
Bugula stolonifera	+	+	+	–
Bugula turrita	–	–	–	–
Bugula uniserialis	–	–	–	–
Caulibugula pearsei	–	–	–	–
Celleporella carolinensis	–	–	–	+
Crisia elongata	+	+	+	+
Cryptosula pallasiana	+	+	+	
Electra bellula	–	–	–	–
Jellyella tuberculata [a]	+	+	–	+
Microporella umbracula	–	–	–	–
Nolella stipata	+	+	+	+
Parasmittina betamorphaea [a]	–	+	+	–
Pourtalesella incrassata [a]	+	+	+	
Savignyella lafontii	–	–	–	–
Schizoporella "unicornis"	+	+	+	+
Scrupocellaria regularis	+	–	+	+
Sundanella sibogae	+	–	–	–
Synnotum aegyptiacum	–	+	–	+
Thalamoporella floridana	–	–	–	+
Vittaticella contei	–	–	–	–
Vittacella uberrima	–	–	–	–
Watersipora subtorquata	–	+	+	+
Zoobotryon verticillatum	–	–	–	–
Additional species found, 1998–1999				
Aimulosia spp	+	–	+	+
Amathia vidovici	+	+	+	+
Celleporaria sp. 2	+	+	+	
Escharoides costifer	–	–	–	+
Exechonella antillea	–	+	+	+
Biflustra arborescens	–	+	–	+
Lichenopora sp.	–	–	–	+
Parasmittina sp. 2	–	–	–	+
Pasythea tulipifera	+	+	–	–
Scrupocellaria "bertholletii"	–	+	–	–

[a] Species for which nomenclature has been revised since Winston (1982).

TABLE 3. Bryozoans found (+) in 1998–1999 four-season resurvey in comparison with those found in original survey at the Johnson House Seagrass Bed, Harbor Branch Oceanographic Institution, Link Port, Fort Pierce, Florida. Dominant species are shown in **bold** type; a dash (–) indicates species not found at this location during the resurvey.

Species	November 1998	February 1999	April 1999	July 1999
Species found in 1974–1975 survey				
Amathia distans	–	–	–	–
Beania klugei	–	–	+	–
Bugula neritina	–	+	+	–
Bowerbankia gracilis	–	–	–	–
Bowerbankia maxima	+	+	+	+
Conopeum tenuissimum	+	+	+	+
Electra bellula	–	–	–	–
Nolella stipata	–	+	+	–
Schizoporella floridana	+	+	+	+
Additional species found in resurvey				
Aetea sica	–	–	+	+
Aeverrillia armata	+	–	–	–
Hippoporina verrilli	+	–	–	–
Scrupocellaria "bertholletii"	–	–	+	–

1974–1975, were also found at one or more censuses in 1998–1999. The three species not found during the restudy, *Amathia distans*, *Bowerbankia gracilis* Leidy, 1855, and *Electra bellula* (Hincks, 1881), were still present in the lagoon at other sites.

A1A Causeway

In addition to the three sites from the original study, one new site was also surveyed quarterly. The site is a shaded spot under the east end of the Route A1A causeway bridge to North Hutchinson Island. This site was chosen because of its position in the Indian River Lagoon, about 3 km north of the mouth of Fort Pierce Inlet, and close to Little Jim Island, where in 1989 a *Scrupocellaria* species previously unrecorded in the region had first been collected (Winston, 1995). Material was collected from bridge pilings, from drift algae, and from submerged wood.

At the November census water temperature was 23.3°C. The most abundant species were *Bugula neritina*, *Caulibugula armata*, *Bugula stolonifera* Ryland, 1960 (the latter two reproductive), and *Zoobotryon verticillatum* (Delle Chiaje, 1828). Medium-sized *Zoobotryon* colonies had some areas of new growth with actively feeding polypides.

At the February census the water temperature was 21.1°C with a cold north wind and turbid water conditions. *Bugula neritina* was again dominant, with large, bright wine red-colored, sexually reproductive colonies. Other abundant species were *Amathia vidovici* (colonies mostly mud coated, but with clean actively growing branch tips) and long stalks of *Caulibugula armata*. *Zoobotryon verticillatum* was present only as short, heavily fouled, and senescent clumps.

At the April census water temperature was 23.2°C, with almost no wind and extremely clear water. *Bugula neritina* was still dominant on bridge pilings, with more mature and senescent colonies than in February. *Zoobotryon verticillatum* was still present, as large colonies drifting among seagrasses and short clumps attached to pilings, all of them heavily fouled, but with some young actively growing branches. *Caulibugula armata* was still present, with large and unfouled colonies. *Amathia vidovici* was still abundant, but colonies were heavily fouled.

At the July census water temperature was 28.8°C, wind calm, with fairly clear water (visibility about 1 m). *Bugula neritina* and *Zoobotryon verticillatum* were absent. Dominant species were *Caulibugula armata* (old, fouled colonies, with many brown bodies in the lower parts of branches, but with zooids containing feeding polypides and ovicelled zooids containing creamy white embryos near branch tips), *Savignyella lafontii*, a delicate branching cheilostome, *Nolella stipata*, and *Amathia vidovici* (as small, heavily fouled colonies).

Twelve species were found at this site (Table 4), making it less diverse than the open coast sites but more diverse

TABLE 4. Bryozoans found (+) in the 1998–1999 four-season survey at the AIA Causeway Bridge, North Hutchinson Island, Fort Pierce, Florida. Dominant species are shown in **bold** type; a dash (–) indicates species not found at this location during the resurvey.

Species	November 1998	February 1999	April 1999	July 1999
Aetea sica	–	+	+	–
Amathia vidovici	+	+	+	+
Beania klugei	–	+	+	+
Bowerbankia gracilis	–	–	–	+
Bowerbankia maxima	+	+	+	–
Bugula neritina	+	+	+	–
Bugula stolonifera	+	–	–	–
Caulibugula armata	+	+	+	+
Nolella stipata	+	+	+	–
Savignyella lafontii	+	+	+	–
Scrupocellaria "bertholletii"	–	+	+	–
Zoobotryon verticillatum	+	–	–	–

than the Johnson House Seagrass Bed site (10 species) further up the lagoon from Fort Pierce Inlet. Species composition was stable; most species found there were collected in at least three of the four censuses. Overall dominants were *Amathia vidovici*, *Bugula neritina*, *Zoobotryon verticillatum*, and *Caulibugula armata*, a species that had not been collected in the area until about 1994.

DISCUSSION

In the 1974–1975 study, 55 species were recorded from all lagoon and shallow coastal sites. Forty-nine species were recorded at the three sites later resurveyed. During the 1998–1999 survey, 39 species were found at those three sites. Thus, 80% of the bryozoan species known originally from those sites were recollected after a 24-year interval, despite a smaller sampling effort (4 versus 12 collections). Seventy percent of the species found originally from all inshore sites combined were also found in the four-site resurvey, again with a much smaller sampling effort involved. There has been remarkable stability in species composition of the bryozoan fauna over the time period.

Sixteen species had additional localities (that is, they were present in the area originally, but occurred at a different site in the second study than that from which they had been recorded in the original survey), indicating that most species were not restricted to one site and could be expected at any or all sites provided the appropriate substratum and environmental conditions were present. Even though most of the species involved have nonfeeding, rapidly settling larvae, there is apparently enough dispersal and recruitment that disappearance from one site would not mean that a species would disappear from the region entirely. Only one species, *Schizoporella floridana*, was limited to one site, the Johnson Seagrass Bed, and to one substratum, *Thalassia testudinum*, and was not collected elsewhere in 1998–1999.

Species new for inshore intertidal sites, but known from offshore hard substrata or algae (Winston and Eisman, 1980; Winston and Håkansson, 1986), included *Aimulosia uvulifera* (Osburn, 1914), *Aimulosia pusilla* (Smitt, 1873), and *Escharoides costifer* (Osburn, 1914). Four species were newly recorded for the region during the study: two species of *Parasmittina*, a species of *Celleporaria*, and a *Lichenopora* species.

Although species composition remained very stable, species abundances changed considerably, not only from season to season but also between the two studies. The most notable changes involved the decline in abundance of the warm temperate species *Bugula stolonifera*, *Cryptosula pallasiana*, and *Hippoporina verrilli*, all of which have western Atlantic distributions extending northward to Long Island or Cape Cod. During the original study period abundant *Bugula stolonifera* colonies were found attached to the proximal portions of *Bugula neritina* colonies. In the re-study only a few colonies were found, and they were not in association with *Bugula neritina*. *Hippoporina verrilli* was a common species on Indian River Lagoon panels (Mook, 1976) and on panels and seagrasses in 1974–1975, and it was also found at two coastal sites

at that time. Reproduction and settlement were heaviest in the cooler months (October–January). In the re-study only a few small colonies were found at the Johnson Seagrass Bed. *Cryptosula pallasiana* is a cosmopolitan temperate fouling species. In 1974–1975 it occurred at four intertidal coastal sites. In the re-survey, however, it was found only at Walton Rocks where it was much less abundant under beach rock stones than originally. Instead, in the underrock habitat the dominant encrusting bryozoans in 1998–1999 included *Exechonella antillea*, a Caribbean species which, in the original study, had been collected only one time, at the North Beach Breakwater site. That original record itself may have indicated a range expansion for the species because a distributional survey by Maturo (1968) reported the species only from Miami south.

The other new species in the study are similarly warm-water species. *Caulibugula armata* was described by Verrill from Bermuda, and it is known from the Tortugas, Puerto Rico, and Brazil, according to Osburn (1940). *Aimulosia pusilla* was described from the Tortugas by Smitt (1873) and *Aimulosia uvulifera* and *Escharoides costifer* from the same locality by Osburn (1914). The typical *Scrupocellaria bertholletii* is a circumtropical species, often associated with coral reefs (Winston, 1986), but Indian River and other western Atlantic specimens show some morphological differences to those from other localities, indicating that *Scrupocellaria bertholletii* is a species complex rather than a single widespread species. It was first recorded in the Indian River lagoon in 1989 and continues to occur at both coastal and lagoon sites. The genera *Celleporaria* and *Parasmittina* contain numerous species that are extremely successful in both tropical fouling and cryptic coral reef communities (Winston, 1986). The addition of species in this group is not surprising.

The increase in warm-water species has continued since the re-study was completed. *Nellia tenella* (Lamarck, 1816), another circumtropical fouling and reef-associated species, was first found in the Indian River area in 1999, in intertidal collections in Fort Pierce Inlet. It has been found every year since then, although its abundance has varied. *Hippopodina irregularis*, a species described form Guanica Harbor, Puerto Rico, by Osburn (1940), was first found on *Syringodium* seagrass in Fort Pierce inlet in the summer of 2001. *Schizoporella pungens* (Canu and Bassler, 1928), the massive dark purple, Caribbean–Gulf of Mexico *Schizoporella*, whose colonies are characteristically found on submerged mangrove roots and in harbor fouling communities, had been on noted on drift plastic items washed ashore in the area for several years, always with an associated fauna of small corals and *Millepora* species that suggested the debris had been colonized further south, perhaps in the Straits of Florida or the Florida Keys. *Schizoporella pungens* colonies first recruited to panels in Indian River Lagoon (Faber Cove), as well as to numerous benthic substrata in Fort Pierce Inlet between July 2002 and July 2003. *Celleporaria sherryae* Winston, 2005, another Caribbean fouling and shallow reef-associated species, has also appeared at some coastal sites (2001) and within the Fort Pierce Inlet (2003).

Reasons for the increase in warm-water species are harder to identify. One explanation might be global warming. As noted by many recent studies, the decade of the 1990s was the warmest on record (Levitus et al., 2000). The effects of warming seawater temperatures on marine organisms, including bryozoans (Kelmo et al., 2004), have been noted worldwide. In addition to direct effects on growth and survival of benthic organisms, changes in water temperature also affect food supply (Menge et al., 2008; Richardson, 2008), as well as producing indirect effects via changes in ocean chemistry and circulation (Harley et al., 2006).

For these collecting sites no records of seawater temperature exist for the entire time period of the two studies (1974–1999). However, as these sites are all intertidal, it seemed reasonable to make use of the published air temperature data that were available for Fort Pierce as a substitute. Although mean annual temperatures and mean annual high temperatures (based on monthly averages) showed no discernible statistically significant pattern (Figure 1A,B), there is a suggestion in the data that mean annual low temperatures (Figure 1C) have increased over the time period. If warm-water species are more susceptible to cold-water shock than high temperatures, as has been shown in studies of Florida fish kills after freezes in the region (Gilmore et al., 1978), warmer winter temperatures might be a factor permitting the invasion and survival of populations of the more tropical species, as has been shown to be the case for some introduced marine invertebrates in other studies (Stachowicz et al., 2002).

However, other factors are involved. The Indian River Lagoon is part of the Intracoastal Waterway, a passage for boat traffic moving up and down the Atlantic coast, as well as in and out of the Gulf of Mexico and the Caribbean. Fort Pierce has a small commercial port with shipping traffic from the Bahamas (especially Freeport, where containers from China and other distant sources are transferred for transshipment into the USA), the Gulf of Mexico, and the Caribbean, as well as U.S. ports along the east coast. Species could be introduced through ballast water exchange by larger ships, as well as by hull fouling of small and large vessels.

Although the stability of the bryozoan fauna over this time period gives a positive picture of the health and stability of the lagoon epifauna overall, there is no way to predict the long-term impact of these factors. The dependence of many bryozoans on living substrata such as seagrasses, hydroids, and octocorals also makes it clear that disturbances affecting substratum organisms would have a major impact on the bryozoans and would probably be more destructive to their local diversity than the environmental fluctuations noted so far.

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The Turtles' Tale: Flagships and Instruments for Marine Research, Education, and Conservation

John G. Frazier

ABSTRACT. Marine turtles are classic flagship species. Their remarkable natural history—large body size, dependence on both terrestrial and oceanic environments, delayed maturity requiring decades to reach adulthood, regular migrations that crisscross ocean basins, massive reproductive output, mammal-like physiology, and other features—make them attractive to researchers and the general public alike. This attraction is further enhanced by the fact that these reptiles are widely recognized as endangered species. They are "biomagnets" for people around the world, from various sectors of society; incredible amounts of time, energy, and resources go into diverse types of investigation, public education, conservation, and international policy directed specifically at these "lowly reptiles." Oceanographers, ecologists, geneticists, marine biologists, and specialists from other related disciplines frequently begin basic research projects on marine turtles. These activities quickly evolve into large multifaceted programs including conservation activities, community-based approaches, and public education together with other forms of development and social projects, and even policy initiatives for promoting regional and global cooperation in the conservation of these shared resources and the habitats on which they depend. Besides enhancing better understanding of the biology and ecology of these animals and nurturing more active and diverse conservation and education initiatives, work on marine turtles also promotes much-needed initiatives in interdisciplinary and international cooperation, which are fundamental challenges to marine work in general. This paper provides a summary of the flagship species concept and gives examples of how work focused on marine turtles has promoted diverse initiatives in marine research, education, and conservation at multiple scholarly, social, and political levels; it argues that this approach serves as a critical integrating force to nurture a wider comprehension and appreciation of the scientific endeavor and its role in society.

FLAGSHIP SPECIES AND THE INCREASE AND DIFFUSION OF KNOWLEDGE

Scientists, educators, and conservationists who specialize on marine organisms and marine environments may all be convinced of the fundamental importance of such things as larval nectophores, pedunculate siphonophores, disappearing zooxanthellae, discharged nematocysts, mitochondrial cytochrome oxidase 1, maximum parsimony, and other indicators of "good science," but what of the rest of society? Marine biodiversity is unique yet poorly understood or appreciated by the general public or decision makers;

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and a central question with which we all must contend is "How can we promote it?"

Many marine organisms have complex, intriguing life histories, and marine turtles, comprising just seven living species, are classic examples. These air-breathing reptiles are typified by highly complex life cycles: they live with fish but nest on land, relying on terrestrial, coastal, benthic, and pelagic environments during different parts of their life cycle; they can occur in extremely dense concentrations both on land and in the sea; they are "highly migratory," crossing ocean basins; they take a decade or more to reach sexual maturity and can live for half a century or more; and they have highly specialized morphological and dietary adaptations, including mammal-like physiology. A single female often lays more than 100 eggs in a nest and can lay several nests in a season. Their large body size (up to 1 ton), striking coloration, and primeval appearance all add to the attractiveness of these marine reptiles. The fact that marine turtles are globally recognized as endangered species adds a further level of importance. Hence, these reptiles are flagship species: ambassadors of the oceans. The attraction has led to not only enormous interest on the part of the general public but also disproportionate attention in academic circles (Frazier, 2003a, 2005a, 2005b): nearly as much research is conducted on just seven species of marine turtles as is carried out for the remaining 300-some species of chelonians.

In addition, marine turtles are widely valued as sources of meat, eggs, oil, skin, and shell, which have been utilized, crafted, and traded for millennia. A global trading network that supplied elite urbanites of the Mediterranean with raw materials from the shores of the Indian Ocean and beyond was well established before the time of Christ, and the most frequently mentioned commodity was tortoise shell (the external keratinous scutes of the hawksbill turtle, *Eretmochelys imbricata* Linn.). Intricately fashioned toilet articles, particularly ornamental combs, some of which were 85 cm wide, as well as a special style of French furniture luxuriously inlaid with tortoise shell and metal ("Boulle"), and religious accoutrements have all been made famous by the tortoise shell used in their creation. In addition to the tremendous diversity of objects crafted from turtle parts, these animals have been portrayed for millennia on a wide variety of media, from cave walls to carved rocks to delicate ceramics to the cylindrical seals of ancient Arabia (Frazier, 2003b, 2004a, 2005c). Hence, they have had very important cultural, social, and spiritual values in many societies. During contemporary times marine turtles have been celebrated in many and diverse forms, ranging from symbols of sacred nature and "pristine" environments to evidence of the evils committed by modern society on the environment (Campbell, 2003). All this conveys upon these animals a wide variety of values, from cultural and historic to economic and spiritual.

ACTIVITIES FOCUSED ON MARINE TURTLE RESEARCH AND CONSERVATION

The national marine turtle program in Brazil, which began as a dedicated study of reproductive biology and natural history, has evolved into one of the best known long-term programs in South America and the world in general, and the attraction of the turtle flagship over the years has resulted in the incorporation of massive efforts in public education and community development, including alternate livelihoods for community residents, training, and facilitated interactions between different sectors of government and society, not to mention national counsel for regional and international policy actions (Marcovaldi et al., 2005). Similarly, multiyear programs in Uruguay (Laporta and Miller, 2005), northwestern Mexico (Delgado and Nichols, 2005), the Caribbean (Eckert and Hemphill, 2005), and Nova Scotia (Martin and James, 2005) conduct research on diverse topics such as feeding ecology, reproductive biology, genetics, migration, and fisheries interactions. All this research, as well as the associated educational and conservation activities, has been greatly facilitated—if indeed not made possible—by the attractiveness of marine turtles and the ease with which researchers have been able to make use of these flagship species to promote interest in collaborating with different research activities. It is not uncommon for fishermen to go out of their way not only to inform researchers about sightings and captures of marine turtles but also to take on extra work, requiring time, effort, and materials to deliver information and specimens to researchers. Frequently this means allowing, or even inviting, researchers to come onboard and make free use of the fishermen's vessels and materials. Swordfish fishermen in Nova Scotia provide their vessels as research platforms for the complicated process of capturing, boarding, measuring, instrumenting, and releasing turtles of half a ton in body weight or more; researchers are very much aware that the success of their work depends on the altruistic behavior of fishermen (Martin and James, 2005). Uruguayan fishermen, many of whom live at a subsistence level, not only invite researchers to make use of their boats but are active collaborators in the research, attending meetings and participating in presentations (Laporta and Miller, 2005). A dramatic example of

the level of dedication to, and investment in, marine turtle projects is *Theeram Pakriti Samrakshana Samiti* (Coastal Ecosystem Protection Committee) in Kolavipalam Village, Kerala, India. A group of artisanal fishermen decided to protect nesting turtles and their eggs, formed the committee, built a modest beach station, and now run nightly beach patrols, maintain an interpretation center with live turtles, and give public education presentations: all these activities have been self-organized and self-motivated, thanks to the attractive power of the turtles (Shanker and Kutty, 2005). This sort of material and moral support is difficult to evaluate adequately in simple financial terms, but it has been absolutely essential in supporting various aspects of basic research, education, and conservation activities. Indeed, many of these activities would not only be far outside the operational budgets of the organizations involved but simply impossible to achieve without the full collaboration of the fishing communities.

Adventure tourism, often referred to as "eco-tourism," has been widely promoted around the world with marine turtles as the central attractants; indeed, there is even an international travel guidebook that is dedicated specifically to marine turtle tourism (Devaux and De Wetter, 2000). In addition to paying their travel costs, it is not uncommon for tourists to actually pay for the privilege of working as volunteers in turtle research projects, some of which have been operating for decades (Campbell and Smith, 2005). In this way the flagship attraction directly supports research through both funding and the availability of trained volunteer assistants.

An incredible diversity of outreach and public education has been developed with marine turtles as the centerpiece, a phenomenon common around the world and far too diverse to summarize easily (Frazier, 2005d). There are national and regional training programs specific to marine turtle biology and conservation, and some of these have been active for more than a decade, during which time they have seeded well-trained and enthusiastic researchers, educators, and conservationists throughout vast areas, such as India (Shanker and Kutty, 2005), the Caribbean (Eckert and Hemphill, 2005), and Latin America (Buitrago et al., 2008; Marcovaldi et al., 2005). In some cases, the activities and festivals organized by conservationists have been appropriated by local people, who have completely taken over what were initially devised to "sensitize" and "motivate" them to collaborate with marine turtle projects. One of the clearest examples of the rapidly increasing and powerful attraction of marine turtles is the Annual Symposium on Sea Turtle Biology and Conservation, an event that is attended by about a thousand people, with representation from scores of countries and hundreds of presentations (Frazier, 2003a). By using the turtles as attractants "to get people in the door," these activities, events, and projects clearly transcend the turtles and provide a wide basis of information on a diversity of marine organisms and environments, thereby promoting greater interest, research, and appreciation for these topics.

There is ample evidence that the flagship attraction can be instrumental for developing popular and political support to affect local policy decisions, such as the creation of special protected areas and tourism management programs (Tisdell and Wilson, 2005). Moreover, international maritime and fisheries policies have been directly affected by international, regional, and national efforts to conserve marine turtles, particularly through such efforts as mitigation of fisheries bycatch (Bache, 2005). In fact, an extraordinary amount of attention has been paid to marine turtles in the field of international environmental law (Frazier, 2002). At present there are two bilateral agreements, an incipient trilateral agreement, a program under a United Nations Environmental Programme (UNEP) Regional Seas convention in the southeast Pacific, a memorandum of understanding for the Atlantic coast of Africa, and another memorandum of understanding for the Indian Ocean (both under the United Nations Convention for Migratory Species), and a "stand-alone" treaty for the Western Hemisphere, all focused specifically on the conservation of marine turtles. Every one of these instruments includes measures of habitat protection, and the term "habitat" is even included in the title of one accord. Hence, through activities to protect marine turtle habitats over vast areas, these instruments have direct relevance to a wide range of marine organisms and environments, again clearly transcending marine turtles.

In addition to these seven agreements specific to marine turtles, there are many other international agreements that are relevant to marine turtle research, conservation, and education: these include such major global treaties as the UN Convention on the Law of the Sea (UNCLOS), Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), Convention on Biological Diversity (CBD), and the Convention on the Conservation of Migratory Species of Wild Animals (CMS) (Wold, 2002). Moreover, intense concern for the conservation of marine turtles has been instrumental in shaping policy and management directions in fisheries and other maritime issues (Bache, 2002). For example, the Inter-American Tropical Tuna Commission (IATTC), which was constituted nearly 60 years ago to develop regional management of tuna stocks in the Eastern Tropical Pacific, has

been dealing specifically with accidental capture of marine turtles since 2003 and has adopted at least eight resolutions to promote mitigation measures on turtle bycatch. Even the United Nations Food and Agriculture Organization (FAO), originally created to enhance the production of food, has become intimately involved in marine turtle conservation. In March 2004 the "Expert Consultation on Interactions between Sea Turtles and Fisheries within an Ecosystem Context" was held in Rome (FAO, 2004), followed by a technical consultation at which guidelines for mitigating turtle bycatch in fisheries were proposed (FAO, 2005). These technical considerations and recommendations were then taken up by the political body, FAO's Committee on Fisheries, where the proposal was adopted at a global level (COFI, 2005). The result is a set of recommendations for all States that are members of FAO (virtually every country that exists). Some of the specific actions that States are supposed to carry out include stock identification and assessment, tagging and genetic studies, testing mitigation techniques, "pay urgent attention . . . to collection of statistics," collect and share information, and harmonize conservation and management initiatives.

At an even greater level of political importance was a dispute brought before the World Trade Organization (WTO), which challenged the right of a Party to the WTO to enact unilateral measures that ban certain imports in an effort to protect marine turtles from capture and mortality in certain fisheries operations. After several years of contentious debate and the production and exchange of thousands of pages of documentation, an WTO Appellate Body decision released on 22 October 2001 concluded that because marine turtles are endangered species, countries can take exceptions to the all-powerful free-trade rules of the WTO and—following certain procedures—enact unilateral measures to protect turtles, including trade embargos (Bache and Frazier, 2006; Frazier and Bache, 2002).

COMPLEXITIES OF FLAGSHIP PERCEPTIONS

It is important to point out, however, that the inappropriate use of a flagship can lead to totally misguided policies and activities, counterproductive to both environmental and social needs. For example, easy access to highly attractive hatchling marine turtles led to an explosion of "sea turtle conservation hatcheries" along the coast of Sri Lanka, generously funded by unknowing tourists, despite the fact that these establishments were illegal and had negative impacts on hatchling recruitment (Tisdell and Wilson, 2005). Conservation programs that focus reflexively on an urgent need to do everything possible to protect marine turtles but ignore local sociopolitical complexities can create tremendous conflict, for different sectors of society often have divergent, even conflicting, views on how to respond to the flagship and what it primarily symbolizes (Shanker and Kutty, 2005; Frazier, 2008). Although conservationists view marine turtles as indisputable symbols of the need for people to cherish and protect the environment, other sectors of society—for example, certain ethnic groups—see the same turtles in very different ways, such as symbols of cultural identity and reclamation. This divergence in perceptions is true both on Pacific islands (Kinan and Dalzell, 2005) and on a Greek island in the Mediterranean, where contradictions in perceived value of the marine turtle flagship have resulted in violence, death threats, and other forms of intense conflict between different sectors of society (Theodossopoulos, 2005).

SHARED RESOURCES—THE ROOT PROBLEM

Because of their life history characteristics (particularly the long lifespan, dependence on a variety of diverse environments, and dispersal and migration across oceanic basins), marine turtles provide a classic case of shared resources, or "common property." Simple, but basic, questions such as "Who owns turtles?" or "Who has rights to turtles?" clearly show that many parts of many societies have direct impacts, rights, and responsibilities relating to these animals (Frazier, 2004b). This contention is easily illustrated by the fact that more than 2 million reproductive turtles were taken from the breeding grounds in Pacific Mexico between 1964 and 1980 (Frazier et al., 2007). Yet, animals from this population migrate widely throughout the eastern tropical Pacific, living at different times within the jurisdiction of different sovereign States or on the high seas (Morreale et al., 2007). Who had the right to slaughter so many reproductive animals that are part of the fauna of a vast region (an action that had enormous implications on the status of a shared population)? The same question can be asked of people who pollute the oceans with oil spills, plastics, or other wastes: What right do they have to contaminate a common resource? Similarly, when endangered species of marine wildlife, such as dolphins, whales, seabirds, and marine turtles, are caught and killed in fishing activities, the question arises: "What right does the fishing industry have to be killing (even if it is accidentally) wildlife species that are valued by the citizens of many nations?"

Dealing with shared resources is the root issue for nearly all questions regarding biological conservation—

particularly in marine environments. Hence, by highlighting the importance of this central problem, work on marine turtles brings even greater attention to this critical issue, and because these reptiles are regarded globally as endangered species, their importance is further enhanced. Investigations on marine turtles that help promote ways to resolve intractable issues of common property have implications that go far beyond chelonian biology and natural history: they bear on the way modern societies interact with the oceans.

CONCLUSIONS: PROMOTING MARINE RESEARCH, EDUCATION, AND CONSERVATION THROUGH FLAGSHIP SPECIES

The attention given to marine turtles spans the entire sociopolitical spectrum, from marginalized, politically insignificant fishing communities to the most politically powerful organizations on the planet. From one extreme of the political continuum to the other, these animals have been given extraordinary importance. These local, national, regional, and global policy decisions have enormous importance in the ways that individuals, governments, and organizations at various levels assign priorities and allocate resources. Even if the intent is only to comply superficially with obligations that are not enforced, the end result is resources and personnel allotted to some aspect of marine turtle research, education, and conservation.

Although the scientific enterprise and its practitioners strive to develop and maintain an objective, unbiased view of the world, there is no escaping the fact that both the enterprise and the practitioners are immersed within complex social and political systems. The result, despite the firmest of desires, is that there is close interplay and interaction between scientific activities and attitudes that dominate in the surrounding society (Rozzi, 1999). In fact, an anthropological study of the scientific establishment shows not that scientists and their practices are unique among humanity, but rather that they are immersed in a world of power struggles, politics, and myths—little different from the world of the lay public that is so often demeaned by the scientific community (Nader, 1996).

There is no inherent reason that information produced by scientific research will be read, understood, appreciated, followed, used, or even recognized in the halls of power; if practitioners of the scientific endeavor want their information to impact society outside the ivory towers of academia, it is essential that we learn how to "package" the information in digestible, understandable, interesting, and convincing ways (Frazier, 2005e). Flagship species greatly facilitate this exercise for they have values that are attractive to the general society. Used efficiently and appropriately, such species are powerful tools for promoting research, education, and conservation of countless marine issues.

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Latitudinal Gradients in Recruitment and Community Dynamics in Marine Epifaunal Communities: Implications for Invasion Success

Amy L. Freestone, Richard W. Osman, and Robert B. Whitlatch

ABSTRACT. Although the latitudinal diversity gradient, where species diversity peaks at low latitudes, is well documented, much less is known about how species life history strategies differ among regions and the implications of these differences for community development trajectories and particularly for invasion dynamics. As a first step in trying to understand these factors, we contrast spatial and temporal variation in recruitment rates and resultant community development of epifaunal assemblages in regions along a latitudinal gradient from the temperate zone to the tropics. We exposed settlement panels in four regions: Long Island Sound (Connecticut), Chesapeake Bay and Virginia's Eastern Shore (Maryland and Virginia), Indian River Lagoon (Florida), and a portion of the Mesoamerican reef in Belize. Panels were deployed for either one to two weeks, to evaluate recruitment patterns, or one year, to monitor community development. We found that both recruitment and community development rates were inversely correlated with diversity, with the highest rates seen in temperate latitudes and the lowest in tropical Belize. Seasonal variability in recruitment also varied latitudinally, with strong summer pulses of recruitment in northern latitudes shifting to low and year-round recruitment at low latitudes. However, species turnover through time in communities becoming established was highest in Belize. We conclude with predictions regarding the implications these patterns may have on invasion dynamics at different latitudes.

INTRODUCTION

Latitudinal patterns in diversity have remained an important theme in ecology for more than a century, yet we still continue to debate the relative contributions of processes that may cause these patterns (Currie et al., 2004; Mittelbach et al., 2007). There are many environmental variables that change with latitude, and it is easy to correlate species distribution patterns with these factors. Unfortunately, it is as easy to find exceptions to these correlations. In addition, with increased transport of nonnative species (Ruiz et al., 2000), species distributions continue to be altered. Although latitudinal gradients in native species diversity are well documented, studies on terrestrial and freshwater systems suggest latitudinal gradients in invasion success occur as well (Sax, 2001). However, little work to date has examined this question in marine systems. Therefore, we have been documenting latitudinal differences in both the recruitment and the community development of

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marine epifaunal invertebrates as a first step in understanding latitudinal differences in species invasions.

The mode by which species successfully invade new habitats is a pressing ecological research issue (Rejmanek and Richardson, 1996; Williamson and Fitter, 1996; Moyle and Light, 1996). Current theory predicting the attributes of successful invaders has largely been developed in terrestrial environments and usually stresses the importance of life history traits associated with rapid reproduction and wide dispersal ability (Rejmanek and Richardson, 1996). In the far more open marine environment, ocean currents can disperse larvae and adults of many species for great distances over relatively short time periods (Jokiel, 1984; Scheltema, 1986). Additionally, man inadvertently transports countless individuals and species between discrete biogeographic provinces (Ruiz et al., 1997; Carlton, 1999). Given the generally good dispersal abilities of marine species, those attributes of new species that allow them to coexist with, or even displace, native species will be as important as dispersal ability to a species invasion potential.

The sessile invertebrate or epifaunal community is an excellent system in which to examine rigorously both the life history attributes that characterize successful invaders as well as those attributes of native communities that govern their susceptibility to invasion. Epifaunal communities occur in all coastal habitats and can be found in all biogeographic regions. These communities contain species with a variety of life histories, yet their principal species are usually permanently attached as adults and are easy to manipulate. Although the species within these communities differ among regions, they function in similar ways. Most have planktonic larvae as the main means of dispersal, feed from the water column, compete for limited available space, and are preyed on by a variety of mobile vertebrate and invertebrate predators. Because epifaunal species are sessile and relatively small in size, natural communities can develop on small discrete substrates, with larval dispersal and recruitment linking communities within a site or habitat as well as within a region. These attributes make them ideal systems that can be experimentally manipulated in the field to test directly hypothetical relationships while maintaining natural levels of abundance, species composition, and diversity.

Among epifaunal communities, a major difference is the number of available species that have some reasonable probability of recruiting to a particular site within a region. Osman and Dean (1987) found that these regional pools of species varied by almost an order of magnitude and that both the mean number of species found on individual substrates and the correlated richness at each site varied greatly among the study sites within each region, with overlap among sites in different regions. These patterns potentially result from (1) the low probability of recruits of many species in the regional species pool actually reaching a particular site during the course of investigation and (2) the high probability of local, within-site dispersal of species already present at a particular site. Alternatively, high predation and local extinction rates at some sites may prevent certain species in the regional species pool from colonizing these sites (Osman and Whitlatch, 1996, 1998). As a first step in trying to understand factors contributing to the local and regional differences in diversity and how these are likely to influence species invasions, we have been contrasting temporal variation in recruitment rates and resultant community development in regions along a latitudinal gradient from tropical to temperate regions.

METHODS

We deployed experimental panels in four biogeographic regions along the eastern seaboard of the United States and in the Caribbean Sea. These regions were Long Island Sound in Connecticut (LIS; 41°N), Maryland and Virginia's Chesapeake Bay and Eastern Shore region (CB; 37°N), the Indian River Lagoon in Florida (IRL; 27°N), and the vicinity of Carrie Bow Cay in Belize (BEL; 16°N). Polyvinyl chloride (PVC) panels, 100 cm^2, were abraded to facilitate settlement of invertebrates and were suspended on racks underneath docks. The panels were held horizontal with the experimental surface facing the seafloor.

Recruitment

To estimate recruitment in all regions, panels were sampled either weekly (LIS) or biweekly (CB, IRL, BEL). At the beginning of each sampling period, four clean panels were exposed at each of the field sites. After the one- or two-week exposure period the panels were collected and new panels were deployed. In the laboratory, all panels were examined under a dissecting microscope, and all attached invertebrates were identified to the lowest possible taxonomic unit (usually species) and counted.

Sample schedules varied by region as necessitated by recruitment patterns and destructive storm activity. Weekly sampling at the LIS Avery Point (AP) site began in 1991 and has continued unabated to the present. In the years 1991–1996 sampling was suspended during the win-

ter months when almost no settlement occurs. From 1997 to the present, sampling was conducted continuously with biweekly sampling during the winter. The remaining LIS sites (Groton Long Point [GLP] and Mystic River [MR]) were added in 2001 and have been sampled on the same schedule as the AP site. Sampling in CB and IRL was begun in 2004 with two sites in each region. The CB sites were at the Smithsonian Environmental Research Center (SERC) in the upper Bay and at the Virginia Institute of Marine Science (VIMS) in the lower Bay. The IRL sites were the Smithsonian Marine Station (SMS) and the Ft. Pierce Inlet (Inlet). Sampling at the VIMS site was discontinued in 2007 after hurricane damage to the dock, and sampling at both IRL sites was suspended from September 2004 until March 2005 because of the loss of docks as the result of two hurricanes. Sampling in BEL began in December 2004 and continued through February 2006.

Data Analysis

Recruitment differences among sites within and across regions were compared by matching means for each sampling time and using paired *t* tests to analyze for significant differences. Wilcoxon signed-rank tests were also conducted for each pairing to eliminate the possible effects of large seasonal differences biasing the results. Because of the species differences among regions, analyses were done for total recruitment of all species, pooled invasive species, and pooled native species. Species identified as cryptogenic were included with the native species. The number of sampling periods varied greatly among the regions, and we conducted the analysis of each pair of stations using the maximum number of sampling periods in common based on the year and week of sampling. Data were corrected for exposure time to account for the one- and two-week sampling periods used in different regions.

Community Development

To measure difference in community development, experimental panels (same as above) were deployed for at least one year and nondestructively sampled for invertebrate richness. Four panels were deployed at each site (three per region) between July and August 2006 to a depth of 0.6 m below LLT and at least 0.5 m above the bottom. Panels in LIS, CB, and IRL were sampled iteratively 1, 3, and 12 months after deployment. Panels in BEL were sampled 3, 6, and 12 months after deployment. Panels were sampled with a dissecting microscope, and attached invertebrates were identified to the lowest possible taxonomic unit. Taxonomic richness on each panel was recorded.

RESULTS

Recruitment

Three types of recruitment patterns are evident. Within sites there are temporal patterns, among sites within regions there are fairly consistent relationships, and together these produce broader patterns among the regions.

Within-Site Temporal Patterns

Within each site there are temporal patterns in recruitment that result from seasonal cycles in reproduction and year-to-year variation in recruitment that can result from a variety of causes. Seasonal variability in recruitment is most evident in the two northern regions, LIS and CB, which experience large variations in temperature. In both regions recruitment is largely absent during the coldest winter months. The three sites in LIS are consistent in exhibiting peak recruitment in the late summer (Figure 1). Recruitment at the GLP site begins earlier and remains consistently higher than at the other sites throughout the whole season. This site is in shallower water and consequently experiences lower winter temperatures and higher summer temperatures (Osman and Whitlatch, 2007) and warms more quickly in the spring. At the CB sites, the majority of recruitment occurs in the spring and early summer, with a second, much smaller peak period in the autumn (Figure 1). Most dominant species in this region such as barnacles, bivalves, and polychaetes are planktotrophic with feeding larvae dependent on the spring and autumn plankton blooms. Recruitment in the remaining two regions, although temporally variable, exhibits no consistent seasonal cycle. Recruitment occurs year round at both IRL sites, with the inlet having somewhat higher recruitment in the summer (Figure 1). Recruitment at the SMS dock is dominated by several species of barnacles and has much more sporadic peaks. Finally, BEL recruitment was extremely low and demonstrated no obvious patterns.

Spatial Variability among Sites within Regions

Within the three regions with multiple sites we have observed fairly consistent differences among the sites. Based on the paired *t* tests of weekly differences in total recruitment over the period 2001 through 2007, the three sites in LIS were significantly different, with GLP > AP > MR

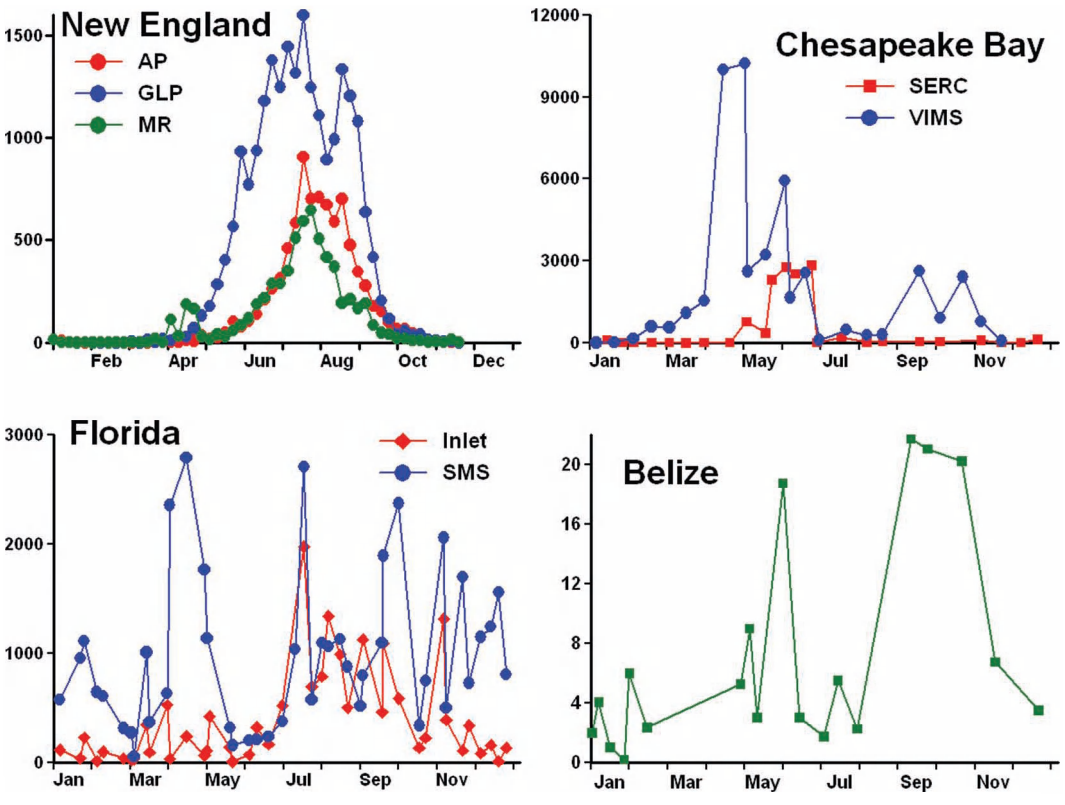

FIGURE 1. Comparison of temporal variation in mean recruitment in the four regions. Individual sites within regions are shown. Means were based on 1–6 years of data depending on region and the periods over which recruitment was measured (see Methods). Sampling sites are as follows: New England: AP = Avery Point, GLP = Groton Long Point, MR = Mystic River; Chesapeake Bay: SERC = Smithsonian Environmental Research Center, VIMS = Virginia Institute of Marine Science; Florida: Inlet = Ft. Pierce Inlet, SMS = Smithsonian Marine Station; Belize: Carrie Bow Cay.

(Table 1). Native species recruitment at the three sites showed the same pattern while the recruitment of invasive species was not significantly different among the sites (Table 2).

A similar analysis of the two CB sites for 2004 through 2006 found that total recruitment at the VIMS site was significantly greater than at the SERC site (Table 1). Recruitment at both sites was dominated by native barnacles, and invasive species recruitment was very low. Nevertheless both native and invasive species exhibited the same pattern as total recruitment (Table 2). Although experiencing similar variability in temperature, these two sites differ greatly in their salinity regimes. The SERC site is in the upper, low-salinity region of CB whereas the VIMS site is in the lower CB with higher salinities. In general fewer species recruit at the SERC site, and barnacle recruitment is much less.

Similarly, the two IRL sites differed significantly (2004–2006) in total recruitment, with SMS greater than Inlet (Table 1). Native and invasive species exhibited the same pattern (Table 2). Although there was little difference between the sites in temperature and salinity, they did differ in dominant species, which resulted in strong differences in total recruitment. Barnacle recruitment (six different species) was consistently much higher at the SMS site and this contributed greatly to the overall site differences. Most species of bryozoans as well as spirorbid worms had higher recruitment at the Inlet site. Figure 2 illustrates these differences.

The nonparametric paired analyses of the data from all three regions were almost identical to those above. The only difference was that in LIS invasive species recruitment was significantly greater at GLP than at either AP or MR.

Regional Patterns

The regional differences in temporal and spatial patterns in recruitment can be seen in Figure 1. In LIS the strong

TABLE 1. Results of paired analysis of mean recruitment between each pair of sites. Recruitment data were paired by sampling time. Mean values are for 2-week sampling periods and vary based on the number of sampling dates in common between any two pairs of sites (*df* = degrees of freedom;). Significant probabilities (Prob) are in **bold.**

			Total Annual Recruitment				
Site 1 [a]	Site 2 [a]	*df*	Mean 1	Mean 2	*t*-ratio	Prob > |*t*|	One-sided
Avery Point	Mystic River	229	474.5	299.2	6.39	**< 0.0001**	**< 0.0001**
	Groton LP	252	446.6	1000.4	8.60	**< 0.0001**	**< 0.0001**
	SERC	21	208.4	550.8	1.44	0.16	0.08
	VIMS	21	251.3	2206.2	2.99	**0.007**	**0.004**
	SMS	38	638.2	1010.0	2.22	**0.03**	**0.02**
	Inlet	36	607.5	607.5	1.37	0.17	0.09
Mystic River	Groton LP	234	296.7	996.8	9.63	**< 0.0001**	**< 0.0001**
	SERC	23	152.3	506.2	1.65	0.11	0.06
	VIMS	23	184.1	2097.8	3.22	**0.004**	**0.002**
	SMS	39	425.4	1068.1	4.15	**0.0002**	**0.0001**
	Inlet	38	451.2	459.9	0.09	0.93	0.46
Groton LP	SERC	21	698.5	550.8	0.52	0.61	0.31
	VIMS	21	761.3	2275.1	2.11	**0.05**	**0.02**
	SMS	39	1288.4	1050.5	0.91	0.36	0.18
	Inlet	37	1192.5	471.3	3.60	**0.0009**	**0.0005**
SERC	VIMS	17	381.6	1519.7	2.11	**0.05**	**0.03**
	SMS	10	341.6	685.3	1.18	0.26	0.13
	Inlet	10	341.6	242.1	0.42	0.68	0.34
VIMS	SMS	15	2694.9	903.9	2.38	**0.03**	**0.02**
	Inlet	15	2694.9	169.6	3.01	**0.009**	**0.004**
SMS	Inlet	71	1045.5	399.6	7.20	**< 0.0001**	**< 0.0001**

[a] Groton LP = Groton Long Point (GLP); SERC = Smithsonian Environmental Research Center; VIMS = Virginia Institute of Marine Science; SMS = Smithsonian Marine Station.

seasonality produces a relatively normal distribution in recruitment centered on the summer months of peak temperatures. Peak periods are relatively broad, with 1,000 to 2,000 recruits per panel per week. This overall pattern reflects the concentration of recruitment by most species in the summer period. Recruitment in CB is also seasonal but generally dominated by a few species, with sharp peaks in recruitment of 3,000 to 10,000 individuals per panel. The pattern in IRL is more diffuse with recruitment occurring throughout the year and several sharp peaks of 2,000 to 3,000 recruits per panel (barnacles) over a background of continuous recruitment. Individual species do have peaks in recruitment but they do not occur at the same time as in the northern regions. Thus, some species recruit in the winter and others in the summer, and this difference is reflected in the continuous total recruitment throughout the year. Finally, recruitment at the BEL site was extremely low, despite the much greater species diversity in the region.

Given these patterns, we examined whether total annual recruitment was influenced by the regional differences in variability and peak abundances. Figure 3 shows the total mean annual recruitment for each of the sites; no general pattern is discernible from these data. Except for BEL, within-region differences in total annual recruitment are as great as, if not greater than, differences among regions. Figure 3 also shows the dominance of barnacle recruitment in both the low-diversity CB and high-diversity IRL regions, whereas bryozoans and ascidians dominate recruitment in LIS. Interregional differences in total recruitment, regardless of strong differences in temporal patterns, exhibited no pattern that could be associated with diversity or latitude.

Results from the paired analyses did show some regional differences (Tables 1, 2; see Figure 3). For total recruitment the VIMS site in CB had significantly greater recruitment than all other sites. The GLP in LIS and SMS in IRL were significantly greater than the Inlet IRL, SERC CB, and AP LIS

TABLE 2. Results of paired analysis of mean invasive and native recruitment between each pair of sites. Recruitment data were paired by sampling time. Mean values are for 2-week sampling periods and vary based on the number of sampling dates in common between any two pairs of sites. Significant probabilities (Prob) are in **bold**.

			Invasive					Native				
Site 1 [a]	Site 2 [a]	*df*	Mean 1	Mean 2	*t*-ratio	Prob > |*t*|	One-sided	Mean 1	Mean 2	*t*-ratio	Prob > |*t*|	One-sided
Avery Point	Mystic River	229	144.9	144.9	0.00	0.99	0.50	329.6	154.3	6.69	**< 0.0001**	**< 0.0001**
	Groton LP	252	133.7	145.9	0.67	0.50	0.25	312.9	854.5	8.91	**< 0.0001**	**< 0.0001**
	SERC	21	66.6	1.3	2.18	**0.04**	**0.02**	141.8	549.5	1.81	0.08	**0.04**
	VIMS	21	95.8	18.8	1.94	0.07	**0.03**	155.6	2187.4	3.14	**0.005**	**0.003**
	SMS	38	218.9	345.5	1.23	0.22	0.11	409.0	666.6	1.84	0.07	**0.04**
	Inlet	36	203.2	191.0	0.39	0.70	0.35	381.3	270.6	1.46	0.15	0.08
Mystic River	Groton LP	234	143.3	149.3	0.31	0.75	0.38	153.4	847.5	9.87	**< 0.0001**	**< 0.0001**
	SERC	23	35.8	1.2	2.27	**0.03**	**0.02**	116.4	505.0	1.86	0.08	**0.04**
	VIMS	23	28.9	24.0	0.60	0.55	0.28	155.1	2073.8	3.24	**0.003**	**0.002**
	SMS	39	199.9	355.9	1.85	0.07	**0.04**	225.4	712.3	4.37	**0.0001**	**<0.0001**
	Inlet	38	200.4	186.1	0.21	0.83	0.42	250.8	273.8	0.37	0.71	0.36
Groton LP	SERC	21	163.7	1.3	2.31	**0.03**	**0.02**	534.8	549.5	0.05	0.96	0.48
	VIMS	21	220.7	18.8	2.47	**0.02**	**0.01**	540.7	2256.3	2.47	**0.02**	**0.01**
	SMS	39	218.9	126.6	2.01	**0.05**	**0.03**	1069.5	705.0	1.50	0.14	0.07
	Inlet	37	203.2	−12.2	0.30	0.77	0.38	989.3	280.3	3.55	**0.001**	**0.0005**
SERC	VIMS	17	1.2	25.6	2.11	**0.05**	**0.02**	380.3	1494.2	2.05	0.06	**0.03**
	SMS	10	0.4	173.1	3.11	**0.01**	**0.006**	341.2	512.2	0.63	0.54	0.27
	Inlet	10	0.4	48.7	1.95	0.08	**0.04**	341.2	193.4	0.61	0.56	0.28
VIMS	SMS	15	11.0	239.4	3.23	**0.006**	**0.003**	2683.9	664.5	2.78	**0.01**	**0.007**
	Inlet	15	11.0	26.6	2.38	**0.03**	**0.02**	2683.9	143.0	3.02	**0.009**	**0.004**
SMS	Inlet	71	374.8	138.7	5.11	**< 0.0001**	**< 0.0001**	670.7	260.9	5.96	**< 0.0001**	**< 0.0001**

[a] R. = River; Inlet = Ft. Pierce Inlet.

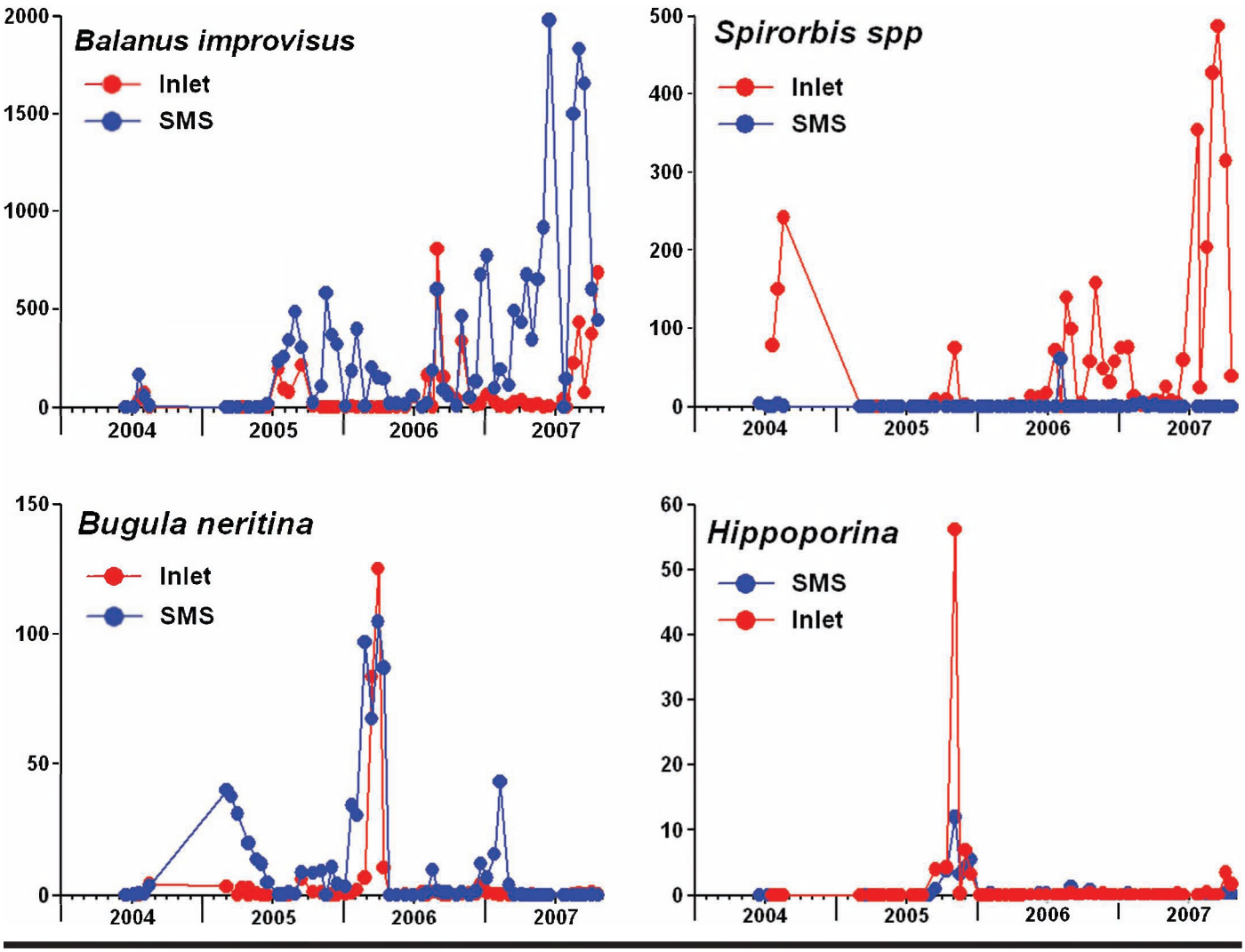

FIGURE 2. Comparison of recruitment at the two sites in Indian River Lagoon, Florida, for the barnacle *Balanus improvisus*, spirorbid polychaetes (*Spirorbis* spp.), arborescent bryozoan *Bugula neritina*, and encrusting bryozoan *Hipporina* sp.

sites, and all were greater than the MR LIS site. Recruitment of native species exhibited similar interregional patterns. However, for invasive species, recruitment was significantly higher in IRL than CB, with the LIS sites intermediate.

Community Development

Spatial and Temporal Variability

The speed of community development varied dramatically with latitude. The primary limiting resource, space, was quickly occupied in the temperate and subtropical regions by three months, compared to the tropical communities, which took close to a year to attain comparable spatial coverage (Figure 4). In the northernmost region, LIS, growth rates were particularly high, and at one site (AP) panels were completely covered after only one month, which is a striking comparison to comparably aged communities in BEL (Figure 5). These productive communities in AP were primarily composed of *Diplosoma listerianum*, an invasive colonial tunicate, and *Mogula manhattensis*, a solitary tunicate. These animals quickly became too heavy to remain attached to the panel and sloughed off, providing another flush of open space to recruiting species. This punctuated seasonal pulse of productivity of *Diplosoma* and *Mogula* did not occur at the other two sites in LIS, but growth rates remained high throughout the region.

Overall, communities in LIS experienced less temporal turnover in species composition than more southerly sites,

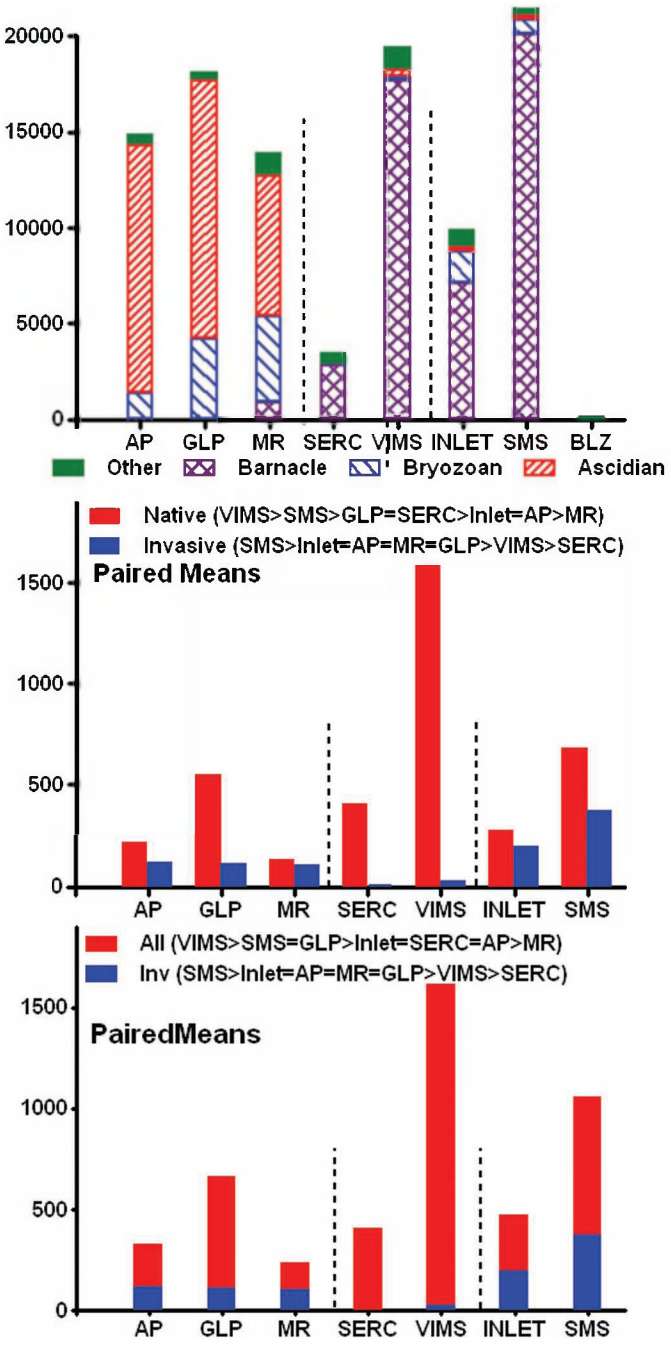

FIGURE 3. Comparison of recruitment among regions. Top: Total annual recruitment at each of the sites within regions. The contributions in each region of major taxonomic groups are represented by colored shading and/or scoring within the histogram bars. Middle: Mean recruitment of invasive (blue) and native (red) species by sites within region; significant differences are based on paired analyses (see Table 2). Bottom: Total mean recruitment by site showing the contribution of invasive (Inv) and native species. Dashed lines separate regions in all graphs.

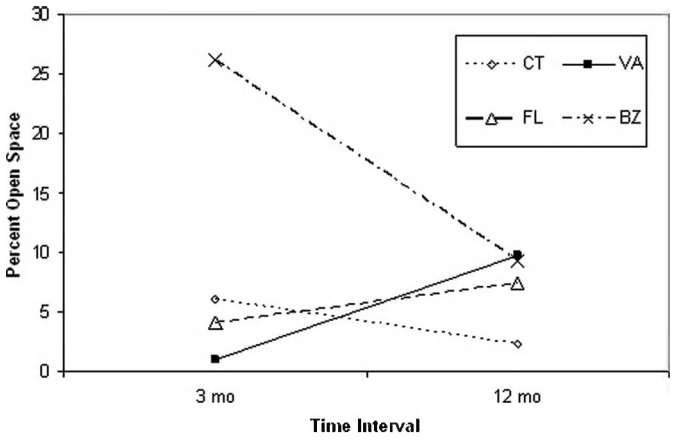

FIGURE 4. Total percent cover of open space on panels in the four regions after 3 and 12 months (CT = Connecticut; VA = Virginia; FL = Florida; BZ = Belize). In Belize, space occupied by algae was included as covered space, so percent cover of invertebrates was even lower than shown.

particularly BEL (Figure 6). Communities in LIS were consistently dominated by bryozoans, particularly *Bugula turrita*, and both solitary and colonial tunicates (Figure 7; personal observations). This observation is in contrast to the higher rates of species turnover that characterized communities in tropical BEL (Figure 6; Freestone, unpublished data).

Epifaunal communities in CB had low temporal and spatial variability in species composition compared to other regions. Barnacle recruitment occurred soon after deployment, in July 2006. After the first month, all panels had 99% to 100% cover (see Figure 4) and were almost completely covered with barnacles, with few other coexisting species. After three months, community structure still closely resembled the one-month communities; however, barnacles began to die and other species, such as *Mogula*, various hydroids, and sabellid polychaete worms recruited (see Figure 7). After one year, the primary layer of barnacles was less visible, having been covered with a thick layer of sediment tubes, mostly from amphipods and worms. Anemones were also common throughout. Panels deployed at the three sites were also very similar. Porifera were least common in CB when compared with other regions throughout the experiment.

Overall, communities in IRL retained almost complete phyla representation through time (Figure 7). All focal phyla were found on all panels in IRL after three months, and only Porifera had a very modest decline by one year. Species in these communities coexisted at very small spatial scales, with the result that IRL had the most diverse invertebrate assemblages at the panel scale after

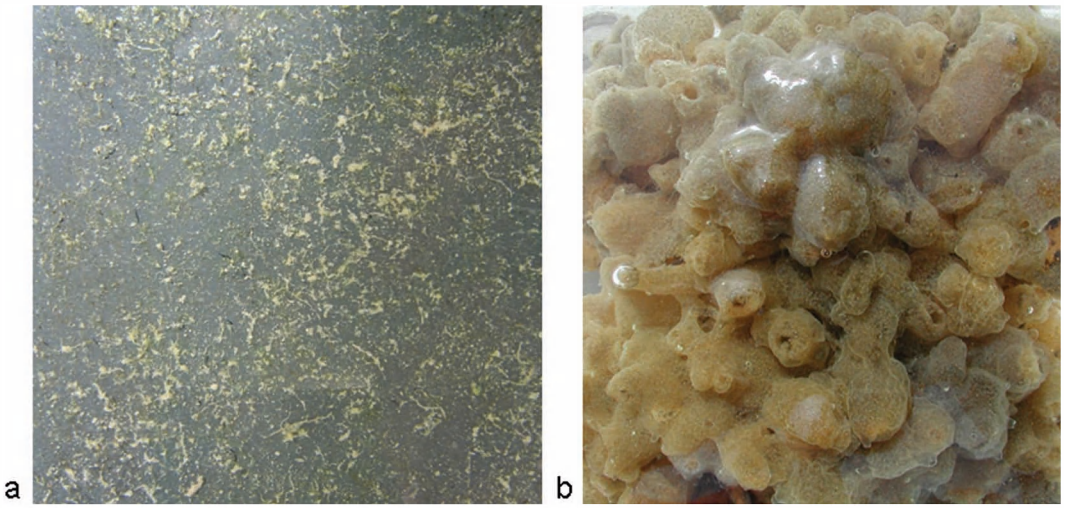

FIGURE 5. Growth rates were higher at northern latitudes, a pattern that is clearly visible in this comparison between (a) a 3-week-old community from Carrie Bow Cay, Belize and (b) a 4-week-old community from Avery Point, Long Island Sound (100-cm^2 panels are shown).

one year (Freestone, personal observation). More species turnover was apparent over the course of the year at IRL than in LIS or CB, but not as much as at BEL.

Communities in BEL were characterized by significant temporal and spatial variability in species composition. Communities developed much more slowly than did more northern communities (see Figure 4), and community composition clearly changed with time. These communities also varied at very small spatial scales, as panels that were deployed within a meter of each other harbored very distinct community assemblages with differing amounts of open space. In contrast to more northern communities, BEL communities were more consistently dominated by polychaetes, Cnidaria (sea anemones, hydroids, coral), and Porifera. After one year, Porifera clearly dominated the panels (Freestone, personal observation). Compared to the bushy and common bryozoan colonies that occur in LIS, bryozoans in BEL were generally very small, delicate, and rare.

Similar to the recruitment study, the largest difference in taxonomic composition of developing communities across all regions was the presence of barnacles. Barnacles were common in temperate and subtropical zones but were completely absent in BEL at 3 months. After 12 months, their dominance was still seen in CB and IRL, but barnacles were less common in LIS. However, only one barnacle on one panel was found in BEL. Although barnacles in temperate and subtropical zones are both intertidal and subtidal, barnacles are almost exclusively intertidal in BEL.

DISCUSSION

Based on our preliminary examination of these ongoing studies, it is clear that there are both strong intraregional and interregional patterns in both the recruitment and the development of epifaunal communities. Seasonality in recruitment clearly varies with latitude. Strong summer peaks coupled with the almost complete absence of any recruitment in the winter were found for most species in the temperate regions (LIS and CB). The strong dominance of barnacles in CB resulted in a bimodal pattern generally associated with the spring–fall plankton blooms upon which barnacle larvae feed. In IRL there was also a strong temporal variability in recruitment but neither a consistent seasonal pattern nor any similarity among species. Finally, in BEL recruitment was too low to discern any pattern. There were also fairly distinct patterns among sites within regions. In the temperate regions, sites showed consistent differences in numbers of recruits but little difference in the species recruiting at any one time or in the relative abundances of these species. In the subtropical IRL, there were greater differences between the two sites in the composition of the fauna recruiting at any one time. Based on the low recruitment and greater community variability among sites in BEL, it would appear that site differences in recruitment in the tropics are likely to be even greater.

The interregional variation in recruitment for native species was influenced by the variation in barnacle dominance, with the CB and IRL sites showing significantly

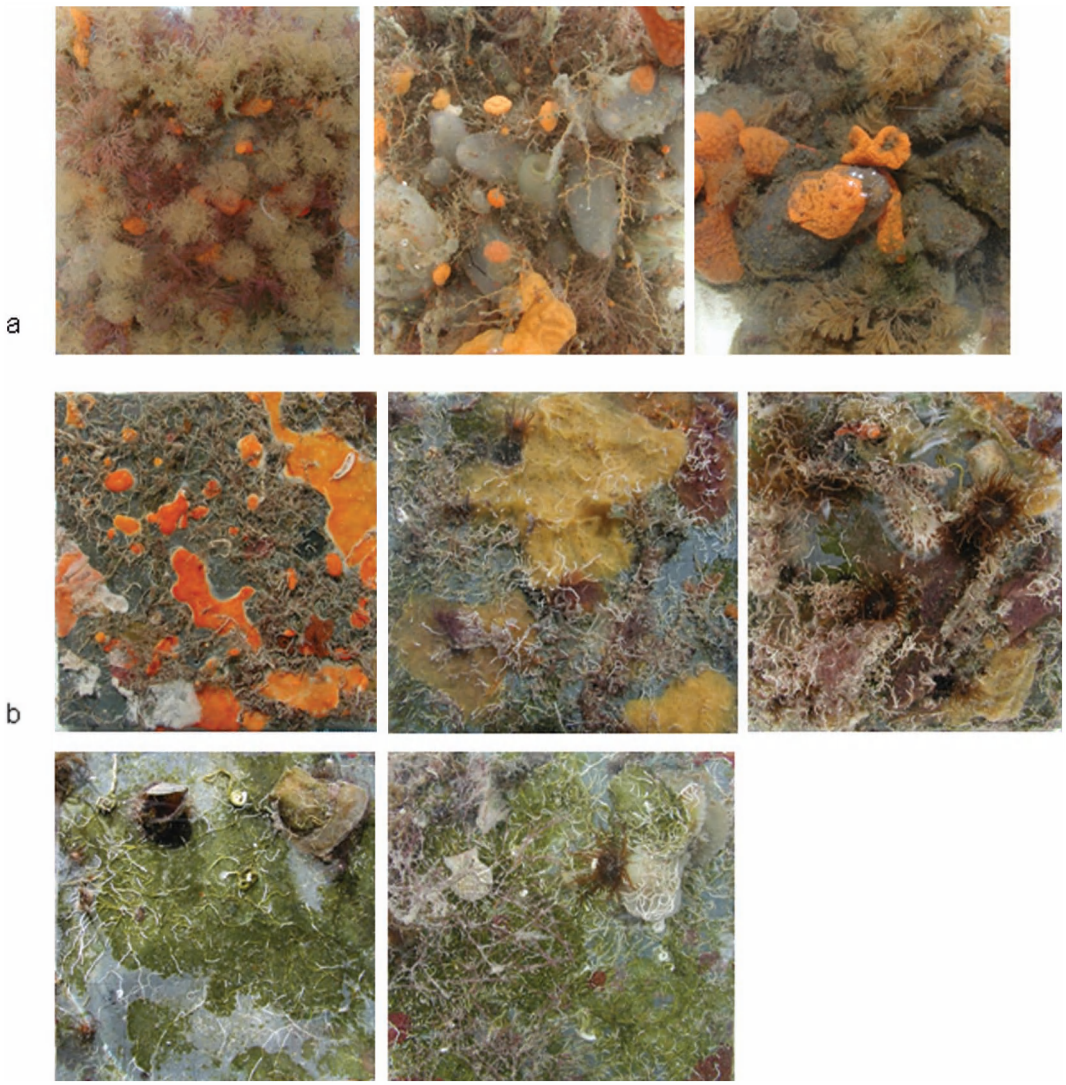

FIGURE 6. Time-series comparison of community development in (a) Long Island Sound after 1, 3, and 12 months and (b) Belize after 3, 6, 9, 12, and 20 months (100-cm^2 panels are shown). Greater species turnover occurred in Belize than in Long Island Sound.

higher recruitment than the LIS sites. However, the recruitment of invasive species varied in a similar manner to patterns described by Sax (2001). The tropical BEL site had little overall recruitment and no recruitment of invasive species, the subtropical IRL sites had the highest recruitment of invasive species, and the higher latitude temperate sites had lower recruitment of invasive species. The combined patterns of native and invasive species resulted in invasive species representing a much higher proportion of overall recruitment in IRL than in all other regions. In the low-diversity estuarine CB, invasive species contributed a very small proportion of total recruitment (see Figure 3).

Finally, even with the strong differences in recruitment reflected in the paired analyses, similarities in total annual recruitment were found between some sites in the three northern regions. This result suggests that the cumulative recruitment in more seasonal regions with strong peaks and in regions with little or no seasonality in recruitment can be similar.

In BEL, the epifaunal communities were quite spatially variable in community composition even at the smallest scale of panels at each site. This pattern is consistent with the spatial heterogeneity hypothesis for the latitudinal diversity gradient, which states that abiotic variability in tropical systems allows more species to coexist (see Davidowitz and

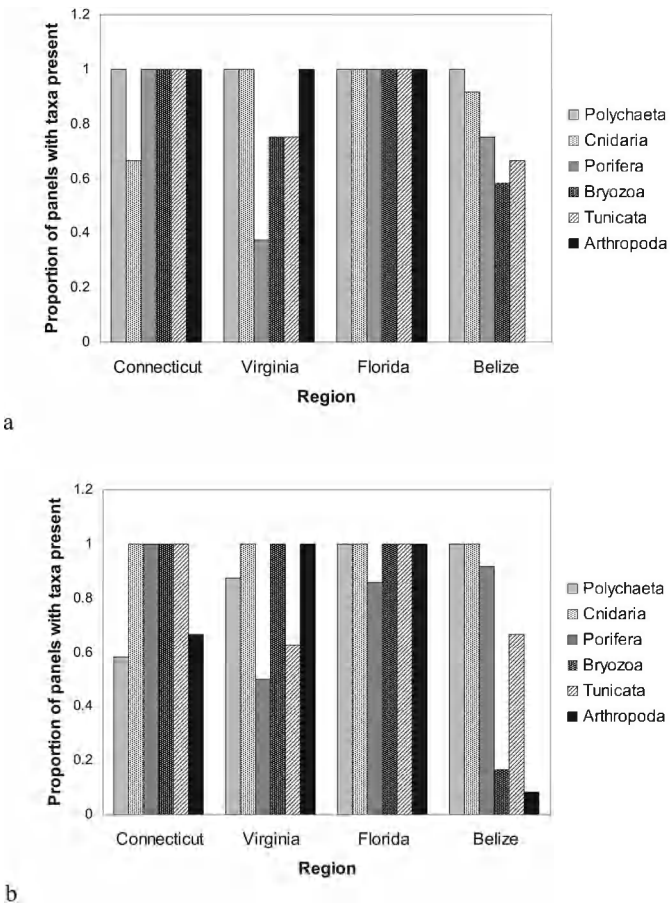

FIGURE 7. Proportion of panels that had listed taxa present at (a) 3 months and (b) 12 months by region.

Rosenzweig, 1998). Interestingly, in contrast to the abiotic variation that characterizes other systems, such as terrestrial plant–soil relationships, the settlement panels were identical in size and material, so substrate composition was not a source of variability. While it is possible that differences in subtle small-scale variation in currents or eddies could drive community variability, a more parsimonious explanation is that propagule supply is very low and sporadic. Community developmental trajectories may therefore be more a result of random recruitment from a limited larval pool rather than spatial variability in abiotic conditions.

Another possible explanation that has strong theoretical underpinnings is that biotic interactions, such as predation, are also strong and spatially variable in the tropics (Schemske, 2002). Although this hypothesis has not been empirically tested in a comprehensive experiment, the idea that biotic interactions are stronger in the tropics has received much theoretical attention (Mittelbach et al., 2007). Visual observations of the communities in BEL support this hypothesis. For example, we commonly observed grazing or saw indirect evidence of grazing (i.e., abrasions) on the panels from indiscriminate consumers, including gastropods, crabs, and fish. While predation undoubtedly occurs in temperate environments (Osman and Whitlatch, 1995, 1998, 2004), overall interaction strengths may be weaker and more spatially predictable in northern latitudes. Sporadic and low larval recruitment, spatially variable predation, and low growth rates in areas of low productivity are all potential contributors to the spatial and temporal variability of tropical epifaunal community development.

Our main goal has been to document and contrast recruitment and community development patterns among regions along a latitudinal gradient to ascertain potential differences in the ability of nonnative species to invade these systems. Except for our sites in BEL, all the regions we have been studying have nonnative species present, and such species are often dominant within these epifaunal communities. In LIS we have found that early recruitment of invasive ascidians in years with warm winters (Stachowicz et al., 2002a) and their dominance at harbor sites without native predators that prey on their recruits (Osman and Whitlatch, 1995, 1998, 2004) have contributed to their successful invasion. The strong and consistent timing of recruitment of native species certainly can create an opening for invaders that can recruit outside this window. In CB recruitment is even more constrained temporally with much higher numbers of recruits, again creating potential temporal windows for invasion. However, as our community development data have shown, the communities in both these temperate systems develop rapidly and thus quickly limit resources for new species. Studies in LIS (Stachowicz et al., 2002b) have shown that as community diversity within these systems increases, the communities become more resistant to invasions, mostly by increasing the likelihood of limiting open space.

In IRL, space was also rapidly occupied and the amount of open space remained low after three months. In addition, the diversity within this system is higher, and some species are recruiting at any time of the year. Based on the results of the LIS study (Stachowicz et al., 2002a) these factors should increase the resistance of this system to invaders. Although we have found several invasive species at our study sites, none of these species appears to be particularly abundant or dominant. Finally, in BEL we have observed much more diverse and spatially variable communities. Recruitment and the rates of community development are low, and this situation certainly allows spatial resource to be available for much longer periods of time, which should create a greater window for species invasion. However, the extremely high diversity of both epifaunal

species and predators may inhibit invasion success. It is also likely that, given the spatial variability in communities, invaders will face completely different communities at each site as well as temporal variability in communities within sites. Although it is much too early in our studies to link latitudinal variation in recruitment or community development to invasion success, our preliminary results do suggest that there is a correlation between decreasing invasion success and increasing diversity, increasing community variability, and the reduction in recruitment windows in less seasonal environments, with species varying greatly in the timing of recruitment.

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Ex Situ Culture of Caribbean and Pacific Coral Larvae Comparing Various Flow-Through Chambers

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ABSTRACT. Coral reefs are some of the oldest and most diverse ecosystems on our planet, yet throughout their range coral reefs are declining precipitously, mainly as the consequence of human activities. In situ conservation practices, such as habitat preservation, are an important way to protect coral reefs. However, reefs now face global threats in addition to local impacts. It is therefore critical that ex situ conservation activities are incorporated into conservation practices for coral reefs. Many coral species reproduce sexually during a limited yearly breeding season. If the resulting larvae are cultured, their husbandry can be very time consuming: time that is often taken away from larval research. Three different types of flow-through larval rearing systems were designed and tested during breeding seasons of the elkhorn coral *Acropora palmata*, the mushroom coral *Fungia scutaria*, and the cauliflower coral *Pocillopora meandrina*. The flow-through systems were tested against static bowl rearing, and no difference was observed in the survival of the larvae in two of the species: $P = 0.12$ for *A. palmata* and $P = 0.99$ for *F. scutaria*. These results suggested that these chambers may result in significant savings of limited research time during a coral spawning event. However, *P. meandrina* larval survival was better in bowls than in the flow-through chamber ($P = 0.03$). Rearing the maximum number of larvae possible with minimal maintenance will enhance opportunities for larval research, settlement, and growth. This is especially important for species that are now threatened, for which time and information are critical during the breeding season.

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INTRODUCTION

Coral reefs are some of the oldest and most diverse ecosystems on our planet. They are essential nurseries and feeding grounds for fish and invertebrates, act as natural storm barriers for coastlines, and are a potential source for novel pharmaceuticals (Colin, 1998). Throughout their range, coral reefs are declining precipitously, mainly because of human activities. These negative influences induce stress and can increase diseases in corals. Even in the most

remote marine bioreserves, such as the northwestern Hawaiian Islands (Maragos et al., 2004), human activities are damaging fragile coral ecosystems (Bellwood et al., 2004). Additionally, other environmental pressures, such as El Niño-Southern Oscillation events, result in bleaching and coral mortality (Glynn and D'Croz, 1990; Glynn, 1996). As greenhouse gases increase, atmospheric and sea-surface temperatures and ocean acidification are also expected to increase (Kleypas et al., 1999; Hoegh-Guldberg et al., 2007). When these effects are coupled with human-induced stresses, reefs will remain in crisis, their existence worldwide increasingly threatened (Hoegh-Guldberg, 1999; Hughes et al., 2003).

Scientists speculate that unless committed efforts are made to remedy this situation functional coral ecosystems may disappear in less than 50 years (World Wildlife Report, 2004; Hoegh-Guldberg et al., 2007). Although all the oceans in the world have corals, reef-building corals in the Caribbean are showing the greatest signs of disease-related mortality, and these corals may have far less than 50 years left to survive (Hoegh-Guldberg et al., 2007). The massive elkhorn coral, *Acropora palmata*, has historically been the most ecologically important reef-building coral in the Caribbean, but its populations have declined 90% to 99% since the mid-1980s, primarily because of disease (Aronson and Precht, 2001). Because of this decline and its critical role for Caribbean reefs, *A. palmata* has been one of the first two corals listed as "threatened" under the Endangered Species Act (*Acropora* Biological Review Team, 2005). As stony corals continue to die, they are being replaced with sponges, gorgonians, and algae (Hughes, 1994; McClanahan and Muthiga, 1998), altering the composition of Caribbean ecosystems.

In situ conservation practices, such as establishment of marine protected areas, are an important way to protect coral reefs. However, reefs now face global rather than just local threats. Therefore it is critical that ex situ conservation techniques are incorporated into conservation actions for coral reefs. Ex situ conservation techniques, defined as protecting organisms outside their native habitat, such as rearing sexually produced larvae in seminatural enclosures for future restoration purposes, hold strong promise for improvements in preserving species and genetic diversity within ecosystems. This stage is particularly needed to help diversify some of the declining endangered populations in Florida where many of the stands of *A. palmata* are genetically identical (Baums et al., 2005).

To address the ex situ conservation needs for coral reefs, SECORE (www.secore.org) was initiated by the Rotterdam Zoo in 2001 with the primary goals of studying sexual coral reproduction, specifically developing ex situ breeding techniques, disseminating techniques among aquarium and research communities through workshops and publications, developing a cooperative international network of public aquariums and research institutions, and establishing breeding programs to help sustain ex situ and field populations. In 2006 and 2007 SECORE members representing several national and international institutions held workshops in Puerto Rico with goals to successfully rear elkhorn coral from spawn produced during the annual mass spawning at Rincón and Bajo Gallardo sites. Gametes were collected and fertilized, producing close to a million larvae, of which hundreds of thousands were raised in the field laboratory and more than 400,000 were brought into captivity, resulting in approximately 2,300 juvenile larval recruits now living in public aquaria around the world (Petersen et al., 2007). These larvae were the first juveniles of this species ever reared in captivity, constituting a major step that will help with the conservation of their genome and restoration of this species in the wild.

Although ex situ conservation practices have yet to be applied to coral populations in conjunction with restoration, extensive work has been conducted in the zoological community on maintaining gene diversity in populations with ex situ techniques (Ballou, 1992; Harnal et al., 2002; Pukazhenthi et al., 2006). In particular, the black-footed ferret was rescued from the brink of extinction, with only 18 individuals remaining in the population, using ex situ conservation practices in parallel with restoration practices (Howard et al., 2003). Enhancing reproductive success of endangered coral through ex situ practices may be key to their future restoration and preservation (Richmond and Hunter, 1990). There are a number of ex situ techniques that have enhanced larval survival and settlement. Heyward et al. (2002) used a seminatural enhancement procedure for maintaining acroporid corals in open floating pools in the ocean. Water was pumped into the pools throughout the larval growth period, and then the contents were pumped into an enclosed area on the sea bottom with conditioned ceramic tiles. Heyward et al. started with $\sim 10.5 \times 10^6$ larvae/pool and after 144 h post-fertilization had $\sim 7.5 \times 10^5$ larvae/pool ($\sim$0.7% survival), resulting in $\sim$1,500 settled recruits in the best treatments versus 0 on the control tiles. Although this settlement rate was relatively low, it was far greater than the natural settlement rate and indicated a robust enhancement of recruits for this area.

Most current coral larval husbandry practices are low-cost efforts, such as bowls or aquaria filled with fil-

tered seawater, and these methods are very successful at rearing larvae (Babcock and Heyward, 1986; Schwartz et al., 1999; Petersen et al., 2007). The problem is that these time-consuming and labor-intensive husbandry practices compete with the limited time available for research during a coral breeding season, especially if the coral species is limited to a single annual breeding, as is *Acropora palmata*. For coral in need of replenishment, rearing the maximum number of larvae possible with the least time invested in husbandry would enhance opportunities for larval growth and settlement (Richmond and Hunter, 1990; Petersen and Tollrian, 2001; Borneman, 2006). The goal of this paper was to design and test simple flow-through systems in the field that would minimize husbandry and yet successfully rear large numbers of coral larvae without compromising survival.

Three species of coral larvae were tested in three different types of rearing chambers. These larvae were selected because they represented a good cross section of coral larval types with different buoyancies, swimming behaviors, and rates of development that might benefit from these chambers. *Acropora palmata* are large lipid-filled floating larvae (Figure 1a) that develop slow swimming ability in the water column after 48 h. *Fungia scutaria* are small negatively buoyant larvae containing modest lipid stores. These larvae develop rapid swimming behavior in the water column within 12 to 24 h (Figure 1b). *Pocillopora meandrina* are negatively buoyant larvae with modest lipid stores (Figure 1c); these larvae develop slow swimming behavior along the bottom after 24 h. In designing and constructing these low-tech chambers, we made an effort to use materials for their components that are affordable and available in most hardware stores throughout the world.

MATERIALS AND METHODS

Larval Collection and Rearing

Acropora palmata eggs and sperm were collected during the annual spawn from Tres Palmas Reserve (Rincon, Puerto Rico) and the offshore submerged bank Bajo Gallardo (Boqueron, Puerto Rico) in August 2007. Egg/sperm bundles were collected in the water over the spawning coral with 1 L plastic Nalgene bottles attached to fine mesh nets. The egg/sperm bundles were brought to shore in the plastic bottles, separated by gentle agitation, and then combined with the eggs and sperm from at least three to four individuals to yield a sperm concentration of approximately 10^6 cells/mL (final concentration in water). The eggs and sperm were gently agitated for 2 h, cleaned with 1 μm-filtered seawater, assessed for fertilization rates, and released into rearing chambers for subsequent development.

Fungia scutaria eggs and sperm were collected from captive animals held in flowing seawater tanks from June through October 2006 at Coconut Island, Hawaii. Animals were prepared for spawning following the methods of Krupp (1983). Briefly, as a female spawned, these eggs were gently moved into a plastic bowl and fertilized with ~150 mL sperm (10^6 cells/mL, final concentration in water) from four or five males. The embryos, resulting from several male and female gametes, were kept in a single plastic bowl (8 L) and left overnight to develop. In the morning the developing larvae were cleaned with four changes of 0.5 μm-filtered seawater and then released into their rearing chambers for subsequent development.

Egg/sperm bundles were collected from *Pocillopora meandrina* fragments in April and May 2008 from Coconut Island, Hawaii. The eggs and sperm were separated

FIGURE 1. Three species of coral were reared in this study. a, *Acropora palmata*, elkhorn coral; inset: larvae at 24 h postfertilization. b, *Fungia scutaria*, mushroom coral; inset: larvae at 96 h postfertilization. c, *Pocillopora meandrina*, cauliflower coral; inset: larvae at 96 h postfertilization. All scales = 50 μm.

by gentle agitation, and then combined with the eggs and sperm from at least three or four individuals to yield a sperm concentration of approximately 10^6 cells/mL. The eggs and sperm were gently agitated for 0.5 h, cleaned with 0.5 μm-filtered seawater, and left overnight to develop. The next morning the developing larvae were cleaned with 0.5 μm-filtered seawater and released into their rearing chambers for subsequent development.

Digital images of the larvae from all three species were captured with an Olympus BX41 microscope with an attached digital camera Sony DFWV300, and the major and minor axes were measured with NIH Image software.

Construction of Rearing Tanks and Measurement of Densities

Larval corals were reared in three different designs of flow-through chambers (Figures 2–4), as well as static bowls that required daily cleaning and water changes. The names of these chambers were chosen to describe the major water movement they provided to the larvae. All developmental times reported throughout the paper are in hours postfertilization.

Up-Flowing Tanks

These tanks were made from 20 L heavy-walled plastic pans (U.S. Plastics Corp., Lima, Ohio) modified by covering the handles in a buoyant foam and removing four panels from the bottom and replacing them with nylon screening (240 μm mesh). A central cross-shaped area was left intact to create an inlet for upward-directed water flow; then additional shear flow was added with four additional adjustable water inlets around the edges approximately 16 cm above the bottom, yielding a final volume in the chambers of ~23 L (see Figure 2). All flow was regulated by valves to optimize the slow tumbling movement of the larvae in the chamber. The floating chambers were immersed in large 2,400 L pools to stabilize their temperature (28°–31°C) and mimic natural temperature cycles throughout a 24 h period. To maintain water quality close to that which the larvae would experience in open water, the chambers were attached to a filtered (1 μm) flow-through system with seawater pumped from the reserve, so that water was completely exchanged in the chambers several times each day. Flow rates through the chambers were maintained at approximately 2 L/min, and the bath of fresh seawater surrounding the chambers was turned over about one to two times per hour. Salinity, temperature, and pH closely mimicked natural conditions without additional effort.

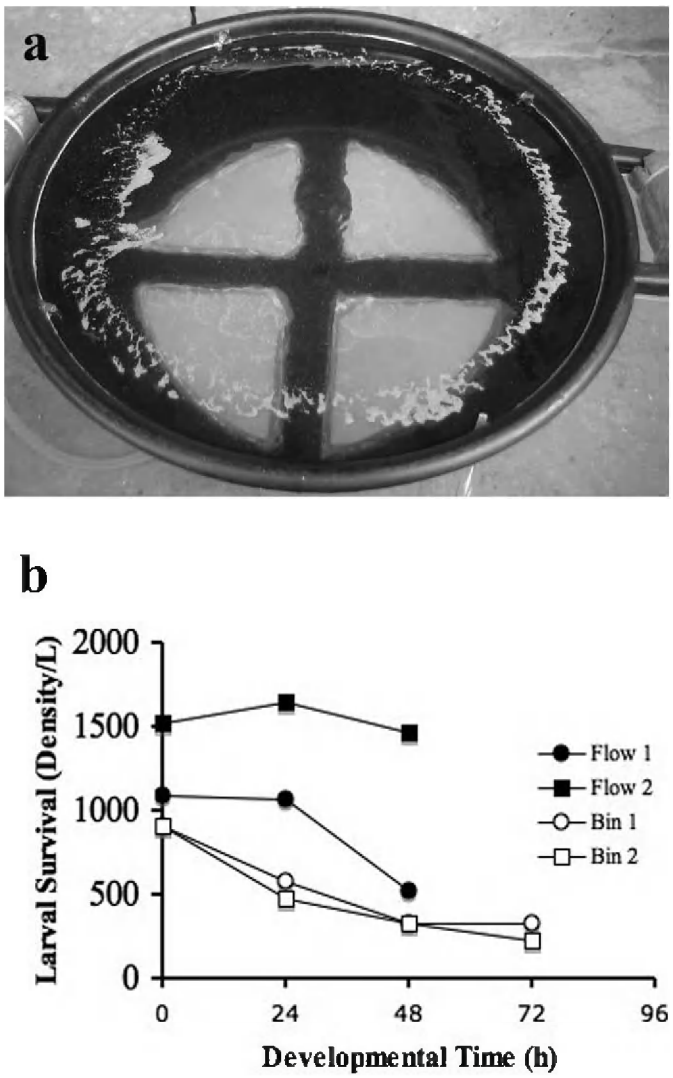

FIGURE 2. Larval rearing of *Acropora palmata* larvae. a, Up-flowing tank used to rear the *A. palmata* larvae. b, There was no significant difference in survival between the up-flowing tanks and static bins (ANCOVA, $P = 0.12$). Points show mean counts for each trial. Larvae in the upward-flow tanks were developing rapidly (~28°C) while those in the static bins were maintained at a slightly lower temperature (~25°C). Larvae in the flow chambers were removed from the experiment one day earlier than those in the bins to ship them to an aquarium for settlement and rearing.

Approximately 1,000 to 1,500 larvae/L were fertilized in 50 mL conical plastic tubes, then placed into either the up-flowing tanks ($n = 2$) or static bins. Counts were taken immediately, and then daily for all groups. Bin density began at ~900 larvae/L, and the two flow chambers contained either 1,100 or 1,500 larvae/L. The fertilization rate for *A. palmata* spawn used for these tests was ~90%.

The static treatments used for comparison with the up-flowing tanks were plastic rectangular bins (length [L] × width [W] = 51 cm × 36 cm) with water depth of 12 cm, yielding a volume of 22 L. These tanks were maintained in an air-conditioned room; the water was maintained at 25° to 26°C and changed twice daily. To keep the floating *A. palmata* larvae from clumping and forming an anoxic layer, the water and floating larvae were stirred every hour with a bubble-wand (2-mm-diameter rigid air line attached to a small air pump) throughout the rearing period. The previous year, a stocking density of approximately 1,000 *A. palmata* larvae/L was used successfully in each bin and we used this same level for these tests. Larvae from the same spawn and bulk fertilization as were used in the chambers were placed in the static bins (n = 2) 2 h after fertilization.

To determine larval survival, the chamber and static containers were stirred to suspend the larvae evenly in the water column, and five 15 mL samples were taken and the number of larvae counted each day. The number of larvae/mL was multiplied by 1,000 to determine the density per liter (density/L). The larvae in both systems were only allowed to develop for two to three days, and then they were packaged for shipment. Approximately 4,000 larvae were placed into a 2 L Nalgene bottle with filtered seawater (FSW); the bottles were filled to the top with FSW and capped leaving no bubbles, taped for security, placed horizontally in a cooler (8–12 bottles were placed in a single cooler), and sent by express mail to aquaria throughout the USA.

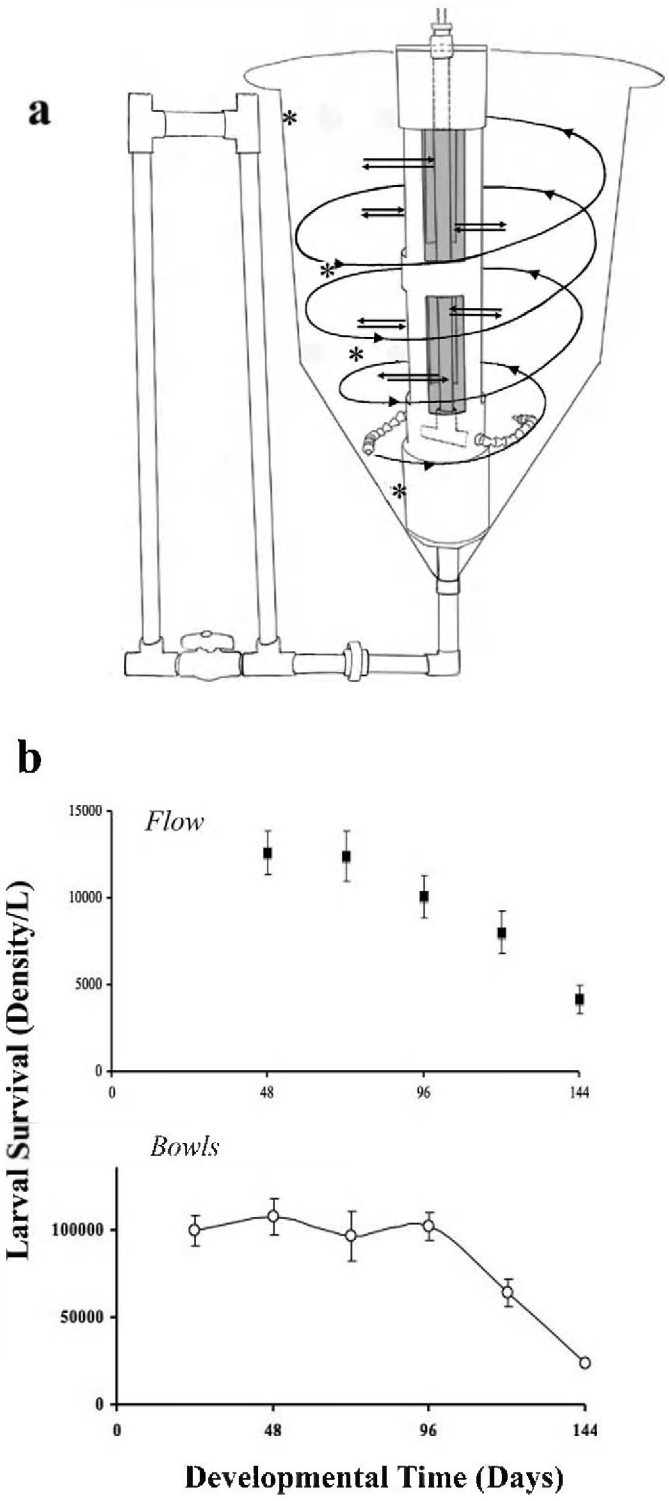

FIGURE 3. Larval rearing of *Fungia scutaria*. a, Drawing of assembled spiral-flowing tank and its flow, mixing, and position of dye experiments. Curved lines with arrowheads indicate direction of water flow spiraling upward from one inlet of a Loc-Line (both inlets had flow, but for simplicity flow from only one is drawn). Double arrows indicate water freely flows into and back out of the mesh areas on the standpipe. Asterisks (*) indicate locations in the water column where dye was injected for dye tests. b, Survival rate of *F. scutaria* larvae maintained in the spiral-flowing tank (upper graph, "Flow") and the static bowls (lower graph, "Bowls") between 24 and 144 h postfertilization. Each point shows mean and standard error. There was no difference in survival of larvae from flow chambers and static bowls (ANCOVA, P = 0.99).

Spiral-Flowing Tanks

Small conical fiberglass tanks (~75 L) were fitted with a central standpipe covered with 40 μm nylon mesh to rear *Fungia scutaria* larvae (see Figure 3). To maintain the water quality close to that which the larvae would experience under natural conditions, the conical tanks were attached to a filtered (0.5 μm) flow-through system with seawater

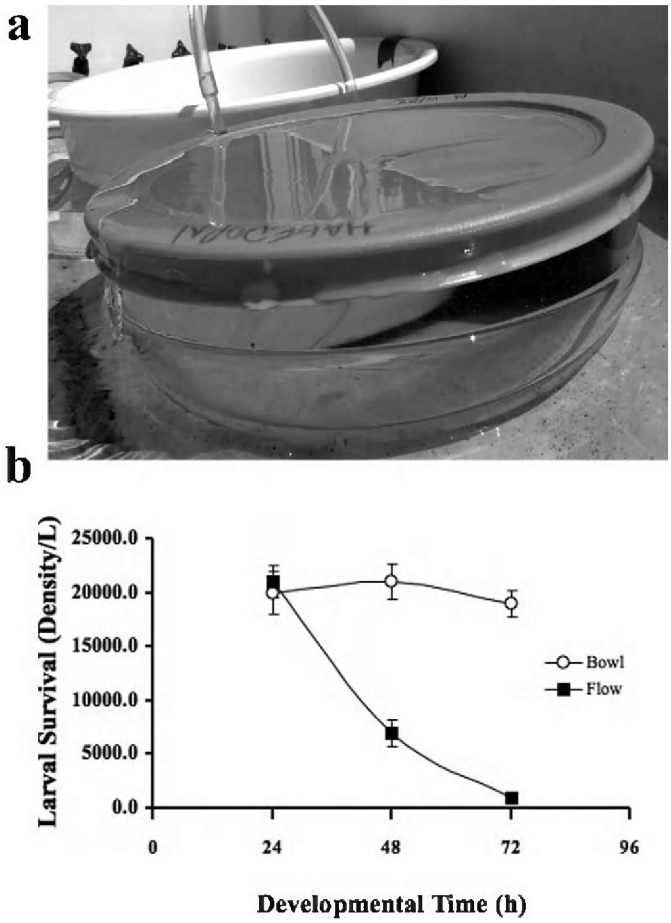

FIGURE 4. Larval rearing of *Pocillopora meandrina*. a, Down-flowing tank used to rear *P. meandrina*. b, Curves for larval survival in the static ("Bowl") and down-flowing ("Flow") tanks did not have the same slope (ANCOVA, $P = 0.03$); bowl rearing for this species produced substantially better survival than the down-flowing tank. Points show mean counts for each trial and standard error of the mean.

pumped from the reef. Flowing filtered seawater entered the top of the central tube and moved through nozzles at the tank base to produce gentle circular movement throughout the water column, and the wastewater exited the tank through the mesh-covered outflow. The flow rate was 150 to 300 mL/min, producing a complete turnover in the tanks approximately every 4 to 8 h. To test whether the conical tank could support the growth and development of *F. scutaria*, approximately 10,000 larvae/L were stocked in the conical tanks ($n = 4$) tanks. To reduce mortality of the early fragile stages (0–24 h postfertilization) from excessive motion in the flow-through chambers, the spiral-flow tanks were tested with 24 h postfertilization larvae. Therefore fertilization rate was not an issue, because all the larvae used for these tests were intact and developing. A comparison of the static bowl method (with daily cleaning) and the flow-through method was then performed to determine survival rate over time (up to 144 h postfertilization).

The static treatments used for comparison to the conical tanks were 3 L plastic bowls that had been the standard for successfully rearing *F. scutaria* larvae for many years (Schwarz et al., 1999). At 24 h postfertilization, *F. scutaria* larvae from 10 bowls were combined and evenly distributed into 2 larger bowls. Counts (see below) were taken for each bowl, and a standard rearing density was redistributed into 8 separate smaller bowls at a larval density of 100,000 larvae/L filtered seawater.

Fungia scutaria larvae were counted each day by gently stirring to homogeneously suspend them in the water column. Ten 1 mL samples were taken midwater from each conical flow-through tank and placed into a Sedgewick-Rafter counting chamber; the larvae were counted with a dissecting microscope and their numbers averaged. In addition, 10 samples (20 μL each) were taken midwater from each bowl, the number of larvae counted under a dissecting microscope, and their numbers averaged. These smaller 20 μL samples were assessed from the *F. scutaria* because of their high densities in the chambers.

Down-Flowing Tank

In April 2008 we attempted to rear *Pocillopora meandrina* larvae in the spiral-flowing tanks, but because of the negatively buoyant nature of the larvae, this method resulted in 100% mortality. Therefore, we developed a down-flowing tank for rearing *P. meandrina* larvae in May 2008 (see Figure 4). The tank was constructed of a 1.65 L glass bowl with a plastic lid. The center of the lid was removed and replaced with 40 μm mesh, leaving a 2 cm ring around the outside in which a hole was made to insert a 2 mm plastic rigid air line attached with air-line tubing to a manifold for controlling water flow. To maintain the water quality close to that which the larvae would experience in the open water, the down-flowing tanks were attached to a filtered (1 μm) flow-through system with seawater pumped from the reef. Flow to the tanks was maintained at 120 mL/min.

The larvae were fertilized in 50 mL tubes, rinsed with sterile filtered seawater, and placed into two bowls at 28°C to develop overnight at a density of approximately 80,000/L. After 24 h postfertilization, larvae were counted and then cleaned using a 40 μm mesh and 0.5 μm-filtered

seawater. One group was placed into the downward-flow chamber at a flow rate of 120 mL/min with a 40 μm mesh top to allow the water to flow out. The other bowl remained static. *P. meandrina* larvae were cleaned (static bowl only) and counted daily from the flow tanks and static bowl (maintained as described for *F. scutaria* above) for comparison.

Statistics

To determine the differences between survival in flow chamber versus static treatment, the data from all experiments was normalized and the *y*-values linearly transformed; analysis of covariance (ANCOVA) was then performed to determine whether the slopes were significantly different, using GraphPad Prism 5 software for the Macintosh GraphPad Software (San Diego, CA).

RESULTS

Rearing chambers were designed for three different coral species exhibiting different larval swimming behaviors, buoyancy, and sizes.

Up-Flowing Tank

Acropora palmata was the largest of the larvae studied (~700 × 500 μm depending on the developmental stage) and had the slowest rate of development (see Figure 1a). *A. palmata* larvae float at or near the surface of the water approximately 48 to 60 h postfertilization (depending on the temperature) until they began swimming. Even once they had begun swimming, they swam at or near the surface and were considered positively buoyant for most of their larval development before metamorphosis and settlement (~144 h). Clearly, all the larvae must become negatively buoyant before settlement; therefore, these categories only apply to the early larval periods (up to ~120 h, depending on the species).

During the first 24 h of development, the larvae developed asymmetrical, small protrusions of cells that could have easily be damaged in the up-flowing tank, but the chamber produced normally developed *A. palmata* larvae (as compared to the bins) because it simulated the gentle tumbling that the larvae would experience in the natural water (see Figure 2). Larval survival in the up-flowing tanks was similar to that in the static bins ($P = 0.12$) (Figure 2a). However, the up-flowing tank did not produce viable larvae for *F. scutaria* and *P. meandrina*. This tank produced 100% mortality in *F. scutaria* larvae within 24 h, even if the larvae were slightly more developed when placed in the chambers.

Spiral-Flowing Tank

Dye injection studies were performed on the spiral-flowing tank, using food coloring to examine the mixing properties of the vessel with an inlet flow of 150 mL/min. Figure 3 illustrates the mixing pattern in the flow-through vessel. Flow into and out of the system was equal, but the open area of the slits in the 10 cm central polyvinyl chloride (PVC) tube dictated the velocity through the screens. The nozzles were angled slightly downward to promote turbulence at the bottom to keep the larvae well mixed. The 180° positions of the nozzles provided rotation within the water column and encouraged mixing. Dye studies with separate injections were made at positions noted by asterisks (*) in Figure 3. At a flow of 150 mL/min, full vertical mixing occurred within minutes.

Developing *F. scutaria* larvae were fairly small (~200 × 100 μm) and fragile during the first 12 h of development and were just negatively buoyant during their early embryonic period (0–12 h postfertilization) (Figure 1b). However, once they began swimming, they were evenly distributed in the water column, and we considered this species to be neutrally buoyant. There was no difference in the survival between the spiral-flowing tank and bowls ($P = 0.99$). Both rearing systems produced similar survival rates in which the densities remained relatively steady through 96 h postfertilization and then dropped off at 120 to 144 h postfertilization (see Figure 3). This decrease in densities may reflect the complete absorption of stored fats (M. Hagedorn, unpublished data), as these larvae did not have zooxanthellae. *P. meandrina* larvae were tested in the spiral-flow tank, but 100% mortality was observed after 48 h postfertilization.

Down-Flowing Tank

Pocillopora meandrina larvae were the smallest of the larvae tested (~120 × 40 μm); they began slow swimming at 24 h but were negatively buoyant for the remainder of their larval development, remaining on or near the bottom (Figure 1c). Similar to *F. scutaria*, *P. meandrina* larvae were relatively susceptible to damage within the first 24 h, so they were reared in 3 L bowls for the first 24 h. The down-flowing tank was used for rearing *P. meandrina*; however, the static bowls appeared superior to the down-flowing tank for this species ($P = 0.03$) (see Figure 4).

DISCUSSION

For many years, large numbers of coral larvae have been reared successfully using simple husbandry methods such as static bowls and tanks. We have demonstrated that species of buoyant and neutrally buoyant coral larvae have similar survival in either static or flow-through chambers (see Figures 2, 3). These devices have proven to be very useful in improving culture conditions to reduce husbandry labor because neither embryos nor fresh water needed to be constantly transferred.

Modified examples of the up-flowing tank have already been used successfully by coral restoration biologists in the field (Margaret Miller, NOAA Southwest Fisheries Center, personal communication). *Montastraea faveolata* and *Diploria strigosa* were reared successfully in the up-flowing tanks and shipped to Columbus Zoo and Aquarium for settlement with 3-month survival as high as 65% and 45% for each species, respectively. Thus, the up-flowing tank has proven to be both practical, in that it can be adapted to the researcher's needs, and valuable, because it reduced husbandry time and facilitated restoration science under field conditions.

In weighing the benefits of each rearing system, one of the biggest factors to consider is time. For species that have only a single breeding season consisting of a few days, time available for conservation and restoration research is precious, and any time savings is a benefit. Moreover, the time remaining for some species that are threatened has become critical, and restoration practices need to be improved. *Acropora palmata* (elkhorn coral) and *Acropora cervicornis* (staghorn coral) were the first corals to be listed as threatened species under the U.S. Endangered Species Act. These major reef-building species once formed dense thickets and stands in the Caribbean. Today, these two species are currently at 1% to 20% of their historical levels throughout their range (Bruckner, 2003). Here we describe only one aspect of an ex situ conservation process, namely improved rearing associated with yielding better time management.

However, both the static and flow-through methods described here have their strengths and weaknesses. The static method was inexpensive to set up in terms of equipment and space. For example, 60 bowls can be maintained in two double-tiered flowing water tables taking up only about 2.5 m^2; however, this method was very expensive in terms of labor needed for cleaning (~5,000 h $year^{-1}$). The flow-through system was more costly to set up because it required a filtered flow-through water system and specially constructed rearing chambers. The amount of salary needed to pay one person for a season cleaning larvae, however, far exceeds the cost of the filtered seawater system and rearing chambers. The flow-through chambers required more space than the bowls, but each flow-through vessel could maintain almost three times the density (in ~0.25 m^2) than was ordinarily maintained in a static bowl and with little maintenance time required.

One of the major issues facing biologists in rearing coral larvae is how to keep them cool (28°–30°C) under field conditions. During daylight hours, static bins left outside without any cooling mechanism can easily reach 31° to 33°C, which is lethal for most species. The rearing data in Figure 3 reflect some of these issues. These data were not exactly comparable, because they did not have the same developmental temperatures. Had the static bins been maintained at 28° to 30°C (as were the flow chambers), possibly their survival would have been far worse, because their water quality would decay so rapidly. Because *A. palmata* is an endangered species, our goal was to produce the most larvae for captive maintenance in public zoos and aquaria (Petersen et al., 2007), this required having static "backup" bins maintained at a slightly cooler temperature to provide the larvae sufficient development time in transit to reach their respective sites before settlement. However, without an air-conditioned room to cool the bins, this would not have been possible, making this impractical under some field conditions.

Within the first 24 h of development, many coral larvae are susceptible to fragmentation by mechanical disruption. However, the water movement within the up-flowing tank and potential contact with the walls did not cause substantial fragmentation of *A. palmata* during early development, even when the *A. palmata* larvae were placed in the chambers within the first few hours after fertilization. In contrast, *P. meandrina* was far more delicate, did not develop strong swimming behaviors, and could not withstand the water movements in the flow chambers. *F. scutaria* larvae are negatively/neutrally buoyant larvae that develop strong swimming behaviors within the first 12 to 24 h, and the spiral-flow system shown in Figure 3 functioned well for them, because the water flow is upward and any disintegrating unfertilized oocytes and larvae passed through the mesh, allowing for the maintenance of excellent water quality in the rearing chambers. However, no one type of rearing chamber can be applied universally across species. Instead, the type of water flow within the chamber must be matched with the buoyancy and early swimming behavior of the larvae. Regardless, these readily built and easily maintained flow-through chambers may be a substantial aid to coral conservation and restoration.

ACKNOWLEDGMENTS

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Worldwide Diving Discoveries of Living Fossil Animals from the Depths of Anchialine and Marine Caves

Thomas M. Iliffe and Louis S. Kornicker

ABSTRACT. Inland (anchialine) and offshore submarine caves in limestone and volcanic bedrock are extreme environments inhabited by endemic, cave-adapted (typically eye- and pigment-reduced) fauna. Specialized cave diving technology is essential for investigating this habitat. A number of new higher taxa are represented herein, including closely related species inhabiting caves on opposite sides of the Earth, thus suggesting an ancient common ancestry. Because many of these species are known from only a single cave, pollution or destruction of caves will result in their extinction.

INTRODUCTION

Definition of Anchialine and Marine Caves

Anchialine caves are partially or totally submerged caves situated within a few kilometers inland from the coast in volcanic or karstic limestone terrain. Tidal marine waters in these caves have a long residence time, of months to years. Such caves are locally termed "cenotes" in the Yucatan Peninsula of Mexico, "blue holes" in the Bahamas and Belize, and "grietas" in the Galapagos Islands. The caves typically possess a highly stratified water column, with surface layers of freshwater or brackish water, separated by a thermo–chemocline from underlying fully marine waters low in dissolved oxygen (Iliffe, 2000). Animals that are restricted to the anchialine habitat and show pronounced morphological, physiological, biochemical, and behavioral adaptations are termed stygofauna or stygobites. In some areas such as Yucatan, freshwater and marine stygobites inhabit their respective water masses within the same caves.

In contrast to anchialine caves, marine caves are located either directly on the coastline (e.g., tidal springs) or are wholly submerged beneath the seafloor (e.g., offshore blue holes) and contain marine waters that freely exchange with the sea on each tidal cycle. The stygophilic fauna of marine caves can also be found in suitable and similar habitats outside of caves (e.g., under rocks or in crevices within the reef) and lack specialized adaptations for subterranean life.

Moderate to strong tidal currents are present in many marine caves. As a result, encrusting and low-growing, filter-feeding animals such as sponges, hydroids, anemones, tube worms, and even some corals may completely cover all

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hard surfaces. Other organisms are swept into caves by tidal currents but can only survive there for short periods of time and are termed accidentals. Some species of fishes, lobster, and mysidaceans seek shelter within marine caves but must venture out into open waters to feed and are classified as stygoxenes.

Some extensive marine caves extend far or deep enough so that a more or less gradual transition to long water residence times takes place and conversion to a true anchialine habitat occurs. Similarly, a number of inland anchialine systems have submerged entrances in the sea, with significant water exchange occurring in the entrance sections but with a transition to anchialine characteristics and fauna taking place as distance from the sea increases and the magnitude and impact of tidally exchanging water decline.

Biological Significance

Anchialine caves contain a rich and diverse, endemic stygobitic fauna (Sket, 1996; Iliffe, 2000, 2004) but, because of the specialized technological demands and potential dangers of cave diving, are relatively unstudied. These habitats serve as refuges to "living fossil" organisms, for instance, members of remiped crustaceans, and to animals closely related to deep-sea species, such as the galatheid crab *Munidopsis polymorpha*. Such stygobites typically possess regressed features including loss of eyes and body pigmentation. For reasons that remain unclear, the invertebrate fauna is dominated by crustaceans and includes the new class Remipedia, plus three new orders, nine new families, more than 75 new genera, and 300 new species. This extraordinary degree of novelty qualifies anchialine habitats as uniquely important. Because anchialine species commonly have a highly restricted distribution, often being found only in a single cave system on one island, pollution or destruction of the caves will result in their extinction.

Stygobitic anchialine fauna often have highly disjunct biogeographic distributions, inhabiting caves in isolated locations on opposite sides of the Atlantic and Pacific Oceans, as well as in the Mediterranean, and are considered Tethyan relicts. Various hypotheses have been proposed to explain the origin of anchialine fauna. In general, these theories invoke either vicariance (geological) or dispersal (biological) processes. Recently initiated molecular genetic comparisons of cave populations from distant locations may help provide data for determining the age and dispersal sequence of anchialine stygobites (Zakšek et al., 2007; Hunter et al., 2008).

Lifestyle Adaptations

The extreme environmental conditions in anchialine caves, such as the absence of light, hypoxia, and limited food reserves, present a unique set of challenges for the organisms that reside there. The lack of light precludes photosynthetic (primary) production of oxygen and food. Without light, organisms receive no visual information for orientation or communication and must function without diurnal timing mechanisms.

Adaptations to anchialine and marine caves can be morphological, behavioral, and physiological (Iliffe and Bishop, 2007). As a result of both food scarcity and hypoxia, there is a high selective advantage for economy of energy observed in many taxa, with possible adaptations including enhanced chemo–mechano-receptors for improved food finding capability, starvation resistance, and reduction in energy demand via reduced metabolism.

METHODS

Diving Investigations

Because anchialine stygobites are commonly found only at significant depths or distances from the water surface, cave diving is an essential component of the collection and study of anchialine fauna (Iliffe and Bowen, 2001). Cave diving requires specialized training, equipment, and techniques because a direct assent to the surface is not possible and divers may be hundreds of meters from outside access. In case of equipment failure or loss of air supply, cave divers must have readily available backups. Special techniques for cave diving may include the use of side-mounted, instead of back-mounted, scuba tanks to allow divers to pass through low bedding plane passages. Closed circuit rebreathers, which recycle the diver's exhaled gases, reduce the amount of percolation, that is, of silt dislodged from cave ceiling or walls by the exhaust bubbles produced in conventional open circuit scuba, and lessen contamination of the cave waters, which are low in dissolved oxygen (Figure 1). Rebreathers allow for much longer dives and generally less decompression time. Deep dives, depths below 40 m, require the use of special breathing gas mixtures that replace part or all of the nitrogen with helium to reduce the effect of nitrogen narcosis. As many cave dives are for longer durations and/or to deeper depths, they frequently involve long decompression.

Sampling and Fixation

The exceptionally clear waters of anchialine caves facilitate visual observation and collection of stygobitic

FIGURE 1. A diver uses a Megalodon closed-circuit rebreather with full face mask to collect a small shrimp, *Typhlatya* sp., from a cave in Yucatan. Rebreathers recycle expired gas so that no bubbles are produced.

species. Collectors generally lead the dive to have undisturbed water in front of them. As they slowly sweep their dive lights back and forth in an arc, observing the water column illuminated by the light beam, animals as small as a few millimeters can be distinguished as white pinpoints, sharply contrasting with the black background of the cave. Specimens recognized in this manner can be collected either individually in clear glass vials or plastic tubes or in larger numbers using a type of suction device known as the "Sket bottle" (Chevaldonné et al., 2008). Plankton nets, of 93 μm mesh with a 30 cm mouth diameter and 1 m length, can be used to collect smaller animals, such as copepods, from the water column. When collecting animals from the surface layer of sediments, divers can gently fan up the sediments with a hand and then sweep the plankton net through the disturbed water. This agitation should be done with care so as not to obscure overall visibility, which could cause the dive team to lose sight of their guideline leading back to the surface. Larger amounts of sediment can be collected in sealable plastic bags for later sorting in the laboratory. Finally, minnow traps or similar funnel-shaped traps made from plastic bottles (Manning, 1986) can be baited with a small amount of fish, crab, or other attractant and left within the cave for 6 to 24 h. If the trap is carefully placed inside a sealed plastic bag when it is recovered, even small invertebrates can be collected.

If temperatures are kept close to cave temperature after collecting, specimens will remain alive for up to 24 h. Photographic documentation of color pattern and natural body position in live specimens is highly desirable. Smaller animals can be photographed using a phototube attachment on a dissecting microscope and larger specimens with the macro setting found on many digital cameras. If animals are too active to be photographed easily, they can be chilled in a small dish placed in a refrigerator or an ice bath until they stop moving. Digital video segments showing swimming and other behaviors can be made in the same manner. Specimens are sorted under a dissecting microscope using small pipettes to transfer them to individual dishes for each taxon. Depending upon the type of animal and its intended use, various fixatives can be used. Most animals are best preserved in 70% to 95% pharmaceutical grade ethanol, which allows them to be used for either morphological or molecular investigations. Specimens for confocal laser scanning microscopy can be fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) buffer (1:1 in seawater), while those intended for scanning or transmission electron microscopy are fixed in 2% glutaraldehyde in seawater.

GEOLOGICAL ORIGINS, AGE, AND DISTRIBUTION OF ANCHIALINE HABITATS

Anchialine caves occur in both volcanic bedrock and karstic limestone. Lava tube caves form during volcanic eruptions of basaltic lava. They typically occur close to the earth's surface and are thus relatively short lived (thousands to a few tens of thousands of years). Anchialine lava tubes may originate on land and extend out under the coastline and beneath the seafloor or can form from submarine eruptions. Anchialine lava tube caves are known from the Canary Islands, Galapagos Islands, Hawaii, and Western Samoa. The longest of these is the Jameos del Agua (Atlantida Tunnel) on Lanzarote in the Canary Islands, the submerged portion of which extends 1.6 km beyond the coastline, reaching a depth of 50 m (Iliffe et al., 2000).

The most extensive of known anchialine habitats are solutionally developed limestone caves that typically contain both freshwater and marine waters. Such caves are sometimes referred to as flank margin caves and were formed by mixing dissolution in a fresh groundwater lens (Mylroie and Carew, 1990). The largest anchialine cave is Sistema Ox Bel Ha located on the Caribbean coast of the Yucatan Peninsula in Mexico; it contains 180 km of

surveyed underwater passages interconnecting 130 cenote entrances. Extensive anchialine limestone caves are also known from the Bahamas, Bermuda, Belize, Dominican Republic, and Bonaire in the Caribbean, plus the Balearic Islands and Sardinia in the Mediterranean. Smaller anchialine caves are present on many islands in the Indo-South Pacific and in Western Australia.

Limestone caves last much longer than lava tubes and can be hundreds of thousands to many millions of years old. Commonly, massive stalactites and stalagmites occur underwater to depths in excess of 50 m in coastal limestone caves. Because speleothems form very slowly and only in air, these caves must have been dry and filled with air for long periods of time when glacial sea levels were as much as 130 m lower than today. The last low stand of Ice Age sea level occurred only 18,000 years ago.

Coastal tectonic faults that extend below sea level constitute another form of anchialine habitat. On Santa Cruz in the Galapagos Islands, vertical faults in coastal volcanic rock are locally called "grietas" (Iliffe, 1991). Wedged breakdown blocks have partially roofed over submerged portions of grietas so that they are in total darkness. Similar faults are present in Iceland. Fault caves also occur in uplifted reef limestone on the island of Niue in the Central Pacific, producing deep chasms containing anchialine pools. The Ras Muhammad Crack in the Sinai Peninsula consists of a water-filled crack in an elevated fossil reef formed by a 1968 earthquake (Por and Tsurnamal, 1973). Many of the offshore ocean blue holes of the Bahamas consist of submarine faults running parallel to the platform edge. Ocean blue holes typically exhibit exceptionally strong, reversing tidal currents created by an imbalance between tides on opposite sides of the islands.

ANCHIALINE CAVE ECOLOGY

Physical and Chemical Characteristics

The water column in most anchialine caves is highly stratified (Iliffe, 2000). The largest changes in chemical and physical parameters typically occur at the halocline where freshwater or brackish water is separated from underlying fully marine waters (Figure 2). It is not uncommon for caves to possess multiple haloclines. On larger islands and in continental regions such as Yucatan and Western Australia, freshwater occurs in the shape of a lens with thickness increasing in a direct relationship with distance inland from the coast. In Yucatan, the depth of the halocline and corresponding thickness of the freshwater lens increases from 10 m at 2 km distance inland to 20 m at 10 km inland.

Water temperature in Yucatan caves generally increases with depth, although in the Bahamas the inverse occurs and water below the halocline is generally cooler than surface water. Warmer waters below the halocline could be caused by geothermal heating at depth or evaporative cooling at the surface.

In the lightless interior of caves, there are no plants and hence no photosynthetic oxygen production; stable and stratified water masses also restrict vertical mixing and exchange of oxygen with surface waters. Thus, cave waters are typically hypoxic to anoxic. Where deeper, water-filled vertical shafts extend to the surface, such as in many cenotes and inland blue holes, input of leaves and other organic detritus has caused the total depletion of dissolved oxygen with resulting anoxia and hydrogen sulfide production. A cloud-like layer of hydrogen sulfide several meters thick occurs just below the halocline and may reduce underwater visibility to near zero, but water clarity improves considerably below the H_2S layer. In some caves, dissolved oxygen levels can recover to 1 mg/L or less and populations of stygobitic animals occur.

A pH minimum generally occurs at the halocline, possibly arising from microbial oxidation of organic matter suspended at the density interface and resulting CO_2 production. Increased acidity at the halocline may explain the dissolution of limestone and the resulting development of cave passages at this depth.

Trophic Relationships

Determination of stable carbon and nitrogen isotopes values from animals, sediments, and other sources of organic matter in Yucatan caves has been used to examine the trophic ecology of these systems (Pohlman et al., 1997, 2000). Four potential sources of organic matter were identified in Yucatan caves: the soil from the surrounding jungle, algae from the cenote pool, chemoautotrophic bacteria, and, to a lesser extent, organic matter originating from marine waters. Stable nitrogen isotope data determined that the food web comprised 2 to 2.5 trophic levels.

The paucity of food in anchialine caves drives organisms toward a generalist diet. Mysids and isopods tend toward omnivory, while ostracods and thermosbaenaceans occupy the roles of detritivores. The thermosbaenacean *Tulumella* and atyid shrimp *Typhlatya* have modified appendages that allow them to filter out even the tiniest particles. Remipedes, fishes, and some amphipods, operating either as top-level predators or as scavengers, feed on ostracods, thermosbaenaceans, copepods, isopods, amphipods, and shrimps.

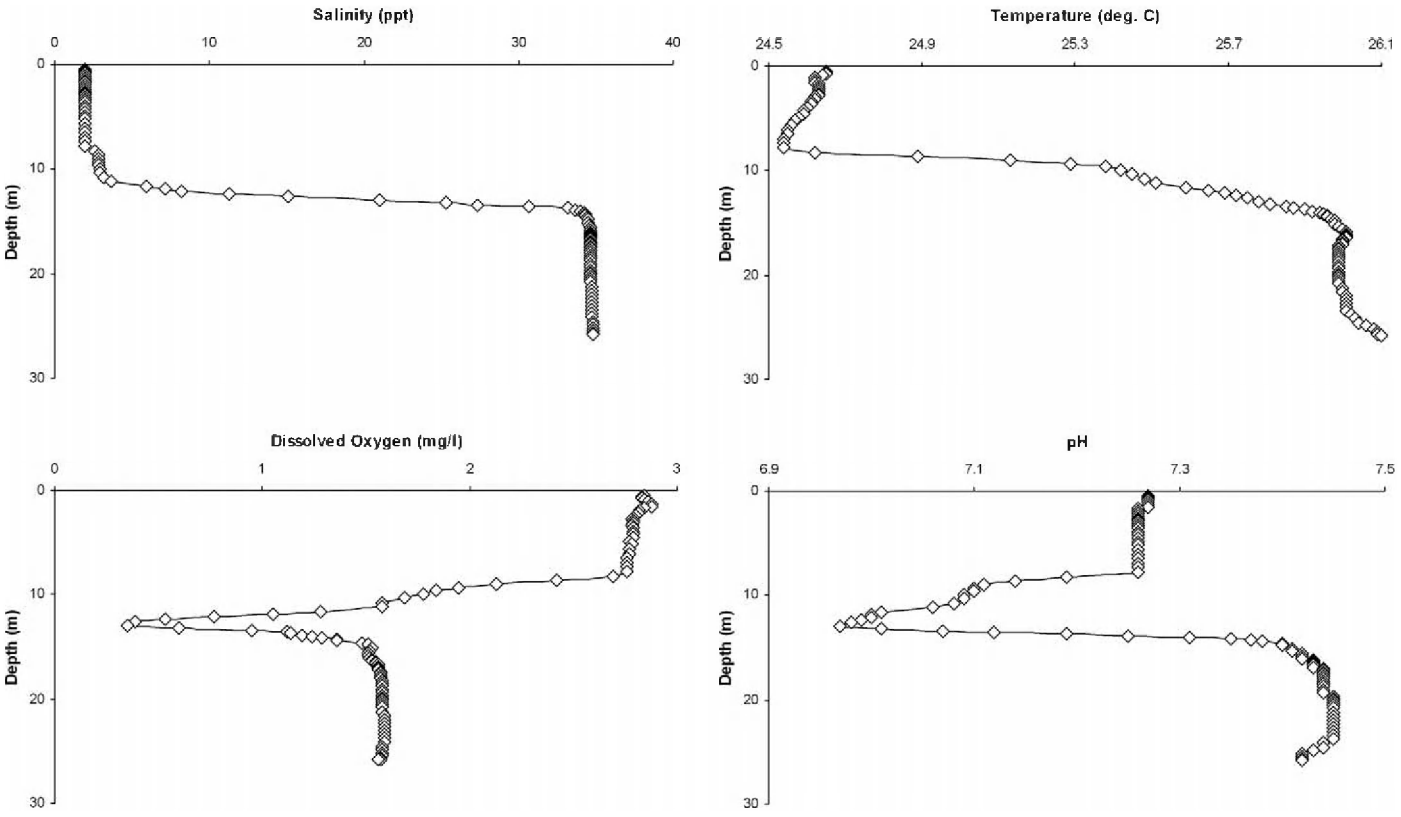

FIGURE 2. Depth profiles of salinity, temperature, dissolved oxygen, and pH from an anchialine cave, Cenote 27 Steps, Akumal, Mexico, 7 December 2003, recorded with a YSI 600 XLM multiparameter water quality monitor. Individual measurements (diamond symbols) were taken at 4 s intervals between the surface and 26 m water depth.

BIODIVERSITY

Fishes

Stygobitic anchialine fishes (Figure 3a) are represented in the families Bythidae (eight species in two genera from the Bahamas, Cuba, Yucatan, and Galapagos Islands), Eleotridae (one species from Northwestern Australia), Gobiidae (three species in two genera from the Philippines and Japan), and Synbranchidae (two species in one genus from Northwestern Australia and Yucatan) (Romero, 2001).

Non-Crustacean Invertebrates

Although most stygobitic anchialine invertebrates are crustaceans, a variety of non-crustacean invertebrate stygofaunal species have been described. Anchialine species include four sponges, one turbellarian, five gastropods, ten annelids, four chaetognaths, one tantulocarid, and three water mites. Although some of these species are questionable stygobites, several are clearly cave adapted. The polychaetes *Gesiella jameensis* from the Canary Islands and *Pelagomacellicephala iliffei* from the Caicos Islands and Bahamas (Figure 3b) conserve energy by slowly swimming in the cave water column, while the chaetognath *Paraspadella anops* from the Bahamas lacks eyes and pigment.

Crustaceans

Crustaceans are the most abundant and diverse group present in both freshwater and anchialine cave habitats. Among the anchialine Crustacea, the largest numbers of species are represented by amphipods, copepods, decapods, ostracods, isopods, mysids, and thermosbaenaceans, approximately in that order.

Remipedia

Remipedes are a class of Crustacea originally described from Bahamian caves by Yager (1981). Although their multi-segmented trunk and paired swimming appendages

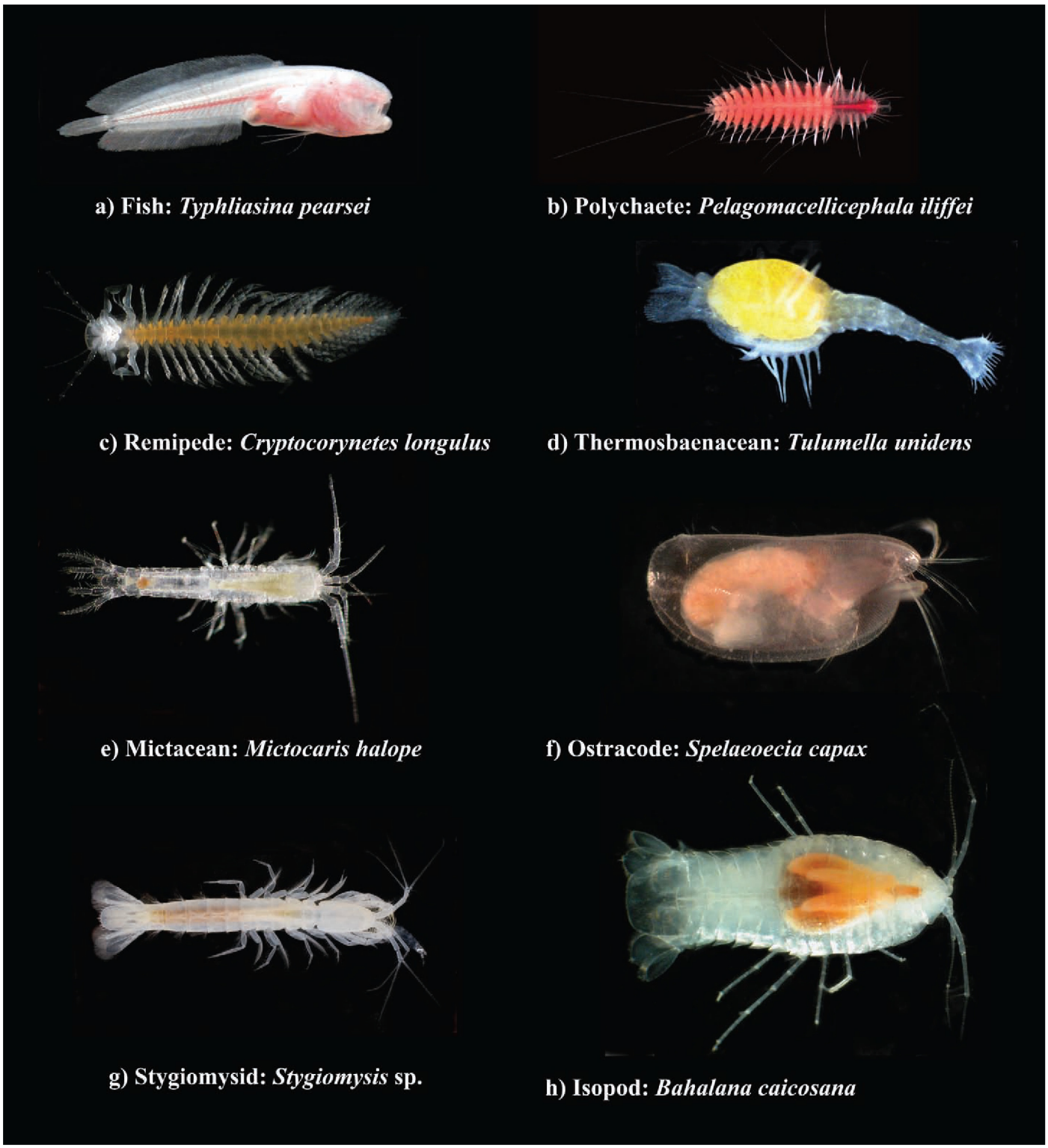

FIGURE 3. Characteristic anchialine cave animals include the (a) Yucatan cave fish *Typhliasina pearsei*; (b) polynoid polychaete worm *Pelagomacellicephala iliffei* from the Bahamas; (c) remipede *Cryptocorynetes longulus* from the Bahamas; (d) thermosbaenacean *Tulumella unidens* from Yucatan; (e) mictacean *Mictocaris halope* from Bermuda; (f) halocyprid ostracod *Spelaeoecia capax* from the Bahamas; (g) stygiomysid *Stygiomysis* sp. from Yucatan; and (h) cirolanid isopod *Bahalana caicosana* from the Caicos Islands.

appear primitive, their head and mouth parts are highly specialized (Figure 3c). Remipedes have paired hollow fangs for capturing prey and are among the top predators in anchialine habitats. They are up to 4.5 cm in length, usually colorless and blind, with elongate, centipede-like bodies. Twenty species of remipedes inhabit fully marine, oxygen-deficient waters in caves from the Bahamas, Caicos Islands, Cuba, Yucatan Peninsula, Dominican Republic, Canary Islands, and Western Australia (Koenemann et al., 2008b; Daenekas et al., 2009). The recent discovery of free-living, nonfeeding remipede larvae promises to yield information on the reproduction and development as well as the evolutionary affinities of this enigmatic group (Koenemann et al., 2007, 2009).

Thermosbaenacea

Thermosbaenaceans (Figure 3d) are small (5 mm or less), eyeless or eye-reduced, anchialine and freshwater peracarid crustaceans with a dorsal brood pouch in females (Wagner, 1994; Jaume, 2008). They include at least 34 species with a wide distribution in caves and thermal springs around the Mediterranean and Caribbean, as well as in Australia and Cambodia.

Mictacea

Mictaceans (Figure 3e) are small (3–3.5 mm), eyeless and depigmented, nonpredatory crustaceans. This peracarid order is represented by only a single species that inhabits anchialine caves in Bermuda (Bowman and Iliffe, 1985).

Bochusacea

Bochusaceans are very small (1.2–1.6 mm), semitransparent, and eyeless peracarid crustaceans that include two anchialine species from the Bahamas and Cayman Islands, plus two deep-sea species (Gutu and Iliffe, 1998; Jaume et al., 2006).

Copepoda

Platycopioid, misophrioid, cyclopoid, harpacticoid, and calanoid (especially epacteriscid and ridgewayiid) copepods inhabit anchialine caves in tropical regions around the globe. They are small (typically 1–2 mm long) and have a short, cylindrical body with head and thorax fused into a cephalothorax. Most are planktonic filter feeders, but some, such as the harpacticoids and cyclopoids, are benthic, while epacteriscids are predators on other copepods.

Ostracoda

Halocyprid ostracods (Figure 3f) include anchialine species with a distribution and co-occurrence similar to that of remipedes (Kornicker et al., 2007). *Danielopolina* is the most widely distributed stygobitic genus with species on opposite sides of both the Atlantic and Pacific, inhabiting caves in the Bahamas, Cuba, Yucatan, Jamaica, Canary Islands, Galapagos, Western Australia, and Christmas Island. More than 300 species of podocopid ostracods have been found in springs, caves, and anchialine habitats.

Mysidacea

Stygobitic mysids are found in freshwater and anchialine habitats in Africa, the Caribbean, Mediterranean, and India. Their distribution suggests that they were stranded in caves by lowering of the sea level in the Tethys and Mediterranean. Recent molecular phylogenies of the mysids suggest that a new order is justified for the stygiomysids (Figure 3g), which inhabit caves in the Caribbean and Italy (Meland and Willassen, 2007).

Isopoda

Stygobitic isopods (Figure 3h) range from several millimeters to several centimeters in length. Anthurid isopods occur in anchialine and freshwater caves in the Canary Islands, Caribbean and Indian Ocean islands, Mexico, and South America. Asellot isopods inhabit anchialine and freshwater caves in the Caribbean, Europe, Galapagos, India, Indonesia, Japan, Malaysia, North and Central America, and Polynesia. Cirolanid isopods have been found in freshwater and anchialine caves clustered in Mexico and the Caribbean (Iliffe and Botosaneanu, 2006), as well as in Europe and the Mediterranean.

Amphipoda

Amphipods occur in freshwater and marine cave habitats. Stygobitic representatives are present in the bogidiellid, crangonyctid, hadziid, and niphargid families of the amphipod suborder Gammaridea. They are very widely dispersed, with large numbers of species inhabiting caves in Central and Southern Europe, the Mediterranean, eastern and southern North America, and the Caribbean.

Decapoda

The anomuran galatheid crab *Munidopsis polymorpha* inhabits an anchialine lava tube in the Canary Islands

(Wilkens et al., 1990). Brachyuran crabs are widely distributed in caves of the tropics and subtropics. Anchialine stygobitic shrimp include representatives from the caridean families Agostocarididae, Alpheidae, Atyidae, Hippolytidae, Palaemonidae, and Procarididae; the stenopodid family Macromaxillocarididae; and the thalassinid family Laomediidae.

Other Crustacean Stygofauna

One tantulocarid, an exceptionally tiny ectoparasite on anchialine harpacticoid copepods, occurs in the Canary Islands (Boxshall and Huys, 1989). A species of stygobitic nebaliacean inhabits anchialine caves in the Bahamas and Caicos Islands (Bowman et al., 1985). Several species of cumaceans and tanaidaceans have been collected from anchialine caves in Bermuda and the Bahamas, but it is not clear whether they belong to the stygofauna.

BIOGEOGRAPHY

Upon examining the biogeography of anchialine fauna, some extraordinary patterns are evident. A number of anchialine genera, including the remipede *Lasionectes*, ostracod *Danielopolina*, thermosbaenacean *Halosbaena*, and misophrioid *Speleophria*, inhabit caves on opposite sides of the Earth and are believed to be relicts whose ancestors inhabited the Tethys Sea during the Mesozoic (Humphreys, 2000). Some anchialine taxa are represented in the Mediterranean, but others, notably remipedes and *Halosbaena*, are absent. The presence of anchialine taxa at all in the Mediterranean is remarkable considering that this basin was completely dry for long periods of time during the Miocene. The aytid shrimp *Typhlatya* shows an especially interesting distribution with 17 known species inhabiting freshwater and anchialine caves in the Mediterranean region, Bermuda, Ascension Island, Caribbean locations including Cuba and Yucatan, and the Galapagos Islands (Alvarez et al., 2005). The shrimp family Procaridae contains one genus with species in the mid-Atlantic and Caribbean, as well as Hawaii.

Based on numbers of stygobitic species, the Bahamian archipelago appears to have been a possible center of origin for anchialine fauna. Among the Remipedia, 15 of 20 described species inhabit caves in the Bahamas (Koenemann et al., 2008b; Daenekas et al., 2009), whereas among anchialine halocyprid ostracods, Bahamian species account for 4 of 11 in the genus *Danielopolina*, 6 of 11 in *Spelaeoecia*, and all 8 species of *Deeveya* (Kornicker et al., 2007).

The Bahamas archipelago consists of a series of broad, shallow-water, highly karstified, carbonate platforms rising abruptly from the deep sea. The islands and cays consist of Pleistocene limestone covered by a thin veneer of Holocene carbonate reefs and sediments. Underlying these younger limestones is a continuous section of Tertiary and Cretaceous limestones and dolomites exceeding 11 km in thickness. If the position of the tectonic plates before the development of the Atlantic Ocean is reconstructed, virtually all the Bahamas overlap the African continent and its continental shelf. This finding suggests that the Bahama platform developed over oceanic crust during the earliest phase of the creation of the Atlantic. The extended shallow-water history of the Bahamas, coupled with the cavernous nature of the limestone, may help to explain its rich and diverse anchialine fauna.

ORIGINS OF ANCHIALINE BIOTA

A number of theories have been proposed to explain the trans-oceanic distribution of many anchialine taxa. The *vicariance model* suggests that plate tectonics served as a mechanism for the dispersal of anchialine fauna (Rosen, 1976; Wiley, 1988). This model mainly describes the Tethyan track of ancient taxa that were rafted on the drifting continents to their present positions (Stock, 1993; Jaume et al., 2001). However, the existence of anchialine fauna on mid-ocean islands such as Bermuda, Ascension, and Hawaii that have never been part of or closer to a continent cannot be explained by this mechanism (Iliffe, 2000).

The *regression model* suggests that sea-level regressions, caused by tectonic uplift or eustatic glacial lowering of sea levels, stranded crevicular or interstitial marine littoral species that subsequently adapted to brackish or freshwater conditions (Stock, 1980). This model is supported by the observed correlation between the distribution patterns of numerous, marine-derived cave organisms and the position of shorelines during the Late Mesozoic or Tertiary seas. Nevertheless, the presence of anchialine fauna in caves that were completely dry and air filled (as evidenced by their now-submarine speleothems) less than 10,000 years ago indicates that these animals can migrate vertically with raising postglacial sea levels (Iliffe, 2000). Also, small islands such as Bermuda offer little chance for marine species to be stranded.

A *deep-sea origin* has been proposed for some anchialine species having close relatives that inhabit bathyal depths (Hart et al., 1985). Both caves and the deep sea are old, climatically stable, lightless, and nonrigorous environments. Anchialine habitats on islands and continental

margins could be connected via a continuum of crevicular corridors extending from shallow depths to the deep sea (Iliffe, 1990). However, evidence against a deep-sea origin of cave faunas includes the questionable ability of deep-sea species to cross the oceanic thermocline, the relatively recent nature of deep-sea species (resulting from the lack of oxygen in Atlantic bathyal waters during the late Oligocene), and phylogenetic analyses of morphological characters supporting independent colonization of deep-sea and anchialine habitats (Stock, 1986; Danielopol, 1990).

The *active migration model* involves the inland dispersal and colonization of subterranean habitats by expansionistic marine species with a high degree of salinity tolerance (Rouch and Danielopol, 1987). This process is independent of climatological and geological variations.

Passive oceanic dispersal of larval or postlarval stages of anchialine species by currents could explain the wide distribution of some anchialine shrimp species within the Indo-Pacific. Rafting on floating objects, such as wood, algae, kelp, and coconuts, or on mobile and migratory animals, for instance, sea turtles, fishes, and larger arthropods, could disperse anchialine species, even those without a free larval stage. However, oceanic dispersal is unlikely for many anchialine groups that produce few offspring or have narrow physiological tolerances.

ADAPTATION TO LIFE IN ANCHIALINE CAVES

Behavioral Adaptations

Behavioral adaptations are the most immediate adaptations for survival and colonization in cave systems. Cave organisms, in particular amblyopsid cave fishes, use a glide-and-rest technique to conserve energy in their search for food. Remipede locomotion is also designed for the economy of movement. Remipedes swim slowly, using less energy for the same distance than if they swam at higher speeds (Koenemann et al., 2008a). The power stroke produces drag by individual legs, but the recovery stroke is completed with the legs folded with other legs to reduce water resistance.

The stygobitic galatheid crab *Munidopsis polymorpha*, inhabiting an anchialine lava tube in the Canary Islands, has a number of specialized behaviors (Parzefall, 1992, 2000). These small crabs are most abundant in a dimly illuminated pool where they hide in rock crevices during the day but come out at night to feed on diatoms. Because of the large numbers of individuals in this pool, they spread in an almost regular pattern determined by the length of the second antennae.

Munidopsis crabs remain aggressive throughout the year. They detect intruders from water movements and attack with extended chelipeds. Male crabs are attracted by a molting hormone released by females. To prevent the females from fleeing, males rhythmically move their chelipeds as they approach, until the female responds by vibrating one of her chelipeds. The male then seems to turn the female over on her back to initiate insemination.

Morphological Adaptations

Regressive Features

The loss of features that in cave environments no longer have a function, such as eyes and pigmentation, is regarded as regressive evolution. There are two main theories explaining the driving force for regressive evolution. In an environment with a depauperate food supply, natural selection should favor reallocating energy from developing unused features, such as eyes and pigment, to growth and survival. A second explanation is that regressive evolution may be the result of nonselective processes such as neutral mutation and genetic drift. Features such as eyes and pigment that abruptly lose their biological function when animals enter caves are free to be turned off by now non-lethal mutations.

Unfortunately, the theory of energy economy by character reduction in stygobites is not well tested, especially with anchialine stygobites, yet the anchialine environment is dominated by blind, depigmented organisms.

Constructive Features

In the case of constructive features, priority is given to life history, metabolism, development, and starvation resistance, with sensory development such as mechano- and chemoreceptors being subordinate. For troglomorphy to occur, two factors must be present: (1) selective pressure in favor of the development and (2) genetic, physiological, or behavioral ability of the organism to respond to the selective pressure. A prerequisite for constructive traits is their genetic availability in epigean forms: if traits are not present in epigean ancestors, they will not be present in hypogean descendants.

There are several areas of the body where constructive features occur. In crustaceans, appendages may be elongated, in particular, the antennae, and in fish, the head may become enlarged or flattened. Corresponding to the morphological changes, there is an increased sensitivity to chemical and mechanical stimulants. As a result of compensatory enhancement of extraocular senses, the signal-processing structures in the brain are altered.

Physiological Adaptations

Adaptations to a Food-Poor Environment

Food in the stygobitic environment may be in general scarce or at best patchy; therefore, the stygofauna need to cope with temporal periodicity of food availability and potentially tolerate long periods of starvation. This adaptation occurs through lipid accumulation or energy economy. In comparison with pelagic crustaceans, anchialine crustaceans sacrifice protein mass for increased lipid stores (Iliffe and Bishop, 2007). Lipids provide neutral buoyancy without energy expenditure, while also serving as an energy reserve when food is limiting. Anchialine stygobites also tend to be smaller than their epigean counterparts. Their small size is a mechanism for energy economy.

Adaptation to Hypoxia and Anoxia

As mentioned previously, the anchialine environment, especially at or below the halocline, is commonly hypoxic or even anoxic. As a result, hypogean organisms tend to have substantially lower oxygen consumption rates than their epigean relatives (Bishop et al., 2004). Many organisms are capable of obtaining energy when faced with a reduction or absence of oxygen, but few are able to survive indefinitely without a return to oxygen. When the oxygen supply becomes inadequate, organisms switch to anaerobiosis to compensate for adenosine triphosphate (ATP) demand.

During periods of anaerobiosis, organisms conserve their energy stores by a loss in physiological functions such as motility, ingestion, and digestion, combined with a dramatic depression of their energy (ATP) demand. When oxygen is temporarily unavailable, many organisms switch to anaerobic glycolysis. Anaerobic glycolysis is, however, a fundamentally inefficient metabolic strategy and thus not an attractive solution for anchialine organisms.

By examining the activities of enzymes critical to metabolism and energy conversion, it is possible to determine the rate at which food is converted to cellular energy (Bishop et al., 2004). Citrate synthase (CS) is an indicator of an organism's maximum aerobic potential, or how fast an organism can aerobically convert glucose to energy. Malate dehydrogenase (MDH) functions in the presence as well as absence of oxygen, whereas lactate dehydrogenase (LDH) contributes to both aerobic and anaerobic metabolic pathways and serves as an indicator of glycolytic potential.

Anchialine organisms are anaerobically poised with both LDH:CS and MDH:LDH ratios tending to be greater than one. The higher the MDH:LDH ratio, the greater is the tolerance to hypoxia. Such high ratios indicate an evolutionary adaptation to the anaerobic anchialine environment.

CONSERVATION

Over the past 25 years, more than 400 new species of anchialine stygobites have been discovered and described. A high percentage of these species are known only from a single cave or cave system. Even within caves, species are characteristically found only at specific depths or locations as defined by a narrow range of environmental parameters. In many parts of the world, tourism development, limestone quarries, and groundwater pollution are either destroying or grossly polluting numerous caves, resulting in extinction of untold numbers of species.

Anchialine species qualify for inclusion on endangered species lists for reasons of their limited distribution and the declining environmental quality of their habitat. In Bermuda, 25 cave species are on the IUCN (International Union for Conservation of Nature) Red List of endangered species. Other cave species from the Yucatan Peninsula are on the official Mexican list of threatened and endangered species.

Maintaining groundwater quality is essential to the environmental health of the subterranean environment. For example, the small oceanic island of Bermuda is the third most densely populated country in the world and has the largest number of private cesspits per capita. Disposal of sewage and other wastewater into cesspits or by pumping down boreholes is contaminating the groundwater and cave water with nitrates, detergents, toxic metals, and pharmaceuticals; depleting the very limited amounts of dissolved oxygen in cave water; and generating toxic levels of hydrogen sulfide.

Some ocean caves such as the Blue Holes of the Bahamas have strong tidal currents sweeping through them for very considerable distances. In one such cave, plastic bottles and other trash have been observed littering the floor of the cave nearly a mile back into previously unexplored passages. Far too many caves and sinkholes are viewed as preferred locations for the dumping of garbage and other waste products.

Another serious environmental problem concerns the destruction of caves by limestone quarries or construction activities. Half a dozen or more Bermuda caves have been totally destroyed by two limestone quarries that produce crushed aggregate for construction purposes. Untold other

caves have been lost to enormous limestone quarries in the Yucatan Peninsula. Many caves have been filled in and built over by golf courses, hotels, and housing developments in Bermuda. Recently, a series of luxury town homes was built directly on top of the largest cave lake in Bermuda.

Sometimes even seemingly innocent activities can threaten caves and cave animals. Along the Caribbean coast of the Yucatan Peninsula, many open water cenote pools are inhabited by the freshwater fish *Astyanax fasciatus*. Some of these fish frequently follow divers into caves, moving in front of the dive team and voraciously darting in to consume any cave fish or crustaceans that are illuminated by the beam of a dive light. Considering the many thousands of cave divers who use these systems each year, it is not surprising that the caves most heavily visited by tourist divers are now essentially devoid of life.

Even the gas exhaled by divers may have adverse effects on cave animals. Because anchialine cave waters typically contain extremely low levels of dissolved oxygen, exhaust bubbles from open circuit scuba could have profound effects on the cave ecosystem. Several anchialine caves in Western Australia with unique fauna are currently off limits to open circuit divers and may only be visited by those using rebreathers (Humphreys et al., 1999).

Some anchialine caves in Bermuda, the Canary Islands, and Mallorca have been developed into commercial tourist attractions. Unfortunately, many of the tourists visiting these sites have viewed the deep clear water cave pools as natural wishing wells in which to throw a coin or two. Copper coins tend to rapidly deteriorate and dissolve in saltwater, producing high levels of toxic copper ions in the cave waters. In one such cave in the Canary Islands, the endemic crab *Munidopsis polymorpha* has shown a marked decline in abundance during the past decade or longer, probably in response to high levels of copper in the cave water.

Acknowledgments

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Decimating Mangrove Forests for Commercial Development in the Pelican Cays, Belize: Long-Term Ecological Loss for Short-Term Gain?

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ABSTRACT. The unique, biologically diverse ecosystems of Pelican Cays, Belize, are in serious danger from sediment suffocation related to the recent clear-cutting of mangroves for commercial development in what is currently designated Southwater Cay Marine Reserve. Field observations in the Pelican Cays in March 2007 revealed extensive clear-cutting of mangroves and covering of exposed peat surfaces with sediment dredged from the adjacent seafloor to create false sand cays. On Manatee Cay, introduction of dredge spoils taken from the nearby seabed resulted in fine sediment plumes spilling into the adjacent ponds, smothering the attached benthic communities on mangrove roots and burying *Thalassia* bottom communities. In addition, comparative studies of microalgal (phytoplankton) assemblages in a Manatee Cay pond before and after mangrove clearing indicate a dramatic loss in this group. This change, related to high turbidity observed in the water column, signals a serious impact to this aquatic ecosystem. In March 2007, clear-cutting, burning, and dredge and fill operations were taking place on Fisherman's Cay, with additional survey lines visible on Fisherman's, Manatee, and Cat Cays. We used a series of aerial photographic surveys from 2003 to 2007 to document the extensive loss of mangroves on both Manatee and Fisherman's Cays. To date, additional clearing of mangroves has occurred on Northeast Cay, Bird Cays, and Ridge Cay, resulting in a total of 15.3 ha or more than 29% of the mangrove community that have been destroyed in the Pelican Cays. Furthermore, several survey lines through still-forested areas on these islands indicated that additional clearing of mangroves was planned. The Pelican Cays ponds contain unique, biologically diverse ecosystems dominated by delicate sessile photosynthetic and filter-feeding populations; these rare communities will be lost as a result of sediment suffocation caused by the clearing and filling of these islands. However, the conversion of mangrove ecosystems for residential, tourism, and commercial uses is both widespread and accelerating in Belize and throughout the global tropics. This pressure is having an adverse effect on the health of coral reefs and the biomass and viability of commercial fisheries, which are essential for both tourism and local livelihoods.

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INTRODUCTION

The Pelican Cays group is an oceanic coral reef boundary environment (Macintyre et al., 2000a), containing a network of coral ridges and semi-enclosed or enclosed ponds (Figure 1) where shallow mangrove cays are immediately adjacent to channels approximately 20 to 30 m deep. The lagoon-like ponds, which

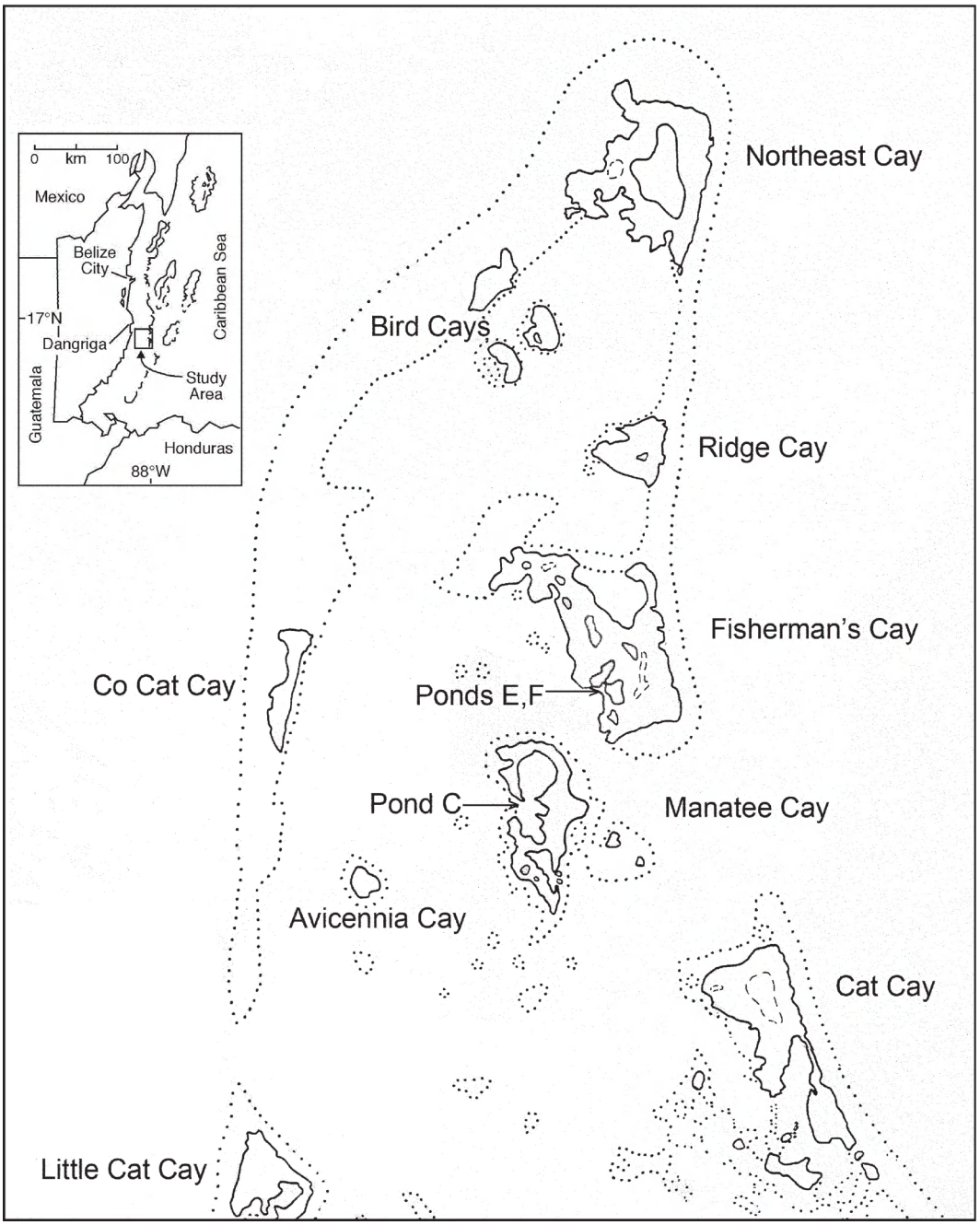

FIGURE 1. Index map showing the major mangrove islands of the Pelican Cays and their ponds.

may be 10 to 12 m deep, harbor high-diversity, low-energy environments dominated by populations of photosynthetic and filter-feeding organisms. These ponds were formed by differential coral accumulations on polygonal karst patterns of the underlying Pleistocene limestone (Macintyre et al., 2000b). The scleractinian coral *Acropora cervicornis*, which is capable of rapid accretion in response to sea-level rise (catch up), exaggerated the underlying karst topography, resulting in greater relief than that of the antecedent karst surface, and created steep-sided ridges that form the ponds. Red mangrove (*Rhizophora mangle*) communities have established on the reef ridges of this area, forming intertidal cays encircling or partially encircling the ponds. The mangrove prop roots extending into the ponds pro-

vide substrate for rich communities of sponges, ascidians, algae, corals, bryozoans, mollusks, and other organisms (Macintyre and Rützler, 2000).

Pelican Cays ponds and associated ridges support a very high level of shallow marine biodiversity within the Belize Lagoon. Examples include 70 species in 30 genera of ascidians (Figure 2A), representing 60% of all known shallow-water species in the Caribbean (Goodbody, 2000). Ten of 52 species of echinoderms found in the ponds and associated space had never been previously reported from Belize waters (Hendler and Pawson, 2000). Of 187 sponge species (Figure 2B) reported for several mangrove island groups along Belize Mesoamerican Barrier Reef, Rützler et al. (2000) found the "most diverse sponge fauna" at Pelican Cays. Of the 147 sponge species at Pelican Cays, 45% were new species or variants special to the pond environments. Manatee Cay had 95 species, Cat Cay had 77 species, and Fisherman's Cay had 90 species. Wulff (2000) attributed sponge community differences to the fact that the Pelican Cays' mangrove roots are embedded in coral reefs rather than thick peat sections as at Twin Cays (Macintyre et al., 2004) and Tobacco Range (Macintyre et al., 1995). The reef substrate may be a preferred environment for spongivorous fishes that determine the distinctive species composition of the Pelican Cays sponge community (Wulff, 2000). Richardson (2000) reported a total of 7 species of epiphytic foraminifera living on turtle grass (*Thalassia testudinum*) blades, of which 2 were new species. Littler et al. (2000) reported 152 species of marine macrophytes, of which 148 were algae and 4 were vascular plants. A total of 31 bryozoan species were found in the Pelican Cays, forming extensive colonies on the mangrove roots (Winston, 2007). Coral species on ridges and in deeper or more open areas of the ponds included *Porites furcata*, *P. divaricata*, *P. porites*, *P. astreoides*, *Acropora cervicornis*, *Siderastrea siderea*, *Agaricia tenuifolia*, *Millepora complanata*, and *Montastrea annularis* (mainly at the opening of Pond E, Fisherman's Cay; see Figure 1). Barnacles and mollusks also inhabit the ponds in significant numbers.

Faust (2000) identified 110 species in 33 genera of planktonic, oceanic, red tide-forming, benthic, and coastal dinoflagellate species from six of the Pelican Cays of great typological diversity. Approximately 50% of these appeared to be new species. Manatee Cay had 93 species, Douglas Cay, 47 species, and Cat Cay, 32 species. Waters in the Pelican Cays allow dinoflagellates to proliferate in a naturally nutrient-enriched environment, protected from prevailing winds by the surrounding mangroves and coral ridge (Faust, 2000).

In March 2000, a special issue of the *Atoll Research Bulletin* on the biology and physical characteristics of the Pelican Cays ponds was published to assist the efforts of the Government of Belize (GOB) to determine if this area should be included in the South Water Cay Marine Reserve (SWCMR). It was hoped that by bringing attention to the unique characteristics of these fragile communities they would be preserved. Based on those studies, the Pelican Cays were incorporated into the SWCMR that extends from Tobacco Cay in the north to Cat Cay in the south. The SWCMR is part of the Belize Barrier Reef Reserve System, which was inscribed on the UNESCO World Heritage List in 1996 (http://whc.unesco.org/). At that time, it was recognized by the World Heritage Committee and the GOB that, except for privately owned cays and those with preexisting leases, the cays of the SWCMR would be protected from development. In the Pelican Cays, such an exclusion would apply to a small area (<1 ha) at the southern tip of Northeast Cay. However, since 1996 most of the mangrove cays within the SWCMR have been leased for proposed resort developments. Based on our recent aerial surveys in April 2008, in most of the islands in the Pelican Cays archipelago, large sections of the mangrove forests have been cut down and covered with dredged marine sediment from the adjacent seafloor. Runoff of the fine fraction of the covering sediment has entered the interior ponds and smothered both the prop root-based and benthic seagrass communities. The extensive land clearing and filling is apparently an attempt to convert these mangrove islands into sandy cays in preparation for new tourist resorts.

METHODS AND RESULTS

Field Observations, March 2007

We visited Manatee Cay (16°39.97′N, 88°11.53′W) in mid-March 2007 to conduct reconnaissance of seagrass and prop root-based benthic communities along the perimeter of Pond C. At a point on the southeast side of Pond C (see Figure 1), we encountered an area of dead mangrove roots and a thick sediment drape covering the steep slope into the center of the pond (see Figure 2C). The seagrass communities lining the pond slope had been effectively buried by the sediment drape. At the top of the slope in this area, the ubiquitous submerged mangrove root epibenthos was conspicuously absent. At the surface, we noted a fringe of dead red mangrove trees behind which was recently cleared land covered in white marine sediment containing numerous coral fragments and mollusk shells.

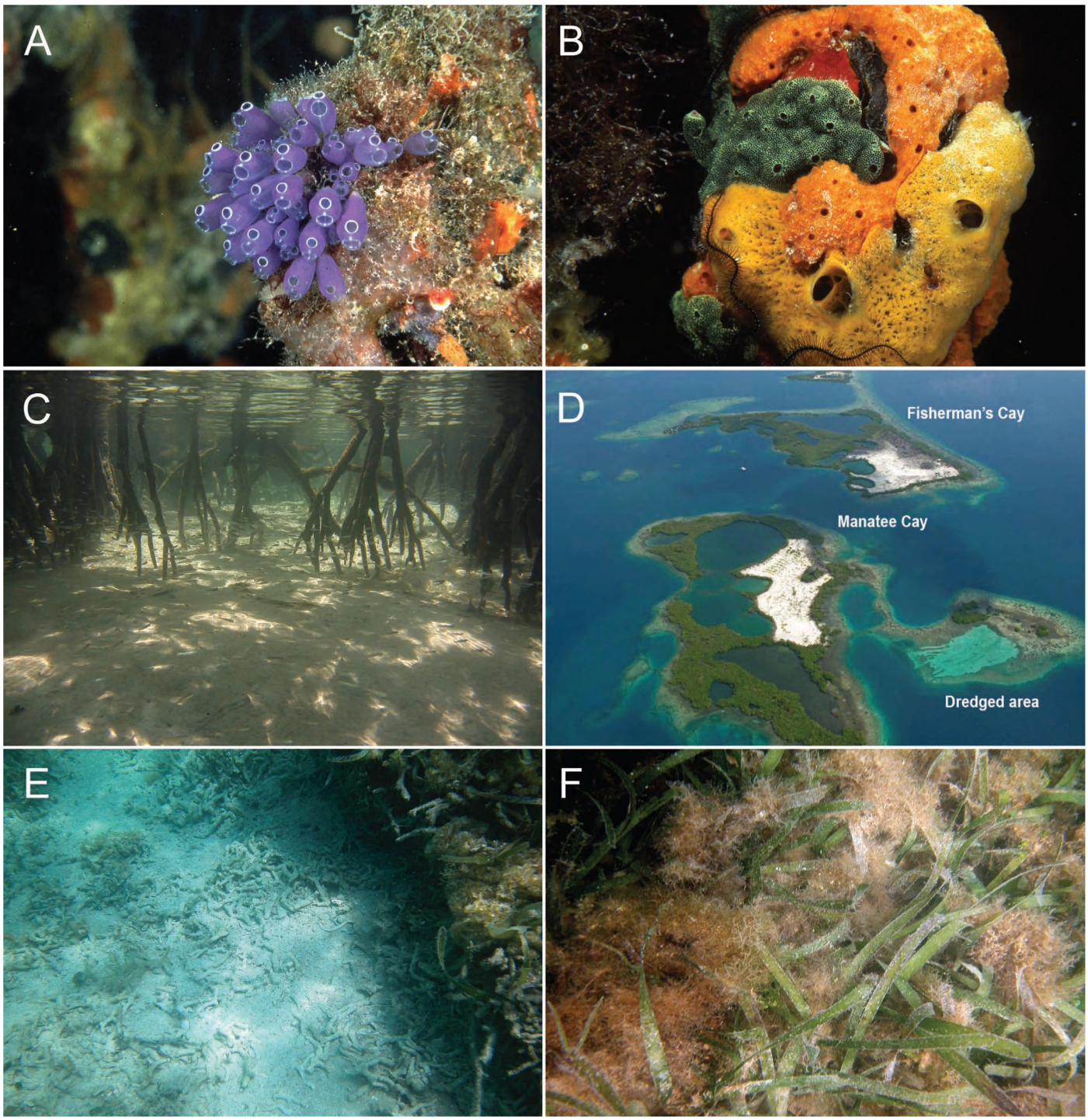

FIGURE 2. Marine communities of the Pelican Cay before (A, B) and after (C–F) dredging operations. A. Pre-dredging example of a rich encrusting community on a mangrove root that is dominated by the purple ascidian *Clavelina puertosecensis*. B. Healthy sponge community dominated by *Mycale* sp. and *Scopalina* sp. on a root before dredging. C. Spillover sediment surrounding bare dead mangrove roots in Pond C, Manatee Cay, after the deposition of dredged lagoonal sediment on clear-cut mangrove peat, March 2007. D. Aerial photograph showing the clear-cut sedimented areas of Fisherman's and Manatee Cays. Note the dredge site in the lagoon at the right of Manatee Cay. E. Dredged *Thalassia–Porites* bottom community in the lagoon east of Manatee Cay. F. Harmful toxic alga *Dictyota caribaea* overgrowing the seagrass *Thalassia testudinum* at the bottom of Pond C, August 2007.

At that time, the dredging work on Manatee Cay had been completed, but on Fisherman's Cay (16°40.25′N, 88°11.40′W), it appeared to be under way. Here, we observed similar clear-cutting and numerous large-diameter plastic pipes laid across the cleared area (Figure 3). The pipes are used to spread the sediment–water slurry over the mangrove peat and stumps. A dredging vessel was anchored just offshore of Fisherman's Cay (Figure 4).

Dredged marine sediment covered the exposed mangrove peat substrate and created the illusion of a sandy cay. However, the introduction of loose, water-laden sediment over peat resulted in runoff, which smothered and killed the mangrove root and lagoonal bottom communities along the edges of the islands. Sediment collected on the mud-covered slope in Pond C (see Figure 2C) was very fine grained (rich in the clay-size fraction), which caused turbidity when it washed off the island. Along the outer edges of the island, runoff of the sediment slurry carried these fine sediments into nearshore waters. In one case, it smothered shallow-water head coral communities along the windward side of Fisherman's Cay and created a muddy plume that extended into the pass between Fisherman's and Manatee Cays. More detailed studies are needed to establish the changes that have occurred in the pond communities as a result of the dredging activity. A brief visit in August 2007 indicated a marked increase in macroalgae, most notably *Dictyota caribaea*, a bushy and toxic brown alga (Littler et al., 2006; see Figure 2F). In addition, further studies of the dredged lagoonal seafloor areas should undertaken to assess the damage. A seagrass–*Porites* community to the east of Manatee Cay had been dredged down to 2 to 3 m below the shallow surface (Figure 2E). We estimate that such destruction will take decades to recover, as indicated by the still bare but shallower seismic line depressions in seagrass beds surveyed in the 1960s between Carrie Bow Cay and Twin Cays.

Aerial Photo Surveys, April 2007

Aerial photos taken in April 2007 indicated extensive clearing of mangroves and dredge-spoil filling over the exposed mangrove peat on Manatee and Fisherman's Cays

FIGURE 3. Stumps of clear-cut mangroves being covered with lagoonal sediment transported by the black pipes (arrow) in the background, March 2007.

FIGURE 4. Dredge boat operating off Fisherman's Cay, March 2007.

(see Figure 2D). An area of disturbed, bare seabed (see Figure 2E) was visible where the dredge removed the sediment near Manatee Cay. The time series of aerial photos since 2003 indicated that Northeast Cay had been partially cleared and had buildings on it before March 2003. Manatee, Fisherman's, Ridge, Bird, and Cat Cays had not yet been cleared in March 2003, but clearing had begun on all but one of the Bird Cays by April 2006. With the pumping of dredge spoil over the exposed mangrove peat and truncated roots, sediment spillover and runoff has resulted in extensive nearshore turbidity, which was also visible from the air along the edges of the ponds and the outer shorelines.

As of April 2007, approximately 15.4 ha (of 53.3 ha, or 29% of the total) of mangrove forests in the Pelican Cays had already been cleared, burned, and filled. Additional survey lines, which are typically the first evidence of development activity, were visible on both Fisherman and Manatee Cays in the aerial photographs taken in April 2007.

Phytoplankton Sampling Along the North Side of Pond C

In May 2007, we conducted a preliminary survey at Manatee Cay to determine the effect of mangrove clearing and dredging in the Pelican Cays on the phytoplankton populations dominated by dinoflagellates, which may form red tides and visibly discolor the water (Morton and Villareal, 2001). Manatee Cay Pond C (see Figure 1) is large, semi-enclosed, separated from open water by coral ridges (Figures 1, 2D), and has distinct hydrographic, chemical, and biological characteristics. With little water exchange from the ocean side, the pond is warmer and more saline than the surrounding waters. This environmental setting allows microplankton, filter feeders, and corals to proliferate (Villareal et al., 2000). We observed that dying mangrove trees edged the pond and the water surface was highly turbid. Dinoflagellates and associated microplankton were collected in the center of Pond C via a vertical tow with a 20 μm pore size plankton net. Specimens were examined in the laboratory with an Axiophot Carl Zeiss light microscope, and dinoflagellate species were identified (Faust, 2000). The water sample included a total of 14 species representing six genera. Oceanic species included *Ceratium* (2), *Protoperidinium* (5), and *Pyrophacus* (2). Coastal planktonic species included *Gymnodinium* (3) and *Peridinium* (2). Benthic species included *Prorocentrum* (3) (Table 1).

TABLE 1. Number of species in the dinoflagellate genera recorded in Manatee Cay, Pond C, Pelican Cays, collected in May 1996 and May 2007.

	Number of species	
Dinoflagellate genus	**1996**	**2007**
Amphidinium	2	–
Bepharocysta	1	–
Bysmatrum	1	–
Ceratium	10	2
Cochlodinium	1	–
Coolia	1	–
Corythodinium	1	–
Dinophysis	3	–
Diplopelta	1	–
Diplopsalis	3	–
Diplopsalopsis	1	–
Gambierdiscus	3	–
Gonyaulax	6	–
Gymnodinium	3	3
Heteraulacus	1	–
Lingolidinium	1	–
Ostreopsis	5	–
Peridiniella	1	–
Peridinium	3	2
Phaeopolykrikos	1	–
Plagodinium	1	–
Prorocentrum	1	3
Protoceratium	2	–
Protoperidinium	15	5
Pyrodinium	2	–
Pyrophacus	2	2
Scrippsiella	2	–
Zygabikonidium sp.	1	–
Total genera: 28	75	17

Live dinoflagellate cells were fewer than expected. Table 1 provides a comparison of the biodiversity and species associations of dinoflagellate assemblages before (May 1996) and after (May 2007) the clearing. In 1996, dinoflagellates included 28 genera and 83 species. In contrast, in 2007 only 6 genera and 14 species were present, and all were reduced in numbers of individuals present.

DISCUSSION

Despite repeated demonstration of their ecological and economic importance, mangroves are one of the world's most threatened ecosystems (Valiela et al., 2001; Alongi, 2002; Rivera-Monroy et al., 2004). Overall, 50% of the world's mangrove forests have been lost in the past 50 years, with at least 35% lost in just the past two decades (Valiela et al., 2001). Duke et al. (2007) predicted the current rate of loss would lead to mangrove extinction in 100 years. Loss of mangroves is occurring faster in some areas. For example, 29% of Guatemala's mangroves were lost in just 6 years between 1992 and 1998 (Abt Associates Inc., 2003). Most of that loss is directly attributed to unfettered clear-cutting for shrimp farm aquaculture, agriculture, mining, and development (Alongi, 2002). Based on growing evidence from around the world, the clearing and filling of mangrove forests for waterfront property to meet the demands of the leisure and tourism market for seaside resorts and retirement homes are also contributing significantly to this loss (Ellison and Farnsworth, 1996; Curran and Cruz, 2002; Barbier and Cox, 2003; Naylor et al., 2002; Choong, 2005). In the Gulf of Honduras along the Caribbean coast of Central America, mangroves have been destroyed to make way for hotels and other tourism infrastructure (CZAI, 2000). In these low-lying areas, such development requires fill material that is dredged from the seabed of nearby subtidal habitats. This action not only destroys corals and seagrass directly but also causes the suspension of sediments (turbidity), reduces light penetration, smothers seagrass and corals, increases nutrient levels, and releases contaminants (Rambøll Consulting Engineers, 2000). In addition, the inadequately disposed solid waste and untreated sewage associated with this coastal development enter waterways and increase unwanted nutrients, thus decreasing water quality. Similar building efforts have been observed all along the Belize mainland coastline where many acres of mangroves have been cut down to make room for numerous large private homes and resorts. In addition, many acres of mangroves have been removed to create extensive areas of shrimp ponds.

Although tourism is the second largest foreign exchange earner for the countries of Belize, Honduras, and Guatemala in the region, this type of development is counterproductive because loss of mangroves leads to a reduction in income from tourism and fisheries, changes in employment, loss of aesthetic value, loss of cultural heritage, conflicts between user groups, and loss of recreational opportunities (Abt Associates Inc., 2003).

Although mangrove forests are apparently considered of little value, recent studies have demonstrated the vital role of mangroves as nursery habitat for several species of reef fish (Mumby et al., 2004; Mumby, 2006). As stated by Mumby et al. (2004; specifically concerning Belize and Mexico), "Current rates of mangrove deforestation are likely to have severe deleterious consequences for the ecosystem function, fisheries productivity and resilience of coral reefs." In particular, the availability of mangroves for fish nursery habitat (intermediate between seagrass beds and coral reefs) is highly correlated to the numbers of reproducing adults and even the continued existence of certain species. According to these authors, parrotfish, which are important herbivores on reefs, have become locally extinct as a consequence of mangrove removal. Commercial species biomass has been effectively halved in areas of mangrove removal. Thus, the health of coral reefs and of fisheries, both essential for both tourism and local livelihood, are deleteriously affected by mangrove loss.

Despite the legislated restrictions on leasing government-owned lands within the Belize Barrier Reef World Heritage Site and the SWCMR, most of these cays have been leased or sold to foreign developers since 1996 by following standard procedures. These procedures involve locating a suitable site and having it surveyed; this requires a permit from the Lands & Surveys Office in Belmopan, Belize, which has apparently ignored the legal protection status afforded areas in marine protected areas. Separate permits from government departments are required to clear the leased areas delineated by survey lines. After the areas have been cleared, a developer must obtain another permit from the government to dredge material from the adjacent seabed to fill the leased areas.

The areas of mangrove clearing and filling with lagoonal sediments on Manatee and Fisherman's Cays (and other islands in the group) are extensive, exposing most of the available island surfaces. As such, sufficiently large areas have been cleared on these cays to account for potentially extensive development of seaside resorts. Disturbingly, the thin veneer of sediment laid over mangrove peat, especially where the mangroves themselves have been cleared, will not prevent significant subsidence as the underlying peat

decomposes and compacts. The substrate will then further subside because of the pressure of any load placed upon it, pilings notwithstanding, which will prove to be a significant long-term problem for construction on mangrove substrate.

In addition to these obvious problems, construction on and habitation of these islands will ensure perpetual pollution of the ponds from continued sediment runoff combined with the eventual addition of sewage outflow and solid waste. Turbidity in the water column from runoff of dredge spoil will continue to deleteriously affect marine communities adjacent to the affected islands. Along the outer shorelines of Manatee and Fisherman's Cays, numerous nearshore coral heads and patch reefs will eventually be smothered by the sediment load noted along the shorelines and in the passes between the Cays. In the Ponds, the rich benthic communities inhabiting the ridges upon which the islands are built and in the seagrass beds lining the slopes of the ponds have been locally decimated by direct sediment runoff. The water column in Pond C is now generally turbid, and it is anticipated that further sediment pumping, runoff, construction waste, and eventually untreated sewage outfall will further impact the pond habitats and cause eutrophication of the water column, which could lead to further losses of species, particularly photosynthetic organisms.

The Pelican Cays, although small in geographic scale, are characterized by great topological diversity in coral reef–mangrove habitats. Biological communities within this system vary markedly from one pond to another. Because of this complexity, some important details about the associations of dinoflagellate species in this ecologically diverse environment have come to light as a result of long-term studies in Pelican Cays (Faust, 2000). Dinoflagellates and microalgae are the primary food source for zooplankton, their primary consumer, including filter feeders and juvenile fish (Frenchel, 1988). Dinoflagellates and zooplankton proliferate in response to their unique physical, chemical, and biological needs (Villareal et al., 2000).

Species associations of dinoflagellates is another important indicator of certain stability in mangrove communities that are constantly threatened. Studies targeting processes in the Caribbean have examined benthic and epiphytic dinoflagellates in the coral reefs of the Virgin Islands (Tindall and Morton, 1998). Mangrove detritus, a unique microcosm, maintains a reservoir of diverse microalgae and meiofauna at Twin Cays, Belize (Faust, 1996). Most species tend to show preference for one habitat, either on sessile macroalgae or free-floating in the water column (Faust, 2004), although some species are found in a wide range of habitats.

Microbial communities can be damaged and species driven to local extinction by external factors; however, the damage is not immediately apparent to the human eye. Recent field observations of microscopic microalgae and zooplankton signaled significantly altered dinoflagellate populations, dead cells, and a greatly changed microscopic food web in Manatee Cay Pond C (see Table 1). This is yet another example of the continuing trend in the Belizean coral reef–mangrove ecosystem observed over 25 years (beginning in 1982) indicating declining abundances of dinoflagellate and zooplankton in the microbial food web caused by human activities (Faust, 2004). This finding in itself has important implications for the ecology and economy of the Belizean Barrier Reef, in that dinoflagellate populations are the primary food source of zooplankton, including fish larvae and juvenile fish.

CONCLUSIONS

Despite the location of Pelican Cays within the SWCMR and the World Heritage Site, development has been accomplished by following a sequence of procedures involving several separate jurisdictions. Investigations began recently into the process of mangrove cutting, clearing, and filling in the Pelican Cays, and it appears that some of this activity was illegal (Melanie McField, Smithsonian Marine Station at Fort Pierce, personal communication, April 2008).

It is highly questionable that the proposed highly vulnerable tourist resort on the Pelican Cays will survive the subsidence related to rotting peat or storms. Indeed, these sea-level structures will be readily destroyed by severe storms, leaving abandoned communities both on land and in the sea in an area originally noted for its unique and unusually high biological diversity.

The future of the unique ecology of the mangrove and seagrass communities in the Pelican Cays appears to be very bleak. The dredging barge is no longer operating off Fisherman's Cay, and a Caribbean Island Brokers website is now offering 37 acres of cleared mangrove on this cay for US $1,750,000. Given this situation, all mangrove islands in this area will be cleared and developed, so that the ponds adjacent to cleared areas will likely suffer the same fate as Pond C of Manatee Cay, as will nearshore marine communities along the outer perimeters of these islands. Lack of foresight, which is disrupting the connectivity between mangroves and the health of the nearshore marine realm, will result in economic losses following the reduc-

tion of commercial and recreational fisheries that rely on mangroves. Much of this collapse is related to the dramatic loss of dinoflagellate assemblages, which provide the base of food webs supporting fisheries in Belize.

Tourism losses will subsequently occur as coral reefs decline without mangroves to support the mangrove-dependent fish species essential to reef herbivory and commerce. Thus, the short-term economic gains from construction will lead to long-term environmental disruption, ecological degradation, local species extinction, and the consequent economic collapse of the tourism and fishing industries all along the Belizean coast and similarly affected areas of Mesoamerican reefs. The government of Belize has instituted a mangrove clearing moratorium to evaluate the situation.

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Using the Panama Canal to Test Predictions about Tropical Marine Invasions

Gregory M. Ruiz, Mark E. Torchin, and Katharine Grant

ABSTRACT. As humans alter the landscape of the Earth and economic globalization expands, biological invasions increasingly homogenize the world's biota. In temperate marine systems, invasions are occurring at a rapid pace, driven by the transfer of organisms by vessels and live trade (including aquaculture and fisheries activities). In contrast, little is known about patterns and processes of tropical marine invasions, although the same species transfer mechanisms are in operation. This disparity may be the result of limited studies of invasions in the tropics relative to temperate regions. Alternatively, the tropics may be less susceptible to invasion than temperate regions for reasons of environmental unsuitability and biotic interactions. This paper provides a brief summary of the current but limited information of marine invasions across latitudes, focusing particular attention on the eastern Pacific north of the Equator. Within this latitudinal framework, the Panama Canal provides an especially important model system for testing predictions about marine invasions in the tropics for reasons of (a) the high level of shipping traffic since the Canal opened in 1914; (b) the permeability of the Canal as a conduit for marine invaders, despite the apparent freshwater barrier; and (c) the current expansion of the Canal that is expected to increase the size and number of ships visiting the region.

INTRODUCTION

Biological invasions are common in coastal marine ecosystems around the world (Cohen and Carlton, 1995; Orensanz et al., 2002; Fofonoff et al., 2008). In fact, reports of new invasions are increasing exponentially in many well-studied regions (Cohen and Carlton, 1998; Ruiz et al., 2000; Hewitt et al., 2004). Although invasions can result from natural dispersal, most contemporary invasions derive from human-mediated transfer associated with a variety of activities. As economic globalization continues to expand, creating a high degree of connectivity through the movement of commodities and people, opportunities for new invasions also increase. Bays and estuaries have been the most invaded marine systems, probably because they are hubs for shipping, aquaculture, and other human endeavors known to transfer organisms (Ruiz et al., 1997; Wasson et al., 2005).

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To date, most human-mediated invasions (hereafter introduced species) in marine habitats have been reported in temperate latitudes (Ruiz and Hewitt, 2008, and references therein). Relatively few introduced species have been reported from tropical or polar regions. This difference across latitudes may result partly from historical research effort and taxonomic knowledge, which are greatest in the temperate zone. However, a small but growing literature for high latitudes suggests that marine invasions may be limited in polar regions by a combination of current low temperatures and low propagule supply (Barnes et al., 2006; Aronson et al., 2007; Ruiz and Hewitt, 2008).

It is evident that marine invasions can occur in tropical marine systems (Agard et al., 1992; Guerrero and Franco, 2008), but the extent to which they occur remains largely unexplored. Few studies have evaluated marine invasions in the tropics. The exceptions are extensive analyses of introduced species on the Hawaiian Islands and Guam (Eldredge and Carlton, 2002; Paulay et al., 2002). It is uncertain whether these island ecosystems are broadly representative of the tropics, including especially mainland sites that may differ from islands in susceptibility to invasion (Elton, 1958; MacArthur and Wilson, 1967; Sax, 2001).

In a preliminary analysis of marine invasion patterns for mainland Australia, Hewitt (2002) reported an increase in introduced species richness with increasing latitude. The study included four tropical and four temperate sites, spanning 13°–38°S latitude. Despite a significant relationship with latitude, there is uncertainty about the taxonomic identification and biogeographic origin of many tropical species, resulting from limited information and relative lack of study for low latitude biotas. For this reason, Hewitt urges some caution and underscores the need for further analyses to interpret the observed pattern. It is nonetheless intriguing that this preliminary analysis provides results similar to those reported for tropical terrestrial systems, where relatively few exotic species of birds, mammals, and plants are established (Sax, 2001).

We have begun to explore latitudinal patterns of marine invasions for the mainland (continental) habitats within the Americas. To date, most of our analyses have focused on bays and estuaries within the United States, particularly on the Pacific Coast. We are currently initiating a research program to compare the number of introduced species, scale of vector operations (propagule supply), and ecology of invasions across temperate and tropical latitudes. Here, we briefly review the current state of knowledge about invasions and invasion processes along the Pacific Coast of the Central and North America and discuss the potential significance of Panama as a model system to evaluate regional and latitudinal patterns of marine invasion.

LATITUDINAL PATTERN OF INVASIONS ALONG THE NORTHEASTERN PACIFIC

Outside of the tropics, there is a clear increase in the number of nonnative species reported with decreasing latitude, from Alaska to California, 61°–32°N (Ruiz et al., 2006a). An extensive review and synthesis of the literature indicate that more than 250 nonnative species of invertebrates and algae are established in coastal waters of California (NEMESIS, 2008). Most of these invasions are attributed to commercial shipping and live shipments of organisms, especially oysters and their associated biota (Cohen and Carlton, 1995; Miller, 2000; Ruiz et al., unpublished data). Some of the California invasions have spread northward through natural dispersal, and other species have been introduced independently to the north. However, compared to California, far fewer nonnative species are known from Oregon, Washington, and Alaska (Cohen et al., 1998; Wonham and Carlton, 2005; Ruiz et al., 2006a).

Although this latitudinal pattern of invasion could result from reporting biases in the literature, particularly in the level of research (search effort) among regions, recent surveys suggest that the pattern is robust for sessile invertebrates in hard substrate fouling communities. Using standardized surveys to sample sessile invertebrates, deRivera et al. (2005) and Ruiz et al. (2006a) found that the number of introduced species increased with decreasing latitude from Alaska to southern California. It appears that the northern spread of many nonnative species from California may have been limited by dispersal as a result of the relatively low level of human activities (and, thus, species transfer opportunities) that have been present historically (Ruiz and Hewitt, 2008).

Similar analyses are not yet available to extend this comparison to lower latitudes along the eastern Pacific. Although there have been some studies reporting introduced marine species in Central America (Rubinoff and Rubinoff, 1969; Lambert and Lambert, 2003; Wysor, 2004, Roche and Torchin, 2007; Roche et al., 2009; Bastida-Zavala, 2008), standardized, quantitative community-level comparisons are lacking. In particular, synthetic studies focused within bays and estuaries of Central America targeting those taxonomic groups for which invasions are often most prevalent do not exist. Even where syntheses from the literature have been attempted, the paucity

of available data limits conclusions about the scope of invasions. For example, Cohen (2006) provides a useful summary of available information on invasions surrounding the Panama Canal, which has received considerable attention for a tropical system. Despite the historical interest on biotic exchange in Panama, Cohen characterizes the current state of knowledge as follows: "The Panama Canal lies in a region of the world where the marine biota is both diverse and relatively poorly known, and there has been remarkably little investigation of the effect that the Canal has had on the distribution of that biota."

With a broad goal to evaluate patterns and processes in marine invasions using a latitudinal framework, we have initiated a research program in Central America (a) to compile available data from the literature on nonnative marine species, as part of our database (NEMESIS, 2008), and (b) to conduct standardized surveys at multiple sites. Our approach will allow direct comparisons with more than two dozen sites surveyed on the Pacific and Atlantic coasts of the USA. Our initial effort is focused primarily on sessile invertebrates (including ascidians, barnacles, bryozoans, hydroids, mussels, and sponges), which comprise a large proportion of marine introductions, are relatively well studied, and are conducive to standardized, quantitative field surveys.

A preliminary review of the literature for barnacles suggests the number of introduced species increases from Alaska to Panama (Figure 1A), consistent with an increase in the magnitude of shipping (see next section). At least four nonnative species of barnacles are reported to occur on the Pacific coast of Panama, including *Amphibalanus amphitrite*, *A. reticulatus*, *Balanus trigonus*, and *Fistulobalanus pallidus* (Matsui et al., 1964; Jones and Dawson, 1973; McCosker and Dawson, 1975; Laguna, 1985). Three introduced barnacles are known from California: *Amphibalanus amphitrite*, *A. eburneus*, and *A. improvisus* (Carlton, 1979; Carlton and Zullo, 1969; Cohen and Carlton, 1995; Cohen et al., 2002). *Amphibalanus reticulatus* has also been detected in recent surveys in southern California, but it is not yet known to be established (Ruiz, unpublished data). Only one introduced barnacle, *A. improvisus*, is reported in Oregon and Washington (Carlton, 1979; Wonham and Carlton, 2005), and there are no introduced barnacles known from Alaska (Ruiz et al., 2006a). It is noteworthy that the reported number of nonnative barnacle species in Panama exceeds that along the western USA, considering the latter is relatively well surveyed. Thus, we expect that strength of this inverse relationship with latitude may increase with additional information.

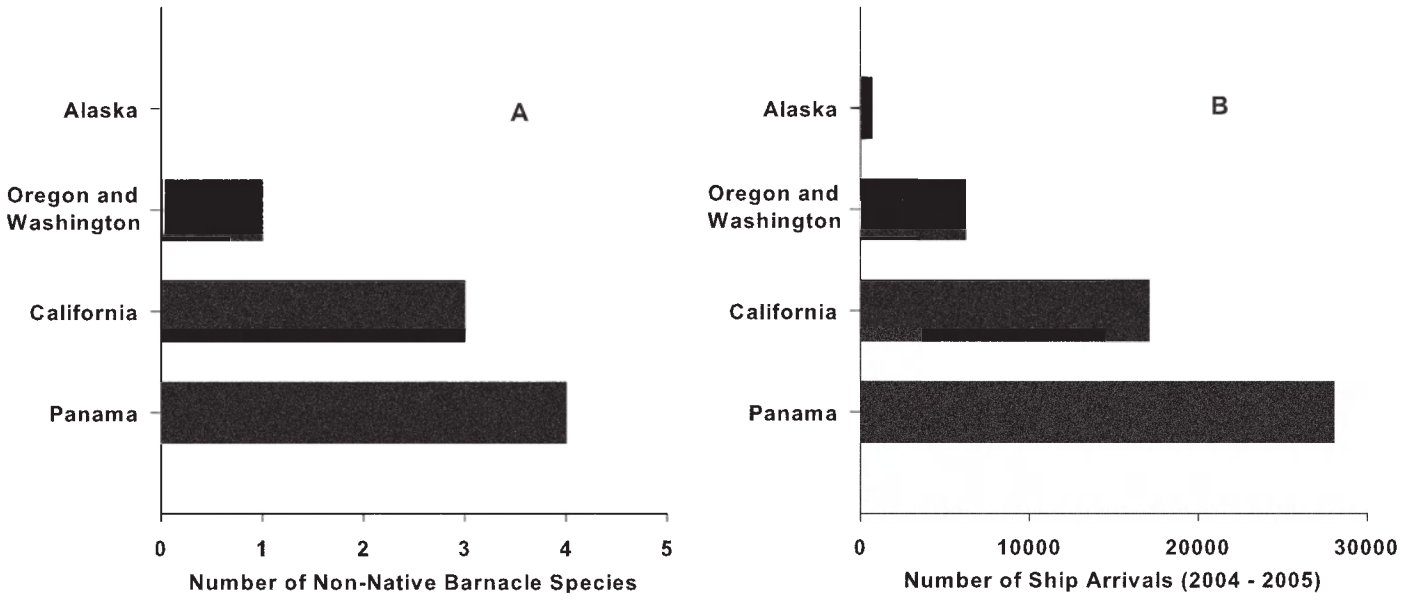

FIGURE 1. A. Number of nonnative barnacle species established by geographic region. Shown are the numbers of nonnative barnacle species reported to be established from Alaska to Panama (see text). B. Number of vessel arrivals by geographic region. Shown are the numbers of commercial vessel arrivals from overseas to different geographic regions, from Alaska to Panama, over a two-year period (2004–2005). Coastwise domestic traffic is excluded from arrivals to U.S. locations. (Data from Miller et al., 2007; ACP, 2008b.)

At the present time, the relationship between introduced species richness and latitude is poorly resolved for the northeastern Pacific and other global regions. The pattern presented in Figure 1A should be considered as preliminary, and it may change with further research. We also caution that these data are restricted to barnacles, a very small subset of species present in the fouling community.

PANAMA: A TEST CASE FOR TROPICAL MARINE INVASIONS

Panama is a potential hotspot for tropical marine invasions, because of the country's historic significance as a hub of world trade since the fifteenth century, expanding greatly since construction of the Panama Canal. The Canal created a new shipping route between the Atlantic and Pacific basins, resulting in a large influx of commercial ships, which have been an important source of introduced species in North America (Cohen and Carlton, 1995; Cohen et al., 1998, 2002; Ruiz et al., 2000; Wonham and Carlton, 2005; see discussion below). Figure 1B compares the magnitude of commercial shipping to several major port systems, indicating that ship arrivals to Panama exceed those to major port systems in the western United States by a large margin. Over the two-year period 2004–2005, nearly twice as many vessels arrived to Panama as overseas vessels arrived to California. In fact, Panama receives more ship arrivals than any of the largest ports in the United States (Ruiz et al., 2006b; Miller et al., 2007).

Since its opening in 1914, the number of Canal transits increased rapidly, with the exception of a brief interruption during WW II, until reaching capacity in 1970 (ACP, 2008a; Figure 2). Currently, the Canal is operating at 90% of its theoretical maximum capacity, servicing 12,000 to 14,000 vessels and carrying approximately 5% of the world's cargo annually (Reagan, 2007). More than 800,000 ocean-going commercial vessels have passed through the Canal since its completion (Ruiz et al., 2006b).

While the number of transits has leveled off, the average size of ships transiting the Canal has continued to increase, allowing for a continued increase in the volume of cargo passing through the Canal (ACP, 2008a; see Figure 2). The average tonnage (based on CPSUAB, a universal system of tonnage for the Panama Canal, or Canal ton, which is equivalent to approximately 100 cubic feet of cargo) per transit has increased from 4,832 in 1955 to 21,963 in 2005 (ACP, 2008a). This change in cargo capacity reflects an increase in the size of vessels over time;

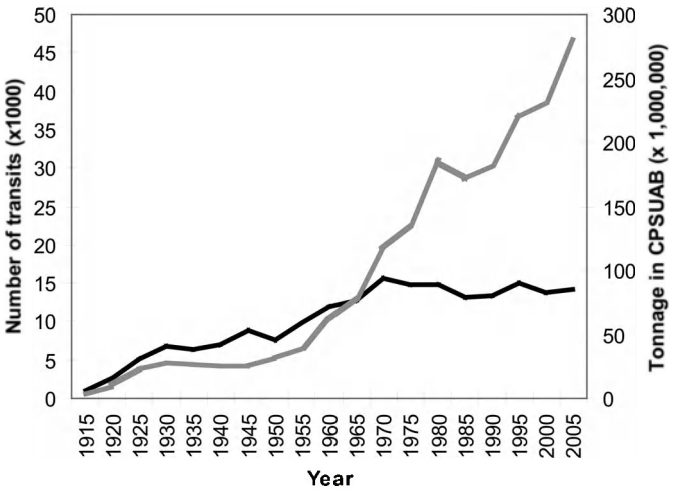

FIGURE 2. Number of commercial vessel transits (black line) through the Panama Canal and associated cargo tonnage (gray line); CPSUAB is a universal system of tonnage for the Panama Canal, or Canal ton, which is equivalent to approximately 100 cubic feet of cargo. (Figure modified from ACP, 2008a.)

these changes are the topic of a future analysis that will characterize changes both in vessel size and in underwater surface area available for colonization by organisms. In recent years, however, the size of vessels has been constrained by the lock dimensions and has been relatively static, with the Panamax ships designed specifically as the largest vessels able to transit the locks (see next section).

Likely consequences of the Panama Canal and ports located at both entrances are an increase in (a) the global transfer of marine organisms, as the canal provides a conduit for worldwide shipping, and (b) regional biological invasions in Central America. Commercial shipping is a major pathway for the movement of species and appears largely responsible for a dramatic increase in the rate of known invasions for many regions in recent time (Ruiz et al., 2000; Fofonoff et al., 2003; Hewitt et al., 2004). Ships move organisms associated primarily with hull and sea chest fouling and with ballasted materials, as an unintended result of normal operations (Carlton, 1985; Minchin and Gollasch, 2003). In general, the likelihood of invasions increases with increasing propagule supply, including the magnitude and frequency of organisms delivered (Ruiz and Carlton, 2003; Lockwood et al., 2005). Thus, the chance of colonization by introduced species in Panama is likely to have increased over time with the high frequency of vessels arriving to Panama from around the globe.

Given the high number of vessel arrivals, we might also expect the relative magnitude of propagule supply and invasions to be high in Panama. However, this remains to be tested, and there are several reasons why this may not be the case. First, different ship types and operational behaviors vary in their potential to transfer marine organisms (Verling et al., 2005; Miller et al., 2007; NBIC, 2008). Second, independent of propagule supply, some sites are less susceptible to invasion for reasons of either environmental conditions or biological interactions (Lonsdale, 1999; Ruiz et al., 2000; Roche et al., 2009).

Past studies have certainly highlighted the potential significance of vessels as a source of invasions to the Panama Canal and surrounding waters (see Cohen, 2006, and references therein for recent review). For example, Chesher (1968) discusses the potential importance of ballast water. Menzies (1968) considers the capacity of vessels to transfer fouling organisms. Hay and Gaines (1984) suggest that small pleasure boats may be especially important in the transfer or organisms across the Isthmus of Panama. A few studies also test the capacity of marine organisms to survive freshwater exposure for the duration of a transit through the Canal (Chesher, 1968; Hay and Gaines, 1984). Despite the long interest and recognition in ship-mediated transfer, the estimates given above are limited to few (if any) data on species composition or direct quantitative estimates of propagule supply (abundance) on vessels. Surprisingly few data exist on biota associated with ballast water or hulls of vessels associated with the Canal. Instead, there are only coarse data available on general operational aspects of vessels that may affect species transport opportunities.

Most commercial ships arriving to Panama will transit the Canal, but some will have considerable time at anchorage before entering the Canal. From 2000 to 2005, the average service time (from arrival to complete transit) of ships passing through the Canal was 16 hours when holding reservations. However, many ships have not had reservations, and average service times for these ships can reach 57 hours (Table 1). Although the proportion of ships holding reservations has increased in recent years, half of all ships still experienced some delay. Such increased residence time is likely to also increase the opportunity for reproduction and colonization of organisms associated with ships' hulls (Minchin and Gollasch, 2003; Davidson et al., 2008), relative to shorter residence times. It is evident that some organisms arrive to Panama on the hulls of vessels (Figure 3). However, a lack of quantitative information on the biota associated with outer surfaces of vessels transiting the Panama Canal and surrounding ports limits any detailed analyses.

For ballast water, we are not aware of any reliable estimates of the historical patterns of ballast water management and discharge of vessels arriving to Panama, including those ships delivering cargo to the terminals and those simply transiting the Canal. Even a coarse estimate of volume is challenging, given large differences in operations among vessels (Verling et al., 2005; but see Chesher, 1968). Presumably, ballast water discharge today is rather limited because many vessels conduct ballast operations to compensate for loading or off-loading cargo. In addition, Panama prohibits ballasting operations in the Canal under most circumstances (ACP, 2008b).

Despite the limited information available, we surmise that propagule supply has been relatively high in Panama, compared to many other temperate and tropical sites. Based solely on the large number of vessel arrivals and their relatively long residence times (see Figure 2, Table 1), it is likely that Panama has received large inocula of nonnative organisms associated with the vessels' hulls and sea chests, which have been historically important sources of invasions in

TABLE 1. Comparison of service time for ships with and without reservations transiting the Panama Canal; *n* = number of ships. (Source: Modified from ACP, 2008a.)

	Mean transit time (hours) through canal					
Year	Reservation (*n*)		No reservation (*n*)		Could not get reservation (*n*)	
2000	16.7	(1,944)	35.7	(6,864)	42.1	(121)
2001	15.7	(5,008)	26.3	(6,590)	43.7	(306)
2002	16.1	(5,692)	29.0	(5,134)	57.1	(1,062)
2003	16.2	(5,527)	24.9	(4,596)	45.1	(1,361)
2004	16.4	(6,419)	30.5	(3,568)	49.8	(2,531)
2005	16.5	(6,972)	27.3	(3,406)	45.8	(2,270)

FIGURE 3. Photograph of a vessel hull upon arrival to Panama showing associated biofouling organisms. Inset: Close up of bow with barnacles.

other regions (Coutts, 1999; Coutts et al., 2003; Coutts and Taylor, 2004; Hewitt et al., 2004).

As a result of its shipping history, Panama provides a unique opportunity to test hypotheses about patterns and processes of invasions to tropical marine systems. If propagule supply drives invasion patterns, we predict that Panama may be a hotspot for invasions. If tropical systems are inherently less susceptible to invasions (Elton, 1958; Sax, 2001), we would expect to see low introduced species richness despite high historical propagule supply. Our current research seeks to estimate nonnative species richness and advance our understanding of historical propagule supply in Panama, in the context of a broader latitudinal comparison as discussed above.

EVALUATING FUTURE CHANGES IN PANAMA

In October 2006, the Republic of Panama passed a referendum to expand the capacity of the existing Canal. The modernization will include (a) two new sets of locks, one at the Pacific entrance and one at the Atlantic; (b) two new navigational channels to connect the locks to existing channels; and (c) deeper and wider shipping lanes (Reagan, 2007). The expansion project is now under way and is scheduled to be completed by 2015 (Reagan, 2007).

When the expansion is completed, the Panama Canal Authority estimates that Canal transits will most likely increase from 12,700 per year in 2005 to approximately 19,600 in 2025, with an optimistic forecast as high as 22,100 transits per year (Figure 4). Further, the largest vessels currently capable of transiting the Canal are Panamax ships reaching 320 m in length that can carry 65,000 tons of cargo. After the completion of the new locks, the Canal will accommodate vessels up to 425 m long, carrying about twice the amount of cargo of today's ships (Gawrylewski, 2007; Reagan, 2007).

While efforts have been made to evaluate potential environmental effects of the Panama Canal expansion (ACP, 2008a), the possible effects of this expansion on invasion dynamics have not received much attention to date. One might expect an increase in propagule supply associated with the increased number and size of vessels transiting the

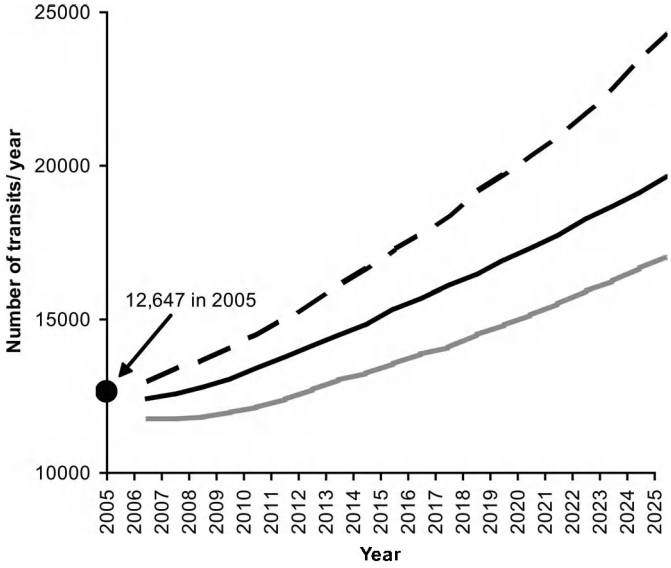

FIGURE 4. Forecast of demand for Canal transits. Solid black line = probable demand, dashed line = high (optimistic) demand, and shaded gray line = low (pessimistic) forecast demand. In 2005, there were 12,647 recorded Canal transits (solid circle). (Figure modified from ACP, 2008a.)

Canal. There may also be shifts in trade routes that could expand the species pool associated with ships' arrivals, resulting from either new markets or previous constraints on the size of vessels that could previously use this corridor. Alternatively, the service time of vessels may decrease as the capacity to accommodate more transits increases. This decrease could reduce the establishment probability of organisms attached to the hulls of arriving vessels, as residence time and likelihood of invasion are thought to be positively correlated (Davidson et al., 2008).

Potential changes in environmental conditions associated with both the ships and the Canal entrances could also influence future invasions. With the international ban on tributyl tin as an antifouling coating now coming into force, some have suggested that biofouling of ships' hulls, and hence ship-mediated propagule supply, may increase (Nehring, 2001). Additionally, changes in the salinity regimes will probably occur at both Pacific and Atlantic entrances to the Canal, as well as in areas within the Canal near the lockages, as a result of increased freshwater discharges into the oceans and potential seawater intrusion into the Canal. Such changes in salinities could alter the susceptibility to invasion for arriving organisms. However, any predictions about directional changes in propagule supply and susceptibility are currently speculative at best, as sufficient information presently is not available.

There is also a regional context for the Panama Canal that deserves consideration. Although the Canal provides a critical corridor across the Isthmus of Panama for global trade, Panama's ports are becoming increasingly important hubs for the regional distribution of commodities. More specifically, cargo that is delivered to Panama's ports is often transferred secondarily by other vessels to surrounding countries in the region. As Panama is a distribution center, any increase in introduced species increases the chances for ship-mediated dispersal to surrounding ports. Conversely, increased commerce with the other countries in the region also enhances the opportunity for delivery of organisms to Panama. The potential significance of such regional dispersal through this hub-and-spoke system of shipping has not been evaluated for the past, present, or future.

We are currently working with the Panama Canal Authority and the University of Panama to evaluate the role of the Panama Canal in regional and global marine invasions. Although the major focus of our efforts is to evaluate past and current levels of invasion, as well as to obtain some coarse estimates of propagule supply to the region, we hope to provide the baseline needed to forecast and evaluate potential impacts of future changes on invasion risks.

CONCLUSIONS

Panama provides exceptional opportunities to test hypotheses about invasions in tropical marine systems. The presence of the Canal and the magnitude of shipping to the region have undoubtedly increased the supply of nonnative species delivered to the shores of Panama. While there is limited information on actual propagule delivery, the Panama Canal Authority has maintained historical records on the number and characteristics of transiting vessels. This information provides a unique view of the magnitude of shipping and changes through time and could be used as an initial coarse proxy for propagule supply. We predict that invasions are common in Panama relative to surrounding regions as a result of the intensity of shipping in the area. If propagule supply is positively correlated to introduced species richness, as the literature suggests, we predict a relatively high number of invasions have occurred. However, if relatively few introduced species are detected in Panama, this suggests that some combination of environmental conditions and biotic resistance may limit invasions in this tropical region.

We have focused attention on Panama as a model system to understand marine invasion dynamics, but a robust analysis must also include comparisons to other locations that differ in the intensity of shipping and other transfer mechanisms. Ideally, such comparisons should be replicated across latitudes. Such a comparative approach is key to untangling patterns of marine invasions in tropical and temperate regions and, ultimately, in determining the processes that drive these patterns.

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Ciguatera Fish Poisoning in the Caribbean

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ABSTRACT. Ciguatera fish poisoning (CFP) is a significant illness in the Caribbean. Local fishers and natives attempt to avoid CFP by applying traditional knowledge concerning where and when certain fish species are likely to be ciguatoxic, but this knowledge is incomplete. Evidence gathered over the past decades indicates that CFP events are increasing and becoming more unpredictable, thereby posing a greater threat to local inhabitants as well as tourists. The current understanding of CFP distribution is from studies nearly a decade old and generated largely by self-reported CFP incidents to a call-in "hotline" in Miami, Florida. To better guide resource allocation and focus future research, an active survey method was used to uniformly query public health professionals and fisheries officials on the occurrence of CFP. Points of contact from each of these two groups were compiled for the 24 Caribbean island countries and territories and 9 mainland countries bordering the Caribbean. An outcome of this project will be to provide public health agencies, resource managers, and others with information they can use in developing CFP tracking systems and effective public education programs. The long-term goal of associated efforts is to provide accurate and affordable monitoring tools for predicting the onset of CFP events.

PREFACE

Ciguatera fish poisoning (CFP) occurs in tropical regions worldwide and is globally the most common nonbacterial food-borne illness (Tester, 1994; CDC, 2007; Figure 1A). The toxic organisms most commonly associated with CFP are benthic dinoflagellates reported to produce ciguatoxins or maitotoxins (Yasumoto et al., 1977; Durand-Clement, 1987; Satake, 2007). Ciguatoxins bioconcentrate in the food chain and reach their highest levels in top predators such as barracuda or other tropical reef fish. These toxins have been found in more than 400 fish species, including groupers, snappers, jacks, mackerels, triggerfish, and surgeonfish (Bagnis et al., 1970). Consumption of tainted fish can lead to gastrointestinal distress followed by neurological (perioral numbness, tingling, temperature sensory reversal) and cardiovascular (arrhythmia, bradycardia, tachycardia, reduced blood pressure) symptoms and, in rare cases, death. The chronic phase of CFP can persist for weeks, months, or years (Freudenthal, 1990), and repeated exposure to ciguatoxins exacerbates the symptoms.

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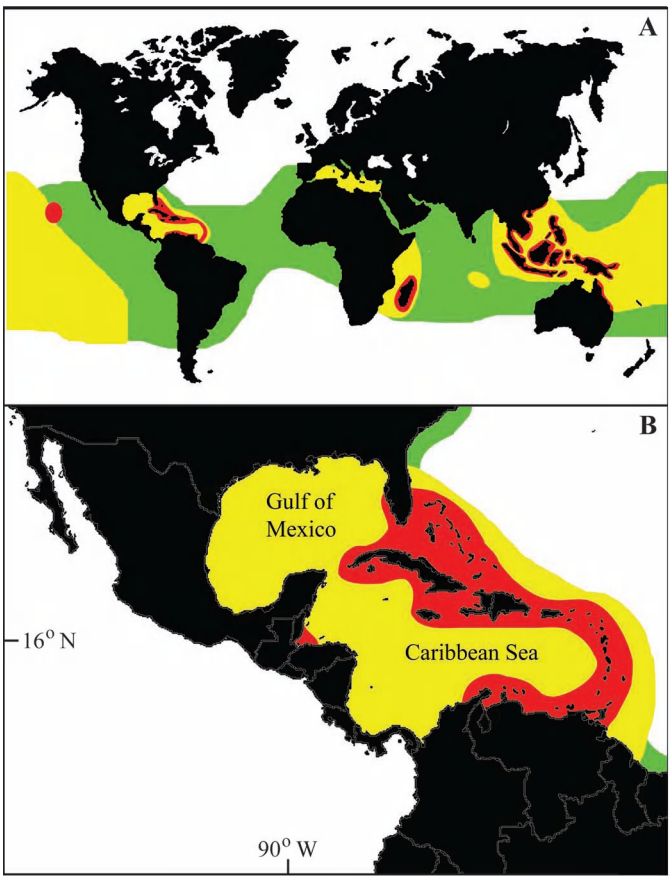

FIGURE 1. A, Potential global distribution of ciguatera fish poisoning (CFP). Red areas indicate regions with a high CFP prevalence, yellow indicates moderate potential exposure, and green indicates regions where the dinoflagellates responsible for the disease are found and represent a potential problem. This map represents a composite of the data obtained from an aquatic biotoxins review by Huss et al. (2003), the CFP distribution map maintained by the journal *Harmful Algae* (WHOI, 2008), and Lewis (2006). B, Potential distribution map of CFP in the Caribbean, as modified from Stinn et al. (2000), combined with some recent incident reports showing the presumed distribution of ciguatera fish poisoning in the Caribbean, mostly collected by passive means; that is, a self-reporting CFP hotline in Miami ("Cigualine" at 1-888-232-8635). Red areas indicate high frequency of CFP reports; yellow indicates regions where CFP is reported less frequently; green indicates infrequent reports of CFP. These maps may not accurately portray the actual CFP distribution because many cases go unreported.

This paper provides the justification for and an overview of our recent efforts to conduct an active survey of public health officials and fishery management professionals on the incidence of CFP in the Caribbean. We currently lack an accurate picture of CFP in the Caribbean because of the difficulty in diagnosing CFP and the absence of uniform reporting criteria or any entity responsible for maintaining this information. Previous information gathered on the incidence of CFP in the Caribbean has relied heavily on self-reporting mechanisms, such as calls to a "hot-line" in Miami, Florida. Because people living closer to Miami are more likely to know about the hot-line, the reported incidence rates could reflect a geographic bias (Figure 1B). Another important aspect of this research has been to focus the joint research efforts of the National Oceanic and Atmospheric Administration (NOAA) and Smithsonian Institution scientists who are working on the molecular and morphological characterization of the toxic dinoflagellates responsible for CFP. Both groups have strong interests in understanding how changes in the distribution and abundance of ciguatera-associated dinoflagellate species relate to the occurrence and severity of CFP.

An important outcome of this project will be to provide public health agencies, resource managers, and others with information that they can use in developing CFP tracking systems and effective public education programs. The long-term goal of associated efforts is to provide accurate and affordable monitoring tools for predicting the onset of CFP events.

INTRODUCTION AND BACKGROUND

Ciguatera fish poisoning is a common disease in the Caribbean, caused by the ingestion of a wide variety of fishes that contain toxins accumulated from the marine food web (Lewis and Holmes, 1993) (Figure 1B). The ultimate sources of these toxins (ciguatoxins and maitotoxins) are small benthic microalgae belonging to the dinoflagellate genera *Gambierdiscus*, *Coolia*, *Ostreopsis*, and *Prorocentrum* (Figure 2) (Steidinger and Baden, 1984). Although ciguatera fish poisoning (CFP) is a threat to public health throughout the Caribbean, it is generally managed by local, traditional knowledge of the native fishers. However, their knowledge of the seasonality of occurrence and locations of ciguatoxic reefs may no longer be accurate because of changing environmental conditions (Tester, 1994; Tosteson, 2004). These environmental changes in turn alter the distribution and abundance patterns of the cells that cause CFP. Some evidence exists that ciguatoxicity may vary seasonally, but not all studies support this view (de Fouw et al., 2001). Tosteson (2004) argued that seasonality of CFP and the correlation of dinoflagellate abundance with CFP intoxications evident before 1990

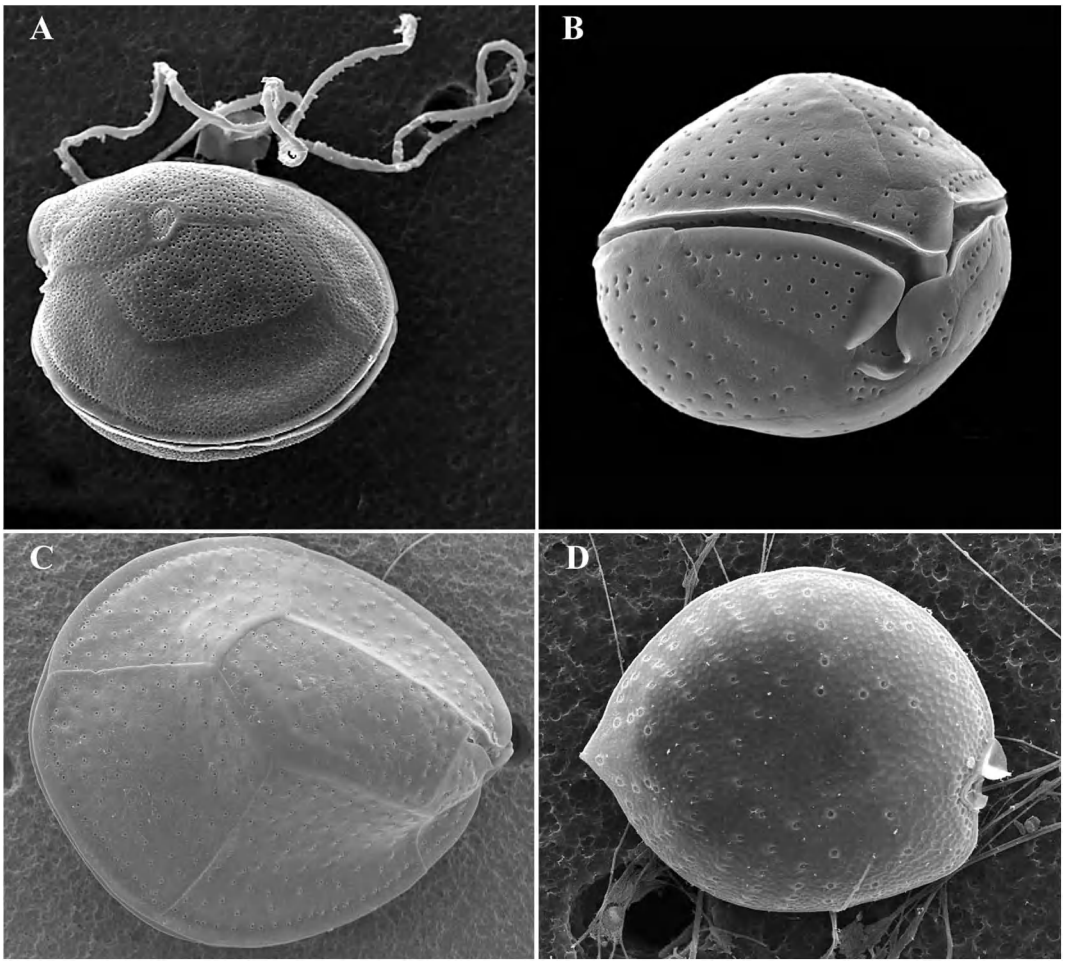

FIGURE 2. Scanning electron micrographs of ciguatera-associated dinoflagellates: A, *Gambierdiscus*; B, *Coolia*; C, *Ostreopsis*; D, *Prorocentrum*.

was not observed in data from 1990–2000. He suggested these changes appeared to be associated with increasing periods of elevated sea-surface temperatures in the Caribbean. Further, the potential for a greater number of people to be exposed to CFP has increased because of more intense exploitation of fisheries and the depersonalization of markets (Olsen et al., 1984). Both trends have been accelerated by tourism and rapidly growing resident populations (CIA, 2008).

The average number of tourist days (excluding ships' passengers) in the Caribbean, 174 million, dwarfs the 38.8 million residents and represents a significant exposure of a naive population to CFP. The most common route of exposure is through consumption of locally harvested fish. Currently, the annual total Caribbean fishery landings exceed 1.6 million metric tons (CRFM, 2008; FAO, 2005, 2008; WRI, 2007), making a strong argument for focused studies on CFP occurrence and on the environmental factors that affect the distribution and abundance of CFP-associated organisms.

As part of its commitment to understand and characterize the diversity, distribution, and abundance of organisms throughout the Caribbean, the Smithsonian Institution has carried out extensive studies on dinoflagellates over the past 20 years (Faust and Gulledge, 2002). Because of this pioneering work, much of the background information and expertise needed to characterize the diversity of ciguatera-causing dinoflagellates are already in place. During the past five years, NOAA (National Oceanic and Atmospheric Administration) and Smithsonian scientists have collaborated to isolate, identify, and genetically characterize the ciguatera-causing dinoflagellates

of the Caribbean, as well as to develop species-specific molecular assays for assessing their abundance. As part of this work, four new *Gambierdiscus* species have been discovered and are being described (Tester et al., 2008; Litaker et al., in press).

We are now in a position to begin systematic studies of the incidence of CFP and distribution and abundance of CFP-causing dinoflagellates throughout the Caribbean. To identify areas of concern from both public health and marine resource perspectives, and to focus the effectiveness of environmental sampling, we needed to identify areas where CFP was most common. Consequently, we initiated active surveys of local fishery managers and public health officials. By examining the CFP incidences among the 24 islands and the 9 mainland countries surrounding the Caribbean, additional insights can be gained into factors that govern the spatial and temporal variations in the prevalence of CFP.

A second objective of this study was to determine how CFP was being monitored and reported throughout the Caribbean, where more than 46% of the tourists are from the United States (United Nations Statistics Division, 2004; CTO, 2008; Figure 3) and the average length of stay is 8.7 days (United Nations Statistics Division, 2004; CTO, 2008; Figure 4). This project represents the first steps toward an assessment of community vulnerability by the identification of susceptible populations and serves as a framework for developing human dimensions research as a cross-cutting priority of ecosystem science supporting marine resource management (Bauer, 2006).

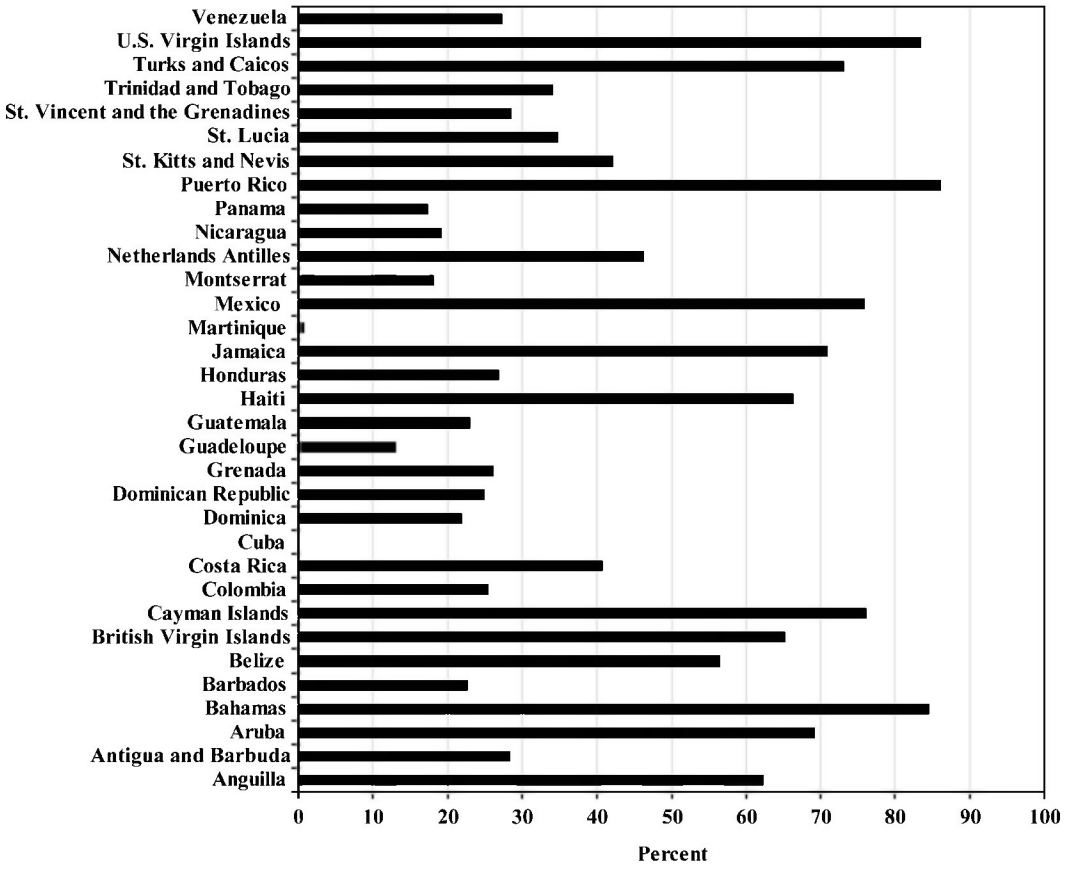

FIGURE 3. Average percentage of American tourists visiting the Caribbean by country (1996–2005). Only data from Cancun and Cozumel were used for Mexico. On average, 46% of tourists who visited all Caribbean countries came from the United States. On average, not counting visits from passengers on cruise ships, tourists spend over 174 million tourist days in this region each year (OAS, 1997; ACS-AEC, 2003; UNSD, 2004; CIA, 2008; CTO, 2008).

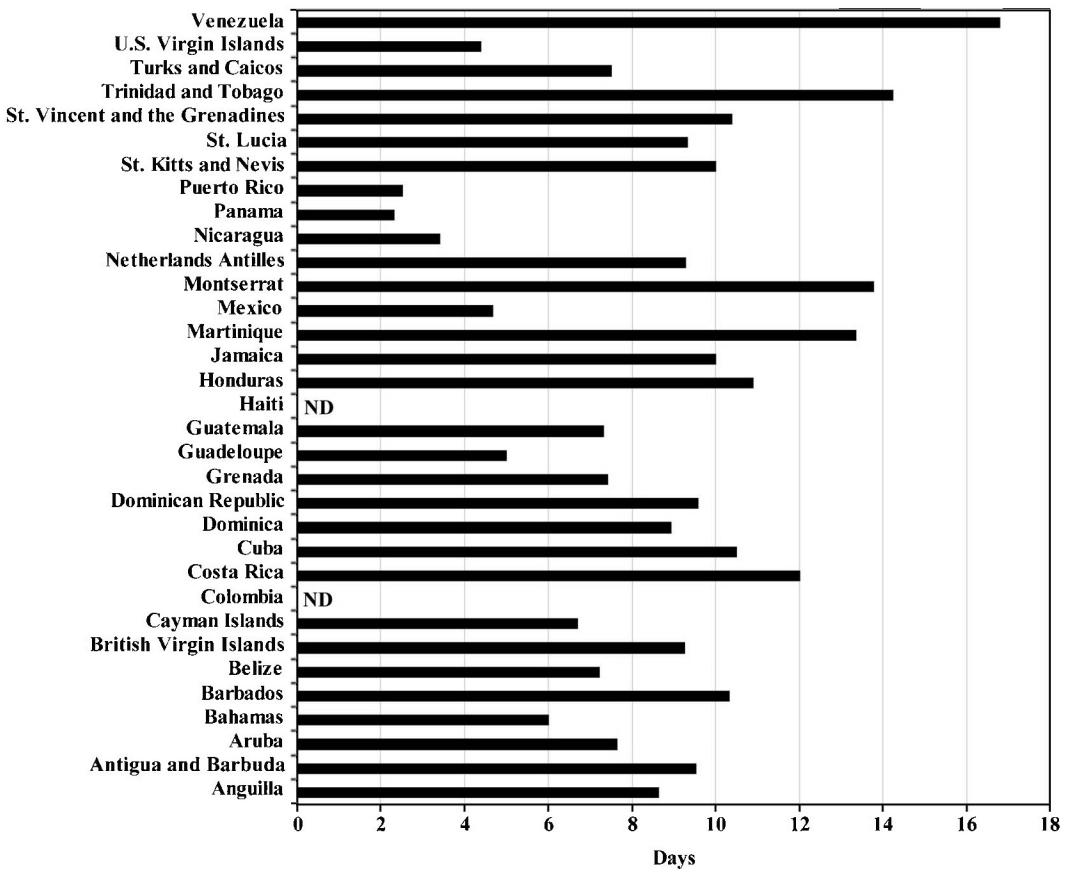

FIGURE 4. Average length of stay for all tourists visiting the Caribbean during 1996–2005, by country. Only data from Cancun and Cozumel were used for Mexico. The average length of stay for all tourists was 8.7 days (OAS, 1997; ACS-AEC, 2003; UNSD, 2004; CIA, 2008; CTO, 2008). ND = no data.

Our ultimate goal is a comprehensive assessment of the environmental, sociocultural, and economic impacts of CFP in the Caribbean and the development of effective detection and monitoring tools to support management decisions and improve inter-island communications among public health officials, marine resource managers, Caribbean residents, and tourists.

METHODS

Based on published cases and self-reporting, it appears that CFP is more prevalent in the eastern Caribbean than the western Caribbean (Stinn et al., 2000; see Figure 1B). To assess whether this is the case or whether the pattern derives from reporting bias, we used an active method to query public health officials and fisheries managers about the occurrence of CFP from 1996 through 2006 in 24 Caribbean island nations and territories and 9 mainland countries bordering the Caribbean. Fisheries and public health officials were contacted separately. One or both agencies could be involved in the surveillance of and response to CFP, although often within different administrative units. Querying two separate agencies was intended to allow corroboration of the data and to measure information-sharing between agencies. The questionnaires used in this study were vetted by a panel of experts with experience in designing human health surveys (see Acknowledgments).

Initial contact was made with public health and fisheries department staff persons by telephone. Introductory conversations were conducted in English, Spanish, or French, depending on the preference of the official

contacted. The following preliminary information and questions were provided during these telephone calls before sending the survey:

- The focus of the project is to gather information about where and how many people are poisoned by eating fish contaminated with ciguatera toxin (ciguatoxin) throughout the Caribbean, including in _____ *(name of country)*. People who eat fish carrying this toxin can develop ciguatera fish poisoning, an illness that affects primarily the digestive and nervous systems.
- Does your office compile information about fish that transmit ciguatera or cases of ciguatera fish poisoning in _____ *(name of country)*?
  (If No) Do you know of another office that does?
  (If Yes) What is the name of that office?
  (If No) Do you know anyone who might be able to help me locate an office that compiles information about ciguatera fish poisoning?

A long-term goal of the research project is to better understand where ciguatera fish poisoning occurs, which could improve the use of resources to monitor and respond to it. The results of our research throughout the Caribbean will be summarized in a report, documented in a database, and displayed on maps that will be available to you and others interested in the project. We will not be collecting names, addresses, or other personal information from people who have ciguatera fish poisoning.

- Are you the best person in your office to provide information about ciguatera in _____ *(name of country)*?
  (If referred to another person or agency) Do you have any contact information for the person you recommend I speak with?

We are asking for your voluntary assistance with our research.

- Would you be willing to answer a few questions?
  (If Yes) Thank you! I would greatly appreciate being able to e-mail you some specific questions I have. May I do so?
  (If No) Why not?

Once an appropriate contact was identified, a written copy of the questionnaire was provided in the appropriate language (or languages, as some participants received the questionnaire in both Spanish and English or French and English). Both questionnaires (the fisheries department version and the public health department version) included the 11 core questions listed in Appendix I, as well as 4 questions that applied to only the fisheries department (Appendix II) or the public health department (Appendix III). Efforts were made, in designing the questionnaire, to allow respondents to qualify how confident they were of the completeness of the data they were providing.

RESULTS AND DISCUSSION

To date, results are preliminary, as not all the questionnaires have been returned. However, some trends have begun to emerge, and it is possible to provide a brief synopsis of these. One of the most striking results was the wide range of concern and knowledge about CFP. Some government agencies have simply asserted that CFP cases do not occur in their jurisdictions and declined to receive or complete the questionnaire. Other agencies acknowledged that a potential problem exists but have been hampered by insufficient resources to institute an organized monitoring system. Still other governments reported making progress toward bringing CFP surveillance programs online, sometimes in response to a recent outbreak of CFP cases.

Some countries had a well-developed mandatory protocol for reporting CFP, including information on the name of the patients, symptoms, and diagnosis. In some instances, public health officials have a high degree of confidence that they are finding 90% or more of the cases, but most public health officials who have responded to date are less confident in their statistics. In some countries, when clusters of CFP cases are observed, the health department issues a press release. At the same time, the department may do a public service announcement for radio and TV about the risk of consuming barracuda.

A wide range of opinions were offered about how aware and concerned local populations and fishers are about the risk that eating certain types of locally caught fish could result in developing CFP. These responses ranged from "Not aware" or "Not concerned," to "Somewhat aware" or "Somewhat concerned," to "Very aware" or "Very concerned." One respondent commented that native-born citizens had a higher level of awareness and concern than people who recently moved to the region. Perceived levels of risk might depend more on being educated about the problem rather than an actual risk of exposure. At least in some regions where CFP is well known, most people seem to understand that if they feel tingling or prickling on their tongues when they are eating fish, they should stop eating it to minimize the risk of becoming sick.

In some countries, the data also suggest a trend toward increasing numbers of CFP cases with time. Public health officials on a few islands attributed this not to environmental change, but to population growth, in some cases as rapid as a doubling of the population in 20 years. As the population has grown, so too has the demand for fish, which could result in an increase in the number of people exposed to CFP. It is generally agreed that CFP is underreported and that this lack could be attributable to a variety of reasons (e.g., because its symptoms resemble those of other diseases when the poisoning is mild). This apparent increase may also be attributable to increased reporting because of heightened awareness, or it may reflect an actual increase in new cases of CFP.

Several public health departments have compiled and reported the months and years when people ate ciguatoxic fish and were diagnosed with CFP. From these limited data it appears that the number of CFP episodes was distributed evenly throughout the year but that the number of cases (people diagnosed with CFP) per episode was greater in September and October (Figure 5).

Overall, public health and fisheries officials indicated that consumption of contaminated barracuda was the most common cause of CFP. Other species frequently identified with CFP include jack, grouper, snapper, hogfish, and mackerel. Some fishermen discard barracuda that do not "put up a fight" when caught, believing that if a fish does not fight, it is sick. However, it should be noted that ciguatoxic status cannot be discerned visually; seemingly healthy fish can be quite toxic. One positive outcome of this research was that some countries provided data indicating geographic locations where ciguatoxic fish were frequently found. This information will guide future sampling efforts.

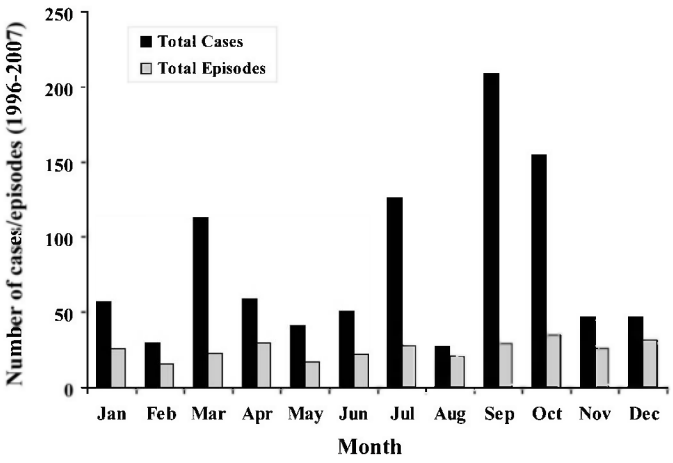

FIGURE 5. Ciguatera episodes and cases in the Caribbean by month from 1996 to 2007. Episodes indicate multiple cases (usually defined by zip code) during the same week.

CONCLUSIONS

The data currently available from Caribbean countries suggest there is wide variability in the amount of attention given to CFP. This variability is probably not entirely attributable to how prevalent CFP is in various areas. The reasons for this include differences in (1) how significant a problem CFP is thought to pose, (2) awareness of the risk of CFP, (3) whether central reporting of CFP cases is mandatory, and (4) resources available for CFP monitoring and education.

Active surveys, such as the one described in this study, can help countries quantify potential risks and establish training and monitoring systems for CFP. This study also provides unique insights into human dimensions of CFP, including perceptions of how significant the risks are in different areas and how frequently health and fisheries departments exchange information concerning CFP. The data from this study were also detailed enough, in some cases, to suggest specific regions in the Caribbean where CFP occurrences are elevated or are relatively rare. This information will facilitate identification of specific sampling sites for future investigations of the factors that affect the temporal and spatial variability in exposure to CFP. The fruitful partnership between the Smithsonian Institution and NOAA continues the Smithsonian's tradition of documenting the diversity of life on earth and NOAA's mission to bring state-of-the-art management tools to the marine community.

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APPENDIX 1

The following core questions were used in both fisheries and health department questionnaires:

1. a. What information does your office compile about cases of ciguatera fish poisoning (for example, number of people diagnosed, locations of people diagnosed, locations where fish were caught, etc.)?
   b. If your office does not compile such information, is there another governmental office or agency that does, and what is its name? ___ Yes ___ No ___ I'm not sure
   Name of other office or agency:
   c. If yes, what types of information do you think that office might have?
   d. Please provide contact information for someone in that office, if possible (contact name, e-mail address, phone number, and fax number).

2. If you receive reports of ciguatera fish poisoning, from whom do the reports come? (*Please check ALL that apply.*)
   ___ Doctors ___ Clinics and Hospitals ___ Fisheries Department [for the health department survey] ___ Fishermen ___ Health Department [for the fisheries department survey]/Other Health Agencies [for the health department survey] (please specify jurisdiction represented and contact person, if available) ___ Restaurants ___ Hotels ___ Individual Citizens ___ Other Sources (please list)

3. Please indicate the total number of reported ciguatera fish poisoning cases per calendar year from January 1, 1996, through December 31, 2006.
   [A table containing one line for each year, a column for the number of cases reported, and a column for any comments was provided here.]

4. To the extent available, please provide the following information for each episode of ciguatera fish poisoning. (For this study, an episode is defined as an occasion when one or more people were poisoned on the same day by one or multiple fish of the same variety, caught in the same place.)
   a. Number of people poisoned
   b. Date of episode (list season and year if date or month is not known)
   c. Date of diagnosis, if date of poisoning (B) is not known
   d. City where fish with ciguatera was eaten
   e. Home city of patient(s), if city where fish was eaten (D) is not known
   f. Type of fish with ciguatera (common name or scientific name)
   g. Describe where fish with ciguatera was caught, in as much detail as possible (with latitudes and longitudes, if available)

5. At this time, is reporting any information about fish transmitting ciguatera or cases of ciguatera fish poisoning voluntary or mandatory? ___ Voluntary ___ Mandatory ___ I'm not sure
   If reporting is mandatory:
   a. What information must be reported?
   b. When did it become mandatory?
   c. What agency receives these reports initially?

6. a. What percentage of ciguatera fish poisoning cases diagnosed each year in _____ (*name of country or territory*) do you think are reported to your office?
   b. How confident are you of this estimate? ___ Very confident ___ Somewhat confident ___ Slightly confident ___ Not at all confident ___ I'm not sure

continued

Appendix 1 continued

7. a. To your knowledge, has your agency or another governmental agency issued any advisory warnings related to consuming fish that might carry ciguatera, such as barracuda or large reef fish?
   ___ Yes ___ No ___ I'm not sure
   b. If yes, please indicate which office issued the advisory.
   c. If applicable, please include or attach the wording of each such advisory and indicate when it was issued. Attach additional pages, if necessary.
   d. If your agency has not issued an advisory, who or what agency would be most appropriate to consult for information on advisories? (Please list the agency name and the following, if available: a contact name, e-mail address, phone number, and fax number.)

8. How often do your department and fisheries department officials [for the health department survey]/health department officials [for the fisheries department survey] exchange information about episodes of ciguatera fish poisoning?
   ___ As cases occur ___ Every month ___ Every 3 months ___ Every 6 months ___ Every year ___ Never
   ___ Other (please specify):

9. How aware do you think local citizens are of the risk that eating certain types of fish could cause them to develop ciguatera fish poisoning? ___ Very aware ___ Somewhat aware ___ Not very aware ___ Not aware
   ___ I'm not sure

10. To what extent do you think local citizens are concerned about ciguatera fish poisoning?
    __ Very concerned __ Somewhat concerned __ Slightly concerned __ Not concerned __ I'm not sure

11. Please provide your contact information for future reference. Thanks again for your assistance!
    Government represented:
    Agency and office:
    Name and title of person completing questionnaire:
    Telephone number, with city code:
    Fax number, with city code:
    e-mail address:
    Date information provided:
    Would you like to receive notification of the results of the study? ___ Yes ___ No

APPENDIX 2

The following questions were directed only to officials representing fisheries departments:

1. a. Is information usually communicated to you about where fish suspected of carrying ciguatera were caught?
   ___ Yes ___ No
   b. Is information usually communicated to you about what types of fish have carried ciguatera?
   ___ Yes ___ No
   c. If yes to either (a) or (b), and if you do not have information in the format provided in Question 4, please provide any information you have about the types of fish, and the locations involved in episodes of ciguatera fish poisoning reported to you, for the years 1996 to 2006. [A table was provided with the following headings: Year, Common or scientific names of fish reported, Locations of fish reported (latitudes/longitudes, if possible, or place names, in as much detail as possible).]

2. Please provide any information you have on economic losses resulting from ciguatera fish poisoning, either quantitative or qualitative (for example, if fishing had ceased at a particular reef because of the suspected presence of ciguatera toxins, there might be an annual loss of $10,000 to the fishing industry). Please include the year(s) your data reflects and note your data sources.

3. To what extent do you think fishermen are aware of the risk of catching certain types of fish that could cause people to develop ciguatera fish poisoning? ___ Very aware ___ Somewhat aware ___ Not very aware ___ Not aware ___ I'm not sure

4. To what extent do you think fishermen are concerned about catching certain types of fish that could cause ciguatera fish poisoning? ___ Very concerned ___ Somewhat concerned ___ Slightly concerned ___ Not concerned ___ I'm not sure

APPENDIX 3

The following questions were directed only to officials representing health departments:

1. Is any information available to you on the cost per year to your government of monitoring or documenting the incidence of ciguatera fish poisoning? ___ Yes ___No
   If yes, please provide the information below and note your data sources.

2. Is any information available to you on the cost per year of medical treatments in _____ (*name of country or territory*) for ciguatera fish poisoning, as an average per person affected by ciguatera fish poisoning and/or annually for _____ (*name of country or territory*)? ___ Yes ___No
   If yes, please provide it below, specify whether it reflects a total or an average per person, and note your data sources.

3. Is any information available to you related to the number of days people have been unable to work due to ciguatera fish poisoning per year in _____ (*name of country or territory*)? ___ Yes ___No
   If yes, please provide it below, specify whether it reflects a total or an average per person, and note your data sources.

4. Would you rank ciguatera fish poisoning as one of the 10 most severe food-borne illnesses in _____ (*name of country or territory*)? ___ Yes ___No ___ I'm not sure
   If yes, would it rank in the top ___ 1 to 5 or ___ 6 to 10?

History of Reef Coral Assemblages on the Rhomboid Shoals of Belize

Richard B. Aronson, Ian G. Macintyre, Anke M. Moesinger, William F. Precht, and Michael R. Dardeau

ABSTRACT. Coral assemblages of the rhomboid shoals of the Belizean barrier reef have undergone dramatic, historically unprecedented changes over the past several decades. Before the late 1980s, the flanks of the shoals exhibited a distinct biological zonation, with branching *Porites* spp. dominant in a shallow zone (0–3 m water depth); the staghorn coral *Acropora cervicornis* dominant in an intermediate zone (3–15 m depth); and large, plating agariciids and the lettuce coral *Agaricia tenuifolia* dominant in a deep zone (15–30 m depth). *Acropora cervicornis* died off catastrophically from white-band disease after 1986 and was replaced by *Agaricia tenuifolia* in the intermediate zone. Push-cores extracted from intermediate depths in previous studies showed that *Acropora cervicornis* was the dominant space occupant and primary framework builder for millennia before the phase shift to *Agaricia tenuifolia*. Cores extracted from the shallow zone showed that *Acropora cervicornis* dominated until several centuries ago, when the tops of the reefs reached approximately 2 m water depth and branching *Porites* spp. replaced it. In contrast, three cores extracted from the deep zone in the present study showed that for millennia the subsurface coral assemblage, like the assemblage on the modern deep-reef surface, was dominated by large, plating agariciids and *Agaricia tenuifolia*. Because white-band disease only affects acroporid corals, the unprecedented phase shift that followed the outbreak was confined to the intermediate zone. High sea temperatures in the summer of 1998 caused coral bleaching and mortality, especially of agariciids in the intermediate and deep zones, but to date this event has not left a geologic signature in the Holocene record.

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INTRODUCTION

Coral reef ecosystems are collapsing at an accelerating rate, jeopardizing the ecosystem services that they provide (Hughes et al., 2003; Wilkinson, 2006; Carpenter et al., 2008). The common presumption that mortality of hard corals (Scleractinia and Milleporina) commenced earlier and was more severe in the Caribbean and eastern Pacific than in other tropical and subtropical regions may not be correct (Bruno and Selig, 2007). Nevertheless, the causes and consequences are best understood for the Caribbean and eastern Pacific.

Coral mortality has been elevated in the Caribbean since the late 1970s (Gardner et al., 2003). The impacts of global change, including increasing sea temperatures, increasing cyclone intensity, and declining aragonite saturation

state (Kleypas et al., 1999; Buddemeier et al., 2004; Hoegh-Guldberg et al., 2007), are sources of grave concern, but coral assemblages throughout the Caribbean have already been severely affected by outbreaks of infectious marine diseases (Aronson and Precht, 2001b; Sutherland et al., 2004; Weil et al., 2006). In particular, white-band disease (WBD), a bacterial infection that is specific to acroporid corals, decimated *Acropora palmata* (elkhorn coral) and *Acropora cervicornis* (staghorn coral) on reefs throughout the western Atlantic from the late 1970s through the early 1990s (Aronson and Precht, 2001a, 2001b). Acroporid populations have been reduced so drastically that the two species are now listed as threatened under the U.S. Endangered Species Act (Hogarth, 2006) and are classified as critically endangered according to the Red List criteria of IUCN, the International Union for Conservation of Nature (Carpenter et al., 2008). Hurricanes, temperature-induced bleaching, declining herbivory, nutrient loading, and predation by corallivores have had additional, interacting impacts on coral mortality and the scope for population recovery (Aronson and Precht, 2006). Emergent diseases, for example, could be related to or exacerbated by global warming and nutrient loading (Harvell et al., 2002; Rosenberg and Ben-Haim, 2002; Bruno et al., 2003, 2007; Sutherland et al., 2004; Kline et al., 2006). Recent changes on Caribbean reefs were novel events in at least the last 3,000 to 4,000 years (Aronson et al., 2002a, 2004, 2005a; Wapnick et al., 2004; Hubbard et al., 2005; Greer et al., 2009), and Pandolfi et al. (2006) drew a similar conclusion about Holocene reef dynamics in Papua New Guinea.

Aronson and Precht (2001a, 2001b, 2006; Precht and Aronson, 2006) argued that because WBD was the primary cause of recent mortality of *Acropora palmata* and *Acropora cervicornis* in the Caribbean, and because the two species were the dominant space occupants at depth ranges of 0–5 and 5–25 m, WBD was clearly one of the most important causes of recent coral mortality in the region. Mass mortality of the acroporids was followed by two types of phase shifts. Where coral mortality exceeded the capacity of herbivores to respond to algal growth on the space that had been opened, macroalgae rose to dominance (Ostrander et al., 2000; Aronson and Precht, 2001a, 2006; Williams et al., 2001; Rogers and Miller, 2006). Where herbivory was sufficient to control the algae, brooding, self-fertilizing corals, primarily of the families Agariciidae and Poritidae, replaced the acroporids (Aronson and Precht, 1997; Greenstein et al., 1998; Bythell et al., 2000; Knowlton, 2001; Green et al., 2008). The shift to macroalgal dominance has not been as widespread as previously supposed (Bruno et al., 2009).

An important exception to the overall Caribbean trend is the Flower Garden Banks (FGB) in the northwestern Gulf of Mexico, where coral cover has held steady at 40%–60% at depths of 17–26 m from the 1970s to the present. Aronson et al. (2005c) explained the persistently high coverage of living corals based on the historical absence of the cold-sensitive acroporids. Coral mortality has been far lower at the FGB than elsewhere in the Western Atlantic region because no acroporids were present to die of WBD. The appearance of *Acropora palmata* at the FGB in the past few years could be related to global warming (Precht and Aronson, 2004).

An ecosystem-level version of this biogeographic argument is that reef zones historically not dominated by acroporids should not have undergone phase shifts at the same time as the adjacent *Acropora*-dominated zones. In this study we examined the millennial-scale history of the coral assemblage near the bases of the rhomboid shoals in the central shelf lagoon of the Belizean barrier reef. We cored the deep-reef framework of two of the shoals, reconstructed the history of the coral assemblage during the late Holocene, and completed a model of reef development over the last several thousand years from present sea level down to the bases of the shoals. Although *Acropora cervicornis* dominated at intermediate depths for millennia until the late 1980s, acroporids apparently did not dominate the deep zone for at least the past 1,500 to 2,000 years, providing an opportunity to test our hypothesis of the occurrence and timing of phase shifts.

ZONATION AND PALEOECOLOGY OF THE RHOMBOID SHOALS

The rhomboid shoals are uncemented, atoll-like reefs lying within the central shelf lagoon of the Belizean barrier reef. The sloping outer flanks of the rhomboid shoals displayed a clear pattern of coral zonation from at least as far back as the early 1970s, when the first rigorous ecological observations were made, until 1986 (Westphall, 1986; Aronson and Precht, 1997; Aronson et al., 1998). A shallow zone (0–3 m water depth) was dominated by branching *Porites* spp., primarily *Porites furcata* and *Porites divaricata*, mixed with the hydrocoral *Millepora alcicornis*. *Acropora cervicornis* dominated an intermediate-depth zone (3–15 m depth), with the blade-forming lettuce coral *Agaricia tenuifolia* as the subdominant. (*Agaricia tenuifolia* recently has been revised to *Undaria tenuifolia*; however, we will retain *Agaricia* as the generic designation in this paper.) A deep zone, extending from 15 m to the lagoon floor at 22–30 m

depth, was dominated by large colonies of plating agariciids (*Agaricia lamarcki*, *Agaricia grahamae*, *Agaricia undata*, and *Leptoseris cucullata*) and *Agaricia tenuifolia*, with scattered massive corals. The total hard-coral fauna consisted of approximately 25 species, most of which were rare (Aronson and Precht, 1997).

In the decade following 1986, the dominant coral at intermediate depths, *Acropora cervicornis*, succumbed to WBD and was replaced by *Agaricia tenuifolia*. This phase shift was mediated by an abundant, herbivorous sea urchin, *Echinometra viridis*, which limited macroalgal growth and promoted the recruitment and opportunistic growth of agariciids on the dead skeletons of *Acropora cervicornis* (Aronson and Precht, 1997). *Agaricia tenuifolia* was the fastest growing of the agariciids that recruited and, therefore, it became the new dominant.

To determine whether the transition was historically unique, Aronson et al. (2002a) extracted push-cores at 5–10 m water depth from stations distributed over a 375 km^2 area of the lagoon (Figure 1). Analysis and radiocarbon dating of the cores revealed continuous dominance of *Acropora cervicornis* and upward growth of the reef for at least 3,000 years before the late 1980s. Spines of *Echinometra viridis* were present throughout the cores, indicating continuously high herbivory. During the past three millennia *Agaricia tenuifolia* grew in small patches (of the order of square meters), which appeared as subsurface layers of skeletal plates that were isolated in time and space (Aronson et al., 2002a). The recent, area-wide phase shift, in contrast, was preserved at the tops of the cores as a layer of *Agaricia tenuifolia* plates overlying a thin layer of taphonomically degraded *Acropora cervicornis*. This signature persisted in the Holocene record despite subsequent hurricanes and bleaching events (Aronson et al., 2000, 2002b, 2005b). Coring in a lagoonal habitat at Discovery Bay, Jamaica, showed that a more common phase shift, in which *Acropora cervicornis* was killed by WBD and replaced by macroalgae as the result of limited herbivory, was similarly unprecedented on a millennial time scale (Wapnick et al., 2004).

Cores extracted from the rims and ridges of the shoals near the present sea level revealed that *Acropora cervicornis* dominated the shallowest portions of these reefs for at least several millennia until approximately 500 years ago (Westphall, 1986; Aronson et al., 1998, 2005a; Macintyre et al., 2000). At that time the reef tops grew to within 2 m of sea level, and branching *Porites* spp. replaced *Acropora cervicornis* as the dominant coral taxon. Since then, the *Porites*-dominated assemblage has kept up with the slowly rising sea level, forming the shallow zone. The shallowing-upward, successional sequence in the shallow zone contrasts with the post-1986, disease-induced replacement of *Acropora cervicornis* by *Agaricia tenuifolia* at intermediate depths.

As part of the worldwide reef-bleaching event of 1997–1998 (Wilkinson, 2000), which was related to the El Niño–Southern Oscillation and probably augmented by global warming, a high-temperature anomaly in the summer of 1998 bleached almost all corals in the intermediate and deep zones of the rhomboid shoals (Aronson et al., 2000, 2002b). *Agaricia tenuifolia* is particularly prone to temperature-induced bleaching (Robbart et al., 2004), and populations of this coral at intermediate and deeper depths experienced nearly complete mortality. Mortality rates were lower, but still very high, for plating agariciids. The dead coral skeletons were colonized primarily by thin algal turfs and the sponge *Chondrilla* aff. *nucula* (Aronson et al., 2002b), which Rützler et al. (2007) have now described as *Chondrilla caribensis*. Agariciid populations had not recovered as of December 2008 (W. F. Precht, personal observation). Branching *Porites* corals in the shallow zone were less affected by the 1998 thermal anomaly. These corals did not bleach to the extent the agariciids did, and as a result they did not experience large-scale mortality (W. F. Precht and R. B. Aronson, personal observation).

MATERIALS AND METHODS

In April 2008, we extracted six push-cores in water depths of 14.0–19.5 m from the reefs at Channel and Elbow Cays, in the center of our 375 km^2 study area (see Figure 1). Push-coring requires less equipment than mechanical techniques such as rotary drilling and percussion vibracoring. By eliminating the need for tripods and other heavy equipment, push-coring offers easier logistics, greater mobility, and a much lower cost per core. Penetration and recovery of cores dominated by branching and foliose corals have been excellent in the shallow and intermediate-depth zones of the rhomboid shoals, as well as on uncemented lagoonal reefs in Panama and Jamaica (Dardeau et al., 2000; Aronson et al., 2004; Wapnick et al., 2004). Rotary drilling is not an option because branching and foliose corals generally are broken up and flushed out of the core barrel. As a result, recoveries are poor in lagoonal and fore-reef environments dominated by fragile corals (Glynn and Macintyre, 1977; Halley et al., 1977; Macintyre et al., 1981; Shinn et al., 1982).

Dardeau et al. (2000) described the push-coring method in detail. Briefly, aluminum tubes, 5 m long and

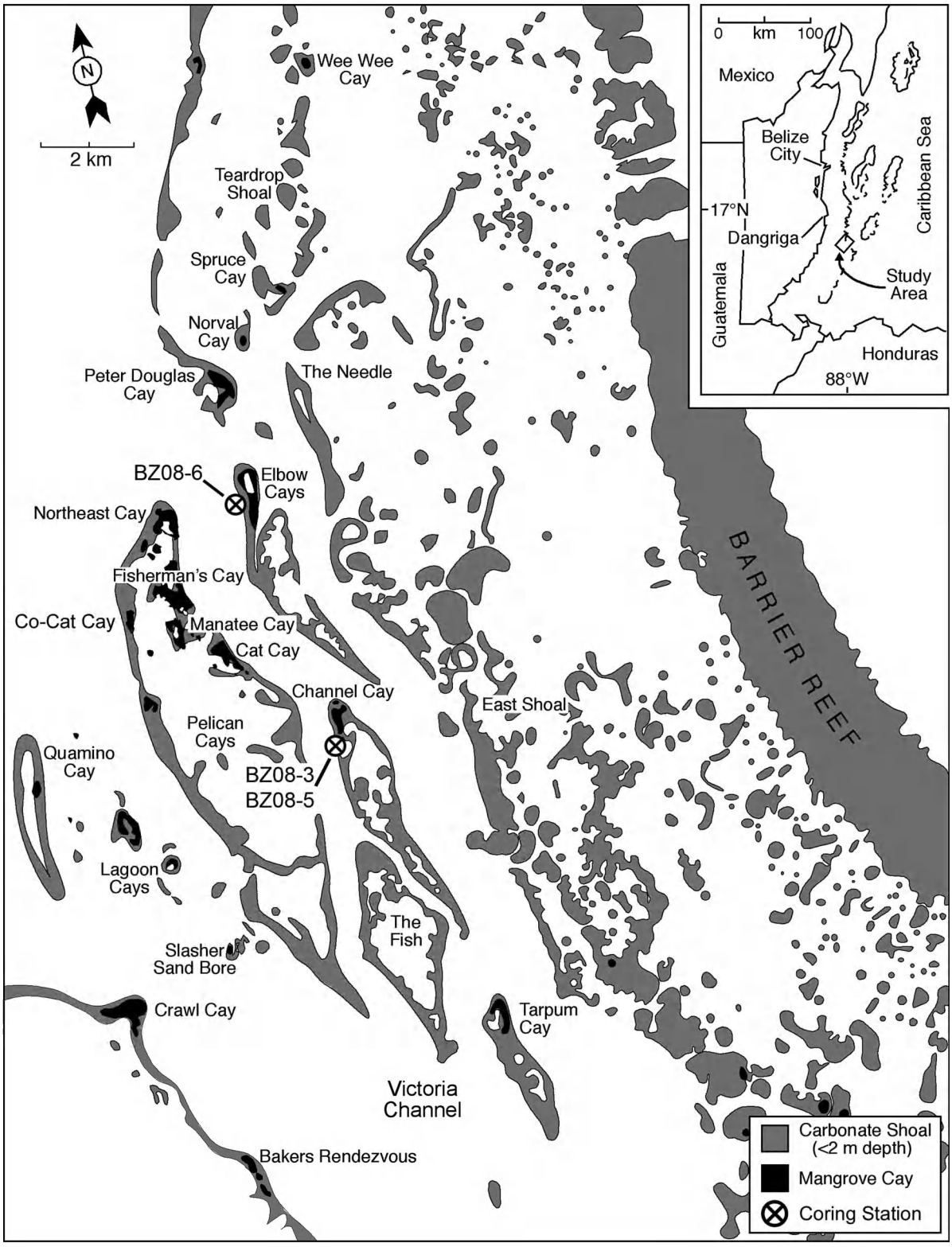

FIGURE 1. Map of the central shelf lagoon of the Belizean barrier reef, showing the rhomboid shoals and the locations of the two coring stations. Three of the six cores extracted were analyzed for this study, as noted on the map. (Modified from Aronson et al., 2002a.)

7.6 cm (3 in.) in diameter with teeth cut into their leading ends, were driven by hand into the uncemented reef framework. The tubes were rotated in using adjustable core slips with handles and tapped with a sliding hammer-weight, sleeved over the top, to aid in penetration. The tubes cut through, penetrated, and captured the loose framework of branching and foliose coral skeletons, and they cored through most massive coral heads as well. Although head corals are rare in the subsurface at shallow and intermediate depths, they are more common in the deep zone. The cores were sealed with plastic caps and electrical tape, extracted from the reef, and transported to the laboratory for analysis.

Estimates of recovery were obtained at intervals during the coring process by dropping a weighted fiberglass measuring tape down the open core barrel. In previous studies these measurements, along with simultaneous measurements of penetration, confirmed that material entered the tubes continuously as they were forced into the reef. In some cases in the present study the tube cored through a massive coral and was plugged by it, preventing further recovery as the tube was forced deeper into the uncemented framework. We used the penetration depth at the point at which the tube was plugged to calculate percent recovery. Comparison of final recoveries measured before extraction with recoveries measured after extrusion in the laboratory showed that little or no material was lost from the bottoms of the tubes during extraction. Of course no material was lost from cores that were plugged at their bases by massive corals.

There were no indications of significant voids in the reef framework. In no case did the tube suddenly drop vertically while we were driving it into the reef. We also saw no reversals in the in situ estimates of recovery, which would have indicated episodic compaction during coring.

Three of the extruded cores were analyzed at intervals of 5 cm. The constituents of each interval retained on a 5 mm sieve were cleaned of matrix, sorted to species, dried to a constant mass, and weighed to the nearest milligram. In earlier studies, we showed from regression analysis that, for the coral constituents, log(mass) was a strong predictor of log(volume), as measured by water displacement.

In the manner described previously by Wapnick et al. (2004), we assessed the degree of taphonomic degradation of the *Acropora cervicornis* material—encrustation, surficial erosion, and internal boring—using a modified version of the rank scales of Greenstein and Moffat (1996). The average taphonomic condition of each coral fragment was rated as good, intermediate, or poor. The good rating was applied to fresh-looking pieces that had little or no encrustation, retained essentially all their surface sculpture, and showed little to no evidence of internal boring. Poor fragments were those with extensive encrustation, surficial erosion, and/or boring; degradation was extensive enough that the structure of the corallites was completely obscured. Fragments were rated as intermediate if their condition, averaged over the three categories, fell between good and poor. A coral taxon, or a taxon in a particular taphonomic condition, was considered dominant in a 5 cm interval if its mass exceeded the mass of each of the other taxa/conditions in that interval.

A coral sample from the bottom of each core was radiocarbon dated by Beta Analytic, Inc. (Miami, Florida), using standard techniques. Measured dates were corrected for isotopic fractionation to generate conventional dates, which are expressed as radiocarbon years before 1950 (^{14}C year). Conventional dates were calibrated to calendar years before 1950 (CalBP).

RESULTS

The cores captured the framework of loose coral skeletons surrounded by a light gray watery matrix of sandy mud. The matrix was almost entirely carbonate, with only a trace of noncarbonate material. It was less compact than the matrix in cores collected from the shallow and intermediate zones (Aronson et al., 1998; Macintyre et al., 2000; Aronson et al., 2002a). X-ray diffraction analysis of sediment samples revealed a notable lack of high magnesium calcite in the sand and silt fractions. The majority of high magnesium calcite was found in the clay fraction, corroborating our earlier conclusion of active precipitation of micritic high magnesium calcite without significant cementation (Macintyre and Aronson, 2006). Spines of *Echinometra viridis* in the matrix indicated that those herbivores were present during the time interval represented by the cores.

Three of the six cores we collected provided penetrations, recoveries, and bottom dates sufficient to analyze temporal trends in the coral assemblage of the deep zone (Table 1). Cores BZ08-3 and BZ08-5 from Channel Cay were both plugged by heads of *Porites astreoides* at penetration depths of 2–3 m. Core BZ08-6 from Elbow Cays penetrated nearly 3.5 m. Bottom samples consisting of *Porites astreoides* from the bases of cores BZ08-3 and BZ08-5, and plating *Agaricia* from the base of BZ08-6, were radiocarbon dated. The remaining three cores yielded recoveries of 65 cm or less and were not analyzed.

TABLE 1. Summary statistics for the three cores.

Core	Water depth (m)	Site	Penetration (cm)	Recovery (cm)	Percent recovery	Basal radiocarbon dates [a]	
						Conventional date (^{14}C year ± SE) [b]	Calibrated date (CalBP) [c]
BZ08-3	14.5	Channel	256	81	31.6%	2,730 ± 50	2,420 (1,860–1,560)
BZ08-5	16.2	Channel	216	78	36.1%	2,130 ± 60	1,710 (2,650–2,320)
BZ08-6	15.3	Elbow	347	109	31.4%	1,290 ± 60	840 (940–700)

[a] Radiocarbon dates are of coral samples from the bases of the cores.
[b] Conventional dates are measured dates corrected for isotopic fractionation, expressed as radiocarbon years before 1950 (^{14}C year) and accompanied by standard errors (SE).
[c] Calibrated dates (CalBP) are expressed as calendar years before 1950, with 95% confidence intervals in parentheses.

The mean recovery for the three cores analyzed was 33.0% of penetration depth (±1.53 SD). This figure is slightly lower than the mean of 35.9% obtained for cores from intermediate depths on the rhomboid shoals and considerably lower than the mean of 62.3% for cores from intermediate depths in Bahía Almirante, a coastal lagoon in Panama (Dardeau et al., 2000). The low recoveries in the present study probably reflect the open reef framework of the rhomboid shoals (compared to Bahía Almirante), combined with the low sediment content of the matrix in the deep zone (compared to intermediate depths on the rhomboid shoals).

All three cores were dominated by agariciid corals (Figure 2). These corals were primarily large, plating forms, which characterized the living community until 1998 and that now characterize the modern, postbleaching death assemblage in the deep zone. *Agaricia tenuifolia* was more common near the tops of cores BZ08-5 and BZ08-6 than lower in those cores. The agariciids were in mixed taphonomic condition, with most intervals containing both intermediate and poor material. The skeletons from the top 20 cm of the cores were in neither better nor worse condition than those further down.

Slope angles in the vicinity of the coring sites, measured with an inclinometer (Aronson et al., 2002a), were 36°–39°. Those slopes were less steep than the critical angle of 45°, above which *Agaricia tenuifolia* skeletons are transported downslope (Aronson et al., 2002a). The critical angle of 45° probably applies to the dead, fragmented skeletons of plating agariciids as well.

Core BZ08-3 contained a layer of *Acropora cervicornis* branch fragments in poor taphonomic condition. This layer could have been the result of downward transport from intermediate depths. On the other hand, *Acropora cervicornis* is less sensitive to slope angle than *Agaricia tenuifolia* (Aronson et al., 2002b), so an autochthonous layer cannot be ruled out. Other coral taxa, including branching *Porites* spp. and *Porites astreoides*, *Montastraea annularis* species complex, *Colpophyllia natans*, *Madracis auretenra* (formerly *Madracis mirabilis*; Locke

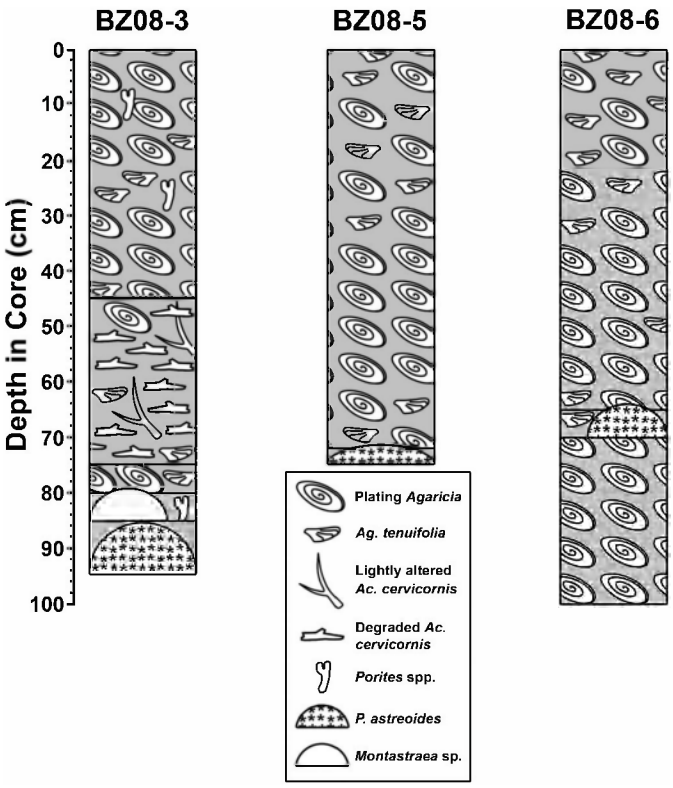

FIGURE 2. Schematic diagrams of the three extruded cores. The lengths of the cores depart slightly from recoveries estimated in the field (Table 1). Gray shading indicates a matrix of watery, sandy mud; *Ag.* = *Agaricia*; *Ac.* = *Acropora*; *P.* = *Porites*.

et al., 2007), and *Stephanocoenia intersepta*, were rare in the cores. None of the cores recorded millennial-scale intervals of actively accreting *Acropora cervicornis* framework, which were represented in the intermediate-zone cores by thick accumulations of *Acropora cervicornis* in good taphonomic condition.

DISCUSSION

Shinn et al. (1979; see also Westphall, 1986) extracted cores from the flanks of the Channel Cay shoal, including one from the base of the reef near our coring station (see Figure 1). Their general statement, that the cores were dominated by *Acropora cervicornis* with agariciids as the subdominants, did not draw distinctions between cores extracted from the different zones. We found that agariciids were the dominant framework constituents in the deep zone.

Core BZ08-3, which contained a layer of taphonomically degraded *Acropora cervicornis* underlying a thick uppermost layer of agariciids, could represent a deepening-upward sequence. This scenario seems unlikely, however, considering that sea level has risen only approximately 2 m during the past 3,000 years (Toscano and Macintyre, 2003). Furthermore, the other two cores showed no such *Acropora cervicornis*-dominated layer. Regardless, none of the three cores suggests a recent transition from millennia of fast-growing and rapidly accumulating *Acropora cervicornis* framework to dominance by agariciid corals, as was observed in the cores from intermediate depths. The layer of *Acropora cervicornis* in BZ08-3 is more likely derived from material that was transported downslope, forming debris fans at the bases of the shoals.

Aronson et al. (2005a) compared late Holocene reef development between the rhomboid shoals and the uncemented reefs of Bahía Almirante in Panama. The shallow and intermediate zones had been cored extensively in both locations, providing an accurate picture of stasis and change in the dominant coral taxa. In both cases, however, the deep zones were poorly characterized. The dearth of push-cores from the bases of the reefs has been primarily a consequence of the greater densities of core-occluding massive corals in the subsurface, compared to the subsurface of the shallow and intermediate zones.

The cores analyzed in this study allow us to present a more comprehensive model of the history of the coral assemblages of the rhomboid shoals (Figure 3). In the shallow zone, catch-up dynamics gave way to keep-up

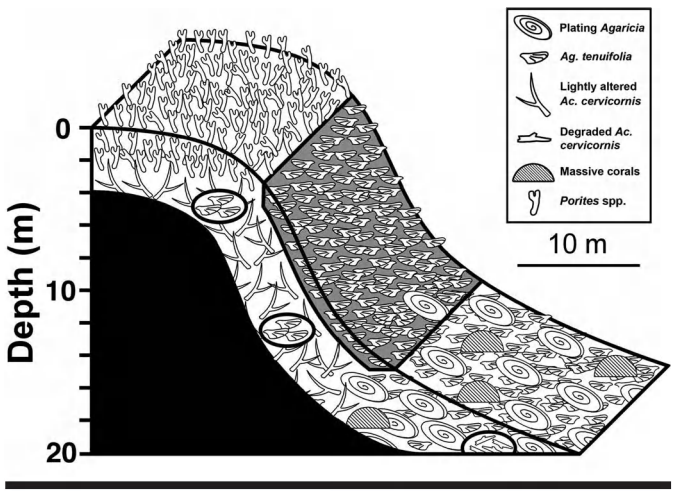

FIGURE 3. Model of reef development on the rhomboid shoals of Belize over the last several thousand years. Gray shading indicates that the coral assemblages at intermediate water depths experienced a recent transition. Black fill represents earlier Holocene and antecedent Pleistocene reef framework at depths not penetrated by the cores. Horizontally oriented, subsurface ellipses indicate spatially isolated layers of *Agaricia tenuifolia* and taphonomically degraded *Acropora cervicornis*. (Modified from Aronson et al., 2005a.)

dynamics: the *Acropora cervicornis* that had dominated for millennia during the catch-up phase was replaced centuries ago by branching *Porites* spp. during the keep-up phase. *Acropora cervicornis* was also dominant for millennia at intermediate depths, but in the late 1980s it was nearly extirpated by white-band disease and then replaced by *Agaricia tenuifolia*. The deep zone, in contrast, appears to have been dominated by agariciids for at least 1,500 to 2,000 years. No recent transitions were evident in the deep zone, a result consistent with the hypothesis that such shifts were predicated on the prior dominance and subsequent mortality of acroporids.

Thus, only the intermediate zone was affected when *Acropora cervicornis* died off regionally in the late 1980s to the early 1990s. The subsequent bleaching event in 1998 killed most of the agariciids on the rhomboid shoals. Cores extracted from the intermediate zone in 2004 did not display a taphonomic signature of that mass mortality event, which would have appeared as a discrete, uppermost layer of taphonomically degraded agariciid skeletons (Aronson et al., 2005b). Similarly, because of the mixed taphonomic character of the subfossil agariciid material in the deep zone, the expected signature of the 1998 event had not been observed in the Holocene record of that zone as of April 2008.

Acknowledgments

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Climate and Hydrological Factors Affecting Variation in Chlorophyll Concentration and Water Clarity in the Bahia Almirante, Panama

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ABSTRACT. Water clarity and productivity are fundamentally important for the distribution of tropical marine organisms. In the Caribbean, changes in nutrient loading that result from rapid development are thought to have caused increased planktonic productivity, reduced water clarity, and reduced reef and seagrass health. Here we analyze chlorophyll *a* concentration and water clarity from eight years of environmental monitoring in Bocas del Toro, Panama. Chlorophyll *a* concentrations did not vary significantly among the six sampled sites and showed no significant temporal changes, despite the recent rapid development in the region, accompanied by scant wastewater treatment. In contrast, water clarity increased significantly during the study period. Because chlorophyll *a* does not vary closely with water clarity, Secchi depths are likely to reflect changes in suspended particulate matter rather than in phytoplankton biomass. Secchi depths decreased with rainfall and wind speed but increased with solar radiation, supporting the idea that clarity was not tightly linked to phytoplankton biomass. The decrease in annual rainfall, but not wind speed, over the past eight years suggests that the long-term trend in Secchi readings is the result of changes in rainfall patterns.

INTRODUCTION

Water clarity and productivity are fundamentally important to the distribution of tropical marine organisms, especially corals. Ocean primary productivity is also important for global geochemistry and carbon sequestration (Falkowski et al., 1998). Global warming and increase in atmospheric CO_2 are expected to influence the distribution of the biota, as well as its abundance, and the photosynthetic activity of phytoplankton (Falkowski et al., 1998). SeaWiFS satellite imagery shows that worldwide oceanic chlorophyll *a* concentrations are about 0.2 mg/m^3 (Yoder et al., 1993) and can reach 5 mg/m^3 in coastal upwelling zones (Falkowski et al., 1991; Walsh et al., 1978). It is difficult to use this method to obtain information on chlorophyll *a* concentrations for many onshore tropical areas because accurate remote sensing is difficult in coastal areas with large sediment input and because many tropical regions have high frequencies of cloud cover. In such areas field measurements of water clarity and chlorophyll *a* concentrations are vital for assessing short-term variation and ground-truthing remote measurements.

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Coral reef environments are particularly sensitive to changes in water quality, especially changes in nutrients, sediment load, and productivity. The paradigm of coral reef biology is that reef development and coral health are greatest in areas with low sedimentation, low primary productivity, low abundances of zooplankton, and high water clarity. These habitats are most conspicuous in the Indo-Pacific and the offshore islands in the Caribbean. In many locations these habitats are suffering from reduction of water quality associated with coastal development (Bell, 1992; Lapointe, 1992). In the Caribbean, most studies of reefs and their waters are conducted in the Bahamas, Puerto Rico, Netherlands Antilles, and other offshore islands (Gilbes et al., 1996; Otero and Carbery, 2005; van Duyl et al., 2002; Webber et al., 2003). In addition there have been some studies of the unusual upwelling sites along the coast of Venezuela and Colombia (Franco-Herrera et al., 2006) and the strongly freshwater-influenced regions around the Yucatan (Herrera-Silveira et al., 2002). However, few studies have examined heavily terrestrially influenced systems without these unusual features in the Caribbean. Here we report the results of eight years of physical climatic and water quality monitoring in Bahia Almirante, an enclosed Caribbean archipelago that is highly terrestrially influenced.

STUDY LOCATION: BOCAS DEL TORO, PANAMA

Three bodies of water surround the Bocas del Toro Archipelago on the Caribbean coast of Panama: the Bahia Almirante and the Laguna de Chiriquí on the landward side, and the Caribbean Sea on the exposed coastal side (Figure 1). The mainland surrounding the region is largely forested, although the completion of a road linking Costa Rica to Bocas del Toro and the rest of Panama in the year

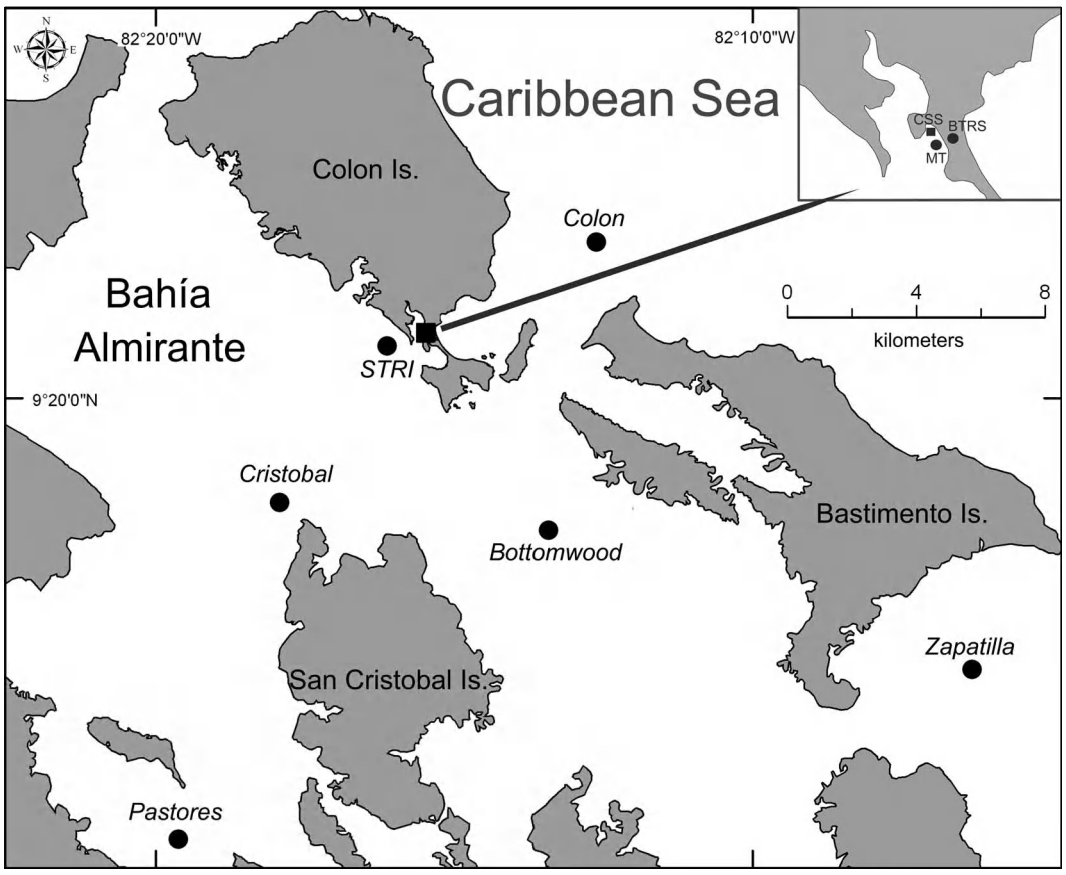

FIGURE 1. Map of the Bahia Almirante region with sampling sites indicated. BTRS = Bocas del Toro Research Station; CSS = CARICOMP seagrass site; MT = instrument platform; STRI = Smithsonian Tropical Research Institute.

2000 has resulted in increased lowland deforestation, as well as land development for small farms and tourism. The landward sides of the islands are fringed with largely intact red mangrove forests, although these are also being cleared from the landward side. The Laguna de Chiriquí, which receives twice the freshwater runoff of Bahia Almirante, has higher nutrient loads and limited coral reef development (D'Croz et al., 2005) and is not discussed further here.

The Bahia Almirante has significantly more oceanic influence than the Laguna de Chiriqui and supports well-developed coral reefs. It receives runoff from only two rivers of any note. High rainfall (about 3 m/year) and runoff from the San San Pond Sac peat swamp forest often result in pronounced haloclines with low-salinity waters (which are often cold) overlying full-salinity bottom waters (Kaufmann and Thompson, 2005). Surface salinities are generally 30–34 PSS (practical salinity scale) but can drop as low as 20 PSS after heavy rains (Kaufmann and Thompson, 2005). Other conditions are relatively aseasonal, with mean sea surface temperatures varying from 27.5°C (January–February) to 29.7°C (September–October). Average wind speed is about 7 km/h but occasionally exceeds 20 km/h (Kaufmann and Thompson, 2005). Despite the high levels of runoff, N:P was always recorded to be below Redfield ratios in a previous survey of the area (D'Croz et al., 2005). This observation suggests that primary productivity could be nutrient limited and that inputs from freshwater runoff or wind mixing could fertilize primary productivity in the Bahia Almirante.

The Smithsonian Tropical Research Institute's Bocas del Toro Research Station is on the Bahia Almirante side of Isla Colon. As part of the development of the scientific knowledge base of the station, and as part of the CARICOMP program, various physical and biological features of the surrounding environment have been monitored since 1999. Here we examine these data (1) to develop a baseline to which future studies can be compared, (2) to determine if the recent rapid development of the region has had an effect on water clarity and phytoplankton biomass, and (3) to explore the physical data to understand what factors influence the variation in these parameters.

MATERIALS AND METHODS

Monitoring History

Isla Colon is the site of the Smithsonian Tropical Research Institute's Bocas del Toro Research Station. At its inception in 1998 a long-term physical and biological monitoring program was initiated. Physical records of air and water temperature, rainfall, salinity, solar radiation, and wind speed have been kept since 1999 (reviewed in Kaufmann and Thompson, 2005). Monitoring of Secchi depths and chlorophyll *a* concentrations was conducted approximately biweekly at five sites (see Figure 1) from 1999 until 2001. The sampling intervals were not equal (ranging from 7 to 28 days), so these data were not appropriate for time-series analyses.

The Secchi depths and chlorophyll *a* monitoring was reinitiated at three of these sites and at an additional site in 2006 and continues to be measured weekly. At one of these, the CARICOMP reef monitoring site (described in Guzmán et al., 2005), the Secchi depths have been recorded weekly since 2000. At the CARICOMP seagrass site, horizontal Secchi readings have been taken weekly since 1999. During the entire period, measurements were made by the same three-person team.

Sampling Localities

Sampling sites (Figure 1; Table 1) were chosen in 1999 to include a range of environments. In 2006 sites were chosen to include an onshore–offshore gradient, in which we expected more oceanic conditions on one end and terrestrially influenced conditions on the other end.

- Colon, 6.3 km northeast of Bocas del Toro Town, is the most exposed site. The bottom at 20.5 m is muddy. Rough conditions occasionally made it impossible to take measurements in this location.
- Cristobal, in the middle of the Almirante Bay, is a site 7 km from the mainland and surrounded by patch reefs. The bottom at 25 m is muddy.
- Pastores is a semienclosed bay, 500 m from the mainland. It is more heavily influenced by continental runoff and creek discharge than the other sites. Depth at the sampling site is 26 m but a nearby coral reef slopes from 5 to 16 m. Jellyfishes are abundant at this site.
- Smithsonian Tropical Research Institute (STRI) is the site closest to the Bocas del Toro Research Station, 500 m from the shore. This site serves as the water monitoring site for the CARICOMP reef site, which is onshore of this location, over a reef that slopes from 5 to 20 m. The bottom is muddy and sandy with isolated patches of coral.
- Bottomwood, between Solarte and San Cristobal Islands, is protected from oceanic influence. The sampling site is near mangrove islets, sand cays, and a shallow coral reef. The reef slopes to a fine sand bottom at 16 m.

- Zapatillas has the highest diversity and abundance of coral and octocoral species of any of our sampling sites. The bottom at about 15 m is mostly covered by patch reefs and fine sand.
- The CARICOMP seagrass site is several hundred meters along the shore to the northwest of the Bocas del Toro Research Station. This shallow (2 m depth) location has extensive *Thalassia* cover, and the small bay is fringed by red mangroves.

Hydrological Measures

Water temperature and salinity were recorded with an YSI 85 multiparameter probe (Yellow Springs Instruments, Yellow Springs, Ohio, USA) at the same time and depth as the seawater was sampled. Measurements were taken at approximately 50 cm. Dissolved oxygen was also measured in 2006–2008. Salinity is expressed in the practical salinity scale (PSS) and dissolved oxygen in milligrams per liter.

Climate Records

Rainfall, solar radiation, and wind speed are monitored continuously at the Bocas del Toro Research Station, as described by Kaufmann and Thompson (2005). These measurements are taken close to the STRI site (see Figure 1). For the purposes of this study average rainfall, solar radiation, and wind speed were calculated for 3 days and 6 days before each sampling day. We chose these periods because Beman et al. (2005) showed that phytoplankton blooms can peak 3 to 5 days after nutrient input from terrestrial runoff.

Annual rainfall was obtained in two ways. First, an hourly tipping bucket measured rainfall from 2002. Because data are incomplete for three of the years (including 2008), we calculated the average daily rainfall to standardize across the years. The second estimates were from the Bocas del Toro airport. These records extend to 1999 and were also converted to annual daily averages.

Secchi Depths

Water clarity was measured by lowering a 30 cm diameter Secchi disk into the water until it was no longer seen and then raised until it reappeared. The Secchi depth was measured according to the length of the submerged rope. This operation was repeated three times at each site during each measurement. At the seagrass site the Secchi was measured horizontally, underwater at 0.5 m depth, and was read with a dive mask.

Chlorophyll *a*

Three replicate water samples were collected by hand at 50 cm below the surface in polyethylene bottles and placed in a cooler for the return to the laboratory. Two liters of each replicate were vacuum filtered on Whatman GF/F (0.7 μm pore size). Filters were wrapped in aluminum foil and stored frozen (−20°C). A Teflon pestle was used

TABLE 1. Study site locations and the data available for each site.

Site	Location	Secchi depths	Chlorophyll *a*
Colon	9°22′37″N	1999–2001	1999–2001
	82°12′37″W	2006–2008	2006–2008
Cristobal	9°18′15″N	2006–2008	2006–2008
	82°17′55″W	–	–
Pastores	9°12′36″N	1999–2001	1999–2001
	82°19′37″W	2006–2008	2006–2008
STRI [a]	9°20′40″N	2000–2008	1999–2001
	82°16′39″W	–	2006–2008
Bottomwood	9°17′47″N	1999–2001	1999–2001
	82°13′25″W	–	–
Zapatilla	9°15′27″N	1999–2001	1999–2001
	82°06′19″W	–	–
CARICOMP seagrass	9°21′06″N	1999–2008	–
	82°15′29″W	Horizontal	

[a] STRI, Smithsonian Tropical Research Institute.

to grind the filters in 5 mL 90% aqueous acetone solution. The slurry was transferred to 15 mL polypropylene screw-cap centrifuge tubes and filled to 10 mL with acetone. The tubes were kept in the dark at −20°C for 24 h. Extracts were centrifuged at 3,000 rpm, and the supernatant was analyzed for chlorophyll *a* following the nonacidification fluorometric method (Welschmeyer, 1994).

Statistical Analysis

Correlation analyses and multiple regression analyses were used to describe the relationships between the variables of interest (Secchi depth and chlorophyll *a* concentration) and the hydrological data and the climate data. Student's *t* test, analysis of variance (ANOVA), and analysis of covariance (ANCOVA) were used to test for differences between sites and sampling periods. Because it is likely that there is a lag in the response of phytoplankton to the input of nutrients from river runoff or turbulence, we looked for correlations between Secchi depths or chlorophyll *a* concentrations and the average of rainfall, solar radiation, and wind speed over the previous 3 and 6 days. Because the 3 day and 6 day results did not differ substantively, only the results using the 3 day average are reported here. Because rainfall and cloud cover are patchy on a local scale, we only examined climatic variables for the STRI and CARICOMP seagrass sites. Time-series autocorrelation analyses were applied to Secchi depth and chlorophyll *a* data. The few weeks of missing data were filled with the averages values for the time series under analysis.

RESULTS

A number of complex relationships were demonstrated between the hydrological parameters, Secchi depths, and chlorophyll *a* concentrations at the different sites. Several of these relationships vary among the sites, and there are a number of interactions between factors; however, the following generalizations can be made. (1) Secchi depths increased with temperature, salinity, and solar radiation, and decreased with rainfall, wind speed, and chlorophyll *a* concentration. (2) Correlations between any hydrological characteristic and Secchi depths or chlorophyll *a* were low (r^2 rarely exceeding 0.10) but were higher for all the climatic variables (rain and solar radiation had r^2 up to 0.22). (3) Chlorophyll *a* concentrations showed no consistent temporal or spatial patterns. (4) Secchi depths were not tightly correlated with chlorophyll *a* concentrations. (5) Secchi depths increased and rainfall decreased throughout the study.

Hydrological Conditions at Each Site

Hydrological parameters varied somewhat among the six sites (Table 2, Figure 2). Salinity was significantly different at all sites (ANOVA with post hoc *t* test; Table 2), with the lowest average salinity in Pastores, the most inland site, and the highest average salinity in Colon, the most oceanic site. For 1999–2001 the average temperature at Pastores was significantly higher than the other sites and the temperature at Colon was significantly lower. The temperature

TABLE 2. Summary of physical and biological data from 1999–2001 and 2006–2008.

Site	Years	Temperature, °C (SD)	Salinity,[a] PSS (SD)	Dissolved oxygen, mg/L (SD)	Secchi depth, m (SD)	Chlorophyll *a*, mg/m^3 (SD)	Significant changes between periods (*t* test)
Colon	1999–2001	28.1 (1.26)	33.0 (1.70)	–	9.1 (3.6)	0.44 (0.19)	Temperature increased
	2006–2008	28.6 (0.89)	33.5 (1.31)	5.80 (0.43)	10.0 (3.6)	0.47 (0.23)	
Cristobal	2006–2008	28.8 (0.95)	32.9 (1.46)	5.83 (0.49)	12.4 (3.2)	0.46 (0.25)	NA
Pastores	1999–2001	28.6 (1.29)	31.9 (2.15)	–	11.0 (3.3)	0.46 (0.24)	Temperature and Secchi depth increased
	2006–2008	29.2 (0.98)	32.4 (1.76)	5.81 (0.51)	13.2 (3.0)	0.49 (0.28)	
STRI	1999–2001	28.3 (1.16)	32.9 (1.33)	–	10.9 (3.9)	0.37 (0.24)	Temperature and Secchi depth increased
	2006–2008	28.7 (0.91)	33.0 (1.34)	5.78 (0.49)	13.2 (3.6)	0.43 (0.23)	
Bottomwood	1999–2001	28.3 (1.24)	32.7 (1.43)	–	11.9 (3.7)	0.36 (0.19)	NA
Zapatillas	1999–2001	28.3 (1.28)	33.0 (2.78)	–	11.3 (2.6)	0.46 (0.22)	NA

[a] PSS = practical salinity scale.

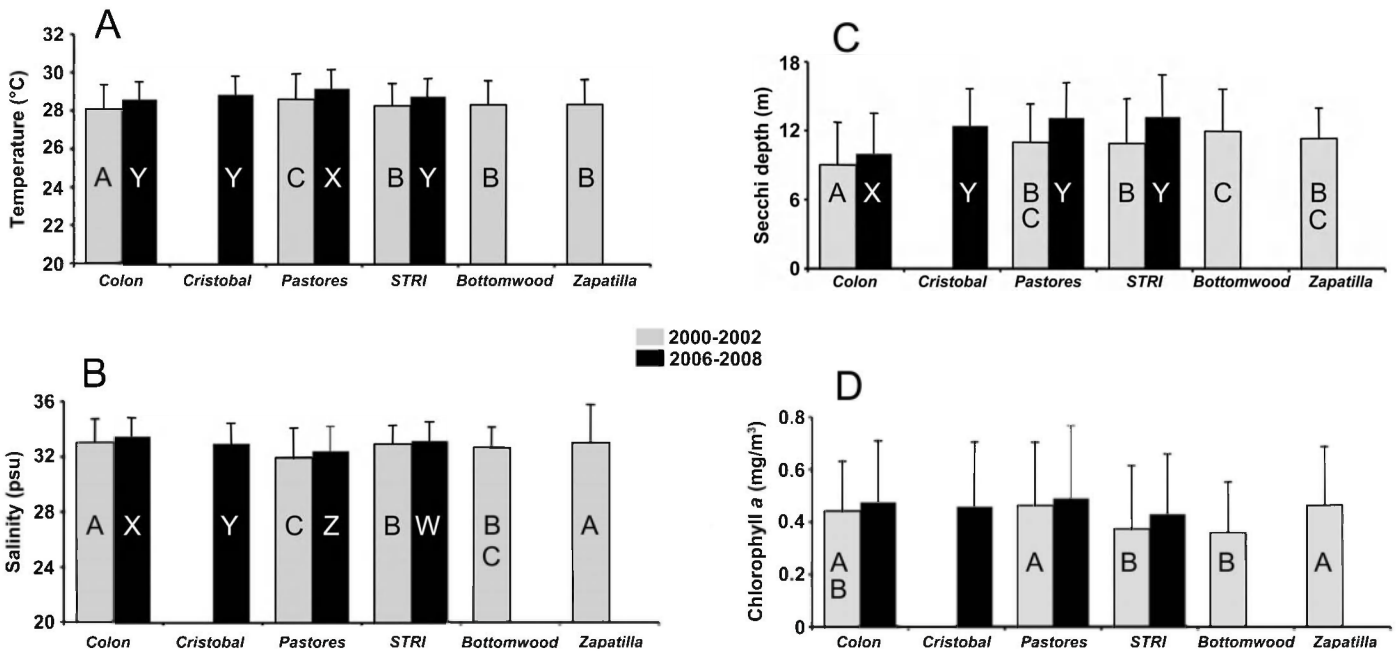

FIGURE 2. Averages of temperature (A), salinity (B), Secchi depths (C), and chlorophyll concentration (D) from the two sampling periods (1999 data excluded). Student's *t* tests showed significant increases in temperature between periods at Colon, Pastores, and STRI and increases in Secchi depth at Pastores and STRI. Single-factor analysis of variance (ANOVA) detected significant ($P < 0.01$) site effects. Significant differences between groups of sites within either sampling period are indicated with letters, so that bars both labeled with "A" are not significantly different from each other but are different from those otherwise labeled with post hoc tests. Specific letters were assigned arbitrarily, but A–C refer to 2000–2002 data and W–Z refer to 2006–2008 data. Bars = one standard deviation (1 SD) of the mean. (Salinity is expressed in the practical salinity scale, PSS.)

at Pastores was also significantly higher than at the other sites in 2006–2008, but there were no significant differences between the remaining sites. Dissolved oxygen did not differ between sites.

Temperature increased significantly between the two time periods at the three sites for which data were available over both periods (*t* test, $P < 0.002$ for each site), despite an overall temperature decrease during the 2006–2008 period. Salinity did not show a significant temporal trend during either time period nor did it differ between the two periods. Dissolved oxygen was only measured for the 2006–2008 period, where it showed no temporal trend. Eight years of data from climatic monitoring at the Bocas Research Station instrument platform shows a downward trend in rainfall, but little change in average solar radiation or average wind speed (Figure 3).

Factors Affecting Secchi Depths

Secchi depths ranged from 2 to 22 m, and the depths varied substantially from week to week (Figure 4). Secchi depths showed significant effects of site, and significant associations with temperature, salinity and chlorophyll *a* concentrations during both the 1999–2001 and 2006–2008 periods (ANCOVA; Table 3). The correlation of any one variable with water clarity was low, with

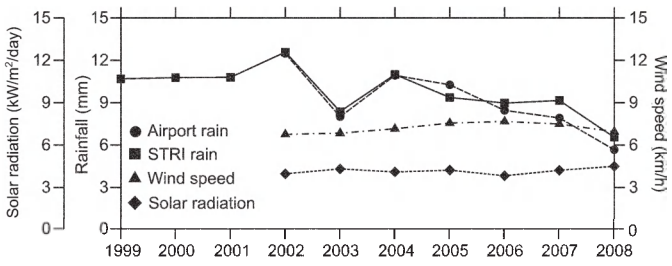

FIGURE 3. Long-term trends in climatic variables. Yearly averages for rainfall, solar radiation, and wind speed during the past 8 years show the decline in average daily rainfall. Daily averages are used because missing data prevent the use of cumulative data. (Rainfall is mm/d.)

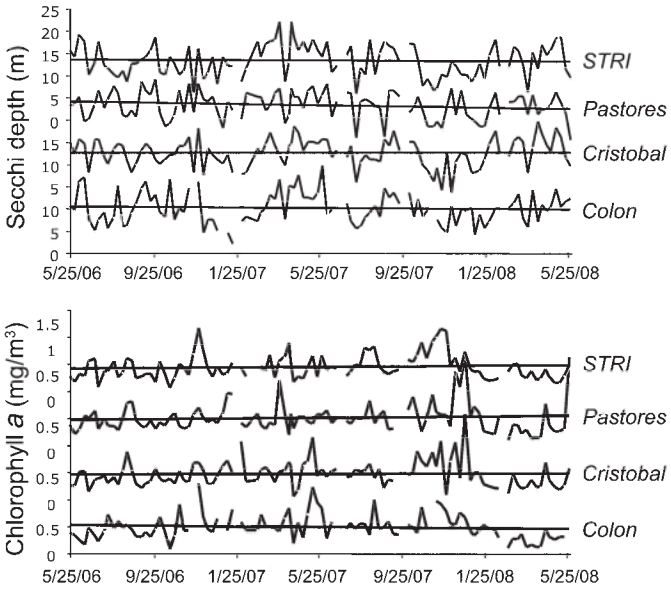

FIGURE 4. Variation in Secchi depths (top graph) and chlorophyll *a* (bottom graph) concentrations over time for the 2006–2008 dataset. Straight line is the trend line of depth or concentration on that date. Superimposed lines show individual variation.

the highest r^2 value for temperature at 0.13–0.17. Secchi depths increased with temperature (Ordinary Least Squares regression [OLS]: 1999–2001, $r^2 = 0.13$, $P < 0.001$; and 2006–2008, $r^2 = 0.17$, $P < 0.001$), and salinity (OLS: 1999–2001, $r^2 = 0.10$, $P < 0.001$; and 2006–2008, $r^2 = 0.06$, $P < 0.001$) and decreased with chlorophyll *a* concentration (OLS: 1999–2001, $r^2 = 0.09$, $P < 0.0001$; and 2006–2008, $r^2 = 0.06$, $P < 0.001$). The average Secchi depth was significantly lower for the exposed Colon site than for the other sites in both time periods (see Table 2, Figure 2). Analysis of the data from the two different periods showed different combinations of interaction effects (see Table 3).

Climatic variables were more tightly correlated with Secchi depth than were the seawater variables. Using the six years of complete climate data from the STRI site, we found that Secchi depths at the STRI site show significant effect of year ($P < 0.0001$), a marginal effect of rainfall ($P = 0.056$), and significant effects of solar radiation ($P = 0.002$) and wind speed ($P < 0.001$), but no significant interactions between these factors. For the CARICOMP seagrass site there was no effect of year, but rainfall and wind speed over the prior 3 days were significant ($P < 0.01$), as well as the interaction between rainfall and solar radiation ($P < 0.0008$). Secchi depth decreased with the amount of rainfall ($r^2 = 0.22$ and 0.23, respectively, with $P < 0.001$) and wind speed ($r^2 = 0.21$; $P < 0.001$) at both sites and increased with solar radiation ($r^2 = 0.21$ and 0.15, respectively, with $P < 0.0001$) at the STRI site but was only significant by its interaction with rainfall in the seagrass site. The interaction at the seagrass site showed that Secchi distances decreased more quickly with rainfall at high levels of solar radiation than at low solar radiation.

Secchi depths increased over the long term: they increased from 1999–2001 to 2006–2008 at Pastores and STRI (*t* test, $P < 0.0001$ for both) but not at Colon. Least squares regression showed a significant increase in Secchi depths ($r^2 = 0.02$; $n = 381$; $P < 0.0002$; slope = 0.3) with date over the 8 years of weekly sampling at STRI. The horizontal Secchi data from the nearby CARICOMP

TABLE 3. Analysis of covariance (ANCOVA) effects of physical variables on chlorophyll *a* concentration and Secchi depth in 1999–2001 and 2006–2008 data after stepwise removal of nonsignificant variables.

Source	*df* [a]	Sum of squares	*F* ratio	*P*
Secchi depth, 1999–2001				
Site	4	235.03	6.78	<0.0001
Temperature	1	100.97	11.65	0.0008
Salinity	1	290.63	33.53	<0.0001
Chlorophyll *a* concentration	1	150.82	17.40	<0.0001
Site*salinity [b]	4	104.02	3.00	0.02
Salinity*temperature [b]	1	67.31	7.76	0.006
Secchi depth, 2006–2008				
Site	3	556.41	21.77	<0.0001
Temperature	1	454.35	53.33	<0.0001
Salinity	1	216.91	25.46	<0.0001
Oxygen	1	2.86	0.33	0.56
Chlorophyll *a* concentration	1	47.15	5.53	0.02
Site*temperature [b]	3	70.34	2.75	0.04
Temperature*oxygen [b]	1	49.58	5.82	0.02
Chlorophyll *a*, 1999–2001				
Site	4	0.38	2.30	0.06
Temperature	1	1.24	30.11	<0.0001
Salinity	1	0.06	1.43	0.23
Site*salinity [b]	4	0.55	3.34	0.01
Salinity*temperature [b]	1	0.31	7.58	0.006
Chlorophyll *a*, 2006–2008				
Temperature	1	0.26	4.90	0.03
Salinity	1	2.41	45.35	<0.0001
Oxygen	1	0.25	4.67	0.03

[a] *df* = Degrees of freedom.
[b] * = Run with only two-way interactions.

seagrass site show no long-term trend. When these values are binned by month, there is a marginal effect of month on the Secchi depths in the seagrass site ($P = 0.07$) and a significant effect of month at the reef site ($P = 0.0007$). Greater Secchi depths were recorded from drier months and sunnier months (Figure 5), a result also found by Kaufmann and Thompson (2005).

Factors Affecting Chlorophyll a Concentrations

Chlorophyll *a* concentration varied between 0.04 and 1.66 mg/m^3. Similar to Secchi depths, concentrations varied substantially from week to week and with no clear seasonal component to the variation (see Figure 4). During the first 14 sampling dates of the 1999–2001 study period, chlorophyll *a* concentrations were measured with a spectrophotometer. An ANOVA testing for effects of site and method showed that the results from the spectrophotometer were significantly higher (site: $F = 3.96$; $df = 4$; $P < 0.005$; method: $F = 26.8$; $df = 1$; $P < 0.0001$; $n = 328$). Therefore values obtained from the spectrophotometer were excluded from the subsequent analyses and this dataset included only data from 2000–2001 or 2006–2008.

Although the average chlorophyll *a* concentrations did not differ between the two periods (*t* test, $P > 0.05$), there were different patterns for the two sampling periods. The only common results were that chlorophyll *a* concentration decreased with temperature, and that the variables examined explained no more than 10% of the variance in chlorophyll *a* concentrations. Data from 2000–2001 showed a significant effect of temperature and a marginal effect of site, but no effect of salinity (ANCOVA; see Table 3). There were significant interactions between site and salinity and between site and temperature (Table 3). Overall, chlorophyll *a* concentrations decreased with temperature (OLS: $n = 328$; $r^2 = 0.06$, $P < 0.0001$). Results from 2006–2008 were different: there were significant effects of temperature and salinity, but not of site or oxygen concentration, nor were there significant interactions (Table 3). Chlorophyll *a* concentrations decreased with temperature (OLS: $n = 402$; $r^2 = 0.03$, $P = 0.002$) and salinity ($r^2 = 0.11$, $P < 0.0001$) but these factors explained very little of the variation.

Climate data were poorly linked to chlorophyll *a* concentrations. Average wind speed for the 3 days before sampling was positively correlated with chlorophyll concentration ($r^2 = 0.19$, $P < 0.005$) but this appeared to be caused by a few periods with extremely high winds. Chlo-

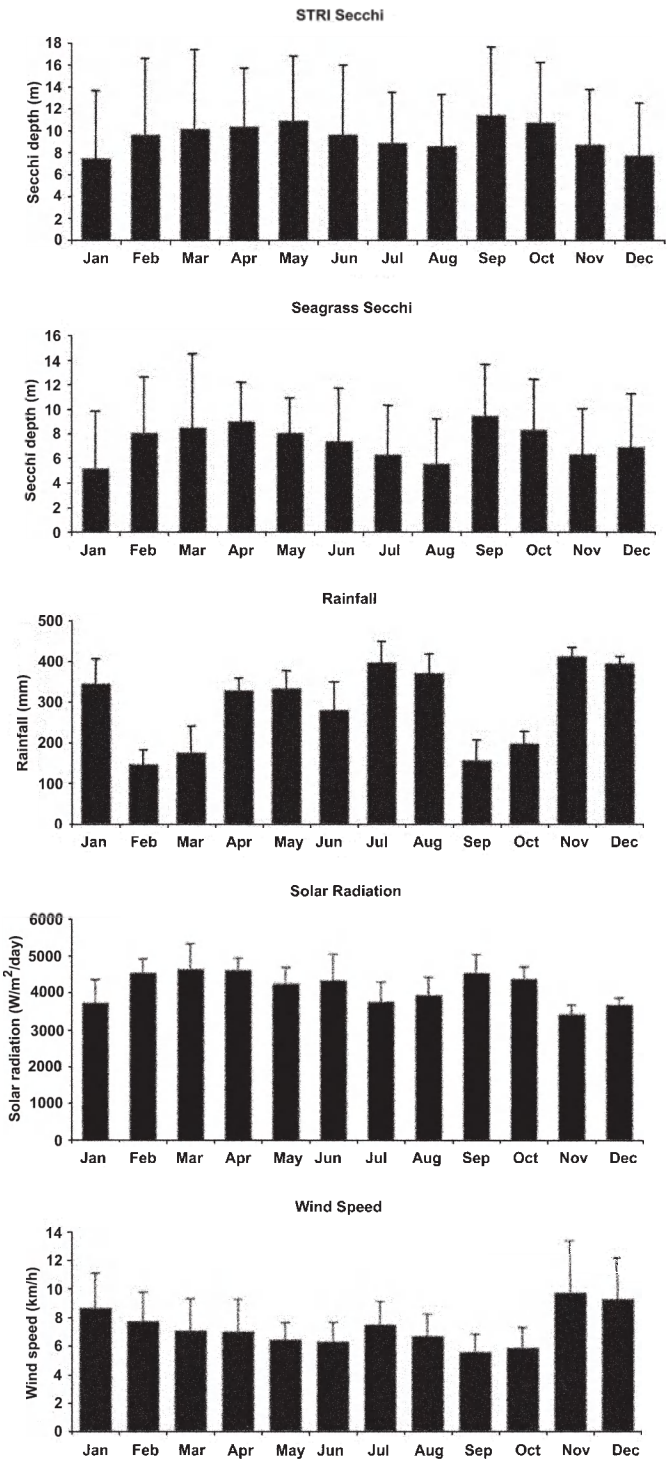

FIGURE 5. Bar graph with monthly averages of Secchi depths, rainfall, solar radiation, and wind speed for the 8-year dataset showing that months with higher average Secchi depths also had lower average rainfall. Bars = 1 SD of the mean.

rophyll *a* concentration was independent of both rainfall over 3 days prior and average solar radiation over the 3 days before the measurements.

Time Series

Both the Secchi depths and chlorophyll *a* concentrations varied considerably from week to week (see Figure 4). To determine if this variation has a temporal autocorrelation, we conducted a time-series analysis. For 1999–2008 Secchi depth is temporally auto-correlated at both the CARICOMP seagrass and the STRI sites (seagrass: Fisher's kappa = 9.9, $P < 0.01$; for coral: Fisher's kappa = 12.2, $P < 0.001$). Over the shorter period, 2006–2008, Secchi depth was temporally auto-correlated at Colon (Fisher's kappa = 8.59, $P < 0.01$), Cristobal (Fisher's kappa = 7.39, $P < 0.02$), and STRI (Fisher's kappa = 6.91, $P < 0.04$) but not Pastores ($P > 0.05$). Chlorophyll *a* concentrations, on the other hand, showed an autocorrelation only for Colon ($P < 0.005$). Examination of the autocorrelation function shows that, over the short term (lag of up to several months), the autocorrelation function appears stationary (Figure 6). However, a peak around the 52 week lag (Figure 6) is evidence of seasonal externally driven periodicity and suggests an annual cycle that is not obvious from plots of the raw data (see Figure 4).

DISCUSSION

The overall values for the data presented here are similar to those reported for the Bahia Almirante by D'Croz et al. (2005), Kaufmann and Thompson (2005), and Carruthers et al. (2005). We report some long-term trends that were not detected by Kaufmann and Thompson, who closely examined the patterns of daily

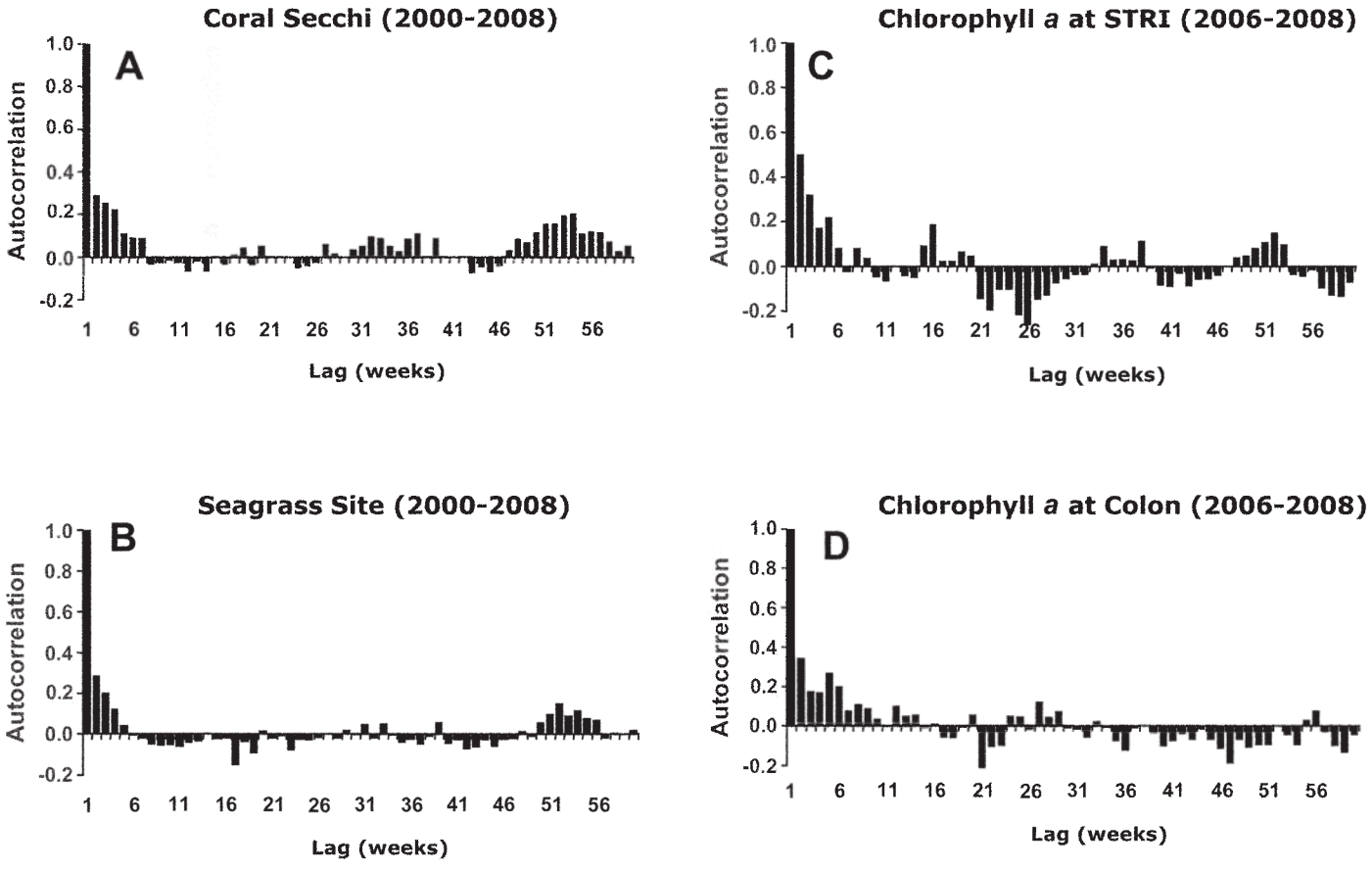

FIGURE 6. Autocorrelation function for 8 years of Secchi data for STRI and CARICOMP seagrass sites and chlorophyll *a* concentrations for 2 years for Colon (significant autocorrelation) and STRI (no significant autocorrelation) sites.

and monthly variation in physical parameters. Comparing the YSI 85 probe measures of temperature and salinity between the 2000–2002 sampling period and the 2006–2008 period, we found significant increases in water temperature and salinity. This finding appears to be associated with the recent trend toward lower rainfall in the region.

Annual rainfall is typically high, in excess of 3,000 mm, in the Bahia Almirante, and the mean freshwater runoff is approximately 1,600 mm per year (IGNTG, 1988). However, average daily rainfall per year dropped from 12.48 mm in 2002 to 7.91 mm in 2007 (see Figure 3). Reduced rainfall likely affected the hydrological conditions in Bahia Almirante, which result from the interaction between river discharge and ocean intrusion (D'Croz et al., 2005). During the 1999–2001 sampling period, rainfall was high and salinity showed the typical increasing trend from Pastores, the site nearest the mainland, to the ocean-exposed sites at Colon and Zapatilla. This pattern in surface salinity is consistent with the expected high dilution at nearshore sites resulting from river discharge into the bay. The inshore-to-offshore salinity gradient was not apparent during the 2006–2008 sampling period, presumably because of the reduction of river discharge and consequent greater influence of salinity from open ocean waters (see Figure 2).

Long-Term Trend in Secchi Depths

The most striking long-term trend we detected was the surprising increase in Secchi disk depths. During the 8 years of monitoring, visibility has increased by 2 m (a rate of 0.25 m/year) for several of the sites. Long-term trends of *decreased* Secchi depths have been reported for monitoring in other areas. For example, in a dataset from the Baltic Sea spanning 77 years, Secchi depths have decreased 0.05 m/year (Sandén and Håkansson, 1996), and a decrease of 0.03 m/year was reported in the Menai Strait in Wales (Kratzer et al., 2003). The few reports of increased Secchi depths were associated with bioremediation or efforts to reduce untreated sewage outfall. For example, Secchi depths increased at 0.05 m/year in Narragansett Bay, Rhode Island, coincident with reductions in anthropogenic total suspended solids (Borkman and Smayda, 1998), as at one of several sampled sites in the Southern California Bight (Convers and McGowan, 1994). Our measures show a much more rapid change in Secchi depths than these previous studies.

The observed changes in Secchi depths were not in the expected direction. A number of complicated, interacting factors can influence water clarity, as measured by Secchi disk, but many of them would indicate a decrease in Secchi depth. The ongoing rapid development of tourism in Bocas del Toro, particularly on Isla Colon, is accompanied by an increase in wastewater input to the Bahia Almirante. Changes in Secchi readings can reflect changes in particulate matter (from runoff or wind-induced turbidity) but can also be caused by changes in phytoplankton biomass or yellow pigments (mostly humic and fulvic acids) in the water. Deforestation and coastal development can affect all three of these factors. Inputs from untreated wastewater as well as runoff from deforested areas can increase the nutrients, particulate matter, and yellow pigments in the water. In addition, increased nutrients often lead to increased primary productivity, which can result in higher standing phytoplankton biomass. These anthropogenic effects have been increasingly affecting coral reef habitats throughout the Caribbean, where wastewater disposal is the leading cause for eutrophication and decreased water clarity (Szmant, 2002). We had expected to see a long-term reduction in water clarity as a result of similar changes in Bocas del Toro.

Secchi depth is often strongly correlated with chlorophyll *a* concentration and has been used as a proxy for productivity in highly seasonal upwelling zones or temperate lakes. This method is often favored because it is cheaper, faster, and easier to obtain than a quantification of chlorophyll *a* concentration. Sandén and Håkansson (1996) reviewed four studies as well as their own data that showed a relationship between Secchi depths and chlorophyll *a* concentrations. The relationships are reported as power functions and show chlorophyll *a* to scale with Secchi depth to the 1.47–2.6 power. Megard and Berman (1989) showed that the proportion of light attenuation caused by chlorophyll concentration differed between neritic and pelagic seawater, but there were clear relationships nonetheless. Here we found that chlorophyll *a* concentration explained only 6%–9% of the variance in Secchi depths. In addition, mean chlorophyll *a* concentrations were relatively low in the Almirante Bay, near 0.5 mg m^{-3}, which is the suggested threshold value for oligotrophic conditions required for coral reef development (Bell, 1992). Therefore, these measures are not consistent with the presence of phytoplankton blooms resulting from anthropogenic nutrient enrichment. They do, however, suggest that even small increases in nutrients or chlorophyll *a* concentrations in this region could result in a shift from coral-dominated to algal-dominated benthic communities.

It seems unlikely to us that there has been a drop in the load of anthropogenic suspended solids and/or nutrients

during the past eight years, despite the probable decrease in the volume of runoff. In fact, it appears that, if anything, these inputs have increased. So, what is the cause of the long-term trend in Secchi depth? The correlation analysis and the monthly trends (see Figure 5) both suggest that rainfall and solar radiation are the most closely associated with Secchi depth. However, rainfall is the only variable showing a strong annual trend consistent with the increased Secchi depths, and rainfall over the three days before the measurements was the variable most highly correlated with Secchi depths of any hydrological or climate variable examined. Solar radiation, although it is positively correlated with Secchi depths on the reef, does not show the pronounced long-term trend that rainfall does. The effect of wind on Secchi depth similarly does not explain the long-term trend. It could, however, explain the fact that Colon, the most exposed site, with the highest winds had consistently lower Secchi depths than the other sites. Wind-induced turbidity, which resuspends bottom sediment, is the likely cause of the limited water clarity at this site.

CONCLUSION

The baseline data reported here will be useful for future studies of anthropogenic effects in the unique Bocas del Toro archipelago. Rapid local development is progressing in the face of little information on the impact of such development and the factors affecting such impacts (e.g., water residence time or currents in the Bahia Almirante). It is likely that anthropogenic inputs of nutrients and suspended particulate matter will contribute to eutrophication of some areas. This study suggests that despite the impact of development, patterns of water clarity and chlorophyll *a* concentrations in the region are currently driven mainly by large-scale climate patterns. There is little evidence of a tight relationship between these measures and features of the local water mass, nor is there evidence of eutrophication at the sites we sampled. Future sampling closer to highly developed areas is necessary to document and monitor the impact of development on water quality.

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Nutrient and Chlorophyll Dynamics in Pacific Central America (Panama)

Luis D'Croz and Aaron O'Dea

ABSTRACT. Strong wind jets from the Caribbean and the Gulf of Mexico cross Central America through topographic depressions in the cordillera during the boreal winter, pushing Pacific coastal waters offshore, lowering sea levels at the coast, and causing coastal upwelling. Where high mountains impede the winds, this phenomenon does not occur. The Panamanian Pacific shelf is an excellent example of this variability. The coast is divided into two large areas, the Gulf of Panama and the Gulf of Chiriquí. To investigate hydrological conditions between the two gulfs, we sampled the water column during upwelling and non-upwelling seasons in each region. In both gulfs during non-upwelling conditions, surface-level nutrients are poor, and the chlorophyll maximum occurs around 30 m where the thermocline intersects the euphotic zone. Oxygen-poor waters (<2 ppm) commonly occurred below the thermocline. During the dry season, wind strength increased and strong upwelling was observed in the Gulf of Panama. The thermocline rose and surface waters became nutrient enriched and chlorophyll *a* levels increased. Well-oxygenated waters were compressed to shallow depths. In the Gulf of Chiriquí, wind strength was weaker, surface waters did not become enriched with nutrients, and surface chlorophyll *a* remained low. We did observe a shallowing of the thermocline in the Gulf of Chiriquí, but in contrast to the Gulf of Panama, wind mixing was not strong enough to result in sea-surface cooling and nutrient enrichment. We postulate that the convergence of a shallow thermocline and internal waves in the Gulf of Chiriquí is the likely mechanism that causes pockets of deep water to occasionally migrate into surface waters, leading to restricted and ephemeral upwelling-like conditions. Although its effects upon shallow-water communities remain to be studied, we propose that the process may be more likely to occur during the boreal winter when the thermocline is shallower.

INTRODUCTION

One of the most pervasive hydrological events to influence the shelf waters of Pacific Central America is upwelling. Intermittent or seasonal upwelling develops in the gulfs of Tehuantepec (Mexico), Papagayo (Costa Rica), and Panama (Legeckis, 1988; McCreary et al., 1989; Xie et al., 2005), driving extensive planktonic productivity and shaping the secondary production of biological communities (Jackson and D'Croz, 1997; O'Dea and Jackson, 2002).

The shelf waters along the Pacific coast of Panama are among the most dynamic in the region. Here, the coastal shelf is naturally divided into two large gulfs by the Azuero Peninsula: the Gulf of Panama (shelf area, 27,175 km^2) and the Gulf

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of Chiriquí (shelf area, 13,119 km^2) (Figure 1). The Gulf of Panama experiences strong seasonal upwelling while the Gulf of Chiriquí exemplifies a non-upwelling environment (Dana, 1975; Kwiecinski and Chial, 1983). This distinction is customarily explained using geographic differences between the two gulfs. Seasonal upwelling in the Gulf of Panama develops during Panama's dry season, corresponding to the boreal winter, when northeast trade winds cross to the Pacific over low areas in the isthmian mountain range, pushing warm and nutrient-poor coastal surface water offshore, lowering the nearshore sea level, and causing the upward movement of colder and nutrient-richer deep water (Smayda, 1966; Forsbergh, 1969; Kwiecinski et al., 1975; D'Croz et al., 1991; D'Croz and Robertson, 1997). The established model proposes that because western Panama has higher mountain ranges that block the winds, surface waters in the Gulf of Chiriquí are not displaced out to the Pacific, and no upwelling as such occurs there.

The structure of shallow biological communities between the two regions supports this inference. Coral reefs, which respond poorly to upwelling conditions, are more extensive in size in the Gulf of Chiriquí than in the Gulf of Panama (Glynn, 1977; Glynn and Maté, 1997), whereas small pelagic fish species from the Gulf of Panama represent a large proportion of the total estimated fishery resource in the country (NORAD, 1988). Satellite imagery shows both wind speeds and chlorophyll content of surface waters to be lower in the Gulf of Chiriquí than the Gulf of Panama during the dry seasons (Pennington et al., 2006).

However, the statement that upwelling does not occur in the Gulf of Chiriquí is supported by sea-surface data derived from satellite imagery analysis or from the measurement of properties in the shallow section of the water column. Hydrological profiles of the water column have documented the shoaling of the thermocline in the Gulf of Chiriquí, yet there appears to be no clear association between the physical forcing of this event with the wind-induced upwelling in the Gulf of Panama. Nevertheless, the movement of pockets of cool water that bring nutrients into the upper layer may be a more common occurrence in the Gulf of Chiriquí than previously suspected (D'Croz and O'Dea, 2007).

It is therefore essential that we obtain detailed and comparable hydrological data from both gulfs if we wish to explain variability in biological communities along the Pacific coast of the Isthmus of Panama today and through geologic time (O'Dea et al., 2007). In this paper we expand the information presented in our previous study (D'Croz and O'Dea, 2007), adding new hydrological and biological data from the Gulf of Chiriquí and the Gulf of Panama, and we further discuss the issue of whether upwelling takes place in the Gulf of Chiriquí.

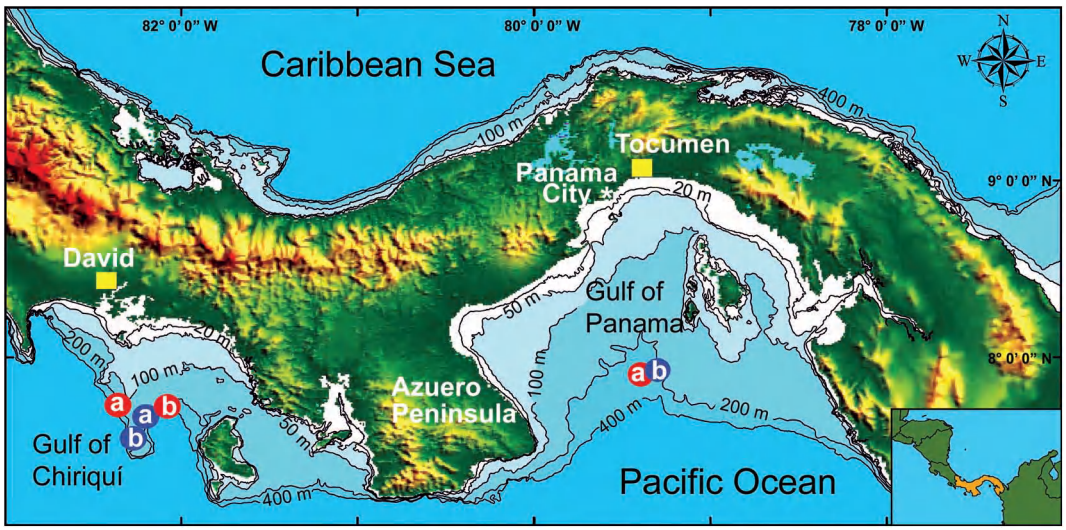

FIGURE 1. Map of the Republic of Panama showing sampling sites. Red dots represent the location of the rainy season samplings in the Gulf of Panama (a = 18 December 2004) and in the Gulf of Chiriquí (a = 13 July 2003; b = 17 December 2004). Blue dots represent the location of the dry season samplings in the Gulf of Panama (a = 29 February 2000) and in the Gulf of Chiriquí (a = 1 March 2000; b = 13 April 2007). Yellow squares indicate the location of the meteorological stations.

MATERIALS AND METHODS

Study Area

Panama's Pacific shelf is located from 07°30′ to 09°01′N and 78°10′ to 82°52′W. The shelf is predominantly occupied by low-salinity surface water, similar to the water mass found over the center of the tropical Pacific Ocean at about 10°N (Wyrtki, 1967; Fiedler and Talley, 2006). The climatology is governed by the Inter-Tropical Convergence Zone (ITCZ), the position of which defines the seasonal pattern of rainfall and winds. The rainy season develops between May and December when the ITCZ is located over or slightly to the north of Panama and winds are light and variable in direction. The dry season develops between January and March when the ITCZ moves south of Panama, a time period characterized by predominating intense northeast trade winds. The mean annual rainfall recorded at meteorological stations near the coast (1999–2004) was 2,760 mm in the Gulf of Chiriquí (David) and 1,880 mm in the Gulf of Panama (Tocumen). Approximately 94% of the annual rainfall in both areas corresponded to the rainy season, the months of September and October being the rainiest in both regions. The estimated sizes of the drainage basins are 11,846 km^2 in the Gulf of Chiriquí and 33,828 km^2 in the Gulf of Panama. River discharges into both gulfs typically follow the seasonal trend described for rainfall. Detailed discussions on wind-stress, rainfall, and river discharge patterns are presented in D'Croz and O'Dea (2007). The tidal regime is semidiurnal, and the sea-level difference during spring tides is 6 m (Glynn, 1972).

Sampling Procedures

Sampling research cruises were conducted in the gulfs of Panama and Chiriquí using the Smithsonian Tropical Research Institute's R/V *Urracá* (see Figure 1). Samplings were scheduled to correspond with different times of the year, representing contrasting hydrological conditions (upwelling and non-upwelling). Surface-to-bottom profiles for salinity, temperature, dissolved oxygen, and chlorophyll *a* were recorded with a CTD (conductivity, temperature, depth) multiparameter profiler (Ocean Seven 316, Idronaut Srl, Milano, Italy). Hydrological casts with the CTD corresponding to the dry season were carried out in both gulfs on 29 February 2000 and 1 March 2000 and in the Gulf of Chiriquí on 13 April 2007. Rainy season CTD casts were carried out in the Gulf of Chiriquí on 13 July 2003 and in both gulfs during 17 and 18 December 2004. The water column was sampled at discrete levels to study nutrient and chlorophyll *a* concentrations. Water samples were collected using Niskin bottles during the dry season of the year 2000 (29 February to 1 March) and during the rainy season of the year 2004 (17 and 18 December). Three replicate water samples per selected depth were collected at each site. Two liters of each individual replicate water sample were immediately sieved through Nitex (350 μm) to exclude zooplankton and vacuum filtered on Whatman GF/F filter (0.7 μm pore size) for chlorophyll *a* analysis. An aliquot from each filtrate was set apart for the determination of dissolved inorganic nutrients. Filters and water samples were stored frozen (−20°C) until analysis. Salinity is expressed using the Practical Salinity Scale (pss) indicated by UNESCO (1981). Results from the chlorophyll *a* analyses were used to check the calibration of the CTD's fluorometer. The depth of the euphotic zone (1% incident radiation) was estimated from Secchi disk readings (Parsons et al., 1984). The light attenuation coefficient was calculated as $K_d = f/zs$ where zs is the Secchi depth and f = 1.4.

Analysis of Samples

Not later than two weeks after sampling, filters holding the phytoplankton were analyzed for chlorophyll *a* using the non-acidification fluorometric method (Welschmeyer, 1994). Water samples were analyzed for $NO_3^- + NO_2^-$ (nitrate + nitrite), $Si(OH)_4$ (silicate), and PO_4^{3-} (phosphate) by colorimetric methods using an Alpkem Flow Solution IV automated analyzer. Minimum detection limits were 0.02 μM for nitrate, 0.01 μM for nitrite, 0.12 μM for silicate, and 0.02 μM for phosphate.

Analysis and Presentation of Data

Water quality variables, namely temperature, salinity, dissolved oxygen, dissolved inorganic nutrients, and chlorophyll *a*, are presented graphically as profiles of the samplings. Overall differences in between the two gulfs were assessed with the Mann–Whitney test (*U*) by taking the median of each variable from samples collected in the top 30 m of the ocean where the highest hydrological variability occurred (Table 1). Water transparency data were compared using the paired *t* test. We followed the practice of taking the position of the 20°C isotherm to represents the depth of the center of the permanent thermocline in the eastern Pacific Ocean (Wyrtki, 1964; Fiedler et al., 1991; Xie et al., 2005). Pearson correlations with Bonferroni adjustment were used to test statistical relationships among variables.

TABLE 1. Average value of hydrological variables in the top water column (30 m) in the gulfs of Panama (GP) and Chiriquí (GC); SE = standard error of the mean. Statistical tests were either Mann–Whitney *U* test or paired *t* test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns = nonsignificant).

Hydrological variables	Dry season values			Rainy season values		
	GP (Mean ± SE)	GC (Mean ± SE)	Statistical value [a,b]	GP (Mean ± SE)	GC (Mean ± SE)	Statistical value [a,b]
Temperature (°C)	17.97 ± 0.92	27.17 ± 0.92	16.0* [a]	26.75 ± 0.54	28.61 ± 0.05	18.0 ns [a]
Salinity (pss) [c]	34.18 ± 0.29	32.98 ± 0.29	12.0* [a]	31.67 ± 0.64	30.48 ± 0.38	3.0 ns
Chlorophyll *a* (μg L^{-1})	1.82 ± 0.65	0.83 ± 0.65	4.0* [a]	0.23 ± 0.13	0.18 ± 0.06	8.5 ns [a]
Dissolved oxygen (ppm)	3.45 ± 0.27	4.78 ± 0.27	13.0* [a]	3.98 ± 0.16	4.38 ± 0.01	4.0 ns [a]
NO_3^- (μM)	14.37 ± 2.48	3.72 ± 2.48	1.0** [a]	0.99 ± 0.34	0.36 ± 0.02	2.5 ns [a]
PO_4^{3-} (μM)	1.08 ± 0.21	0.39 ± 0.21	1.0** [a]	0.43 ± 0.07	0.24 ± 0.03	4.0 ns [a]
N:P ratio	12.82 ± 1.10	7.77 ± 1.10	1.0** [a]	2.11 ± 0.36	1.49 ± 0.10	3.0 ns [a]
$Si(OH)_4$ (μM)	8.99 ± 1.03	4.40 ± 1.03	5.0* [a]	5.40 ± 0.71	4.87 ± 0.47	13.0 ns [a]
Secchi depth (m)	4.20 ± 0.00	14.80 ± 0.00	−1591.0*** [b]	20.00 ± 0.00	19.00 ± 0.00	2.0 ns [b]
Euphotic zone (m)	13.8 ± 0.00	48.63 ± 0.00	−1394.2*** [b]	65.71 ± 0.00	62.43 ± 0.00	188.4 ns [b]

[a] Mann–Whitney *U* test.
[b] Paired *t* test.
[c] pss = practical salinity scale.

RESULTS

Thermohaline Structure

Both the Gulf of Panama and the Gulf of Chiriquí exhibit the typical tropical coastal ocean water structure of cool deep waters leading upward to a shallow thermocline topped by warm surface waters. However, significant differences occur between the two gulfs with respect to climatic variability. During the rainy season, the thermal structure in both gulfs is remarkably similar (see Table 1). Sea-surface temperatures (SSTs) are invariably warm (27°–28°C), and the thermocline sits at approximately 60 m (Figure 2).

During the dry season, thermal conditions become dissimilar between the two regions (Table 1). In our observations, the thermocline in the Gulf of Panama rose sharply and nearly broke at the surface, resulting in a significant cooling of surface waters to 22°C (Figure 3a). Simultaneously, the thermocline in Gulf of Chiriqui rose to around 30 m, compressing warm SSTs into shallow waters (Figure 3b). However, the shoaling of the thermocline in Chiriquí was not as intense as that seen in the Gulf of Panama and did not result in SST cooling.

In general, salinity profiles in both regions revealed a sharp gradient from high-salinity deep water to fresher surface waters. Seasonal variability in surface salinities in both gulfs was very similar (Table 1). During the rainy season, both regions experienced high freshwater dilution in the upper-layer waters, with surface salinities below 30 on the pss (see Figure 2). The halocline was located at 60 m depth, coinciding with the thermocline. During the dry season, lower rainfall led to increased salinities in the surface waters of both gulfs (Figure 3). However, the effect was more striking in the Gulf of Panama as the halocline shoaled and salinity in surface waters reached 34.

In April 2007, the thermohaline structure in the Gulf of Chiriquí departed drastically from the typical condition as the thermocline/halocline shoaled to 20 m. Despite this condition, however, SSTs remained warm (Figure 3c).

Chlorophyll

Concentrations of surface chlorophyll were always below 0.30 μg/L in both gulfs during the rainy season (Table 1), but a deep chlorophyll maximum developed from 30 m to 50 m, lying above the thermocline (Figure 2). The deep chlorophyll maximum contained most of the chlorophyll *a* in the water column in both gulfs, concentrations reaching 1 μg/L during the rainy season. The dry season upwelling changed this pattern in the Gulf of Panama, as the chlorophyll maximum moved into shallower waters, where concentrations surpassed 4 μg/L (Figure 3a). Surface chloro-

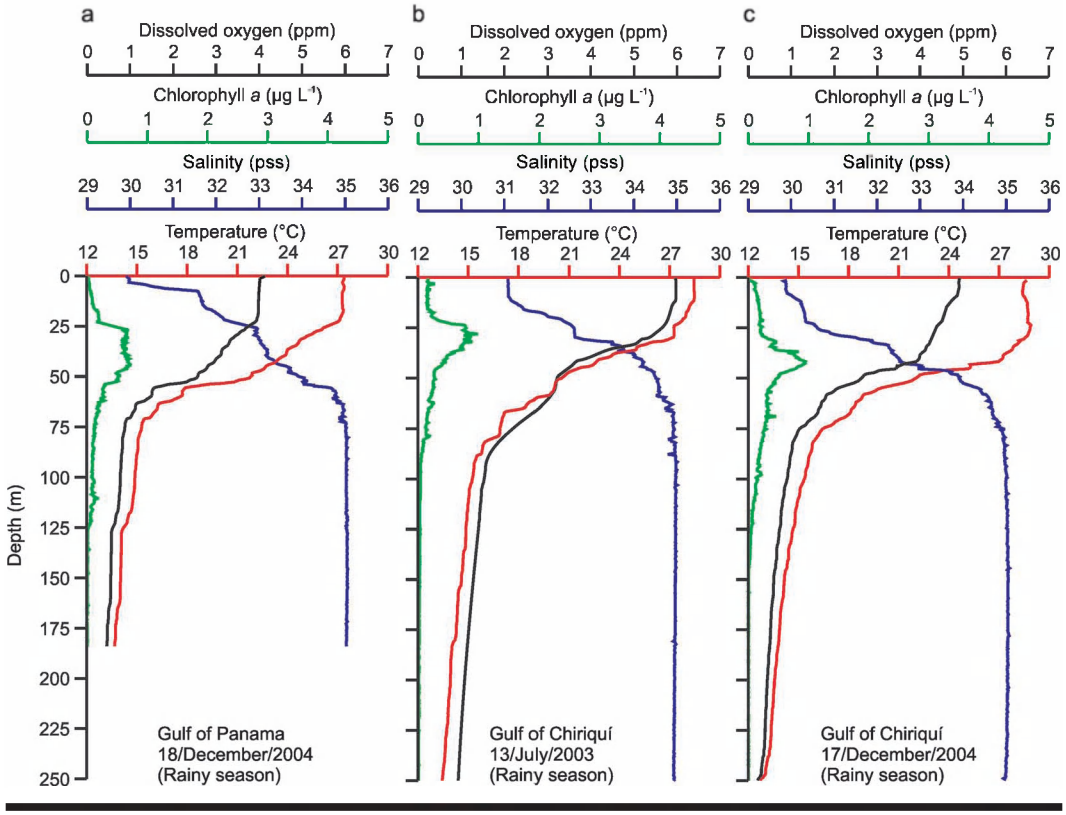

FIGURE 2. Profiles of dissolved oxygen, chlorophyll *a*, salinity, and temperature in the Gulf of Panama and the Gulf of Chiriquí during the rainy season. a = Gulf of Panama, 18 December 2004; b = Gulf of Chiriquí, 13 July 2003; c = Gulf of Chiriquí, 17 December 2004.

phyll *a* remained at very low values in the Gulf of Chiriquí during the dry season, but the deep chlorophyll maximum became remarkably intense at 30 m where concentration reached 3 µg/L (Figure 3b).

Dissolved Oxygen

Dissolved oxygen profiles followed the typical pattern of well-oxygenated surface waters lying on top of deeper oxygen-poor waters. During the rainy season, severe hypoxic conditions (<2 ppm) were recorded below the strong oxycline, at 50 m and nearly coincident with the thermocline (see Figure 2). Oxygen concentrations in waters above the thermocline were strongly correlated with temperature in both the Gulf of Panama ($r = 0.91$; $P < 0.001$) and the Gulf of Chiriquí ($r = 0.89$; $P < 0.001$) during the rainy season. This arrangement, however, had strong seasonal variation in the Gulf of Panama during the dry season, as the oxycline rose to 25 m and compressed the oxygenated waters into shallow depths (Figure 3). Dissolved oxygen below this depth rapidly declined to less than 1 ppm (Figure 3a), whereas waters in the Gulf of Chiriquí only became hypoxic below the 50 m oxycline (Figure 3b). No correlations were confirmed between dissolved oxygen and temperature in any of these regions during the dry season.

Dissolved Nutrients

Both gulfs exhibit a strong vertical gradient of upwardly decreasing nutrient concentrations. Nitrate in surface waters was depleted in both gulfs during the rainy season, with values below 0.5 µM (Figure 4). During the dry season, nitrate concentrations at the surface were observed to increase 10 fold in the Gulf of Panama when the nutricline shoaled to around 10 m (Figure 5a). No similar surface enrichment was detected in the Gulf of Chiriquí, where a strong nutricline was developed at 60 m (Figure 5b).

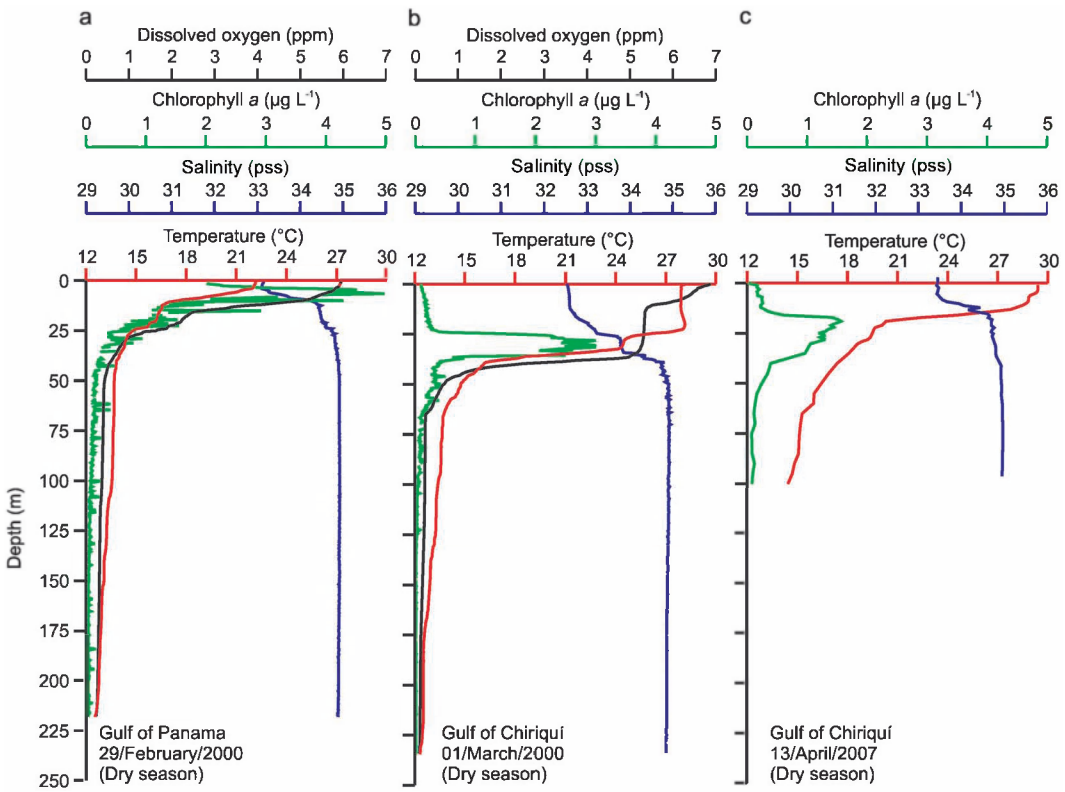

FIGURE 3. Profiles of dissolved oxygen, chlorophyll *a*, salinity, and temperature in the Gulf of Panama and the Gulf of Chiriquí during the dry season. a = Gulf of Panama, 29 February 2000; b = Gulf of Chiriquí, 1 March 2000; c = Gulf of Chiriquí, 13 April 2007.

Overall, the patterns of phosphate resembled those of nitrate, but concentrations were lower by an order of magnitude. Concentrations of phosphate in excess of 1 µM were usually found below 30 m depth. Phosphate concentrations in surface waters remained relatively low (<0.3 µM) in the Gulf of Chiriquí during both climatic seasons (Figures 4b, 5b). However, phosphate enrichment of surface waters clearly occurred in the Gulf of Panama during the dry season when the nutricline shoaled and phosphate concentrations in the top of the water column reached about 1.0 µM (Figure 5a).

Silicate profiles followed similar trends to that of the nitrate and phosphate (Figures 4, 5). Although silicate concentrations were similar in surface waters in both gulfs during the rainy season, they doubled in the Gulf of Panama during the dry season (Table 1).

Dissolved nutrients in the upper 50 m had a high degree of relationship with temperature and salinity. During the rainy season, nitrate concentrations were inversely correlated to temperature in both the Gulf of Chiriquí ($r = -0.78$; $P < 0.001$) and the Gulf of Panama ($r = -0.97$; $P < 0.002$). In the dry season, nitrate in the Gulf of Panama was negatively correlated to temperature ($r = -0.98$; $P < 0.044$) and directly related to salinity ($r = 0.98$; $P < 0.049$). Nitrate was negatively correlated to temperature in the Gulf of Chiriquí during the dry season ($r = -0.89$; $P < 0.016$), but not to salinity ($r = 0.67$; $P > 0.159$). Phosphate was negatively correlated to temperature during the dry season in the Gulf of Panama ($r = -0.98$; $P < 0.038$) and in the Gulf of Chiriquí ($r = -0.97$; $P < 0.036$). Dry season phosphate was also correlated to salinity in the Gulf of Chiriquí ($r = 0.98$; $P < 0.05$).

The extremely low nitrate to phosphate ratios (N:P) suggest that phytoplankton growth in both regions was under severe nitrogen limitation during the rainy season (Figure 6). The N:P ratio was below 2:1 in surface water and increased with depth, surpassing the value of 10:1 below the depth of 50 m. During the dry season, N:P ra-

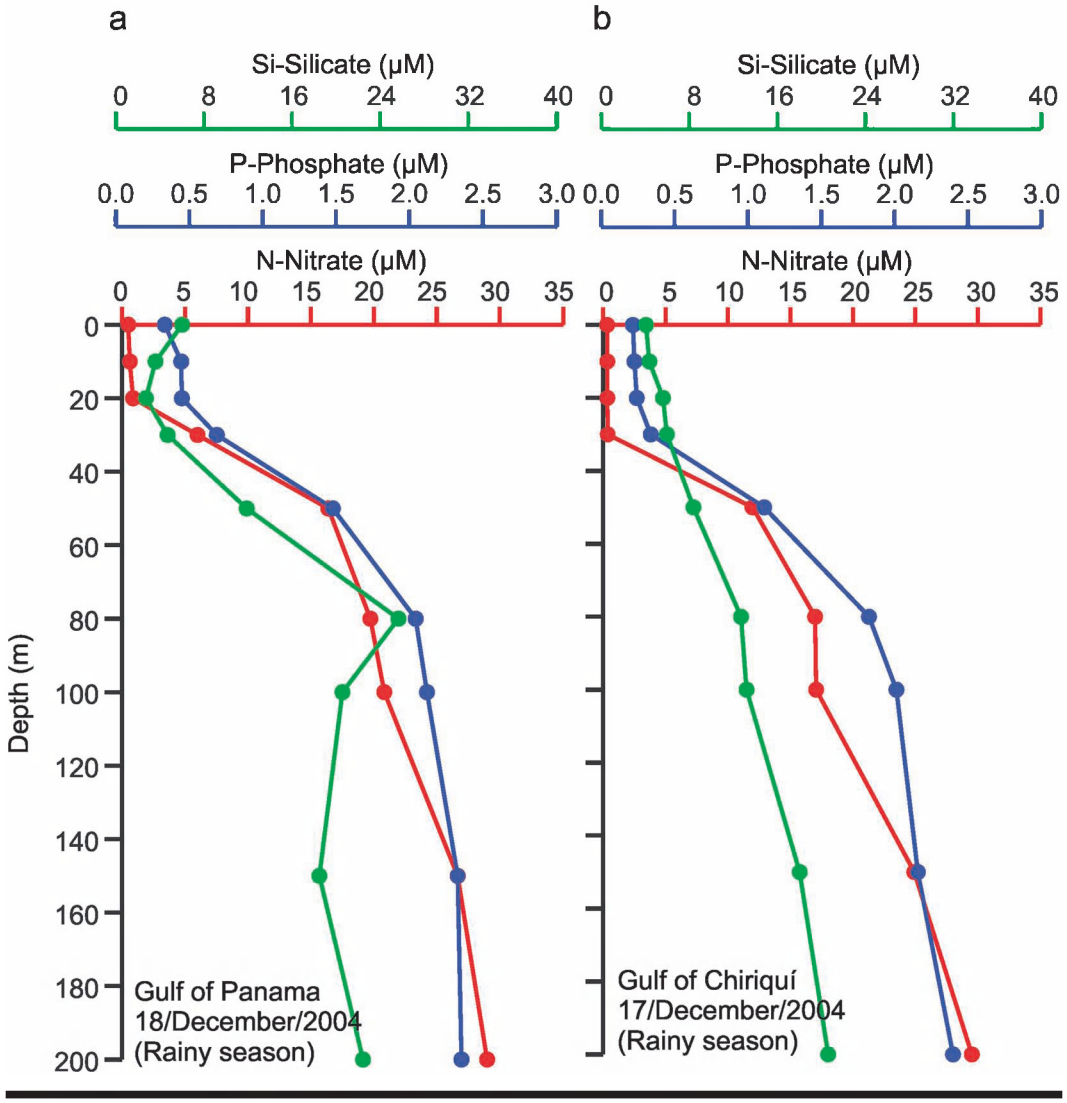

FIGURE 4. Mean profiles of silicate (Si), phosphate (P), and nitrate (N) in the Gulf of Panama and the Gulf of Chiriquí during the rainy season. a = Gulf of Panama, 18 December 2004; b = Gulf of Chiriquí, 17 December 2004.

tios within the euphotic zone largely increased in both regions, becoming closer to the N:P ratio of 16:1 suggested as favorable for phytoplankton growth (Redfield, 1958).

Water Transparency

Water transparency was seasonably stable in the Gulf of Chiriquí but varied considerably in the Gulf of Panama (see Table 1). Water transparency in both gulfs was higher during the rainy season when the euphotic zone was approximately 60 m deep, in contrast to the limited transparency and shallow euphotic zone (14 m) observed in the Gulf of Panama during the dry season upwelling.

DISCUSSION

Our data on bottom-to-surface profiles reveal the dynamics of hydrological conditions along the Pacific coast of Panama during times of both upwelling and non-upwelling. During the non-upwelling rainy season, both gulfs exhibit extremely similar hydrological structures dominated by the

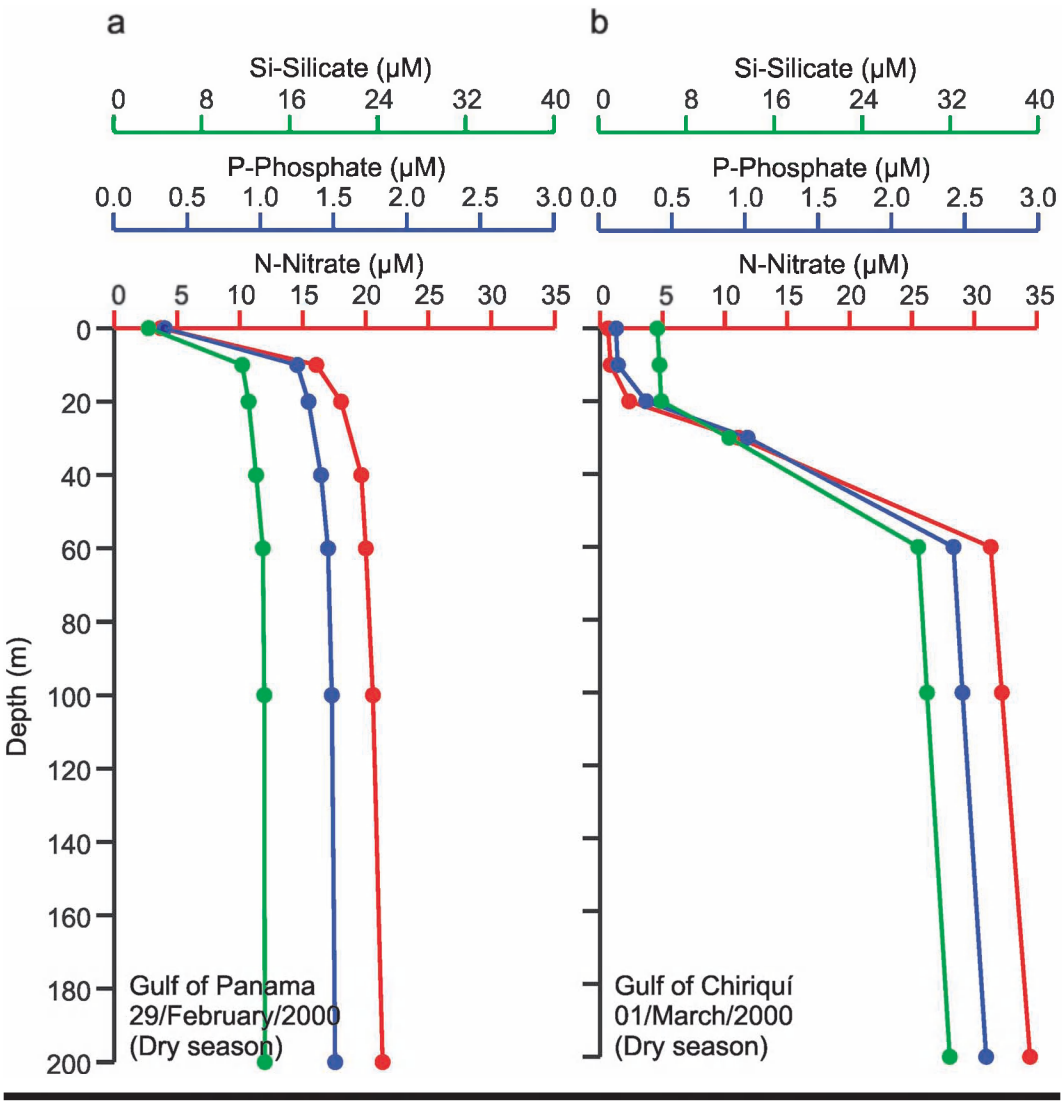

FIGURE 5. Mean profiles of silicate (Si), phosphate (P), and nitrate (N) in the Gulf of Panama and the Gulf of Chiriquí during the dry season. a = Gulf of Panama, 29 February 2000; b = Gulf of Chiriquí, 1 March 2000.

development of an intense thermocline at approximately 60 m. Surface waters tend to have low salinities and are warm and nutrient depleted. Low N:P ratios in surface waters during the rainy season suggest that phytoplankton growth is strongly nitrogen limited. Consequently, the standing stock of chlorophyll *a* is maintained at relatively low levels in surface waters. Phytoplankton does however peak at subsurface levels as the nutrient-rich thermocline waters intersect the euphotic zone, increasing N:P ratios and favoring algal growth. The strong inverse correlation between nutrients and sea temperature is consistent with the coincidence of a shallow thermocline and strong nutricline typical of the eastern tropical Pacific Ocean (Enfield, 2001). As such, the seasonal movement of the thermocline represents a key source of nutrients for phytoplankton. Our sampling sites were far offshore and therefore silicate concentrations were not as high as previously reported for the inner shelf (D'Croz and O'Dea, 2007) even though the concentration of silicate in the Gulf of Panama is reported to be the highest in the eastern Pacific as a consequence of the intense runoff in the area (Pennington et al., 2006).

During the dry season, the hydrological patterns of the two gulfs become dissimilar. In the Gulf of Panama strong upwelling of cold deep waters into coastal and surface wa-

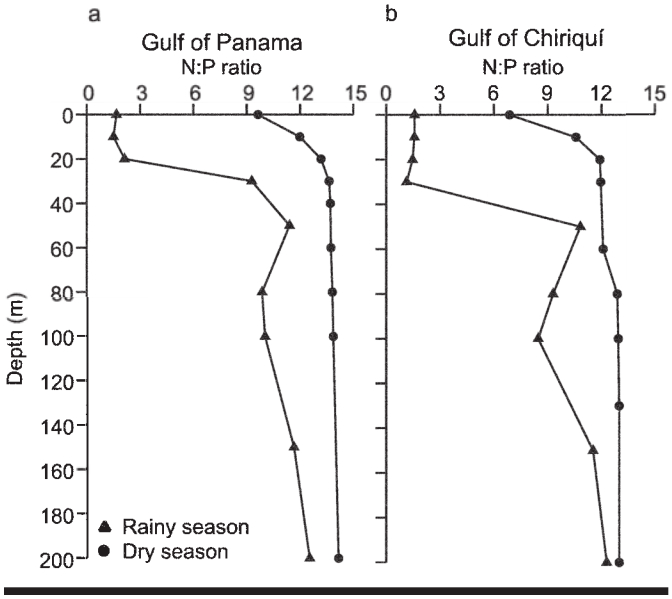

FIGURE 6. Profiles of average nitrate to phosphate ratios (N:P) in rainy (triangles) and dry (circles) seasons: a = Gulf of Panama; b = Gulf of Chiriquí.

ters drives significant changes in the hydrological properties of the water column. The thermocline migrates vertically upward, leading to cooling, increased salinity, and nutrient enrichment on surface waters. Surface N:P ratios become closer to the Redfield value and, as a result, phytoplankton growth intensifies, leading to a reduction in water clarity. A shallow oxycline also develops and oxygen concentration below the oxycline is reduced, often leading to severe hypoxic conditions. In contrast, the oxycline in the Gulf of Chiriquí is deeper and deep water remains hypoxic. Low oxygen minima are nonetheless typical in the eastern tropical Pacific as a combination of high algal growth at the surface, a strong pycnocline that impedes the ventilation of waters below, and the sluggish circulation of deep waters (Fiedler and Talley, 2006). The report of large filamentous *Thioploca*-like sulfur bacteria on shallow sediments in both regions strongly suggests that the inner shelf is exposed to episodes of reduced oxygen (Gallardo and Espinoza, 2007).

A significant relationship between wind-stress index (calculated from the sum of northerly winds) and sea level provides an explanatory mechanism for upwelling in the Gulf of Panama (Schaefer et al., 1958; Legeckis, 1988; Xie et al., 2005). Surface waters are displaced into open ocean by strong northerly winds during the dry season, and deep waters move vertically upward to replace them (Fleming, 1940; Smayda, 1966; Forsbergh, 1969). Consequently, wind stress is inversely related to SST in the Gulf of Panama during the dry season but not during the rainy season (D'Croz and O'Dea, 2007).

Data from the Gulf of Chiriquí are scant but did suggest that upwelling does not occur, because wind stress during the dry season is normally one-third of that of the Gulf of Panama (Kwiecinski and Chial, 1983) and it does not displace surface waters offshore. High mountain ranges running along western Panama impede the flow of northerly winds across to the Gulf of Chiriquí (see Figure 1), whereas mountain ranges in central Panama are low, allowing strong wind jets to form toward the Gulf of Panama. Despite this clear distinction, our data show that similar hydrological changes to those that occur in the Gulf of Panama do take place in the Gulf of Chiriquí. During the dry season, and concurrent with strong upwelling in the Gulf of Panama, we observed deeper waters rise toward shallower depths in the Gulf of Chiriquí. This movement led to a substantial compression of the mixed layer and the corresponding rise of available nutrients within the euphotic zone, shifting the chlorophyll maximum above the shallow thermocline. Although direct evidence of prolonged surface water cooling was not observed, we postulate that cooling and nutrient-enrichment episodes in the Gulf of Chiriquí may occur and that their intensity is dependent upon the depth to which the thermocline reaches in the eastern Pacific during the boreal winter. Nonetheless, the process is clearly much less intense than that in the Gulf of Panama. Despite substantial shifts in deeper water conditions in the Gulf of Chiriquí, surface waters remain warm and nutrient poor, presumably because wind stress is not strong enough to cause the advection of deep, cool, and nutrient-rich waters to the surface (D'Croz and O'Dea, 2007). However, ocean forces such as internal waves might change the oceanographic structure in the Gulf of Chiriquí, causing brief periods of advection of deep cold water to the surface layer (Dana, 1975). Long-term records from data loggers deployed in coral reefs give evidence of such brief SST drops in the Gulf of Chiriquí that are possibly related to internal waves (D'Croz and O'Dea, 2007). This effect might be more evident as the internal waves approach the shallow coasts around the islands in the Gulf of Chiriquí and may be more likely to occur during times of thermocline shallowing.

In conclusion, although the Gulf of Chiriquí does not experience the intense seasonal upwelling characteristic of the Gulf of Panama, deeper waters do migrate upward in synchrony with Gulf of Panama upwelling. This movement is probably caused by an overall shallowing of the thermocline across Central America. The difference in intensity of upward movement of the thermocline between

the two gulfs strongly influences the phytoplankton community, with seasonal blooms occurring in the Gulf of Panama but not in the Gulf of Chiriquí. Deeper waters do nonetheless experience similar patterns of seasonal hydrographic change, and shallow waters of the Gulf of Chiriquí can be exposed to brief pulses of cold and nutrient-rich waters by advection. However, the effects of thermocline migration and advection on the shallow-water communities of the Gulf of Chiriquí remain to be studied in detail.

Acknowledgments

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Growth and Nutrient Conservation in *Rhizophora mangle* in Response to Fertilization along Latitudinal and Tidal Gradients

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ABSTRACT. Mangroves form heterogeneous marine ecosystems with spatial differences in structural complexity, biodiversity, biogeochemistry, and hydrology that vary at local and regional scales. Although mangroves provide critical ecosystem goods and services, they are threatened globally by human activities, including nutrient over-enrichment. Our goal was to determine if enrichment with nitrogen (N) or phosphorus (P) interacts with forest structure and latitude to alter growth and nutrient dynamics. We established a series fertilization experiments across more than 2,000 km and 18° of latitude from the Indian River Lagoon (IRL), Florida, to Twin Cays, Belize, to Bocas del Toro, Panamá. At each site, we fertilized individual trees with one of three treatment levels (control, +N, +P) in two intertidal zones (fringe, scrub) and measured their responses for four years. We tested the effects of nutrient over-enrichment on growth, resorption efficiency, and resorption proficiency of the red mangrove *Rhizophora mangle*. All sites were nutrient limited, but patterns of nutrient limitation varied by zone and latitude. At IRL, growth was N limited; at Twin Cays, the fringe was N limited, but the scrub forest was P limited; at Bocas del Toro, the fringe was N limited, but the scrub forest was both N- and P limited. Nutrient enrichment had dramatic and complex effects on nutrient conservation. Adding nutrients to mangrove ecosystems affected growth and the nutrient recycling, but the pattern depended on location, site characteristics, and the nature of nutrient limitation. Predicting how forests will respond to nutrient over-enrichment requires an assessment of spatial heterogeneity at multiple scales of response.

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INTRODUCTION

Mangrove ecosystems are coastal wetlands dominated by woody plants that span gradients in latitude (30°N to 37°S), tidal height (<1 m to >4 m), geomorphology (oceanic islands to riverine systems), sedimentary environment (peat to alluvial), climate (warm temperate to both arid and wet tropics), and nutrient loading (oligotrophic to eutrophic). Throughout their distribution, mangroves are critical not only for sustaining biodiversity in these intertidal forests but also for their direct and indirect benefit to human activities. As a detritus-based ecosystem, the leaf litter from these trees provides the basis for adjacent aquatic and terrestrial food webs (Odum and Heald, 1975). Mangroves function as nurseries for many of the sport and commercial fishes found in deeper waters and provide feeding grounds

for large reef fishes (Nagelkerken et al., 2000; Mumby et al., 2004). As a result, mangrove-assimilated energy and nutrients are exported to surrounding coral reefs (Dittmar and Lara, 2001). Besides supporting and renewing coastal fishing stock, mangroves also benefit human economic development by stabilizing shorelines. This stabilization is a critical function in tropical coastal areas that may be battered periodically by tropical storms, hurricanes, and tsunamis (Danielson et al., 2005; Barbier, 2006).

Despite repeated demonstration of their ecological and economic importance, mangroves are one of the world's most threatened ecosystems (Valiela et al., 2001; Alongi, 2002; Barbier and Cox, 2003; Rivera-Monroy et al., 2004; Duke et al., 2007). In addition to direct destruction, increasing input of human-caused nutrient pollution is widely recognized as one of the major threats to mangroves and other marine environments worldwide (NRC, 1995, 2000, 2001; Duce et al., 2008). However, system-specific attributes may lead to large differences among coastal and estuarine systems in their sensitivity and susceptibility to these increasing nutrient levels (Cloern, 2001). The complex suite of direct and indirect responses in coastal systems to nutrient over-enrichment include changes in water chemistry, distribution and biomass of plants, sediment biogeochemistry, decomposition processes, nutrient cycling, nutrient ratios, phytoplankton communities, habitat quality for metazoans, and ecosystem functions.

Relatively little is known about how the structure and function of mangrove ecosystems are altered by nutrient enrichment. In temperate salt marshes and mangroves, ecological processes have been shown to be nitrogen- (N) limited (Valiela and Teal, 1979; Feller et al., 2003b). The few tropical and subtropical mangrove wetlands that have been studied were shown to be both phosphorus- (P) and N limited (Boto and Wellington, 1984; Feller, 1995; Feller et al., 1999, 2003a, 2003b; Lovelock and Feller, 2003; Lovelock et al., 2004). Because mangroves are responsive to processes operating at multiple spatial scales, comparisons along a broad latitudinal gradient in climate and across narrow tidal gradients will improve our understanding of the relative impacts of global versus local factors on the structure and function of these ecosystems. In this study, we focused on the mangrove *Rhizophora mangle* (red mangrove), an evergreen tree that has a large geographic range throughout the Atlantic-East Pacific region (Duke, 1992). Along the Atlantic coasts of North and South America, its distribution is continuous and spans almost 60° of latitude from its northern limit along the coast of Florida at 29°42.94′N (Zomlefer et al., 2006) to its southern limit along the coast of Brazil at 27°53′S (Shaeffer-Novelli et al., 1990). In this study, our goals were to determine how nutrient availability varies among *R. mangle* forests spanning a temperate to tropical gradient and how nutrient over-enrichment affects plant growth and nutrient conservation. We manipulated nutrient availability and measured responses of trees fertilized with nitrogen (+N) or phosphorus (+P) growing along intertidal gradients in similar habitats at three locations along this latitudinal gradient to test the following hypotheses.

1. Nutrient availability varies along a latitudinal gradient with a decreasing supply of P relative to N toward the tropics (Vitousek, 1984; Vitousek and Sanford, 1986; Crews et al., 1995). This hypothesis predicts increasing P limitation in mangrove forests at lower latitudes and N limitation at higher latitudes (Güsewell, 2004; McGroddy et al., 2004; Reich and Oleksyn, 2004; Kerkhoff et al., 2005).
2. Delivery, uptake, or assimilation of P is more strongly affected by tidal flushing and concomitant factors that vary spatially than is that of N (Smith, 1984; McKee et al., 2002). This hypothesis predicts differences in N versus P limitation within mangrove forests at different intertidal elevations (Ross et al., 2006). Specifically, N limitation is predicted for the low intertidal where tidal flushing is greater (residence time is shorter) than in the high intertidal where P limitation is predicted.
3. Because of difference in growth rates along climatic gradients, the mechanisms used by plants to recycle and conserve nutrients will be more efficient at higher latitudes (Oleksyn et al., 2003). This hypothesis predicts increased nutrient conservation by mangroves growing near their temperate limit (Lovelock et al., 2007).
4. As nutrient availability increases, nutrient conservation mechanisms become less efficient (Shaver and Melillo, 1984; Vitousek, 1984; Schlesinger et al., 1989; Escudero et al., 1992). This hypothesis predicts that the effects of nutrient loading on mangrove forests will differ depending on whether a system is N- or P limited, with the expectation that the limiting nutrient will be more efficiently and tightly conserved (Feller et al., 1999).

MATERIALS AND METHODS

Site Descriptions

We compared the effects of nutrient over-enrichment on plant growth and nutrient dynamics in *Rhizophora mangle* L. at three locations along the Atlantic and Caribbean coasts from Florida to Panamá spanning a climatic gradient of more than 2,000 km and 18° of latitude (Figure 1):

FIGURE 1. The three study sites used in this study span more than 18° of latitude and extend from the Indian River Lagoon (IRL), Florida, in the north, to Twin Cays, Belize, and to Bocas del Toro, Panama, in the south.

(1) Indian River Lagoon (referred to hereafter as IRL), Florida; (2) Twin Cays, Belize (referred to hereafter as Twin Cays); and (3) Bocas del Toro, Republic of Panamá (referred to hereafter as Bocas) (Table 1). Table 2 provides a summary of the characteristics for the three locations (Koltes et al., 1998; McKee et al., 2002; Feller et al., 2003a; Feller and Chamberlain, 2007; Lovelock et al., 2005). Forest structure at the three locations was heterogeneous and characterized by complex gradients in tree height that included a narrow seaward fringe of uniformly tall (~4 m) trees dominated by *R. mangle*, varying in width from 5 to 20 m (Figure 2). Tree height decreased rapidly to landward with interior areas dominated by old-growth stands of low stature, or "scrub," trees (~1.5 m) (Table 3). The black mangrove (*Avicennia germinans* L.) and the white mangrove (*Laguncularia racemosa* (L.) Gaertn. f.) were also present in each of these locations, typically near the landward ecotone. The hydrogeomorphic settings were variable among the three locations. IRL and Bocas were continental in contrast with Twin Cays, which is a low oceanic island. However, Twin Cays and Bocas were more similar in mineralogy (Phillips et al., 1997; Macintyre et al., 2004; Coates et al., 2005), with mangrove forests atop a carbonate platform and deep peat deposits. All sites were microtidal with mixed semidiurnal tides (Kjerfve et al., 1982; Kaufmann and Thompson, 2005). The fringe zones at the three locations were similarly well flushed, but the hydrological conditions of the scrub zones varied. At Twin Cays, these interior portions of the forest were completely inundated and waterlogged (McKee et al., 2007). In contrast, the Bocas scrub zone drained completely at low tide (Lovelock et al., 2005). At IRL, the scrub zone drained completely at low tide during the summer but remained inundated for days during the winter (Feller et al., 2003b).

In the IRL, our experimental sites were situated on the lagoonal side of two barrier islands. The fringe site was in Avalon State Park on North Hutchinson Island, St. Lucie County; the scrub site was in the Hobe Sound National Wildlife Refuge on Jupiter Island, Martin County. In this area, soil was composed primarily of marine sand with mangrove forests adjacent to coastal strand vegetation and maritime hammocks. Descriptions of forest

TABLE 1. Hydrogeomorphic characteristics of the study sites along a latitudinal gradient from the Indian River Lagoon (IRL), Florida, to Twin Cays, Belize, to Bocas del Toro (Bocas), Panama.

Characteristic	IRL	Twin Cays	Bocas
Latitude	27°33′N, 80°13′W	16°50′N, 88°06′W	9°09′N, 82°15′W
Freshwater inflow	Medium	Low	High
Type of landscape	Continental	Oceanic	Continental
Topographic relief	Medium	Low	High
Nutrient flux	High	Low	Medium
Mineralogy	Siliclastic/carbonate	Peat/limestone	Peat/limestone
Annual rainfall	1.3 m	2.8 m	3.5 m
Mean temperature range [a]	12.4°–23.6°C (w) 22.6°–31.9°C (s)	18.3°–29.9°C (w) 22.2°–31.3°C (s)	20.1°–31.1°C (w) 21.9°–31.8°C (s)
Mean tidal range	37 cm	34 cm	19 cm
Major disturbances	Anthropogenic, hurricanes	Anthropogenic, hurricanes	Anthropogenic, flooding

[a] w = winter; s = summer.

TABLE 2. Characteristics of the mangrove forest structure in the fringe and scrub zones at the Indian River Lagoon (IRL), Florida, to Twin Cays, Belize, to Bocas del Toro (Bocas), Republic of Panama. Data are from Koltes et al. (1998), McKee et al. (2002), Feller et al. (2003a), Lovelock et al. (2005), and Feller and Chamberlain (2007).

Location	Zone	Salinity (‰) (mean ± SE)	Species	Tree height (m) (mean ± 1 SE)	DBH (cm) (mean ± 1 SE)	Stem density (stems·0.1 ha^{-1})	Basal area (m^2·0.1 ha^{-1})
IRL	Fringe	32.7 ± 0.7	*Rhizophora mangle*	3.9 ± 0.1	4.5	3,9536.4	
			Laguncularia racemosa	3.2 ± 0.3	6.1	1,3433.9	
			Avicennia germinans	3.8 ± 0.3	4.8	6711.2	
	Scrub	32.4 ± 0.5	*Rhizophora mangle*	1.7 ± 0.1	2.5 ± 0.2	2,3861.5	
			Laguncularia racemosa	4.5 ± 0.4	4.6 ± 0.7	4771.0	
			Avicennia germinans	1.6 ± 0.0	1.2 ± 0.0	730.01	
Twin Cays	Fringe	36.9 ± 1.2	*Rhizophora mangle*	3.2 ± 0.2	7.3 ± 0.4	4012.1	
			Laguncularia racemosa	2.2 [a]	2.9 [a]	3 [a]0.2 [a]	
			Avicennia germinans	2.2 [a]	4.0 [a]	3 [a]0.01 [a]	
	Scrub	39.4 ± 1.2	*Rhizophora mangle*	0.8 ± 0.1	2.4 ± 0.2	8970.4	
Bocas	Fringe	34.4 ± 0.6	*Rhizophora mangle*	3.9 ± 0.1	5.3 ± 0.6	8501.6	
	Scrub	33.3 ± 1.9	*Rhizophora mangle*	0.7 ± 0.1	1.5 ± 0.1	3,3570.7	

[a] Based on occurrence of a single tree in each zone.

structure, hydro-edaphic conditions, growth, nutrient dynamics, and photosynthesis at the Avalon State Park site were previously reported (Feller et al., 2003a; Lovelock and Feller, 2003).

At Twin Cays, our fringe and scrub sites were located on the two largest islands of this 92-ha mangrove archipelago, 10 km offshore. Descriptions of forest structure, biogeochemistry, ecophysiology, growth, and nutrient dynamics were previously reported (Rützler and Feller, 1996; McKee et al., 2002; Feller et al., 2003b, 2007; Lovelock et al., 2006a, 2006b, 2006c, 2006d). These oceanic mangroves islands are underlain by deep deposits of mangrove peat 8 to 12 m thick (Macintyre et al., 2004; McKee et al., 2007).

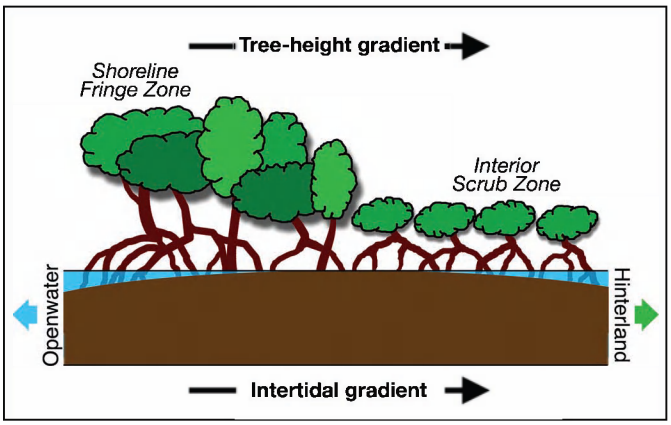

FIGURE 2. Mangrove forests at each of our study sites are characterized by a distinctive tree-height gradient with tall trees fringing the shoreline and scrub trees in the interior.

At Bocas, fringe and scrub sites were located on three islands (San Cristobal, Solarte, Isla Popa) in Almirante Bay and the Chiriqui Lagoon in a vast network of mangrove islands and mainland peninsulas covering approximately 2,885 km^2 (De Croz, 1993; Guzman and Guevara, 1998; Guzman et al., 2005; Lovelock et al., 2004, 2005). Here, mangroves occurred adjacent to tropical rainforests and grew on peat approximately 5 m deep atop ancient coral reef limestone (Phillips and Bustin, 1996; Phillips et al., 1997). This location was outside the hurricane belt, but flooding was common. Earthquakes are episodic (Phillips et al., 1994, 1997; Phillips and Bustin, 1996) and are likely to be the major nonanthropogenic disturbance regime influencing these forests.

Experimental Design

Fertilization experiments were set up at IRL in January 1997, at Twin Cays in January 1995, and at Bocas in January 1999. To compare responses, we used a three-way factorial analysis of variance (ANOVA) design (i.e., 3 nutrient enrichment treatment levels [Control, +N, +P] × 2 zones [fringe, scrub] × 3 locations [IRL, Twin Cays, Bocas] × 3 sites per location × 3 replicate trees per site, for a total of 162 trees). Nutrient treatment was randomly as-

TABLE 3. Three-way factorial analysis of variance (ANOVA) results on the seven response variables: shoot elongation (Growth), N-, P-, and K-resorption efficiencies (NRE, PRE, KRE), and N-, P-, and K-resorption proficiencies (NRP, PRP, KRP). The kind of transformation conducted on response variables for normalization and homogeneity of variances is given in the second line of column headings. Results are in the form of *F* statistical values for each effect and the corresponding level of significance: $***P < 0.001$; $**P < 0.01$; $*P < 0.05$; and ~ for $P < 0.1$.

		Growth *Log(x)*		NRE *Exp(x)*		PRE *Exp(4x)*		KRE *Exp(x)*		NRP *Log(x)*		PRP *Log(1000x)*		KRP *Log(x)*	
Factor	*df*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Location (L)	2	24.2	***	28.6	***	126	***	16.5	***	86.1	***	373	***	18.9	***
Zone (Z)	1	0.11		65.2	***	4.57	*	0.96		75.4	***	0.29		5.18	*
Treatment (T)	2	23.4	***	10.4	***	75.4	***	14.6	***	40.3	***	108	***	36.8	***
L × Z	2	12.0	***	3.21	*	1.21		0.78		2.88	~	7.15	**	1.98	
L × T	4	12.9	***	4.37	**	12.5	***	5.60	***	2.16	~	23.6	***	8.13	***
Z × T	2	17.8	***	3.27	*	4.36	*	8.97	***	4.77	**	13.4	***	25.0	***
L × T × Z	4	4.9	**	4.35	**	1.30		4.27	**	7.95	***	4.21	**	8.61	***
Residuals	140														

signed within each zone and site. Trees were amended with 150 g N as NH_4 (45:0:0), or P fertilizer as P_2O_5 (0:45:0), per centimeter diameter breast height, as described in Feller (1995). Doses (150 g) of fertilizer were sealed in dialysis tubing and placed in each of two holes 30 cm deep, cored into the substrate on opposing sides of a tree beneath the outermost margin of its canopy, and sealed. Experiments at IRL and Twin Cays were fertilized twice per year. Because of limited access, the Bocas experiment was fertilized once per year. Thus, growth responses were normalized to the annual rate of fertilizer application. For controls, holes were cored and sealed but no fertilizer was added. Direct fertilizer application to the root zone of our target trees was used because all sites were flooded at high tides and fertilizer broadcasted on the surface would have washed away.

Tree Growth

To quantify growth, we measured the length of five initially unbranched shoots in sunlit positions in the outer part of the canopy of each tree at the three locations. To compare growth responses among the three locations, we calculated the annual shoot elongation based on the amount fertilizer added per location ($cm \cdot year^{-1}\ kg^{-1}$).

Leaf Nutrient Dynamics

To determine the relative effects of nutrient overenrichment on the ability of *R. mangle* to conserve nutrients invested in foliage, we measured N, P, and potassium (K) concentrations in green and senescent leaves. For green leaves, we sampled the youngest, fully mature green leaves from penapical stem positions in sunlit portions of the canopy. Fully senescent yellow leaves with well-developed abscission layers were taken directly from the trees. Leaf area was determined with a Li-Cor 3000 Leaf Area Meter (Lincoln, Neb., USA). Leaf samples were dried at 70°C in a convection oven and ground in a Wiley Mill to pass through a 40 mesh (0.38 mm) screen. Concentrations of carbon (C) and N were determined with a Model 440 CHN Elemental Analyzer (Exeter Analytical, North Chelmsford, Mass., USA) at the Smithsonian Environmental Research Center, Edgewater, Md. Concentrations of P and K were determined using an inductively coupled plasma spectrophotometer by Analytical Services, Pennsylvania State University, Pa. Nutrient concentrations expressed on a leaf area basis ($mg \cdot cm^{-2}$) were used to calculate N, P, and K resorption efficiencies (NRE, PRE, KRE), as below (Chapin and Van Cleve, 1989):

$$\text{resorption efficiency} = \frac{\text{N, P, or K (mg} \cdot \text{cm}^{-2})_{\text{green leaf}} - \text{N, P, or K (mg} \cdot \text{cm}^{-2})_{\text{senescent leaf}}}{\text{N, P, or K (mg} \cdot \text{cm}^{-2})_{\text{green leaf}}} \times 100$$

The absolute levels to which N, P, and K were reduced (% dry mass) in senesced leaves (indicated as $\%N_{senesced\ leaf}$, $\%P_{senesced\ leaf}$, and $\%K_{senesced\ leaf}$, respectively) were used directly as indices of N, P, and K resorption proficiencies (NRP, PRP, KRP), as below (Killingbeck, 1996):

resorption proficiency = the level to which N, P, or K has been reduced in senescent leaves (% dry mass).

Note that low levels for $\%N_{senesced\ leaf}$, $\%P_{senesced\ leaf}$, and $\%K_{senesced\ leaf}$ are indicative of high resorption proficiency whereas high levels indicate low resorption proficiency. Concentrations less than 0.7% are considered complete resorption for N and concentrations less than 0.04% are considered complete resorption for P (Killingbeck, 1996). Higher values indicate incomplete resorption. In this study, we considered values less than 0.3% N and less than 0.01% P as the ultimate resorption potential for *R. mangle*, as proposed by Killingbeck (1996). Comparable values for K resorption potential have not been determined.

Statistics

Our data were grouped by nutrient treatment (Control, +N, +P) × zone (fringe, scrub) × location (IRL, Twin Cays, Bocas), to compare seven response variables of *R. mangle*, including growth responses, N-, P-, and K-resorption efficiencies, and N-, P-, and K-resorption proficiencies. Three-way factorial analyses of variance (ANOVA) were applied for each response variable. When an ANOVA found significant effects, Tukey's honestly significant difference (HSD) tests were applied to examine pairwise differences within and among the treatment levels. To respect the assumptions of heterogeneity of variances and normality, the response variables were transformed using logarithms and exponentials. To investigate relationships between nutrient content of green and senescent leaves as well as among nutrient resorption proficiencies, we used the Spearman rho (ρ) correlation test on the ranked row values. These analyses were conducted using the R software 2.7.0 (R Development Core Team, 2008).

RESULTS

Tree Growth

There was a significant three-way interaction of nutrient enrichment × location × zone on growth rates of *R. mangle* trees (see Table 3; Figure 3). For control trees in the fringe zone, the rate of shoot elongation at IRL was signifi-

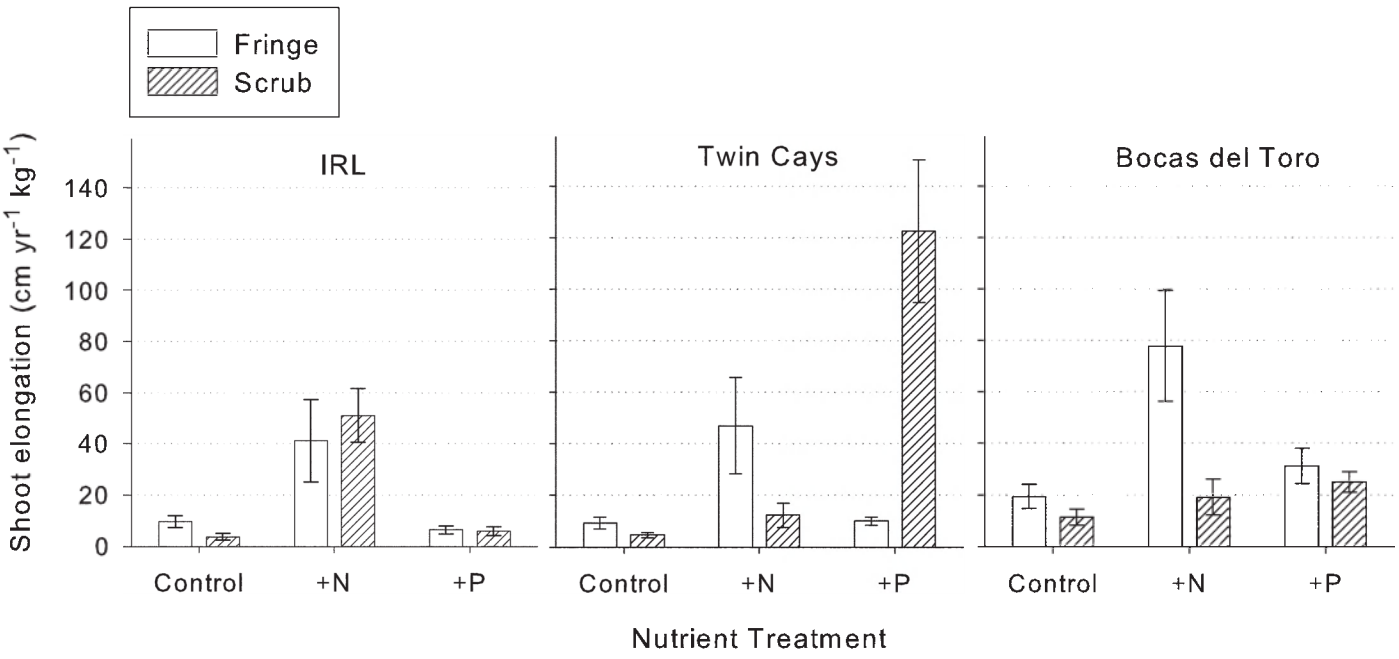

FIGURE 3. *Rhizophora mangle* growth (cm · year^{-1} · kg^{-1}) measured as elongation of individual shoots per year (normalized to fertilizer application at each site) at Indian River Lagoon (IRL), Twin Cays, and Bocas del Toro, in two zones (fringe, scrub), and in response to nutrient enrichment with nitrogen (+N) or phosphorus (+P). (IRL and Twin Cays data from Feller et al., 2003a, 2003b).

cantly lower than at Bocas (HSD adjusted $P < 0.001$) but similar to those at Twin Cays (HSD adjusted $P = 0.070$), which had similar values. There were no significant differences in shoot elongation rates for control trees in the scrub zone among all the locations. +N caused significant increases in shoot elongation rates for fringe and scrub trees at IRL, but only for fringe trees at Twin Cays and Bocas. However, shoot elongation for +N fringe trees in the IRL was lower than observed at Bocas (HSD adjusted $P = 0.089$). +N caused similar increases in shoot elongation in the fringe at Bocas and Twin Cays. In the scrub zone, +P increased growth at Twin Cays (HSD adjusted $P < 0.001$) and Bocas (HSD adjusted $P = 0.095$), although the rates were much higher for Twin Cays (HSD adjusted $P = 0.047$). +P had no effect on growth in either fringe or scrub zones at IRL. The +N treatment had no effect on growth rates in the scrub zones at Twin Cays and Bocas.

Nutrient Conservation

The impact of fertilization on N-, P-, and K-resorption efficiencies varied by location and zone (Figure 4a–c). For N-resorption efficiency (NRE), there was a significant three-way interaction among location, zone, and nutrient enrichment (see Table 3; Figure 4a). Values ranged from 26% to 68%. In control trees at all locations, NRE was consistently highest for the fringe. At IRL, +N caused a slight decline in values for fringe but not scrub trees. At Twin Cays, +N had no effect on NRE in the fringe where growth was N limited. However, +P caused an approximately 40% increase in NRE for the P-limited scrub trees (HSD adjusted $P < 0.001$). Although +N had no effect on the growth of scrub trees at Twin Cays, it did result in a slight increase in NRE. Overall, values for NRE were lowest at Bocas.

There were significant two-way interactions among nutrient enrichment × location and nutrient enrichment × zone on P-resorption efficiencies. However, the three-way interaction among nutrient enrichment × location × zone was not significant (see Table 3, PRE; Figure 4b). PRE values ranged from 36% to 80%. Overall, IRL had the lowest PRE. Here, values for control fringe and scrub trees were approximately half those at Twin Cays and Bocas where values were similar. +N caused a slight increase in PRE for IRL fringe and scrub trees. At Twin Cays and Bocas, +N had no effect in either zone, but +P caused an approximately 50% decrease in PRE for scrub trees and an approximately 25% decrease for fringe trees.

For K-resorption efficiency (KRE), we found a significant three-way interaction of nutrient enrichment × location × zone (see Table 3; Figure 4c). In the IRL, values were uniformly low but positive in both zones, and nutrient enrichment had no effect. For control fringe trees at all locations, KRE was consistently positive. Overall, the lowest KRE values occurred at Twin Cays. The negative values for senescent foliage from control scrub trees at Twin Cays and Bocas indicated that K accumulated in leaves rather than being resorbed by the plant during senescence. At Twin Cays and Bocas, +P caused a significant increase in KRE by scrub trees, but had little effect on fringe trees. However, +N had no significant effect on KRE in either zone.

Fertilization also had striking and complex effects on resorption proficiencies, measured as the $\%N_{\text{senesced leaf}}$, $\%P_{\text{senesced leaf}}$, and $\%K_{\text{senesced leaf}}$, that varied by location and zone (Table 3; Figure 5a–c). Concentrations of N, P, and K in senesced leaves were positively associated with their concentrations in green leaves (Spearman ρ values for N, P, and K = 0.52, 0.87, and 0.65, respectively, all significantly different than 0 with $P < 0.0001$). There was no relationship between $\%N_{\text{senesced leaf}}$ and $\%P_{\text{senesced leaf}}$ (Spearman $\rho = 0.03$, $P = 0.66$), but $\%K_{\text{senesced leaf}}$ was significantly correlated with $\%N_{\text{senesced leaf}}$ (Spearman $\rho = 0.19$, $P = 0.02$) and with $\%P_{\text{senesced leaf}}$ (Spearman $\rho = -0.43$, $P < 0.0001$). For NRP, there was a significant three-way interaction among location, zone, and nutrient enrichment (see Table 3; Figure 5a). The $\%N_{\text{senesced leaf}}$ ranged from a low of 0.28% for +P scrub trees at Twin Cays to a high of 0.91% in +N fringe trees at Bocas. For control trees from the fringe and scrub zones, values were similar at IRL and Bocas but were significantly lower at Twin Cays, which indicated increased NRP. +N caused an increase of 20% in $\%N_{\text{senesced leaf}}$ from the fringe at IRL but had little effect on fringe trees at the other locations. In the scrub zone, +N had no effect on $\%N_{\text{senesced leaf}}$ at IRL and Twin Cays, but significantly higher values at Bocas resulted in a decrease in NRP. +P had little effect on either fringe or scrub zones at IRL and Bocas, but it caused a dramatic decrease in $\%N_{\text{senesced leaf}}$ and a corresponding increase in NRP in scrub trees at Twin Cays.

We found the highest levels of $\%P_{\text{senesced leaf}}$ (~0.06%) in the control trees in both zones at IRL, which indicated low PRP compared to Twin Cays and Bocas. Fertilization with +N or +P had no detectable effect on these levels at IRL (Figure 4b; all HSD adjusted $P > 0.5$). Very low levels (~0.01%) of $\%P_{\text{senesced leaf}}$ in both zones at Twin Cays and Bocas indicated high PRP in the range of maximal P resorption (Figure 6). +N had no effect on values in either zone at Twin Cays or Bocas. +P caused the most dramatic increase in $\%P_{\text{senesced leaf}}$, with a concomitant decrease in

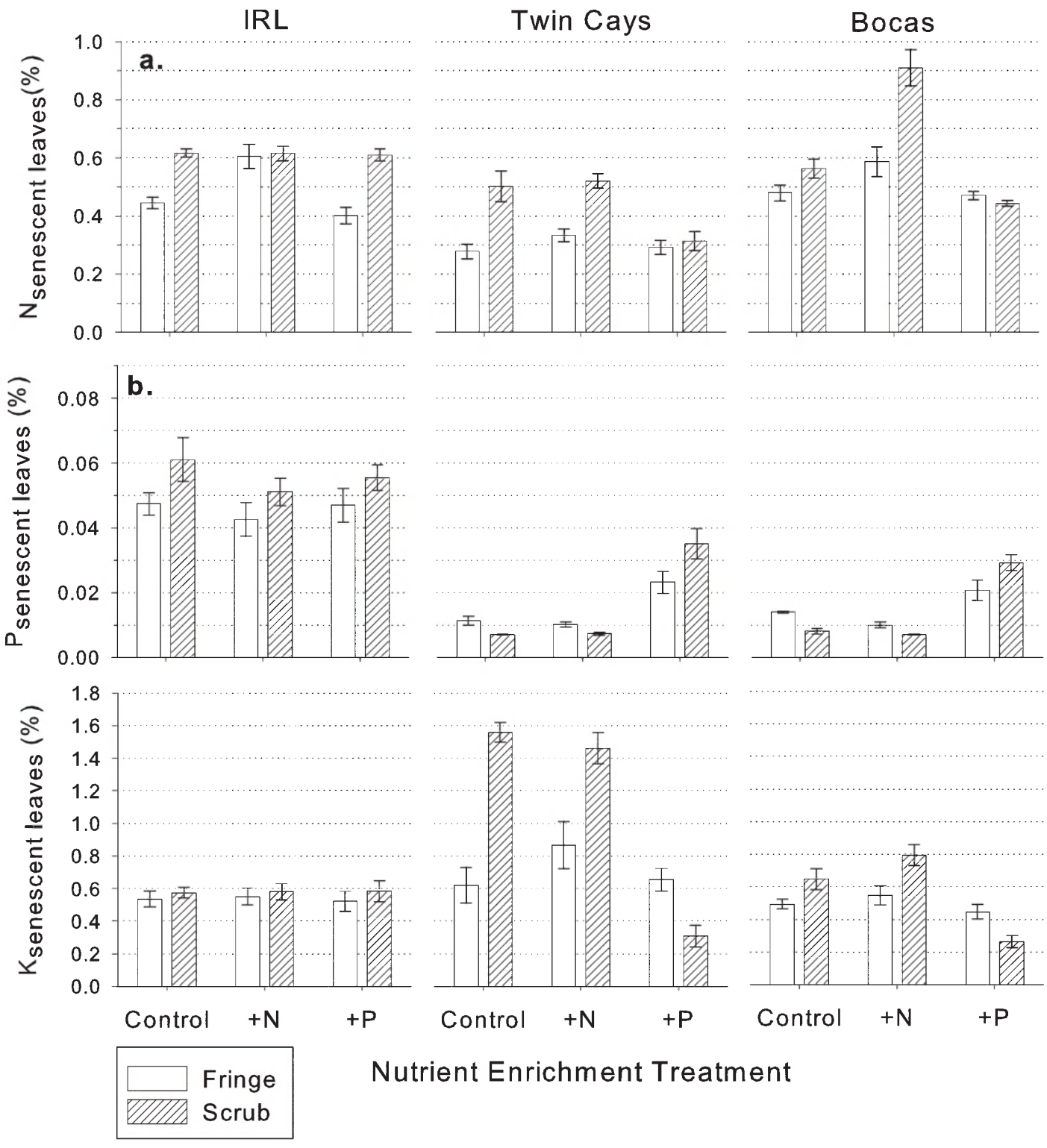

FIGURE 4. Resorption efficiencies for (a) nitrogen (N), (b) phosphorus (P), and (c) potassium (K) at Indian River Lagoon (IRL), Twin Cays, and Bocas del Toro (Bocas) in two zones (fringe = open bars, scrub = hatched bars), and in response to nutrient enrichment with nitrogen (+N) or phosphorus (+P). (IRL and Twin Cays data from Feller et al., 2003a, 2003b).

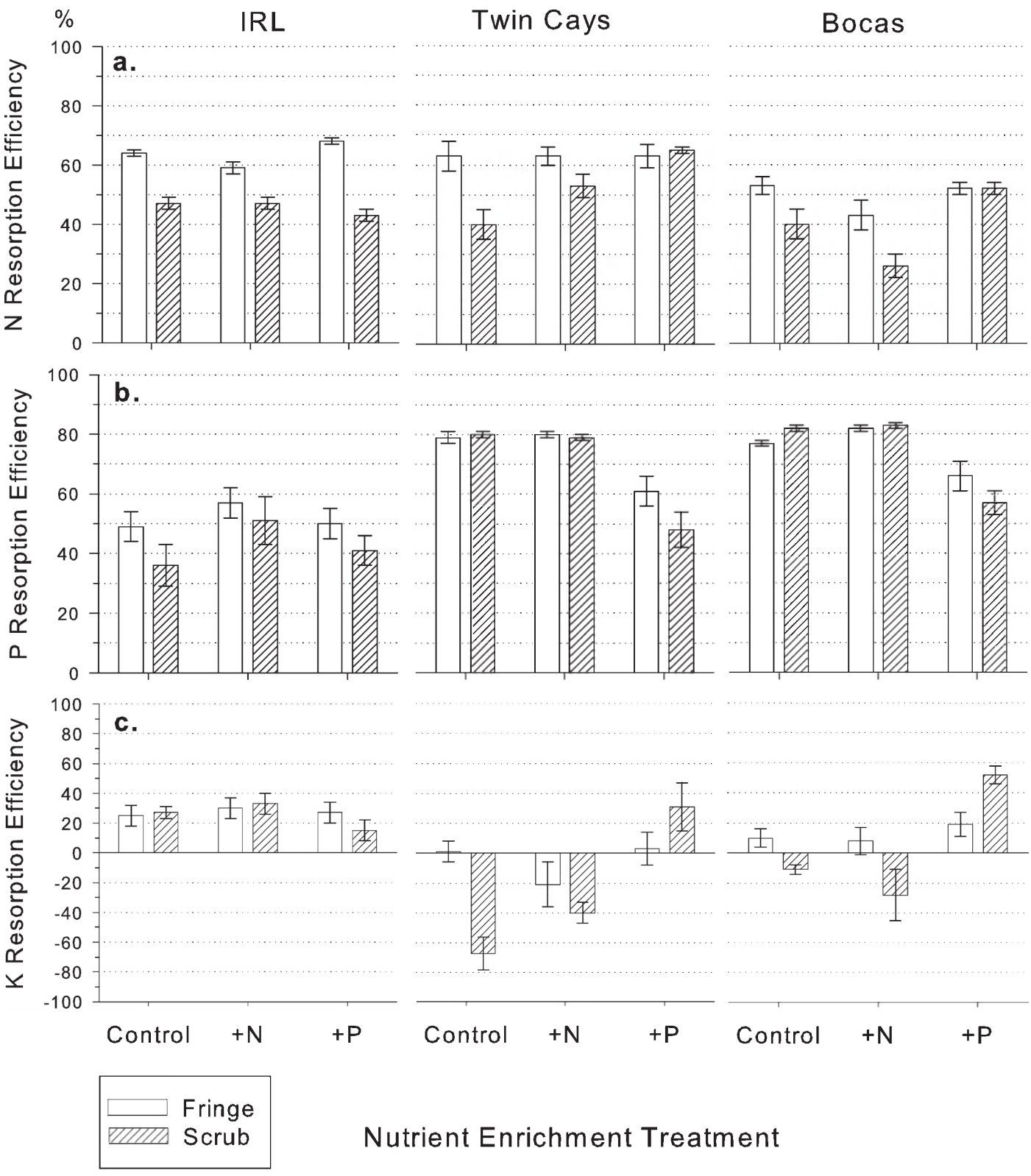

FIGURE 5. Resorption proficiencies for (a) nitrogen, (b) phosphorus, and (c) potassium at Indian River Lagoon (IRL), Twin Cays, and Bocas del Toro (Bocas) in two zones (fringe, scrub), and in response to nutrient enrichment with nitrogen (+N) or phosphorus (+P).

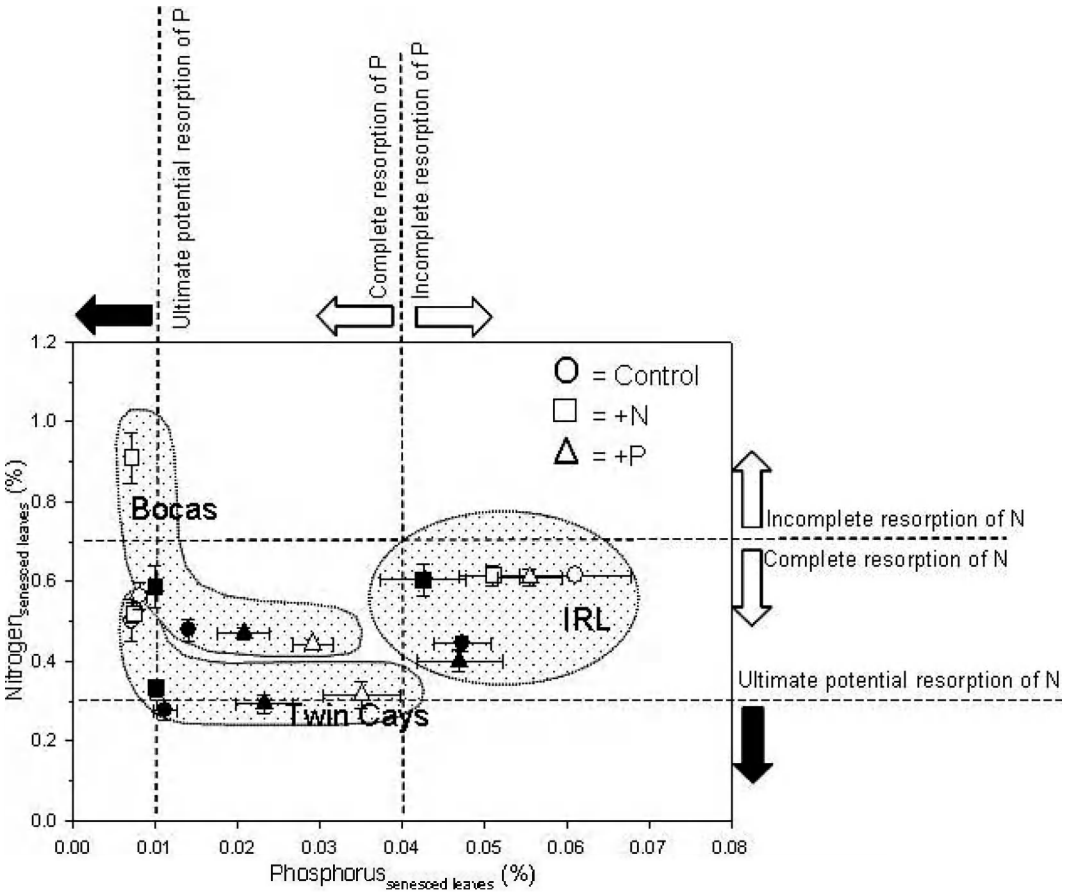

FIGURE 6. N-resorption proficiency ($\%N_{senesced\ leaf}$) versus P-resorption proficiency ($\%P_{senesced\ leaf}$) for *Rhizophora mangle* by location (Indian River Lagoon [IRL], Twin Cays, Bocas del Toro), nutrient enrichment treatment level (control [○], +N [□], +P [△]), and intertidal zone (fringe [closed symbols] or scrub [open symbols]).

PRP, for both the fringe and scrub zones at Twin Cays (both HSD adjusted $P < 0.001$). At Bocas, there was a similar increase in $\%P_{senesced\ leaf}$ with +P in the scrub zone (HSD adjusted $P < 0.001$), whereas the response in the fringe zone was comparatively smaller and not significant (HSD adjusted $P = 0.694$).

There was also a significant three-way interaction among location, zone, and nutrient enrichment on KRP (see Table 3; Figure 5c). Values for control trees in both zones at IRL and Bocas were similar with no differences between zones. Neither +N nor +P had any effect at IRL, but +P caused a significant decrease in $\%K_{senesced\ leaf}$ in both zones at Bocas. The $\%K_{senesced\ leaf}$ ranged from a low of 0.26% for +P scrub trees at Bocas to a high of 1.56% in control scrub trees at Twin Cays, which was more than double the K concentrations in senescent foliage of fringe trees. In the Twin Cays scrub zone, +P caused a fourfold decrease in $\%K_{senesced\ leaf}$, resulting in an associated increase in KRP.

DISCUSSION

Long-term fertilization experiments at IRL, Twin Cays, and Bocas del Toro demonstrated that these three locations, which were arrayed along a latitudinal gradient, were nutrient limited. However, system-specific attributes resulted in significant differences in patterns of nutrient limitation and responses to fertilization. Although the mangrove ecosystems at these locations exhibited similar tree-height gradients dominated by *Rhizophora mangle*, they differed in several hydrogeomorphic and structural

features (see Tables 1, 2). The locations also differed in substrate types; that is, the soil at IRL was composed of Pleistocene marine sands while the soils at Twin Cays and Bocas site were deep deposits (6–12 m) of mangrove peat formed during the Holocene (Phillips and Bustin, 1996; Lovejoy, 1998; Macintyre et al., 2004). Our experimental site in the IRL was in a young forest, less than 40 years old, in an abandoned mosquito impoundment (Rey et al., 1986). In contrast, the experiments at Twin Cays and Bocas were in old-growth forests. Although no data are available for a direct comparison, it is likely that the forests at Bocas are older than at Twin Cays because of differences in their exposures to hurricanes (Stoddart, 1963; Carruthers et al., 2005). Overall, stem density was lowest at Twin Cays. Stem density in the IRL fringe was approximately 10 times greater than at Twin Cays and 4 times greater than at Bocas. On the other hand, the density of trees in the scrub forest was highest at Bocas.

Growth of *R. mangle* stems, which we used as a bioassay of nutrient limitation in our fertilization experiments, varied among IRL, Twin Cays, and Bocas. However, the responses did not support Hypothesis 1 of increasing P limitation toward the tropics (Vitousek, 1984; Vitousek and Sanford, 1986; Crews et al., 1995). This hypothesis predicted that P limitation would be greatest at Bocas, which was located at the lowest of the three latitudes compared in this study. Instead, shoot elongation indicated an order that ranged from N limitation in both fringe and scrub zones at IRL, to N limitation in fringe and scrub as well as P limitation in scrub at Bocas, and to N limitation in fringe and P limitation in scrub at Twin Cays. The magnitude of the growth responses to fertilization with the limiting nutrient at each location was also consistent with this order, that is, IRL < Bocas < Twin Cays, with the most severe P limitation and the greatest growth response to P fertilization in the scrub zone at Twin Cays.

The differences in growth responses that we observed at the three locations suggest that nutrient limitation within and among mangrove ecosystems is likely determined by several features of their geomorphology, including sediment/nutrient flux, tidal range, and substrate type. These findings contrast with other studies that attribute P limitation in the tropics mainly to differences in the age of soils between tropical and temperate regions, with the most P-limited forests on the oldest soils (Vitousek, 1984; Vitousek and Sanford, 1986; Crews et al., 1995; Güsewell, 2004; McGroddy et al., 2004; Reich and Oleksyn, 2004; Kerkhoff et al., 2005).

Based on findings from Twin Cays (Feller, 1995; Feller et al., 2003a, 2007), McKee et al. (2002) hypothesized that the shift from N limitation in fringe zone around the periphery of the island to severe P limitation in scrub zone in the interior was the result of differences in factors associated with tidal flushing. Our results from the other two locations compared in this study partially support this hypothesis. Although all locations were N limited in the fringe, growth in the scrub zone at Bocas was limited by both N and P. This finding again differs from the IRL where growth was N limited in both zones (Feller et al., 2003b). These patterns along tidal gradients indicate that differences in nutrient limitation among the three locations are the result of variations in tidal flushing, external nutrient supply, substrate, and endogenous biological processes. The scrub forests in the interior areas have a low tidal exchange and a low supply of exogenous nutrients, whereas the fringe zones are well flushed with a higher net exchange of nutrients. Mangroves at the IRL and Bocas locations are in continental settings with medium to high relief, freshwater inflow, and nutrient flux. However, their tidal regimes and underlying soils differ dramatically. In contrast with IRL where mangroves are growing on sandy soils, mangroves at Twin Cays and Bocas are growing on peat. Although both of these locations are associated with low-nutrient coral reef ecosystems, Twin Cays receives negligible terrigenous inputs of freshwater or sediments whereas Bocas mangroves experiences a high flux of nutrients from several rivers draining into the archipelago. In addition, patterns of nutrient limitation in these systems may be affected by local patterns of N_2 fixation (Joye and Lee, 2004; Borgatti, 2008).

Resorption of phloem-mobile nutrients from leaves during senescence is an important nutrient conservation strategy for plants that influences many ecological processes, including primary production, nutrient uptake, competition, and nutrient cycling (Chapin, 1980). To resolve the relative degree to which latitude and nutrient enrichment affect the ability of *R. mangle* to conserve nutrients invested in foliage, we examined resorption of N, P, and K. Across location, zone, and nutrient treatment levels, our results indicate that a major control of the nutrient concentrations in senesced leaves was nutrient concentration in green leaves, which is consistent with a global dataset compiled by Kobe et al. (2005). Specifically, concentrations of N, P, and K in senesced leaves were positively associated with their concentrations in green leaves. In contrast to Oleksyn et al. (2003), who predicted that nutrient resorption efficiencies should increase with latitude, we found the lowest efficiencies at IRL, our northernmost location, consistent with Lovelock et al. (2007). We also found the most efficient nutrient conservation for

N and P at Twin Cays, the location positioned at the intermediate latitude. Although the levels to which nutrients were conserved varied by nutrient, location, and zone, the patterns did not fall clearly along a latitudinal gradient. All experimental trees at the three locations, except for the +N trees in the scrub zone at Bocas, had less than 0.7% N concentrations in their senescent leaves, which is within the range of complete resorption in the model proposed by Killingbeck (1996) (see Figure 6). In the Twin Cays fringe and the +P-fertilized scrub trees, the N concentration in senesced leaves was less than 0.3%, which was found to be the maximal level to which N can be reduced in senescent leaves of evergreen species and is regarded by Killingbeck (1996) as the ultimate potential resorption for N. In Killingbeck's model, less than 0.04% $P_{senesced\ leaf}$ represents complete resorption of P for evergreens. All experimental trees at Twin Cays and Bocas had values below this threshold and thus exhibited complete P resorption. Moreover, control and +N trees in the scrub and fringe zones at Twin Cays and Bocas had 0.01% $P_{senesced\ leaf}$ or less, which is the maximal level to which P can be reduced in senescent leaves in evergreens representing the ultimate potential resorption of P. Comparable levels of $\%P_{senesced\ leaf}$ have been reported for mangroves elsewhere (Alongi et al., 2005). In contrast, all the trees at IRL had values for $P_{senesced\ leaf}$ greater than 0.04%, which represents incomplete resorption. In contrast to suggestions by Aerts and Chapin (2000), the results presented here indicate there are nutritional controls on nutrient resorption in *R. mangle*. Nutrient enrichment clearly altered resorption of N and P at Twin Cays and Bocas but had no effect at IRL. Enrichment with +P resulted in increases in N and K resorption efficiency and proficiency at Twin Cays and Bocas but had the opposite effect on P resorption. Similarly, +N decreased N resorption, but only in the N-fertilized trees in the scrub zone at Bocas. These findings suggest that P enrichment may have either increased the requirements for N and K in *R. mangle* or it may have increased its physiological capacity to conserve these nutrients during leaf senescence. Increased resorption of N and K in response to +P may also indicate that under P-limiting conditions these nutrients become limiting when P is added to the system. Although we found no relationships between growth and N or P concentrations in green leaves, we did observe a weak but significant relationship between $\%K_{green\ leaf}$ and growth rates ($r = 0.230$, $F = 8.723$, $P < 0.01$). These results indicate that K availability may be important to the structure and function of some mangrove forests (Kathiresan et al., 1994), which warrants further study.

In conclusion, our results indicate that nutrient overenrichment of the coastal zone will alter forest structure and nutrient dynamics in mangrove ecosystems. We showed that fertilization altered growth and nutrient conservation in *R. mangle*, but the patterns did not correspond with a latitudinal gradient. Growth was consistently N limited for trees in fringing forests, which have higher water exchange rates compared to scrub forests, supporting the hypothesis of Smith (1984) and McKee et al. (2002) that open systems are more likely to be N limited than P limited. In the IRL, scrub trees in the interior of the forest were also N limited. Patterns of nutrient limitation became more complex at lower latitudes. Phosphorus limitations characterized the scrub zone at Twin Cays whereas both N and P limitations were widespread in the scrub zone at Bocas. Our results clearly indicated that the phenotypic potential of *R. mangle* to resorb N, P, and K from senescing leaves varied as a function of nutrient availability, which was driven by differences in hydrology and substrate along latitudinal and tidal gradients.

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Underwater Spectral Energy Distribution and Seagrass Depth Limits along an Optical Water Quality Gradient

Charles L. Gallegos, W. Judson Kenworthy, Patrick D. Biber, and Bret S. Wolfe

ABSTRACT. We measured in situ inherent optical properties and seagrass maximum depth distribution in widely differing optical water types, including turbid green waters of the Indian River Lagoon (IRL, Florida, USA), a mix of turbid and clear waters in Panama, and very clear waters in Belize. We used Hydrolight to model in situ spectral energy distributions and measured leaf absorbance spectra (*Thalassia testudinum*) to distinguish between photosynthetically available radiation (PAR) and photosynthetically usable radiation (PUR). Attenuation coefficients for PAR and PUR were nearly indistinguishable in Belize and Panama and differed only slightly in the IRL. Grass grew to depths of penetration of 33% of PAR in the IRL, 14% in Panama, and approximately 5% in Belize, although we expect the value for Belize is an underestimate because conditions more turbid than are typical were prevailing at the time of the measurements. Corresponding percentages for PUR were 27%, 12%, and 5% for IRL, Panama, and Belize, respectively. These regional differences in light requirements were striking, and less than half of the difference could be attributed to latitudinal variations in incident light. We conclude that factors other than spectral energy distribution that covary with water clarity control site-specific light requirements of seagrasses. Possibilities include epiphytes and sediment quality.

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INTRODUCTION

Seagrasses are important primary producers that play a role in the stability, nursery function, biogeochemical cycling, and trophodynamics of many coastal and estuarine ecosystems and as such are important for sustaining a broad spectrum of organisms (Hemminga and Duarte, 2000). Seagrasses are potentially sensitive indicators of declining water quality because of their high light requirements (11%–37% surface irradiance) compared to those of other aquatic primary producers with much lower light requirements (<1%) (Dennison et al., 1993; Zimmerman, 2003). Seagrass communities have declined in coastal regions worldwide (Orth et al., 2006), which is usually attributed to reductions in water clarity brought about, at least initially, by accelerated eutrophication in the coastal zone (Krause-Jensen et al., 2008).

Management efforts aimed at preserving and restoring seagrass systems generally focus on improving water clarity (Batiuk et al., 2000; Kenworthy and Haunert, 1991; Steward and Green, 2007), based on the high light requirements

of seagrasses and the reduction in light penetration associated with eutrophication (Ralph et al., 2007). Deciding on the extent of water quality improvements (or limit of allowable deterioration) requires more detailed knowledge of the wavelength-specific light requirements of seagrasses. Based on a survey of available literature, Carter et al. (2000) determined that mesohaline and polyhaline submerged grass communities in Chesapeake Bay require a long-term average of 22% of surface irradiance at the deep edge of the grass meadow for survival. Gallegos and Kenworthy (1996) determined a similar requirement for mixed beds of *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme* in the Indian River Lagoon (IRL) near Ft. Pierce, Florida. In contrast, Steward et al. (2005) found 20% to be near the minimum for the IRL, while the average light requirement was 33% of annual incident irradiance, similar to the wide range (24%–37%) reported for the southern Indian River Lagoon (Kenworthy and Fonseca, 1996). More recently, Duarte et al. (2007) analyzed 424 reports of seagrass colonization depths and light attenuation and found generally higher light requirements for plant communities growing in shallow, turbid waters than in clear, deep waters. The authors suggested that large differences in light requirements between shallow- and deep-growing seagrasses may be partially attributed to differences in the quality of light. Seagrasses may grow deeper in clear water because there is more high-energy blue light available for photosynthesis, whereas in shallow turbid water the shorter blue wavelengths are rapidly attenuated.

The wavelength specificity of light absorption by seagrasses has implications for setting water quality requirements needed to protect or restore these plants in eutrophic waters that are dominated by inefficient green wavelengths. The absorption of light by the complement of pigments (chlorophyll *a* and chlorophyll *b*) in seagrasses is highly wavelength selective, with absorption peaks in the blue (centered around 450 nm) and red (centered around 670 nm) regions of the visible spectrum, and a broad absorption minimum in the green between 500 and 600 nm (Drake et al., 2003; Zimmerman, 2003). Wavelengths of light that are poorly absorbed by the plant are relatively inefficient at driving photosynthesis (Drake et al., 2003; Falkowski and Raven, 2007).

Light requirements of seagrasses that have been determined to date (Batiuk et al., 2000; Kenworthy and Fonseca, 1996) have been based on photosynthetically available radiation (PAR, 400–700 nm) because of the widespread availability of underwater quantum sensors. PAR measurements weight quanta of all visible wavelengths equally. By contrast, measurements of photosynthetically *usable* radiation (i.e., PUR; see Morel, 1978) weight quanta in proportion to the efficiency with which they are absorbed. There are no sensors for direct measurement of PUR; it must be calculated from the underwater spectrum (measured or modeled) weighted by the relative absorption spectrum of the plant of interest.

Using a bio-optical model of light penetration in the mesohaline Chesapeake Bay, Gallegos (1994) determined that the 22% surface PAR requirement for seagrasses occurred at the same depth as the penetration of 16% of surface PUR. The distinction is potentially important because the penetration of PUR is more sensitive to the concentration of phytoplankton chlorophyll (i.e., eutrophication) than is the penetration of PAR, for the reason that phytoplankton chlorophyll absorption selectively removes those same wavelengths most efficiently used in photosynthesis by seagrass. Thus, by basing light requirements on PUR rather than on PAR, we would predict greater restoration benefit from chlorophyll reduction, and greater seagrass losses from chlorophyll increases, than by light requirements based on PAR (Gallegos, 1994).

The objective of this work was to determine whether the distinction between PAR and PUR requirements could be determined from in situ depth distributions of seagrass communities. The distinction cannot be drawn from depth distributions at a single site such as Chesapeake Bay or the IRL, because within these systems the underwater spectrum is peaked in the green, and thus there is insufficient spectral variability in available light to differentiate between depth limits based on PAR compared with PUR. The gradient of optical water quality types across locations of the Smithsonian Marine Science Network, however, offers a potentially ideal scenario for making this determination. All three of the dominant seagrass species found in the IRL also occur in the tropical waters of Carrie Bow Cay, Belize, and Bocas del Toro, Panama. In optically clear waters, the underwater spectrum peaks in the blue, near an absorption peak of chlorophyll *a* or *b*. In blue water, therefore, PUR penetrates deeper than PAR, and plants should grow to relatively deeper depths in blue tropical waters if PUR rather than PAR is the determining factor. To investigate this distinction, we surveyed seagrass distributions and measured inherent optical properties (IOPs), from which we calculated underwater light spectra at the deep edges of grass beds, to test the hypothesis that across the optical water quality gradient seagrass would grow to a consistent depth of penetration of PUR but a variable percentage of PAR.

METHODS

Study Sites

Station locations are shown in Figure 1. We occupied stations in the clear tropical waters off Carrie Bow Cay, Belize (station Blue Ground Range, BGR), and in Bahia Almirante, Panama (station STRI [Smithsonian Tropical Research Institute]), a station receiving colored-water discharge from a nearby creek in Panama (station SN03), and the more eutrophic waters of the Indian River Lagoon, Florida (ICW194; see Figure 1). Detailed characteristics of these sites are given by Lang (2009) in the Introduction to this volume.

Optical Properties

We measured in situ profiles of IOPs, the spectral absorption and beam attenuation coefficients, at nine wavelengths (412, 440, 488, 510, 532, 555, 650, and 715 nm) using a WETLabs ac-9 instrument with a 0.1 m pathlength, equipped with a pressure sensor to measure depth. A Seabird SBE-5T pump provided water flow to the ac-9 and a WETLabs MPAK unit that controlled pump and instruments and logged data.

Measured absorption and beam attenuation coefficients were corrected for temperature according to the manufacturer's protocols. We corrected absorption coefficients for scattering errors (Kirk, 1992) by the Zaneveld et al. (1994) algorithm that subtracts a fraction of measured scattering coefficient from absorption (Equation 1):

$$a_{t-w}(\lambda) = a_m(\lambda) - \varepsilon(c_{t-w}(\lambda) - a_m(\lambda)) \quad (1)$$

where $a_{t-w}(\lambda)$ is the scattering-corrected absorption coefficient less pure water absorption at wavelength λ, a_m is the measured non-water absorption coefficient subject to scattering error, c_{t-w} is the measured non-water beam attenuation coefficient, and ε is a coefficient that accounts

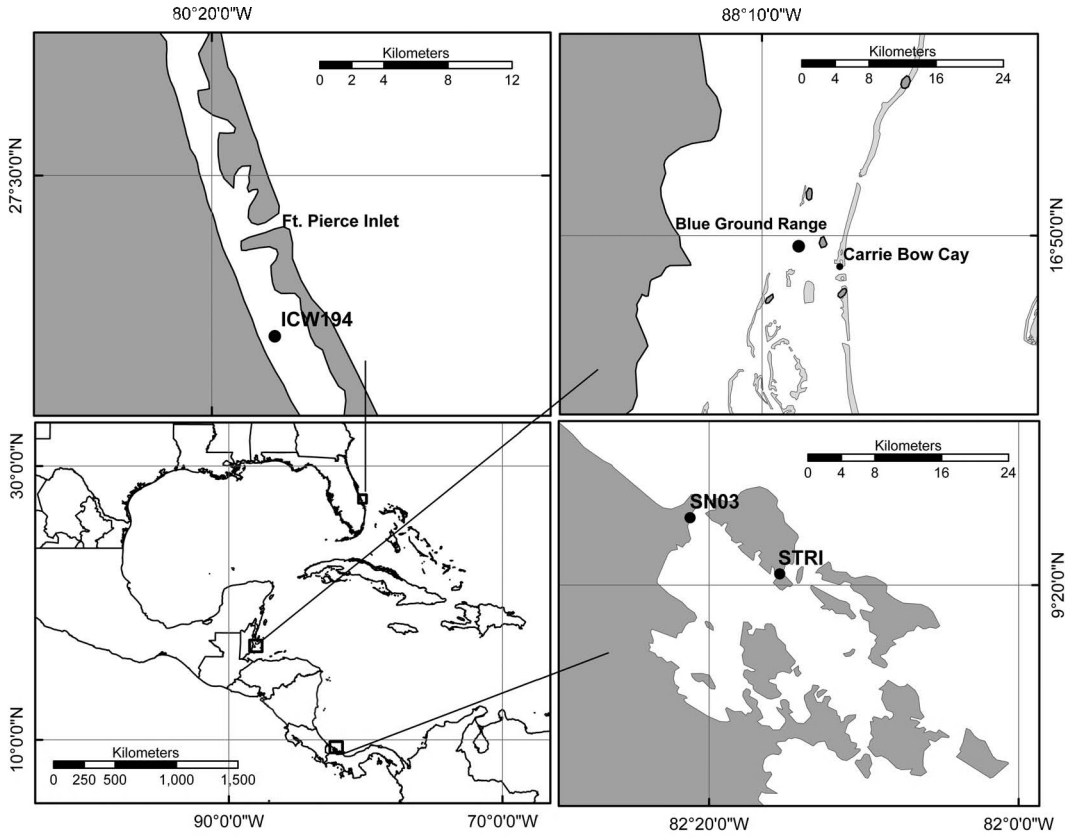

FIGURE 1. Locations of stations in the Indian River Lagoon, Florida (upper left), Belize (upper right), and Panama (lower right). Lower left panel shows overview of Caribbean. Light gray shading in Belize panel indicates coral reef habitat.

for overall errors with the reflective tube absorption meter of the ac-9 that result from a failure to collect all scattered light (Kirk, 1992). In this work we verified the assumption that non-water absorption at the longest ac-9 wavelength (715 nm) was not measurable in the laboratory (Tzortziou et al., 2006). Thus, we calculated ε by Equation 2:

$$\varepsilon = \frac{a_m(715)}{c_{t-w}(715) - a_m(715)} \tag{2}$$

We measured the absorption spectrum of *Thalassia testudinum* leaves in an integrating sphere (LICOR 1800-12S) interfaced to an Ocean Optics USB2000 spectrometer. A clean segment of leaf was placed on a microscope slide over the opening to the sphere and illuminated with a fiberoptic microscope light source. Black tape on the slide obscured the portion of the opening not covered by the leaf. Percent transmittance (%T) of the leaf was calculated referenced to the slide and tape without a leaf in place. Absorbance was calculated as $-\ln(\%T)$, and the spectrum was normalized to the value at the absorption peak at 675 nm. Measurements on eight leaves collected from the deep edge at the site in Belize were averaged. Similar measurements made in Panama had similar results.

Radiative Transfer Modeling

To calculate spectral diffuse attenuation coefficients and underwater light spectra, we used the commercially available radiative transfer model, Hydrolight 4.2, which is extensively documented by Mobley (1994). User input consists of specifications for IOPs, boundary conditions, and assumptions on inelastic scattering processes. We used the pure-water absorption coefficients of Pope and Fry (1997) and pure-water scattering coefficients for freshwater from Buiteveld et al. (1994). We used in situ estimates of absorption, attenuation, and scattering coefficients binned at 0.5 m intervals. Following Tzortziou et al. (2006), we used the Fournier–Forand scattering-phase function, the shape of which was shown by Mobley et al. (2002) to be well specified by the backscattering ratio. We omitted inelastic scattering processes because our interest is in downwelling irradiance, and these processes primarily affect only calculations of upwelling radiance. For incident irradiance and the distribution of total irradiance between direct and sky irradiance we used the built-in RADTRAN routine for the time, location, and estimate of approximate cloud cover.

From the simulations of spectral downwelling irradiance we calculated PAR according to its definition (Equation 3):

$$PAR(z) = \int_{400}^{700} Q(\lambda, z)\, d\lambda = \int_{400}^{700} \frac{E_d(\lambda, z)}{h\eta} d\lambda \tag{3}$$

where Q is the quantum flux, E_d is the spectral downwelling irradiance in energy units, h is Planck's constant, λ is the wavelength and $\eta = 2\pi c/\lambda$ is the frequency of light, and c is the speed of light in vacuum. PUR was calculated in an analogous manner, weighted by the plant absorption spectrum, measured at the deep edge of the Belize site:

$$PUR(z) = \int_{400}^{700} Q(\lambda, z)\tilde{a}_{Th}(\lambda)\, d\lambda \tag{4}$$

where $\tilde{a}_{Th}(\lambda)$ is the absorption spectrum of *T. testudinum* normalized to its peak at 675 nm and to unit sum. For comparison of attenuation rates, PAR and PUR were both normalized to their values at the surface.

Seagrass Surveys

At each sampling site a pair of scuba divers entered the water to visually confirm the seagrass bed (*T. testudinum*) deep edge, defined as the visible transition between vegetated and unvegetated bottom. Once the physical boundaries of the meadow edges were identified underwater, the divers laid out two 10 m long transects parallel to the edge of the seagrass bed. At 1.0 m intervals along each transect, the divers visually estimated seagrass cover in a 0.25 m^2 quadrat using the Braun-Blanquet scale (1965). The Braun-Blanquet cover abundance scale is a visual assessment technique for estimating the canopy cover. Values are 0.1 = solitary shoot, with small cover; 0.5 = few shoots, with small cover; 1 = numerous, but less than 5% cover, 2 = 5%–25% cover, 3 = 25%–50% cover, 4 = 50%–75% cover, and 5 = more than 75% cover.

At the same location each diver counted the number of seagrass short shoots in either a 0.25 m^2 or 0.0625 m^2 quadrat, depending on the shoot density. Short shoot counts were multiplied by the appropriate scaling factor and averaged for the 10 quadrats to obtain an estimate of the number of short shoots per square meter. For comparison of deep edge seagrass characteristics, we also surveyed relatively shallow sites at the Blue Ground Range station in Belize (2.4 m) and the STRI station in Panama (1.8 m). At SN03 in Panama we only surveyed at the deep

edge. Deep edge data for the IRL are from annual surveys by the South Florida Water Management District (http://my.sfwmd.gov/gisapps/sfwmdxwebdc/dataview.asp?query=unq_id=1797).

RESULTS

Seagrass Depth Limits

At the Blue Ground Range station in Belize, the deep edge of the *Thalassia testudinum* meadow was located at 10–11 m. The deep edge was a distinct transition from a sparse cover of *T. testudinum* to unvegetated, fine carbonate mud. Recently germinated seedlings of the small opportunistic species *Halophila decipiens* were observed just outside of the deep edge of the *T. testudinum* meadow. Braun-Blanquet cover values ranged from 0.5 (a few individual short shoots) to 1 (<5%). *Thalassia testudinum* short shoot densities ranged from 0 to 48 shoots m^{-2}, averaging 22.4 shoots m^{-2}. At the shallow Blue Ground Range transect, *T. testudinum* Braun-Blanquet scores ranged from 3 to 4, indicating that cover generally varied from 25% to 75%, while densities ranged from 176 to 416 shoots m^{-2}, averaging 310 shoots m^{-2}. At the shallow station *T. testudinum* was 14 times more dense than at the deep edge. No other seagrass species were observed at this station.

At the STRI station in Panama we located the deep edge of the *T. testudinum* at 8.5 m. The transition edge of the *T. testudinum* meadow was distinct; however, there was considerably more *H. decipiens* just downslope of the edge than there was at the Blue Ground Station in Belize. *Thalassia testudinum* short shoot densities ranged from 0 to 56 shoots m^{-2}, averaging 18 shoots m^{-2}, similar to the deep edge at the Blue Ground Range Station in Belize. Braun-Blanquet values ranged from 0 to 1, indicating that cover was generally less than 5%. We also observed three quadrats with a relatively sparse cover of *Halodule wrightii*. At the shallow STRI station (1.8 m), *T. testudinum* densities ranged from 160 to 528 shoots m^{-2} with an average of 465, 25 times the density at the deep edge and more dense than the shallow station at Blue Ground Range in Belize. Braun-Blanquet values ranged from 3 to 4, similar to the shallow station at Blue Ground Range (BGR) in Belize.

At the SN03 site in Panama, the deep edge of the *T. testudinum* bed was located at 2.4 m. Short shoot densities ranged from 0 to 288 m^{-2}, with a mean value of 114. The deep edge of the *T. testudinum* meadow was marked by a transition from *T. testudinum* to unvegetated sediment. Braun-Blanquet scores ranged from 0 to 3, indicating cover values less than 50%.

Seagrass depth limits in the IRL at the site where optical measurements were made in 2001 were reported as 0.92 m for beds described as continuous and dense, with a lower limit of 50% to 60% cover.

Optical Properties

A wide range of optical properties was observed among the four sites (Figure 2a). Based on absorption spectra, Belize had the clearest water while the most turbid water occurred in the IRL. The two sites in Panama were intermediate. The rank order of sites was different for scattering coefficients (Figure 2b), with scattering coefficients at the Panama shallow site (SN03) being the highest and the Panama deep site (STRI) the lowest.

Absorption Spectrum

Normalized absorption by *T. testudinum* was similar to measurements by other investigators (Zimmerman, 2003), having peaks in the red wavelengths (~680 nm), a broad maximum at blue wavelengths (400–490 nm), and a trough at green wavelengths (~525–625 nm) (Figure 3, solid line). This spectrum was used to calculate PUR from simulated downwelling spectral irradiance according to Equation 4. However, even at the local minimum at 555 nm, measured absorption was still 37% of the red peak. On considering that *T. testudinum* has no chlorophyll pigments that absorb green wavelengths (Zimmerman, 2003), we also constructed a hypothetical photosynthetic action spectrum based on chlorophyll absorption alone, consisting of Gaussian curves with peaks at 410, 430, 455, 642, and 680 nm for an alternate calculation of PUR (see Figure 3, dashed line). The hypothetical action spectrum is expected to produce the maximal separation between PAR and PUR, especially in turbid green water, because the trough in the hypothetical chlorophyll absorption spectrum at green wavelengths is much more pronounced compared with the measured absorption spectrum, which includes an unquantified contribution by photosynthetic carotenoids. This hypothetical chlorophyll-based action spectrum serves as a site-independent sensitivity test for the greatest possible difference between PAR and PUR for a higher plant. We did not measure absorption spectra in the IRL, so they are unknown. The hypothetical spectrum allows a comparison among sites in the absence of measurements at all sites.

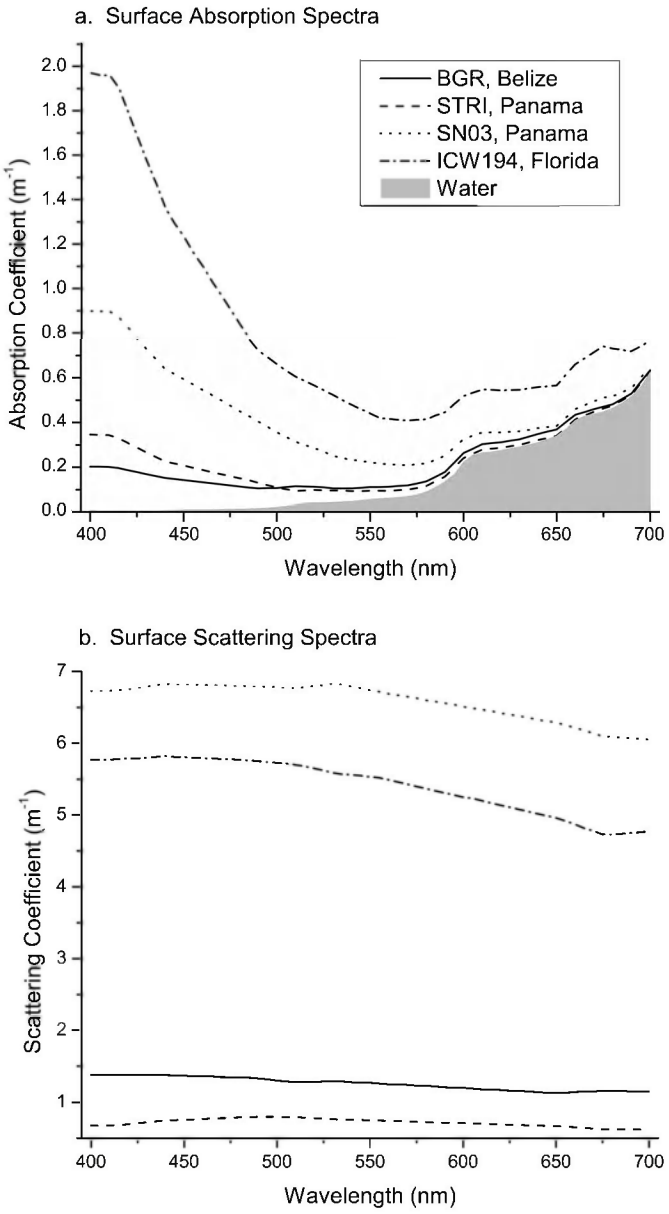

FIGURE 2. Surface water absorption spectra (a) and surface water scattering spectra (b) at sites sampled in Belize, Panama, and Florida.

PAR and PUR Profiles

Profiles of normalized downwelling PAR and PUR based on the measured absorption spectrum (PURm) and PUR based on the hypothetical action spectrum (PURh) are shown for the stations having the least and the most separation between PAR and PUR in Figure 4. The diffuse attenuation coefficients for each of the three quantities are reported for all stations in Table 1. At the Blue Ground Range station in Belize, diffuse attenuation coefficients for PAR and PURm were indistinguishable, while that for PURh was only 7% higher than for PAR (Table 1). The largest differences among the three attenuation coefficients occurred at the IRL. The relative differences between attenuation coefficients for PAR and PURm (13%) and between PAR and PURh (31%) were similar for the IRL and SN03 site in Panama, although the absolute coefficients were smaller at SN03 (Table 1).

The percentages of surface light remaining at the deep edges of the seagrass beds varied widely among the locations, from about 5% at the Blue Ground Range site in Belize to about 30% at the IRL (see Table 1). The percentages based on PUR were, as expected, lower than those based on PAR, but the differences among sites was still large (Table 1). Because of the extremely large differences among sites in the percentage of light at the seagrass bed deep edge, the calculation of PUR did not yield a consistent value across sites. The overall range was, however, somewhat smaller for PUR than for PAR (Table 1). Spectra of downwelling irradiance at the deep edges calculated by Hydrolight are shown in Figure 5. The overall fraction of surface irradiance remaining at the deep edges at the different locations follows the percentages in Table 1. Qualitative differences in the spectra of light remaining at

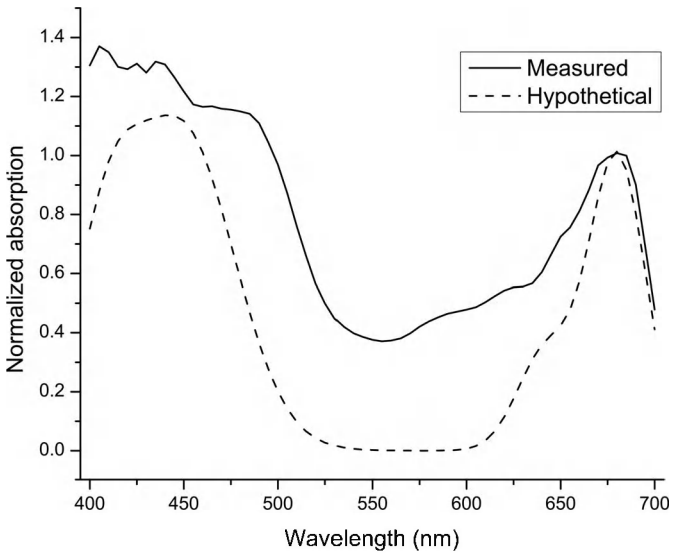

FIGURE 3. Normalized absorption spectra used for calculating photosynthetically usable radiation, based on absorption spectrum measured on *Thalassia testudinum* leaves (solid line), and a hypothetical action spectrum derived by assuming only light absorbed by chlorophylls *a* and *b* drive photosynthesis in *Thalassia* (dashed line).

the deep edges also occur. Because of absorption by water, virtually no light is present at wavelengths greater than 600 nm at the BGR location in Belize and very little at STRI in Panama. Increasing amounts of red wavelengths are present at the SN03 and IRL sites as a result of the shallower depths of the deep edges. The peaks of the in situ spectra shift progressively toward green wavelengths along the progression from BGR to IRL, and the greatest similarities are at 400 to 410 nm, where the percentage of surface irradiance remaining ranges from 2% to 6%.

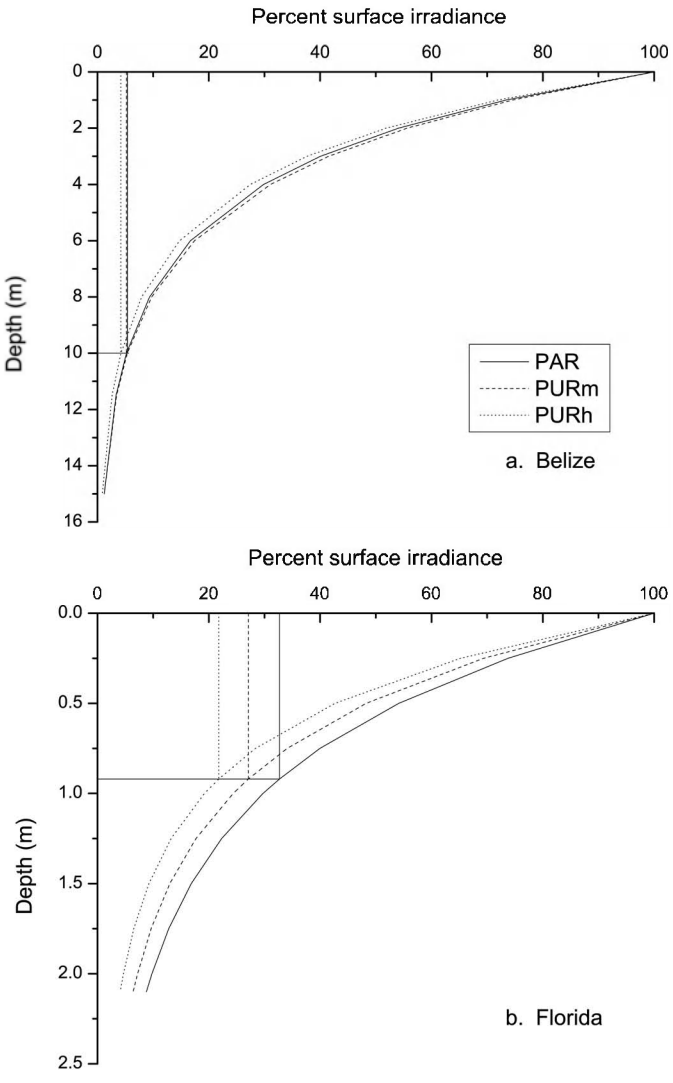

FIGURE 4. Vertical profiles of photosynthetically active radiation (PAR, solid line), and photosynthetically usable radiation (PUR) based on measured absorption spectrum (PURm; dashed line) and hypothetical action spectrum (PURh; dotted line) in (a) Belize and (b) the Indian River Lagoon (IRL), Florida. Profiles were normalized to the irradiance incident at the surface (100%).

TABLE 1. Depths of seagrass deep edge (Z_{max}) and attenuation coefficients for photosynthetically active radiation (K_{PAR}) and photosynthetically usable radiation (PUR) weighted by measured absorption spectrum of *Thalassia testudinum* leaves (K_{PURm}) or weighted by a hypothetical action spectrum (K_{PURh}; see Figure 2). Percentage of surface light penetrating to the seagrass deep edge is given in parentheses.

Site [a]	Z_{max} (m)	K_{PAR} (m^{-1})	K_{PURm} (m^{-1})	K_{PURh} (m^{-1})
BGR, Belize	10	0.293 (5.2%)	0.293 (5.4%)	0.314 (4.2%)
STRI, Panama	8.5	0.232 (13.6%)	0.247 (12.0%)	0.304 (7.4%)
SN03, Panama	2.4	0.836 (14.1%)	0.945 (11.0%)	1.098 (7.7%)
IRL, Florida	0.92	1.157 (32.7%)	1.301 (27.1%)	1.52 (21.8%)

[a] BGR, Blue Ground Range; STRI, Smithsonian Tropical Research Institute; SN03, Panama creek station; IRL, Indian River Lagoon.

DISCUSSION

At all three study sites we were able to locate a distinct deep edge of the *Thalassia testudinum* meadows, characterized by a transition from moderate and sparsely vegetated seagrass to either unvegetated substrate or patches of the smaller, low light adapted seagrass *Halophila decipiens*. Where we were able to sample shallower sites in Belize and Panama, there were substantially higher densities of *T. testudinum*. The presence of *H. decipiens* at the Blue Ground Range (BGR) station in Belize and the STRI site in Panama further confirmed that we were sampling at light-limiting edges of the *T. testudinum* distribution. *Halophila decipiens* is a small, ruderal species of seagrass commonly found growing in deep or turbid water and has lower light requirements than *T. testudinum* (Kenworthy, 2000; Gallegos and Kenworthy, 1996; Kenworthy et al., 1989). The presence of *H. decipiens* at these two stations was a good indication of light-limiting conditions for *Thalassia*. Although we did not record *H. decipiens* at SN03 in Panama, a thorough visual examination by divers at deeper depths than the observed *T. testudinum* distribution confirmed there were no seagrasses growing beyond 2.4 m depth.

Attenuation coefficients for PAR and PUR were nearly indistinguishable in Belize and Panama and differed only slightly in the IRL. Based on these one-time profiles, we calculated that seagrass grew to depths of penetration of

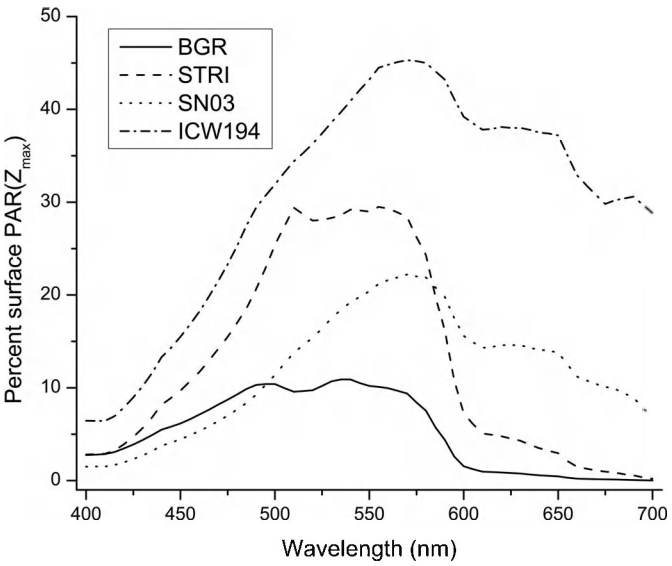

FIGURE 5. Spectra of photosynthetically active radiation (PAR) at the depth of the seagrass deep edge (Z_{max}) in Belize (BGR, solid line), Panama (STRI, dashed line, and SN03, dotted line), and Florida (IRL [ICW194], dot-dashed line).

33% of PAR in the IRL, 14% in Panama, and approximately 5% in Belize. Corresponding percentages for PUR were 27%, 12%, and 5% for IRL, Panama, and Belize, respectively. The accuracy of these estimates depends on the degree to which the profiles were measured under conditions that are typical for their respective growing seasons. We are fairly certain this was *not* the case in Belize, where strong northerly winds, atypical for the season, blew for several days before and on the day of sampling. Horizontally sighted Secchi disk visibility at a seagrass bed near Twin Cays was 5.5 m during the time of our measurements, compared with annual means of 10.1 m (±0.38 m SE) for 2004 and 8.9 m (±0.25 m SE) for 2005 (see Koltes and Opishinski, 2009: fig. 6, this volume). If the water column were more strongly stirred with higher than typical concentrations of particulate matter, then our estimates for Belize would be biased low, as we suspect they are. The estimated PAR light requirements for the IRL are, however, based on more frequent visits and are in agreement with other published estimates (Kenworthy and Fonseca, 1996; Steward et al., 2005). The limitation of our approach was the inability to determine the integral of light requirements for the whole growing season from only a few days of measurements. Because of this limitation, it is unlikely that the observed depth distribution of the seagrasses is fully captured by PAR and PUR percentages calculated, and repeating this study during another season could yield different percentages.

Nonetheless, assuming that the light requirements for seagrasses at Belize are similar to those in Panama, the regional differences in light requirements between the IRL and the two tropical sites remain striking. Qualitatively, the differences are consistent with the observations of Duarte et al. (2007) that seagrasses growing in shallow, turbid waters (e.g., IRL) have higher light requirements than those growing in clear, deep water (Panama, Belize). Calculation of PUR closed the gap only slightly, leading us to conclude that factors other than spectral energy distribution contribute substantially to site-specific light requirements of seagrasses, especially at the deep edges. An extended growing season in the more tropical locations of Belize and Panama could possibly account for some of the difference. The tropical sites receive about 7% more incident radiation annually than the IRL site, most of which occurs during winter months (November through February) when temperatures are also more favorable in the tropics. Other possible differences between sites include leaf-shading epiphytes, sediment quality (e.g., grain size or organic matter content), and possible periods of low oxygen in thermally stratified deeper waters. These latter factors have management implications because they are all affected by coastal eutrophication. Improved understanding of the factors accounting for site-specific differences in seagrass light requirements is, therefore, urgently needed.

Acknowledgments

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Interannual Variation in Gelatinous Zooplankton and Their Prey in the Rhode River, Maryland

Eileen S. Graham, Danielle M. Tuzzolino, Rebecca B. Burrell, and Denise L. Breitburg

ABSTRACT. The lobate ctenophore *Mnemiopsis leidyi* is an important predator of zooplankton and ichthyoplankton both within and outside its native range, and it is a dominant consumer within the Chesapeake Bay food web. We sampled the Rhode River, a subestuary of Chesapeake Bay, during 2004 and 2005 to quantify the abundances of *M. leidyi*, its scyphomedusan predators, and its mesozooplankton prey, and conducted ctenophore egg production experiments in 2004. Despite low mesozooplankton densities, ctenophores produced up to 9,380 eggs individual^{-1} day^{-1}. Temporal patterns, as well as peak abundances, of copepods, ctenophores, and sea nettles (*Chrysaora quinquecirrha*; the major predator of *M. leidyi*) varied considerably between years. This interannual variation may have been caused by direct and indirect effects of physical factors, especially low salinities during 2004, on all components of the food web. In 2004, zooplankton abundances peaked in June, *M. leidyi* abundances steadily increased throughout the summer, and *C. quinquecirrha* was rare. In contrast, during 2005, *C. quinquecirrha* density increased during midsummer. As this medusa increased in abundance, *M. leidyi* numbers declined and copepod abundances increased. Shallow systems with salinities near the minimum threshold for *C. quinquecirrha* ephyra production may exhibit more extreme interannual variability than deeper, higher-salinity systems and may serve as models to provide insight into factors controlling gelatinous zooplankton dynamics.

INTRODUCTION

The lobate ctenophore *Mnemiopsis leidyi* is native to Atlantic and Caribbean estuaries and coastal waters from Massachusetts to southern Argentina and has been introduced to several Eurasian systems including the Black, Caspian, Baltic, and North Seas (Purcell et al., 2001; Kube et al., 2007). *Mnemiopsis leidyi* can tolerate a wide range of temperatures, salinities, and dissolved oxygen (DO) concentrations. It occurs in waters with salinities ranging from less than 5 to more than 36 (Purcell et al., 2001; Purcell and Decker, 2005) and can survive exposure to DO concentrations of 0.5 mg L^{-1} for at least 4 d (Decker et al., 2004). Optimal temperatures for *M. leidyi* reproduction are approximately 18°–20°C (Costello et al., 2006).

In late spring and early summer, *M. leidyi* can be abundant in Chesapeake Bay and its tributaries, where it is a dominant consumer, potentially capable of clearing much of the daily standing stock of zooplankton and ichthyoplankton

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(Cowan et al., 1992; Cowan and Houde, 1993; Purcell et al., 1994; Purcell and Decker, 2005). In mesohaline portions of the Chesapeake Bay system, the major predator of *M. leidyi*, the scyphomedusa *Chrysaora quinquecirrha*, usually becomes abundant in early July and persists through the end of summer (Cargo and King, 1990). As *C. quinquecirrha* population densities increase, *M. leidyi* abundances typically decline and zooplankton populations rebound (Purcell and Cowan, 1995). However, in years when *C. quinquecirrha* populations are low, *M. leidyi* may exert much greater and prolonged control within the food web. *Chrysaora quinquecirrha* polyps are generally found in salinities of 7 to 20 and strobilate when temperatures exceed 17°C (Cargo and Schultz, 1967; Cargo and King, 1990). Medusae are most abundant at salinities of 10–16 (using the Practical Salinity Scale) and temperatures of 26°–30°C (Decker et al., 2007). Thus, interannual variation in salinity and temperature can strongly affect the timing and spatial distribution of *C. quinquecirrha* and its control of *M. leidyi*.

The Rhode River is a small, shallow subestuary on the western shore of Chesapeake Bay (Figure 1) characterized by summer salinities that vary interannually in both absolute maxima and timing of these maxima. Similar to other tributaries in the Chesapeake Bay system, this estuary supports a gelatinous zooplankton food web throughout late spring and summer months. The most abundant gelatinous species are the zooplanktivorous *M. leidyi* and its scyphomedusan predator and competitor *C. quinquecirrha*. Average spring–summer salinity in the Rhode River is near the lower limit required for strobilation by *C. quinquecirrha*. In addition, interannual variation in water temperature has the potential to cause variation in the timing of initial and peak occurrences of these gelatinous species and their prey. As a result, the Rhode River can have two distinct gelatinous food webs: one in which the top predator (*C. quinquecirrha*) exerts control over the intermediate consumer (*M. leidyi*) and one in which the intermediate consumer is not controlled by predation.

The objectives of this study were to examine temporal and spatial patterns in abundances of *M. leidyi* and *C. quinquecirrha* within and near the Rhode River and to examine how those patterns varied in relationship to water temperature, salinity, and the abundance of mesozooplankton prey. We also examined temporal and spatial variation in egg production by *M. leidyi*. This study was conducted during the summers of 2004 and 2005, years with very different temporal patterns of *M. leidyi* and *C. quinquecirrha* densities.

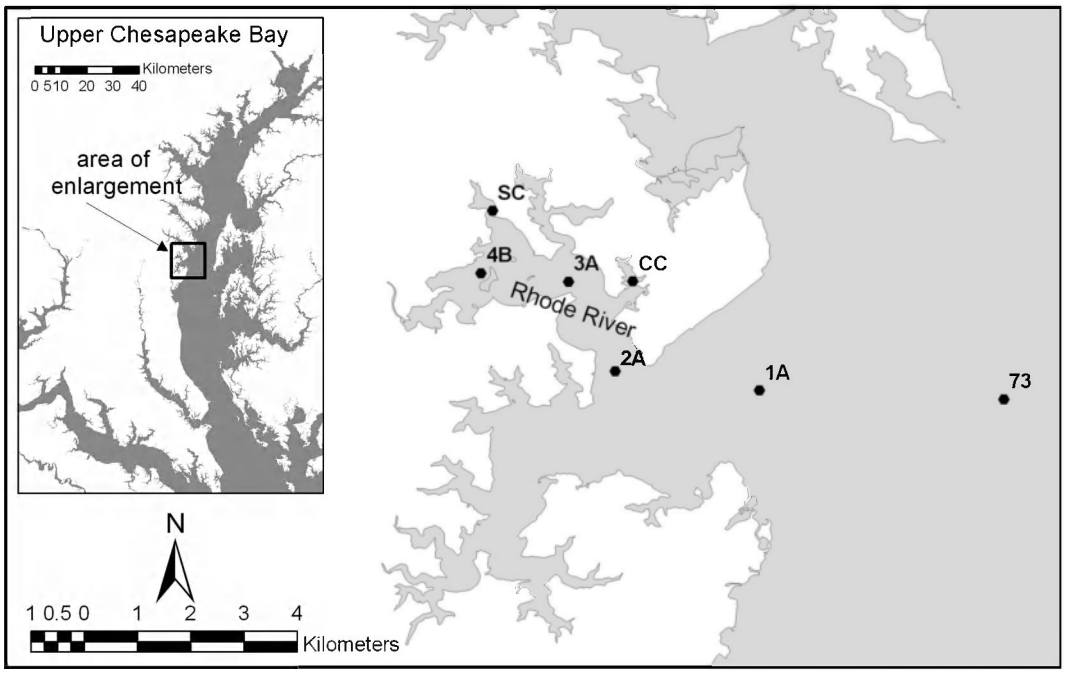


FIGURE 1. The Rhode River and its location in the Chesapeake Bay. Dots indicate location of sampling sites; the SERC dock is located directly inshore (northwest) of site 4B.

METHODS

We sampled seven sites: six within the Rhode River and one just beyond the mouth of the river in the mainstem Chesapeake Bay. Sites were chosen based on prior research conducted in the Rhode River and designed to cover its entire length. At each site, weather conditions were noted and temperature, DO, and salinity were recorded at the surface and subsequent 1 m depth intervals with a YSI 600QS meter. Additional temperature, DO, and salinity data were available from the monitoring station located at the dock of the Smithsonian Environmental Research Center (SERC) in the Rhode River, which was equipped with a YSI 6600 meter (C. Gallegos, SERC, unpublished data).

Gelatinous zooplankton samples were collected in duplicate 3 min stepped oblique tows using a 0.5 m diameter, 202 μm mesh hoop plankton net towed at approximately 2 knots and equipped with a General Oceanics flowmeter (model 2030). Excess water was strained from the sample, total volume of gelatinous zooplankton was measured, and all individuals were identified to species and enumerated. Bell diameters of *C. quinquecirrha* and the oral to aboral lengths of up to 15 *M. leidyi* were recorded. Remaining specimens of *M. leidyi* were classified as either larger than or equal to or less than 3.0 cm.

Mesozooplankton samples were collected using 0.3 m diameter, 202 μm mesh paired hoop nets. Samples were rinsed through a 2 mm sieve to remove gelatinous zooplankton and preserved with 10% buffered formalin; mesozooplankton species were subsequently identified and enumerated.

Whole water column chlorophyll data were collected by another research group (C. Gallegos, SERC) at the four central Rhode River sites (1A, 2A, 3A, 4B; see Figure 1) on different days during each sampling week. Chlorophyll *a* (chl *a*) was measured with a Spectronics Genesis 5 spectrophotometer and converted into micrograms per liter ($\mu g\ L^{-1}$).

Mnemiopsis leidyi egg production assays were conducted in 2004 using established methodology (Kremer, 1976; Grove and Breitburg, 2005). Undamaged individuals covering the size range from each site (3–8 cm) were randomly assigned to jars containing 3 L filtered Rhode River water and left overnight at ambient water temperatures. At approximately 0900 the following morning, adult ctenophores were removed and lengths and volumes recorded. Water from each jar was strained through a 35 μm sieve, preserved with 10% acid Lugol's solution (Sullivan and Gifford, unpublished data; Grove and Breitburg, 2005), and eggs were enumerated. Egg production was normalized by ctenophore volume to facilitate comparisons among individuals.

Data were analyzed using analysis of variance (Proc GLM: SAS v. 9.1) on rank-transformed data. Student–Newman–Keuls tests were used for a posteriori comparisons. Regression models were used to examine the effects of ctenophore volume, site, date, and interactions between these factors on egg production. Nonsignificant interaction terms with $P \geq 0.25$ were dropped from statistical models.

RESULTS

Physical Parameters

Temperature, salinity, and DO all varied among sites and between years (Table 1; Figure 2; two-way analysis of variance [ANOVA]). Surface water temperature varied among sites ($F = 38.21$, $P < 0.01$), and was cooler adjacent to, and near the mouth of, the Rhode River and at the deeper sites. Surface salinity also varied significantly among sites ($F = 3.55$, $P < 0.01$), and was generally highest at the Bay site (Site 73) and at sites near the mouth of the Rhode River. Minimum DO concentration varied among sites ($F = 7.33$, $P < 0.01$) and was significantly lower at the Bay site than elsewhere.

Measurements at the SERC dock indicated that surface water temperatures reached 25°C more than 3 weeks earlier in 2004 than in 2005 but exceeded 30°C only during 2005. Salinity remained below 8 except for a brief period in 2004 but exceeded 8 for most of the summer in 2005. Daytime low DO concentrations (<2 mg L^{-1}) were occasionally recorded in the bottom waters during cruises; all low daytime DO measurements in 2004 and all but one in 2005 were recorded at the Bay site. The continuous YSI 6600 monitor at the SERC dock indicated that low DO concentrations occurred near the surface within the Rhode River in the early morning hours of both years (C. Gallegos, SERC, personal communication, 2004). Analysis of our weekly sampling data indicated that temperature ($F = 5.38$, $P = 0.02$), salinity ($F = 135.18$, $P < 0.01$), and DO concentrations ($F = 6.39$, $P = 0.01$) were all significantly higher in 2005 than in 2004.

2004 Biota

Chlorophyll *a* concentrations peaked in early June, declined, and then rose continually during the period sampled from mid-June through early September 2004 (see

TABLE 1. Mean environmental conditions measured at each site sampled for 2004 and 2005. See Figure 1 for site locations. Chlorophyll *a* concentrations are whole-water integrated values (C. Gallegos, SERC); minimum dissolved oxygen (DO) values are based on near-bottom measurements; temperature and salinity are from surface waters (<1 m depth); NA = site not sampled.

	2004				2005			
Site	Chlorophyll *a* ($\mu g\ L^{-1}$)	Minimum DO ($mg\ L^{-1}$)	Temperature (°C)	Salinity	Chlorophyll *a* ($\mu g\ L^{-1}$)	Minimum DO ($mg\ L^{-1}$)	Temperature (°C)	Salinity
73[a]	NA	2.31	25.40	7.76	NA	3.46	26.26	9.79
1A	24.55	5.20	25.95	8.20	21.37	6.61	26.64	9.26
2A	24.76	5.77	26.66	8.04	37.02	6.88	27.58	9.44
CC	NA	4.70	29.14	7.35	NA	5.14	28.91	9.10
3A	32.43	4.73	27.54	7.94	28.20	5.21	28.00	8.87
SC	NA	4.23	28.09	7.78	NA	5.31	27.97	9.38
4B	44.34	5.22	28.41	7.29	32.29	5.19	28.62	9.08

[a] Because of sea conditions, site 73 was not sampled during mid- to late summer 2004 as frequently as other sites; thus, averages are not necessarily representative of physical conditions at site 73 relative to other sites measured on the same dates.

Figure 2). Mesozooplankton samples in both years were dominated (>95% of individuals) by the calanoid copepod *Acartia tonsa*. During 2004, mesozooplankton densities varied significantly among dates ($F = 6.28$, $P < 0.01$). Peak densities of 4–7 individuals L^{-1} occurred on 21 June and 7 July and then declined to approximately 1.0 individuals L^{-1} for the rest of the season (see Figure 2).

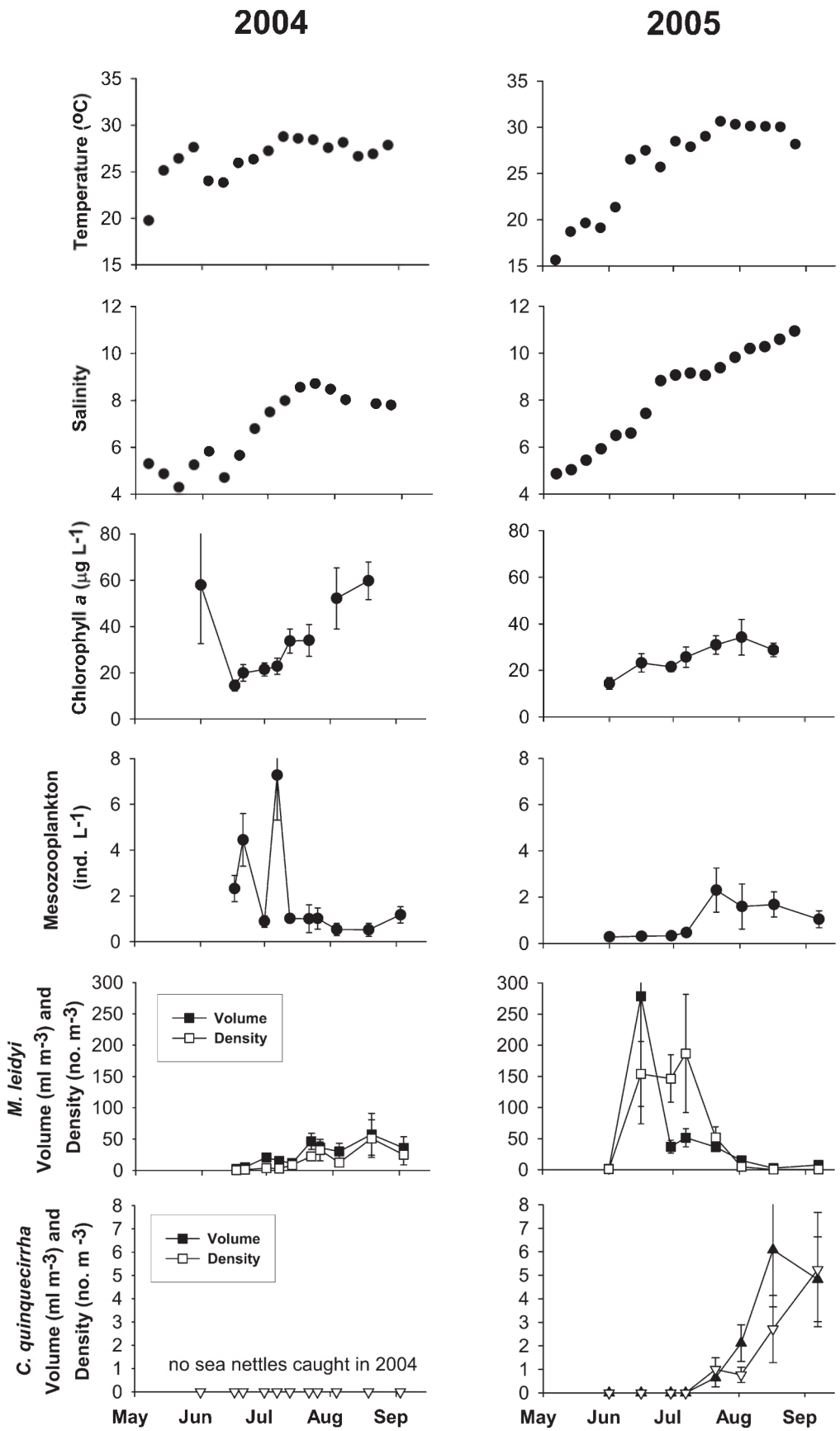
Mnemiopsis leidyi volumes also varied significantly among dates (one-way ANOVA on ranks; $F = 6.08$, $P < 0.01$). Numerical densities and volumes were lowest in mid-June ($\leq 0.62 \pm 0.25$ individuals m^{-3} and $\leq 2.3 \pm 0.77$ mL m^{-3}, respectively), and then gradually increased to a maximum of 51 ± 30.2 individuals m^{-3} and 58 ± 33.5 mL m^{-3} on 19 August (see Figure 2), the date that coincided with highest densities of "recruits" (individuals ≤1 cm in length). Regression analyses indicated a significant relationship between the zooplankton density of the prior week and both *M. leidyi* volume ($r^2 = 0.13$, $P < 0.01$) and the density of recruits ($r^2 = 0.21$, $P < 0.01$). However, the previous week's chl *a* concentration explained a greater percentage of the variation in both these measures of *M. leidyi* abundance for the sites at which chl *a* data were available (1A, 2A, 3A, 4B) (volume: $r^2 = 0.33$, $P < 0.01$; density of new recruits: $r^2 = 0.25$, $P < 0.01$). *Chrysaora quinquecirrha* abundances were low during 2004. A few medusae were seen in the field during August and early September but were never caught with either the 0.5 m diameter hoop net or the larger 1 m^2 neuston net, which was deployed in an attempt to more accurately sample the low-density *C. quinquecirrha* population.

2005 Biota

Temporal patterns and peak abundances of most biota in 2005 differed from those in 2004 (see Figure 2). Mid-June chl *a* concentrations in 2005 were similar to those in the corresponding time period in 2004, and as in 2004 generally increased during the remainder of the season. However, sampling did not detect an early June chl *a* peak in 2005, and maximum chl *a* concentrations in late summer 2005 reached only about two-thirds the concentrations reached in 2004 (Figure 2). Mesozooplankton densities varied among dates (one-way ANOVA on ranks, $F = 4.87$, $P < 0.01$). The 21 July peak density of 2.3 individuals L^{-1} was both later and lower than peak densities in 2004. Early June through early July mesozooplankton densities remained below 1 individual L^{-1} and were similar to mid-July–early September densities in 2004.

The timing of the increase in mesozooplankton densities in 2005 corresponded to a decrease in *M. leidyi* densities and the appearance of *C. quinquecirrha*. *M. leidyi* densities varied significantly among dates (one-way ANOVA on ranks: $F = 13.98$, $P < 0.01$). Peak *M. leidyi*

FIGURE 2. (*facing page*) Weekly mean temperature (°C) and salinity at the SERC dock (C. Gallegos, unpublished data), and river-wide mean (±SE) chlorophyll *a* concentration ($\mu g\ L^{-1}$), mesozooplankton abundance (number L^{-1}), and *Mnemiopsis (M.) leidyi* and *Chrysaora (C.) quinquecirrha* abundance (volume, mL m^{-3}; density, number m^{-3}) for 2004 (left) and 2005 (right).


2004
2005
Temperature (°C)
Salinity
Chlorophyll a (µg L-1)
Mesozooplankton (ind. L-1)
M. leidyi Volume (ml m-3) and Density (no. m-3)
Volume
Density
C. quinquecirrha Volume (ml m-3) and Density (no. m -3)
no sea nettles caught in 2004
May
Jun
Jul
Aug
Sep

densities were higher and occurred earlier in 2005 than in 2004. Volumes peaked on 16 June (279 ± 205 mL m^{-3}), declined substantially by the 21 July sample date, and then remained low throughout the rest of the season (Figure 2). Medusae of *Chrysaora quinquecirrha* were first caught in our sample nets on 18 July 2005, and numbers continually increased over the season, reaching a maximum on the last sample date, 7 September (Figure 2). *Mnemiopsis leidyi* densities declined as *C. quinquecirrha* abundances increased. Regression analysis was run on *C. quinquecirrha* density and the prior week zooplankton density and chl *a* concentrations. Partial r^2 values indicated that *C. quinquecirrha* number explained 41% of the variation in the number of *M. leidyi* recruits whereas prior week zooplankton explained only 17% and 13% of the variation in number of recruits and *M. leidyi* volume, respectively.

Mnemiopsis leidyi Egg Production

Egg production assays were performed on three dates in July 2004. *Mnemiopsis leidyi* produced between 0 and 668 eggs mL^{-1} of ctenophore. There was a significant positive correlation between *M. leidyi* volume and the number of eggs produced, both on each date and for the three dates combined (Figure 3). Egg production on each date in 2004 differed significantly from all others. Egg production was highest on 7 July, 355 ± 28.2 eggs mL^{-1} (n = 36); lower on 1 July, 274 ± 25.7 eggs mL^{-1} (n = 33); and lowest on 22 July, 50 ± 8.71 eggs mL^{-1} (n = 35) (see Table 2).

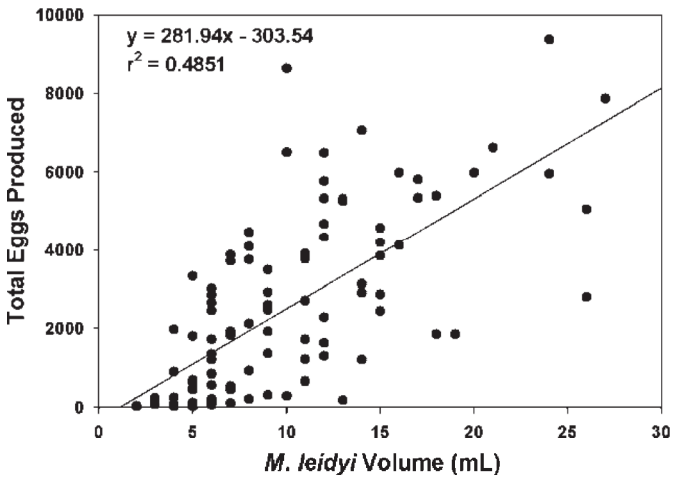


FIGURE 3. Total number of eggs produced and volume (mL) of each *M. leidyi* in all three reproduction experiments.

TABLE 2. Mean egg production rate (eggs mL^{-1} ctenophore ± SE) and 2-week mean mesozooplankton density (number L^{-1} ± SE) for each reproduction study date.

Date	Egg production	Zooplankton density
1 July	274 (± 25.7)	2.72 (± 0.56)
7 July	355 (± 28.2)	4.13 (± 1.03)
22 July	50 (± 8.7)	1.03 (± 0.34)

Mesozooplankton prey density during the week leading up to the reproduction assays was estimated by averaging mesozooplankton densities measured in samples collected on the day of the egg production assay and from the previous week to include food immediately available as well as prey quantity potentially affecting prior growth and reproduction. Two week average zooplankton densities were 4.13 ± 1.03 (n = 6), 2.72 ± 0.56 (n = 7), and 1.03 ± 0.34 (n = 7) individuals L^{-1} for 7 July, 1 July, and 22 July, respectively (see Table 2). These zooplankton densities corresponded directly with the ranked egg production rates on these dates. ANOVA indicated that the total number of eggs produced per individual increased significantly with ctenophore volume (F = 201.24, $P < 0.01$) and average zooplankton density (F = 34.87, $P < 0.01$), and varied among sites (F = 5.70, $P < 0.01$), dates (F = 13.82, $P < 0.01$), and the interaction between sites and dates (F = 3.39, $P < 0.01$); the model r^2 was 0.80 ($P < 0.01$).

DISCUSSION

Temporal patterns of mesozooplankton, *M. leidyi*, and *C. quinquecirrha* in the Rhode River differed strongly between 2004 and 2005. In 2004 mesozooplankton abundances peaked in early summer and then declined as ctenophores gradually increased throughout the season. *C. quinquecirrha* medusae were rare, and their appearance did not result in a decline in ctenophore density or biomass. In contrast, in 2005, late spring through early summer mesozooplankton densities were low and ctenophore density and biomass were high. As *C. quinquecirrha* abundances increased in late summer, *M. leidyi* decreased and mesozooplankton densities increased. Peak densities of *M. leidyi* measured during this study in the Rhode River (approximately 200 individuals m^{-3} and nearly 300 mL m^{-3}) are higher than those reported in the Pamlico River,

North Carolina (just over 60 mL m^{-3}; Miller, 1974) or the mid-Chesapeake Bay (Purcell et al. 2001), but similar to abundances reported for Narragansett Bay, Rhode Island (Deason, 1982; Sullivan et al., 2001). Peak Rhode River densities measured in this study were lower, however, than those reported for systems such as the Black and Caspian Seas to which *M. leidyi* has been introduced (Kideys and Romanova, 2001; Bilio and Niermann, 2004).

Interannual variation in salinity likely contributed to observed interannual differences in gelatinous zooplankton densities and food web interactions, but the effect of interannual variation in water temperatures is less clear. Low salinities in 2004 likely resulted in the low densities of *C. quinquecirrha* in that year. *Chrysaora quinquecirrha* polyps are generally not found in salinities less than 7 and become more abundant as salinities increase to between 7 and 10 (Cargo and King, 1990). During 2004, surface salinity did not reach 5 until mid-June, or 7 until July, and never reached 10. In contrast, surface salinity reached 7 by mid-June and 10 by early August in 2005. We suggest that salinities below 5 in May and early June also delayed or reduced early-season *M. leidyi* reproduction in Rhode River in 2004 (Purcell et al., 2001). We were unable to find published studies that report *M. leidyi* reproductive rates at salinities below 5. However, if this hypothesis is correct, there is a very narrow margin between salinities that prevent recruitment of *C. quinquecirrha* and allow *M. leidyi* populations to grow unchecked by predation and salinities that hinder *M. leidyi* populations by limiting reproduction. The combined effects of salinity on these two gelatinous species in Rhode River in 2004 appears to have resulted in a persistent *M. leidyi* population that did not become abundant until mid- to late July but then remained abundant at least through early September.

Although surface waters warmed earlier in the season during 2004 than during 2005, the effect of this warming on gelatinous zooplankton seasonal abundances is not clear and may have been overwhelmed by other factors. Spring temperatures were 5°C higher in 2004 than in 2005. By early May of both years, however, temperatures exceeded the 9°–13°C minimum temperature required for *M. leidyi* reproduction (P. Kremer, University of Connecticut, unpublished data), and by mid-May of both years, temperatures exceeded the 17°C threshold required for strobilation by *C. quinquecirrha* (Cargo and King, 1990; Purcell and Decker, 2005). In addition, there are no data to suggest that temperatures that occurred during the warmer 2004 spring should have reduced growth or reproduction of either gelatinous species. By late July 2005, surface water temperatures exceeded 30°C, the temperature at which *M. leidyi* suffers mortality in laboratory experiments (D. Breitburg, unpublished data). However, *M. leidyi* could have avoided high midday surface temperatures by moving lower in the water column, and the appearance of predatory *C. quinquecirrha* is a more parsimonious explanation as the major cause of the seasonal ctenophore decline during 2005, given the high percentage of *M. leidyi* with damage indicative of encounters with medusae (Purcell and Cowan, 1995; Kreps et al., 1997).

With a mean depth of 2 m, the shallow bathymetry of the Rhode River may limit the potential for coexistence of *M. leidyi* and *C. quinquecirrha*. In the Rhode River, densities of *M. leidyi* averaged less than 2 mL m^{-3} in August and September 2005 when *C. quinquecirrha* densities reached an average of 2–6 mL m^{-3}. In contrast, Keister et al. (2000) found 26.6 mL *M. leidyi* m^{-3} in the Patuxent River, Maryland, when *C. quinquecirrha* density averaged 11.8 mL m^{-3}. The deeper water column of the Patuxent, which includes a bottom layer with variable and sometimes severely hypoxic DO concentrations (Breitburg et al., 2003), may provide greater opportunity for spatial separation of *M. leidyi* and *C. quinquecirrha* and increase survival of *M. leidyi* at moderate *C. quinquecirrha* densities.

Prey availability could limit *M. leidyi* abundance and production, but our data do not suggest that low mesozooplankton densities were likely to have caused the large interannual variation in ctenophore abundances. Mesozooplankton densities were higher in 2004 than in 2005, and the temporal pattern of mesozooplankton and ctenophore abundances was more suggestive of ctenophore control of mesozooplankton than the reverse. An inverse relationship between copepod densities and ctenophore abundance has been noted previously in both Chesapeake Bay (Feigenbaum and Kelly, 1984; Purcell and Cowan, 1995) and Narragansett Bay (Sullivan et al., 2001). In both years of our sampling, high densities of *M. leidyi* recruits were found in the Rhode River during periods of lowest mesozooplankton densities. We did not sample microzooplankton, however, and cannot rule out their potential influence on ctenophore abundance.

The maximum egg production we measured in the Rhode River (9,000 eggs $individual^{-1}$ *M. leidyi* day^{-1}) was lower than the maximum reported value of 14,000 eggs $individual^{-1}$ day^{-1} (Kremer, 1976; Reeve et al., 1989) but well within the range of values reported elsewhere. *Mnemiopsis leidyi* egg production in the Rhode River was similar to that of field-collected ctenophores from elsewhere in Chesapeake Bay (Purcell et al., 2001), including the Patuxent River (D. Breitburg and R. Burrell, unpublished data). *Mnemiopsis leidyi* from

the Patuxent produced a maximum of 610 eggs mL^{-1} of ctenophores at mesozooplankton abundance of 1 individual L^{-1}, which is very close to the rate found in this study of 668 eggs mL^{-1} at 2.2 mesozooplankton individuals L^{-1}. Variation among dates in the relationship between zooplankton density and egg production suggests an interesting pattern of trade-offs in energy allocation to somatic growth versus reproduction, or nutritional constraints.

Predicted changes in sea-surface temperatures and rainfall throughout the world may lead to changes in the geographic ranges of many aquatic organisms. The Rhode River provides an interesting model that may aid predictions of climate change-related shifts in ranges and predator–prey dynamics because it is often near the threshold of salinity tolerances and the dynamics of the system can fluctuate markedly from year to year. These characteristics of the Rhode River allowed us to examine the gelatinous zooplankton food web within the river during two distinct years: one with, and one without, strong influence by a top predator. Differences in species abundances and food web interactions observed here may help to predict dynamics in other systems as environmental conditions, and the range of *C. quinquecirrha*, change. Although generally considered a nuisance species by swimmers and fishermen, *C. quinquecirrha* may benefit fisheries and habitat by controlling densities of *M. leidyi*, which is an important predator of oyster larvae—a prey not utilized by *C. quinquecirrha* (Purcell et al., 1991; Breitburg and Fulford, 2006).

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Patterns of Water Quality and Movement in the Vicinity of Carrie Bow Cay, Belize

Karen H. Koltes and Thomas B. Opishinski

ABSTRACT. Meteorological and oceanographic conditions have been monitored at the Smithsonian Field Station at Carrie Bow Cay, Belize, since 1993 through the Caribbean Coastal Marine Productivity (CARICOMP) program, and since 1997 through an automated monitoring system operated by the Caribbean Coral Reef Ecosystems Program (CCRE). Collectively, the two datasets represent a unique resource that provides a mechanism to improve our understanding of changing environmental conditions on the Mesoamerican Barrier Reef and, particularly, the conditions governing water quality and movement around Carrie Bow Cay. Especially evident is the broad influence on water quality of seasonal climate patterns as well as short-term events such as cold fronts and major storms. Among several variables examined, wind direction appears to be a good indicator of water quality conditions. From March to June, prevailing northeasterly airflow and limited rainfall result in higher water quality along this portion of the Belize Barrier Reef. Under decreased trade or increasing westerly winds, especially during periods of higher rainfall from October to January, turbid coastal water moves (drifts or is pushed) out onto the reef from the lagoon. The most significant finding, however, has been a dramatic loss of water quality along this portion of the Belize Barrier Reef since monitoring began at Carrie Bow Cay in 1993.

INTRODUCTION

Carrie Bow Cay, Belize, has been the site of extensive biological, geological, and ecological study as part of the Smithsonian Institution's Caribbean Coral Reef Ecosystem (CCRE) program (Rützler and Macintyre, 1982). Despite more than three decades of multidisciplinary research, however, relatively little is known about the complex interaction of physical factors that influence the reef environment, including winds, tides, temperature, and rainfall. These physical parameters provide the context for understanding and predicting relationships between reef organisms and their environment, but such parameters require accurate and consistent measurement over long time periods to establish a reliable description of baselines and trends. This is particularly true of water quality and movement, as the constant mixing and motion of water masses, bathymetry, and proximity to sediment inputs lead to significant spatial and temporal variability.

Establishing baselines and trends for environmental conditions has also become increasingly important as Belize, as well as neighboring countries, experience rapid

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urban and economic growth from the recent expansion of agriculture, aquaculture, and tourism. As the coastline and mangrove cays of Belize have experienced accelerated development over the past few decades, the barrier reef environment has been subjected increasingly to chronic and acute disturbances from terrigenous inputs. Poorly managed exploitation of coastal and offshore natural resources, extensive land modifications from dredging, land reclamation, deforestation, and conversion, and effluents from sewage and agriculture/aquaculture are delivering increased loads of sediments, nutrients, pesticides, herbicides, and other man-made chemicals to the central lagoon (Gibson and Carter, 2003). Eroded sediments and the residues of fertilizers and pesticides are also entering Belize's coastal waters from the more than 300,000 hectares of banana, oil palm, sugar cane, citrus, and pineapple crops cultivated across the wider Mesoamerican region (Burke and Sugg, 2006).

Early qualitative observations suggested that a period of heavy rainfall in the central portion of Belize was followed within 1 to 2 days by the appearance of a plume of low-salinity, turbid water over the fore-reef at Carrie Bow Cay (CBC). For major storms, the lens could be significant in duration and thickness. Rainfall from Hurricane Mitch, a Category 5 storm, approached 2 m over Central America between 29 October and 1 November 1998, causing severe flooding, landslides, and mudflows. Much of the storm discharge entered the Gulf of Honduras, where it flowed north as a highly turbid, plankton-enriched water mass, reaching the Belize shelf on 3 November 1998 (Andréfouët et al., 2002; Sheng et al., 2007). On 15 November 1998, K. Koltes observed this surface lens to be about 15–20 m in thickness in the waters adjacent to CBC. Further in situ characterization of the lens was not possible because of the closure of the field station, but recent numerical modeling of satellite images of terrestrial runoff plumes in the Gulf of Honduras confirms that influxes of sediments and nutrients are reaching the central reefs from local and more distant origins (Andréfouët et al., 2002; Tang et al., 2006; Chérubin et al., 2008; Paris and Chérubin, 2008).

Consistent monitoring of environmental variables began at Carrie Bow Cay in 1993 as part of the Caribbean Coastal Marine Productivity Program (CARICOMP). CARICOMP is a regional scientific effort to study land–sea interaction processes, to monitor for change on a local and regional scale, and to provide appropriate scientific information for management (Kjerfve et al., 1999; http://www.ccdc.org.jm/frontpage.html). In 1997, an automated environmental monitoring system (EMS) was installed at Carrie Bow Cay that provides an independent set of weather and water quality measurements. It was one of the earliest monitoring systems to process and transfer real-time data from a remote geographic location to a website for public access (http://nmnhmp.riocean.com). To our knowledge it is still the only automated system continuously monitoring oceanographic and meteorological conditions on the outer Mesoamerican Barrier Reef. In December 1997, a catastrophic fire destroyed the field station, suspending all monitoring except the automated water temperature measurements under the CARICOMP program. The full complement of CARICOMP measurements resumed after the station reopened in late 1999, and the automated measurements resumed after a new EMS was installed in September 2000.

Together, the CARICOMP and EMS datasets have provided a nearly continuous record of climatic and oceanographic conditions along the central portion of the Belize Barrier Reef. The 15 years of water temperature and Secchi disk measurements likely now constitute the longest continuous records for the Belize Barrier Reef and are among the longest for the entire Mesoamerican region. The data serve as an important resource for researchers to examine long-term trends, episodic events, and short-term and seasonal cycles (McField and Kramer, 2004). The data also allow comparative studies with other reef ecosystems to assess biodiversity and correlate environmental factors with biological phenomena. Data have been used to characterize prevailing conditions (Koltes et al., 1998) as well as the anomalous conditions that occur during extreme climatic events such as the El Niño–Southern Oscillation (ENSO) of 1997–1998 (Aronson et al., 2002) and powerful storms such as the Category 5 hurricanes Mitch, Keith, Iris, Dean, and Wilma.

These long-term datasets are beginning to yield reliable descriptions of meteorological and oceanographic conditions around Carrie Bow Cay. We report here on preliminary analyses of the patterns, trends, and relationships that are emerging from these long-term records, with special reference to factors that control water quality and movement around Carrie Bow Cay.

METHODS

Carrie Bow Cay is a small island (0.7 acres) located in the central province of the Belize Barrier Reef (Burke, 1982) about 18 km from the mainland (16°48′N and 88°05′W; Figure 1). Carrie Bow Cay lies on the barrier reef proper between two tidal passes, a relatively rare occurrence along the otherwise nearly continuous barrier

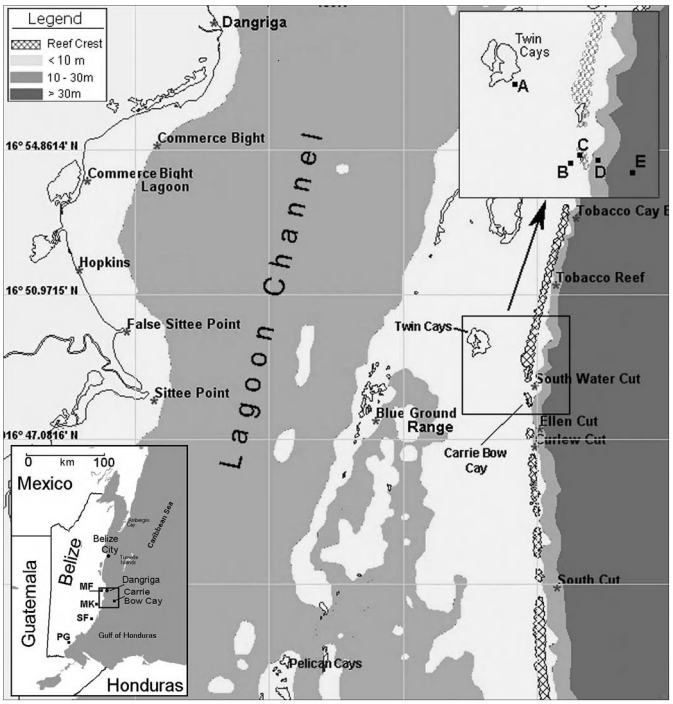


FIGURE 1. Map of the central province of Belize showing the location of the Smithsonian Institution's Field Station (16°48′N and 88°05′W) at Carrie Bow Cay. Note the Lagoon Channel that separates the outer lagoon platform from the mainland. Inset upper right: Location of the CARICOMP permanent monitoring sites in the seagrass beds near Twin Cays (A = "lagoon") and Carrie Bow Cay (B), on the inner fore-reef slope (D) and seaward of the barrier reef (E = "drop-off"), and (C) the Environmental Monitoring System on Carrie Bow Cay. Inset lower left: The Belize National Meteorological Service stations at Melinda Forest (MF), Maya King (MK), Savannah Forest (SF), and Punta Gorda (PG).

reef. It is in close proximity to deep ocean water (>300 m) to the east. A line of cays to the west that includes Twin Cays is part of a fault-block ridge that separates the shallower back-reef lagoon from the deeper Lagoon Channel that parallels Belize's shoreline. Although shoal formations are the outcome of a variety of factors such as currents and sea level changes, the present-day patterns of parallel shoals, reefs, and mangrove islands in this area resulted principally from faulting along a NNE trend during the Pliocene era (Dillon and Vedder, 1973; Macintyre and Aronson, 1997).

CARICOMP Measurements

Scientific monitoring of meteorological and oceanographic conditions under the CARICOMP program has included daily measurement of precipitation (mm) and air temperature (°C) at Carrie Bow Cay ("C" in Figure 1) and weekly measurement of surface water temperature (°C) and salinity (‰) at a permanent monitoring station in the seagrass beds adjacent to Twin Cays ("lagoon"; "A" in Figure 1; water depth ≈1.2 m) and in the ocean seaward of the drop-off ("drop-off"; "E" in Figure 1; water depth >300 m). Bottom water temperatures have been recorded continuously at intervals of 15–48 min at the permanent CARICOMP monitoring sites in the lagoon ("A" in Figure 1), in the seagrass beds adjacent to Carrie Bow Cay ("B" in Figure 1; water depth ≈2m), and on the inner fore-reef ("D" in Figure 1; water depth ≈13.5m) using Onset Corporation's model HOBO, StowAway, and TidbiT data loggers (±0.2°C).

Water quality characteristics that are associated with water transparency have been measured by Secchi disk as horizontal distance (m) taken 0.5 m below the surface in the lagoon ("A" in Figure 1) and vertical distance (m) at the drop-off ("E" in Figure 1). From 1993 to 1997, water transparency was measured once a week between 1000 and 1200 using a 30 cm diameter disk with black and white quadrants. In 1999, the CARICOMP protocol was modified to take advantage of the 20 cm diameter black and white disk that is more available commercially. The difference in the diameters of the two Secchi disks has little effect on the measurements, particularly compared to other sources of error such as sun angle, cloud cover, sea state, wind, currents, and observer difference (Steel and Neuhausser, 2002; Hou et al., 2007). In 2002, the Secchi disk measurements were increased to two times per week to more accurately characterize water quality trends. No water transparency measurements were made during adverse weather conditions, particularly over the drop-off, during closure of the station in the fall of 1993 and 1994, and for approximately two years following destruction of the field station from the fire in 1997.

To better characterize water quality, especially during storm events, light intensity loggers were mounted on a cinder block (water depth ≈13.5 m) at the permanent CARICOMP monitoring site on the inner fore-reef (note "D" in Figure 1). Onset Corp.'s model StowAway LI was used from 2002 until 2005 when Onset ceased manufacturing this model; Onset's model HOBO UA-002-64 Pendant Temp/Light has been used since 2005. Light intensity (lumens/ft^2) was recorded at intervals from 5 s to 15 min over periods ranging from days to weeks from 2002 to 2008. While deployed, the light logger was kept free of sediment and epibionts by periodically wiping the surface of the housing. The StowAway LI was designed to measure relative light levels (e.g., sun versus shade) and was calibrated for incandescent sources (spectral response, ≈200–1,100 nm; range, ≈0.001–1,000 lumens/ft^2). The

HOBO UA-002-64 Pendant Temp/Light was designed to measure relative light levels indoors or outdoors (spectral response, ≈200–1,200 nm; range, ≈0–30,000 lumens/ft^2). Our objective was to establish patterns in relative light levels, and hence water transparency, by comparing in situ irradiance on the inner fore-reef ("reef irradiance") to that at the surface ("incident irradiance"). No attempt was made to relate these measurements to biologically active wavelengths.

Environmental Monitoring System

The EMS continuously records meteorological and oceanographic conditions at Carrie Bow Cay. A marine-grade wind monitor (RM Young model 05106), LI-COR model LI-200 pyranometer, temperature/relative humidity sensor (Vaisala model HMP50), and barometric pressure sensors monitor meteorological conditions. The weather sensors, mounted on an aluminum tower above the main laboratory, are approximately 13 m above ground level ("C" in Figure 1). Rain is measured with a Texas Electronics solid-state tipping bucket rain gauge (model 525USW). The pyranometer features a silicon photovoltaic detector designed to measure solar radiation under conditions of unobstructed natural daylight. Measurements of wind speed (mph) and direction (0°–360°), solar radiation (W/m^2), rain accumulation (mm) and rate (cm/h), barometric pressure (mbar), air temperature (°C), and relative humidity (%) are recorded every 10 min.

Oceanographic conditions are monitored via a YSI model 6600EDS multiparameter water quality sonde, mounted inside a stilling tube. The sonde is mounted on the dock on the west side of Carrie Bow Cay about 0.6 m below the surface of the water ("C" in Figure 1). Every 10 min, measurements are taken of water level (m), water temperature (°C), salinity (‰), dissolved oxygen (% saturation), pH, and turbidity (NTU).

Data acquisition of both oceanographic and meteorological systems is managed automatically by a datalogger and control system and transmitted by radio to a server on the mainland. A regular program of maintenance, including calibration of sensors to manufacturer's standards (Eaton et al., 2005) and minimization of fouling, is followed to maintain the best possible data quality. Data are subjected to a quality assurance/quality control (QA/QC) process to remove outliers and invalid and suspect data before they are included in the historical archives. The QA/QC process also incorporates established criteria and procedures to correct for sensor and fouling drift (Wagner et al., 2006).

The data have contributed to new and existing research studies, publications, and management programs for both Smithsonian researchers and an increasing number of organizations from the region (e.g., Renken and Mumby, 2009).

Analysis

The annual means and standard deviation of Secchi disk measurements from 1993 to 2008 for the lagoon (n = 787) and drop-off (n = 727) were calculated, and the 15-year trend in water quality was determined by least-squares regression analysis. To evaluate seasonal patterns in water quality, monthly means were calculated for the Secchi measurements from the lagoon because horizontal measurements are generally more reliable than vertical measurements (Steel and Neuhausser, 2002) and were more often taken during adverse weather conditions.

To examine annual variation in wind patterns, monthly wind rose plots were generated for Carrie Bow Cay using approximately 350,000 wind measurements collected between 2003 and 2008. A wind rose plot is a combination of a polar plot and a histogram that depicts the distribution of wind speed and the frequency of occurrences that wind blows from 1 of 16 cardinal directions (N, NNE, NE, etc.). Seasonal and geographic patterns of rainfall were derived by calculating monthly mean precipitation for Carrie Bow Cay and four coastal stations (Melinda Forest, Mayaking, Savannah Forest, and Punta Gorda; see Figure 1, lower inset) maintained by the Belize National Meteorological Service (2003–2008).

Previous oceanographic studies at Carrie Bow Cay suggested that tides play a major role in controlling water movement, and hence water quality, around Carrie Bow Cay (Greer and Kjerfve, 1982; Kjerfve et al., 1982). To examine the role of tides, reef irradiance was compared to meteorological and oceanographic variables using a harmonic regression analysis model based on linear regression algorithms to compute constituents. Specifically, we compared reef irradiance to incident irradiance and then wind direction and intensity, tidal stage, and precipitation from all stations. The objective of the analyses was to extract harmonic constituents to judge the relative strength of the tidal forcing on the light patterns. The analyses were inconclusive because tidal components were small relative to the strong solar forcing inherent to the measurements of light and from the lack of a strong diurnal or semidiurnal signal in the "microtides" of Belize (Kjerfve et al., 1982).

To establish annual changes in light, monthly means were computed for reef and incident irradiance from 2003

to 2008. The analyses included more than a half million reef irradiance samples and approximately 350,000 measurements of incident irradiance. The monthly means were normalized with respect to the maximum monthly mean observed in each set of light measurements; this established a common datum reference and allowed comparisons of monthly and seasonal variations and patterns. Additional qualitative analyses of the various time series suggested that a consistent correlation existed between patterns of reef irradiance and both seasonal and short-term changes in wind direction. A comparative analysis of conditions during periods of normal weather conditions and specific weather "events" was undertaken to further define the localized influence of wind direction and other parameters on water quality over the fore-reef and to identify external (regional) events that are observed in the Carrie Bow Cay measurements.

To establish annual changes in light, monthly means were computed for reef ($n \approx 500{,}000$) and incident irradiance ($n \approx 350{,}000$) from 2003 to 2008. The monthly means were normalized with respect to the maximum monthly mean computed for each set of light measurements; this established the maximum mean for each set as a common reference point and allowed comparisons of monthly and seasonal variations and patterns. Additional qualitative analyses of the various time series suggested that a consistent correlation existed between patterns of reef irradiance and both seasonal and short-term changes in wind direction. A comparative analysis of conditions during periods of normal weather conditions and specific weather "events" was undertaken to further define the localized influence of wind direction and other parameters on water quality over the fore-reef and to identify external (regional) events that are observed in the Carrie Bow measurements.

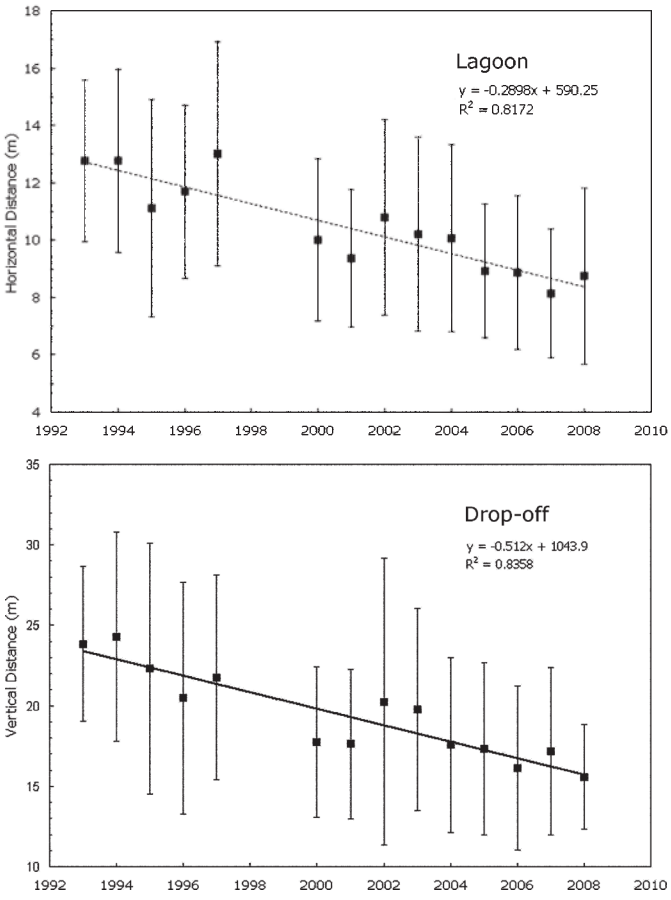
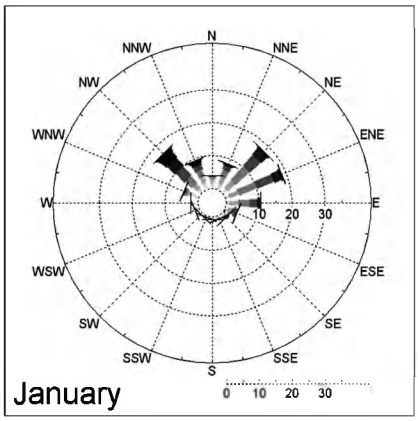


FIGURE 2. Annual mean and standard deviation of Secchi disk distance (horizontal, m) in the lagoon (top) and over the drop-off seaward of Carrie Bow Cay (vertical, m; bottom) from 1993 to 2008. There has been a dramatic and significant ($P < 0.001$) loss of transparency in the waters around Carrie Bow Cay during the past 15 years.

RESULTS

Significant temporal and spatial variability exists in the records of the meteorological and oceanographic variables. Particularly evident are longer-term seasonal patterns as well as signatures of short-term events such as cold fronts and major storms. The most significant finding, however, has been a dramatic decline in water clarity along this portion of the Belize Barrier Reef since monitoring began at Carrie Bow Cay in 1993 (Figure 2). Mean annual Secchi distance (horizontal) declined from 12.8 m in the lagoon in 1993 to 8.7 m by 2008, a loss of almost 0.3 m/year. During the same period, mean annual Secchi distance (vertical) declined from 23.8 m to 15.6 m over the drop-off or by about 0.5 m/year.

Comparisons of monthly means of water transparency, wind direction, and precipitation show that seasonal weather patterns strongly influence water quality (Figure 3). From February through May, the prevailing northeasterly airflow (Figure 3, left) is associated with a uniformly dry pattern across all stations (Figure 3, right). Monthly rainfall amounts average less than 75 mm. By June, a divergence can be seen among the stations, with those to the south receiving increasingly greater amounts of rain relative to the stations to the north, including Carrie Bow Cay. The sharp onset of the rainy season in the south is partly the result of the intrusion of the Inter-Tropical Convergence Zone as it migrates northward (http://hydromet.gov.bz/Climate_Summary.htm).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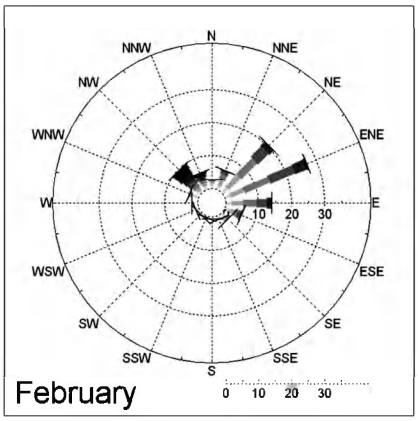
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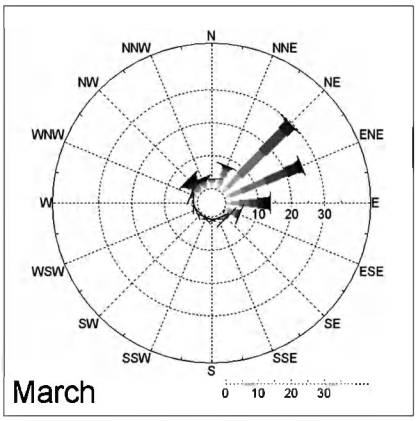
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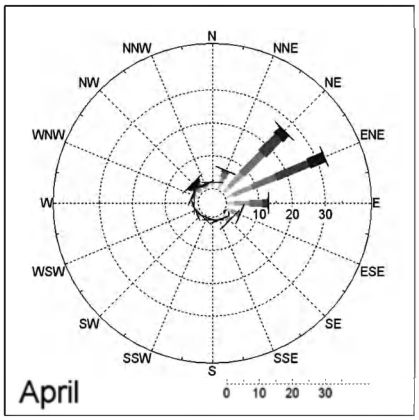
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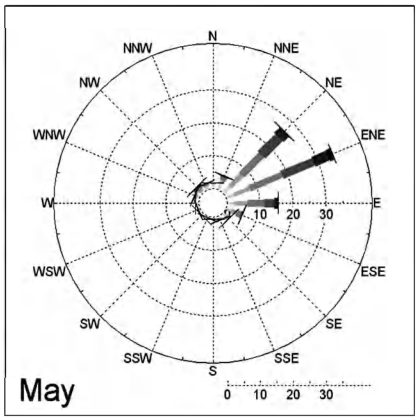
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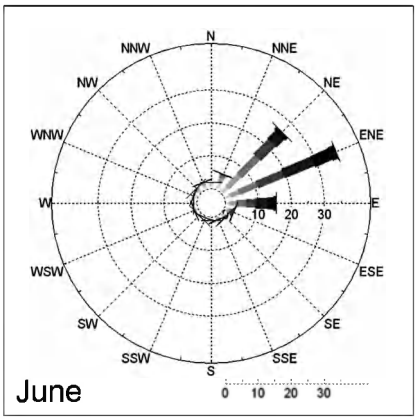
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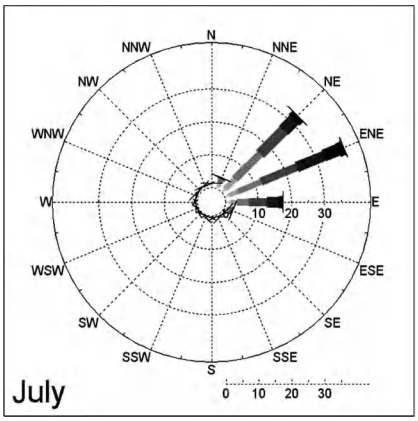
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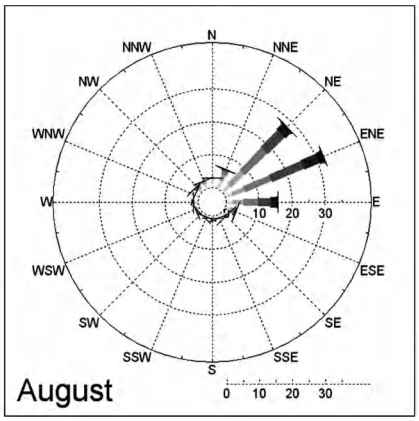
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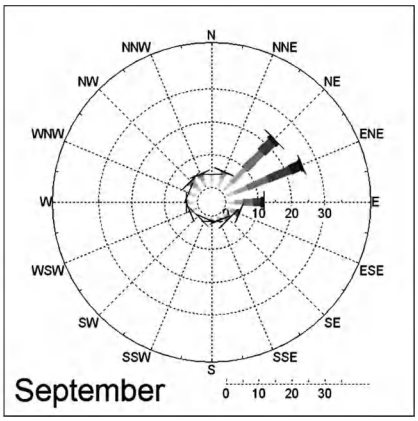
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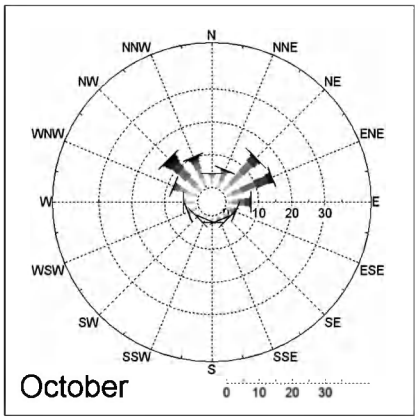
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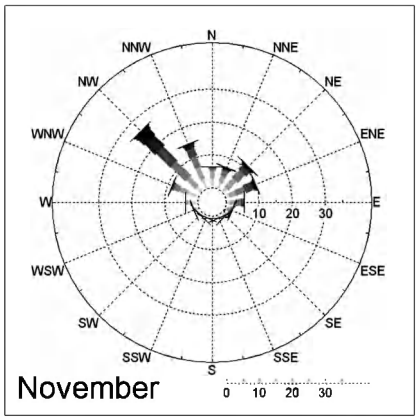
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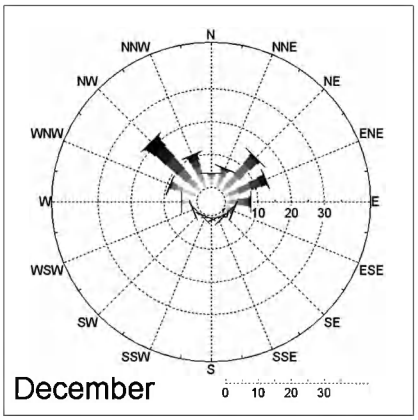
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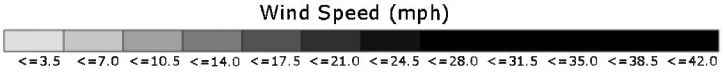
Wind Speed (mph)
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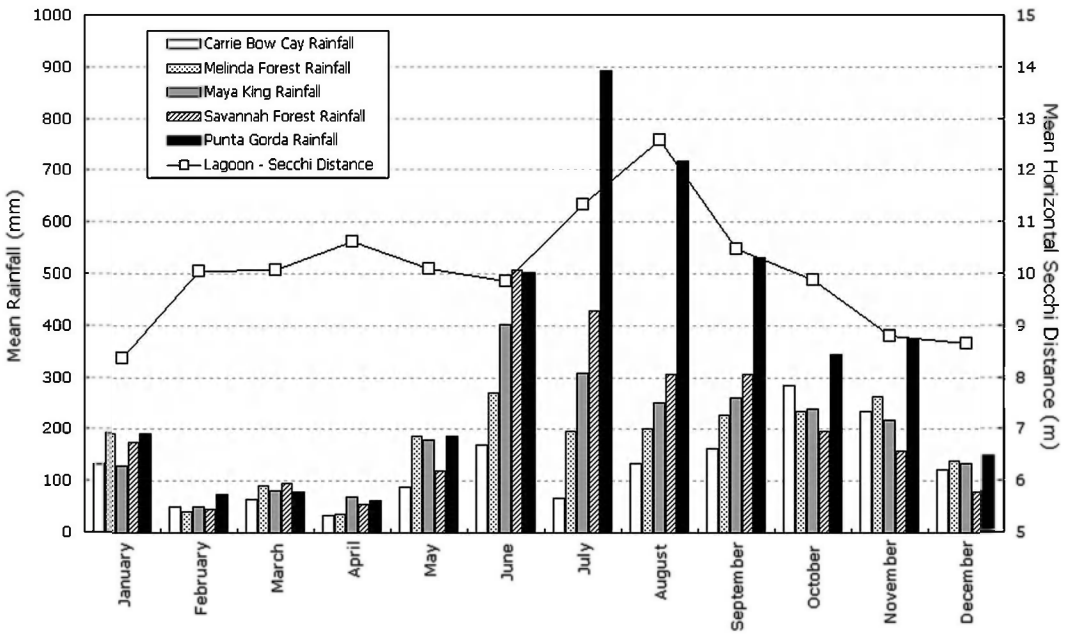

FIGURE 3. Left (*facing page*), Monthly means of wind speed and direction at Carrie Bow Cay for 2003–2008. The wind rose depicts the distribution of wind speed and direction: the length of each "spoke" indicates the percentage of time that winds blow from 1 of 16 cardinal directions (N, NNE, NE, etc.); the categories within the spoke represent the speed. Trade winds are the dominant pattern from March to September. Wind patterns are most variable during the transitional months of October/January/February. **Right** (*above*), Monthly means of rainfall at Carrie Bow Cay and the four Belize National Meteorological Service stations (left axis), and water transparency in the lagoon (horizontal Secchi disk distance [m], right axis) from 2003 to 2008.

Rainfall maxima also diverge in terms of timing. Mean monthly rainfall peaks in June–July in southern Belize, with approximately 900 mm at Punta Gorda; the maximum at Carrie Bow Cay (about 290 mm) occurs in October. The marked shift in rainfall patterns beginning in June is accompanied by consistent northeasterly winds and tropical waves moving westward from June to November (http://hydromet.gov.bz/Climate_Summary.htm). Cold fronts, or "northers," occur frequently from December to February and are associated with the southerly extension of the North American high-pressure system. During the peak season of December and January, cold fronts pass through Belize approximately every 10 days, the signatures of which appear in the temperature profiles on the fore-reef (Figure 4).

Monthly Secchi disk measurements in the lagoon (see Figure 3, right) correlate with the seasonal shift in climate patterns. Water transparency peaks in August (12.6 m), while incident irradiance is high (Figure 5) and the winds are still predominantly from the NE (see Figure 3, left). This peak also corresponds to a break in the rainy season on the mainland known as the "Mauga" (http://hydromet.gov.bz/Climate_Summary.htm). In contrast, the break in the rainy season occurs in July at Carrie Bow Cay (see Figure 3, right). Water transparency reaches a minimum in January (8.4 m) around the winter solstice and the period when cold fronts, characterized by strong NW winds, reach a peak (Figure 3, left). This period of increased storm activity and resulting high sea states also interrupts routine Secchi disk measurements such that the winter means may be biased upward.

The seasonal pattern of reef irradiance is similar to that observed for water transparency measured by Secchi disk (see Figure 5) and is largely governed by changes in incident irradiance. Maximum irradiance occurs around the solstice, with reef irradiance showing a peak in June compared to August for Secchi disk distance (see Figure 2) and July for incident irradiance. However, reef irradiance attenuates more rapidly toward the winter solstice relative to incident irradiance. By December, reef irradiance has fallen to less than 40% of its summer peak whereas incident irradiance has fallen only to 60% of its summer

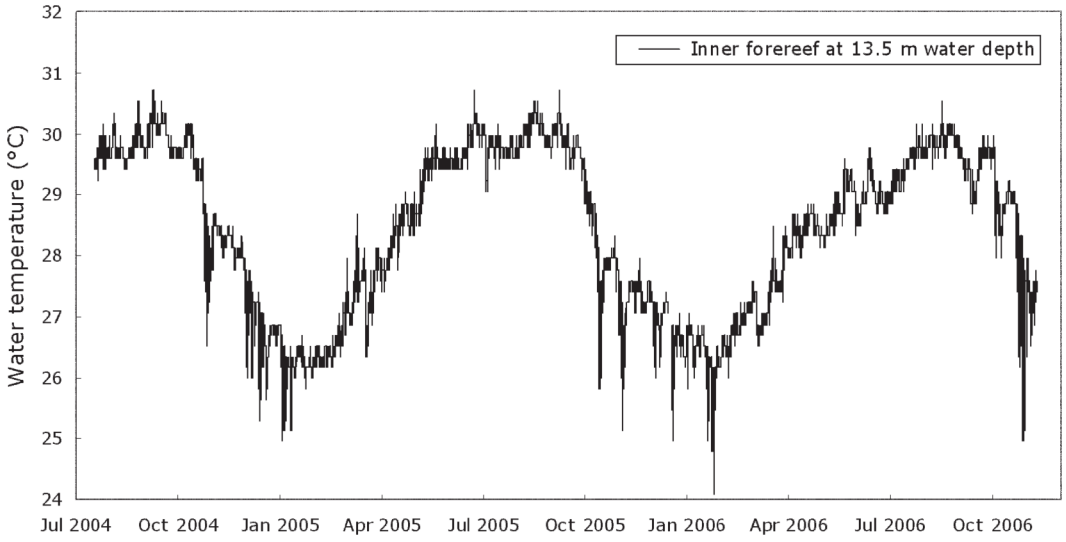

FIGURE 4. Water temperature on the inner fore-reef at 13.5 m from 29 July 2004 to 11 December 2006. Note the signatures of cold fronts in the temperature profile between October and January and a late season cold front that passed 1–3 April 2005.

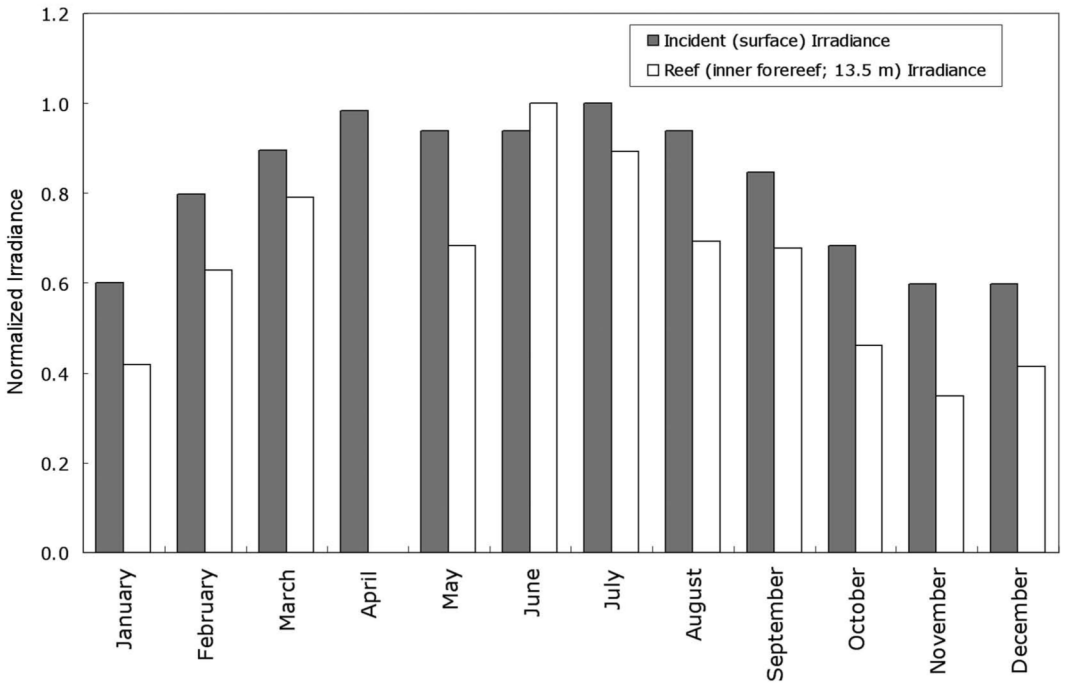

FIGURE 5. Monthly mean of reef irradiance (inner fore-reef, 13.5 m) and incident (surface) irradiance (2003–2008), normalized to allow comparisons among the light recording instruments. Reef irradiance attenuates at an accelerated rate relative to incident irradiance approaching the winter solstice.

peak. Decreased light levels around the winter solstice may be caused by lower sun angle, increased turbidity and/or higher sea states associated with the winter climate pattern.

Flow of turbid water into the area can also be seen in finer-scale comparisons of reef and incident irradiance (Figure 6) that suggest turbidity is transported from more distant locations. Following two days of light winds that had shifted from NNE first to the south and eventually to the NW, turbid water flowed on to the fore-reef, driving light levels down by about 50% despite nearly full incident irradiance. At least some of the flow may be related to tides (Figure 7), but as previously mentioned, the mixed, semidiurnal microtide of this region has made it difficult to analyze the role of tides.

DISCUSSION

The constant mixing and motion of water masses, localized inputs of sediments, and changing meteorological conditions produce strong spatial and temporal variations in conditions along the Belize Barrier Reef. These factors present a challenging analysis, especially when combined with Carrie Bow Cay's complex geomorphology that includes cuts in the reef to the north and south where disparate bodies of water mix together. Nevertheless, initial analyses have shown significant correlations between certain weather conditions, both episodic and seasonal, and water properties. Particularly evident are longer-term seasonal patterns, as well as signatures of discrete events such as the passage of cold fronts and other changes from

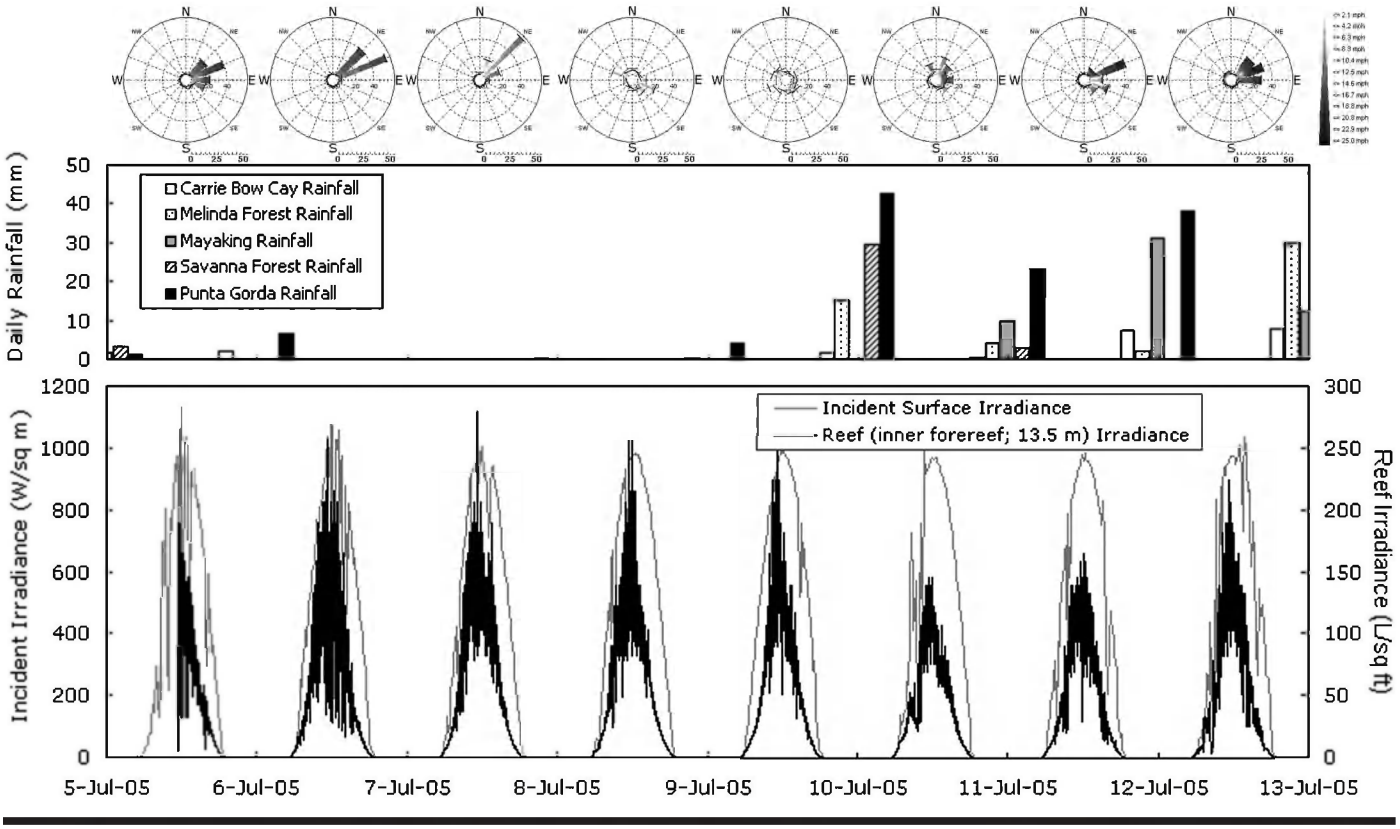

FIGURE 6. Progression of a turbidity event: Early in July 2005, light levels on the reef were among the highest recorded for reef irradiance (bottom, right axis). Beginning on 7 July, winds that had been blowing from the northeast at 15 mph began to taper off and remained calm through 9 July, as shown in the daily wind rose (top; see Figure 3 for description of wind rose). On 10 July, the winds increased at Carrie Bow Cay and heavy rainfall occurred on the mainland, particularly to the south (middle; stations as in Figure 3, right). Incident irradiance (lumens[L]/ft^2; bottom, left axis) remained high, but light levels on the reef dropped by nearly 50%. The drop in reef irradiance is attributed to a sediment plume, possibly from the south, that drifted over the fore-reef under conditions of little or no wind. Winds shifted around again to the northeast by 11 July, and subsequent mixing of water returned light levels over the reef to near maximum by 13 July.

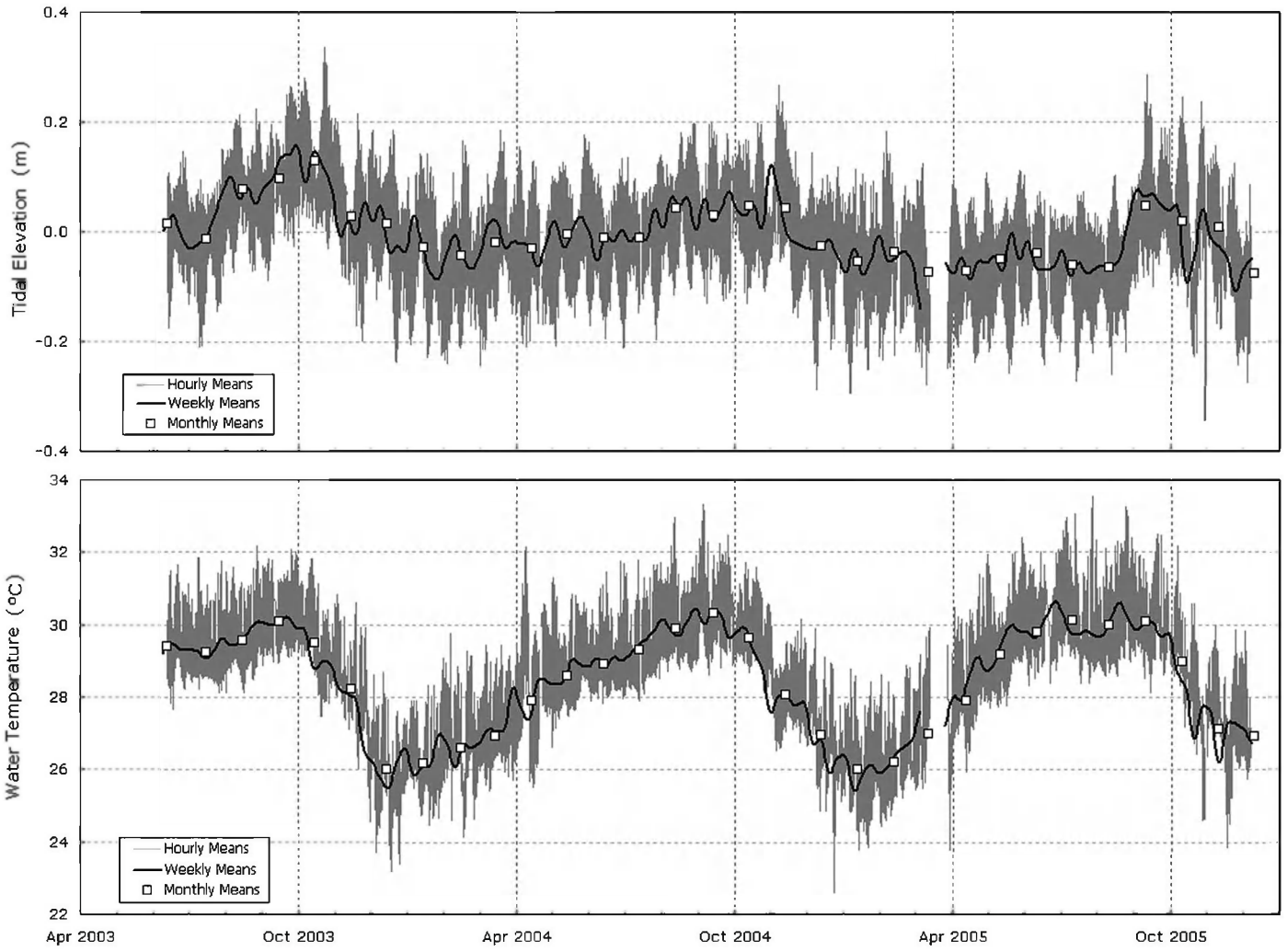

FIGURE 7. Hourly, weekly, and monthly means of tidal elevation (m) and water temperature (°C) measured at the dock at Carrie Bow Cay. Tidal elevation (top) reveals a complex pattern with a seasonal increase in elevation in October, when water temperature (bottom) reaches a maximum, that is typical of the Caribbean.

the typical weather patterns. Strong correlations are also apparent during less prominent variations in weather and particularly changes in wind direction such as those observed in July 2005. We found several other instances in which light levels on the reef dropped significantly when winds simply tapered off completely during the 24 to 48 hour "calm" period that often preceded a cold front. We also document a substantial decline of water quality around Carrie Bow Cay over the past 15 years.

Patterns of water transparency are driven largely by incident solar radiation, reflecting the strong seasonal cycle of solar radiation. However, variations in those patterns are useful at tracking short- and long-term changes in water transparency. The pattern of decreased light intensity on the fore-reef as a function of incident solar radiation in December versus June likely reflects the higher rainfall, consistent northwesterly winds, and frequent "northers" of the winter months and is consistent with recent simulation models showing locally higher turbidity during the winter months (Paris and Chérubin, 2008) from increased precipitation. This seasonal cycle is also apparent in the record of Secchi disk measurements. The difference observed between the timing of the maximum quality measured by Secchi disk and those measured by light logger may reflect the differences in the sampling period (1993–1998 versus 2002–2008). The disparity may also reflect the fact that the light logger data have not been collected as often during the spring–summer months as they have been during fall–winter months.

Although additional modeling and analysis are required, particularly of tidal currents, preliminary results suggest a strong link between climate patterns and water quality and movement along the barrier reef in the central province of Belize. We propose that, under the typical pattern of prevailing trade winds, sediment-laden riverine input is pushed shoreward and held along the coast where it flows south in the Lagoon Channel. A southerly flow of water immediately adjacent to the shoreline is consistent with circulation models that have been developed for the Gulf of Honduras (Ezer et al., 2005). We also suggest that the fault-block ridge along the eastern boundary of the Lagoon Channel forms a natural "sill" or "dam," facilitating the segregation of turbid coastal water driven shoreward by wind forcing. Under the influence of "oceanic" water, turbidity over the fore-reef at Carrie Bow Cay is generally lower.

Localized turbidity events are triggered when the northeasterly flow of the trade winds changes in either speed or direction. Turbid water that is normally contained along the coast spills over the submarine ridge of the Lagoon Channel and drifts or is pushed out across the outer (back-reef) lagoon and onto the fore-reef. Changes in the prevailing weather patterns are also frequently accompanied by periods of rain on the mainland that discharge additional sediment and freshwater into the lagoon, increasing the volume and degree of turbid coastal water and spillover onto the reef platform.

The dramatic loss of water quality at Carrie Bow Cay over the past 15 years also suggests longer-term effects of increasing sediment and nutrient loads to the lagoon from the rapid modifications of the Belizean coastline and that of neighboring countries. Recent hydrological models of the Mesoamerican Barrier Reef estimated that runoff and associated river discharge have doubled and sediment delivery has increased 20 fold under present-day land use changes compared to a hypothetical "natural" (unaltered) state (Burke and Sugg, 2006). Although the modeling suggested that Belize contributed only about 10%–15% of the sediment load to the region, the Belize River was identified as a significant contributor of sediments and nutrients to the Mesoamerican Barrier Reef. In the central and southern portions of Belize, large amounts of fertilizers used to cultivate citrus and bananas and the direct discharges of domestic sewage produce high nutrient levels in several areas along the coast (Gibson and Carter, 2003). Shrimp and other aquaculture operations are also discharging effluents directly to the lagoon. These sediments appear to become entrained in the Gulf of Honduras gyre and, over the long term, are driving down water quality across the region.

Increasing turbidity also appears to be related to a wider regional increase in sedimentation and nutrient enrichment in the Gulf of Honduras (Burke and Sugg, 2006). Hydrological models indicate that sediment delivery increases southward along the coastline of Central America with Honduras contributing an estimated 80% of the sediment and half of the nutrients to the region. Circulation models of the Gulf of Honduras suggest that these sediments and nutrients are carried north along the Mesoamerican Barrier Reef by the Caribbean Current (Thattai et al., 2003). Recent modeling of satellite images indicate that runoff from watersheds in northern Honduras can extend as far north as Glovers Reef atoll (Andréfouët, 2002; Paris and Chérubin, 2008). Based on modeling of satellite images and the hydrological models of Burke and Sugg (2006), Chérubin et al. (2008) concluded that concentrations of buoyant matter from terrestrial runoff into the Gulf of Honduras were high from October to January. Plumes were transported by a cyclonic gyre toward the Yucatan, creating seasonal variation in the concentration of runoff loads along the Mesoamerican Barrier Reef. The influence of terrestrial runoff was maximal from October to January and minimal from March to April.

Long-term in situ measurements of the sort presented here are relatively rare. Although the results of our analyses are preliminary, they already demonstrate the value of these measurements for advancing our understanding of the range and complexity of interactions of natural and human-induced variables governing the conditions around Carrie Bow Cay and across the region. These in situ data are also critical to ground-truthing remotely sensed data such as the satellite-generated sea-surface temperature (SST) records used to calculate bleaching thresholds during the 1998 ENSO (Aronson et al., 2002). Finally, our data are beginning to yield reliable descriptions of water quality conditions in the central portion of the Belize Barrier Reef, including those conditions that have accompanied the rapid modifications of the Belizean coastal zone during the past few decades. Most significantly, the dramatic loss of water quality documented by these long-term records has significant biological, management, and economic implications for Belize and the other countries of the Mesoamerican Barrier Reef.

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Global Change and Marsh Elevation Dynamics: Experimenting Where Land Meets Sea and Biology Meets Geology

J. Adam Langley, Marc V. Sigrist, James Duls, Donald R. Cahoon, James C. Lynch, and J. Patrick Megonigal

ABSTRACT. Coastal marshes must accumulate soil to keep up with rising sea levels. It is unknown how the response of these ecosystems to global change will influence their ability to continue to keep up with sea-level rise. Here, we describe an in situ experimental chamber approach for manipulating key environmental variables, such as atmospheric CO_2 and soil N availability, in a brackish marsh. We outfitted each chamber with surface elevation tables (SETs) to closely monitor soil elevation change, a sensitive indicator of marsh vulnerability to sea-level rise. Further, the design facilitates measurements of ecosystem exchange of CO_2, plant productivity, porewater chemistry, and other environmental parameters.

INTRODUCTION

Projecting the impacts of climate change, eutrophication, and other perturbations on ecosystems requires experimental manipulations. Large experimental facilities have been built and operated in all types of ecosystems over the past decades to provide such data. There are at least six characteristics that complicate experimental manipulations in tidal wetlands. First, such ecosystems can be quickly and irreversibly damaged by heavy foot traffic, so boardwalks must be built to minimize long-term impacts on vegetation and soils. Second, because many wetlands have deep, low-density, peaty soils, the permanent infrastructure, such as boardwalks and chambers, must be well anchored for stability. Third, tidal wetlands are often inundated by tides and can be under more than a meter of water during storm surges, which dictates that all buoyant equipment must be soundly fixed in place. All electrical service and sensitive equipment must be positioned high and be easy to remove during extreme flooding events. Further, emergency shutoff systems must be in place to cut off the electrical supply and gas exchange equipment during flood events. Fourth, the water that floods brackish marshes is saline and corrodes most metals. Fifth, the lack of shade means that UV-sensitive materials will degrade. Care must be taken to select UV-resistant materials, and even those must be monitored and frequently replaced. Sixth, high-latitude marshes may experience cold winters. Ice formation can severely damage even rigid and well-anchored infrastructure. Here we

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describe a global change experiment in a brackish marsh that was designed to overcome these substantial technical challenges.

SITE DESCRIPTION

This study took place at Kirkpatrick Marsh, which is located on the Rhode River, a subestuary of Chesapeake Bay at the Smithsonian Environmental Research Center in Edgewater, Maryland. The site is dominated by the C_3 sedge, *Schoenoplectus americanus* (formerly *Scirpus olneyi*), and less so by two C_4 grasses, *Spartina patens* and *Distichlis spicata*. The soils at this site are organic (80% organic matter) to a depth of approximately 5 m. Mean tidal range is 30 cm. The high marsh zone is 40–60 cm above mean low water level and is inundated by 2% of high tides. Salinity averages 10 parts per thousand (ppt) and ranges from 4 to 15 ppt seasonally. Average daily low air temperature is −4°C in January, and the average daily high is 31°C in July.

To examine the interactive effects of elevated CO_2 and nitrogen addition, we identified 20 plots of similar plant composition in summer 2005. Each plot consisted of one octagon (2 m across) that would be enclosed in an experimental chamber to allow for atmosphere manipulation and an adjacent rectangular portion (2 × 1 m) that served as a reference plot to account for spatial variation and to gauge potential chamber effects.

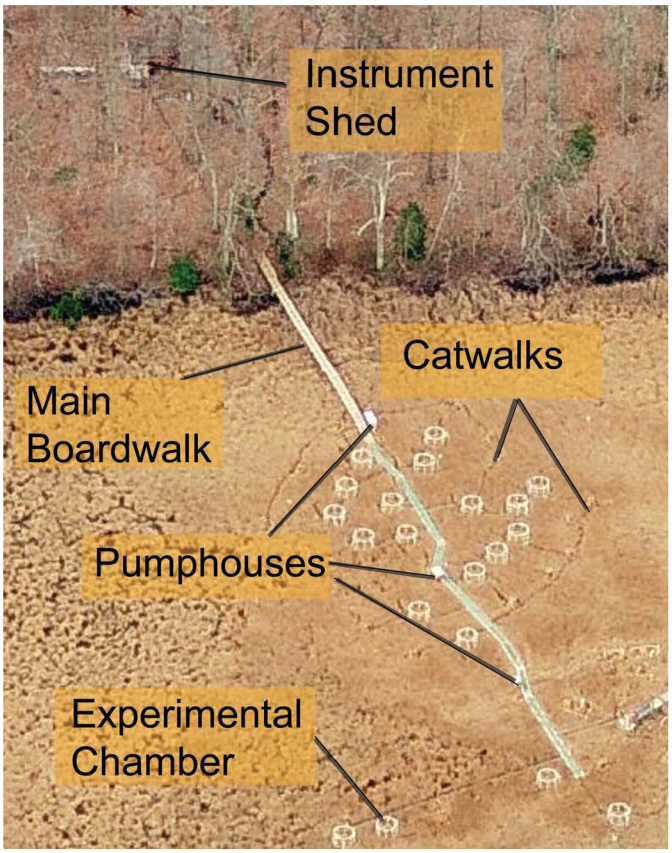

FIGURE 1. An overhead image of the entire CO_2 site. The main boardwalk connects to 20 experimental plots by smaller catwalks, the paths of which are visible. Gas samples are pumped via the pump houses to the analytical shed on the bank.

CONSTRUCTION

Walkways and Equipment Housing

A main boardwalk and a series of thinner, lighter "catwalks" were built to access each plot without continually walking on the marsh (Figure 1; see also Figures 4, 5). The main boardwalk, built perpendicular to shore, roughly bisected the experimental plots. Most of the horizontal surfaces of the boardwalks were fiberglass grating (50% open), which allowed light to penetrate through the boardwalks, sustaining plant life and providing excellent traction. The supports for the main boardwalk were built of 10 × 10 cm posts sunk 2 m into the ground. The catwalks departed from the main boardwalk, forming a perimeter around each experimental chamber (Figure 2). These catwalks were less than 30 cm above the ground to avoid shading the plots. They were built of fiberglass grating (30 cm wide planks) laid flat on supports built of 2.5 cm polyvinyl chloride (PVC) that were anchored more than 1 m into the marsh using segments of 2.5 cm PVC pipe. After all walks were in place, the marsh surface was rarely stepped upon directly.

Three small pump houses, which housed air sample pumps and remote datalogging equipment, were built alongside the main boardwalk. An analytical shed was constructed on the bank 5 m above sea level to house sensitive analytical equipment.

Open-Top Chambers

The chamber design followed that of a previous open-top chamber study in the same marsh (Drake et al., 1989), but with several major innovations to enhance durability and plot accessibility (Figures 2–5). In 2006, the chambers consisted of four major components: base, manifold, chamber skeleton, and chamber panels. The octagonal shape of the chamber was a compromise between two design goals. It approximated a cylinder, which was ideal

for uniform air mixing inside the chamber and minimizing dead spots. The flat surface of each side allowed us to enclose the chamber with eight flat panels that can be removed easily for access to the inside of the chamber.

The base of the chamber was an aluminum octagon (0.5 cm thick, with an L-shaped cross section) implanted 10 cm into the marsh surface. In the portion of the base that was implanted into the soil, 2 cm holes were cut to allow root growth to further stabilize the base. A hollow octagonal manifold (cross section, 30 cm high × 6.35 cm wide) was attached to the base to distribute inflowing air equally around the chamber (see Figure 2). Manifolds were built from welded aluminum (grade 6061-T5) covered with transparent acrylic panels that allowed light transmittance.

Mounted to the top of the manifold was the "skeleton," consisting of eight vertical legs supporting an octagonal ring oriented horizontally at the top. The skeleton was built from 2.5 cm diameter PVC pipe. The only custom pieces in the skeleton were three-way fittings on the octagonal ring that join two PVC pipes in the ring with one leg. The joint was made by tapping female thread into the side of a 45° elbow. The legs of the skeleton sat in welded supports on the top of the manifold.

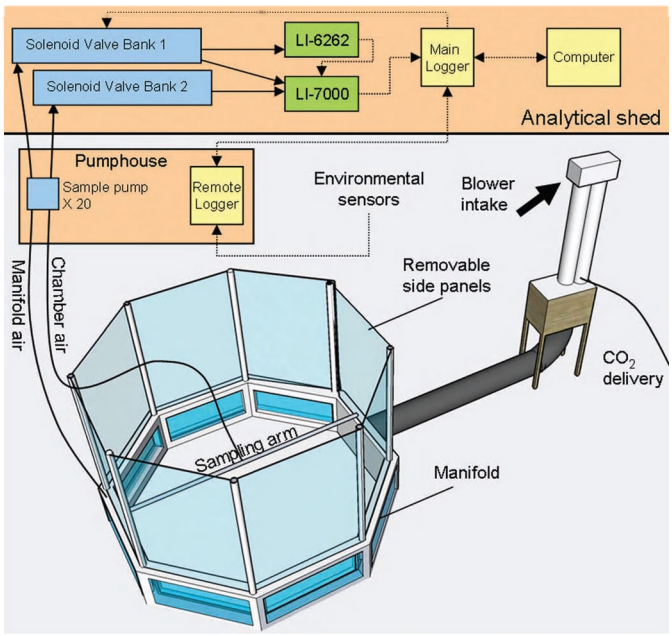

FIGURE 2. Schematic of an experimental chamber and gas sampling system. The ambient CO_2 chambers are the same except there is no CO_2 delivered into the blower stream. Solid (black) arrows represent air flow; dotted arrows (appearing light gray) represent information flow.

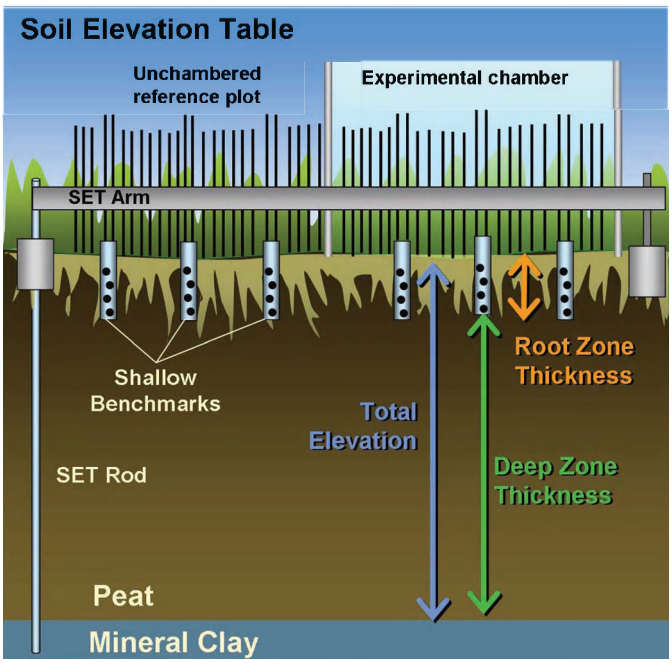

FIGURE 3. Schematic of the surface elevation table (SET) design. The SET arm is periodically connected to the SET rod benchmark, which has been anchored into the mineral clay underlying the peat profile. Pins are placed through holes in the SET arm to the soil surface to measure changes in elevation occurring over the entire profile. Any change in elevation of the shallow benchmarks must occur as a result of processes occurring beneath the root zone. Root zone changes are calculated by subtracting deep zone elevation change from total elevation change.

Removable rectangular panels were made from aluminum (grade 6063-T52), covered with infrared (IR)-transparent film (Aclar 22A, Honeywell) that was taped on using transparent UV-resistant tape (3M, 851). The film does not absorb IR radiation as do other films and therefore heat is not allowed to accumulate. The panels were attached to the PVC frame with custom-fitted PVC snaps so that any of the eight panels could be removed to access any portion of the plot. Further, panels were removed in the winter to prevent damage while CO_2 fumigation was terminated.

Finally, after the 2006 growing season, to conserve CO_2 and achieve a more stable CO_2 concentration by reducing wind incursions, an octagonal frustum, or wind foil, was added to the chamber design (see Figure 5). The frustum was constructed of 1.9 cm PVC, angled inward at 45°, and covered with the same film to reduce wind incursions. The final dimensions of the chamber were 1.5 m in height, 2 m in diameter, and with 1 m sides; the volume is 6.5 m^3.

FIGURE 4. Photograph of SET measurements being made in summer 2006. Each pin is gently lowered to the soil surface, while applying minimal pressure so that the pin does not depress the soil surface. (Photograph by M. V. Sigrist.)

To move ambient air through the open-top chamber, a blower (Dayton, 5C095) was mounted on a stand 1.5 m above the marsh surface to avoid high tides. The blowers were placed at least 6 m away from each chamber and oriented to avoid shading the study area (see Figures 1, 2, 4). PVC chimneys (two 15 cm pipes per blower) were affixed vertically to the top of the blower intake so that the blowers would take in air from 4 m above the ground that was not influenced by biological activity on the ground and thus had relatively stable [CO_2]. The chimneys were capped to prevent rainwater from entering the blower. A 20 cm diameter duct fed air from the blower to the chamber manifold. Two hundred fifty-two 1 cm diameter holes (the same total area as the intact pipes) were drilled on the inside of the manifold, so that air would flow in the chambers equally from each side of the octagon. The blowers forced 12.5 m^3 per minute through the chambers, resulting in an approximate chamber air turnover rate of 2 min^{-1}.

Surface Elevation Tables

To take repeatable measurements of soil elevation, each plot was outfitted with a rod surface elevation table (SET; see Figures 3, 4) (Cahoon et al., 2002) modified to accommodate plot dimensions. Outside each experimental chamber, a posthole was dug roughly 15 cm in diameter and 20 cm deep. A 30 cm long PVC pipe (15 cm diameter) was placed vertically into the hole. In the center of the PVC pipe, a series of attachable stainless steel rods was driven with an electric hammer through the entire profile of organic matter (4–5 m depth) and anchored to the point of refusal (6–7 m) into the subsurface mineral clay underlying the marsh. Concrete was poured into the PVC pipe to secure the top of the SET rod.

To isolate the influence of root zone processes on elevation, we implanted "shallow benchmarks" to a depth of 30 cm. The vertical movement of these benchmarks results from processes that occur below the top 30 cm of soil. The benchmarks were made of aluminum pipe (5 cm diameter by 40 cm long). Several 1 cm diameter holes were drilled into the sides of the lower 10 cm of the pipe to allow roots to grow through and anchor the benchmarks in place. Six benchmarks were implanted to a depth of 30 cm under the path of the SET arm in each chamber, three inside the chamber and three outside. After

FIGURE 5. Photograph showing a chamber with a frustum that was added to all chambers before the 2007 growing season. The tubing leading to one set of porewater wells, the gas sampling tube, and the catwalk is also visible. (Photograph courtesy J. A. Langley.)

placement, solid caps were placed on the top of each pipe. All these perturbations, as well as boardwalks to service each plot, were completed in the summer of 2005, at least 9 months before the beginning of the experiment.

At intervals ranging from 1 to 3 months, the modified horizontal aluminum SET arm (4 m long compared to less than 0.5 m long for the original rod SET design) was attached to the top of the SET rod benchmark, leveled precisely, and affixed to an aluminum post at the other end. The arm provided a horizontal reference of known elevation across the soil surface; changes in the distance from this reference surface to the soil surface were a sensitive measure of changes in soil elevation. Fiberglass pins (3 mm in diameter), all exactly 91.0 cm in length, were placed through precision-drilled holes in the SET arm at 1 cm increments. Approximately 40 individual measurements were made in each chamber and 40 in each adjacent, unchambered reference plot. Each pin was carefully lowered to the soil surface and gently placed so that no litter or live plant obstructed the pin. The height from the SET arm to the top of each pin was measured to the nearest millimeter (mm), providing a measurement of total elevation. Changes in absolute soil elevation were partitioned into either the root zone (top 30 cm of soil) or the deep zone (below 30 cm). To measure elevation changes occurring in the deep zone, we lowered 2 to 4 pins to the surface of each of the six shallow benchmarks (three inside and three outside each chamber) and measured in the same manner. We calculated the change in elevation resulting from processes occurring in the root zone, ΔE_R, from the two measured variables following the equation $\Delta E_R = \Delta E_T - \Delta E_D$, where ΔE_T represented the change in total elevation and ΔE_D represented elevation change attributable to change in thickness of the deep zone. Surface accretion was also measured using feldspar marker horizons in each plot (Cahoon et al., 1995). To eliminate compaction during coring, the deposition rate was estimated by taking cryocores (Cahoon et al., 1996) and measuring the amount of soil deposited on top of the marker horizon.

Total soil elevation was strongly related to innate spatial and temporal variability of deep zone dynamics. Specifically, changes in the thickness of the deep zone followed mean monthly sea level through time, and distance from the bank predicted the amplitude of that oscillation. To isolate treatment effects on various soil elevation parameters, we accounted for variation by referencing SET measurements in experimental chambers to those in the adjacent, unchambered reference plots, so that relativized ΔE = experimental plot ΔE − reference plot ΔE, where E = the elevation parameter of interest. We used a repeated-measures multivariate analysis of variance (MANOVA) to test for changes in elevation through time; we used *t* tests to liberally detect chamber effects on elevation parameters at individual dates and a two-way analysis of variance (ANOVA) to test for treatment differences in surface accretion.

TREATMENT APPLICATION

CO_2 Delivery and Sampling System

Carbon dioxide was delivered to each of the 10 elevated CO_2 chambers at a rate of approximately 6 L min^{-1} to achieve a target concentration of 720 ppm, which is nearly double the current ambient concentration of 380 ppm. Each CO_2 delivery line was controlled with metered valves and fed into the intake chimney on the blower for each respective elevated chamber. Adding the CO_2 upstream of the blower ensured sufficient mixing before air entered the chamber through the manifold.

Two sample lines continuously pumped air from each of 20 chambers to instruments located in a nearby shed: one line sampled manifold air and the other sampled the chamber atmosphere. To achieve a representative sample of the chamber atmosphere, air was sampled with a 2 m long pipe oriented horizontally across each chamber. The pipe was 1.3 cm diameter PVC with caps on both ends and a series of 2 mm diameter holes at geometrically increasing intervals away from the center of the pipe. The geometry allowed air drawn from the center of the pipe to be a composite sample representing each point on a transect through the chamber equally. The sampling pipe was positioned horizontally and adjusted to roughly half the green canopy height to best represent the air that photosynthetic tissue experienced.

Air was pulled under negative pressure from each chamber a short distance to a Teflon-coated double diaphragm pump (Thomas Industries, 2107-CA14-TFE), from which it was pushed under positive pressure to the analytical shed (see Figure 1). To avoid drawing water into the pumps, they were plugged into normally closed float switches (Dayton 3BY75) that cut the power supply when the water level approached the height of the gas sampling lines. Each of the 40 lines entered a bank of solenoid valves (model 3V1, Sizto Tech Corporation), then flowed into a common line, one for manifold lines and one for chamber lines. The two solenoid valves controlling each chamber opened simultaneously, one with manifold air and the other with chamber air; the other solenoid valves remained closed so that the contents of only one chamber at a time passed through the common lines to the gas

analyzers. Each chamber was sampled for 2 min to allow ample time for air in the common portion of the system to be flushed out before measurements were logged, which meant that each chamber was sampled at least once every 40 min.

One infrared gas analyzer (IRGA) measured the difference between a chamber's manifold air (i.e., incoming air) and a dry, zero-CO_2 reference gas. A second IRGA measured the difference between manifold air and chamber air. This configuration maximized our ability to precisely measure absolute CO_2 concentration and to accurately measure the CO_2 concentration difference between two locations. A LI-6262 (Licor, Lincoln, NE) had dry, zero-CO_2 air cycling through the reference cell and the manifold air passing through the sample cell. A Li-7000 (Licor) had the manifold air passing through the reference cell and chamber air passing through the sample cell. Cell A of the LI-7000 was referenced to an analog signal from the LI-6262 as the absolute concentration of CO_2 and H_2O in the manifold air.

We monitored the manifold line to determine how much CO_2 was being delivered to each manifold. The chamber air sampling line allowed us to monitor the actual chamber atmosphere and to fine tune the CO_2 delivery rate to achieve our target concentration in the chamber atmosphere, accounting for photosynthetic drawdown and wind incursions.

Nitrogen Fertilization

A total of 25 g N $year^{-1}$ was applied to each high-N plot. Ammonium chloride was dissolved in 5 L brackish water from the nearby Rhode River, the subestuary adjacent to the site. At five dates (approximately monthly, avoiding high tides) throughout the growing season we used backpack sprayers to deliver the fertilizer (equivalent to 5 g N) solution to 10 plots. Then, the fertilizer solution was rinsed from standing vegetation with another 5 L unamended river water applied with backpack sprayers. Each fertilization treatment simulated 5 g N m^{-2} in the equivalent of 0.5 cm river water. The 10 unfertilized chambers received 10 L unamended river water applied in the same manner. The river water was taken from the tidal fetch area adjacent to the marsh. Mean annual [NH_4^+] in that water ranges from 32 to 82 μg L^{-1}, with a mean of 52, and salinity has ranged from 4.0 to 10.6 ppt, with a mean of 6.7, over the past 20 years (growing season means from biweekly sampling; Thomas Jordan, unpublished data). Assuming the added NH_4Cl integrated into the top 40 cm of porewater (as our sampling indicates), and excluding losses from the ecosystem or plant uptake, we estimated that this fertilization would have increased porewater salinity by a maximum of 0.05 ppt, less than 1% of normal salinity.

MEASUREMENTS

The chambered experimental plots consisted of two halves, one-half geological and one-half biogeochemical. All sampling that involved disturbance of soil was performed on the biogeochemical half. All elevation measurements, which were considered to be more sensitive to soil disturbance, were performed on the geological half.

Aboveground Biomass

We estimated peak aboveground biomass with a combination of allometry and harvested subplots (Erickson et al., 2007). At the end of July of each year, eight 30 × 30 cm quadrats were placed in prescribed locations in each plot, six inside the chamber and two in an adjacent unchambered control plot. In the quadrats, each *Schoenoplectus americanus* stem was counted and nondestructively measured for total height, green height, and width at half-height. In the corner of each quadrat, we clipped and removed all vegetation and litter in a 5 × 5 cm area. Vegetation was sorted according to species. We measured the clipped *S. americanus* stems for total height and width. Clippings were dried for 72 h at 60°C and weighed. We measured length and width on a subset of freshly clipped stems. We used the calculated relationship between linear dimensions and dry mass ($r^2 > 0.9$) to estimate the mass of each live *S. americanus* stem. To estimate *Spartina patens* and *Distichlis spicata* mass, we scaled up from mass in the clipped areas to total chamber area.

Root Productivity

Three soil cores (30 cm depth × 5 cm diameter) were taken from each plot and replaced with cylindrical ingrowth bags (30 cm height × 5 cm diameter). The bags were constructed from 1 cm mesh and filled with milled, moistened peat so as to achieve the bulk density of in situ peat, 0.12 g cm^{-3}. Bags were implanted in winter and removed in November the following year. Contents were washed over a 1 mm sieve. Large organic fragments were picked out by hand. Root mass was separated into fine (<2 mm diameter) and coarse (>2 mm) categories, dried for 72 h at 60°C, and weighed.

Porewater Wells

We implanted nine porewater wells (three replicates at each of three depths: 20, 40 and 80 cm) in each experimental plot. We built wells from 0.6 cm internal diameter rigid Teflon tubing (GE Polymershapes) plugged at the bottom with silicon caulk and open at the top, which extended 10 cm above ground. Sixteen holes (1 mm diameter) were drilled into the bottom 10 cm of the Teflon tube to allow ample conductance of porewater into the well. A vinyl hose (6 mm [OD], 3 mm inner diameter [ID]) was fastened to the top of each well and draped over the chamber for easy access from outside the chamber. Wells were flushed with 60 mL, equivalent to more than total well volume, and sampled monthly for a suite of chemical parameters using syringes.

Net Ecosystem Exchange (NEE)

The chambers were also designed to allow for measurement of net ecosystem exchange (NEE) of CO_2 between the atmosphere and the enclosed ecosystem (Figure 6). Periodically throughout the growing season, octagonal caps were placed on a subset of chambers. The purpose of the caps was not to render the chamber airtight, but to eliminate wind incursions and generate a consistent, predictable pattern of air flow through the chambers. The caps were octagons with a crossbeam built from 1.9 cm PVC pipe covered with the same IR-transparent film for the chamber panels. The film was perforated with 2 cm diameter holes. The gas sampling pipe, described above, was raised to a height roughly 30 cm below the cap and aligned with the cap perforations. This arrangement allowed us to measure the $[CO_2]$ of air exiting each chamber after it had been influenced by soil and vegetation.

To estimate flow rate of air through the chamber, we cut a slit in the air delivery ducts from each blower and measured air velocity using a handheld anemometer (AM 4822, Mastech; www.p-mastech.com). We initially measured the velocity at a range of distances from the duct wall and determined that the mean of two measurements (centered at 4 cm and 9 cm from the duct wall) adequately estimated the average velocity for the entire cross section. Multiplying velocity (cm s^{-1}) and cross-sectional area (cm^2)

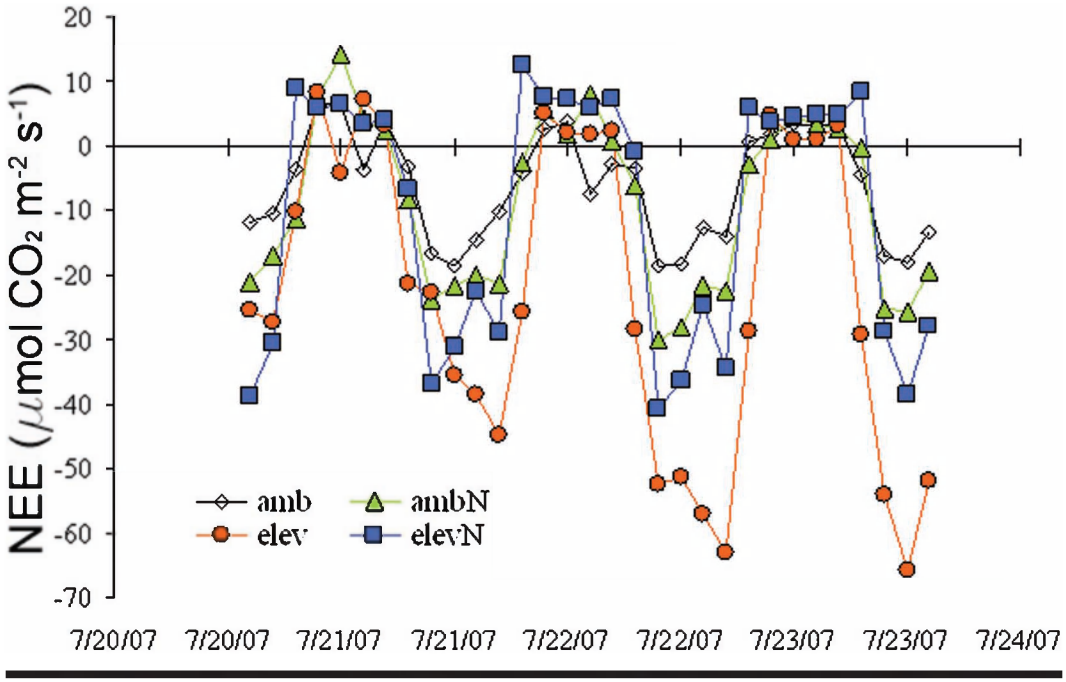

FIGURE 6. Net ecosystem exchange (NEE) of CO_2 over three days in July 2007. Negative values represent net uptake by the ecosystem. Generally, values are negative in summer daytimes when photosynthetic rate surpasses respiration rate. Each point represents the means of approximately 12 individual measurements from each of two replicate chambers binned into 2.4-h intervals. amb = ambient; elev = elevated; ambN = ambient N; elevN = elevated N.

yielded volumetric flow rate ($cm^3\ s^{-1}$). The volumetric flow rate was converted to mass flow using air temperatures from the site. We calculated NEE as $([CO_2]_{in} - [CO_2]_{out}) \times$ flow rate.

Because we did not want to incur chamber effects such as warming or rain exclusion, we measured NEE on a rotating subset of chambers balanced by treatment, for variable intervals from 3 to 7 days. These data will be used to calculate NEE light-response curves for net CO_2 uptake during the day and NEE temperature-response curves for net CO_2 release during the night. The response curve models will be driven with continuous measurements of soil temperature and photosynthetically active radiation to extrapolate up to integrated NEE for a complete growing season (Rasse et al., 2003). The gas sampling program was adjusted to increase the frequency with which NEE chambers were sampled to increase resolution for these low signal-to-noise NEE measurements, compared to the relatively stable absolute atmospheric $[CO_2]$ data when all chambers are sampled equally.

Environmental Variables

Soil temperature was measured at 5 and 15 cm depth using type-T thermocouples. Wind speed was monitored with an anemometer (O14A-L, Campbell Scientific, Logan, UT). Water level was recorded using a differential pressure transducer (PS-9805, Northwest Technologies) placed at the bottom of a 0.5 m well. All environmental data were logged on a combination of a multiplexor (AM32T, Campbell Scientific) for temperature and a datalogger (CR10X, Campbell Scientific), which were positioned remotely in the marsh to minimize analog signal degradation. Information was then relayed digitally between the marsh and main datalogger (CR1000, Campbell Scientific) using multidrop interfaces (MD485, Campbell Scientific).

RESULTS AND DISCUSSION

Treatment Application

Average daily mean $CO_2 \pm$ SE was 394 ± 1.2 ppm in ambient and 707 ± 6.0 ppm in elevated chamber atmosphere in 2007, a treatment difference of 313 ppm. The standard deviation among daily means for individual chambers averaged 21.9 and 59.0 ppm for individual ambient and elevated chambers. The variation in means between days was driven by differences in wind speed (Figure 7). High winds resulted in incursions of ambient air into elevated chambers, thereby diluting the elevated $[CO_2]$. On the other hand, stillness allowed respired CO_2 to accumulate overnight, which increased background $[CO_2]$ in ambient and elevated chambers. Because this buildup affects each treatment equally, the difference between ambient and elevated chambers persisted. However, wind incursions drove down concentrations in elevated chambers only, which decreased the difference between ambient and elevated $[CO_2]$.

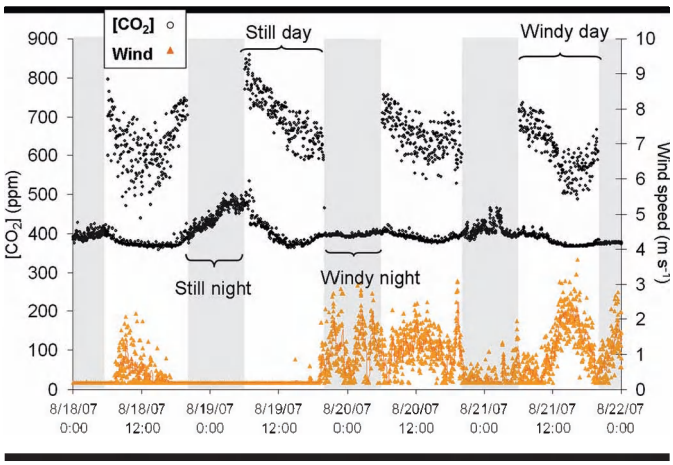

FIGURE 7. The CO_2 treatment and wind speed from four varying days in August 2007. The shaded areas represent hours of darkness when the CO_2 delivery was shut down. During those off hours, all chambers were at ambient $[CO_2]$. During still nights, ambient $[CO_2]$ approached concentrations much higher than well-mixed atmospheric $[CO_2]$ as respired CO_2 accumulated relatively near the ground.

In 2006, before chambers were equipped with frusta, ambient and elevated chambers $[CO_2]$ were 395 and 669, a difference of 274 ppm. Although the mean $[CO_2]$ could have been elevated in the chamber without adding frusta, the fluctuations with wind would have been extreme, and the expense of the additional CO_2 was deemed prohibitive.

The $[NH_4]$ in porewater was successfully increased by the N addition by a factor of 2.9, from 17 to 64 $\mu mol\ L^{-1}$ averaged over the growing season in 2006. The factor by which N addition increased porewater $[NH_4]$ was much higher early in the season and declined as growing plants took up N.

Measurement Validation: Chamber Effects

Elevation

To examine the possibility of chamber effects on elevation, we examined the measurements in the ambient CO_2,

low-N (no added N) treatment (Figure 8). The in-chamber measurements were very similar to those in the reference plots. Both sets of data revealed significant changes in elevation through time (repeated-measures MANOVA, $P < 0.05$). Most notably, all plots experienced a dip of roughly 0.8 cm in total elevation during March 2007, followed by a strong recovery. This dip was driven entirely by dynamics in the thickness of the deep zone. Compared to absolute changes in elevation (range, >1.2 cm), the differences between in-chamber and reference elevation were relatively small (range, <0.2 cm). There was a trend of a chamber effect on total elevation driven by deep zone dynamics. This effect was significant in summer 2007 but has vanished since then. The relativized root zone thickness in ambient CO_2, low-N chambers never differed from zero (t test, $P > 0.40$ for all dates), which indicated that there was not a detectable chamber effect in this stratum where we expected treatment effects to be manifested.

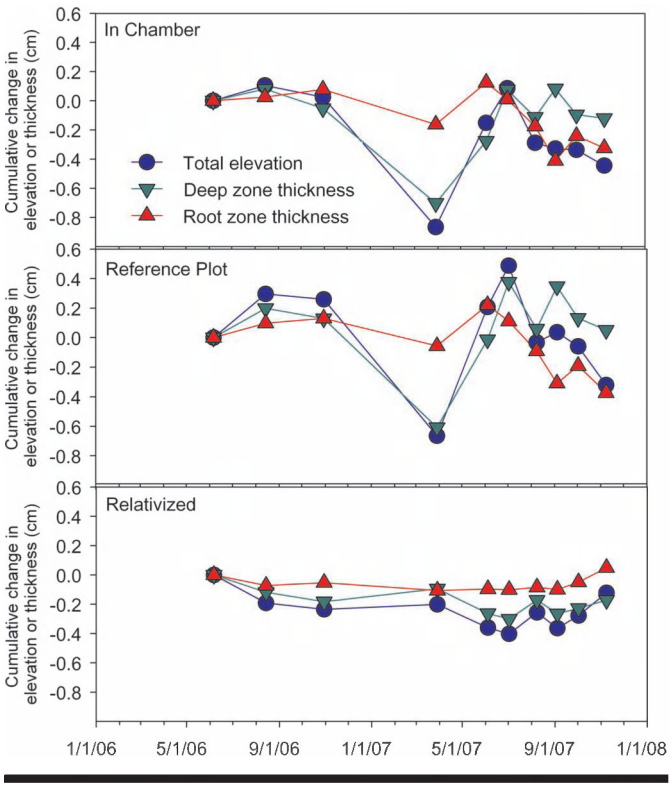

FIGURE 8. Elevation data from the five ambient CO_2, low-nitrogen (N) plots. Change in total elevation is partitioned between changes in thickness of either the deep zone or root zone. Top panel: elevation and thicknesses from inside the experimental chambers; middle panel: from the adjacent reference plots; bottom panel: difference between the in-chamber and reference measurements (relativized). There was a slight chamber effect on total elevation, driven by contraction of deep zone thickness. Root zone did not differ from zero.

Surface Accretion

One criticism of the design was that the chambers, by enclosing plots, may have excluded sediments from being deposited on the marsh surface. The difference between in-chamber and reference accretion measured with cryocores in November 2007 was small (0.058 cm) and did not differ significantly from zero (95% confidence interval: −0.18 to 0.07, $n = 20$). The treatment means also did not differ from each other (two-way ANOVA: CO_2, $P > 0.10$; N, $P > 0.10$) or from the reference plots (chamber effect: $P > 0.10$; Figure 9).

CONCLUSIONS

The design of our field experiment proved robust to a number of challenges unique to tidal salt marsh environments, including saltwater corrosion and deep tides. More importantly the chamber design allowed us to consistently elevate atmospheric CO_2. The frustum was a key feature of the chamber because it average-stabilized and raised the $[CO_2]$ in the elevated treatment, likely resulting in saved

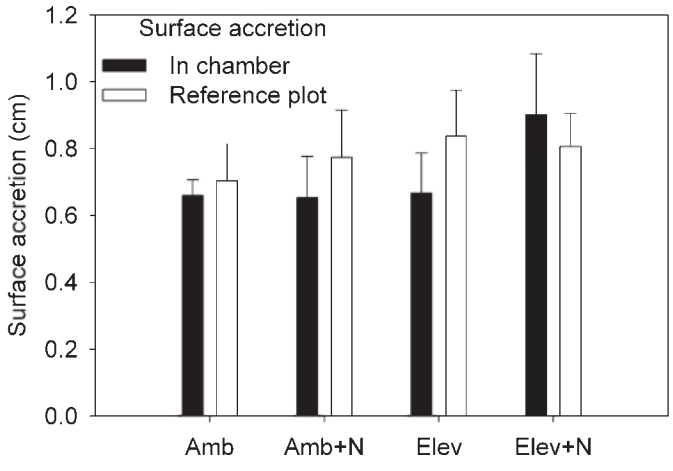

FIGURE 9. Surface accretion measured as the accumulation of matter on top of the marker horizon inside the chamber versus that outside the chamber. It was thought the chambers may exclude exogenous sediment, but there was no difference between in-chamber (black bars) and outside-chamber (reference plot, white bars) accretion rates. Amb = ambient; Elev = elevation.

CO_2. N addition yielded higher porewater N concentrations as expected, but further chemical analyses are needed for a more precise estimate of the magnitude of the N treatment. The SET design allowed for sensitive measures of soil elevation change. The chambers, perhaps by virtue of their mass, appeared to slightly depress soil elevation. However, there was no chamber effect in the root zone where the most important treatment effects are expected to occur. Further, the size of the chamber effect on elevation was small (0.2 cm; Figure 8, bottom panel) relative to the natural range of variation in those elevation parameters (1.2 cm; Figure 8, middle panel).

Acknowledgments

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Herbivory, Nutrients, Stochastic Events, and Relative Dominances of Benthic Indicator Groups on Coral Reefs: A Review and Recommendations

Mark M. Littler, Diane S. Littler, and Barrett L. Brooks

ABSTRACT. Threshold levels (i.e., tipping points where the probability of community phase shifts is increased and the potential for recoverability is reduced) for critical bottom-up interactions of productivity (e.g., nutrients) and those for top-down disturbances (e.g., herbivory) must be known to manage the competitive interactions determining the health of coral-dominated reefs. We further posit that latent trajectories (reduced resiliencies/recoverability from phase shifts) are often activated or accelerated by large-scale stochastic disturbances such as tropical storms, cold fronts, warming events, diseases, and predator outbreaks. In highly diverse and productive reef ecosystems, much of the overall diversity at the benthic primary producer level is afforded by the interaction of opposing nutrient-limiting/nutrient-enhancing and herbivory controls with the local physical and spatial variability, such that a mosaic of environmental conditions typically occur in close proximity. Although the relative dominance model (RDM) appears straightforwardly simple, because of the nature of direct/indirect and stimulating/limiting factors and their interactions it is extremely complex. For example, insufficient nutrients may act directly to limit fleshy algal domination (via physiological stress); conversely, abundant nutrients enhance fleshy algal growth, with the opposite effect on reef-building corals (via toxic inhibition or increased diseases). Furthermore, the effects of controls can be indirect, by influencing competition. Even this seemingly indirect control can have further levels of complexity because competition between algae and corals can be direct (e.g., overgrowth) or indirect (e.g., preemption of substrate). High herbivory (via physical removal) also acts indirectly on fleshy algae through reduced competitive ability, whereas lowered herbivory and elevated nutrients also indirectly inhibit or control corals and coralline algae by enhancing fleshy algal competition. Other ecologically important bottom-up factors, such as reduced light, abrasion, allelopathy, disease vectoring, and sediment smothering, also result from indirect side effects of fleshy algal competition. These factors tend to selectively eliminate the long-lived organisms in favor of weedy fast-growing species, thereby reducing desirable complexity and biodiversity.

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INTRODUCTION

There has been an exhaustive debate in the coral reef literature over the relative importance of forces that regulate community structure and resilience (i.e., the potential to resist stresses and to recover following disturbances). The expansion of the human population and associated increases in destructive fishing

(Hughes, 1994) and nutrient loading (Lapointe, 1999), compounded with ocean warming (Hoegh-Guldberg, 1999) and stochastic environmental effects (Precht et al., 2005), have been broadly debated to explain the increasing degradation of coral reefs worldwide. Because human population growth is not expected to abate, discriminating among various stressors is critical to determine conservation strategies and to eventually ameliorate the accelerating degradation of coral reefs. What has been lacking is the ability to rigorously test and differentiate among the possible acute versus chronic stressors—leading to ongoing controversy. In an attempt to address this problem, several workers (Mora, 2008; Burkepile and Hay, 2006) have conducted broad correlative and statistical assessments of communities over large regional scales. These studies have suggested a clear interaction between eutrophication in conjunction with declining herbivorous organisms as direct causes for maintaining present undesirable phase shifts on coral reefs.

Such phase shifts have been devastating to the many uniquely specialized benthic photosynthetic symbionts dominating tropical reefs, which are responsible for some of the most productive natural ecosystems known. Four major space-occupying groups of benthic primary producers combine to create high coral-reef primary productivity: reef-building corals (containing symbiotic algae), crustose coralline algae, algal turfs (fleshy filamentous and low-growing prostrate forms, and frondose macroalgae. Of these, photosynthetic corals create much of the structural heterogeneity and complexity and, with coralline algae, are primarily responsible for accretion of $CaCO_3$ into the reef matrix—making them the most desirable functional groups from a management perspective.

A basic objective in management ecology is to determine the mechanisms by which natural and anthropogenic factors maintain or alter structure and interactions in biotic communities. Anthropogenic eutrophication and destructive overfishing (i.e., herbivore removal by trapping, netting, poisoning, blasting) are the most tractable factors correlated with the marked global decline in tropical reef communities over the past two decades (see reviews in Ginsburg, 1994; Birkeland, 1997; papers in Szmant, 2001). The theoretical framework involving "top-down" regulation by predators and "bottom-up" control by resource availability in terrestrial systems was first proposed by Hairston et al. (1960), concepts that were later used (Atkinson and Grigg, 1984) to describe mechanisms that regulate the structure of coral-reef communities. These factors provide a valuable perspective (Figure 1) to assess and manage the human activities that affect the interactive mechanisms controlling stable states, tipping points, phase shifts, and recovery among the dominant functional groups of primary producers on tropical reefs.

In healthy coral-dominated reefs, nutrient concentrations are extremely low and attachment space is occupied by a broad diversity of three-dimensional overgrowing organisms. Given these conditions, the major tenets of the management model proposed by Littler and Littler (2006: fig. 1, relative dominance model [RDM]) are (1) that competition for space and light is crucial in determining the relative abundances of major benthic photosynthetic organisms, and (2) that the outcome of competition for these resources is most often, but not exclusively, controlled by the complex interactions of biological factors (top-down controls such as grazing) and environmental factors (bottom-up controls such as nutrient levels). As suggested by Grime (1979) for terrestrial plants and expanded for marine macroalgae (Littler and Littler, 1984; Steneck and Dethier, 1994), primary producer abundance and evolutionary strategies are controlled by physical disturbances (i.e., factors that remove biomass) coupled with physiological stresses (i.e., factors that limit metabolic production). In the conceptual relative dominance model (RDM; see Figure 1), grazing physically reduces biomass (top-down) and nutrients control production (bottom-up). The complex natural interactions between herbivory and nutrients are most dramatically impacted by large-scale catastrophic disturbances such as tropical storms (Done, 1992), warming events (Macintyre and Glynn, 1990; Lough, 1994), cold fronts (Precht and Miller, 2007), diseases (Santavy and Peters, 1997), and predator outbreaks (Cameron, 1977). These events serve to trigger or accelerate the ultimate long-term phase shifts postulated in the RDM. Such stochastic events selectively eliminate the longer-lived organisms in favor of faster-growing fleshy macroalgae, which are often competitively superior (Birkeland, 1977). However, nutrients and herbivory, in the absence of large-scale disturbances, are both sufficient to maintain phase shifts independently or in concert (Smith et al., 2001; Armitage and Fong, 2004; Littler et al., 2006a).

On undisturbed oligotrophic coral-reef habitats, the effects of well-documented top-down physical controls via intense herbivory prevail, where changes in grazing intensity often show acutely rapid effects. Conversely, bottom-up stimulatory controls are more chronic, the result of lack of nutrient availability, overcompensation by grazers, and a slower growth response, compared with acute physical destruction by herbivory. However, under persistent elevated nutrient conditions (relative to low [near-undetectable] concentrations), consistent coral declines can occur, con-

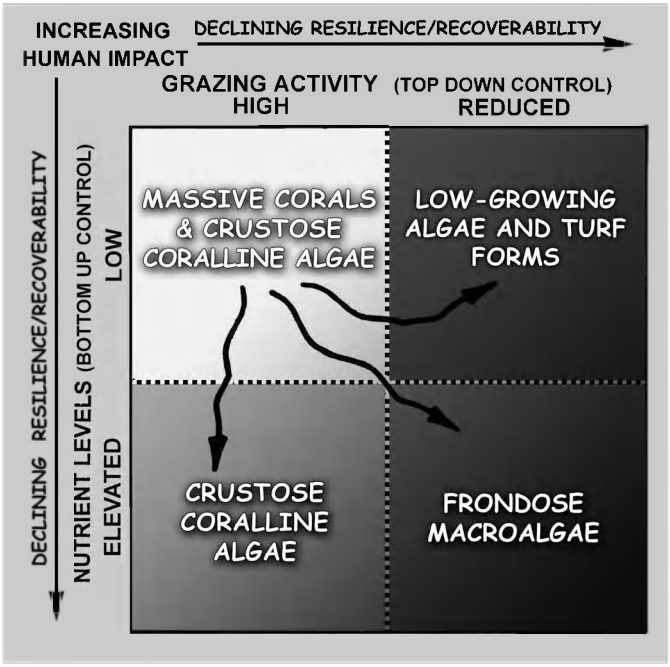

FIGURE 1. The competition-based relative dominance model (RDM). All the functional indicator groups occur under the conditions of every compartment of the model; however, the RDM predicts which group will most often *dominate*. Light to dark shading indicates declining desirability of each functional group from a management perspective. Crustose coralline algae are posited to be competitively inferior and dominate mainly by default, that is, where fleshy algae are removed by herbivores and some corals are inhibited by nutrients. The dashed lines approximate tipping points where declining herbivory and increasing nutrients reach critical levels that begin to reduce resilience to and recoverability from phase shifts. One vector can partially offset the other; for example, high herbivory can delay the impact of elevated nutrients, or low nutrients may offset the impact of reduced herbivory. As a baseline for healthy coral-reef habitats, herbivore population abundances and diversity should be high and palatable test plants should show at least greater than 50% loss 6 h^{-1} (i.e., <6 h half-life) during a replicated series of midday in situ exposures. Hypothetical nutrient tipping points (i.e., thresholds that sustain algal growth) are thus far indicated to be quite low (i.e., ~0.1 μM soluble reactive phosphorus [SRP], ~1.0 μM dissolved inorganic nitrogen [DIN]), as suggested by laboratory growth experiments, case studies for macroalgal overgrowth of coral reef communities, and in situ experimental nutrient enrichment research (Bell, 1992; Bell and Elmetri, 1995; Lapointe et al., 1993, Bell et al., 2007). We further posit that latent trajectories (reduced resilience/recoverability from phase shifts) are often triggered or accelerated by large-scale stochastic disturbances such as tropical storms, cold fronts, warming events, diseases, and predator outbreaks. Although these are events from which coral reefs have recovered for millions of years in the absence of humans, when tipping points remain surpassed, less-desirable stable states can persist.

comitant with algal increases that may lead to enduring states throughout all combinations of herbivory (Littler et al., 2006a). Changes in bottom-up controls and their interactions not only alter the dominance patterns of the major benthic functional groups on coral reefs but, hypothetically, could have profound long-term consequences mediated through structural transformations and chemical modifications to reef systems and their herbivorous fish populations. In other words, excessive nutrient enrichment not only increases the productivity and biomass of weedy macroalgae via bottom-up controls that alter patterns of competitive dominance (Littler et al., 1993) but, over the long term, may lead to coral habitat degradation through (1) reduced spatial heterogeneity by overgrowth (Johannes, 1975; Pastorok and Bilyard, 1985; Szmant, 1997) and (2) nighttime anoxic conditions (tolerated by macroalgae, but not by coral competitors and herbivorous predators; Lapointe and Matzie, 1996) that could indirectly reduce top-down grazer effects. Furthermore, fleshy macroalgal blooms, irrespective of how they are induced, decrease the growth and reproductive capacity of the more structurally complex reef-building corals (Tanner, 1995; Miller and Hay, 1996; Bellwood et al., 2006; Hughes et al., 2007), as well as inhibit coral larval recruitment (Birkeland, 1977; Tomascik, 1991; Ward and Harrison, 1997) and survival (Lewis, 1986; Hughes et al., 1987; Hughes, 1989; Wittenberg and Hunte, 1992). Such complicated feedback loops following eutrophication (e.g., anoxia) are known to occur in seagrass meadows (Sand-Jensen and Borum, 1991; Duarte, 1995) and could also explain decreases in fish populations on coral reefs with long-term histories of eutrophication.

CORAL-REEF MANAGEMENT

The data relevant for long-term reef management consist of (1) many important short-term caging and feeding experiments (in the case of exceedingly well-documented top-down herbivory effects), (2) circumstantial evidence (Hallock et al., 1993), (3) correlative biogeographic surveys contrasting oligotrophic versus eutrophic systems (Littler et al., 1991; Verheij, 1993; Mora, 2008), (4) comparative experiments on systems containing natural nutrient gradients (Lapointe et al., 2004, 2005b; Vroom et al., 2005), (5) physiological assays (Littler and Littler, 1990; Lapointe et al., 1997), and (6) logistically complicated, in situ, long-term, experimental/causality studies, in the case of bottom-up nutrient controls (Smith et al., 2001; Littler et al., 2006a). Top-down control by abundant populations

of large mobile herbivores is particularly well studied for coral reefs, beginning nearly five decades ago with the caging study of Stephenson and Searles (1960 As examples, Sammarco et al. (1974), Ogden and Lobel (1978), Sammarco (1980), Carpenter (1986), Lewis (1986), Morrisson (1988), and numerous other workers (see review by McCook et al., 2001) have demonstrated that lowering herbivory in low-nutrient habitats (usually assumed) often results in rapid increases in low-growing stages of fleshy macrophytes.

In the study of Lewis (1986) on the same reef flat studied by Littler et al. (2006a, 2006b), increases in a dominant vegetative algal turf form (*Vaughaniella* stage) with its upright fertile *Padina* blades, *not* blooms of mixed macroalgae, followed short-term (11 week) reductions of herbivorous fish grazing under conditions of low nutrient levels. Lewis' (1986) table 4 (although pseudoreplicated) shows statistically significant, but relatively small, increases (26%) in the above *Vaughaniella*-turf stage and its reproductive *Padina* blades; however, in contrast to several literature citations, no significant increases occurred in any of the abundant upright macroalgal dominants such as *Turbinaria turbinata* and *Halimeda* spp. Such low mats are unique in containing an abundance of nitrogen-fixing blue-green algae that can enrich other members within the low-growing algal community (Adey and Goertmiller, 1987; Adey, 1998). In presumably higher-nutrient environments, herbivore removals usually result in dramatic blooms of larger frondose macroalgae (Bellwood et al., 2006: fig. 4; Hughes et al., 2007).

Throughout the past decade, many biologists and managers have not recognized the importance of chronic nutrient enrichment and associated eutrophication problems facing coral reefs. A recent study (Littler et al., 2006b) provided a detailed review and discussion of the misinterpretations, misunderstandings, and suboptimal experimental designs that pervade the literature in regard to nutrient enrichment and the health of coral reefs. Overgrown 0.5 L porous clay-pot diffusers ("mini-reefs," following a decade of recruitment, colonization, and competition) were utilized (Littler et al., 2006b) to evaluate protocols for studies of controlled nutrient enrichment on coral reefs. A commonly used nutrient source, Tree Food Stakes containing up to 6% chlorine, resulted in a significant 11-fold and 20-fold decrease of fleshy algae and calcareous coralline algae, respectively, relative to the control treatments, while blue-green algae (Cyanobacteria) became significantly (6 fold) more abundant. Osmocote-filled mini-reefs showed no significant differences from the controls for any of the indicator groups. By avoiding the pitfalls of suboptimal study areas, insufficient duration of colonization/competition studies, inadequate nutrient detection limits, and inappropriate sources of enrichment in future research, the potential to provide new insights into the nutrient status of coral reefs will be greatly improved. Nutrient research is logistically difficult and, because the growth responses are relatively slow (i.e., chronic), requires more emphasis on multifaceted approaches carried out over sufficiently long time periods. Optimally, studies should include in situ enrichment experiments that test the long-term competitive interactions of functional indicator groups on healthy coral-dominated reefs, in addition to precisely monitoring water column nutrient levels, tissue C:N:P ratios, and algal physiological response assays.

Although nutrient data are typically lacking in coral-reef herbivory studies, natural background levels in conjunction with ample water motion are usually assumed to exceed levels that are limiting to macroalgal growth (Fong et al., 2003). As pointed out by Lewis (1986), large frondose macroalgae such as *Sargassum* and *Turbinaria* do occur in oligotrophic reef areas adjacent to coral colonies (see also Littler et al., 1986; McCook et al, 2001; Vroom at al., 2005); however, many of these frondose forms occupy microhabitats that generate increased current acceleration, such as the reef crest and tops of patch reef rocks, implicating higher nutrient fluxes (Atkinson et al., 2001). Also, large biomass/standing stocks of slow-growing perennial macroalgae (e.g., rockweeds) can develop over time under low inorganic nutrient concentrations; rainforests are good illustrations of this as well. Furthermore, *Sargassum* spp. can coexist with corals in oligotrophic waters by utilizing particulate organic sources of nutrients (Schaffelke, 1999); therefore, in this particular situation, large plant biomasses of low diversity do not necessarily indicate detrimentally abundant dissolved nutrients. Tissue analyses of mid-shelf *Sargassum* transplants on the great barrier reef (McCook, 1999) revealed a C:N ratio of 32:1 and a C:P ratio of 1261:1, exceeding values for pelagic *Sargassum* in the nutrient-impoverished Sargasso Sea (C:P = 877:1; Lapointe, 1995), which are compelling for substantial N limitation and severe P limitation. A further consideration is the now-ubiquitous presence of significant anthropogenic nitrogen sources (from burning fossil fuels) in rainfall worldwide (Vitousek et al., 1997), making the term "pristine" relative, at best. The demise of copious coral cover (Pollock, 1928) and concomitant rise in frondose algae (Doty, 1971) and coralline algae (Littler, 1971) on the reef flat at Waikiki, Hawaii, was the first phase shift from coral to macroalgal domination that was postulated (Littler, 1973) as caused by increases in eutrophication (bottom-up control).

Eutrophication affects coral reefs to different degrees and on varying scales. Several studies (Atkinson et al., 1995; Grigg, 1995; Steven and Broadbent, 1997; McCook, 1999; Bongiorni et al., 2003) indicated no substantial adverse responses of coral species to elevated nutrients. However, other laboratory and field experiments (Pastorok and Bilyard, 1985; Tomascik and Sander, 1987; Muscatine et al., 1989; Stambler et al., 1991; Jokiel et al., 1994; Koop et al., 2001) have concluded that corals are negatively affected by increased levels of nutrients and that diversity suffers. Numerous in situ observations exemplify the types of shifts from coral dominance to algal dominance that suggest linkages with chronic nutrient loading, including case studies in Hawaii (Littler, 1973; Banner, 1974; Smith et al., 1981; Maragos et al., 1985; Grigg, 1995), Venezuela (Weiss and Goddard, 1977), the Red Sea (Mergener, 1981; Walker and Ormond, 1982), Barbados (Tomascik and Sander, 1985, 1987), American Samoa (Green et al., 1997), Reunion Island (Cuet et al., 1988; Naim, 1993), Bermuda (Lapointe and O'Connell, 1989), the Great Barrier Reef (Bell, 1992), the Florida Keys (Lapointe et al., 1994), Martinique (Littler et al., 1993), and Jamaica (Goreau et al., 1997; Lapointe et al., 1997).

In a number of cases, herbivory patterns alone (similar to nutrient levels) do not explain the distribution and abundance of benthic algae on coral reefs (Adey et al., 1977; Hay, 1981; Hatcher, 1983; Hatcher and Larkum, 1983; Carpenter, 1986). Several studies (Hatcher, 1981; Schmitt, 1997; Lirman and Biber, 2000) found no significant correlation between grazing intensity and algal biomass. A dramatic increase in algal biomass resulting from eutrophication, without any simultaneous reduction in herbivore populations, was reported (Fishelson, 1973). The importance of the very low nutrient levels involved in eutrophication (i.e., nutrient threshold hypothesis, NTH), either natural or anthropogenic, has only recently come to light (Bell, 1992; Lapointe et al., 1997; Small and Adey, 2001; Bell et al., 2007) regarding the potential for phase shifts from corals toward macroalgal dominance. These kinds of biotic phase shifts also have been attributed to overfishing of herbivore stocks (see Hughes, 1994 on Jamaican reef trends), in concert with cultural eutrophication (Goreau et al., 1997; Lapointe et al., 1997). It is now clear (Burkepile and Hay, 2006; Mora, 2008) that both herbivory and nutrient levels interact on large scales as major factors in maintaining or degrading coral-reef health.

We hasten to point out that individuals of all the functional indicator groups can and do occur under the conditions of every compartment of the RDM (see Figure 1); however, the model predicts which group most often will *dominate* (as does the very similar fig. 2a in Bellwood et al., 2004). Such apparent presence/absence anomalies, on closer inspection, are often scientifically logical but have led to different perspectives. Following large coral bleaching events and die-offs in Belize, we have observed dramatic increases in chemically defended sponges (e.g., *Chondrilla*) and Cyanobacteria (blue-green algae) under high levels of grazing by sea urchins and fishes. Other observations that appear counterintuitive include some corals growing in high-nutrient habitats, some large fleshy macroalgae growing under low nutrients, certain turf algae exposed to high herbivory, and the frequent coexistence of crustose corallines and the other functional groups. We agree with these observations and have addressed such anomalies herein.

The general applicability as well as the limitations of the RDM can be demonstrated further in relationship to a number of recent studies. For example, nutrients and herbivory are not independent, and the positive effects of nutrients on marine plant productivity and growth can actually make plants more palatable and susceptible to grazers (McGlathery, 1995; Boyer et al., 2004). Furthermore, nutrient increases are sometimes associated with coral inhibition (Koop et al., 2001) as well as coral diseases (Harvell et al., 1999, 2002; Bruno et al., 2003), and algal blooms can serve as disease vectors (Nugues et al., 2004). The sophisticated enrichment study (ENCORE) on a large and carefully controlled scale (Larkum and Koop, 1997; Encore Group, 2001) did not produce supportive results because (1) ambient nutrient levels within the lagoon at One Tree Island are well above tipping-point concentrations that may be inhibitory to some corals, while being more than sufficient to support luxuriant frondose macroalgal growth (Bell, 1992; Larkum and Koop, 1997; Bell et al., 2007) and (2) the test organisms were isolated on raised grids to measure growth rates, precluding natural encroachment, overgrowth, or other competitive interactions crucial to testing the RDM. However, all increases in nutrient levels did adversely affect coral reproduction (Koop et al., 2001). Additionally, several short-term (<4 months) studies (Thacker et al., 2001; Belliveau and Paul, 2002; Miller et al., 1999; McClanahan et al., 2002) reported lack of algal stimulation following nutrient enrichment, further documenting the low ambient nutrient concentrations sustaining ample algal growth.

In contrast, two in situ experimental studies conducted over longer time scales in healthy coral-reef settings (Smith et al., 2001; Littler et al., 2006a), in conjunction with natural successional and competitive interactions,

provided the most relevant causality data demonstrating the importance of both nutrient and herbivory influences; the present review builds on these findings. The paper by Lapointe (1997) was the first to put forth a convincing case for the effectiveness of the RDM in addressing harmful algal bloom issues on coral reefs. Additionally, highly diverse living model systems of coral-reef communities (i.e., mesocosms), operated for decades (Small and Adey, 2001), clearly have demonstrated that minute increases in nitrogen and phosphorus reduce coral growth (sometimes causing substantial die-backs). Such self-contained systems require continuous removal of nutrients by algal-turf scrubbers or protein skimmers in combination with an abundance of fish and invertebrate grazers to maintain a high coral and algal diversity. The burgeoning awareness of coral-reef degradation worldwide (see Ginsburg, 1994; chapters in Birkeland, 1997; Gardner et al., 2003), particularly from coastal eutrophication (Bell, 1992; Windom, 1992; Nixon, 1995; Lapointe, 1997, 1999) and destructive overfishing (Hughes, 1994; Jackson et al., 2001), makes this management perspective relevant and opportune (see Figure 1).

Although harmful macroalgal blooms on coral reefs have long been attributed to nutrient enrichment and eutrophication (Littler, 1973; Banner, 1974; Johannes, 1975; Smith et al., 1981; Lapointe, 1997; Lapointe et al., 2005a, 2005b), some reef biologists have countered that such changes in benthic community structure routinely result primarily from natural stochastic events (Precht et al., 2005), overfishing of herbivorous fish stocks (Hughes, 1994; Pandolfi et al., 2003; Lesser, 2004), or loss of keystone grazers, such as the long-spined sea urchin *Diadema antillarum* (Jackson et al., 2001). Although generally supported, these last observations are not typical of the majority of grazer reduction experiments in extreme oligotrophic environments (see Lapointe, 1999), most of which have reported an expansion of small low-growing algal forms rather than macroalgal blooms (as predicted in Figure 1). It is encouraging that the critical role of excess nutrients on coral reefs has begun to receive attention in recent review papers (Scheffer et al., 2001; Hughes et al. 2003; Bellwood et al., 2004; Pandolfi et al., 2005; Burkepile and Hay, 2006; Mora, 2008). Some scientists (e.g., Precht et al., 2005) downplay declining resilience issues, instead emphasizing fundamental stochastic factors such as upwellings, hurricanes, and cold fronts (see caption, Figure 1). These occurrences represent unmanageable events from which coral reefs have recovered for millions of years, but not in the presence of modern human influences such as destructive overfishing and nutrient pollution (see Mora, 2008). There are strong interactions between catastrophic stochastic factors and the roles of herbivores and nutrients that strongly impact reefs. For example, coral mortality following hurricanes and coral bleaching events opens up large amounts of new two-dimensional space readily colonized by fast-growing algae. Such increases in productivity and the area available for grazing hypothetically satiate the herbivore pressure over large areas, assuming that natural herbivore populations have an upper limit in the amount of reef area that they can graze effectively (Williams et al., 2001; Mumby, 2006). This diluted grazing pressure and reduction in suitable shelter could in turn lead to further increases in algal cover and a decline in the recovery capacity (i.e., resilience) of coral communities. Thus, stochastic processes are unquestionably important factors in determining the trajectories of reef health and interact with the processes discussed herein.

To establish the baseline conditions and detect subsequent changes, a combination of environmental, survey, inventory, and bioassay data are essential to characterize and monitor the ambient nutrient and herbivory environments and antecedent nutrient history of a given management area. Valid and reliable data are the cornerstone needed to prioritize among different management strategies and motivate the local populace and politicians/lawmakers to support and implement the goals necessary for responsible management. The RDM provides a clear visual depiction that is easily understood and, therefore, can serve as a convincing illustrative aid. It is essential that assessment and monitoring methods should be both simple and rapid to use. Chlorophyll *a* concentration (determined by fluorometric or spectrophotometric methods; see Bell and Elmetri, 1995) is an especially useful ancillary indicator of water column enrichment because phytoplankton blooms can rapidly attenuate critical light energy while buffering inorganic nutrient pulses. Along with nutrient levels, chlorophyll *a* serves as a valuable tipping-point indicator, where levels in excess of 0.2–0.3 $\mu g\ L^{-1}$ indicate approaching overabundances of nutrients (Bell et al., 2007).

Water column nutrient concentrations represent the net sum of internal cycling, algal assimilation, and external inputs, relative to macroalgal growth demands (Lapointe, 1997), and therefore offer the most direct method to assess nutrient excesses on any given coral reef. Consequently, a nutrient threshold model based on nutrient concentrations (rather than on nutrient fluxes) is not only valid but is likely the best index of nutrient status. Low-nutrient tipping points, where increasing nutrients reach hypothetically critical levels that begin to reduce recoverability from phase shifts (i.e., ~1.0 μM dissolved inorganic nitrogen

[DIN] = nitrogen: 0.014 ppm N or 0.040 ppm NO_3 and ~0.10 μM soluble reactive phosphorus [SRP] = phosphorus: 0.003 ppm P or 0.007 ppm PO_4), have been broadly corroborated (in developing the nutrient threshold hypothesis [NTH]; Bell, 1992; Lapointe et al., 1993; Bell et al., 2007) for sustaining macroalgal overgrowth of seagrass beds and coral reefs. The physiological/kinetic basis for such low-nutrient tipping points is the hyperbolic Monod relation (Droop, 1985; Bell et al., 2007), which is also supported by controlled, high-flux, continuous-culture laboratory experiments (Caperon et al., 1971 DeBoer et al., 1978; Lapointe and Tenore, 1981). In our experience, if modern analytical instruments can detect measurable nutrient levels, so can growth-limited macroalgae.

Additionally, a wealth of in situ coral-reef studies carried out in areas characterized by nutrient levels only moderately above the putative 0.1 μM SRP and 1.0 μM DIN tipping points (Larkum and Koop, 1997; Miller et al., 1999; Thacker et al., 2001) have reported minimal algal stimulation following experimental nutrient enrichment, further documenting the low natural nutrient concentrations required for ample algal growth and their widespread applicability. Some corals can tolerate high levels of DIN and SRP; however, nutrient tipping points not much above the present analytical limits of detection represent levels of resource availability at which resilience begins to be reduced (Scheffer et al., 2001), such that stochastic or other disturbances and stresses can trigger coral-reef ecosystem shifts toward sustained dominance by macroalgal stable states. Moreover, the macroalgal overgrowth experimentally stimulated (Smith et al., 2001; Littler et al., 2006b) in reduced-grazing/elevated-nutrient treatments demonstrates that ambient nutrient concentrations inhibitory to growth under the natural turbulence levels found on coral reefs are similar to those reported above for other tropical marine algae. It should be noted that the remote reef in the northwestern Hawaiian Islands studied by Smith et al. (2001) had nutrient levels at or above the hypothetical levels needed to sustain macroalgal growth (i.e., 1.1 μM DIN and 0.2 μM SRP). This system, with its present lack of macroalgae and dominance by unbroken thickets of three branching and one massive coral species, may be the result of overcompensation by intense grazing and, consequently, could be susceptible to a future relative dominance reversal.

Littler et al. (2006a: tbl. 1) give typical baseline herbivorous fish assay and population density data contrasting natural Belize Barrier Reef sites of low and high herbivory. Based on similar experiments conducted worldwide on coral reefs by a range of workers (Hay, 1984; Lewis and Wainwright, 1985; Paul et al., 1987; Sluka and Miller, 2001; Littler et al., 2006a), Littler and Littler (2006 posited that less than a six hour half-life (>50% mean loss per 6 h for palatable algae) during a series of in situ, midday, assay periods is indicative of a healthy level of herbivory for the particular habitat(s) tested. Herbivore abundances also should be enumerated by counting numbers of individuals (by species), from midmorning to midafternoon throughout a typical day for weather (Littler et al., 2006a, see their table 1), at fixed distances on either side of random replicates of standardized transect lines. Video transects are quick; enumeration can be done later in the laboratory, and the videos provide a permanent record of the target species (Littler et al., 1986).

FUNCTIONAL INDICATOR GROUPS

The fast growth and turnover rates of fleshy algae compared to other reef organisms suggest their value as early-warning indicators of reef degradation. Representatives of ubiquitous algal form/function groups (from Littler and Littler, 2006) are increasingly encountered as dominants on reefs, particularly those subjected to human activities (see Littler and Littler, 2006: fig. 2).

Reef-Building Corals (Cnidaria)

A predominance of diverse corals and calcareous coralline algae are universally accepted as the most desirable components of biotic reefs because of (1) their three-dimensional architecture, which provides habitats for a myriad of other reef organisms (largely responsible for much of the heterogeneity/high biodiversity), (2) their roles in producing the massive carbonate structure of reefs, and (3) their aesthetic qualities. The vertical structure and horizontal canopies of branching forms allow abundant populations of shade-dwelling crustose coralline algae to co-occur. Reef-building corals, while preyed upon by a few omnivorous fishes and specialist invertebrates (e.g., crown-of-thorns sea star), generally achieve dominance under the top-down control of intense herbivory (Lewis, 1986; Lirman, 2001) and extremely low nutrient concentrations (Bell, 1992; Lapointe et al., 1993). Massive corals are resistant to grazing at the higher levels of herbivory (Littler et al., 1989). Hard mound-shaped forms show relatively little colony mortality under high grazing pressure, even though occasionally rasped by parrotfishes. Contrastingly, some delicately branched corals such as *Porites porites* are quite palatable and readily eaten by

parrotfishes (e.g., *Sparisoma viride*; Littler et al., 1989; Miller and Hay, 1998). Nutrient increases are sometimes associated with coral diseases (Harvell et al., 1999, 2002; Bruno et al., 2003). As mentioned earlier, numerous corals tolerate elevated nutrient levels (Atkinson et al., 1995; Steven and Broadbent, 1997; Bongiorni et al., 2003), but their diversity suffers. Conversely, others are physiologically inhibited by increases in nitrate (e.g., *Montastrea annularis* and *Porites porites*: Marubini and Davies, 1996), ammonium (e.g., *Pocillopora damicornis*: Stambler et al., 1991; Muller-Parker et al., 1994), and orthophosphate (e.g., *Porites compressa*: Townsley, cited in Doty, 1969; *P. damicornis* and *Stylophora pistillata*: Høegh-Guldberg et al., 1997). Nutrient inhibition of coral larval settlement also has been shown for *Acropora longicyathis* (Ward and Harrison, 1997). During the extensive ENCORE program on Heron Island, all increases in nutrient levels adversely affected coral reproduction (Koop et al., 2001).

Macroalgae

With an increase in nutrients, the growth of harmful fleshy algae is favored over that of the slower-growing but highly desirable corals (Genin et al., 1995; Miller and Hay, 1996; Lapointe et al., 1997), and the latter become inhibited by competition for space and light, increased diseases, and physiological inhibition. On healthy oligotrophic coral reefs, even very low nutrient increases may exceed critical levels that can shift relative dominances by stimulating macroalgal production while inhibiting corals. As indicated earlier, large biomass, or standing stocks, of slow-growing perennial macroalgae (e.g., rockweeds) can develop over time under low inorganic nutrient concentrations (McCook, 1999), and *Sargassum* spp. can coexist with corals in oligotrophic waters by utilizing particulate organic sources of nutrients (Schaffelke, 1999). Therefore, in this particular situation, large plant biomasses do not necessarily indicate detrimentally abundant dissolved nutrients. Filamentous and frondose algae can outcompete corals (Birkeland, 1977; but see McCook et al., 2001), many of which are inhibited under elevated nutrient levels (reviewed in Marubini and Davies, 1996). Fast-growing algae are not just opportunists that depend on disturbances to release space resources from established longer-lived populations but become the superior competitors (Birkeland, 1977) when provided with sufficient nutrients. As a result, frondose macroalgae as a group are now generally recognized as harmful to the longevity of coral reefs because of the linkage between excessive blooms and coastal eutrophication (ECOHAB, 1997). Potential competitive dominance of fast-growing macroalgae is inferred from their overshadowing canopy heights, as well as from inverse correlations in abundances between algae and the other benthic producer groups (Lewis, 1986), particularly at elevated nutrient concentrations (Littler et al., 1993; Lapointe et al., 1997). Macroalgae, such as *Halimeda* spp., also gain competitive advantage by serving as carriers of coral diseases (Nugues et al., 2004). The fleshy macroalgal form-group has proven to be particularly attractive to herbivores (see Hay, 1981; Littler et al., 1983a, 1983b) and only becomes abundant where grazing is decreased or swamped by excessive algal growth (chemically defended forms, e.g., Cyanobacteria, are exceptions). Such overcompensation by herbivory may explain some of the reported cases (Crossland et al., 1984; Szmant, 1997; Smith et al., 2001) of specific corals surviving high-nutrient reef environments.

Crustose Coralline Algae

The predominant members of this indicator group, the coralline algae, tend to be slow-growing, competitively inferior taxa abundant in most reef systems (Littler, 1972). However, they span a spectrum of morphotypes from thin sheet-like crusts to thick massive pavements to upright branched and columnar coral-like heads that contribute to both cementation and bulk. This functional group is highly resilient and is able to recover or restore the coral-reef system relatively more quickly, given that some crustose coralline algae chemically attract and facilitate the survival of coral larvae (Harrington et al., 2004) whereas the other two algal functional groups inhibit larval settlement. Because crustose corallines continually slough upper surface layers, they play a key role, as do filter-feeding corals, in physically preventing the settlement and colonization of many undesirable fleshy fouling organisms on coral reefs (Littler and Littler, 1997). Crustose corallines, because of their slow growth rates, tolerate low nutrient levels and generally are conspicuous, but not dominant, under low concentrations of nutrients and high levels of herbivory (Littler et al., 1991). Accordingly, they do well under both low and elevated nutrients; that is, most are not inhibited by nutrient stress and many are maintained competitor free by surface cell layer shedding (Johnson and Mann, 1986), even at lower levels of grazing (Littler and Littler, 1997). Therefore, crustose coralline algae do not require elevated nutrients, as might be inferred from the RDM (Figure 1); instead, their rise to dominance is largely controlled indirectly by the factors influencing the abundances of the other groups, primarily corals and

fleshy macroalgae. The key point is that crustose corallines predominate mainly by default (i.e., under conditions of minimal competition), where either corals are inhibited by elevated nutrients or fleshy algae are removed by intense herbivory. In independent corroboration of the herbivory portion of the RDM, a gradient of frondose- to turf- to coralline algal groups was closely correlated with escalating herbivory on coral reefs (Steneck, 1989).

Low-Growing and Turf Algae

The turf algae are mostly dense filamentous and low-growing frondose members of all four algal phyla and tend to become dominant under minimal inhibitory top-down and stimulatory bottom-up controls. Domination by low-growing algae suggests desirably low nutrient levels but an inadequate herbivory component. Their relatively small size and rapid perennation results in moderate losses to herbivory at low grazing pressures. They have opportunistic life history characteristics, including the ability to maintain substantial nutrient uptake and growth rates under low-nutrient conditions (Rosenberg and Ramus, 1984), and also contain an abundance of nitrogen-fixing Cyanobacteria (Adey and Goertemiller, 1987; Adey, 1998) that can enrich other low-growing members of the dense turf community. Algal turfs have been shown to be favored under reduced nutrient-loading rates (Fong et al., 1987) or infrequent nutrient pulses (Fujita et al., 1988) and can form extensive horizontal mats.

DISCUSSION

This paper directly addresses the goals of an imperative research agenda (ECOHAB, 1997) by providing a management perspective and assessment strategies for the mechanisms that initiate and sustain harmful blooms of algae that degrade coral-reef ecosystems. The complex interactions of herbivory and nutrients can change gradually with no apparent effects to induce subtle declines in resiliency and recoverability of coral/coralline-dominated reef systems (Scheffer et al., 2001). As mentioned, these systems then become vulnerable to catastrophic impacts by large-scale stochastic disturbances that typically trigger or accelerate such low-resilience reef systems (Scheffer et al., 2001; Bellwood et al., 2004). Most importantly, recovery to coral domination cannot occur unless tipping points are returned to healthy levels, and even then alternative stable states may persist. For example, when catastrophic events selectively eliminate the longer-lived organisms in favor of early-successional fleshy algae (Littler and Littler, 1984), the settlement of coral planulae is prevented and the algae persist as competitively superior states (Birkeland, 1977; Lewis, 1986). For completeness, we also point out the obvious devastating effects of toxic spills, carbonate mining, land-fill, and sediment inundation, some of which also are associated with nutrient pollution and algal blooms.

Because of global-scale degradation of coral-reef ecosystems (Ginsburg, 1994; Wilkinson, 1999), it is important to obtain relevant information on tipping points for both top-down herbivory (relatively fast acting, acute) and bottom-up nutrient controls (slower acting, chronic), both of which are reemphasized. As the first approximation, we posit that on a healthy reef system, herbivore abundances and diversity should be high, and palatable test plants should show at least a 50% mean loss per six hours (i.e., <6 h half-life) during a series of midday in situ assays. Table 1 in Littler and Littler (2006 summarizes baseline assay and critical fish population data of this sort for two natural coral-reef zones of low and high herbivory.

Nutrient threshold points (where increasing water column nutrients reach critical resilience levels such that they reduce recovery from phase shifts) have been widely postulated (as $\sim$1.0 μM DIN and $\sim$0.10 μM SRP [NTH]; Bell, 1992; Lapointe et al., 1993; Bell and Elmetri, 1995) for potential macroalgal overgrowth of coral-reef communities. As mentioned earlier, a further useful tipping-point indicator is water column chlorophyll *a*, where levels in excess of 0.2–0.3 $\mu g\ L^{-1}$ also indicate detrimental overabundances of nutrients (Bell and Elmetri, 1995).

CONCLUSIONS

Assessment protocols for determining and monitoring the status of any given coral reef are suggested: these include (a) herbivore population assessments, (b) herbivory assays, (c) water column nutrient levels, and (d) standing stocks of functional indicator groups. These measurements can reveal quantitative tipping-point levels beyond which resilience to and recovery from undesirable phase shifts begin to become critically reduced. Tipping-point approximations are reviewed and posited both for inorganic nutrients and for herbivory.

This review specifically addresses the relatively acute top-down effects of herbivory and the more chronic bottom-up effects of nutrient enrichment on critical indicator groups of benthic primary producers: reef-building corals, crustose coralline algae, dense turf algae, frondose macroalgae, and herbivore associates.

A predominance of massive corals and calcareous coralline algae relative to frondose macroalgae and low-growing algae indicates a healthy spatially heterogeneous condition reflecting low nutrients and high herbivory. With a few exceptions, an abundance of frondose macroalgae illustrates the least desirable condition of elevated nutrient levels and reduced herbivory, possibly reflecting eutrophication in concert with destructive herbivore fishing practices. A high coverage of coralline algae suggests healthy high herbivory levels but also suggests problems with elevated nutrients that may be inhibitory to some corals. Domination by dense low-growing and turf algae indicates desirably low nutrient levels but also suggests an inadequate herbivory component.

From a management perspective, levels of herbivory and herbivore populations and of nutrients rank among the most useful quantitative indicators of coral-reef resilience and recoverability, whereas the degree of health, degradation, and mortality are inferred by the relative abundances of functional indicator groups.

The bioassay and indictor group monitoring approaches provide powerful perspectives and essential measurement criteria to enable resource managers to protect coral reefs and similar coastal systems from eutrophication, destructive overfishing, and initiation of harmful algal blooms. Human population growth has always been accompanied by changes in land and sea use and by increased exploitation of natural resources, attitudes that continue to cause broad alterations in the structure of coral-reef communities. Unless curbed, anthropogenically induced phase shifts will expand geographically at an accelerated pace. However, solutions are available, which include the use of Marine Protected Areas, banning of destructive fishing practices (e.g., trapping, poisoning, blasting, netting), and regulations protecting keystone herbivorous fish species (e.g., parrotfish, surgeonfish) from market exploitation. Fisheries controls must be backed up by strategies to regulate the effects of pollution along with an international commitment to reduce the emission of greenhouse gases and, finally, the implementation of long-term strategies to reduce or stabilize the ultimate cause of all these stressors, the world's human population growth.

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Impacts of Human Disturbance on Soil Erosion Potential and Habitat Stability of Mangrove-Dominated Islands in the Pelican Cays and Twin Cays Ranges, Belize

Karen L. McKee and William C. Vervaeke

ABSTRACT. The Mesoamerican Barrier Reef System (MBRS) is the longest unbroken reef in the Western Hemisphere and contains hundreds of mangrove-dominated islands. These islands provide critical habitat supporting marine biodiversity and create a self-sustaining system that counterbalances sea-level rise. Undisturbed mangrove islands build vertically through accumulation of organic matter (peat), which forms a strong, erosion-resistant matrix. Clear-cutting and dredging activities for development of tourist resorts, fishing camps, and "improved land" for resale, however, threaten mangrove-dominated islands and adjacent seagrass and coral reef assemblages. Effects of mangrove disturbance were examined on four islands in the designated marine preserves of Twin Cays and the Pelican Cays, Belize. Mangroves were clear cut (1.0–6.2 ha), and marine sediment was dredged from nearby reef flats and seagrass beds to raise land elevations to support beach vegetation and buildings. Removal of mangroves and especially addition of dredged fill significantly altered soil characteristics and decreased shear strength and aggregate stability of soil surfaces. Deep cores collected at both island ranges also revealed underlying deposits of peat (1.5–10.8 m thick), which influence local land subsidence. Although infilling with dredged material temporarily raised elevations, the inexorable subsidence of peat through natural processes of compaction and decomposition and sea-level rise will ultimately submerge such areas. Our findings thus show that soil erosion potential is increased and that long-term stability of islands may be compromised by mangrove clearing and dredging activities. Degradation of key biophysical components and critical habitat will ultimately impact ecotourism activities that depend on a healthy, natural environment.

INTRODUCTION

Persistence of oceanic mangrove islands is dependent upon maintenance of soil elevations relative to sea level. Mangrove-dominated islands can counterbalance rising seas by accumulating organic matter (mangrove-derived peat), which gradually builds land vertically (McKee et al., 2007a). In addition, biodiversity of intertidal and subtidal ecosystems in the Caribbean Region is dependent upon the presence of mangroves because a number of marine species are exclusively associated with the mangrove habitat (Ellison and Farnsworth, 1992; Goodbody, 2000; Taylor, 2000; Rocha et al., 2005). Mangroves also serve as nurseries for many reef fish and other marine organisms (Mumby et al., 2004). Consequently,

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changes in mangrove extent may have a cascading effect on habitat stability and capacity to keep pace with sea-level rise, as well as on marine biodiversity, in regions with mangrove-dominated islands and adjacent seagrass and coral reef assemblages.

The Mesoamerican Barrier Reef System (MBRS) off the coast of Belize, Central America, contains hundreds of mangrove islands in association with extensive seagrass beds and coral reefs (www.mbrs.org.bz; accessed 11 June 2008). Unfortunately, clear-cutting of insular mangroves has greatly accelerated in Belize and other locations in recent years for development of tourist resorts, fishing camps, and "improved land" for resale (K. L. McKee, personal observation). Even if such areas are ultimately abandoned and allowed to recover, recolonization by mangroves may be extremely slow, if it occurs at all. For example, a site on Twin Cays, an island range that was clear cut in 1992, is slowly recovering, but regenerating mangroves are still sparse and have taken 15 years to reach sapling size (~1–2 m tall) (McKee et al., 2007b). In addition to removal of mangroves, bottom sediments are dredged from adjacent seagrass beds and reef flats and pumped onto cleared mangrove areas to raise elevations sufficiently to support beach vegetation and buildings. This type of disturbance thus destroys multiple ecosystems, which require many years for recovery. In the interim, there may be additional consequences from the loss of habitat stability and increased erosion.

The specific objectives of this study were to assess the potential for changes in soil erosion and habitat stability on mangrove islands subjected to clear-cutting and dredging activities. The work focused on the designated marine preserves of Twin Cays and the Pelican Cays ranges, which have been highlighted as critical habitat for marine biodiversity in the region (Macintyre and Rützler, 2000; Macintyre et al., 2004a).

STUDY SITE

The MBRS is the longest unbroken reef in the Western Hemisphere and extends 220 km from the southern part of the Yucatan Peninsula to the Bay Islands of Honduras. Two main areas where clear-cutting and filling of mangrove islands has occurred were studied: Twin Cays and the Pelican Cays. Twin Cays, which consists of two larger and two smaller islands, is located in the central part of the barrier reef system and about 2 km west of the reef crest (Figure 1). The Pelican Cays archipelago is located 21 km south of Twin Cays and contains multiple mangrove-dominated islands (Figure 1). Twin Cays and the Pelican Cays ranges have been a major focus of mangrove research by the Smithsonian Institution (Macintyre and Rützler, 2000; Macintyre et al., 2004a). These mangrove islands are far from the mainland, and peat cores contain no terrigenous sediment (Cameron and Palmer, 1995; McKee and Faulkner, 2000; Purdy and Gischler, 2003; Macintyre et al., 2004b). The only source of freshwater is rainfall, and the entire landform is intertidal (mean tide range [neap] = 0.2 m). The vegetation on undisturbed islands is dominated by *Rhizophora mangle* L. (red mangrove), which is the most common mangrove species in the Caribbean Region. The area has been impacted by numerous hurricanes and tropical storms.

Twin Cays occurs in the Central Province of the barrier reef (16°50′N, 88°06′W) (see Figure 1). Mangrove communities were established at Twin Cays about 8,000 years ago on the Pleistocene surface of the Belize Barrier Reef Platform when it was flooded during the Holocene Transgression (Macintyre et al., 2004b; Purdy and Gischler, 2003). Deep deposits of peat (as much as 11 m thick) have developed as Twin Cays accreted vertically with rising sea level (Macintyre et al., 2004b; McKee et al., 2007a). Cores collected through these deposits indicate that the primary means of vertical land movement is accumulation of mangrove organic matter (mostly root matter) (McKee et al., 2007a). At Twin Cays, five areas ranging in size from 0.1 to 6.2 ha have been cleared and elevations raised by infilling with dredged material, beginning in the early 1990s and continuing until the present; small fishing camps consisting of one or more buildings have been established. Our study targeted the largest site on East Island that was clear cut and filled in 1995.

The Pelican Cays occur in the south central lagoon of the reef system (16°39.8′N, 88°11.5′W). Here, mangroves have developed as part of a complex network of coral ridges that surround deep circular ponds (Macintyre et al., 2000). There are several mangrove cays, including Northeast Cay, Fisherman's Cay, Manatee Cay, Cat Cay, Ridge Cay, Avicennia Cay, Co Cat Cay, the Bird Cays, and several unnamed smaller cays. Mangroves established at the Pelican Cays only within the past 1,000 years (Macintyre et al., 2000; McKee et al., 2007a) but have accumulated deposits of peat as much as 1.5 m thick (McKee and Faulkner, 2000). Disturbance at the Pelican Cays began in the mid-1990s with mangrove clearing on Northeast Cay. Aerial surveys conducted in April 2006 showed disturbed areas on Northeast Cay, Co Cat Cay, and Ridge Cay, and a follow-up survey in April 2007 showed new areas of mangrove clearing and dredging at Manatee Cay and Fisherman's Cay (I. C. Feller, personal communication).

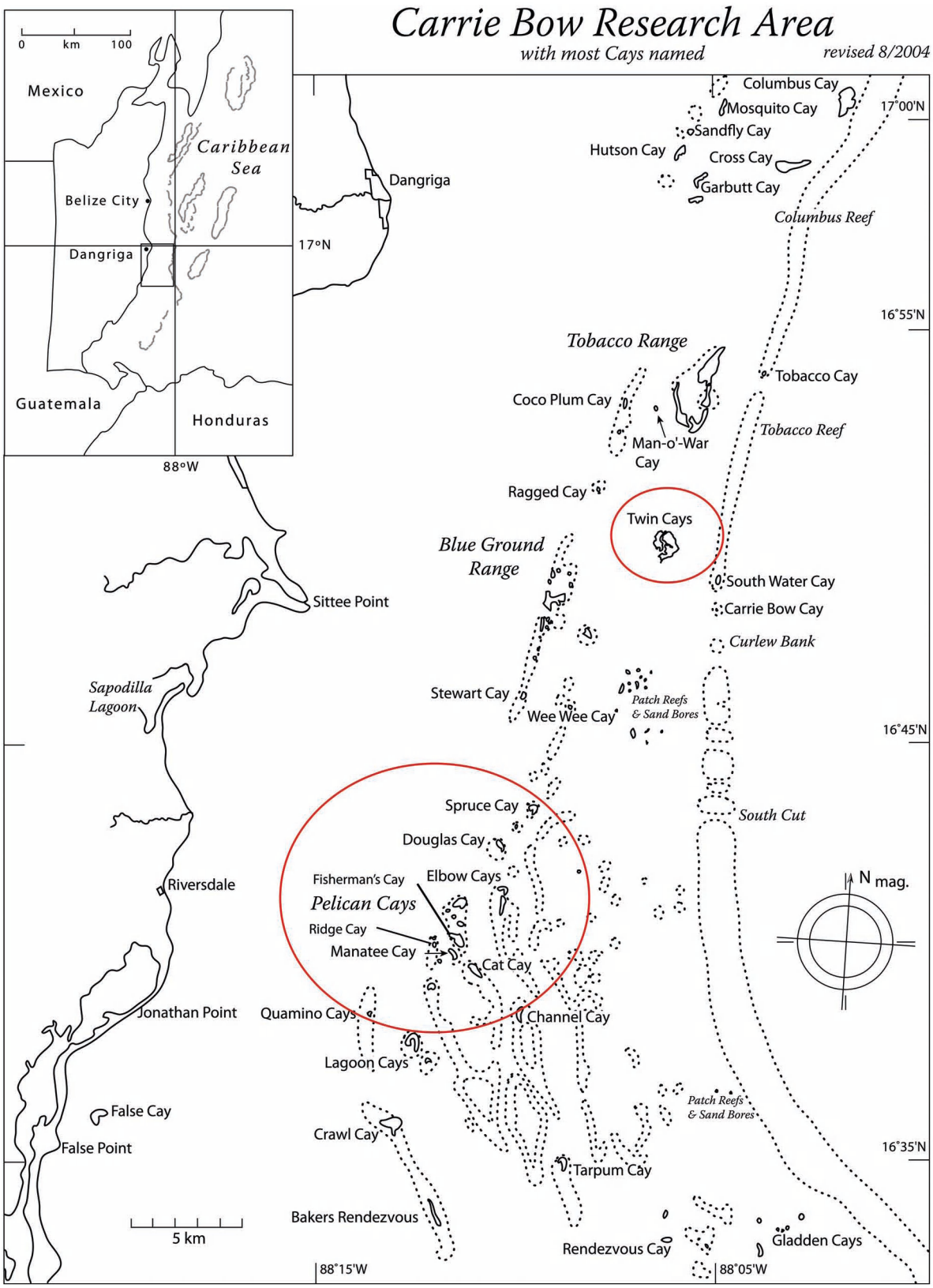

FIGURE 1. Map of research area. The Twin Cays and Pelican Cays ranges are circled.

MATERIALS AND METHODS

Experimental Design

Sampling of Pelican Cays and Twin Cays was conducted during May 2008. The sampling design was a split-plot in which the main plot was disturbance type (fixed effect) and the subplot was spatial position relative to the shoreline (fixed effect). Two disturbance levels were designated: undisturbed and disturbed. Undisturbed, reference areas exhibited no visible signs of human activity. Disturbed areas were characterized by removal of mangroves by clear-cutting followed by deposition of dredged marine sediment. Islands with a disturbed area of this type were identified initially by aerial photography. Four islands were selected for soil sampling based on disturbance history and accessibility: one in the Twin Cays range (East Island) and three in the Pelican Cays range (Manatee, Fisherman's, and Ridge Cays). Extent of natural and disturbed areas at each study location was estimated from satellite imagery (Landsat 7, May 2008; http://landsat.usgs.gov) and aerial photographs (April 2006, 2007; I. C. Feller, unpublished) and confirmed by ground-truthing (May 2008).

At each island, two transects were established perpendicular to the shoreline and traversing the island to a distance of 100 m inland. One transect was located in the disturbed area and the other in an adjacent undisturbed forest. Fourteen to sixteen sampling stations were preselected along each transect in a stratified-random design; that is, two to four stations occurred within each of five intervals (0–10, 11–30, 31–50, 51–70, and 71–100 m from the shoreline). In the disturbed areas, some portions were not clear cut (but may have been buried by dredged fill) or were clear cut and remained free of dredged fill; these zones were sampled as well. The reference transect originated along the same shoreline and was oriented in the same direction as the disturbed transect, although the length was not always the same (as a consequence of variation in island configuration). Each island was considered to be a replicate block.

At each sampling station, percent cover of herbaceous vegetation and mangrove canopy was estimated visually. The following soil variables were measured: bulk density, texture, shear strength, organic matter content, particle size distribution, and aggregate stability (as described below). A surface core (30 cm depth) was also collected at each disturbed island to determine the thickness of dredged material. Also, deeper cores were collected at one island at Twin Cays (West Island) and one undisturbed island in the Pelican Cays (Cat Cay) to determine the stratigraphy and composition of deposits beneath these islands.

Analyses

Soil Shear Strength

Soil shear strength was determined with a Torvane device (H-4212 1, Humbolt Manufacturing Company, Durham Geo-Enterprises), which measures the torque required to shear or deform the soil (McGinnis, 1997). Soil strength was measured at the soil surface, and the only selective criterion was flatness, because the Torvane required a flat or nearly flat surface for accurate measurements. Five replicate measurements were made at each sampling station and averaged.

Soil Aggregate Stability

Duplicate soil cores (2 cm diameter × 10 cm long) were collected at each station. The cores were carefully extruded onto a board, and the upper 1 cm was severed with a knife, providing a total soil volume of 3.14 cm^3 per sample. One core was used for stability testing and the other was placed into a Ziplock bag for determination of soil bulk density and texture (described below).

Soil aggregate stability was determined based on a modification of standard methods (Angers and Mehuys, 1993; Herrick et al., 2001) to better assess the substrates (peat, marine sediment) and the types of erosive forces (waves, currents) typical of the mangrove habitat. In the field, cores were placed in collection boxes, which protected them from disturbance until processing. Because the soils in this study were naturally moist to saturated, samples were not dried before measurement. Each core was transferred to a sieve (#20 mesh, 850 µm) and gently lowered into a container of water, then scored as to initial structural integrity. The core was then gently agitated by repeated dipping (five times) in water and again assessed. Initial stability (based on slaking or disintegration of the core) was scored as 0 (soil too unstable to sample), 1 (50% of structural integrity lost upon immersion or less than 10% of soil remained on sieve after five dips), 2 (10%–25% of soil remained), 3 (25%–75% of soil remained), or 4 (75%–100% of soil remained). After initial assessment, 200 mL water was poured over the sample; the material remaining on the sieve and that washed through the sieve (including the portion from the initial assessment) was transferred to separate bags for drying and weighing. Samples were oven dried at 60°C for 24 h and weighed. The percent by weight of material retained on the sieve was calculated. These two measures were designated as Stability Index 1 and 2, respectively.

Soil Water Content, Bulk Density, and Texture

At the laboratory, the soil was weighed wet, dried at 60°C to constant mass, and reweighed to determine moisture content (percent water in soil sample). Dry bulk density was calculated by dividing the dry mass by the volume (g cm^{-3}). The dried soil was ashed at 550°C for 6 h to determine mineral mass after organic loss on ignition. Percent organic matter content was calculated as 100 minus the percent ash. Particle size distribution (PSD) was determined (only for mineral sediments) based on a micropipette method (Burt et al., 1993). Subsamples (three or four) collected within each zone along a transect were combined to provide sufficient mass for PSD.

Peat Coring

Deep cores were collected to the point of refusal with a Russian peat corer, which extracts uncompressed cores in sections 0.5 m long (McKee et al., 2007a). At Twin Cays, seven cores were collected across a transect traversing West Island (west to east) to depths to 10.8 m. At Cat Cay, nine cores were similarly collected, but the maximum depth was 1.5 m because the peat layer was thinner. A deep core was also collected at Manatee Cay (disturbed area). Each core was extracted and transferred to a half-section of polyvinyl chloride (PVC) pipe, wrapped with plastic wrap, and refrigerated until processing. At the field station, each core section was logged, photographed, and thicknesses of major strata measured. Subsamples were taken at intervals of approximately 10 cm and washed on a 1 mm mesh sieve; plant fragments were identified to species using a key as described previously (McKee and Faulkner, 2000). At all disturbed sites, shallow cores (5 cm diameter × 30 cm deep) were collected with a piston corer to determine the thickness of the dredged fill.

RESULTS

General Observations

Large areas of Twin Cays, Fisherman's Cay, Manatee Cay, and Ridge Cay were clear cut (Table 1), and marine sediment had been dredged from a nearby reef flat and pumped to the island interior. Scars on the seafloor were visible from the air (Figure 2), showing that large areas (0.6 to 1.0 ha) of reef flat had been disturbed. The mangrove areas disturbed at these four study sites varied from 1.0 to 6.2 ha, accounting for up to 34% of the mangrove area per island (Table 1). In most cases, the woody debris from clear-cutting had been burned and only stumps remained to mark the past presence of mangrove trees. All four disturbed sites had received varying amounts of dredged marine sediment that had created a relatively flat, homogeneous landscape of dry, highly reflective, inorganic substrate (Figure 3). The dredged material varied in depth from 12 to 25 cm and contained coral, shells, and sand that indicated the marine origin of the materials. All the sites examined at the Pelican Cays were filled with material dredged from nearby reef flats, as evidenced by the presence of carbonate sand (*Halimeda* spp.), shells, and coral fragments. At Twin Cays, the dredged fill was composed of

TABLE 1. Summary of natural and disturbed mangrove areas at two island ranges: Twin Cays (East and West) and Pelican Cays (Manatee Cay, Fisherman's Cay, and Ridge Cay). A dash (–) indicates data were not obtained.

	Twin Cays		Pelican Cays		
Measurement	East Island	West Island	Manatee Cay	Fisherman's Cay	Ridge Cay
Total island area (ha)	41.4	17.6	12.6	24.3	3.4
Undisturbed area (ha)					
Natural ponds	9.8	2.0	4.3	6.2	0.1
Mangrove	28.7	13.0	6.3	11.9	2.3
Disturbed area (ha)					
Clear-cut only	1.2	1.4	–	–	–
Clear-cut + filled	1.7	1.2	2.0	6.2	1.0
Percent disturbed area					
Island	7%	14%	16%	26%	29%
Mangrove only	9%	17%	24%	34%	30%

FIGURE 2. Aerial photograph of Fisherman's Cay and Manatee Cay in April 2007 showing mangrove areas that were clear cut and filled with marine sediment dredged from an adjacent reef flat (indicated by red circle and arrow pointing to white filled area on the cay). (Photograph by I. C. Feller.)

quartz sand, indicating a mainland origin. With the exception of a narrow fringe of uncut trees and occasionally a lone tree, no mangrove canopy remained in these disturbed areas. In some cases, dredged material had buried the aerial roots of intact trees along some shorelines, and these trees had subsequently died (Figure 4). At all disturbed sites, mangrove associates (*Conocarpus erectus*) and common coastal beach species were present, but total cover was low (<10%). The most common herbaceous species included *Batis maritima* L., *Sesuvium portulacastrum* (L.) L., *Distichlis spicata* (L.) Greene, *Paspalum distichum* L., *Salicornia virginica* L., *Spartina spartinae* (Trin.) Merr. ex A. S. Hitchc., *Rhabdadenia biflora* (Jacq.) Muell.-Arg., *Cyperus* spp., *Ipomoea pescaprae* ssp. *Brasiliensis* (L.) van Ooststr., *Ageratum littorale* Gray, and *Typha* sp. In unvegetated areas, a biological crust of unidentified composition had sometimes formed a thin surface layer on top of the dredged fill.

In contrast, the reference sites contained intact mangrove canopy (dominant species = *R. mangle* with subdominants = *Avicennia germinans* (L.) L. and *Laguncularia racemosa* (L.) Gaertn. f.), low herbaceous cover (<5%; most commonly *B. maritima*), and undisturbed substrate that was dark in color, saturated with water, and composed of live and dead mangrove roots and other organic matter. Abundant aerial roots of intact mangrove vegetation (prop roots and pneumatophores) formed an interlacing network that contributed to the overall structural integrity of the reference areas and also served as substrate for a variety of epiphytes and epibionts. The forest floor was usually covered by algal-microbial mats (Rhodophyta, Chlorophyta, Cyanophycota, Bacillariophyta), typical of mangrove forests in this region (K. L. McKee, unpublished data).

Surface Soil Characteristics and Erosion Potential

Major differences in surface soil characteristics and potential for erosion occurred between reference and disturbed areas (Table 2). The surface soil of reference forests was peat composed of a matrix of live and dead mangrove roots, filamentous algal-microbial mats, and

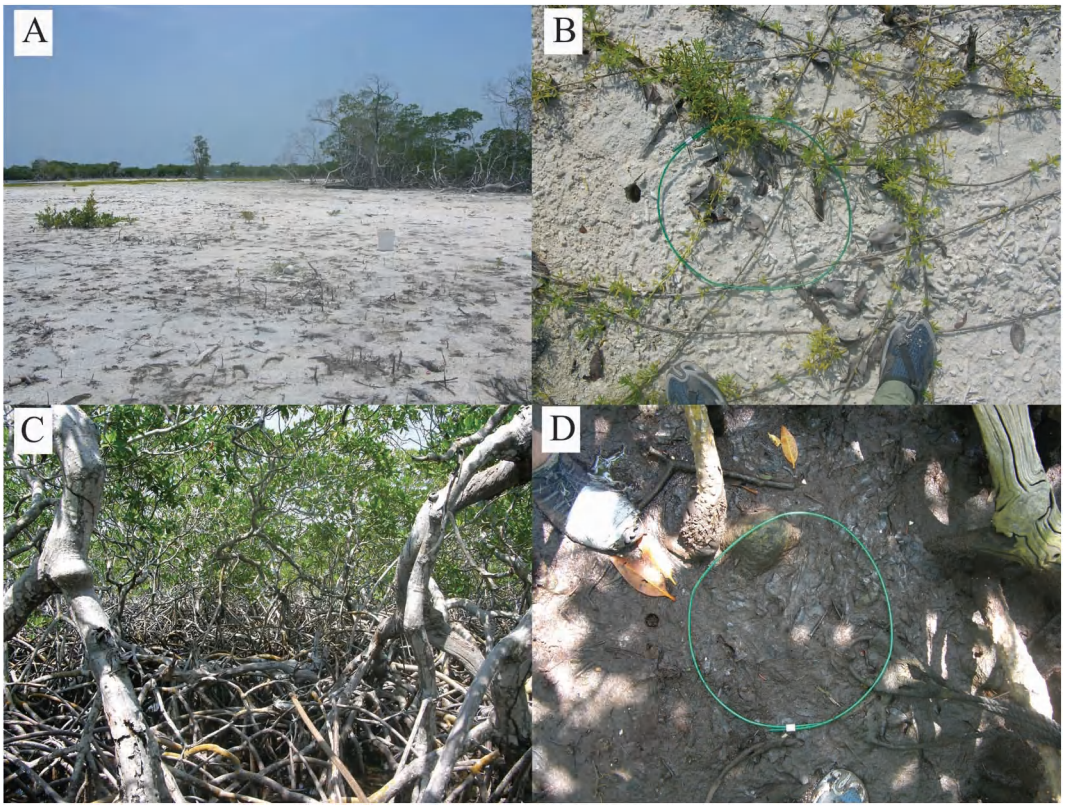

FIGURE 3. Views of disturbed (upper panels, A and B) and reference (lower panels, C and D) areas showing detail of soil surfaces. A circular quadrat in panel D (0.1 m^2) provides scale.

trapped organic matter that retained its structural integrity even when disturbed by sampling. Bulk density was low (0.17 g cm^{-3}), and water (68%) and organic contents (60%) were high. Despite its organic nature, the reference soil (peat) had high shear strength (overall mean, 0.084 kg cm^2), which varied little spatially (Figure 5). Core samples retained their shape and showed little or no slaking upon immersion in water (Stability Index 1 = 3.98) and little loss of material upon repeated agitation (Stability Index 2 = 87%). When stability indices were plotted as *x–y* coordinates, the reference sites grouped together, indicating little difference among islands (Figure 6).

In contrast, the surface soil in disturbed areas was composed of inorganic carbonate particles derived primarily from calcareous algae (*Halimeda* spp.), coral fragments, and shells (PSD showed that >90% of the mass was sand or larger particles). This material had a high bulk density (0.72 g cm^{-3}) and low water content (30%) and organic content (9%). Soil shear strength (0.044 kg cm^2) in disturbed areas was lower overall compared to reference areas and varied spatially (see Figure 5). Aggregate stability was lower overall (Stability Index 1 = 2.14, Stability Index 2 = 47%), and differed among island locations (see Figure 6). Many cores were friable and readily disintegrated when disturbed mechanically. Where vegetation or biological crusts had developed on the dredged material, the shear strength was higher, but aggregate stability remained low; that is, cores typically did not retain their integrity and exhibited a high degree of slaking in water. The mass retained on the sieve was composed of particles greater than 1 mm in diameter (*Halimeda* chips, coral fragments, shells). Shear strength increased overall with increasing distance along some disturbed transects because of the absence of dredged fill at interior stations where the old peat surface remained exposed (e.g., Twin Cays). In such cases, the exposed peat substrate retained high shear strength and high aggregate stability despite the removal of the mangroves.

Peat Stratigraphy

Mangrove islands in the Twin Cays and Pelican Cays ranges were underlain by deposits of peat, varying in maximum thickness from 1.5 m (Cat Cay and Manatee

FIGURE 4. Red mangrove trees along the shoreline of disturbed areas: some trees died as a result of burial with dredged fill (right).

Cay) to more than 10 m (Twin Cays) (Figure 7). At Cat Cay, a series of cores traversing the island showed that peat thickness was greatest on the southern, leeward side and decreased toward the northern, windward shore. Botanical matter in the peat consisted predominately of mangrove roots with fragments of leaves and wood. Beneath the peat layer was sand and/or coral. Deeper peat layers were dominated by *R. mangle*, whereas upper layers (0–50 cm) in the island interior contained remains of *A. germinans*. At Twin Cays, cores across an east–west transect were 7.5 to 10.8 m thick, with two of the cores reaching the limestone platform underlying this range. These cores also consisted

TABLE 2. Summary of analysis of variance (ANOVA) results for soil characteristics. The main plot factor (disturbance) was tested with within-subject error (island); subplot factor (spatial position) and interactions were tested with residual error. Values are the *F* ratio; significance: *P = 0.05, **P = 0.01, ***P = 0.001, ****P = 0.0001, ns = not significant.

		Aggregate stability					Vegetative cover	
Source of error	Shear strength	Index 1	Index 2	Bulk density	Organic matter	Water content	Herbaceous	Mangrove
Disturbance	50.24****	180.0****	86.4****	184.0****	353.2****	350.0****	6.78*	191.3****
Island (block)	6.23***	7.01****	1.93 ns	21.2****	5.52**	2.42 ns	7.80****	7.95****
Position	1.36 ns	4.85**	3.53**	1.23 ns	0.68 ns	3.02*	1.87 ns	1.99 ns
Disturbance × position	5.70***	3.95**	1.29 ns	6.58****	2.82*	8.67****	1.70 ns	1.59 ns

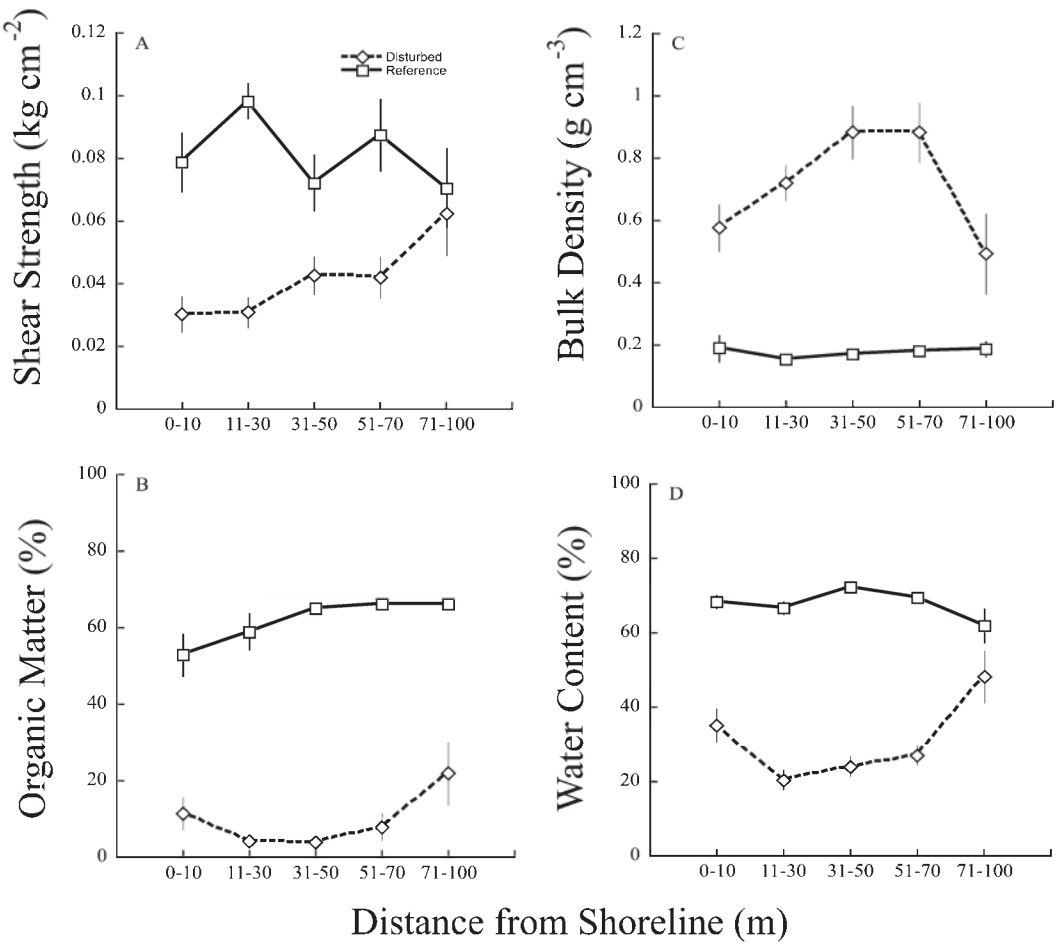

FIGURE 5. Spatial variation in soil shear strength (A), organic matter (B), bulk density (C), and water content (D) in reference (squares) and disturbed (circles) mangrove areas. Values are the mean ± SE (note some SE bars are smaller than the symbols).

of mangrove peat, predominately *R. mangle*. In the island interior, surface layers of *A. germinans* peat varied in thickness from 50 to 100 cm, similar to the pattern observed at Cat Cay.

DISCUSSION

The MBRS is a unique and valuable resource to the Central American countries of Belize, Honduras, and Guatemala (www.mbrs.org.bz; accessed 11 June 2008). Destruction of mangrove islands has rapidly accelerated in this system as a result of attempts to transform these sensitive and fragile habitats into environments more attractive to tourists. Because there are few sand-based islands underlain by shallow limestone platforms and suitable for development, mangrove-dominated islands have been targeted for conversion. In addition to the two ranges included in this study (Pelican Cays, Twin Cays), other mangrove ranges in the vicinity also have undergone similar mangrove clearing and filling (e.g., Blue Ground Range, Tobacco Range, Coco Plum) (K. L. McKee, personal observation). Survey lines found during this study indicate plans for further development at many of these island ranges.

Effects of Mangrove Removal and Dredged Fill on Erosion

Although the direct and indirect effects of mangrove clear-cutting and marine dredging are many and varied, our study focused on the specific consequences for erosion and

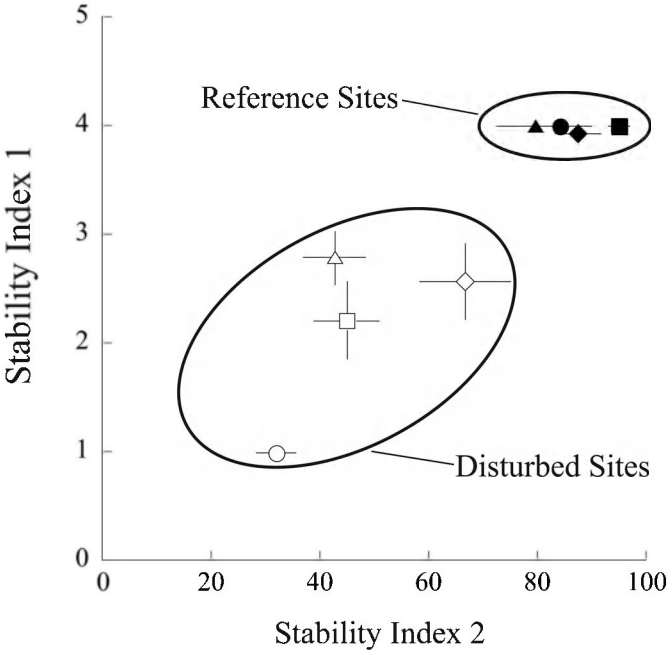

FIGURE 6. Aggregate stability of soils from reference sites (closed symbols) and disturbed mangrove areas (open symbols) at Twin Cays (diamond), Fisherman's Cay (square), Manatee Cay (triangle), and Ridge Cay (circle). Stability indices (mean ± SE) are based on maintenance of soil structure upon immersion in water (Index 1) and percent of soil retained on a 850 μm sieve (Index 2). Probability ellipses (90% confidence curves) are drawn for each group.

long-term loss of elevation. Removal of mangroves and alteration of the soil surface by dredged fill significantly altered the potential for erosion and substrate stability at Twin Cays and the Pelican Cays. The natural substrate in undisturbed mangroves comprised a strong matrix composed of living and dead fibrous roots as well as filamentous algae, which formed mats on the soil surface. This material was extremely resistant to shearing and retained its integrity even when repeatedly agitated by submersion in water (see Figure 6). Although some interior areas contained natural deposits of flocculent material (e.g., microbial mats) that were soft and friable, they were underlain by solid peat. Work in other locations, such as the Bay Islands of Honduras, found similarly high resistance of mangrove peat soils to shearing (McKee and McGinnis, 2002; Cahoon et al., 2003). These results demonstrate the high resistance to soil erosion afforded by intact mangrove peat.

Removal of mangroves by clear-cutting did not by itself appear to have an immediate effect on soil shear strength or aggregate stability in the areas sampled. In a few cases, clear-cut areas that were not covered by dredged material were encountered during surveys (e.g., at Twin Cays), and here shear strength was equal to that in reference areas; these were all areas that had been previously occupied by *R. mangle* and had only been altered by removal of trees. Similarly, some mangrove areas in the Bay Islands of Honduras killed by Hurricane Mitch retained shear strength up to two years following mortality because of the strong matrix of *R. mangle* roots forming the peat substrate (Cahoon et al., 2003). However, those areas that had been dominated by *Avicennia germinans* lost soil integrity and collapsed following mortality of the trees. Eventually, however, the lack of live roots and algal mats may lead to loss of shear strength wherever mangroves have been removed.

A review of sediment burial effects on mangroves suggests that some species are more sensitive and may suffer mortality when subjected to excessive rates of sedimentation (Ellison, 1998). We also found that live trees (*R. mangle*) exposed to dredged fill often died—presumably the result of smothering of aerial roots. This outcome was particularly evident where a narrow (<10 m wide) fringe of trees was left intact along the shoreline and the dredged material overflowed into this zone (see Figure 4). In cases where the shoreline tree zone was wider (20–30 m), there was a higher survival. Without a protective mangrove buffer along the shoreline, these islands may rapidly erode. Observations at older sites (Twin Cays) showed rapid shoreline retreat (up to 0.3 m per year) where mangroves had been removed in 1992 (McKee et al., 2007b).

Long-Term Consequences for Island Stability

Oceanic islands are generally vulnerable to disturbance because of their low-lying position and potential for submergence as well as exposure to tropical storms, hurricanes, and tsunamis that generate strong erosive forces. Mangrove islands in the MBRS have developed and built vertically over thousands of years through deposition of peat derived from mangrove organic matter (McKee and Faulkner, 2000; McKee et al., 2007a). This process occurs in the intertidal zone where abundant mangrove roots are produced and accumulate biomass because of their slow decomposition in the anaerobic environment (Middleton and McKee, 2001). Other biogenic processes include formation of algal and microbial mats on the soil surface (intertidal and subtidal) and carbonate sand formed from calcareous algae (subtidal) in mangrove ecosystems (McKee et al., 2007a; McKee, unpublished data). Growth of mangrove root-algal mats and other biofilms not only contributes to vertical accretion but also stabilizes islands

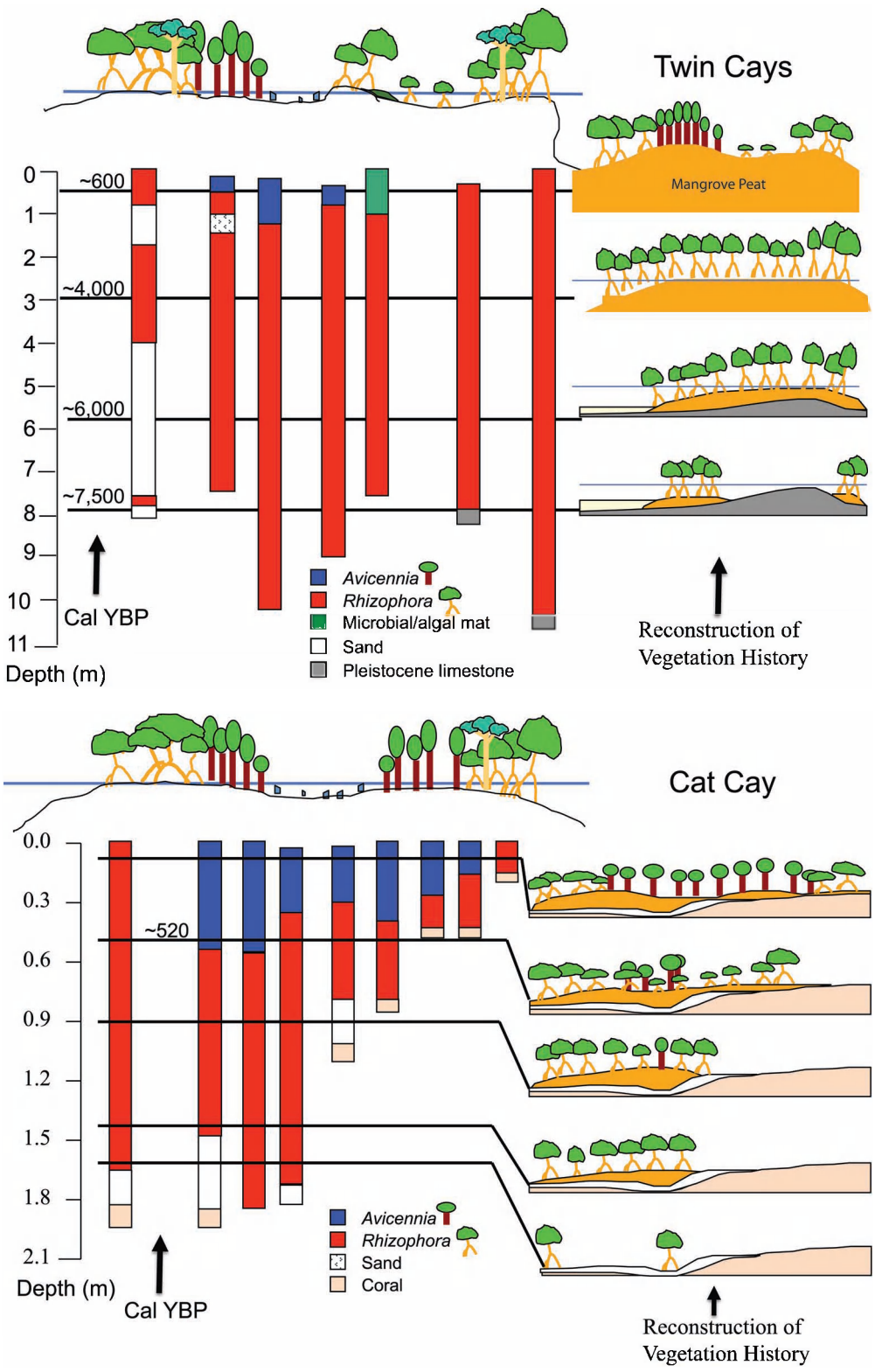

FIGURE 7. Peat stratigraphy across Twin Cays (top) and Cat Cay (Pelican Cays) (bottom) and reconstruction of vegetation history. Radiocarbon dates (calendar years before present [Cal YBP]) are based on previous work (McKee et al., 2007a).

by trapping and consolidating organic and inorganic sediment that is deposited.

To persist, such oceanic islands must accrete vertically to counterbalance both sea-level rise and local rates of subsidence, which vary depending on geomorphology, isostacy, and tectonic movements. Mangrove-dominated islands have the capacity to self-adjust to subsidence and sea-level rise through peat formation. Previous work has shown that mangrove islands in Belize and other Caribbean areas have kept up with changing sea level for thousands of years through the slow accumulation of mangrove roots and other organic material and that vertical building rates are determined by the health and productivity of the mangrove community (McKee and Faulkner, 2000; Middleton and McKee, 2001; McKee et al., 2007a). Peat subsidence rates determined at Twin Cays in vegetated areas averaged 7 mm $year^{-1}$ (McKee et al., 2007a). Similar rates of subsidence were found in the Bay Islands, Honduras (Cahoon et al., 2003). Island subsidence combined with eustatic rise in sea level (3.5 mm $year^{-1}$; Rahmstorf, 2007) means that the relative rise in sea level in this area is at least 10.5 mm $year^{-1}$ (assuming negligible deep subsidence). On undisturbed cays with intact mangroves, vertical building from peat accumulation should maintain surface elevations within the intertidal zone, unless sea-level rise accelerates beyond the capacity of the system to compensate.

Removal of mangroves by clear-cutting eliminates the main mechanism of peat formation and also may alter the environmental conditions necessary for the survival of algal and microbial mats that contribute to sediment trapping and resistance to erosion. Cays disturbed by mangrove clearing and dredged fill deposition will continue to subside, but peat formation will cease. Even if these areas become revegetated with coastal beach vegetation, peat cannot form because of oxidizing conditions (caused by the higher elevations) and lack of the primary peat builder—*R. mangle*. Although disturbed island surfaces have been temporarily raised by dredged fill, the inexorable subsidence of underlying peat and rising seas will lead to submergence. At current rates of peat subsidence and sea-level rise, the elevation gain from dredging will be offset within 20 years.

Implications for Sustainable Ecotourism

The Caribbean Region, and in particular the MBRS, is a major destination for "eco-tourists," who are attracted to the tropical climate, clear waters, and abundant marine life (Uyarra et al., 2005; Diedrich, 2007). A prerequisite for sustainable ecotourism is maintenance of a pristine natural environment and protection of all biophysical components necessary for healthy ecosystems and habitat stability (Casagrandi and Rinaldi, 2002). Unregulated ecotourism enterprises threaten the very features that underpin this industry and, in addition, lead to the degradation of natural resources essential to the livelihood of citizens (e.g., sport and commercial fisheries) (Burger, 2000; Hall, 2001). Although "charismatic" ecosystems such as coral reefs receive much attention by conservationists and tourism regulators (Diedrich, 2007), less emphasis is placed on mangroves. The mangrove destruction occurring in the Belize reef system likely reflects a general misperception that the land beneath mangrove-dominated islands is stable, as well as a failure to recognize mangroves as essential components contributing to habitat stability and marine biodiversity—which is what attracts eco-tourists in the first place (Uyarra et al., 2005).

Our work suggests that the alterations occurring on mangrove islands in the MBRS are inconsistent with sustainable ecotourism. In their natural state, mangroves build a peat substrate that is resistant to erosion and counterbalances subsidence and sea-level rise. In fact, this dynamic peat-building process has allowed mangrove islands such as Twin Cays to persist for the past 8,000 years (Macintyre et al., 2004a, 2004b; McKee et al., 2007a). Attempts to convert mangrove islands to sand islands, with white beaches and coconut palms, will ultimately fail because the underlying peat subsidence and rising seas will eventually prevail. Filling with marine sediment temporarily raises elevations, but without repeated dredging, eventually these cleared areas will become submerged, ultimately reducing the total land area of islands in the MBRS.

Aerial roots of mangroves additionally provide one of the few natural hard substrates for growth of many marine organisms in the MBRS (Ellison et al., 1996; Goodbody, 2000; Macintyre et al., 2000) and also create a permeable barrier that dampens wave energy, decreasing shoreline erosion (Alongi, 2008). Loss of mangrove fringes directly decreases the abundance of marine organisms dependent on mangrove roots for substrate as well as that of reef species dependent on the mangroves as nurseries (Mumby et al., 2004). Although the direct and indirect effects of dredging on reef flats and seagrass beds were not examined in this study, these effects are likely to be substantial, given the sensitivity of such systems to disturbance and sedimentation (Nugues and Roberts, 2003; Erftemeijer and Lewis, 2006).

Future work should examine the long-term consequences of human activities on the resilience of mangrove islands to global change and the contribution of mangroves to terrestrial and marine biodiversity and fishery productivity. In addition, cost–benefit analyses of man-

grove clearing and dredging and the consequences for sustainable ecotourism should be conducted to provide economic rationales for conservation and management of mangroves and associated habitats.

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An Overview of Symbiont Bleaching in the Epiphytic Foraminiferan *Sorites dominicensis*

Susan L. Richardson

ABSTRACT. Populations of *Sorites dominicensis*, an epiphytic foraminiferan that possesses dinoflagellate endosymbionts (*Symbiodinium*), were sampled from seagrass meadows located in Florida and Belize and surveyed for evidence of bleaching. Symbiont bleaching was first documented in *S. dominicensis* populations in the Indian River Lagoon, Florida, in August 2003. Subsequent surveys indicated high rates of bleaching in August 2004, followed by a near eradication of the epiphytic foraminiferal population as a result of the 2004–2005 hurricane seasons. Two contrasting sites in Belize, seagrass beds on the reef flat at Carrie Bow Cay and in Boston Bay, Twin Cays, were surveyed in 2005 and 2006. High rates of bleaching characterize the *S. dominicensis* populations living on turtle grass on the reef flat off Carrie Bow Cay, although freshwater runoff from summer storms during the rainy season may trigger localized bleaching events. Moderate rates of bleaching were also observed in *S. dominicensis* populations in Florida Bay in July 2007. Symbiont bleaching in *S. dominicensis* appears to be triggered by multiple environmental factors: increased water temperatures, high levels of irradiance, and influx of freshwater during storm events. Seasonal summer bleaching events may leave already compromised *S. dominicensis* populations vulnerable to periodic disturbance by hurricanes.

INTRODUCTION

Sorites dominicensis Ehrenberg, 1839, is one of several living foraminiferal species that are host to algal endosymbionts (Hallock, 1999; Lee et al., 1979). Benthic foraminiferans with algal symbionts occur in several different clades (Soritacea, Alveolinacea, Nummulitacea, Calcarinidae, and Amphisteginidae) and are widely distributed in shallow-water, tropical to subtropical reef-associated marine ecosystems (Langer and Hottinger, 2000). As a group, foraminiferans host a diverse array of endosymbionts, most of which are microbial eukaryotic taxa, including stramenopiles (diatoms and chrysophytes), unicellular rhodophytes, unicellular chlorophytes, and alveolates (dinoflagellates) (Lee, 2006; Hallock, 1999). Cyanobacterial endosymbionts have also been isolated from two different soritid taxa collected from the Red Sea and the Great Barrier Reef (Lee, 2006). Foraminiferans with photosymbionts possess enhanced calcification rates, as well as endogenous sources of nutrition (algal photosynthates) that allow them to allocate more of their energy resources to cell growth and

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maintenance (Lee, 2006; Hallock, 1999; Duguay, 1983; Kremer et al., 1980; Lee and Bock, 1976). The algal endosymbionts presumably benefit from the mutualism as well, gaining access to nutrients that are scarce in oligotrophic environments and to refuge from predation (Lee, 2006; Hallock, 1999).

The mutalistic association of *Sorites* and other taxa in the more inclusive foraminiferal clade Soritida, with dinoflagellate endosymbionts in the *Symbiodinium* clade, is of particular interest to the marine biological community because this clade comprises the zooxanthellae in stony corals, soft corals, gorgonians, anemones, jellyfish, bivalve mollusks, nudibranchs, sponges, and ciliates (Baker, 2003; Douglas, 2003; Glynn, 1996). Originally considered to be a single pandemic species that was symbiotic with a broad range of marine taxa, *Symbiodinium microadriaticum* is now known to be part of a more inclusive and genetically diverse clade composed of eight major subclades, identified by the letters A–H (Pochon and Pawlowski, 2006; Coffroth and Santos, 2005; Baker, 2003; Rowan, 1998; Rowan and Powers, 1991, 1992). *Symbiodinium* symbionts from foraminiferal hosts are found in clades C, D, F, G, and H, with clades F and H being composed almost exclusively of *Symbiodinium* isolated from soritid foraminiferans (Garcia-Cuetos et al., 2005; Pochon and Pawlowski, 2006; Pawlowski et al., 2001; Pochon et al., 2001, 2004, 2006; Rodriguez-Lanetty, 2003). Although there is relatively high specificity between *Symbiodinium* clades F, G, and H and Foraminifera, there appears to be very little congruence between host and symbiont phylogenies, indicating that coevolution has not taken place, at least not at the taxonomic levels sampled to date (Garcia-Cuetos et al., 2005; Pochon and Pawlowski, 2006; Pawlowski et al., 2001; Pochon et al., 2001, 2004, 2006). Although DNA sequences have not yet been obtained from the endosymbionts of either the Belizean or Indian River Lagoon populations of *Sorites dominicensis*, *Symbiodinium* sequences from Florida Keys specimens fall within either clade F (subclade F4) or H (Garcia-Cuetos et al., 2005; Pochon and Pawlowski, 2006; Pochon et al., 2006). In all phylogenies published to date, clade H, the dominant phylotype isolated from the Florida Keys, branches as the sister group to clade C, a clade that is widely distributed in the Indo-Pacific, and exhibits more sensitivity to bleaching than the other *Symbiodinium* clades (Garcia-Cuetos et al., 2005; Pochon and Pawlowski, 2006; Pawlowski et al. 2001; Pochon et al., 2001, 2004, 2006; Rowan, 1998, 2004).

The morphological characteristics of *Symbiodinium* symbionts isolated in culture from specimens of *Sorites dominicensis* collected from the Florida Keys have been described by Lee et al. (1979, 1997). Symbionts are distributed throughout the foraminiferal cytoplasm, with the highest densities occurring in the intermediate chambers and the lowest densities occurring in the outer chambers where the digestive vacuoles are concentrated (Richardson, 2006; Müller-Merz and Lee, 1976). Similar to other species of foraminiferans, *S. dominicensis* is multinucleate and possesses two different types of nuclei: generative nuclei that participate in reproduction only, and vegetative nuclei that are transcriptionally active and coordinate the day-to-day activities of the cell (Müller-Merz and Lee, 1976). In *S. dominicensis*, the generative nuclei are localized in the central initial chambers of the test (external shell), which are the chambers with the lowest densities of dinoflagellates, whereas the transcriptionally active foraminiferal nuclei are distributed throughout the cytoplasm in regions with the high symbiont densities (Müller-Merz and Lee, 1976).

Estimates of symbiont population size per cell vary depending on the methodology employed (Richardson, 2006; Doyle and Doyle, 1940). Doyle and Doyle (1940) estimated the population of dinoflagellates in a 2-mm sized individual of *S. dominicensis* to be approximately 1.6×10^4 using light microscopy. In contrast, confocal microscopy of a 2-mm sized individual of *S. dominicensis* collected from Jupiter Sound yielded an estimated 4×10^3 dinoflagellates, equivalent to a density of 1.27×10^5 endosymbionts cm^{-2} of cytoplasm (Richardson, 2006) (Figure 1). Hemacytometer estimates of endosymbiont densities in live individuals of *S. dominicensis* collected from Jupiter Sound indicate that symbiont densities range from 6.1×10^2 to 4.8×10^5 dinoflagellates cm^{-2}, with an average of 6.5×10^4 dinoflagellates cm^{-2} ($n = 85$, $\sigma = 7.9 \times 10^4$, $\sigma^2 = 6.2 \times 109$) (Ross and Richardson, unpublished data). Endosymbiont populations linearly increase with test size: the average number of symbionts per foraminiferal cell is estimated to be 1,469 ($n = 85$, $s = 2{,}919$, $s^2 = 8{,}523{,}907$) for an individual with a test diameter of 1.42 mm ($n = 85$, $s = 0.62$, $s^2 = 0.39$) (Ross and Richardson, unpublished data).

Live individuals possess a dark yellowish-brown coloration to their cytoplasm as a result of the dense populations of *Symbiodinium* in each cell (Figure 2). In healthy individuals, the coloration is evenly distributed throughout the test, except for the outer chambers, which appear colorless because of the low density or absence of endosymbionts from the zone of cytoplasm where digestion takes place (Figure 2). The distinctive coloration of the foraminiferal cytoplasm makes it easy to recognize bleached or mottled individuals, as described below.

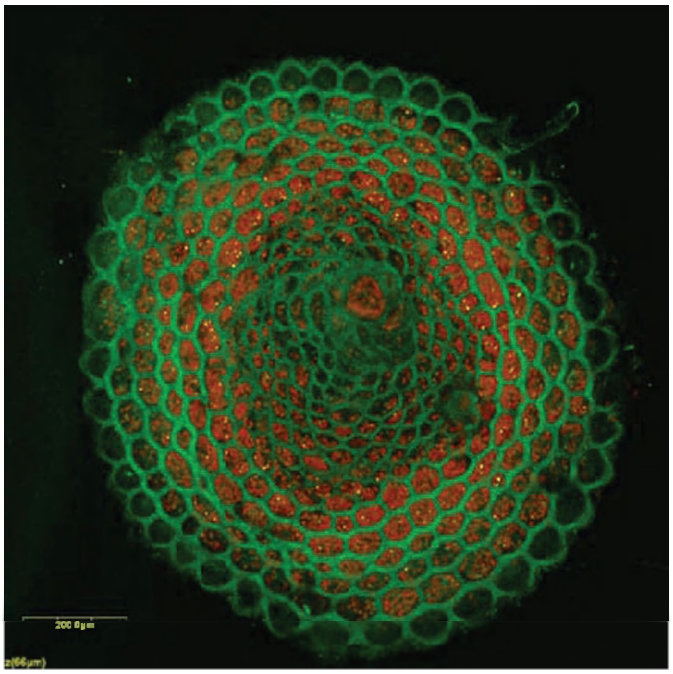

FIGURE 1. Confocal image of live individual of *Sorites dominicensis* from Jupiter Sound, Florida. The foraminiferal test is subdivided into hexagonal chamberlets. The dinoflagellate endosymbionts are most densely packed into the intermediate chambers. Scale bar = 200 μm.

FIELD OBSERVATIONS OF BLEACHING IN *SORITES DOMINICENSIS*

Symbiont bleaching has been observed in field surveys of epiphytic foraminiferal populations from Florida (Indian River Lagoon and Long Key, Florida Keys) and Belize (Carrie Bow Cay and Twin Cays). Bleaching in *Sorites dominicensis* was first documented in epiphytic populations attached to *Thalassia testudinum* (turtle grass) growing in Jupiter Sound in August 2003 and August 2004, followed by field surveys of populations in Belize in July 2005 and July 2006. Bleaching was also observed in epiphytic populations of *S. dominicensis* surveyed from the Florida Keys in July 2007 (Richardson, unpublished data). Although each of the collecting sites studied hosts seagrass meadows dominated by *T. testudinum*, each locality is subject to different physical factors (salinity, temperature, water clarity, and subaerial exposure), as well as differing levels of anthropogenic impact. Detailed descriptions of the field sites in Florida and Belize are given by Richardson (2006). Although experimental studies of bleaching in *S. dominicensis* have yet to be carried out, field observations indicate that symbiont bleaching may occur in response to a number of environmental stressors, including increased water temperature, freshwater influx, subaerial exposure during extreme low tides, and periodic disturbance by hurricanes.

Field Methods

Only epiphytic specimens of the foraminiferan *Sorites dominicensis* that were attached to blades of the seagrass *Thalassia testudinum* were examined in the studies described below. Blades of *T. testudinum* were harvested by wading or snorkeling. Seagrass leaves were removed at the base of the blade, submerged in seawater in a Ziploc bag, and stored in a cooler until return from the field. Both sides of each seagrass blade were examined for the presence of epiphytic foraminiferans using a binocular dissecting microscope (Leica M5). All specimens of the species *S. dominicensis* were removed from the blade using a fine paintbrush or dental pick, measured, and stored on cardboard microslides for additional study and reference material. The cytoplasmic condition (healthy, pale, mottled, totally bleached) and reproductive state (nonreproductive, presence of brood chambers, presence of embryos in brood chambers, or postreproductive)

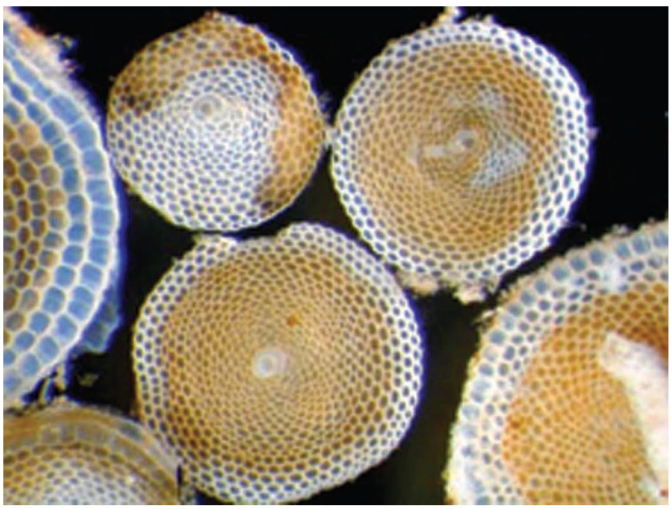

FIGURE 2. Live specimens of *Sorites dominicensis* from Belize, Central America. The two individuals in the upper part of the image show patches of bleached cytoplasm. Note that all specimens, except for the individual in the lower left, possess few, if any, endosymbionts in the outer two or three chambers. The specimen in the lower left is a reproductive individual preparing to undergo multiple fission. The specimen on the upper right is approximately 2 mm in diameter.

of each specimen were noted. Specimens were measured using an optical micrometer calibrated to a stage micrometer. Micrographs of representative individuals (healthy, mottled, and bleached) were taken using a Nikon Coolpix camera with an MCool (Martin Optics) phototube.

Live individuals were recognized by their distinctive cytoplasmic coloration as described below, and/or by the presence of pseudopodial arrays emanating from around the periphery of the protist's test. Bundles of bifurcating pseudopodia in live individuals are usually covered with a light dusting of fine-grained sediment, giving the specimens a star-shaped appearance. Individuals were recorded as having healthy cytoplasm if the cytoplasm possessed an evenly distributed, yellowish-brown coloration (see Figure 2). Individuals were recorded as having a mottled cytoplasm if the cytoplasm contained white-colored patches, interspersed with yellowish-brown sections of cytoplasm (Figure 2). Mottled individuals contained patches of white cytoplasm that were visible on both sides of the disk-shaped test. Specimens were recorded as being totally bleached if the test was completely white. The tests of postreproductive individuals, that is, individuals that had undergone reproduction by multiple fission, were not included in the tallies of bleached specimens. Postreproductive tests are easily distinguished from bleached tests by the presence of fragmented brood chambers, undisseminated embryos, and clusters of dispersed juveniles in close proximity to the parental test. It is assumed that few, if any, of the totally bleached tests had undergone gametogenesis, as microspheric tests (tests formed by syngamy) have never been observed in any of the populations of this species surveyed by the author.

Water Temperature and Bleaching

Studies conducted at both the Jupiter Sound and Belize sites indicate that elevated water temperature, or a combination of elevated water temperature and subaerial exposure, can induce symbiont bleaching in *S. dominicensis*. Bleaching was first observed in the Jupiter Sound populations in 2003 during August (Table 1), when water temperatures are typically at their maximum, often reaching extremes as high as 31°C (RiverKeeper Data, Loxahatchee River District). A relatively low abundance of bleached individuals was recorded in late July 2004; however, a resampling of the site a few weeks later in August indicated that the incidence of bleaching had risen 14 fold (Table 2). In July 2004, water temperatures recorded at the Jupiter Sound site ranged from 30° to 31°C between 1:00 PM and 3:30 PM during an extremely low spring tide that resulted in the subaerial exposure of major portions of the seagrass bed. No water temperature data are available for Jupiter Sound in August 2004, although the water was uncomfortably hot to the touch at the time of collection (Richardson, unpublished). Bleaching was undetectable in surveys of the *S. dominicensis* populations conducted at other times of the year in both 2003 and 2004 (Richardson, unpublished data).

In Belize, water temperatures were recorded using HOBO Tidbit (Onset) submersible temperature loggers deployed for three days in July 2005. One logger was deployed on the reef flat at Carrie Bow Cay and the other in Boston Bay, Twin Cays. The range of water temperatures recorded for both sites are listed in Table 3 and Figure 3. Although the overall mean temperatures were identical for both sites (s = 32°C), the reef flat off Carrie Bow Cay experienced a wider range of temperatures (29°–40°C), with higher maximum temperatures recorded during the late afternoon and lower minimum temperatures recorded at night (Figure 3; Table 3). Correspondingly, the rate of bleaching recorded from the reef flat at Carrie Bow Cay was almost five times higher than that observed in Bos-

TABLE 1. Relative abundance of bleached individuals of *Sorites dominicensis* from Jupiter Sound, Florida, during August 2003 (n = total number of tests examined).

	Percent of tests	
Test condition	2 Aug 2003 (n = 580)	12 Aug 2003 (n = 147)
Mottled cytoplasm	1.0%	1.0%
White cytoplasm	15%	12%
Total bleached	16%	13%

TABLE 2. Relative abundance of bleached individuals of *Sorites dominicensis* from Jupiter Sound, Florida during July and August 2004 (n = total number of tests examined).

	Percent of tests	
Test condition	29 Jul 2004 (n = 446)	19 Aug 2004 (n = 14)
Mottled cytoplasm	2.0%	29%
White cytoplasm	0%	0%
Total bleached	2.0%	29%

TABLE 3. Characteristics of two collecting sites in Belize.

Characteristic	Carrie Bow Cay	Twin Cays
Water depth	<0.5 m	1.0 m
Exposure	Exposed during low tides	Subtidal
Water clarity	Very clear	High tannins and mangrove detritus
Water movement	Swift current	Sheltered with slower current
Temperature range (1–4 July 2005)	30°–35°C	29°–40°C

ton Bay, Twin Cays (Table 4). Although the water temperatures recorded in Boston Bay, Twin Cays, were not as extreme as those recorded off Carrie Bow Cay, they still were higher than the HotSpot (28.9°C) and bleaching (HotSpot + 1°C) thresholds derived by NOAA/NESDIS for Glovers Reef (Opishinski, 2006). The same sites were resurveyed in July 2006, and the incidence of bleaching on the reef flat at Carrie Bow Cay was observed to be 11 times higher than the incidence of bleaching recorded in Boston Bay, Twin Cays, which exhibited almost negligible levels of bleaching (Table 5).

Freshwater Influx and Bleaching

In July and August 2006, continued sampling of the Carrie Bow Cay and Twin Cays field sites in Belize yielded results that indicate that symbiont bleaching in *S. dominicensis* can also be triggered by an influx of freshwater during storm events. In July 2006, field collections were suspended during a three-day period of intense rain then restarted after the storms subsided. After the rainstorms, the incidence of bleaching recorded at both sites rose in all three categories (pale cytoplasm, mottled cytoplasm,

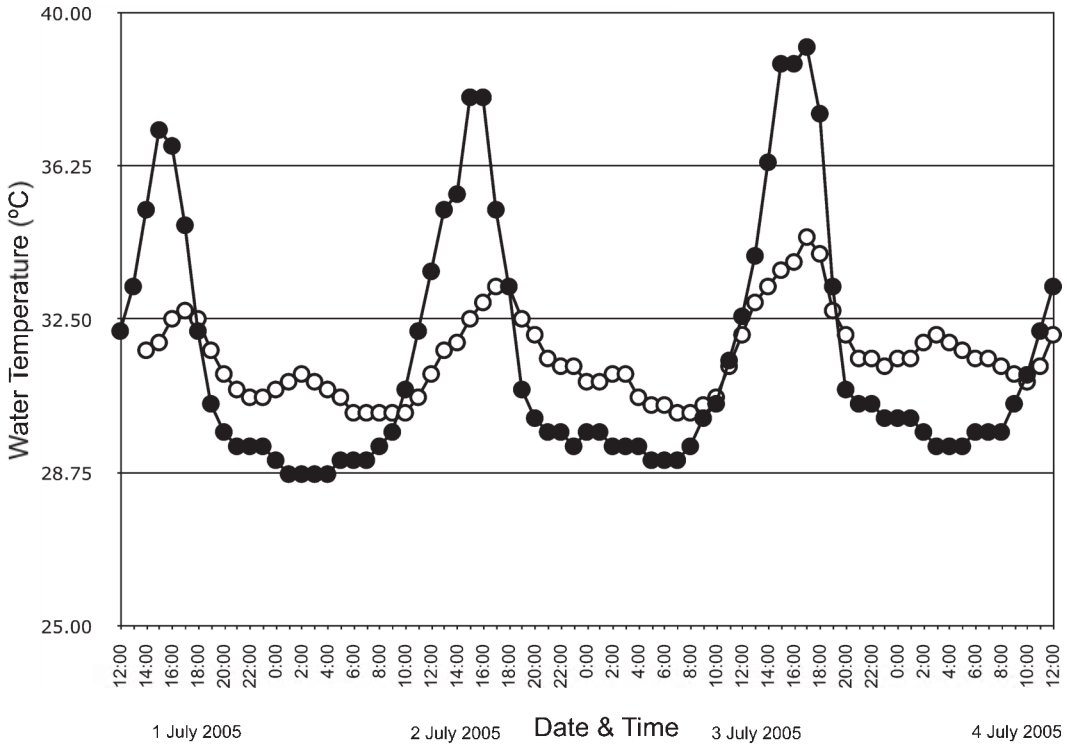

FIGURE 3. Water temperature variations on the reef flat at Carrie Bow Cay and in Boston Bay, Twin Cays, Belize, as measured at noon and every 2.5 hours thereafter during 1–4 July 2005.

TABLE 4. Relative abundance of bleached individuals of *Sorites dominicensis* from two localities in Belize during July 2005 (n = total number of tests examined).

	Percent of tests	
Test condition	Carrie Bow Cay (n = 797)	Boston Bay, Twin Cays (n = 685)
Mottled cytoplasm	4.3%	2.5%
White cytoplasm	14%	1.5%
Total bleached	19%	3.9%

TABLE 5. Relative abundance of bleached individuals of *Sorites dominicensis* from two localities in Belize during July 2006 (n = total number of tests examined). All specimens were collected before a three-day period of intense rain.

	Percent of tests	
Test condition	Carrie Bow Cay, 21 Jul 2006 (n = 62)	Boston Bay, Twin Cays, 23 Jul 2006 (n = 349)
Pale cytoplasm	0%	0%
Mottled cytoplasm	3.2%	0.29%
White cytoplasm	4.8%	0%
Total bleached	8.1%	0.29%

TABLE 6. Relative abundance of bleached individuals of *Sorites dominicensis* from two localities in Belize during July and August 2006 (n = total number of tests examined). All specimens were collected after a three-day period of intense rain.

	Percent of tests	
Test condition	Carrie Bow Cay, 1 Aug 2006 (n = 132)	Boston Bay, Twin Cays, 27 Jul 2006 (n = 369)
Pale cytoplasm	1.5%	0.27%
Mottled cytoplasm	0.76%	2.4%
White cytoplasm	8.3%	16% [a]
Total bleached	11%	19%

[a] Of 60 individuals, 34 were juveniles from the same brood.

and white cytoplasm) (Table 6). Although bleaching on the reef flat at Carrie Bow Cay was slightly higher than the prestorm levels (11% vs. 8.1%), the total poststorm incidence of bleaching in Boston Bay was observed to be more than 65 times higher than that observed just a few days earlier (Table 6). Although the waters in Boston Bay are normally of open ocean marine salinities, during heavy rains and slack tides cold, brackish water drains off Hidden Lake in the Twin Cays and empties into Boston Bay through Hidden Creek (Rützler et al., 2004). Interestingly, juveniles were disproportionately impacted by the bleaching event: 34 of 60 of the tests with white cytoplasm appeared to be individuals from the same brood (Table 6).

IMPACT OF HURRICANES AND RECOVERY

Seasonal bleaching events cause increased mortality in *S. dominicensis*, resulting in compromised populations that are more sensitive to periodic disturbance by hurricanes. Monthly surveys in 2001, 2003, and 2004 indicate that *S. dominicensis* populations normally plummet in the late summer, stay low throughout the winter, and eventually recover and bloom the following spring in late April and May (Richardson, unpublished data). In September 2004, the Jupiter Sound site was traversed by two hurricanes, Jeanne and Frances (Beven, 2005; Lawrence and Cobb, 2005). The Jupiter site was situated in the south eyewall for both storms, and experienced high winds and storm surges and extensive freshwater inundation. Dark, cloudy, turbid water continued to characterize the site for several months following the hurricanes. Other impacts included loss of shading because of downed trees and overgrowth of the seagrass by cyanobacterial blooms. The entire epiphytic foraminiferal community at the Jupiter Sound site was impacted by the 2004 hurricane season (Richardson, unpublished data). Initially, a dramatic reduction in species diversity and abundance was observed, with two species comprising 92% of the community in April and May 2005. By August 2005 the community had rebounded to 2001 levels of species diversity and density, with the exception of the apparent local eradication of *S. dominicensis* (Richardson, unpublished data). *Sorites dominicensis* is the only species at this site to possess photosynthetic endosymbionts and thus is sensitive to the reduced transmission of light in the water column that resulted from the months of increased turbidity following the 2004 hurricanes.

In October 2005, Jupiter Sound was impacted by Hurricane Wilma (Pasch et al., 2006), although this time the region experienced the high winds of the north eye-

TABLE 7. Relative abundance of bleached individuals of *Sorites dominicensis* from Jupiter Sound, Florida, 4 April 2008, as determined from examination of 446 tests.

Test condition	Percent of tests (n = 446)
Pale cytoplasm	5.9%
Mottled cytoplasm	7.8%
White cytoplasm	9.8%
Total bleached	24%

wall of the storm. Individuals of *S. dominicensis* were not recovered from the Jupiter Sound site until the summer of 2007 and did not reach their pre-hurricane densities until April 2008 (Richardson, unpublished). A survey of 446 individuals of *S. dominicensis*, collected in April 2008, yielded a high incidence of bleached individuals (24% total), an unusual event for the spring (Table 7). The trigger for this event is unknown; the rainfall during this period was below average as the region was experiencing an extended seasonal drought. It is also not known whether the population recovered through the reproduction of relict populations of *S. dominicensis* that survived the hurricanes of 2004 and 2005 or whether the site was repopulated through immigrants transported by the Gulf Stream from the Florida Keys and/or the Caribbean.

DISCUSSION

The results from the field studies described above document the occurrence of bleaching in *Sorites dominicensis*, a dinoflagellate-bearing foraminiferan, and delineate some of the environmental stressors that trigger bleaching. As has been observed in corals, bleaching in epiphytic specimens of *S. dominicensis* may be triggered by multiple environmental factors, such as increased irradiance during subaerial exposure at low tide, increased water temperatures, influx of freshwater runoff during storm events, and catastrophic disturbance during hurricanes. The symptoms of bleaching in *S. dominicensis* include decrease in intensity of coloration (pale appearance), the patchy loss of cytoplasmic coloration (mottled appearance), and the total loss of cytoplasmic coloration (white tests). Symbiont bleaching in *S. dominicensis* can be distinguished from the loss of cytoplasmic coloration that occurs during the process of reproduction through multiple fission as the symbiont-rich cytoplasm moves from the central region of the test to the periphery where the brood chambers and embryos will form. Studies are currently underway to link qualitative observations of bleaching in *S. dominicensis* to quantitative studies of symbiont density in bleached specimens using staining techniques that differentiate necrotic or apoptotic algal cells.

The relatively high water temperatures recorded on the reef flat at Carrie Bow Cay in July 2005 are not unusual for tropical seagrasses, which may experience annual fluctuations in seawater temperatures ranging from 19.8° to 41°C (Campbell et al., 2006). Unusually high water daily temperatures (40°–43°C) have been recorded in seagrass beds growing in shallow water off Papua New Guinea (Fred Short, University of New Hampshire, personal communication, January 2006). In addition to high temperatures, tropical seagrasses growing in shallow-water pools in the intertidal zone are subject to desiccation, extremely high levels of photosynthetically active radiation, and high levels of ultraviolet radiation (Campbell et al., 2006; Durako and Kunzelman, 2002).

Although the underlying mechanisms of bleaching in *S. dominicensis* are unknown, it is hypothesized that several of the proposed mechanisms for bleaching in corals may function in foraminiferans as well, such as reduced efficiency of photosystem II resulting from increased irradiance (Venn et al., 2008; Smith et al., 2005), and the production of damaging reactive oxygen species via several different pathways (Lesser, 2006; Smith et al., 2005).

Soritid foraminiferans have the potential to serve as a model system for bleaching, the need of which was recently emphasized by Weis et al. (2008). Not only do *S. dominicensis* and other soritids possess *Symbiodinium* endosymbionts that are closely related to the zooxanthellae in corals and other metazoans, but the small size of *S. dominicensis* facilitates investigation of symbiont bleaching *in hospite*, using methods such as in situ hybridization, immunofluorescence, and other imaging techniques. Future research will focus on developing culture methods for *S. dominicensis* and on exploring cytological methods that will facilitate the visualization of the cell processes underlying the bleaching response in foraminiferans.

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New Perspectives on Ecological Mechanisms Affecting Coral Recruitment on Reefs

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ABSTRACT. Coral mortality has increased in recent decades, making coral recruitment more important than ever in sustaining coral reef ecosystems and contributing to their resilience. This review summarizes existing information on ecological factors affecting scleractinian coral recruitment. Successful recruitment requires the survival of coral offspring through sequential life history stages. Larval availability, successful settlement, and post-settlement survival and growth are all necessary for the addition of new coral individuals to a reef and ultimately maintenance or recovery of coral reef ecosystems. As environmental conditions continue to become more hostile to corals on a global scale, further research on fertilization ecology, connectivity, larval condition, positive and negative cues influencing substrate selection, and post-settlement ecology will be critical to our ability to manage these diverse ecosystems for recovery. A better understanding of the ecological factors influencing coral recruitment is fundamental to coral reef ecology and management.

INTRODUCTION

Coral reefs are facing unprecedented human impacts and continuing acute and chronic threats that can impact community structure (Nyström et al., 2000). Their ability to resist such changes or to recover from them defines their "resilience" (sensu Holling, 1973). Unfortunately, coral reef ecosystems can be resilient in either the more desirable coral-dominated phase or in the less desirable algal-dominated phase (Hughes et al., 2005). Although we know much about what causes undesirable "phase shifts" (Done, 1992; Hughes, 1994; Pandolfi et al., 2005), we know relatively little about what drives coral community recovery (Connell, 1997).

Scleractinian corals are uniquely important to coral reef ecosystems as ecosystem engineers that structure the habitat (Jones et al., 1994, 1997). The abundance of live coral drives key ecological processes in the wider coral reef community, such as providing recruitment habitat for reef fish, lobsters, and sea urchins (Lee, 2006; Mumby and Steneck, 2008). In the past 30 years, the percent cover of live coral has decreased on a global scale (Gardner et al., 2003; Bruno and Selig, 2007), raising the question: How can we increase the number of corals in these ecosystems for recovery?

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Larval settlement (when they first attach to the benthos) and subsequent survival (recruitment) are processes that can control marine population dynamics (Gaines and Roughgarden, 1985; Doherty and Fowler, 1994; Palma et al., 1999). Although corals can reproduce clonally (Fautin, 2003; Baums et al., 2006), recruitment resulting from sexual reproduction is the primary means of recolonization for most species (Connell et al., 1997) and adds genetic variation to coral populations, which may increase survival of a species. Coral settlement followed by subsequent recruit survival and growth maintains coral populations and is necessary for coral reef recovery. For this cycle to occur on any given reef, larval survival and recruitment are dependent on a sequence of three phases: (1) larval availability, which integrates gamete production, fertilization success, and connectivity; (2) settlement ecology, which relates to larval condition and substrate selection behavior; and (3) post-settlement ecology, including substrate-specific survival and growth (Figure 1).

This review summarizes existing information on ecological factors affecting scleractinian corals during these first three phases of their life, covering the period from gamete release to juvenile coral colonies (typically described as <40 mm). We discuss factors that are critical for coral recruitment success, and where insufficient data exist, we draw parallels to concepts that have been developed for other marine larvae or adult corals and briefly discuss their relevance for the early life history stages of corals.

LARVAL AVAILABILITY

Larval supply to a reef depends on sequential processes of gamete production, fertilization success, and larval transport (i.e., larval dispersal and connectivity). Basic life history traits of corals can greatly influence the range of strategies that are used to ensure larval availability. Scleractinians have two main reproductive modes: brooding, where sperm are released into the water column and taken in by conspecifics for internal fertilization, and broadcast spawning, wherein both egg and sperm are released into the environment so that fertilization occurs externally, that is, in the water column (Figure 2; Fadlallah, 1983; Szmant, 1986; Richmond and Hunter, 1990; Richmond, 1997).

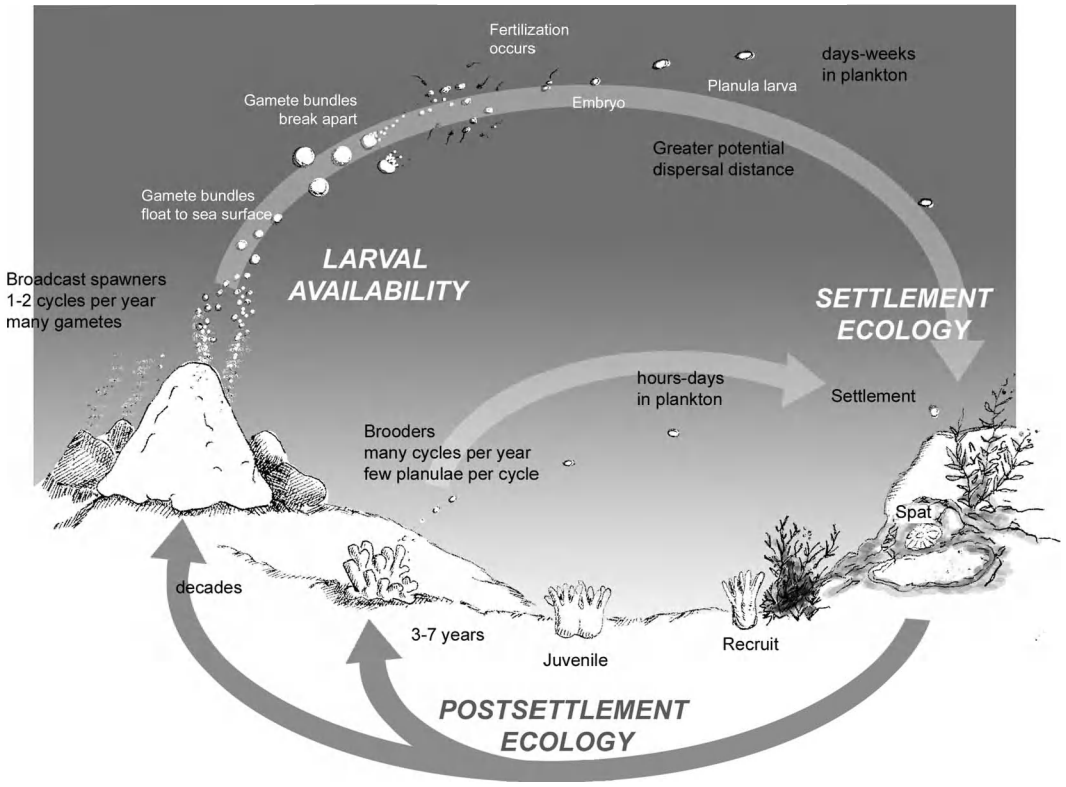

FIGURE 1. Three sequential phases necessary for successful coral recruitment starting with larval availability, progressing to settlement ecology, and ending with post-settlement ecology. (Drawn by Mark Vermeij.)

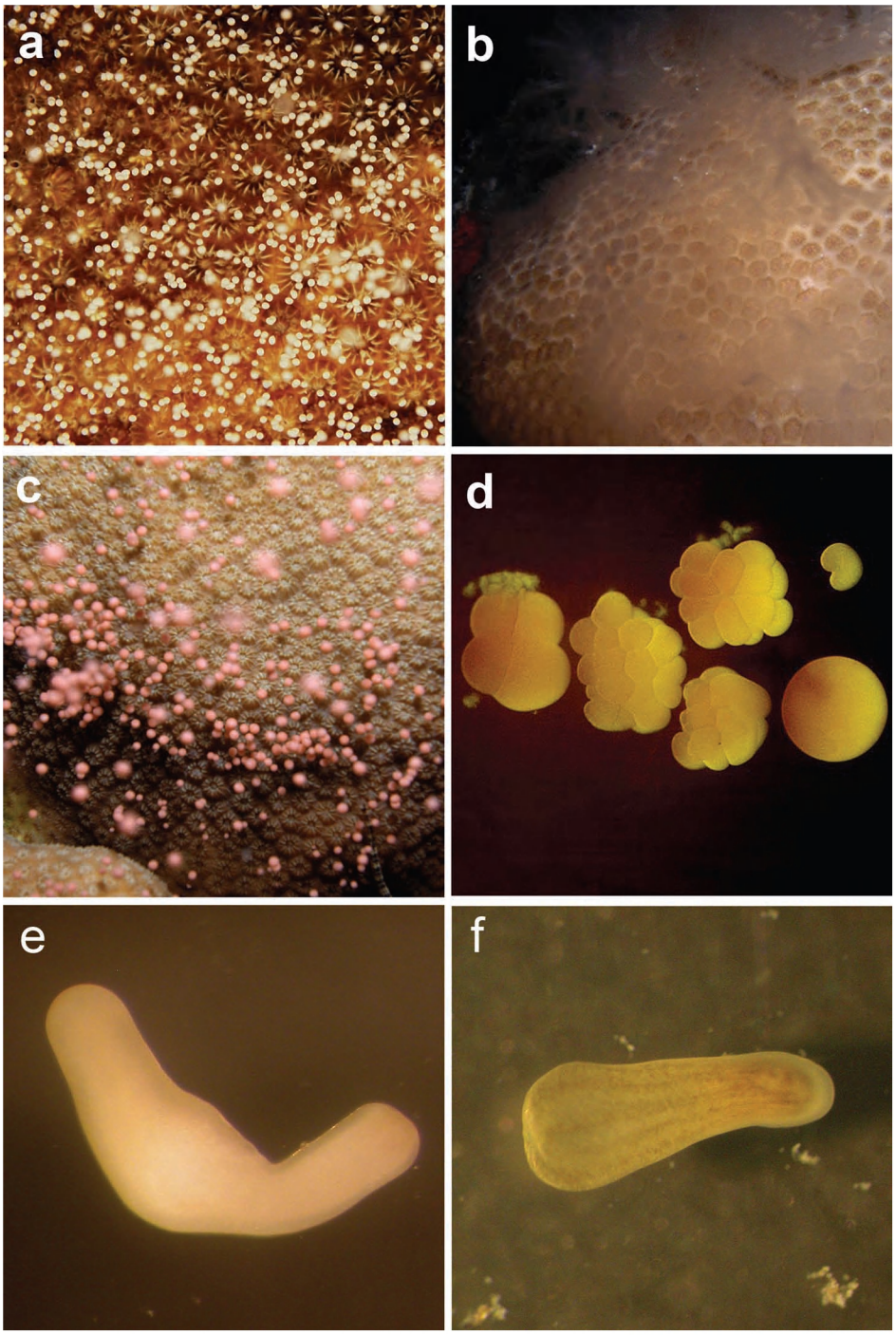

FIGURE 2. Different modes of reproduction influence larval supply in coral species. a, Female *Stephanocoenia intersepta*, a gonochoric spawner, releases eggs. b, A male *S. intersepta* releases sperm. c, The hermaphrodite *Montastraea faveolata* releases eggs and sperm as bundles that float to the surface, where they break apart for fertilization. d, For *Acropora palmata* (and other spawners), fertilization of coral eggs occurs in the water column. e, A larva of *Acropora palmata* completes development in the water column. f, In contrast, a larva of *Porites astreoides* (a brooder) is fully developed when it is released from its parent and contains zooxanthellae. (Photographs a, b, by Mark Vermeij; c, e, f, by Raphael Ritson-Williams; d, by Nicole Fogarty.)

A minority of reef-building coral species worldwide are brooders, but brooding is the dominant reproductive mode found in the Caribbean Sea (Szmant, 1986; Richmond and Hunter, 1990; Smith, 1992). Broadcast spawning is a more common reproductive mode in coral species, and in Australia more than 100 coral species may spawn on a single night (Harrison et al., 1984; Willis et al., 1985; Babcock and Heyward, 1986). Species representing these modes differ in colony size, gametic cycles, larval competency, dispersal distance, and zooxanthellae transmission (Richmond and Hunter, 1990). Brooders are typically smaller than spawning corals and have multiple planulating cycles per year, as opposed to one or two cycles in broadcast spawners (Szmant, 1986).

Fecundity

Reproductive mode determines the frequency of larval release; however, both abiotic and biotic factors can influence the amount of gametes produced in corals. The production of gametes is only possible when a coral has reached an age, and perhaps more importantly a size, capable of reproduction (Hughes, 1984; Szmant, 1986). It is difficult to measure the impact of stressors on gamete production because it is naturally variable both temporally and between individuals within a species (Chorneskey and Peters, 1987). As coral cover declines in both the Caribbean Sea and the Pacific Ocean (Gardner et al., 2003; Bruno and Selig, 2007) there are fewer and often smaller adult colonies. This change could reduce coral fecundity because small body size reduces gamete production (Szmant, 1986) and low population densities reduce fertilization success (see Fertilization section, below). Even with relatively high adult coral densities the fecundity of individual colonies can be decreased by many stressors before and during gametogenesis.

Coral bleaching has been observed to stop gametogenesis (Szmant and Gassman, 1990), reduce the number of gametes produced (Fine et al., 2001), and decrease fertilization rates in *Acropora* corals (Omori et al., 2001). Nutrients added to the water column decreased the number of successfully developed embryos that were formed in the corals *Acropora longicyathus* and *A. aspera* (Koop et al., 2001). Changes in salinity and sedimentation can also reduce gamete production and fertilization success in corals (Richmond, 1993a, 1993b). Guzman et al. (1994) suggested that the increase in injury levels and slower growth in corals exposed to an oil spill further reduced gamete size, viability, and fecundity. The presence of macroalgae adjacent to coral colonies can decrease fecundity (including the number and size of eggs) in the corals *Montastraea annularis* and *Montipora digitata* (Hughes et al., 2007; Foster et al., 2008). Impacts on fecundity are perhaps best summarized by Rinkevich and Loya (1987), who suggested that because reproductive activity involves such high energy expenditure, any stress that diminishes energy reserves will have an effect on adult fecundity.

Fertilization Ecology

Because broadcast spawners only have one or two planulating cycles a year, it is imperative that fertilization be successful. In any broadcast species, fertilization success is highly variable and largely depends on the synchronization of gamete release, gamete compatibility (Palumbi, 1994; Levitan et al., 2004), gamete age (Oliver and Babcock, 1992; Levitan et al., 2004), and abundance of spawning adults (Levitan et al., 1992, 2004). However, the health of the spawning colony and environmental conditions during the spawning event also affect fertilization success (Richmond, 1997; Humphrey et al., 2008).

During multispecies spawning events, synchronized gamete release and species-specific gamete recognition are critical for fertilization success and reducing the probability of interspecific fertilization (hybridization), which may result in reduced offspring fitness (Mayr, 1963); however, Willis et al. (2006) suggest a role for hybridization in range expansion and adaptation to a changing environment. Species with overlapping spawning times typically display low interspecific fertilization success in laboratory crosses (Willis et al., 1997; Hatta et al., 1999; Levitan et al., 2004). Interspecific fertilization success is usually higher among morphologically similar species, suggesting they are more closely related or possibly the same species (Willis et al., 1997; Hatta et al., 1999; Wolstenholme, 2004), but interspecific fertilization can also occur between *Acropora* species that have very different branching morphologies (Hatta et al., 1999). Fertilization success during a mass spawning event could be the result of sperm attractant molecules produced by coral eggs (Coll et al., 1994; Babcock, 1995) but could also be regulated by gamete recognition proteins, such as those that ensure species-specific fertilization in spawning sea urchins (Zigler et al., 2005).

If coral colonies spawn asynchronously or encountered gametes are not compatible, eggs may go unfertilized for extended periods of time or sperm may lose its viability. The effect of age on gamete viability and fertilization success differs among coral species; *Platygyra sinensis* showed reduced fertilization after three hours (Oliver and

Babcock, 1992), but in *Acropora* spp. reduced fertilization success occurred after seven to eight hours (Willis et al., 1997; Omori et al., 2001). With increasing gamete age, fertilization success is reduced in conspecific crosses, but aging effects on gamete viability differ between sperm and eggs. *Montastraea* spp. sperm lose viability after two hours but eggs stay viable for more than three hours (Levitan et al., 2004). Another consequence of gamete aging is an increase in the likelihood of interspecific fertilization. Hybridization rates between *Montastraea faveolata* eggs and *M. annularis* and *M. franksi* sperm increased when eggs had aged at least 75 minutes (Levitan et al., 2004). Increased interspecific fertilization may be caused by a breakdown in gamete recognition proteins, but the specific mechanisms remain to be determined.

The density of spawning individuals plays a critical role in fertilization success. If reproductive individual densities are too low, fertilization success will be limited (also referred to as the allee effect) (Levitan and McGovern, 2005). Coma and Lasker (1997) found that fertilization success in gorgonians was influenced by the density of gametes, which was determined by nearest neighbor distances (approximately 10 m), synchronous gamete release, or hydrodynamic processes. These factors probably influence scleractinian fertilization success; however, it is difficult to directly measure species-specific sperm concentrations in situ because a number of coral species spawn synchronously. Field studies examining sperm concentrations have used either of two methods: (1) measuring the percent of fertilized eggs collected at different times and locations on the reef or (2) determining the fertilization potential of collected surface water samples by adding them to unfertilized eggs and recording the proportion of eggs fertilized (Oliver and Babcock, 1992; Levitan et al., 2004). When lower production or dilution resulted in locally lower than normal sperm concentrations, fertilization success was reduced (Oliver and Babcock, 1992; Willis et al., 1997; Omori et al., 2001; Levitan et al., 2004). These studies showed peak fertilization potential during or shortly after coral species spawn (Oliver and Babcock, 1992; Levitan et al., 2004). Hence, synchronized gamete release is a mechanism for the high gamete density needed to ensure fertilization success.

High gamete concentration brings with it a potential risk as well; as sperm densities increase so does the probability of polyspermy, whereby eggs become fertilized by more than one sperm cell, which results in lowered fertilization rates and developmental failure (Styan, 1998; Tomaiuolo et al., 2007). Reduced fertilization success at high sperm concentrations has been described for several coral species (Oliver and Babcock, 1992; Willis et al., 1997; Levitan et al., 2004), suggesting polyspermic fertilization can occur in scleractinian corals. These findings suggest a trade-off between spawning synchronously (i.e., high gamete density) with other conspecifics to increase fertilization and the potential risk of polyspermy. Polyspermy may therefore act as a negative density-dependent mechanism. Despite the evidence for polyspermy in coral laboratory crosses, field fertilization rates never reached 100% during mass spawning events (97% maximum; Levitan et al., 2004), suggesting that polyspermic conditions are unlikely to occur in nature. In light of recent decreases in adult coral populations, reduced adult density and gamete aging are perhaps the greatest threats to larval production.

Larval Transport: Dispersal and Connectivity

After gamete fertilization, developing planula larvae transport typically away from reproductive populations (called "dispersal") and to reefs where they recruit (called "connectivity") (Levin, 2006). The density of planulae arriving to a reef determines recruitment strength. Larval survival during dispersal varies by means of a combination of hydrodynamic processes, larval energetics, predation pressure (Fabricius and Metzner, 2004), and water quality (Richmond et al., 2007).

Reproductive modes can provide insight into dispersal potential, even though the planktonic duration of coral species can be highly variable and remains undocumented for the majority of scleractinian species. For example, brooders generally settle within hours after release (Carlon and Olson, 1993), whereas broadcast spawners such as *Acropora* spp., *Goniastrea* spp., *Platygyra* spp., and *Montastraea* spp. have planktonic period of 4 to 7 days before they are competent to settle and metamorphose (Babcock and Heyward, 1986; Szmant, 1986). Larvae of the broadcast spawners *Acropora muricata* and *A. valida* settled within 9 to 10 days (Nozawa and Harrison, 2008), but larvae of the spawning corals *Platygyra daedalea* and *Goniastrea favulus* can settle between 2 and 3 days after fertilization, which is sooner than some brooding corals, suggesting that dispersal of these species might be of shorter duration than has been assumed from survival estimates (Miller and Mundy, 2003). In the absence of settlement substrate, a small percentage of *Acropora latistella*, *Favia pallida*, *Pectinia paeonia*, *Goniastrea aspera*, and *Montastraea magnistellata* larvae survived for 195 to 244 days in the water column (Graham et al., 2008). Planulae larvae can probably survive drifting in the plankton for long durations until they encounter suitable settlement substrate;

however, the length of the planktonic period will partially depend on whether the larvae have acquired zooxanthellae, which give them additional energy reserves, from the parent colony (Richmond, 1987).

The frequency of recruitment as a function of distance from a reproductive source population is called a "dispersal kernel" (Steneck, 2006). For most planktonic larvae it was assumed that relatively long larval survival potential in combination with oceanographic transport would generally prevent settlement close to a reproductive source (Cowen et al., 2006). Recent reviews suggest that even though many marine invertebrate larvae have the potential (energy reserves) for long-distance dispersal, they often settle locally because of a combination of oceanographic conditions, larval behavior, and increasing mortality associated with planktonic conditions (Cowen et al., 2000; Strathmann et al., 2002; Levin, 2006). The shape of most dispersal kernels is now thought to be skewed toward the reproductive source, that is, increased rates of local recruitment (Figure 3; Steneck, 2006). Most dispersal and connectivity research to date has focused on fishes; however, one study measured ecological connectivity of coral larvae via a field experiment conducted around the isolated Helix Reef in Australia (Sammarco and Andrews, 1988). They reported that 70% of coral recruitment occurred within 300 m of the larval source and that rates of recruitment declined with distance downstream from the reef (Sammarco and Andrews, 1988). Further, as expected, broadcasters dispersed farther than did species of brooding corals, but the estimated ecologically relevant dispersal kernel for both species was remarkably local. A recent review discusses the limited dispersal kernel of coral planulae (Steneck, 2006); however, there is little experimental evidence for the mechanisms that determine coral ecological connectivity.

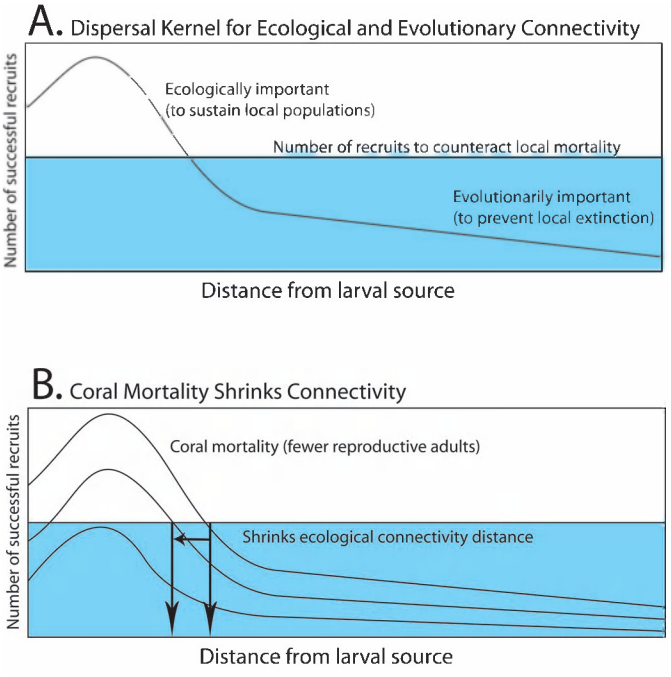

FIGURE 3. Dispersal kernels determine potential connectivity distance between reproductive populations and offspring. A, Distinction between ecologically important recruitment necessary to balance against local mortality and evolutionarily important recruitment to balance against local extinction. B, Shrinking dispersal kernels resulting from adult coral mortality. (After Steneck, 2006.)

Recruitment rates must equal or exceed rates of adult mortality to sustain a local population. Most dispersal kernels show high rates of recruitment near the reproductive source, with recruitment decreasing as distance increases (Figure 3). Although that tail is important for gene flow, that low density of settlement is not sufficient to sustain populations. That is, the ecologically relevant portion of a dispersal kernel reflects the sustained rate of recruitment necessary to compensate for rates of mortality. The critical level of settlement to sustain populations (i.e., horizontal line above each shaded half of Figure 3) is not known; however, colonization rates of the introduced orange cup coral *Tubastraea coccina* can provide some real-world insights into the scale of ecological and evolutionary connectivity. This brooding species was first introduced to the Netherlands Antilles in 1943 and then spread from island to island through the Caribbean, taking 50 years to reach the Bahamas and 60 years to reach Florida (Fenner and Banks, 2004). Once in a region, local populations grew rapidly. This finding is consistent with the concept that the biogeographic spread results from the evolutionarily important "long tail" of the dispersal kernel, whereas the ecologically and demographically significant portion of the dispersal kernel controlling local colonization is much smaller and more local (Figure 3A). Observations of the spread of *T. coccina* are conservative because some of the spread of this species probably resulted from colonized ships moving among the regions (Fenner and Banks, 2004).

Ecological connectivity necessary to sustain populations against chronic mortality is much more difficult to measure than is evolutionary connectivity. Evolutionary or genetic connectivity can be directly measured using a variety of molecular genetic techniques (reviewed in Hellberg, 2007). In Japan, gene flow between islands 30 to 150 km apart was determined to be consistently higher for the spawner *Acropora tenuis* than for the brooding

species *Stylophora pistillata*, but both coral species had unique genotypes across islands separated by 500 km (Nishikawa et al., 2003). In the Caribbean, a genetic break was detected for *Acropora palmata*, roughly dividing populations from the Greater Antilles and western Caribbean from populations in the Lesser Antilles and the southern and eastern Caribbean (Baums et al., 2005). On the relatively contiguous Great Barrier Reef (GBR), high rates of genetic connectivity were observed for both brooders and spawners. For example, gene flow was detected in all the spawners and three of the five brooders despite being separated by 500 to 1,200 km (Ayre and Hughes, 2000). However, the same species of corals were genetically distinct on Lord Howe Island, which is separated from the GBR by 700 km (Ayre and Hughes, 2004). This observation suggests that coral larvae can use islands within the evolutionarily important tail of the dispersal kernel as "stepping stones" to maintain genetic connectivity between distant reefs separated by long distances (Steneck, 2006).

Although dispersal kernels are useful for visualizing how larval availability declines with distance from a source, their ecological effect can be variable. For example, without changing the shape of the kernel but reducing the number of recruits as a consequence of reduced reproductive output following an adult mortality event (Figure 3B), the range of both the ecological and evolutionary parts of the kernel can shrink. If this happens, connectivity among distant reefs could sever, making recovery following an acute disturbance difficult or impossible.

SETTLEMENT ECOLOGY

As local and global threats continue to decrease coral cover, it is likely that fewer coral larvae will be supplied to reefs that may or may not have appropriate settlement habitat. For corals, the transitional stage from planktonic planula larvae to sessile benthic juveniles involves a two-step process of settlement and metamorphosis. Settlement is the behavioral response of a larva when it stops dispersal and selects substrate for recruitment. Metamorphosis includes the subsequent morphological and physiological changes that pelagic larvae undergo to become benthic juveniles. Settlement of coral larvae can be influenced by habitat qualities that facilitate or inhibit settlement and metamorphosis of larvae supplied to a reef (Figure 4). Larval settlement behavior can be determined by the conditions the larvae experienced in the plankton or by the presence of positive or negative cues on the benthos or in the water overlying the reef.

Larval Condition upon Arrival

As coral larvae disperse in the plankton they are exposed to water quality conditions that may affect larval health, behavior, survival, and settlement success (Vermeij et al., 2006). Experiences during early life stages (i.e., depleted energy reserves, nutritional stress, environmental stressors, and pollutant exposure) have latent effects on later life stages in numerous marine larvae across different phyla (reviewed in Pechenik, 2006). Even short-term exposure to stressors or a slight delay in metamorphosis can reduce fitness in juveniles and adults (i.e., decrease growth rate, lower competitive ability, reduce survival, and decrease fecundity) (Pechenik, 2006). Although the mechanisms through which latent effects are mediated are not known, it is suspected that transcriptional or translational processes or direct DNA or key enzyme damage are responsible (Pechenik et al., 1998; Heintz et al., 2000; Pechenik, 2006). As very few studies have tested latent effects in coral larvae, we describe some of the patterns found in other marine organisms to highlight how pre-settlement stress might impact post-settlement coral growth and survival.

Marine invertebrate larvae often rely on external cues to trigger metamorphosis. Without these cues, the larval period can be prolonged (reviewed in Pechenik, 1990), and post-settlement fitness may be reduced (Pechenik, 2006). For some invertebrates, including abalones, tunicates, and bryozoans, delayed metamorphosis slowed post-metamorphic development (Wendt, 1998; Roberts and Lapworth, 2001; Marshall et al., 2003). Depleted energy resources during the larval stage may also be an important contributor to post-settlement growth and survival. Bennett and Marshall (2005) found that depleted energy reserves caused by increased activity in larvae of the ascidian *Diplosoma listerianum* were more costly energetically than extending the larval period or completing metamorphosis. Food limitation during the larval period can reduce size, total organic content, energy reserves of metamorphosed animals, juvenile growth rates, and survival (Miller, 1993; Pechenik, 2002; Thiyagarajan et al., 2003; Chiu et al., 2007, 2008).

Water quality conditions can directly reduce coral larval survival and settlement but also may cause latent effects for new recruits. Salinity reductions during pre-settlement periods can reduce post-metamorphic growth rates and survival for various marine invertebrates (Pechenik et al., 2001; Thiyagarajan et al., 2008). Vermeij et al. (2006) tested salinity stress on *Montastraea faveolata* larvae and how that influenced subsequent post-settlement

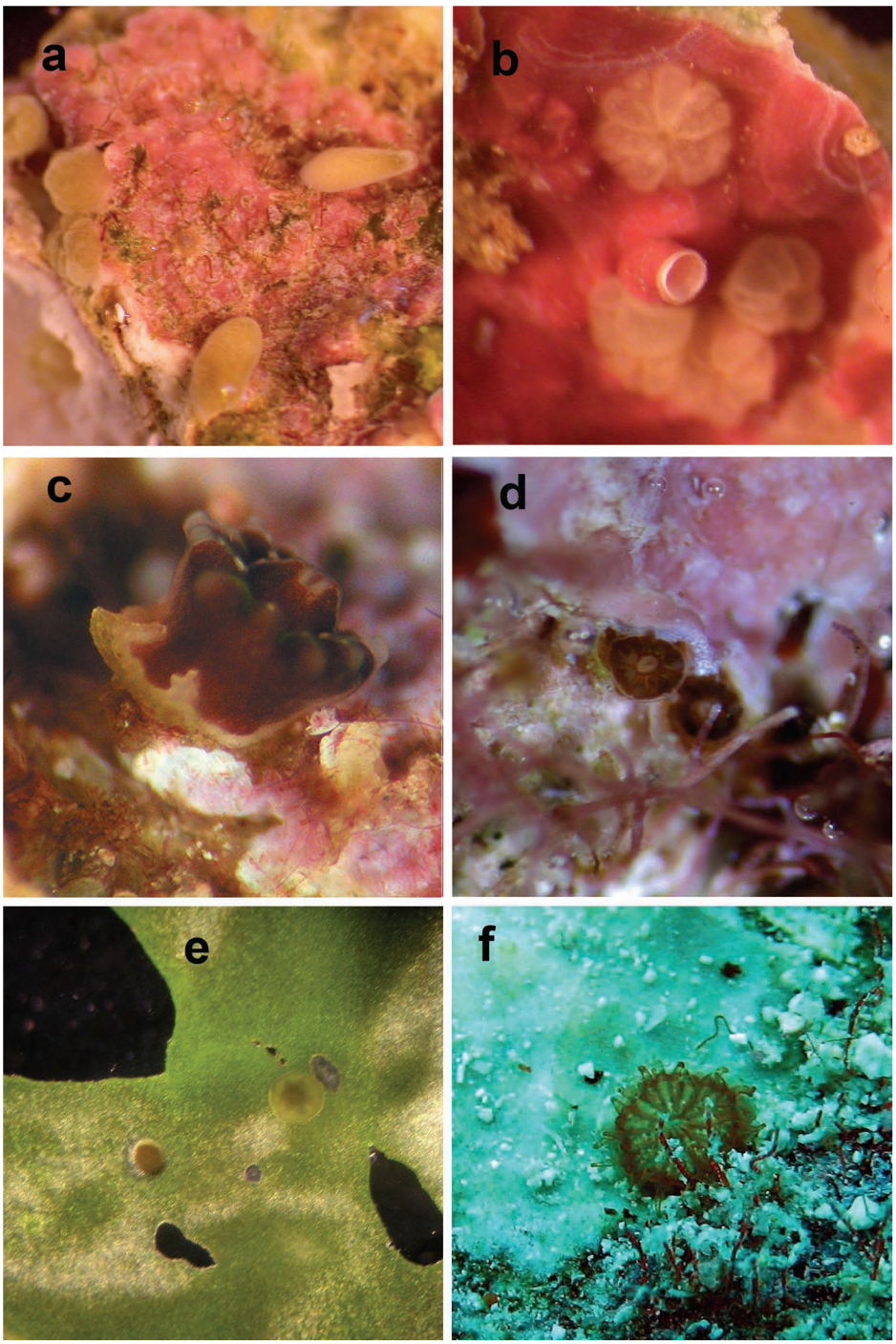

FIGURE 4. Coral larval substrate selection is critical to post-settlement survival. a, *Favia fragum* larvae explore the benthos for a suitable settlement site; some larvae have already attached and are beginning to metamorphose. b, *Acropora cervicornis* settlers are attached to *Titanoderma prototypum* and have metamorphosed. c, A new recruit of *Montastraea faveolata* has settled on coralline algae, which has started to slough its outer layer of tissue, knocking the coral recruit off the substrate. d, *Montastraea faveolata* recruits are being overgrown by a coralline alga. e, *Montipora capitata* larvae have settled on *Ulva* sp., an ephemeral substrate. f, A *Siderastrea radians* recruit has settled in a high-sedimentation environment. (Photographs a, b, by Raphael Ritson-Williams; c, d, by Nicole Fogarty; e, f, by Mark Vermeij.)

performance. Lower than normal seawater salinity caused increased pre- and post-settlement mortality and increased the mobility of coral planulae. It was suggested that the increased activity of the larvae in the lower salinities was an attempt to escape the unfavorable conditions. With increased activity, energy reserves were depleted, which was suggested to be the cause of pre-settlement mortality, smaller post-settlement size, and lower post-settlement survival. Planulae in the lower-salinity treatments settled on a greater range of substrate types. This study emphasized the importance of planktonic conditions on the performance of settling coral larvae, which could then influence post-settlement ecology.

Larval Behavior in Overlying Water

Coral larvae possess a wide array of behaviors that allow them to enhance the likelihood of successful settlement, including, but not limited to, sensitivity to light (Lewis, 1974; Mundy and Babcock, 1998), depth (Carlon, 2001, 2002; Baird et al., 2003; Suzuki et al., 2008), and chemical cues (Morse et al., 1994). One field study showed that multiple behavioral choices determined the larval settlement site of the Caribbean brooder *Agaricia humilis* (Raimondi and Morse, 2000). The larvae swam down when restricted to 3 and 8 m but swam toward the surface when restricted to 25 m. In further field experiments, larvae settled in response to the coralline alga *Hydrolithon boergesenii* but would only settle directly on the coralline alga when it was on the underside of a settlement tile. This study showed that coral larvae are capable of complex behaviors, which are determined to some extent by their ability to detect and discriminate between positive and negative settlement cues in their habitat.

Positive Settlement Cues

Many marine invertebrate larvae use chemical cues to determine the appropriate habitat for settlement (Pawlik, 1992; Hadfield and Paul, 2001). Chemical cues are implicated for both settlement and metamorphosis of corals and may be released by conspecifics and other organisms that indicate appropriate habitat for survival and growth. Research in the Caribbean showed that a membrane-bound carbohydrate complex from the coralline red alga *Hydrolithon boergesenii* induced settlement and metamorphosis in the brooded larvae of *Agaricia humilis* (Morse and Morse, 1991; Morse et al., 1994). It was suggested that many corals require an algal cue for the induction of settlement, indicating a common chemosensory mechanism for settlement and metamorphosis among coral larvae (Morse et al., 1996).

Both the larvae of *Acropora millepora*, a common Indo-Pacific coral species, and coral larvae collected from natural slicks after mass spawning events used coralline algae for settlement and metamorphosis (Heyward and Negri, 1999). Four species of crustose coralline algae, one non-coralline crustose alga, two branching coralline algae, and the skeleton of the massive coral *Goniastrea retiformis* induced metamorphosis. Chemical extracts from both the crustose red alga *Peyssonnelia* sp. and the coral skeleton were highly active, inducing up to 80% larval metamorphosis. Coral larvae can also distinguish among species of coralline algae. The Australian spawning coral *Acropora tenuis* had different rates of settlement in response to different species of coralline algae (Harrington et al., 2004). Settlement choice resulted in higher rates of post-settlement survival on the preferred coralline algae, illustrating the recruitment consequences of larval selectivity. Chemical cues appeared to be involved in this selective behavior, because methanol extracts of the coralline red algae *Titanoderma prototypum* and *Hydrolithon reinboldii* both induced metamorphosis of *A. tenuis*.

Comparative studies have revealed that settlement and metamorphosis in response to crustose coralline algae is not an obligate trait of all coral species. Two brooding Australian corals were compared for their settlement selectivity (Baird and Morse, 2004). *Acropora palifera* larvae only metamorphosed in the presence of coralline red algae, but *Stylophora pistillata* larvae showed some metamorphosis in unfiltered seawater and also metamorphosed onto glass coverslips. A study in Guam found that larvae of the spawning species *Goniastrea retiformis* preferred substrate covered with crustose coralline algae (CCA), but the reef-flat brooding coral *Stylaraea punctata* preferred biofilmed rubble (Golbuu and Richmond, 2007).

Coralline algae have been identified as a positive settlement cue for some corals, but it is unclear if the biofilms present on these algae or the algae themselves are responsible for the observed settlement behavior (Johnson et al., 1991; Webster et al., 2004). Biofilms were isolated from the coralline alga *Hydrolithon onkodes*, and one strain of bacteria alone was enough to induce settlement and metamorphosis of *Acropora millepora* larvae (Negri et al., 2001). When *H. onkodes* was sterilized in an autoclave and treated with antibiotics, it still induced significantly more settlement and metamorphosis than seawater or terracotta tiles. Additionally, coral larvae can distinguish between tiles conditioned at different depths, which could be related to depth-related differences in bacterial community composition of biofilms that formed on tiles (Webster et al., 2004). Whether the coralline algae or its biofilm is producing the inductive compound(s) may depend on the

coral and the coralline algae species tested. The specificity of bacterial communities to different coralline algal species has rarely been investigated (Johnson et al., 1991). With the recent development of more refined genetic techniques it is possible to compare different microbial communities, which might enable the identification of the microbe(s) that can induce coral larval settlement and metamorphosis.

Negative Settlement Cues

Water quality and substrate conditions impact fertilization rates and also may inhibit some coral larvae from normal settlement and metamorphosis. Low coral recruitment is commonly documented in the field, yet surprisingly few studies have experimentally tested which substrate characteristics might deter coral larval settlement. Coral larval survival and settlement can be reduced by many environmental stresses, such as elevated temperatures (Edmunds et al., 2001), variation in salinity (Vermeij et al., 2006), sedimentation (Hodgson, 1990; Gilmour, 1999), and UVB radiation (Kuffner, 2001; Gleason et al., 2006). Survival and settlement are reasonable ecological metrics for the effects of stress, but an important gap in our knowledge is how sublethal stress influences larval behavior and post-settlement health and success (Downs et al., 2005). New techniques including cellular biomarkers and differential gene expression using microarrays should provide important techniques to measure sublethal stress in coral larvae.

Water quality conditions that are known to impact adult corals also have dramatic effects on larval supply and settlement. Of the physical conditions that negatively influence larval settlement, elevated temperature has received the most attention and has the potential to increase in frequency and duration as ocean temperatures continue to warm. Larvae of the Caribbean brooding coral *Porites astreoides* were killed and had low densities of zooxanthellae when exposed to elevated temperatures for 24 hours (Edmunds et al., 2001, 2005). High temperatures (36°C) killed *Acropora muricata* larvae within 40 hours (Baird et al., 2006), and temperatures of 32°C killed *Diploria strigosa* larvae and reduced their settlement (Bassim and Sammarco, 2003). However, at elevated temperatures (29°C) larvae of *Stylophora pistillata* had the same settlement as at 25°C (Putman et al., 2008), and more larvae settled on the CCA in 25°C than in 23°C. Many of these studies used different experimental conditions, making it difficult to compare the effects of temperature on different species of coral larvae. Temperature is one stress that is relatively well studied, but more research is necessary to understand other physical stressors, such as ocean acidification (Albright et al., 2008), that will affect coral larvae in the future.

Larval interactions with the biological inhabitants of reef communities can also reduce larval settlement. Algal turfs, macroalgae, and benthic cyanobacteria can negatively impact the settlement of coral larvae (Kuffner and Paul, 2004; Birrell et al., 2005; Kuffner et al., 2006; Birrell et al., 2008a). In the Florida Keys, two brown algae, *Dictyota pulchella* and *Lobophora variegata*, reduced the total number of *Porites astreoides* settlers (Kuffner et al., 2006). In the Philippines, the algae *Sargassum polycystum* and *Laurencia papillosa* decreased larval settlement of *Pocilloproa damicornis*, but water conditioned with these algae increased settlement over the seawater controls (Maypa and Raymundo, 2004). In Australia, water conditioned with the foliose brown alga *Padina* sp. reduced larval settlement of *Acropora millepora*; however, water conditioned with the brown alga *Lobophora variegata* increased settlement (Birrell et al., 2008a). The cyanobacterium *Lyngbya majuscula* reduced the survivorship of *Acropora surculosa* larvae and settlement and metamorphosis of *Pocillopora damicornis* in studies conducted on Guam (Kuffner and Paul, 2004), and in Florida, the cyanobacterium *Lyngbya polychroa* caused *Porites astreoides* to avoid settling adjacent to it on settlement tiles (Kuffner et al., 2006). Some macroalgae and cyanobacteria can act as settlement inhibitors for coral larvae, but this was not true for all the algae tested. A surprising contrast was observed for *Favia fragum* larvae, which had high rates of settlement and metamorphosis onto live *Halimeda opuntia* when offered with coral rubble (Nugues and Szmant, 2006). Coral larvae of *Montipora capitata* were observed to settle onto *Ulva* sp. (Figure 4e; Vermeij et al., 2009). Why these larvae would settle directly onto blades of algae is unclear as this substrate is ephemeral, thus probably increasing post-settlement mortality. Little research has been done on the mechanisms that algae use to inhibit settlement, but algal qualities such as natural products, shading and abrasion, serving as vectors of bacteria, and releasing dissolved organic matter may contribute to the negative impacts of algae on larval settlement.

Competition from other members of coral reef communities also influences larval behavior. Tissue of the scleractinian coral *Goniopora tenuidens* suspended in seawater inhibited metamorphosis of *Pocillopora damicornis* larvae and reduced the growth of new recruits over seven days (Fearon and Cameron, 1996). The tissue from *Goniopora tenuidens* also caused increased mortality of larvae from *P. damicornis*, *Platygyra daedalea*, *Fungia fungites*, and *Oxypora lacera*. Increased research on the types of benthic

organisms and the mechanisms they use for competition with coral larvae is an important area for further study. An integrated approach to larval stress, physiology, and the physical and biological characteristics of settlement substrata will reveal the impact of benthic organisms on coral larval behavior, settlement, and post-settlement survival. Determining what benthic habitat characteristics are necessary for increased settlement will be a critical step for managing reef habitats for increased coral recruitment.

POST-SETTLEMENT ECOLOGY

Corals, and most benthic marine organisms, suffer high rates of mortality soon after settlement because they are small and vulnerable. Post-settlement processes from the time corals settle (i.e., attach to the benthos) to recruitment (i.e., survive to some later phase) determines much of coral demography (Vermeij and Sandin, 2008). This concept is consistent with the tenet of clonal population biology that states as clonal organisms grow the probability of their death declines but the probability of injury increases (Hughes and Jackson, 1985). Thus, the two rates of early post-settlement mortality and growth can strongly influence the local abundance of corals.

Post-Settlement Mortality

Coral recruits can die from a myriad of causes including chronic disturbances such as competition and predation and pulse disturbances such as bleaching and disease. However, the chronic disturbances probably drive most post-settlement mortality and thus are serious impediments to reef recovery. Caribbean reefs are a case in point, with incidences of recovery much lower than Indo-Pacific reefs as a result of setbacks from chronic disturbances (Connell et al., 1997).

Algae, encrusting invertebrates, and sediment have all been shown to have deleterious effects on newly settled corals (Figure 5; Rylaarsdam, 1983). Settling corals, with limited stores of energy to invest in competitive interactions, are particularly vulnerable when faced with a well-developed benthic community structure and limited space (Jackson and Buss, 1975; Sebens, 1982; Connell et al., 1997). However, the *mechanisms*, or causes, of reduced growth and mortality of newly settled larvae, recruits, and juveniles have, for the most part, only recently been investigated.

Encrusting invertebrates (particularly sponges) can be especially inhospitable for newly settled corals. In cryptic habitats, newly settled corals are likely to lose out by overgrowth of fast-growing heterotrophic groups such as sponges, bryozoans, and bivalves (Vermeij, 2005). Aerts and van Soest (1997) determined the impact of sponges on coral survival to be greatly species specific. Physical, chemical, and biological properties of benthic invertebrates may inhibit coral growth and survival. Some studies used chemical extracts of sponges (Sullivan et al., 1983; Pawlik et al., 2007) to show that allelopathy can negatively impact adult corals. Coral recruits are even more susceptible to stress, yet surprisingly few studies have examined secondary metabolites for their impact on the early life history stages of corals. A field study by Maida et al. (1995) suggested that allelopathy reduced recruitment of corals adjacent to the octocorals *Sinularia flexibilis* and *Sarcophyton glaucum*, and both the live octocorals and settlement plates with dichloromethane extracts of *S. flexibilis* inhibited coral settlement and survival. More long-term, small spatial scale (millimeters to centimeters) studies are needed to determine the effect of benthic invertebrates on post-settlement survival (Edmunds et al., 2004; Vermeij, 2006).

Areas of high algal biomass are known to be poor nursery habitats for settling corals (Birkeland, 1977; Bak and Engel, 1979; Harriott, 1983; Birrell et al., 2008b; Vermeij and Sandin, 2008; Vermeij et al., 2009). There are several mechanisms by which algae may be deleterious to corals. Algae may interfere with larval settlement by simply preempting available settlement space (Mumby et al., 2006; Box and Mumby, 2007). At least one species of turf algae alone (without sediment) has reduced settlement of corals in laboratory experiments (Birrell et al., 2005). More direct physical interactions including algal shading, abrasion, or basal encroachment can result in reduced coral growth or increased mortality (Lirman, 2001; McCook et al., 2001). Shading by the encrusting brown alga *Lobophora variegata* over six months caused a 50% increase in morality of juvenile *Agaricia agaricites* (less than 20 mm diameter), and the mere presence of *L. variegata* around the coral reduced colony growth by 60% (Box and Mumby, 2007). However, shading by *Dictyota pulchella* resulted in no direct mortality but caused a 99% decrease in coral growth. Other studies have determined that *Lobophora variegata* (in the absence of grazing) is a superior competitor to Caribbean corals, including *A. agaricites*, *A. lamarcki*, *Meandrina meandrites*, *Mycetophyllia aliciae*, and *Stephanocoenia intersepta*, and to at least one species of Pacific coral, *Porites cylindrica* (De Ruyter van Steveninck et al., 1988; Jompa and McCook, 2003). Thus, it is likely that community phase shifts to high algal biomass decrease recruitment by reducing larval settlement and post-settlement survival (Hughes and Tanner, 2000; Kuffner et al., 2006).

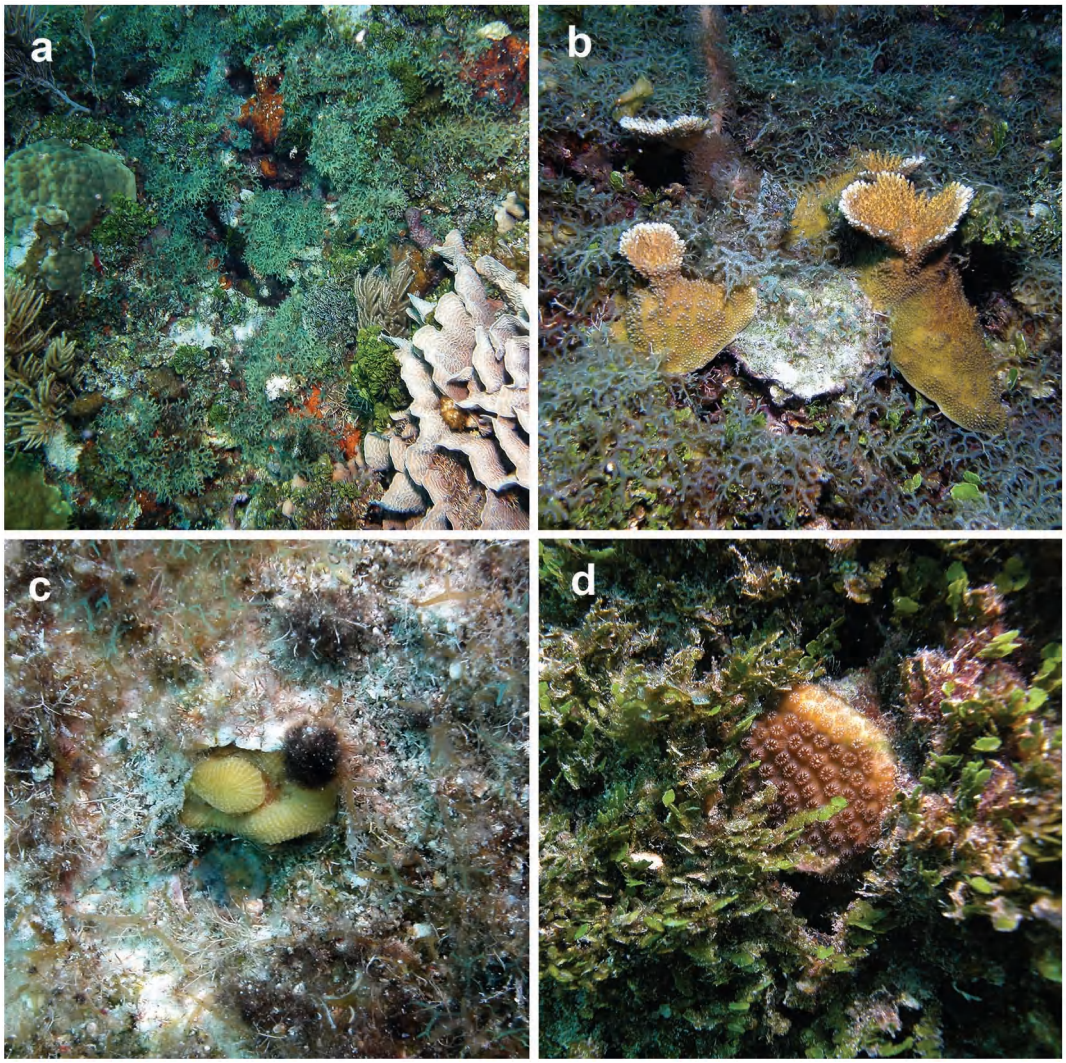

FIGURE 5. Macroalgae can be a dominant space occupier on degraded reefs and can inhibit coral recruitment at multiple life history stages. a, The macroalgae *Dictyota* spp. and *Halimeda opuntia* covered most of the benthos on this Belize reef, potentially inhibiting coral settlement. b, Recruits of *Acropora palmata* surrounded by *Dictyota* sp. c, A new recruit of *Diploria* sp. surrounded by *Gelidiella*, *Jania*, *Dictyota*, and the cyanobacterium *Dichothrix* sp. d, *Montastraea annularis* overgrown by *Halimeda* sp. (All photographs by Raphael Ritson-Williams.)

Reduced coral recruitment in algal-dominated reefs (Edmunds and Carpenter, 2001; Birrell et al., 2005) is thought to be in part the consequence of chemically induced mortality or the increased biomass of fleshy algae actually functioning as a reservoir for coral pathogens (Littler and Littler, 1997; Nugues et al., 2004). Bak and Borsboom (1984) proposed that the reduction in water flow adjacent to macroalgae could cause increased coral mortality through changes in the flow regime and increased allelochemical concentrations. Most recently, enhanced microbial activity caused by algal exudates has been proposed as a mechanism of competition (Smith et al., 2006; Vermeij et al., 2009). Kline et al. (2006) determined that elevated levels of dissolved organic carbon, which can occur in areas of high algal biomass, increased the growth rate of microbes living in the mucopolysaccharide layer of corals. These studies all suggest that the detrimental effect of algae on corals could be mediated by several properties of macrophytes.

On modern reefs, algal-related post-settlement mortality probably decreases the population density of coral

recruits. Vermeij (2006) compared his recruitment study in Curacao from 1998 to 2004 to that of Van Moorsel (1989) from 1979 to 1981, using the same method in the same location. Recruit densities on the topsides of settlement panels in the more recent study were 5.16 times lower and recruitment on the undersides was 1.14 times lower than the 1979–1981 study. Macroalgae had replaced CCA as the dominant topside space occupier, creating a less-suitable habitat for coral recruitment compared to the crustose algae that had dominated the same site roughly 20 years earlier. In places where *Diadema* urchin recovery and grazing have reduced algal abundance, the population density of juvenile corals has increased (Edmunds and Carpenter, 2001; Aronson et al., 2004; Macintyre et al., 2005).

While herbivory can improve the recruitment potential by keeping reefs relatively free of algae, it can also be a potential cause of mortality for newly settled corals. Grazing rates on exposed outer surfaces of shallow reefs are extremely high, exceeding thousands of bites per square meter per day (Carpenter, 1986; Steneck and Dethier, 1994; Steneck and Lang, 2003). Bites, especially from parrotfish that graze deeply into carbonate substrates, would easily kill a newly settled coral. Few studies have documented recruit mortality resulting from fish grazing (Mumby et al., 2006), although it has been suggested as the cause of the low number of recruits observed on the top surface of settlement plates (Adjeroud et al., 2007). The herbivorous sea urchin *Diadema antillarum* was shown to be a significant agent of mortality for newly settled corals (Sammarco and Carleton, 1981). The highest mortality of newly settled corals is likely to occur on outer exposed surfaces where algal growth rates and herbivore grazing rates are greatest and rates of sedimentation are highest. In shallow reef habitats where algal growth and herbivory rates are greatest, coral recruitment is greater in subcryptic microhabitats (Bak and Engel, 1979). However, which microhabitats increase post-settlement survival has rarely been tested (but see Babcock and Mundy, 1996).

Post-Settlement Growth Rates

Given the vulnerability of small size classes, the adaptive advantages of rapid growth rates are obvious. Coral recruit survival is not merely a function of the attributes of the settlement substrate but also of the coral's ability to resist overgrowth by neighboring encrusting invertebrates and algae (Richmond, 1997). As new corals grow, their mortality rates decline (Vermeij and Sandin, 2008), and they are less likely to be overgrown by competitors (Hughes and Jackson, 1985). Often, however, the slow growth rates of newly settled corals make this a losing battle, and early post-settlement mortality is generally high (Figure 6; Bak and Engel, 1979; Edmunds, 2000; Vermeij and Sandin, 2008). Even in a controlled environment, laboratory studies showed that a coral that remains less than 3 mm in diameter for two or three months has only a 20% chance of survival (Rylaarsdam, 1983). Field studies report a huge amount of variance in early post-settlement mortality. Babcock (1985) found post-settlement survivorship over the first three to six months ranged from 16% to 71%, whereas more recently Box and Mumby (2007) determined a monthly estimated mortality rate for *Agaricia agaricites* to be 3.5% per month. Annual juvenile coral survivorship estimates range from 0% to 77% (Smith, 1992; Wittenberg and Hunte, 1992; Maida et al., 1994; Smith, 1997; Edmunds, 2000).

Different species of corals have distinctly different rates of growth and ability to recover following a disturbance (Wakeford et al., 2008). Specifically, some of the Indo-Pacific acroporid corals (e.g., *Acropora tenuis*) are extremely "weedy" and are capable of growing nearly 6 cm in 1.5 years (Omori et al., 2008); this translates to an average growth rate of 3.2 mm/month compared to the much slower growth rates reported for *Oxypora* sp. as ranging between 0.2 and 0.5 mm/month (Babcock and Mundy, 1996).

Settlement habitat also influences growth rates of newly settled corals. Subcryptic habitats protect coral recruits from stresses and disturbances common on outer reef surfaces, but they will invariably have lower productivity potential. Diameters of *Platygyra* sp. and *Oxypora* sp. settlers increased one-quarter to one-half as fast in cryptic undersides than they did on upper exposed surfaces for the two species, respectively (Babcock and Mundy, 1996). Importantly, however, new recruits that selected subcryptic microhabitats had higher survivorship despite their slower growth rates (Babcock and Mundy, 1996).

VARIABILITY OF POST-SETTLEMENT SURVIVAL AND GROWTH: THE ROLES OF BIODIVERSITY AND LIFE HISTORY STRATEGIES

Before the disease-induced *Acropora* spp. decline in the Caribbean, fundamental differences existed between acroporid-dominated reefs of the Caribbean and Indo-Pacific regions. Caribbean reefs are largely built by two species of *Acropora*. Both species recruit rarely (Rylaarsdam, 1983;

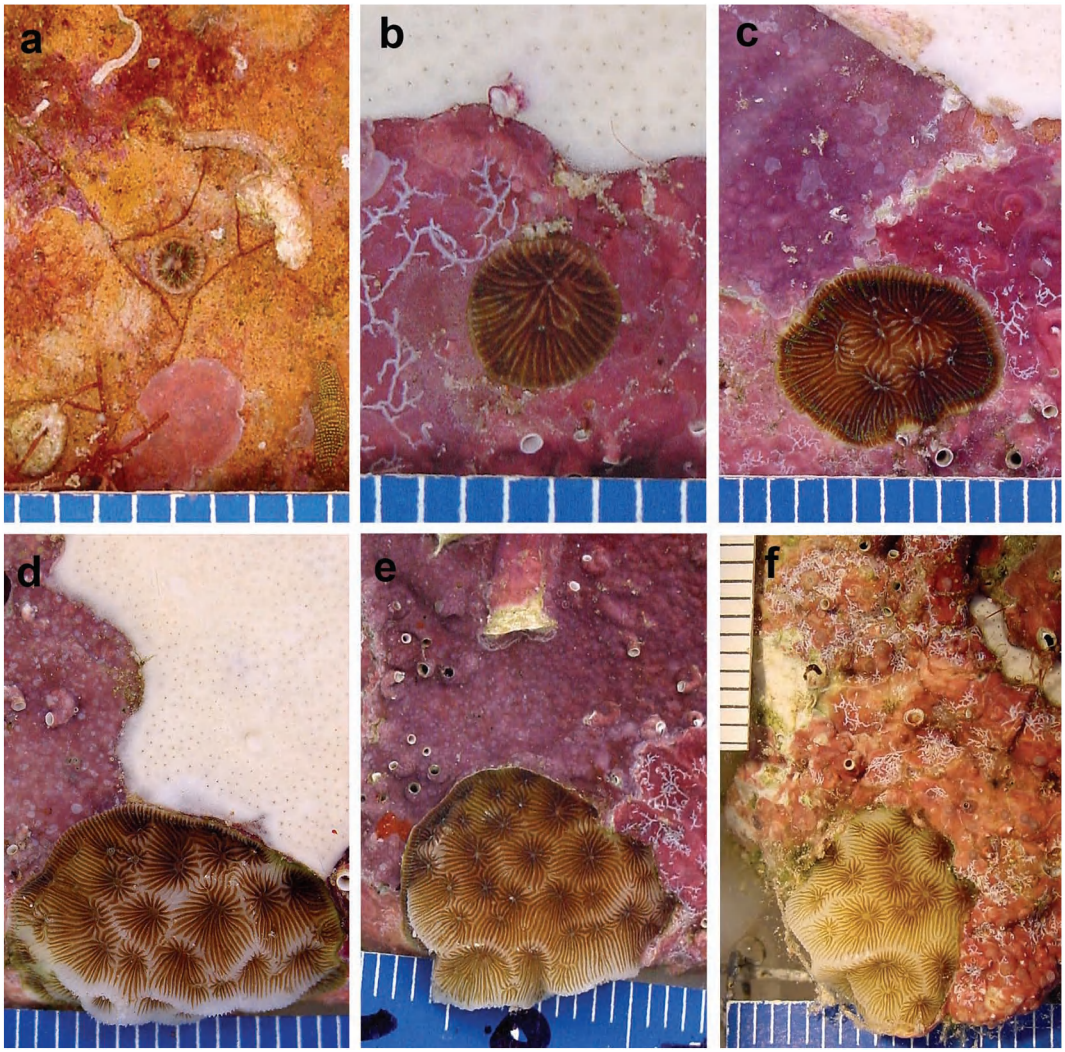

FIGURE 6. A time series of the growth of *Agaricia* sp. settled on a terracotta tile in Bonaire over 3.75 years. After March 2007 (e), this recruit is being overgrown by the coralline alga *Titanoderma prototypum*, a known settlement-facilitating species, illustrating just how hazardous the settlement environment can be. a, June 2004, recruit diameter is 1.3 mm; b, March 2005, 3.4 mm; c, July 2005, 8.4 mm; d, June 2006, 15.2 mm; e, March 2007, 16.0 mm; f, March 2008, 12.0 mm. One segment on the scale bar = 1 mm. (All photographs by Suzanne Arnold.)

Sammarco, 1985), but their clonal growth created massive monocultures of rapidly growing reefs capable of keeping up with rising sea level (Adey, 1978). In contrast, there are two orders of magnitude more species of *Acropora* on Indo-Pacific coral reefs, and the population density of their recruits are also orders of magnitude greater on Indo-Pacific reefs than on Caribbean reefs (Hughes et al., 1999).

Although the high diversity of acroporid corals in the Pacific spans the spectrum of life history characteristics from weeds (i.e., high reproductive output and rapid growth rate; Omori et al., 2008) to trees (i.e., competitively dominant, large colonies; Baird and Hughes, 2000), the two acroporid species comprising Caribbean reefs require long adult lives and considerable clonal propagation. However, since the acroporid die-off in the early 1980s, Caribbean reefs have fundamentally changed. Because of the resultant algal phase shift (Hughes, 1994), acroporid reefs have become hostile to the rare acroporid recruits, and they have lost their receptivity for reattachment of encrusting fragments (Williams et al., 2008). These changes on many

Caribbean reefs may be the primary reason why they appear less capable of recovering from widespread disturbances such as coral disease and bleaching.

The massive, slow-growing coral *Montastraea annularis* is also a broadcast spawner and framework builder in the Caribbean. It also has very low rates of recruitment (Hughes and Tanner, 2000) and thus requires long adult life to establish its dominance. Although it dominates Caribbean reefs today (Kramer, 2003) and is relatively hardy, it too has shown elevated levels of disease in recent years (Pantos et al., 2003) and has increased susceptibility to disease after bleaching (Miller et al., 2006). Again, the long-term prognosis for this Caribbean reef builder is poor.

Weedy, brooding species such as *Agaricia* spp. and *Porites* spp. are the thrust behind the current rates of coral recruitment in the Caribbean. The Caribbean brooder *Agaricia agaricites* is often the most abundant recruit on Caribbean reefs in recent times (Bak and Engel, 1979). This species has well-documented high rates of recruitment and adequate sediment-rejection capabilities yet regenerates poorly from lesions and is often outcompeted by other corals (Bak and Engel, 1979). In the past 30 years *Agaricia tenuifolia* has replaced other corals to dominate the community on two reefs that had historically different community compositions (Aronson et al., 2004).The increasing community dominance observed for *Porites astreoides* at six sites in the Caribbean is being driven by a constant recruitment rate coinciding with reduced percent cover of other coral species (Green et al., 2008).

It is possible that these life history-related differences are fundamentally changing Caribbean reefs. Are Caribbean reefs today following the paths of forests and other marine ecosystems in their shift to weedy, stress-tolerant species? (see Knowlton, 2001). A recovery such as seen in Palau following the 1998 bleaching event, where sexual recruitment and remnant regrowth were equal contributors (Golbuu et al., 2007), has yet to be recorded in the Caribbean. Success stories of Caribbean recoveries led by broadcast spawning species are scarce (but see Idjadi et al., 2006). Thus, the relative importance of sexual versus asexual reproduction to recovery in the Caribbean needs to be addressed by long-term observations with particular focus on recovery following large-scale disturbances such as major storms and bleaching events.

Thus, it seems that Caribbean reefs were built by corals that have been successful since the Pleistocene (Pandolfi and Jackson, 2006) with a strategy of low recruitment, considerable clonal growth, and low post-settlement mortality. However, that strategy may not be broadly viable today, given the global climate trajectory (Hoegh-Guldberg et al., 2007) and patterns of human activities. While Indo-Pacific reefs are not immune to declines in rates of coral recruitment in recent years (Wakeford et al., 2008), the higher biodiversity and range of recruitment and post-recruitment strategies (e.g., high rates of growth) allow reefs there to be more resilient.

CONCLUSIONS

Coral mortality has increased in recent decades, making coral recruitment more important than ever before in sustaining coral reef ecosystems and contributing to their resilience. We identified three critical sequential phases to the recruitment process of corals: larval availability, larval settlement, and post-settlement ecology. All three factors are necessary for coral recruitment and, ultimately, for maintenance or recovery of coral reef ecosystems.

Most coral planulae available for recruitment are probably from relatively local reproduction and relatively short-distance connectivity. As adult coral abundance declines, both fertilization success and the effective dispersal distance of corals (see Figure 3B) will likely decline as well. Physiological stress on reproducing corals might also result in fewer and possibly weaker coral larvae arriving, thereby reducing the per capita rate of settlement success.

Once in the vicinity of a coral reef, settling corals respond to a hierarchy of environmental cues both in the water and from the reef. Several studies have identified organisms that facilitate or inhibit the settlement and metamorphosis of corals. Crustose coralline algae can facilitate coral settlement but, disturbingly, this group of algae is becoming rarer on coral reefs as macroalgae become increasingly dominant. Macroalgae are known inhibitors of settlement, which may result from their ability to rapidly occupy settlement habitat, their suite of secondary metabolites, their microbial communities, or a combination of some or all of these mechanisms.

Stressors that impact multiple life history stages of corals have the most potential to greatly reduce coral recruitment. Poor water quality (such as sedimentation and increased temperatures) and the increased abundance of macroalgae are known to decrease coral recruitment and negatively impact corals at many different life history stages. Human impacts on the water quality of marine systems continue to grow, and few locations remain untouched (Halpern et al., 2008). These and other stressors may decrease the reproductive output of corals, physiologically stress the larvae, block subcryptic nursery habitats, create negative settlement cues, and result in increased post-settlement mortality.

Globally, many Indo-Pacific reefs have higher rates of settlement, recruitment, and recovery from disturbances, which could be the result of higher biodiversity in the region. In contrast, Caribbean reefs may have evolved a strategy of low recruitment and considerable clonal growth, with low post-settlement mortality for its few reef-building acroporid corals. Unfortunately, that strategy may be ineffective in the future given the global climate trajectory of higher ocean temperatures, acidification, and greater disturbance from tropical storms, which will continue to physiologically stress corals. Because Indo-Pacific reefs have two orders of magnitude more acroporid species, weedy and potentially resilient strategies could succeed. If current trends continue on modern reefs, it is possible that reefs in the future will differ from those of the recent past.

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Do Indian River Lagoon Wetland Impoundments (Eastern Florida) Negatively Impact Fiddler Crab (Genus *Uca*) Populations?

Bjorn G. Tunberg

ABSTRACT. Quantitative sampling of fiddler crabs was performed in June–July between 1992 and 1994 along transects at three St. Lucie County mosquito impoundments, Florida, running from the Indian River Lagoon (IRL) shore and across the impoundment perimeter dikes, and in one impoundment across the perimeter ditch. A total of 929 specimens representing four species were found: *Uca pugilator*, *Uca rapax*, *Uca speciosa*, and *Uca thayeri*. The quantitative sampling showed that there was no correlation between the number of *Uca* burrow openings on the sediment surface and the actual number of crabs in the sediment. Differences were recorded in abundance and distributional patterns between impoundments, but no correlation was recorded between substrate organic content and species distributional patterns. The male/female ratio was close to 1 for all species, except for *U. thayeri*; the males dominated for this species (ratio, 1.8:1). High water temperatures potentially lethal to fiddler crabs occurred in the impounded marsh in the summer. *U. pugilator* and *U. rapax* were unlikely to be impacted by the impoundment flooding as they are highly motile and not very site specific. *U. speciosa* and *U. thayeri* were more restricted to the very soft, dark, and wet substrate along perimeter ditch banks and may therefore be impacted during periods of flooding because they are dependent on nonflooded areas for feeding and reproduction.

INTRODUCTION

Burrowing crustaceans, such as fiddler crabs, impact the ecology of associated infaunal communities and, consequently, the ecosystem as a whole (Crane, 1975; Montague, 1982; Dittman, 1996). According to Montague (1980), fiddler crabs are the most abundant macrobenthic crustacean inhabitants of North American estuaries. Their impacts on bioturbation activity and oxygenation of the substrate are considerable (Bertness, 1985). Fiddler crabs may also play an important role in recycling nutrients (Macintosh, 1982; Bertness, 1985). They feed by scraping up and ingesting surface sediment (Crane, 1975; Kraeuter, 1976; Heard, 1982; Macintosh, 1982; Weis and Weis, 2004) and are in that respect very important in overturn of substrates. Fiddler crabs are also an important food source for birds, fish, and mammals (Peterson and Peterson, 1979; Montague, 1980; Grimes et al., 1989; Gilmore et al., 1990). There is a relatively diverse *Uca* species assemblage within the Indian River Lagoon (IRL) region, with seven species reported in the IRL (Salmon, 1967; Kerr, 1976; M. Salmon, Florida

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Atlantic University, personal communication, 1992), four tropical species, *Uca rapax* (Smith), *Uca thayeri* Rathbun, *Uca speciosa* (Ives), and *Uca mordax* (Smith), and three temperate species, *Uca pugnax* (Smith), *Uca pugilator* (Bosc), and *Uca minax* (Le Conte). Only four species were found during these studies: *Uca pugilator*, *U. rapax*, *U. speciosa*, and *U. thayeri*.

That the impoundment of 90% of the marginal wetlands (primarily for mosquito control) of the IRL has a potential negative impact on regional *Uca* populations has been a controversial issue for many years. Each impoundment and the management procedures are described in detail in Rey and Kain (1991). Preliminary studies by Gilmore et al. (1991) revealed that no *Uca* spp. were observed from marsh-mangrove habitats in flooded (short-term and long-term) impoundments, while they were present in large numbers at unimpounded sites adjacent to impoundments. This difference could be associated with a number of factors, because many aspects of the reproduction of *Uca* (including courtship, female receptivity, egg maturation, and hatching) are closely synchronized with the semidiurnal and semilunar tidal cycles (Fingerman, 1957; Barnwell, 1968; Wheeler, 1978; Zucker, 1978; Montague, 1980; DeCorsey, 1983; Salmon et al., 1986). However, according to Fingerman (1957), the tidal rhythm differs between species (*U. pugilator* and *U. speciosa*). The exclusion of natural tidal cycles within several impoundments may therefore have serious impacts on populations of *Uca* spp. In addition, prolonged periods of inundation that usually occur from May to September (management for mosquito control) may displace *Uca* spp., which need periods of exposure of the burrow entrances for survival. Periods of heavy precipitation, mainly during the summer, may also drastically reduce the salinity within these impoundments.

The main objectives of this study were (a) to evaluate survival and adaptation of *Uca* populations to manipulated ecological conditions along the impoundment perimeter ditches (compared with the natural IRL conditions), (b) to determine if these potential adaptations differed among species, and (c) to elucidate zonation patterns of each species (from the IRL shore, across the dike road, down to the impoundment ditch).

METHODS

Figure 1 shows the location of the three studied impoundments, which are described in detail in Rey and Kain (1991). Blue Hole Point (impoundment [Imp.] #23) is a 122 ha breached impoundment. The 20 m breach in the western dike allowed natural tidal access between the impoundment and the IRL. This breach was a result of a severe 1981 winter storm and was left open to the natural tidal cycles of the IRL. The main reason for not repairing the breach was that this impoundment could be used as a reference/control site for numerous impoundment studies (James R. David, St. Lucie County Mosquito Control District, personal communication, 1992). Jack Island State Preserve (Imp. #16A) is a 161 ha impoundment divided into four cells. This impoundment was open via culverts to the IRL during the winter months but was artificially flooded during the summer months (early May through August). Bear Point (Imp. #1) is a 255 ha impoundment. Since August 1993, the culverts here were left open to tidal exchange.

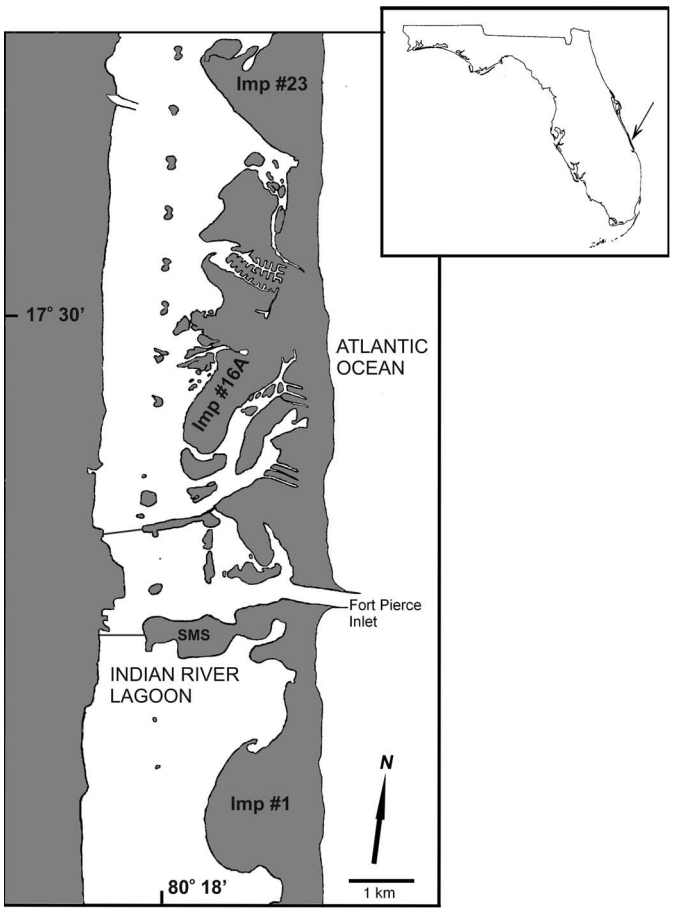

FIGURE 1. The three sampling impoundments (Imp) within St. Lucie County, Florida; SMS = Smithsonian Marine Station.

Quantitative sampling was performed in these impoundments, Imp. #23 and Imp. #16A in June-July 1992,

1993, and 1994 and Imp. #1 in July 1994 along a portion of transect lines previously established for burrow counts (Gilmore et al., 1991). One transect line had been established in each impoundment. These transects ranged from the edge of the IRL (0 m), continued across the artificial dike, and ended at the impoundment perimeter ditch (Figure 2). Four permanent metal stakes indicated the sampling sites (see below). Because of the very hard substrate on top of the actual dike (the road), it was impossible to sample these sites (10 m and 15 m) quantitatively (see below). The 0 m stake was placed at the waterline (low tide) on the IRL side, and the other three stakes (markers) were placed at 5 m intervals across the dike, with the 0 m stake as the starting point. The 15+ m site was between the 15 m stake and the upper bank of the perimeter ditch (Gilmore et al., 1991; see Figure 2). Additional sites were also established for the studies: site A was at the edge of the water (low tide) on the dike side of the perimeter ditch and site B in the corresponding area of the impoundment marsh side of the ditch (Figure 2). It was not possible to establish a site B in Imp. #16A, because of the summer artificial flooding, or in Imp. #1, because it was flooded naturally. The width of the perimeter ditches was about 5 to 6 m. The ditch shores in all impoundments had a very dense (but only about 1.5 to 2 m wide) mangrove vegetation (primarily *Rhizophora mangle*). Two additional sites were established in Imp. #23: site C, about 2 m into the impoundment marsh from site B, immediately behind the dense mangrove vegetation along the ditch shore (see Figure 2), and site D on the sand flat within the marsh (25 m from the ditch). The distance from site 0 m to site C was about 25 m and to site D about 47 m. The sampling sites were 2 × 2 m permanent squares situated at each marker (sites 0 m, 15+ m, C, and D), within which four replicate samples were randomly collected on each sampling

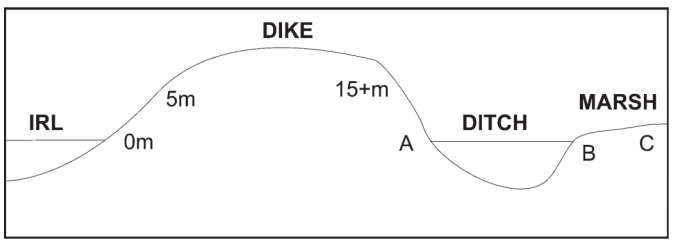

FIGURE 2. Cross section of a typical mosquito impoundment in the Indian River Lagoon (IRL), showing locations of the fiddler crab sampling sites. The dike road was approximately 1.5 m above low tide level in the IRL at three investigated sites (site D is not shown).

date. Sites A and B were sampled the same way (on the exposed substrate at low tide) close to the dense mangrove vegetation at each side of the perimeter ditch. The Imp. #23 impoundment marsh was never flooded during my studies. The random sampling was performed by means of a stainless steel cylinder (0.1 m^2, 40 cm high) with a sharpened bottom edge. Sampling was always performed at low tide and when no, or very few, specimens were observed on the sediment surface. Sampling was never performed when many crabs were observed out of their burrows. Sampling at such times would have resulted in erroneous quantitative results because *Uca* spp., when disturbed on the sediment surface, seek shelter in the closest burrow or even migrate out into the water. The cylinder was forced down to a sediment depth of at least 25 cm. The number of *Uca* burrows within the cylinder area was recorded, and then the sediment was removed with a shovel (with a straight edge). The uppermost fraction (0–10 cm) was sieved (in the field) in seawater through a 2 mm stainless steel mesh sieve. In the remaining fraction (10–25 cm) the crabs were removed by hand in the field. This procedure was deemed acceptable as small crabs only occurred in the uppermost layer of the sediment. The specimens were transferred to plastic bags and kept in a cooler in the field. In the laboratory, the samples were either processed immediately or stored in a freezer for later processing. The crabs were sorted by hand in a tray filled with seawater. They were then placed in labeled glass jars in a solution of 5% borax-neutralized formalin, diluted in seawater. After 4 to 5 days the formalin was replaced with 70% ethanol. All specimens larger than 5 mm carapace width (CW) were later identified and weighed (wet weight) and have been archived for possible future studies. All individuals smaller than 5 mm were regarded as "juveniles." It was not possible to identify these to species level with certainty. The literature sources used for species determination were Tashian and Vernberg (1958), Salmon (1967), and Crane (1975). A total of 140 quantitative samples were collected during the entire study period: 84 in Imp. #23, 40 in Imp. #16A, and 16 in Imp. #1.

Water temperature was measured midafternoon on 26 July 1993 and 1 August 1994 within the marsh of Imp. #16A (which was artificially flooded), in the middle of the adjacent perimeter ditch, and in the IRL (about 5 m from the shore). The measurements were taken at 5 cm water depth.

Because many impoundments are closed for natural tidal exchange to the estuarine waters of the IRL during the artificial flooding periods (impoundment pumps), salinity may drop rapidly during periods of heavy rainfall.

An experiment was therefore performed to investigate tolerance to rapid salinity changes among the four *Uca* species. The laboratory setup consisted of twenty 2 L round plastic containers equipped with a lid. A separate air supply was provided to each container. Four treatments and one control (four replicates per treatment) were established: 100%, 75%, 50%, 25%, and 0% seawater. Laboratory-supplied seawater was diluted with distilled water. The salinities of the different treatments were 100% = 36–37 ppt (parts per thousand), 75% = 27–29 ppt, 50% = 19 ppt, 25% = 9–10 ppt, and 0% = 0 ppt, measured with an ocular refractometer. The water temperature was very stable during the experimental period, 24.0°–26.0°C. Each experiment lasted for seven days. The crabs were collected 48 hours before each experiment and acclimated in 100% aerated seawater during this period. Seven randomly selected female crabs of each species were placed in each experimental container. It was not possible to find enough specimens of *U. thayeri* during the period for these studies. Therefore only 25% and 0% seawater were used as treatments, and each replicate contained five crabs. The experiments were monitored twice a day, and any dead crabs were removed. Water was changed only in the containers where dead crabs were found. These experiments were performed between 4 July and 27 July 1994.

Sediment samples for analysis of organic content (loss on ignition) were collected in 1994 along the three transects. Three sediment cores (inner diameter, 30 mm) were collected to a depth of 5 cm at randomly chosen points at each site. As stated above it was not possible to establish a site B in Imp. #16A or in Imp. #1. The sediment was treated in the laboratory according to the procedures described in Holme and McIntyre (1971).

A one-way analysis of variance (ANOVA) (Holm–Sidak method) was performed to compare the respective monitored sites in the three impoundments regarding organic content (LOI) in the sediment (Table 1).

RESULTS

Abundance

Abundance data from the three transects sampled in 1992, 1993, and 1994 at Imp. #23 and Imp. #16A are presented in Figures 3 and 4, and the one transect sampled in 1994 at Imp. #1 in Figure 5. High water levels prevented sampling 0 m (IRL) at Imp. #16A in 1992 and site A (ditch shore) in 1994.

The results from Imp. #23 were similar the three sampling years (Figure 3). *U. pugilator* and *U. rapax* were relatively evenly distributed across the transect, and a few specimens of *U. rapax* were sometimes observed on the dike road (DIKE; see Figure 2). *U. speciosa*, the dominant species, was found only at site 0 m, and in very high densities in the wet, soft, and dark mud on both sides of the perimeter ditch (sites A and B). *U. thayeri* was also found on both sides of the perimeter ditch (sites A and B), in addition to a few specimens at site 0 m in 1992.

In contrast, at Imp. #16A (Figure 4), *U. pugilator* dominated in abundance at site 5 m whereas *U. rapax* was most abundant at site A (perimeter ditch shore). *U. speciosa* was almost exclusively found at site A and *U. thayeri* at site 0 m.

At Imp. #1 the distributional patterns were similar to the other impoundments. However, *U. pugilator* was found in comparatively low densities, whereas *U. rapax* was abundant at both sites 0 m and 5 m. *U. speciosa* was found in high densities in the wet muddy areas at site 0 m and at site A. *U. thayeri* was found at the 0 m site and to even a greater extent at site A (dike side of the ditch).

No statistical tests were performed to elucidate any potential difference between years at each site, but it was of higher interest to statistically compare abundance patterns between impoundments. Therefore, correlation analyses (Pearson product moment correlation) were performed on the mean abundance data (1992, 1993, 1994) for sites 0 m

TABLE 1. One-way analyses of variance (Holm–Sidak method) concerning differences in organic content (LOI) between the different impoundment and sampling sites. Significant differences (*P* values) are in ***bold italic***.

	Site					
Impoundment no.	**0 m**	**5 m**	**15+**	**A**	**C**	**D**
23 vs. 16A	***0.025***	0.068	0.158	***0.0002***	***0.0001***	***0.001***
23 vs. 1	0.148	***0.044***	0.123	0.124		
16A vs. 1	***0.004***	***0.003***	***0.014***	***0.00006***		

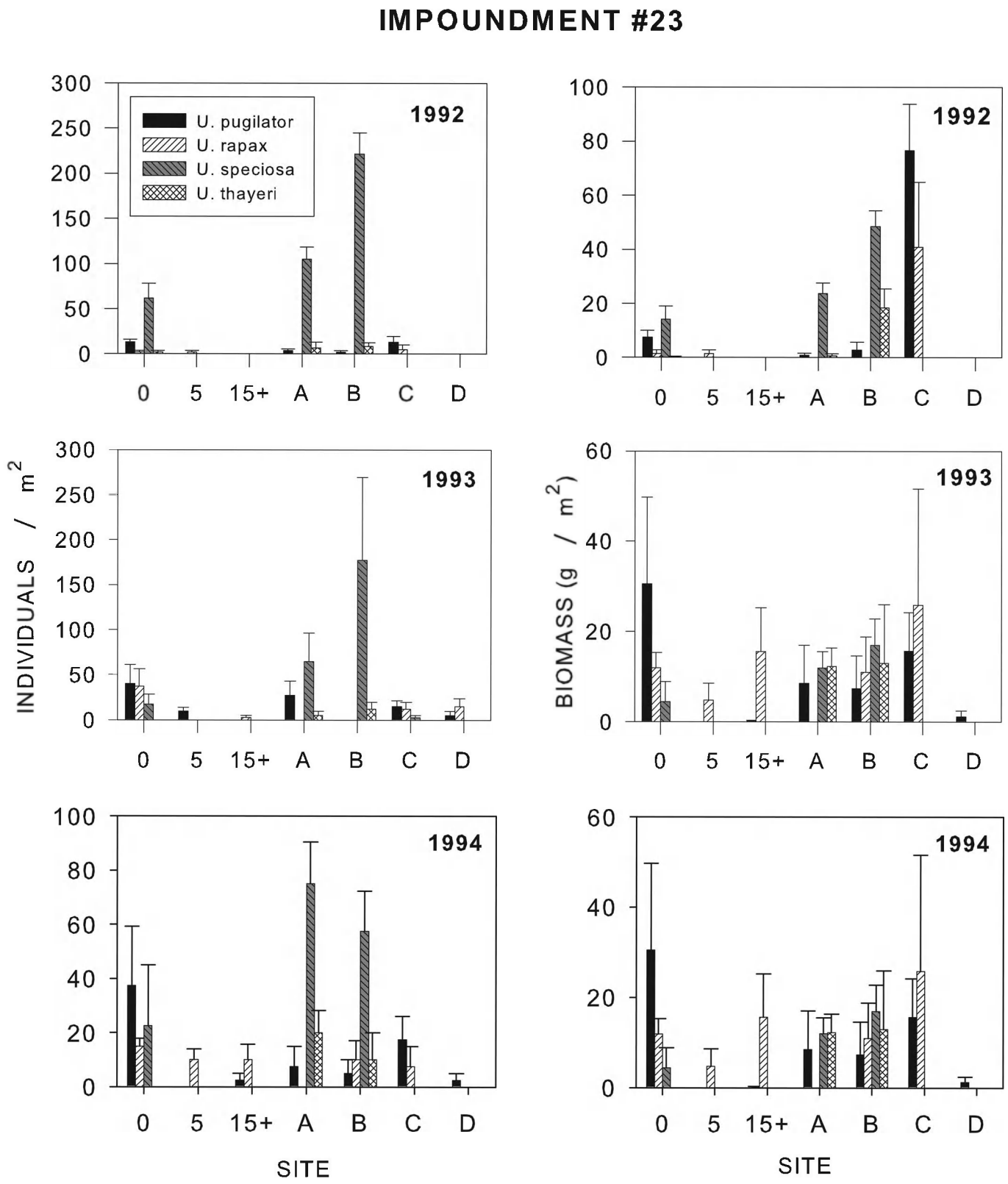

FIGURE 3. Abundance and biomass (wet weight) between 1992 and 1994 of the four fiddler crab species along the impoundment #23 transect. Error bars represent + standard error values ($N = 4$). Note the different scaling on the y-axes.

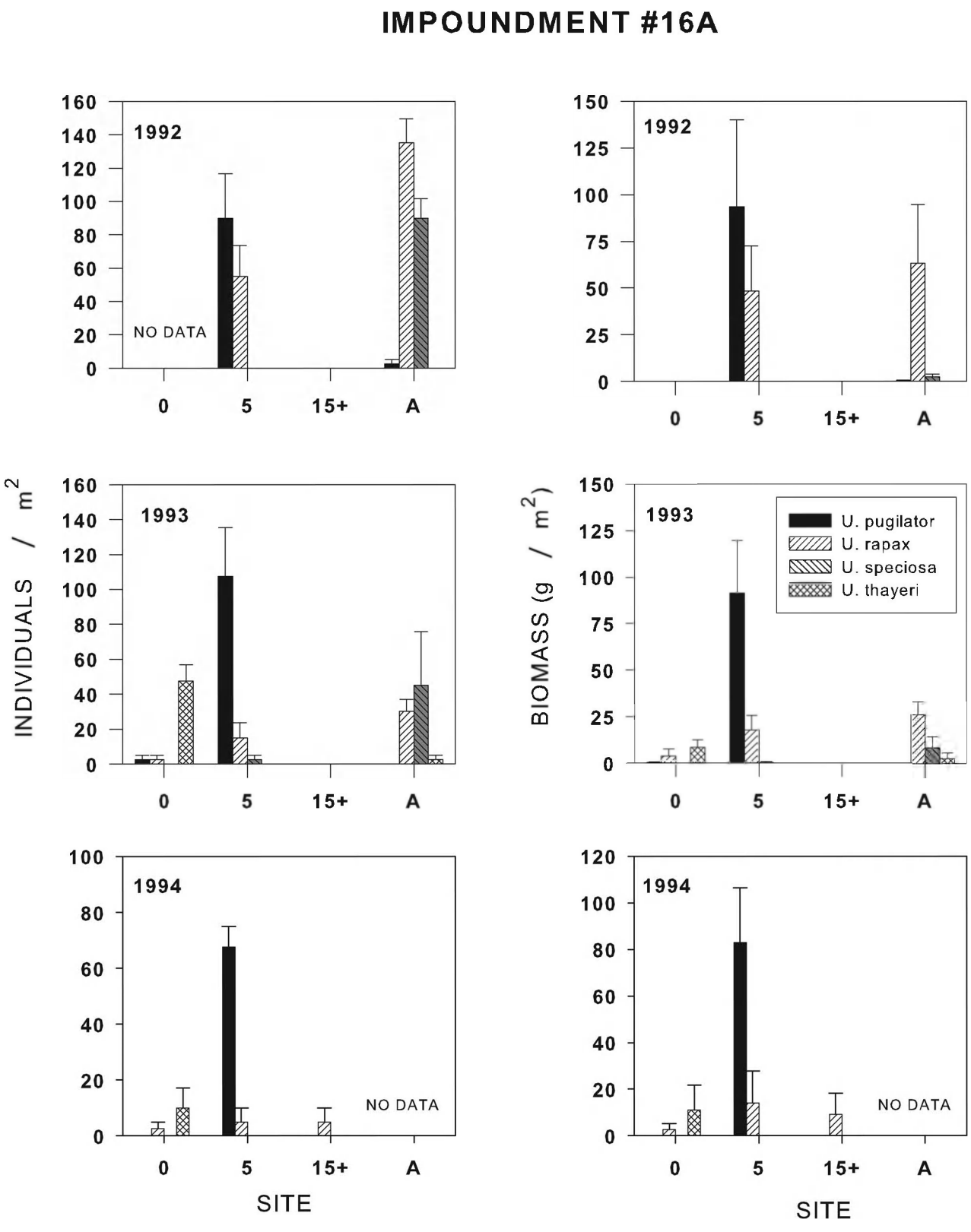

FIGURE 4. Abundance and biomass (wet weight) between 1992 and 1994 of the four fiddler crab species along the transect within impoundment #16A. Error bars represent + standard error values ($N = 4$). Note different scaling on y-axes.

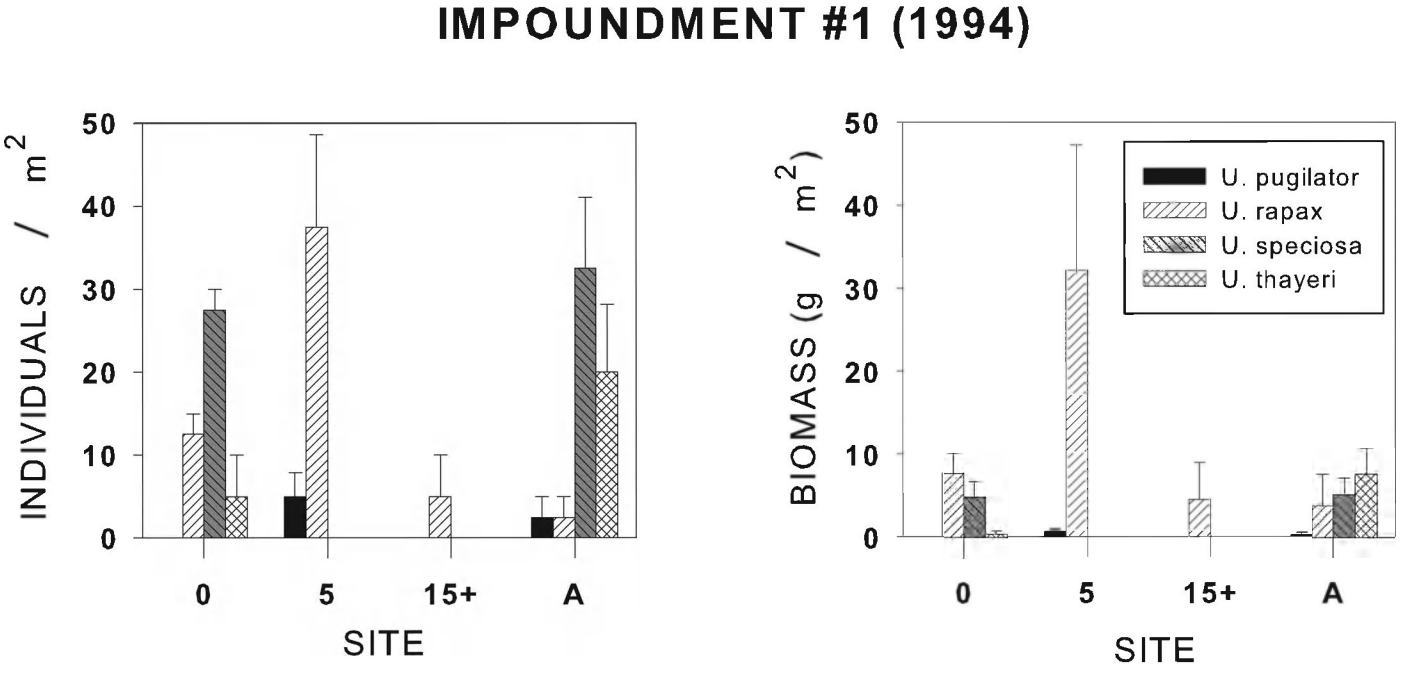

FIGURE 5. Abundance and biomass in 1994 of the four fiddler crab species along the transect within impoundment #1. Error bars represent + standard error values ($N = 4$).

to A for each of the four species separately between Imp. #23 and Imp. #16A. However, no correlation ($P > 0.05$) could be found for any of the species. The same analyses were performed for the 1994 data from Imp. #23, Imp. #16A, and Imp. #1. The only correlation (positive) found was for *U. thayeri* between Imp. #23 and Imp. #1 (correlation coefficient, 0.968; *P* value, 0.031).

Biomass

The biomass (g wet weight) measurements are presented in Figures 3–5. No significance tests were performed concerning the biomass difference among the three years for each species.

However, the biomass calculations for the three years in Imp. #23 (Figure 3) indicate that changes took place, but these changes are based on subjective observations. High biomass values were recorded for *U. pugilator* in 1992 at site C and in 1993 and 1994 at site 0 m. High values were recorded for *U. rapax* throughout the entire transect, especially in 1993 and 1994, except at site D. The highest biomass values for *U. speciosa* were recorded on both sides of the perimeter ditch (sites A and B), especially on the marsh side of the ditch (site B). High *U. thayeri* biomass values were recorded along the perimeter ditch (sites A and B).

At Imp. #16A the biomass values for *U. pugilator* were high at site 5 m all three years (see Figure 4). Relatively high biomass values were recorded for *U. rapax* at sites 5 m and A in 1992. However, data for site A in 1994 are not available. Low biomass values were recorded for *U. speciosa* at site A in 1992 and 1993. *U. thayeri* was only recorded at low biomass values at site 0 m in 1993 and 1994 and at site A in 1993 and at site 0 m in 1994.

At Imp. #1 low values were observed for *U. pugilator* throughout the transect (see Figure 5), but *U. rapax* was, by far, the most dominant (biomass) species across the entire transect. The only exception was site A, where the values for *U. speciosa* and *U. thayeri* were somewhat higher.

Reproduction and Sex Distribution

The percentage of "juveniles" found in 1993 and 1994 at the different sites within Imp. #23 and Imp. #16A is presented in Figure 6. More juveniles were found at site 0 m at Imp. #16A compared with Imp. #23. Many juveniles were also recorded along these impoundment ditch shores (sites A and B in Imp. #23 and site A in Imp. #16A). The sex distribution among adults of the four species from the 1992, 1993, and 1994 (combined) collections (June–July) is presented in Figure 7 with the number

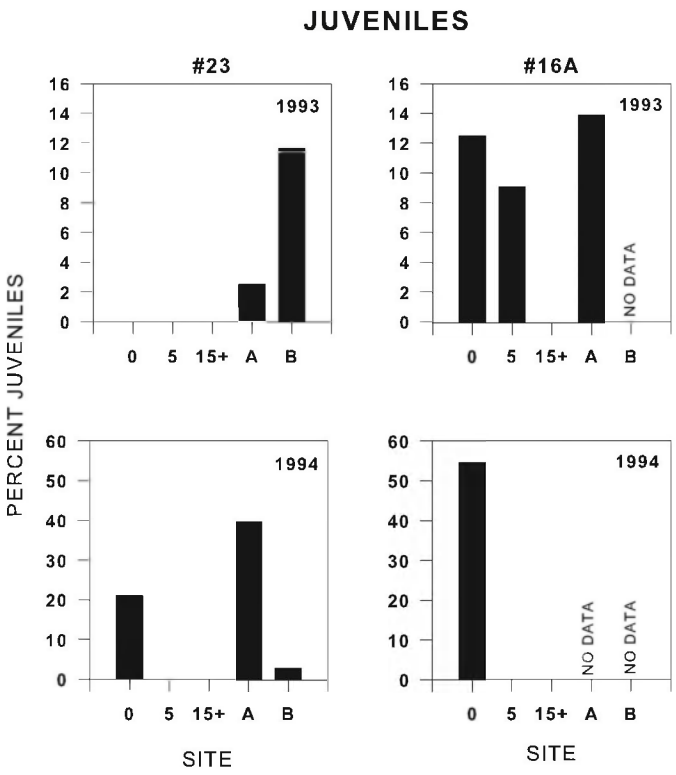

FIGURE 6. Percentage specimens having a carapace width (CW) less than 5 mm (juveniles) of all collected individuals of *Uca* spp. from each site in 1993 and 1994. Note different scaling on *y*-axes.

of ovigerous females. As shown, the sex ratio was near to 1.0 among all four species except for *U. thayeri* where the male/female ratio was approximately 1.8:1. The highest ovigerous rate was found among *U. pugilator* (22.0%) and the lowest among *U. rapax* (3.4%). The corresponding figures for *U. speciosa* and *U. thayeri* were 6.9% and 10.0%, respectively.

Burrows

The correlation between the number of burrows and the actual number of crabs found within each sample in 1992 and 1993 is presented in Figure 8. A Wilcoxon signed-rank test showed that there was no correlation between these two parameters: $P = 0.097$ (linear regression: $R^2 = 0.02$, P [analysis of variance] = 0.23). This finding has also been reported by Colby and Fonseca (1984). The same lack of correlation was also found by the author in a larger and more detailed multiyear study at Merritt Island impoundments (close to Cape Canaveral, eastern Florida.).

Temperature and Salinity Tolerance

The summer water temperatures within Imp. #16A, the perimeter ditch, and in the IRL is presented in Table 2. The water temperature was higher within the impoundment marsh than in the perimeter ditch and in the IRL.

The laboratory experiment showed that no species showed any disturbance or mortality in 100%–25% seawater. However, the reaction toward 0% seawater was severe (Figure 9). *U. speciosa* and *U. thayeri* showed very low tolerance toward 0% seawater while *U. pugilator* showed the highest tolerance. The reaction from *U. rapax* was intermediate.

Sediment

The results of the sediment analyses are presented in Figure 10. The loss on ignition (organic content) was higher in Imp. #16A than in Imp. #23 and Imp. #1 (see

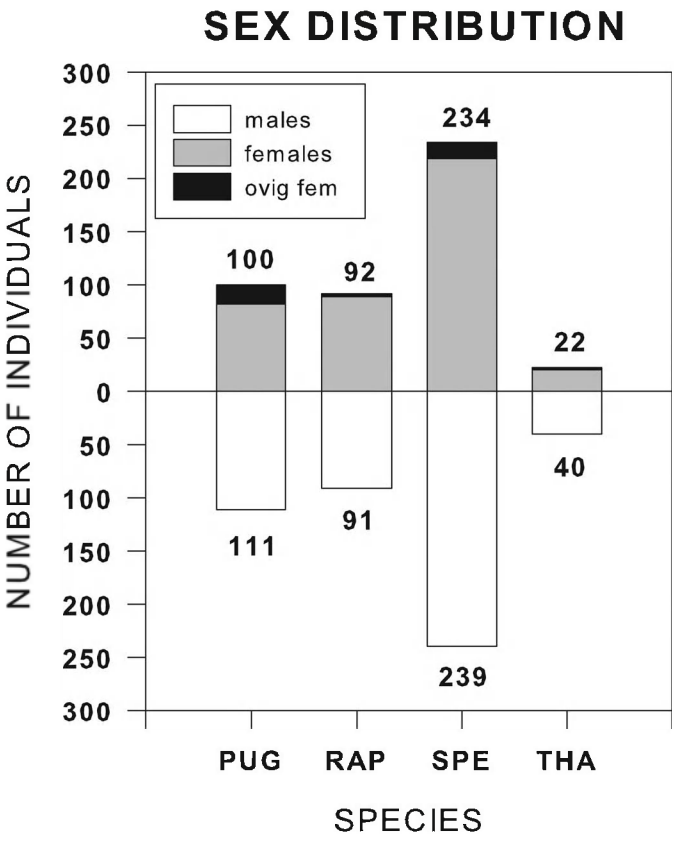

FIGURE 7. Sex distribution of all fiddler crab individuals larger than 5 mm collected during 1992–1994 combined within impoundments #23, #16A, and #1. The bars show sex distribution for each species found throughout the study period; PUG = *Uca pugilator*; RAP = *U. rapax*; SPE = *U. speciosa*; THA = *U. thayeri*.

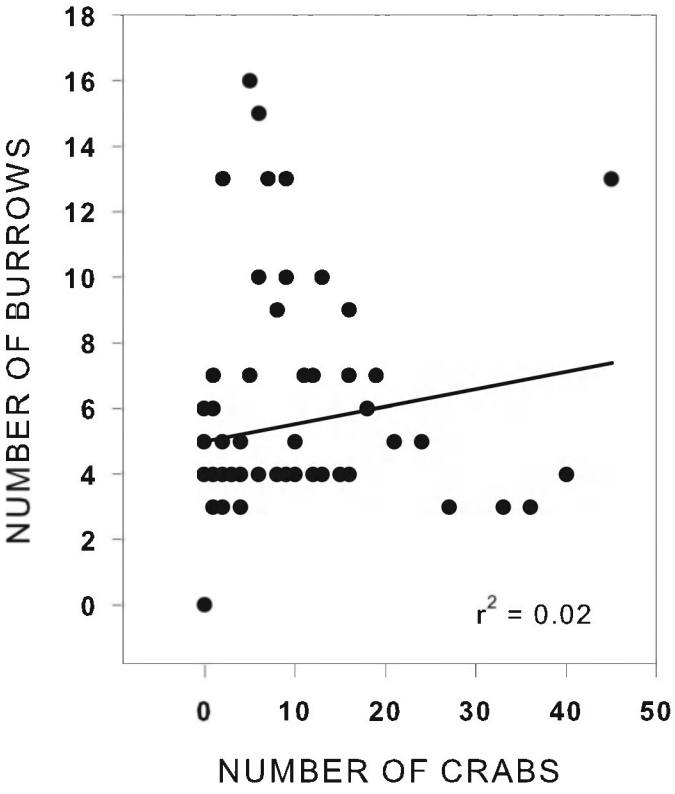

FIGURE 8. Relationship between number of burrows and number of fiddler crabs found in each quantitative sample.

Table 1). The lowest values on the IRL side (site 0 m) were recorded from Imp. #1 and the highest from Imp. #16A. At the ditch (site A) the highest organic value was recorded at Imp. #16A and the lowest at Imp. #1. Within the marsh (site C) (Imp. #23 and Imp. #16A only), the loss on ignition was very high within Imp. #16A and very low within Imp. #23. As indicated in Table 1, Imp. #16A deviated significantly the most from the other two impoundments, with generally the highest organic content (LOI).

TABLE 2. Water temperatures (°C, 5 cm water depth) at impoundment site #16A, measured in midafternoon during July 1993 and July 1994.

Location [a]	1993	1994	Mean
Marsh	44.3	42.4	43.4
Ditch	37.1	36.1	36.6
IRL	37.5	35.2	36.4

[a] Marsh = impoundment marsh; ditch = impoundment perimeter ditch; IRL = Indian River Lagoon.

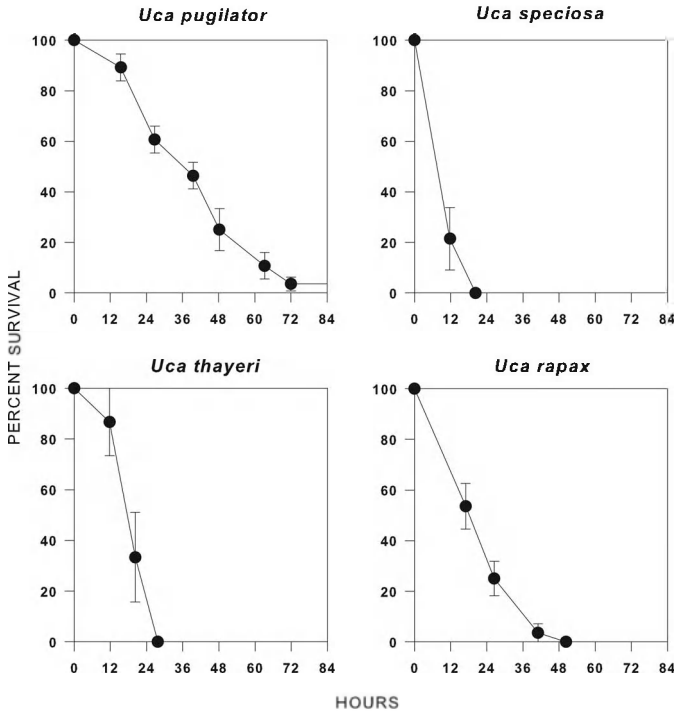

FIGURE 9. Percent survival in fresh (distilled) water of the four fiddler crab species. Error bars represent standard error values ($N = 4$).

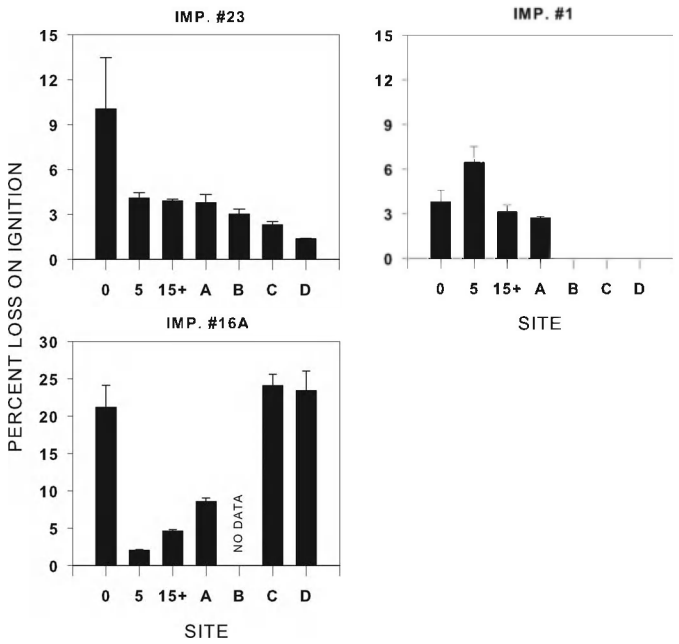

FIGURE 10. Sediment organic content (percent weight loss on ignition) at the sampling sites within the three impoundments in 1994. The error bars represent standard error values ($N = 3$). Note different scaling on y-axes.

Pearson product moment correlation tests were used to elucidate any potential correlation between the organic content of the sediment (LOI) and the distributional pattern (along the transects) of the four different species. All tests were performed on the mean values of each parameter. Only transects where a significant amount of information was available were used for these tests: Imp. #23 in 1993 and 1994, and Imp. #16A in 1994 (see explanation above). No significant correlation ($P > 0.05$) was recorded for any species between abundance and organic content, except for *U. pugilator* at Imp. #23 in 1994, with a correlation coefficient of 0.797 and a P value of 0.032.

DISCUSSION

Different species of fiddler crabs prefer different substrates and salinities for reasons of their specific physiological tolerances and environmental preferences (Teal, 1958; Vernberg et al., 1973). However, the artificial environment of the impoundments in the IRL, with the perimeter ditch and dike, poses a completely new, different type of environment for the *Uca* populations. The occurrence and distributional patterns of the different species of fiddler crabs in these impoundment dikes and perimeter ditches have not been investigated earlier, so this is a first basic study on these populations. The results from this study indicate that more detailed studies are needed in the future in these very extensive artificial environments in the IRL.

Uca speciosa and *U. thayeri* were the most "site- and substrate-specific" species within these environments, whereas *U. pugilator*, and in some cases also *U. rapax*, were more "generalists." *Uca speciosa* and *U. thayeri* were almost exclusively found in the very soft, black, and wet substrate close to the water (primarily sites A and B), which is clearly demonstrated by the data presented from Imp. #23. The highest abundances of *U. speciosa* were here recorded on the perimeter ditch "shores" (sites A and B). Given the rich mangrove vegetation, it was expected that the very fine, wet, and muddy sediment on the ditch perimeter shores (sites A and B) would have a comparatively high organic content, but this was not the case (see Figure 10). Because no correlation between the organic content of the substrate and the abundance pattern of the different species were found, the grain-size distribution, water content, the chemical content of the sediment, root mat density, physiological tolerances, and interspecific interactions may be more important factors in fiddler crab distributional patterns (Teal, 1958; Ringold, 1979; Bertness and Miller, 1984). No root mat areas were investigated in this study. The large differences in substrate organic content (LOI) between Imp. #23 and Imp. #16A are noteworthy (see Table 1).

Large numbers of *U. rapax* (and to some extent also *U. pugilator*) were quite often observed on the dike roads, but *U. speciosa* and *U. thayeri* were never seen there. Thompson et al. (1989) have also demonstrated that some species of desiccated fiddler crabs, among these *U. pugilator*, can rehydrate on damp sand.

When the impoundments are being flooded, it appears that *U. rapax*, and most likely also *U. pugilator*, are able to migrate under water, across the perimeter ditch (often anoxic and with H_2S in the sediment), to more suitable areas. It is, however, important to note that this has so far been confirmed only for *U. rapax*. Therefore, the ability to relocate to more suitable habitat may be the decisive factor in survivorship among *Uca* species. The banks of the perimeter dike (immediately above site A) may therefore act as a temporary "refuge" for some species during periods of impoundment flooding. It is also possible that further migration takes place toward the IRL shores (*U. rapax, U. pugilator*). However, this question does not apply to the rim and road of the dike because of the unsuitable substrate. Furthermore, the distance to the water table is also too great (at least 1 m).

The two species *U. rapax* and *U. pugilator* are probably not adversely affected by impoundment management. Visual observations, and also in situ experiments, have revealed that these species are highly motile within the impoundment areas. According to Thurman (2003) *U. rapax* is typically collected in brackish water. Yoder et al. (2005) have also found that the "herding behavior" in *U. pugilator* is a water-conserving group effect, and this behavior makes them less vulnerable to desiccation. Many specimens of *U. pugilator* and *U. rapax* have been observed (by the author) to migrate over long distances within and outside the impoundments (marked individuals, not reported here). However, further studies need to be performed to clarify these patterns.

Although *U. pugilator* and *U. rapax* thrive in these areas, the fate of the other two species is more uncertain. According to the quantitative sampling results and intensive visual in situ studies, *U. speciosa* and *U. thayeri* are confined to substrate-specific areas of the impoundments, and this may have a negative effect on the populations of these species when the impoundments are being managed (flooded). However, the results from Imp. #16A, which was flooded frequently for mosquito control, seem to contradict this assumption. In spite of this management, a rich community of *U. speciosa* was recorded on the ditch shore (site A), but with low densities of *U. thayeri*.

Even though the data on the occurrence of juveniles are limited, they indicate that fiddler crab reproduction (species unknown) occurs also in the impoundment perimeter ditch (site A and B). As shown in Figure 6, juveniles were, as expected, mainly found close to the water (sites 0 m, A, and B).

The dilution experiments indicate that none of the four species is sensitive to low salinities, a situation that rarely occurs within the impoundments. During this experiment *U. pugilator* was the most tolerant species. Thurman, (2003) investigated the osmoregulation of eight *Uca* species and found that *U. speciosa* and especially *U. pugilator* are able to withstand high "osmotic challenge." Additionally, Thurman (2005) reported that *U. rapax* is best equipped for living in brackish habitats and that *U. thayeri* and *U. speciosa* are best suited physiologically to inhabit low and moderately saline habitats. This observation may explain why the latter two species are able to successfully inhabit the impoundment ditch "shores" (sites A and B). *U. rapax,* and most likely also *U. pugilator* are, as discussed earlier, able to migrate over long distances, for example, across the perimeter ditches (when the impoundments are being flooded) and dikes (studies by the author on ~1,200 of marked *U. rapax* individuals). However, this is most likely not the case with *U. speciosa* and *U. thayeri.* As *U. speciosa* is a comparatively small species, it may therefore be more vulnerable to desiccation than the other three species (Pellegrino, 1984).

High summer temperatures in the shallow impoundment water (see Table 2) pose a threat to the fiddler crab populations. Even though the temperature measurements only were performed twice within Imp. #16A (Table 2), they show that the temperature in the shallow (flooded) areas in the impoundment marsh may reach at least 44°C, which is significantly higher than in the nearby IRL. Large numbers of dead individuals were observed in very shallow water during these high temperature periods (within the marsh of Imp. #16A), but never at lower temperatures, and it was assumed that death was the result of short-term hyperthermia. Replicated laboratory experiments on *U. pugilator* and *U. rapax* collected inside Imp. #23 showed that lethal water temperatures (LD_{50}) on individuals from this area are 41°–42°C. Teal (1958) reported a lethal temperature (LD_{50}) between 39.5° and 40.0°C for *U. pugilator*, *U. minax*, and *U. pugnax*, and Vernberg and Tashian (1959) found that *U. rapax* was more resistant to temperatures of 42°–44°C than was *U. pugnax*. Wilkens and Fingerman (1965) performed a thorough study on lethal temperatures for *U. pugilator* in both saturated and dry air. LD_{50} in saturated air was reached at 40.7°C, which corresponds well to the results from my observations. Powers and Cole (1976) have also demonstrated that burrow temperature decreased rapidly with depth, proving the major heat refuge for *U. panacea* on open sand flats during a study on Mustang Island, Texas. Edney (1961), in a study on a number of fiddler crabs at Inhaca Island, Mozambique, found that the temperature within the burrows during the warmer months was considerably cooler than the sand on the surface. Preliminary results within this study (not presented) also indicate that the temperature drops significantly with sediment depth in the mosquito impoundments.

Genoni (1985), on a study on *U. rapax* in Florida, reported that there were more burrows than fiddler crabs in the sediment. Even if there was no correlation between fiddler crabs (all species) and burrows in the present study, the results were often the opposite from the results by Genoni (1985). Mouton and Felder (1996) investigated the quantitative distribution of *U. spinocarpa* and *U. longisinalis* by quantitatively counting the number of *Uca* burrows along transects in a Gulf of Mexico salt marsh. However, no studies were performed regarding the number of individuals (and species) living in these burrows. Excavating the substrate is a very labor-intensive procedure but obviously necessary to be able to evaluate the actual fiddler crab species distribution and abundance within specific areas (see Methods, above). The studies performed by the author in the three St. Lucie County impoundments and at Merritt Island (Cape Canaveral) impoundments (not reported here) did not produce any correlation between burrows and number of *Uca* specimens. Actually, in several cases when no burrows at all were found on the sediment surface within the 0.1 m^2 sampling area, large amounts of fiddler crabs were found in deeper areas when excavating the substrate within the sampling area according to the description above. Therefore, only counting *Uca* burrows does not seem to give correct data regarding *Uca* population abundance and species distributional pattern. Further detailed studies are therefore needed to elucidate this relationship.

In conclusion, these studies in the St. Lucie impoundments do not indicate that the construction and management of IRL mosquito impoundments pose a serious threat to fiddler crab populations. However, the impoundments may change the distributional patterns of the different species. It is important to note that new, highly suitable habitats were created when the impoundments were constructed, such as the perimeter ditch margins (sites A and B), especially preferred by *U. speciosa* and *U. thayeri* in impoundments with tidal access to the IRL. However, the

fate of these two species at the marsh side of the perimeter ditch (site B) during the prolonged artificial summer flooding is still unknown.

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Dynamic Hydrology of a Mangrove Island: Twin Cays, Belize

Daniel W. Urish, Raymond M. Wright, Ilka C. Feller, and Wilfrid Rodriguez

ABSTRACT. The hydrology of an overwashed mangrove island is shown to be both complex and dynamic, with a strong interaction between tide-induced flow and the resident red mangrove (*Rhizophora mangle* L.) root system. A topographic map of the tidally flooded area of the island was made and related to the tide-induced water levels. The flooded area approximately doubled during the usual tidal event. The bottom topography is highly irregular with a maximum channel water depth of about 1.5 m, but much of the flooded area experiences a water depth of less than 0.5 m. Water elevations were recorded by automatic water level loggers for periods of time up to 9 months. The usual symmetrical parabolic tide signal was transformed into a highly asymmetrical form as it moved landward through the tangled root system of the red mangrove forest. A normal tide range of 13 cm at the island margin attenuated to 3 cm at a distance of 200 m landward, with a lag time of 2 h for highs and 6 h for lows. Maximum flow velocities of 5 cm/s were measured in the main channels with marked reduction in regions of dense mangrove root and shallow water depth. The combined frictional resistance of the bottom and associated mangrove roots is characterized by a Manning's roughness coefficient, n, that ranged from 0.084 to 0.445. The changing flow pattern within the flooded mangrove swamp was mapped during a 7 h high-to-low tide period using aerial photography to track the movement of slugs of visible dye placed at three locations. Analysis of the sequential time-related photos showed limited lateral dispersion in the tortuous main channel but strong tidally controlled flow direction changes and dispersion along the channel axis. A strong circulatory pattern is observed in a shallow pond at the south central terminus of the tidally affected flow system. This large shallow pond is sparsely populated by dwarf red mangrove and is some 350 m from a primary connection with the surrounding lagoon. Poor flushing of the pond creates water temperatures ranging from 25°C in the winter to 40°C in the summer. High surface water evaporation creates a hypersaline condition of 45 ppt salinity in summer. In winter, with the infusion of fresh rainwater, salinity of surface water in the pond can be less than 5 ppt. Because of its role in the transport of nutrients and detritus, and its flushing action, the dynamic hydrological system of the mangrove island is a highly important ecological feature of the overwashed mangrove island.

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INTRODUCTION

Mangrove forests are tropical wetlands with a specialized vegetation adapted to waterlogged and saline conditions (Lugo and Snedaker, 1974; Hutchings and Saenger, 1987; Ball, 1988). These forests provide energy-absorbing buffers from

hurricane-driven seas, prevent coastal erosion, provide a protective habitat for many fish juveniles, and are a nutrient source for the surrounding waters (Odum and Heald, 1972, 1975; Twilley, 1988; Danielson et al., 2005; Barbier, 2006, Constanza et al., 2008), as well as a filtering mechanism for sediments and pollution (Alongi and McKinnon, 2005). Under natural conditions mangroves live in a highly dynamic environment and in synergistic balance with their natural neighbors. Mangroves have evolved features that enable them to cope with an ever-changing regime of tidal water ranges, variable salinity and temperature, and anoxic soil conditions, but within limits (Tomlinson, 1986). The biggest enemy appears to be man, who can directly or indirectly destroy, in days, whole mangrove communities that have taken thousands of years to develop (Alongi, 2002; Macintyre et al., 2004; Rodriguez and Feller, 2004; Taylor et al. 2007; Duke et al., 2007).

It is widely recognized that hydrological patterns determine mangrove structure and function at the ecosystem scale (Lugo and Snedaker, 1974; Forman and Godron, 1986; Twilley, 1995), and general models of mangrove hydrodynamics have been developed (Wolanski et al., 1992). In these coastal wetlands, tidal flooding and surface drainage influence many ecological processes, including habitat quality, water movement, filtration, and nutrient cycling (Forman and Gordon, 1986). Water flow also influences the dispersal and establishment of mangrove propagules (Mazda et al., 1999).

The significant role of vegetation and the effect of intertidal root density on tidal movement in mangrove channels has been described by Wolanski et al. (1980) and over the broader mangrove swamp environment by Wolanski et al. (1992), Furukawa and Wolanski (1996), Mazda et al. (1997), and Mazda et al. (2005). Thus, there is a synergistic relationship for the development and growth of a mangrove forest that depends on the dynamics and magnitude of tidal inundation into the swamp. Concurrently, the frictional resistance of the mangrove roots controls the degree of tidal inundation and patterns of movement in the mangrove swamp (Wright et al., 1991).

Based on long-term experiments on offshore mangrove islands in Belize, hydrodynamics have been linked to distinct patterns of nitrogen (N) and phosphorus (P) limitation across the intertidal flow system (Feller et al., 2003). Lovelock (2008) suggested that differences in tidal inundation also influence soil respiration and belowground carbon sequestion via root production, which is the source of the deep peat deposits underlying these islands. McKee et al. (2007) predicted that the ability of islands such as Twin Cays to keep pace with rising sea levels is dependent on the tight coupling between peat formation and hydrology.

Although these and other studies based at Twin Cays have identified tidal flooding as an important drive of ecological processes, there is limited knowledge on the specific pattern of water movement across these islands. Thus the objective of this research was to conduct a detailed analysis of tidal characteristics and flushing patterns of West Island, the smaller of the two main islands in the Twin Cays Archipelago.

Location

The Twin Cays Archipelago lies some 22 km off the coast of Belize (Figure 1) on the edge of the Belizean Barrier Reef (16°50′N, 88°06′W). Islands of the Barrier Reef and its surrounding waters have been the locations for scientific ecosystem studies by the Smithsonian Institution since 1972 (Rützler and Macintyre, 1982). Because of their pristine condition and relative isolation from anthropogenic effects, the islands and contiguous waters of Twin Cays were selected for detailed scientific research of oceanic mangroves and associated marine ecosystems (Rützler and Feller, 1996). Field studies of the dynamic hydrology of the Twin Cays mangrove ecosystems were begun in 1986 and have continued since that time. This particular study focuses on the surface hydrology of West Island of Twin Cays (Figure 1), a 21.5 ha kidney-shaped landmass approximately 900 m long and 400 m wide. According to the classification of Lugo and Snedaker (1974), the island is an "overwashed mangrove island," one frequently overwashed by tides and with high organic export.

Island Characteristics

The land cover on West Island and effect of man are shown in Figure 2, which depicts the natural mangrove growth and the man-made clear-cut and dredge-fill as mapped by I. C. Feller of the Smithsonian Institution in 2002. Since then even more mangrove destruction has occurred on the east side of the island. The island is dominated by the red mangrove, *Rhizophora mangle* L., with black mangrove (*Avecennia germinans* L.) on somewhat higher topography in the intertidal zone and white mangrove (*Laguncularia racemosea* L.) above the intertidal zone (Rützler and Feller, 1988; Rodriguez and Feller, 2004). It is to be noted that the density of the mangrove is far from uniform, with sparse dwarf red mangrove dominating the interior, and much more vigorous red mangrove growth on the island perimeter

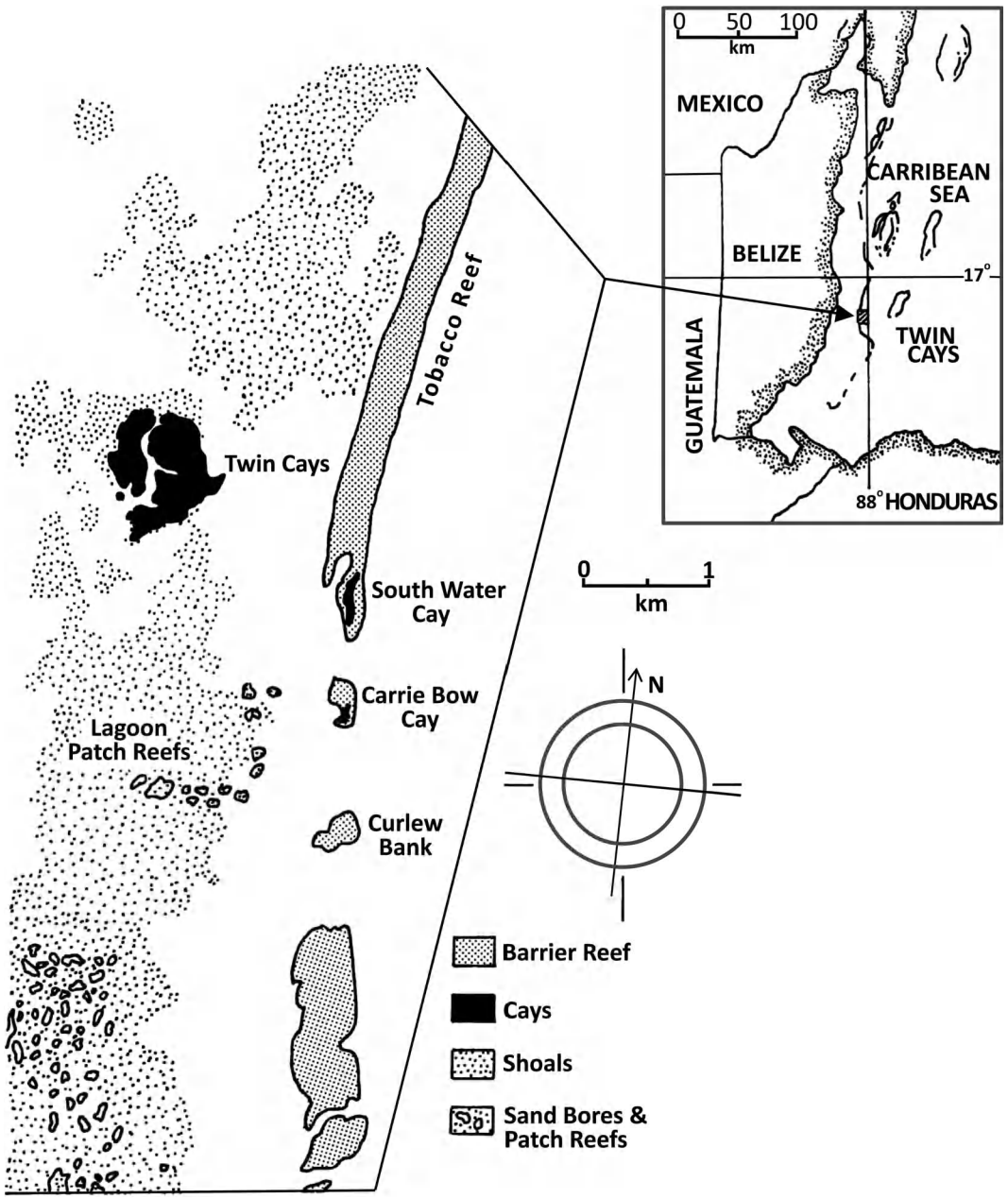

FIGURE 1. Location map of Twin Cays, Belize, Central America. (Adapted from Rützler and Macintyre, 1982.)

and in areas of greater tidal movement. Figure 3 is a photograph taken from the island interior showing the dwarf red mangrove in the foreground and the distant background of taller dense red mangrove growth that characterizes the island perimeter. Figure 4 provides a botanical rendering of the cross section of the scrub red mangrove, showing the relationship between mangrove foliage, stem and root structure, average tidal range, and hydrogeologic strata. It is to be noted that the typical low tide level is near the top of the organic ooze.

The mangroves of Twin Cays have developed on an ancient limestone plateau over the past 8,000 years (Macintyre et al., 2004). During this time 9–12 m of Holocene mangrove deposits have accumulated on the underlying limestone substrate and kept pace with rising sea level (Toscano and Macintyre, 2003; Macintyre

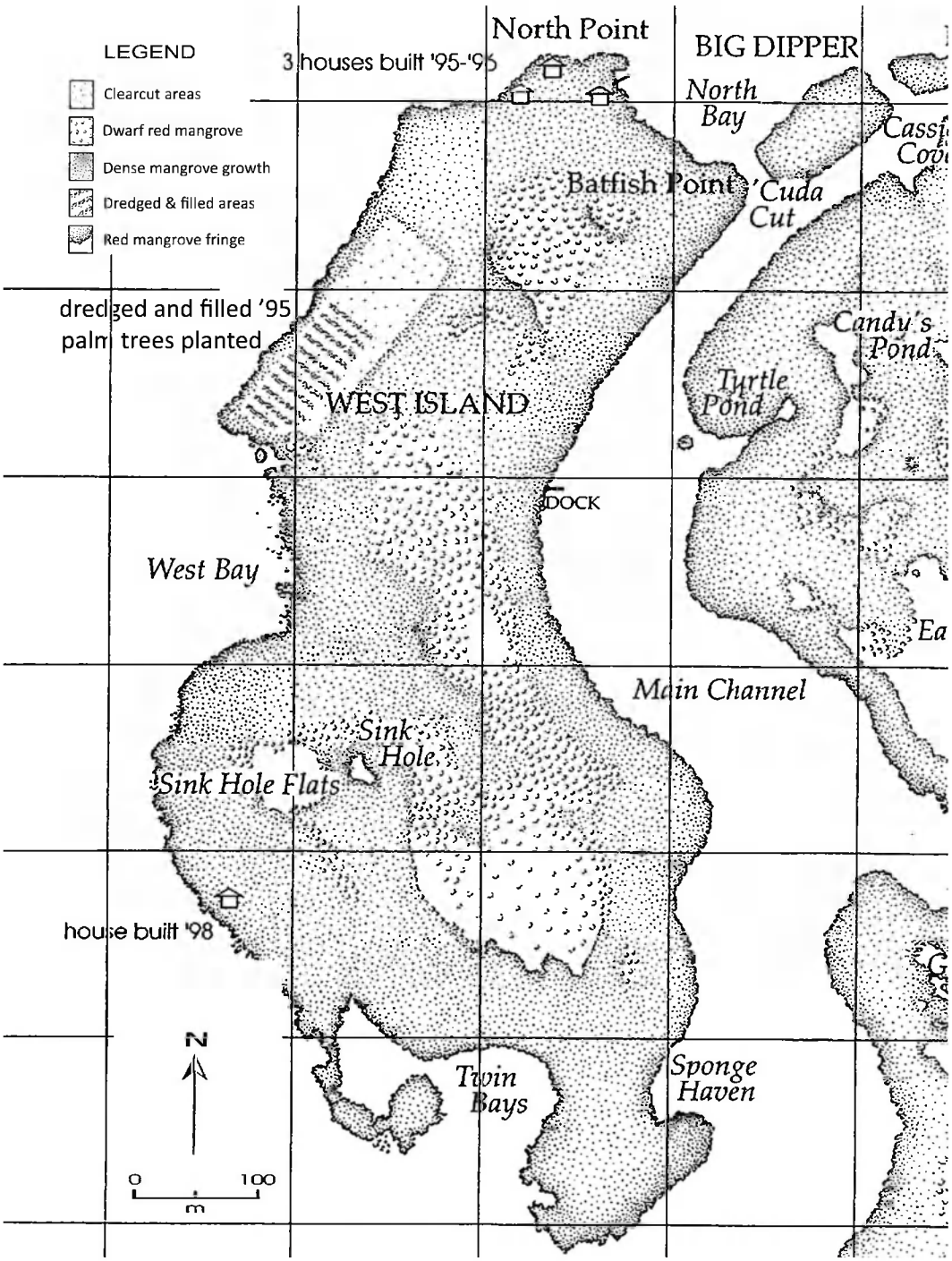

FIGURE 2. Land cover characteristics of West Island, Twin Cays, Belize, based on aerial photographs taken in 2002 that show mangrove density and clear-cut areas. (Drawn by Molly K. Ryan of the Smithsonian Institution in 2002.)

FIGURE 3. Photograph of West Island, Twin Cays, showing dwarf red mangrove of island interior with much more vigorous fringe red mangrove growth in distance along periphery of the island.

et al., 2004; McKee et al., 2007). Macintyre and Toscano (2004) found Pleistocene limestone at depths of 8.3 to 10.8 m below mean sea level in cores at West Island. As sea level rose to cover a subaerially eroded limestone plateau fringing the coastline, mangrove peat appears in the stratigraphic record. The highest topography of the island is on the seaward side where deposited sand is no more than 1 m above mean sea level. The limestone is now found at depths of 9 to 10 m below present sea level (Macintyre et al., 2004). The swamp bottom is composed largely of soft silty organic detritus. Exceptions of harder bottoms are found in the nearshore swamp channels where stronger tide-induced velocities have scoured the channel bottoms.

The climate of Twin Cays is marine tropical with air temperatures ranging during the year from 24°C in January to 29°C in June; humidity averages about 78% (Rützler and Ferraris, 1982). Lagoon water temperatures range from 23°C in the winter to 31°C in the summer. The microclimate of West Island, particularly that of the interior water, has a much greater range. The estimated annual precipitation at Twin Cays is about 1,885 mm, based on 4 years of complete records at the climatological station on Carrie Bow Cay, 4 km away. This precipitation is about 80% of the annual precipitation of the nearest mainland climatological station, the Melinda Forest Station, 30 km to the northwest. The monthly pattern is much the same for both stations. Hurricanes cause seawater to completely overwash the low-lying island. However, the natural mangrove ecosystem seems resilient and well suited to survival from natural events, viz. hurricanes. No significant adverse effects have been observed on Twin Cays; the same cannot be said for the response to man-made features, where mangrove clearing results in severe coastal erosion.

Tides in the lagoon area surrounding the island are microtidal with an average range of about 15 cm and are of the mixed semidiurnal type (Kjerfre et al., 1982). The tides exhibit semidiurnal high and lows with a tidal cycle periodicity of approximately 12 h and 25 min, but display a marked asymmetry with a large tide range following a smaller one. In some cases the larger range is as much as 40 cm, followed by a range of only 10 cm. At times the smaller range is so small as to appear nonexistent. In other cases certain components of the tide occur simultaneously and create a range as great as 50 cm. Once the tidal signal enters the tangled root system of the mangrove, the signal changes from a form that is approximately parabolic to a highly asymmetrical pattern, in which the rising limb of the flood tide is much steeper than the falling limb. Concurrently the amplitude is attenuated, and the highs and lows of the tidal signal lag the open lagoon tide. The spring–neap tidal cycle is about 29.5 days and can cause monthly tidal ranges that completely "dry up" the interior of the island.

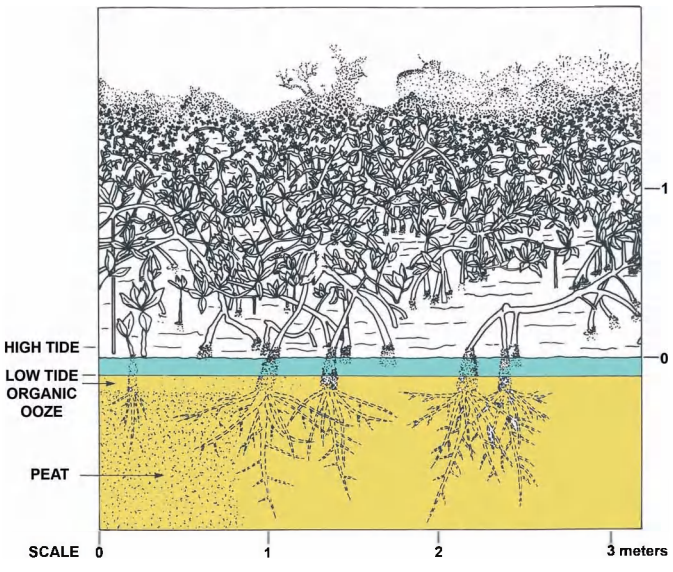

FIGURE 4. Botanical rendering of cross section of dwarf red mangrove showing relationship between mangrove foliage, stem and root structure, average tide range, and hydrogeologic strata. (Drawn by Molly K. Ryan of the Smithsonian Institution in 1989.)

METHODOLOGY

The information required for a study of the dynamic hydrology of West Cay encompassed both spatial and temporal data and a wide variety of methods. These methods included field surveying techniques for obtaining the island topography and bathymetry, automated water level recorders for water levels, automated temperature loggers, electromagnetic water current meters, conductivity meters for determination of water salinity, and aircraft for photographic recording of dye flows, among a host of lesser equipment and measuring devices that were employed over the study period of 18 years (1988–2006).

Topography

Topography was determined for the tidal flood region extending from open lagoon water at the west side of the island along the 350 m long channel and the southern interior pond (Urish et al., 2003; Wright et al., 1991). Some 36 semipermanent monitor locations were established in 1988 in the intertidal swamp to obtain water level and water quality measurements. The locations were marked with 2 cm diameter polyvinyl chloride (PVC) pipes driven into the ground in a grid pattern. Horizontal control was established by field measurement with a 35 m long tape and conventional level and transit surveying techniques (Wolf and Ghilani, 2006), later located with Global Positioning System (GPS) technology using a Garmin GPS 76. These data were later used for georeferencing of all island features (Rodriguez and Feller, 2004). Vertical control for land and water measurements was determined from a primary datum reference point on the east side of the island to which an arbitrary datum was assigned. The initial elevation assigned to this reference point was 3.05 m with all readings later adjusted to an approximate mean lower low water (MLLW) after several years of time segments of about 2 weeks; one long record of 9 months of tidal data was obtained. A datum lower than the typical terrestrial datum of "mean sea level" was used to maintain both topography and water level values positive to the extent possible.

Two principal surveying transects across the island were established: (1) from the lagoon to the bend in the channel along an east-to-west run including 6 points (F1 to A1) and (2) from the bend in the channel to the south pond along a north-to-south run of 12 more points (A1 to A12). In addition, 3 to 5 points were determined perpendicular to each transect point. These secondary points were spaced approximately 15 m apart. These established points, as located on Figure 5, were the primary location references for all subsequent data collection.

Automated pressure transducer water level loggers (In-Situ Environmental Data Logger Model SE 1000c with pressure transducer probes) were employed at five locations for short-term (1–2 weeks) measurements. These units were vented to automatically compensate for ambient atmospheric pressure. Later in the study period 12 of these locations became long-term monitoring stations with automated self-contained water level loggers (Remote Data Systems, Navassa, N. C.) that remained in place for as long as 12 months to record data at 30 min intervals with an accuracy of about 3 mm. Self-contained automated temperature loggers (Optic Stowaway by Onset Computer Corporation) were also deployed to record temperatures at 30 min intervals for as long as 9 months. In addition to the monitor locations, stilling wells consisting of slotted 15 cm diameter PVC pipe for both manual and instrumented tide measurements were established at both shorelines of the island, and later in the study these were correlated with a primary oceanographic/climatological data collection station established at the Smithsonian Research Station on Carrie Bow Cay, 4 km southeast. The tops of the stilling wells were initially assigned an elevation based on the same arbitrary datum as used at the key datum reference points. Elevations were established on the tops of all reference station pipes using survey leveling techniques with a Topcon Automatic Level (model ATF–1A). The coordination of tides at West Island and Carrie Bow Cay was accomplished by comparison of a series of six separate short-term tidal cycle measurements taken concurrently at both stations.

Hydrology

Water flow direction and velocities during various positions of the tide cycle were determined using conventional stream gauging techniques along channel cross-sections, or "reaches:" section A–A′ was defined between survey points A1 and D1 and reach B between survey points D1 and E1. The measurements were taken at various times during the tidal cycle using a Marsh-McBirney electromagnetic current velocity meter and standard stream channel cross-sectioning methods (Watson and Burnett, 1995). Velocities and water depths were measured at 0.6- to 1.5 m intervals perpendicular to the flow to provide 25 to 50 individual measurements at each cross section. These measurements were then plotted to determine flow volumes and flow friction factors and to examine trends.

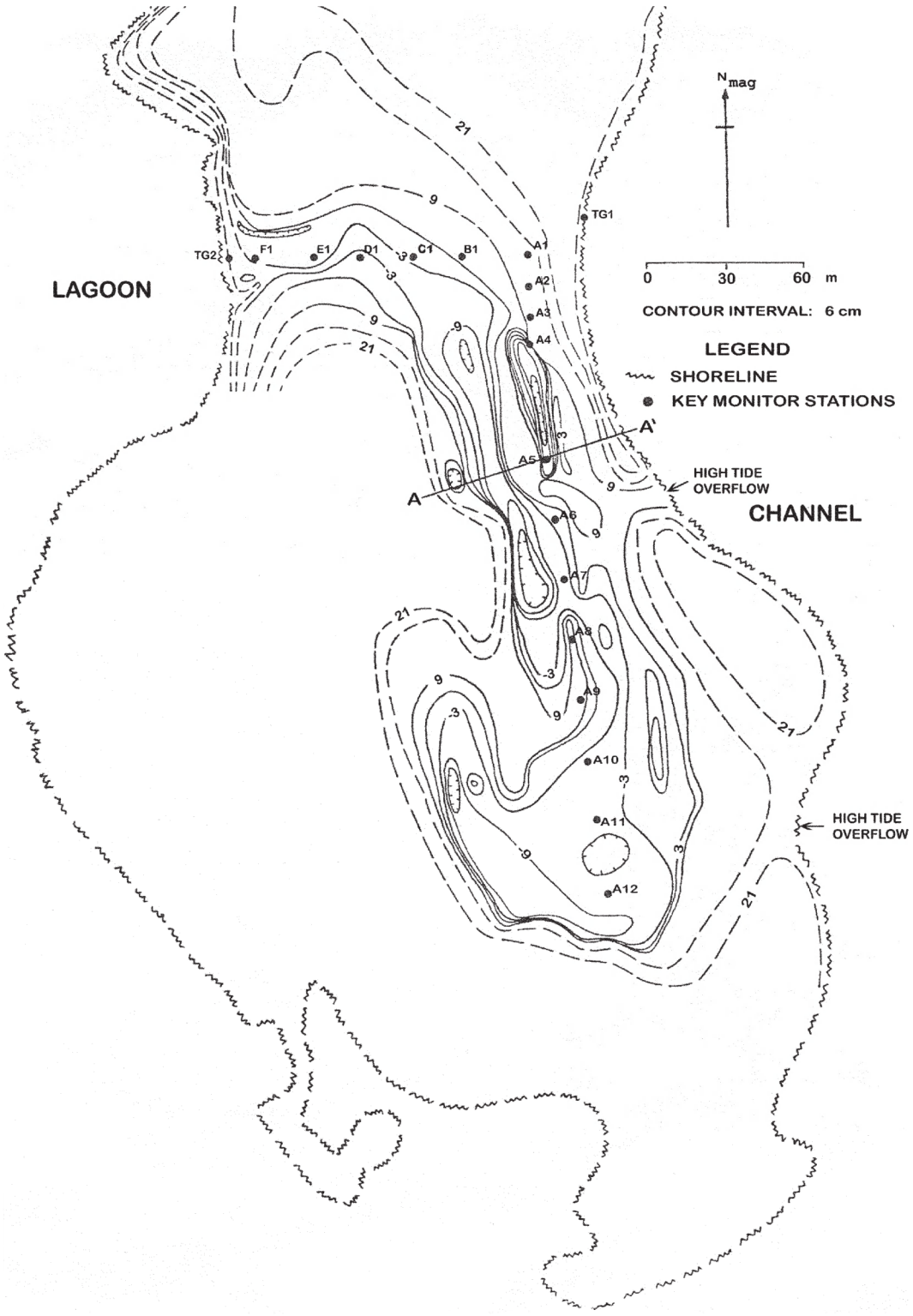

FIGURE 5. Topographic contour map of interior of West Island showing locations of key monitoring stations and representative channel cross section A–A′. Elevation datum = mean lower low water (MLLW). Contour interval = 6 cm.

Water salinity (±0.1 ppt), conductivity (±0.1 uS), and temperature (±0.1°C) were measured with a YSI 30 S-C-T (Yellow Springs, OH) in the field and in the laboratory. Salinity measurements were also made in the field using a refractometer (model 366ATC; Vista) with an accuracy of ±1 ppt.

Flow patterns were also observed and evaluated by use of dye studies, both at the surface water level and by helium balloon low-level photography in 1990 and 1991, and later by high-level aircraft photography during a tidal cycle in 1993. Although the balloon photography was only of limited value because upper air wind currents caused the balloon to drift off the island, aircraft photography was highly successful. Large targets, approximately 1 × 2 m in size, were marked and placed at each station for dye movement referencing. Continuing runs at 0.5 h intervals were made across the island on the same flight path at an altitude of 150 m. Photographs were taken during each run with a SLR camera with AF Zoom 35-70 mm lens (Minolta 5000 MAXXUM), thus enabling both the flow directions and dispersion within the mangrove system to be observed. Slugs of Rhodamine fluorescent dye, a highly visible but nontoxic dye, were placed at three stations at the start of the observation period. The dye remained highly visible during one tidal cycle. Continuing, but diminishing, levels of the fluorescence were measured in the laboratory on water samples taken during three subsequent tidal cycles. The series of photographs taken from the aircraft runs were reduced to a time sequence of plots and then used by George L. Venable of the Smithsonian Institution to produce an animated video of the dye movement for further study.

RESULTS

Topography

A topographic map of the intertidal flood zone region of West Island is shown as Figure 5. The highly irregular nature of the bottom is evident by inspection of the contour pattern. The region of tidal flow and flooded area is characterized by relatively flat areas with water depths frequently only about 25 cm over much of the flow system, but highlighted by sections more than 1 m in depth, such as occur between stations A4 and A5. Such deep holes are not necessarily coherent with the main flow channel. A cross-section (A–A′) plot (Figure 6) at station A5 depicts the extreme changes in channel bottom that exist at this location. In contrast, the other significant feature of the system is a very large shallow pond of about 2.2 ha at stations A10 to A12 at the south central part of the island. This region contains only sparse dwarf red mangrove with a flooded depth of about 0.25 m. Additionally, examination of the topographic map shows ground level at the east shoreline of this pond is about 6 cm lower than the rest of the island periphery. At high tides these limited lower topographic zones allow lagoon water from outside to enter the internal swamp flow system. In particular, high tide waters overtop the island perimeter at two other locations on the east side into the central region, causing short-term hydrological anomalies of temperature and salinity, as well as a somewhat irregular tidal signal, in the system.

Hydrology

Figure 7 is a five-day plot showing the typical tidal signal as it enters the swamp system at TG2. As the signal enters the mangrove system, the frictional resistance of the roots cause attenuation in tide amplitude as well as a time lag in the highs and lows.

The unique hydrological nature of the flow in the mangrove ecosystem is characterized by a very shallow water depth that averages only about 0.5 m at low tide to 0.67 m at high tide, although great variations exist. However, because of the very flat topography, even this low tidal fluctuation causes an extensive and significant hydroperiod of wetting and drying with important implications to the mangrove ecosystem. As depicted on Figure 8, the area typically covered by water during high tide is about double that covered by water during low tide. Doyle (2003) states that his controlled field experiments

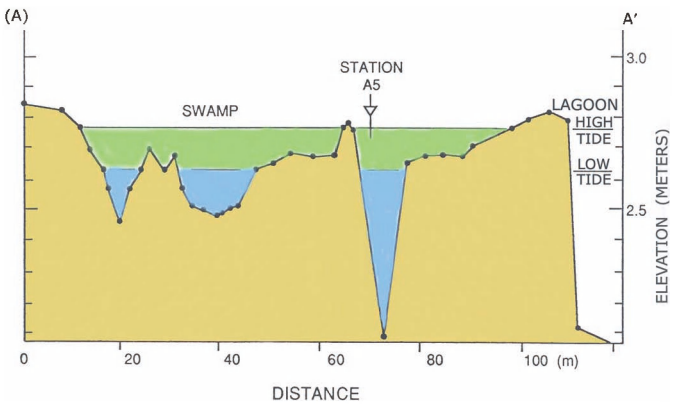

FIGURE 6. West Island cross section A–A′ at station A5 showing relative relationship of high and low tides to bottom contours. Green = range of flooding from the tide; blue = low tide flow. Elevation datum is arbitrary.

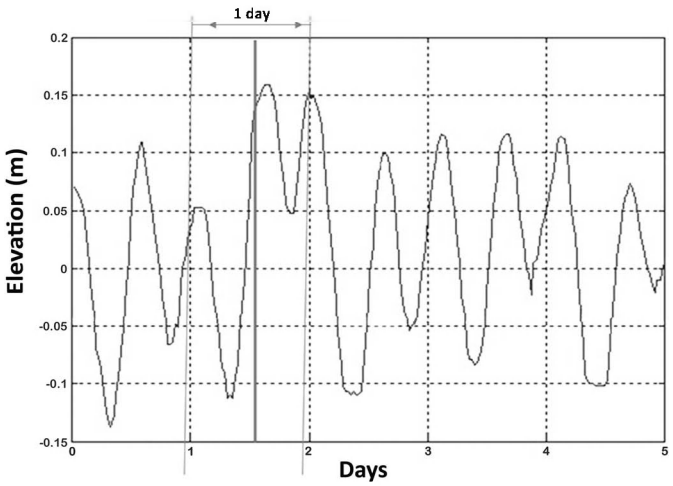

FIGURE 7. Plot of five-day tide sequence showing relative ranges and asymmetry of the tide at West Island. Elevation datum = approximate mean sea level.

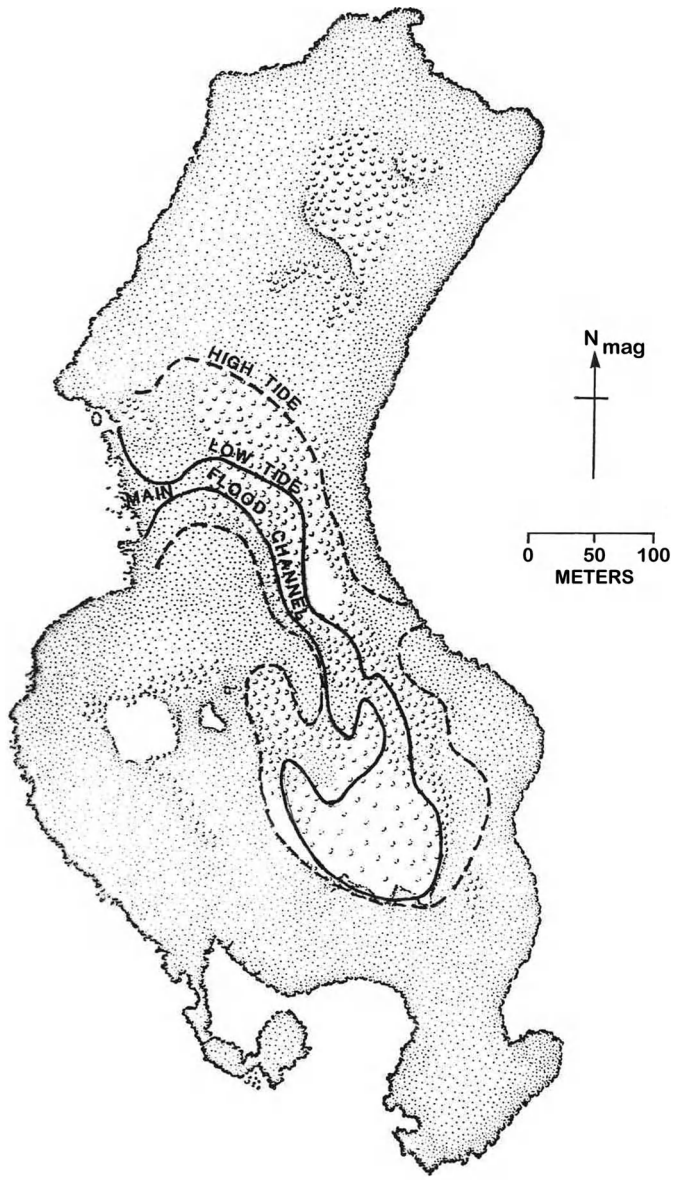

FIGURE 8. Plan of West Island showing aerial extent of tidal flooding between average daily low and high tides. Stippling on map shows relative density of vegetation. (Adapted from drawing by Molly K. Ryan of the Smithsonian Institution in 2002.)

"suggest that the hydroperiod—the rate and level of tidal exchange—plays a much more important role in determining mangrove growth and success than previously documented." Figure 9 presents a conceptual plot showing the wetting–drying cycle during the sequential phases of the tide. Perhaps even more important, the tide range is sufficient to cause reversal of flow direction and velocity throughout the system during each cycle.

Figure 10 shows the changing characteristics of the tidal signal at three stations as it moves inland in a tortuous path through the mangrove ecosystem. A tide range of 13 cm at the island margin is attenuated to 8 cm at a location 50 m landward and to 3 cm at a location 200 m landward in the main flow channel. Concurrently, there is a lag time of 1 h for high tide and 2 h for low tide at 50 m landward, and of 2 h for high tide and 6 h for low tide at 200 m landward. The great difference in lag time between highs and lows is caused by the much greater influence of root density during a receding tide; this is also illustrated by the asymmetrical characteristic of the tidal signal as it transposes landward.

The seasonal climatic variations had a profound effect on the monthly hydrological budget, especially when the high evapotranspiration was considered. Figure 11 shows the approximate seasonal relationships of precipitation, surface water evaporation, and vegetation transpiration (evapotranspiration), assuming a total annual rainfall of 1,885 mm. This value and the estimated monthly values are based on limited (about 5 years) available data that have been collected at Carrie Bow Cay and correlated with the longer-term record at the mainland Melinda Forest Station. The potential evapotranspiration values for each month were calculated from the Thornthwaite equation (Dunne and Leopold, 1978; Thornwaithe and Mather, 1987) using a partial record of temperature and solar radiation available for Carrie Bow Cay. Examination of the water budget shows a deficit of precipitation as

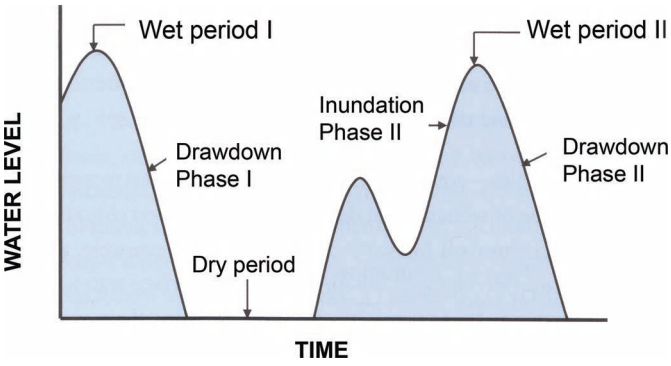

FIGURE 9. Conceptual plot of tidal phases during mixed semidiurnal hydroperiod event. (Adapted from Boulton and Brock, 1999.)

compared with evapotranspiration February through June during the "dry" season and a surplus during the "wet" season months from July through January. This "dry" season water deficit has an extreme effect on the surface water in the semienclosed interior swamp system, especially in the poorly flushed shallow pond at the south end of the island. Figure 12 is a composite plot of water temperature and salinity measured along the main flood channel over several years during the "dry" and "wet" seasons. Near the inflowing/outflowing location at station F1 the values approach those of lagoon water at the periphery of the island, but in the shallow pond the value ranges are much more extreme, with salinity ranging from 5 to 45 ppt and temperatures ranging from 25°C to 40°C. Some temperature and salinities even exceed these values in particularly isolated locations.

The elevations of the water surfaces and velocities at three stations within the first 150 m inland from the shore, as compared with the primary tide signal at shore station TG2, are shown in Figure 13. It is to be noted that at all stations the maximum velocities occur during the middle of the falling tide, with the highest velocities found nearest shore. This pattern is comparable with the tidal asymmetry and velocity patterns found by Bryce et al. (2003) in mangrove creek systems in Australia. The distribution of flow and variations of velocity for a typical channel section are illustrated in Figure 14 for a cross section at station A4. The data were acquired during a mid-tide flood tide at a time of maximum velocity. The upper part of Figure 14 shows depths of water across the section at the specific time of the velocity measurements shown in the lower part. The velocities shown are an average determined from a series of velocities measured at a series of depths over the shortest time period possible. As indicated the velocity changes dramatically, from 2.0 to 0 cm/s, across the section, although there is a general pattern of greater velocity at the deeper parts. However, this is contradictory to the observation that the deeper part on the right side does not

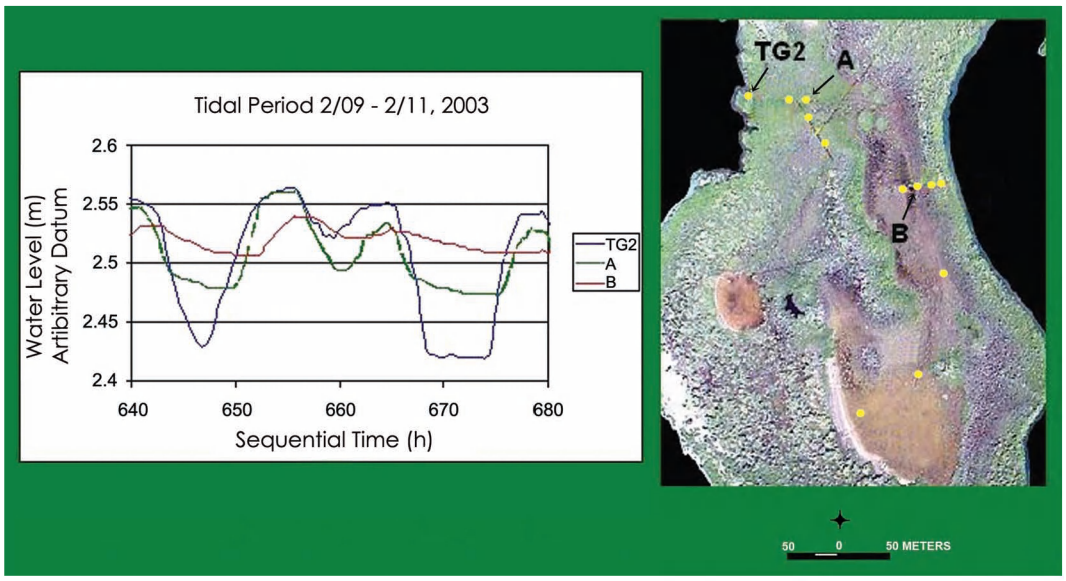

FIGURE 10. Tidal fluctuation plots at three interior monitoring stations—TG2, A, and B, as shown on photograph at right—during maximum velocity of flood tide on 27 May 1988. Yellow dots on the photograph are locations of monitor stations. Elevation datum is arbitrary.

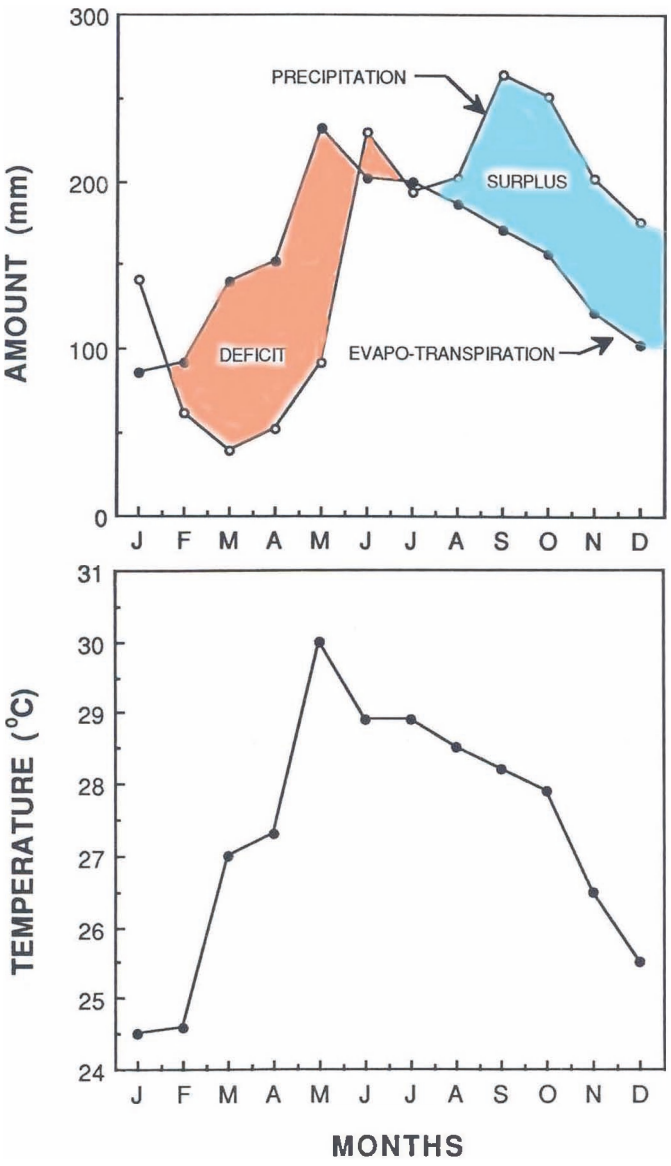

FIGURE 11. Hydrological budget for West Island showing annual pattern of precipitation, evapotranspiration, and temperature. Shaded orange area = the "dry season" with net deficit of water from evapotranspiration; blue shaded area = the "wet season" with a surplus of precipitation.

have a high velocity. Inspection of the topographic plan (see Figure 5) provides the explanation: The deeper right-hand feature is a part of a closed depression, whereas the deeper part on the left is continuous with the main channel flow.

The average flow velocity between points in the swamp is reflective of both the tortuous path of flow through the mangroves and the frictional resistance of the mangrove root system and the channel bottom. This resistance can be quantified by inverse calculation of the Manning equation for stream flow (Watson and Burnett, 1995). Although the Manning equation was originally developed for stream flow, it has a logical deterministic relationship that has been used successfully by other researchers for mangrove flow characterization and modeling (Wolanski et al., 1980). The Manning equation in MKS unit format (Lindsley and Franzini, 1979) is

$$V = 1/\mathrm{n}\, R^{2/3}\, S^{1/2},$$

where V is the average velocity, n is the Manning roughness coefficient, R is the hydraulic radius (cross-section area divided by wetted perimeter), and S is the slope, or hydraulic gradient, of the water surface.

Manning's roughness coefficient, n, was determined at various locations in the flow system and at various times in highly fluctuating stream depth and current direction. The determined values of n for these measurements ranged

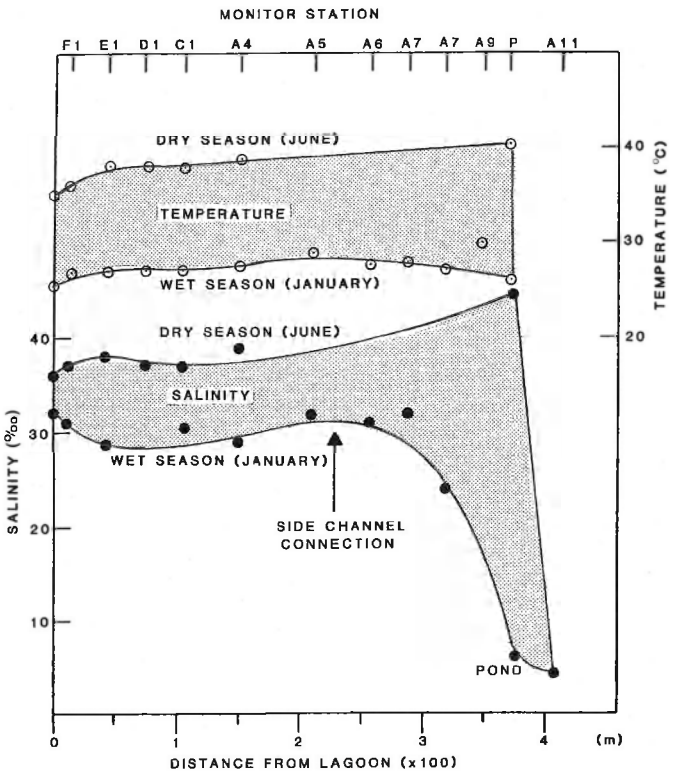

FIGURE 12. Plot showing ranges of salinity (black dots) and temperature (circles) during "dry" and "wet" season conditions at the southern shallow pond on West Island. Monitoring stations (see Figure 5) with distances from the open water lagoon at the west periphery of the island are indicated.

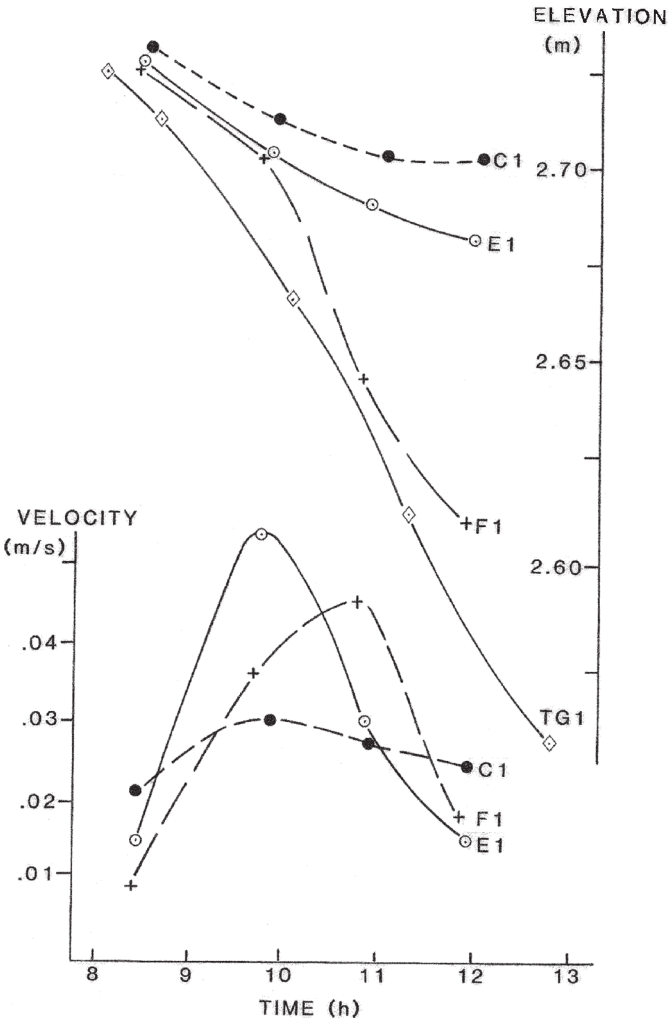

FIGURE 13. Plot of relative elevations (arbitrary datum) and velocities during falling limb of tide at monitor stations C1, E1, and F1 (see Figure 5).

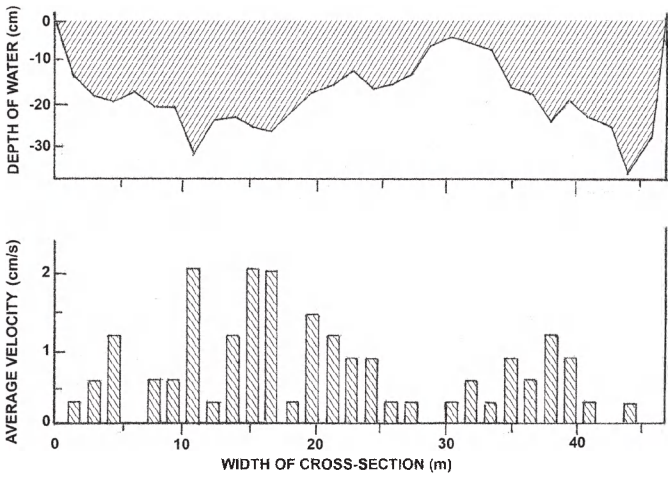

FIGURE 14. Water depths and velocities at cross-section A–A′, at monitoring station A4 (see Figure 5) during a flood tide with maximum velocities, on 27 May 1988.

from 0.084 to 0.445, far higher than the typical 0.035 found even for natural channels with stones and weeds. These values however are comparable to those ranges of 0.2 to 0.7 determined by Wolanski et al. (1992) for flow in southern Japan mangrove systems. Based on earlier studies, Wolanski et al. (1980) had earlier suggested that n is of the order of 0.2–0.4 in mangrove swamps. Table 1 shows the parameters and results for calculation of the Manning coefficient for section A–A′, with depth and velocity characteristics as depicted in Figure 14. It is to be noted in Table 1 that the value of n is greatest at shallow water depth and lower velocities. Again, this is comparable to the findings of Wolanski et al. (1992).

TABLE 1. Summary of hydraulic parameters for cross-section A–A′ at Station A4 (see Figure 5).

Observation [a]	Average water depth (cm)	Hydraulic slope, S (m/m)	Average velocity (cm/s)	Flow (m^3/s)	Manning's coefficient, n
1	11.9	0.000117	0.6	0.034	0.415
2	13.4	0.000110	0.6	0.037	0.445
3	14.3	0.000102	1.3	0.085	0.206
4	14.6	0.000098	1.1	0.069	0.261
5	16.4	0.000086	1.3	0.097	0.210
6	20.3	0.000055	1.6	0.145	0.159

[a] Each observation with the associated calculations is based on 30 measurements across cross-section A–A′ as detailed in Figure 14.

A dye flow study was accomplished during a high tide period on 5 June 1993. Single slugs of Rhodamine fluorescent dye, which produced a distinctive red color, were placed at three locations (monitor stations D1, A6, and A10; see Figure 5) along the main channel early in the morning. Large visual targets were placed at the monitor stations in the mangrove swamp to enable referencing the dye positions during movement.

The movement of dye was documented by aerial photography from an aircraft flown in a fixed flight pattern, and at a fixed altitude of 150 m (500 ft.), Runs were made at 0.5 h intervals. Figure 15 is a series of drawings made from 11 of these runs, depicting the leading edge of the dye with time. The 7 h period of measurement relative to the position of the tide at the exterior lagoon is depicted on the inset tide plot of Figure 15. Figure 16 is a high oblique photograph of West Island taken from an altitude of about 600 m (2,000 feet) showing the position of the dye at 9:10 AM, shortly after high tide. The aircraft run pattern starts over monitor station TG 2 and progresses east, turning south to proceed over the large pond at the south end of the island. The series of photographs have been converted to an animated visual program by George L. Venable of the Smithsonian Institution (URL http://www.uri.edu/cve/dye.mov) that clearly shows the oscillation of the water of the mangrove swamp water as the dye at station D1, some 70 m from the lagoon, first went to the east, then reversed to finally discharge into the lagoon. The dye flow at station A6 also oscillated, then merged with the outgoing dye from station A10 approximately 120 m south of A6, toward the pond. Interestingly, the dye placed at A10, at the north margin of the pond, also moved into the pond and then flowed in a circulatory pattern. This pattern may be caused by new lagoon water overflowing the rim of the pond to the east because the tide during the period of observation was a relatively high spring tide. Finally the dye from D1 discharges into the lagoon, and the merged A6/A10 dye moves north. Previous water level studies with measured levels of fluorescent dye indicated that this dye persists at continuing reduced levels in the central locations

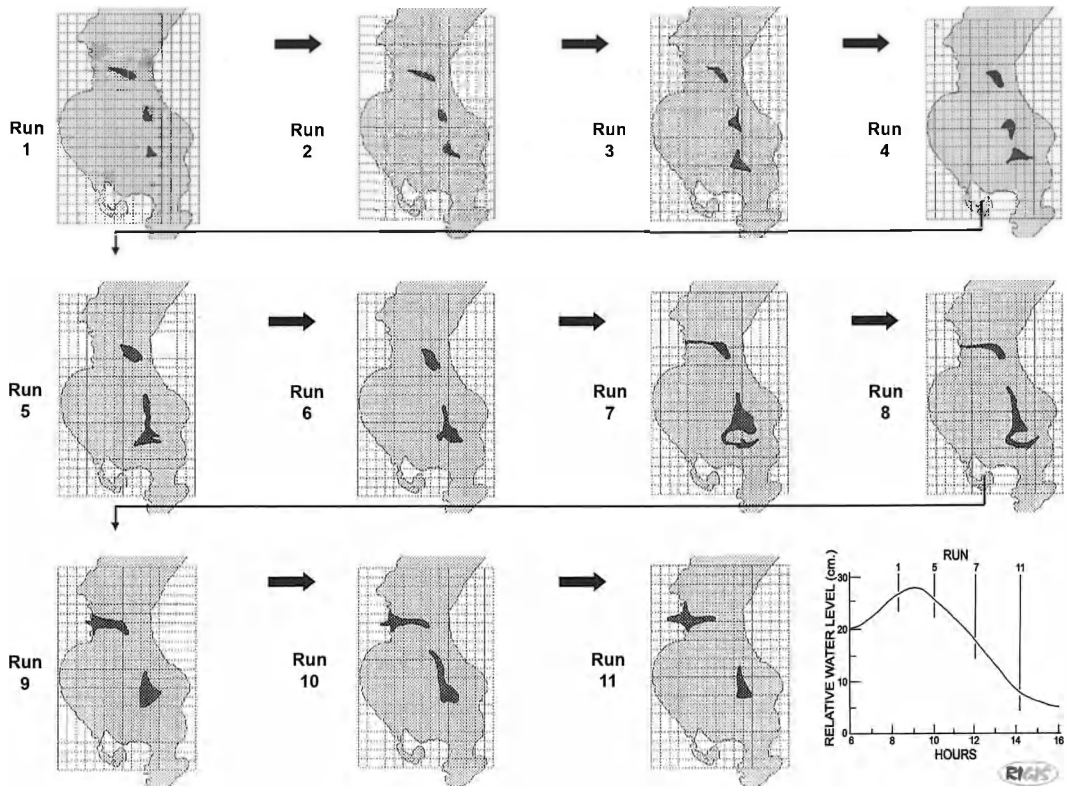

FIGURE 15. Sequential plots of dye flow patterns over a 7 h period from high tide through low tide, during dye flow test on 5 June 1993. The plot in the lower right-hand corner shows the relative time position of runs with tide levels at station TG2.

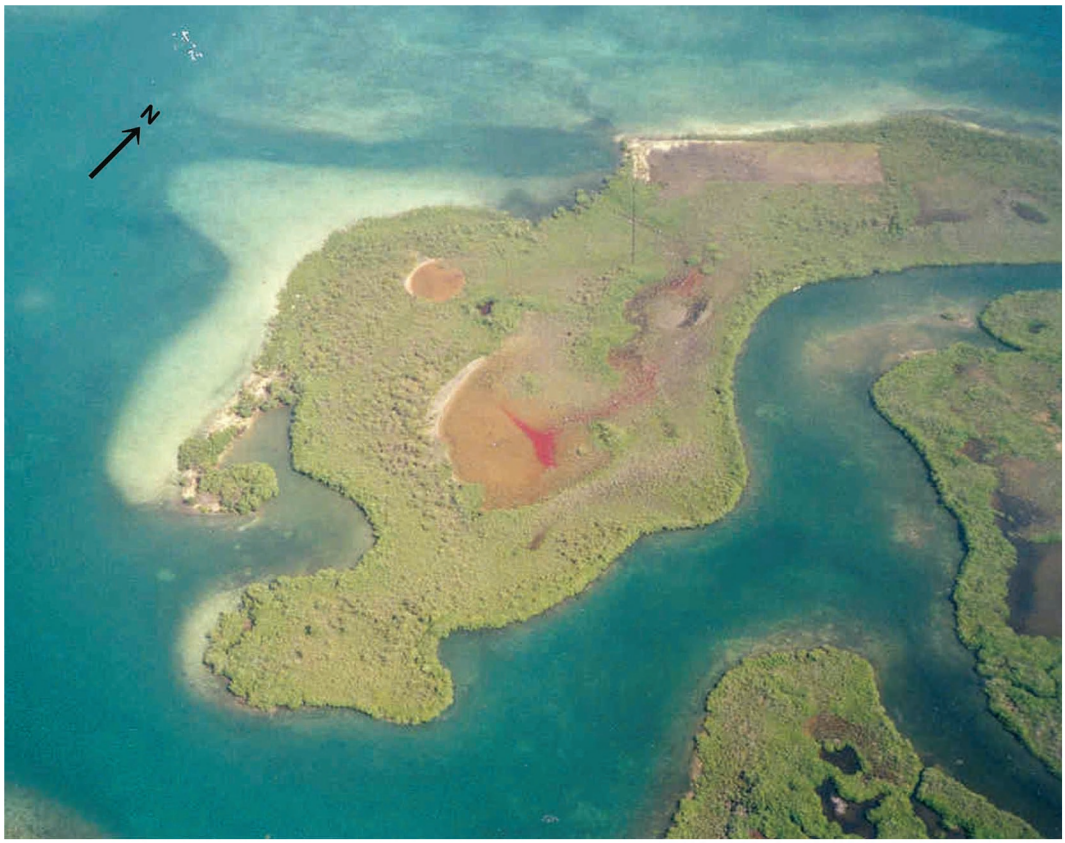

FIGURE 16. Aerial photograph of West Island looking to the northwest from an altitude of 600 m during the 5 June 1993 dye flow test, corresponding approximately to the time of run 4 of Figure 15. The dye is evident as the red configuration in the south pond.

for several tidal cycles before finally being flushed and dissipated into the lagoon.

DISCUSSION

An intimate knowledge of the topography of the flow and flood area is essential to an understanding of the ecosystem of a semiclosed mangrove hydrosystem such as West Island. The interior water system of West Island is primarily tide-induced flow, with modifications caused by precipitation and seasonal climatic change. Hence, it is also very important that the climatic factors be considered in conjunction with the hydrographic characteristics.

The tidally induced hydrodynamics of the water flow in tidal channels and ponds of an overwashed mangrove island, in conjunction with the topography, greatly affect the ecosystem and vitality of the resident mangrove systems. Analysis of the temporal and spatial characteristics of a 21.5 ha island hydrological flow system shows that where flooding and current flow becomes more vigorous, the growth of the red mangrove is enhanced. It was found, during the 18-year period of this study, that hydrological changes such as increased tidal flow, from anthropogenic as well as natural causes, enhanced the growth of the red mangrove. On the other hand, observations of relict tree remains indicate that historically the interior of the island experienced a transition in mangrove species from black mangrove to dwarf red mangrove, possibly because of higher water levels and with poor flushing in the island interior. This concept is in concurrence with findings of Knight et al. (2008) in studies of patterns of tidal flooding within a mangrove forest in Southeast Queensland, Australia, that "mangrove basin types represent a succession in mangrove forest development that corresponds with increasing water depth and tree maturation over time."

Detailed mapping of the topography of the intertidal interior region of the island reveals poorly defined flow

channels that vary greatly in depth and width. Within this system anomalous deep sections exist, further contributing to the complexity of flow. The root structure of the mangroves and the irregular bottom result in a frictional resistance to the flow, quantified by Manning's n as being as much as 10 times greater than that of conventional terrestrial stream channels. In studies in mangrove swamps on Iriomote Island, Japan, Kobashi and Mazda (2005) stress the importance of the hydraulic resistance of mangrove vegetation in determining the flow patterns, especially in reducing the velocity component perpendicular to the main channel. Accordingly, the interaction of the mangrove itself is a determinate factor in stream flow and the resulting flushing action, important to the vitality of the mangrove. It appears to be particularly relevant to the transport of nutrients and other physicochemical conditions important to the growth of the mangrove. The driving force for the flow within the mangrove hydrosystem is the ever-changing hydraulic gradient induced by the tide. Accordingly, the flow moves, at varying velocities, in and out of the interior mangrove swamp with the tide. As a result the seawater entering the mangroves not only follows a constantly changing path, but is regularly reversed in direction, and consequently it takes at least several tidal cycles for flushing of the island interior. There are indications that the central part of West Island is flooding more over a span of years, causing commensurate changes in the mangrove types capable of surviving in the changed regime, a process described by Knight et al. (2008). In other areas the geomorphology of the land is changing consequent to sediment transport, detrius deposition, and subsidence from peat compaction. In this regard Bryce et al. (2003), in studies of a small mangrove creek system near Townsville, Australia, evaluated the role of hydrodynamics in the sediment transport process. Importantly they observed that sediment transport appears to be a seasonal phenomenon, with net flux going either landward or seaward, but they conclude that the net sediment transport for the overall system may be close to long-term equilibrium. They do state that mangrove swamp areas (in the tidal overflow regions) are most likely to be places of sediment accumulation; if so, the more shallow areas of West Island, when flooded at high tide, may experience accumulation from redistribution of sediment within the system as well as from direct leaf drop and in situ detritus accumulation.

The data showed that the annual pattern of precipitation and temperature greatly affects temperature and salinity in the poorly flushed interior pond. On an annual basis there was a net discharge of water from island to the exterior lagoon because of precipitation. However, when the monthly climatic factors are considered it is apparent that during the "dry period" of February to May there is a net loss of freshwater in the island water budget, with high evaporation creating high-temperature hypersaline water in the interior. When the island is in the rainy season, July through December, the reverse is true, with the interior water becoming cooler and fresher from the rains (see Figure 12). In the extreme case, as described by Wolanski et al. (1992) for tropical mangrove systems on the coast of northern Australia, "The balance between rainfall and evaporation, in conjunction with tidal variations, is the key factor in determining if the upper levels of the swamp are (tidally flushed) swamp or (hypersaline) tidal flat." A further important implication for the shallow pond in the south part of West Island is, as stated by Wolanski et al. (1992), "rainfall significantly affects porewater salinity and it is likely that it also affects nutrient levels within the swamp substrate, particularly in areas where regular flooding by the tides does not occur." On West Island, during the "dry period" of February through May, evapotranspiration is approximately three times that of precipitation. However, during the "wet period" of June through December, conditions are reversed with evapotranspiration being approximately one-half that of precipitation (see Figure 11). Thus, the net effect on the poorly flushed interior areas of the West Island mangrove system is that of greatly increased salinity during the "dry season" and short periods of nearly fresh water from rain storms in the wet season (see Figure 12).

The effects of human intrusion into the natural ecosystem are illustrated by Figure 17, an aerial photograph showing the survey lines newly cut in 1993. At that time the strongest flow from the coastal seawater was some 25 m south of the west–east running survey cut, as identified by the darker, more vigorous vegetation. By 2003 the main flow had moved north to the survey cut itself as the cutting as well as foot traffic in the cut had deepened that area. During the course of the investigations, it was observed that the previous dwarf red mangrove trees alongside these survey lines initiated signs of vigorous growth as a result of the increased flushing, as illustrated by the photograph in Figure 18.

Although previous studies have shown that for the past 8,000 years the mangrove growth has managed to keep up with rising sea level because of peat accretion, the future may be in doubt because of anticipated greatly increasing sea-level rise rates (Mckee et al., 2007). The ability of the island to adjust to rising sea level has important implications for a future that will include sea levels rising

FIGURE 17. Aerial photograph taken in 1993 (looking south) showing survey lines that were newly cut in 1993. The original principal tidal flow path is evident as the darker green vegetation approximately 30 m south of the 1993 east–west survey line.

at a rate much greater than that experienced over the past 8,000 years when mangroves first appeared and flourished on Twin Cays. As McKee et al. (2007) have stated, "Rates of subsurface plus subsurface (root) accretion in fringe, transition and interior zones at Twin Cay were 10.4, 6.3, and 2.0 mm/year. Fringe mangroves have kept up and could accommodate eustatic sea level rise of 4 mm/year if current rates of accretion were maintained. If eustatic rates exceed 5 mm/year then these mangrove islands would not be likely to persist, assuming all other conditions remain unchanged."

The islands of Twin Cays, with a history of comprehensive ecological research, remain an important location for measuring and evaluating changes in the mangrove and associated ecosystems because they occur in a world of dramatic coastal change. Much analytical work remains to link the dynamic hydrology of the mangrove island to the physiological parameters essential to mangrove growth. The research site of Twin Cays, with three decades of baseline data and research, is a very important asset for better understanding the ecosystem of the mangrove. It is very important that this work continue and build on the substantial foundation of information that now exists.

CONCLUSIONS

Overwashed mangrove islands are extremely complex ecosystems. They are essentially self-dependent, and the vitality of the resident mangrove species is primarily a result of the tide that produces the essential hydrological functions of flushing and nutrient transport. The topography, the geomorphology, and even the existence of a mangrove island are products of the island vegetation itself. This interaction affecting the island configuration is constantly changing as the mangrove forest with its multiple species adjusts to higher sea levels and the resultant changes in hydrological flow and flooding parameters.

The interior of the island is subject to extremes of temperature (20°–40°C) and salinity (5–45 ppt) with limited flushing that may adversely affect the vitality and existence of the mangrove, as well as the natural selection of mangrove species. A comparison of the hydrological parameters and flow regimes in the regions of vigorously growing red mangrove with that of dwarf red mangrove strongly suggests that enhanced communication with external lagoon water is best for the vitality of the red mangrove on Twin Cays.

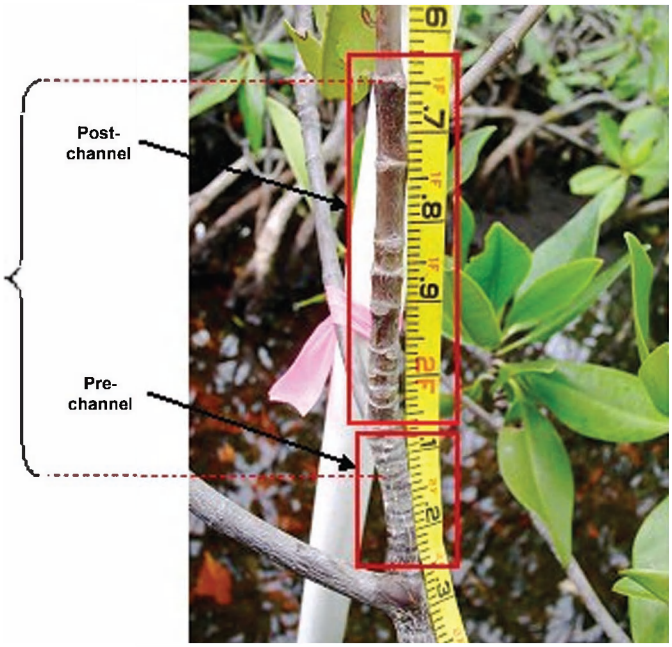

FIGURE 18. Photograph of red mangrove branch taken in 2003 near monitoring station D1 (see Figure 5) showing progressive increase of growth between sequential growth rings after survey lines were cut approximately 10 years previously. Note that the tape is marked decimally in feet.

The flow within the island is strongly influenced by the substantial frictional resistance of the mangrove root system. This dense root system serves to greatly attenuate the tidal amplitude as it progresses into the island, creating a reduced hydraulic gradient for water movement. The resultant reduced flow creates a poorly flushed island interior with poor mangrove growth.

Extensive land clearing, especially along the coastal margins, has long-term continuing effects of mangrove loss from which the island may never recover (Macintyre et al., 2009). In contrast, limited incursions such as the observed survey line cutting may shift, but enhance, channel flow, promoting more vigorous red mangrove growth. In extensive field research (Feller et al., 2003), it was found that the patterns of nutrient availability within and among mangrove ecosystems are complex. Feller et al. (1999) showed the dramatic effects of nutrient enrichment on mangrove growth as well the changes in nutrient limitations that can take place within relatively short distances in swamp ecosystems. At least in the case of the nutrient-poor (P-limitation) condition of the sparse red mangrove in the interior of the island, the cause of nutrient limitation seems to be poor flushing, which limits the refreshing of the system with phosphorus-rich lagoon water from tidal flooding.

A further concern is that of the effect of rising sea level on the ability of the mangrove to survive. The hydrodynamics of the mangrove system greatly influences the mangrove ecosystem both by transport of nutrients and sediment and by the direct ability of the geomorphology of the island to develop to keep pace with rising sea level as it has in the past (McKee et al., 2007). At the least it appears that differential growth of mangroves will occur as flooding occurs and the hydrodynamics of the system changes.

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Ecological Characteristics of *Batis maritima* in Florida and Belize

Dennis F. Whigham, Michael C. Whigham, Ilka C. Feller, Wilfrid Rodriguez, and Ryan S. King

ABSTRACT. *Batis maritima*, a low-growing perennial species with woody stems and succulent leaves, occurs in mangroves and, to a lesser degree, in salt marshes in the Neotropics. It spreads by clonal growth, occurs in a wide range of habitats, and at times forms monotypic stands. Sites that are permanently flooded or are flooded regularly by tides and salt pans are the only mangrove habitats in which *B. maritima* does not occur or occurs as a few scattered plants. On mangrove-dominated islands in Belize, the coverage and height of *B. maritima* were highest in open habitats, including sites disturbed by human activities. In a mangrove-dominated mosquito impoundment in Florida, *B. maritima* occurred in all habitats sampled and, similar to observations in Belize, coverage and height were greatest in the most open habitats. The abundance and, at times, dominance of *B. maritima* suggests that it may play an important role in the dynamics of mangrove ecosystems, especially in the recruitment and establishment of mangrove seedlings. Mangrove seedlings and saplings were present in most of the plots that were sampled in Belize and Florida, but there was no relationship between the percent cover of *B. maritima* and the density of seedlings and saplings.

INTRODUCTION

Batis, the only genus in the family Bataceae, has two species. *Batis maritima* L. occurs in the Neotropics in coastal salt marshes and mangroves from Georgia and Brazil on the Atlantic coast and California to Peru on the Pacific Coast of North and South America. The species is widely distributed in the Caribbean basin. The second species, *Batis agrillicola* P. Royan, is endemic to coastal areas of northern Australia.

Batis maritima, a low-growing C_3 perennial species with woody stems and succulent leaves, is associated with saline soils and has been described as a species that responds to disturbance in mangroves and salt marshes (Rey et al., 1990; Pennings and Richards, 1998; Pennings and Callaway, 2000). An important ecophysiological characteristic of *B. maritima* is the ability to adjust photosynthetic rates to increasing soil salinity by making adjustments to leaf sap osmolalities (Lüttge et al., 1989). The ability to propagate clonally (Pennings and Callaway, 2000) is another characteristic that enables it to respond rapidly to altered environmental conditions.

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Despite its widespread distribution, there have been relatively few ecological studies of *B. maritima*. In Georgia salt marshes, Pennings and Richards (1998) found a positive relationship between the presence of wrack (accumulated litter) and the abundance of *B. maritima*. Pennings and Callaway (2000) found that clonal integration was an important factor in the ability of the species to colonize bare salt pans. The responses of *B. maritima* to altered hydrological conditions appear to vary with differing environmental settings. In hypersaline coastal wetlands in Texas, *B. maritima* cover expanded following inundation with freshwater (Alexander and Dunton, 2002). Conversely, the opposite occurred in Baja California (México) where its cover increased following the construction of a dike that eliminated tidal flooding and increased soil salinity (Ibarra-Obando and Poumian-Tapia, 1991). *Batis maritima* cover was also dynamic in mangroves in Florida that were impounded for mosquito control. The cover of all herbaceous halophytic species, including *B. maritima*, decreased following the construction of dikes and the subsequent impoundment and flooding of mangroves in the Indian River (Rey et al., 1990). Several years later, when tidal exchange between the impoundment and estuary was restored, *B. maritima* recolonized areas that were no longer flooded continuously. Another important ecological feature of *B. maritima* is its inability to tolerate prolonged periods of shade in mangrove-dominated wetlands (López-Portillo and Ezcurra, 1989). Along Florida's Gulf Coast, Milbrandt and Tinsley (2006) observed a greater number of black mangrove (*Avicennia germinans* (L.) Stearn) seedlings in existing *B. maritima* patches compared to surrounding mudflats. They hypothesized that this improved seedling success was the result of a slight increase in elevation provided by the *B. maritima* root system. In contrast, McKee et al. (2007) found that on offshore islands in Belize *B. maritima* did not appear to have an effect on recruitment of red mangrove (*Rhizophora mangle* L.) seedlings.

Other than the experimental research on coastal salt marshes (Pennings and Richards, 1998; Pennings and Callaway, 2000), little is known about the ecological role of *B. maritima* in coastal wetlands, especially in mangroves where it most frequently occurs. Is it a fugitive species that only persists because it is capable of responding to changing environmental conditions? Alternatively, is it an important species in mangroves because of its impact on patterns of nutrient cycling or its ability to influence the establishment of mangrove trees (i.e., *R. mangle*, *A. germinans*, *Laguncularia racemosa* (L.) Gaertn. f. [white mangrove], *Conocarpus erectus* L. [buttonwood])? Although *B. maritima* is a common component of mangrove forests throughout the Neotropics, there is limited knowledge on distribution patterns within the intertidal landscape or on the ecological roles of this species across a range of mangrove habitats.

Our objective was to describe the distribution of *B. maritima* in Florida and Belize as part of our overall goal to determine its ecological role in mangrove ecosystems. Here we describe our initial efforts to characterize the ecology of *B. maritima* at two of the Smithsonian's long-term research sites (Figure 1) that also represent the range of conditions (subtropical and tropical) where this species associates with mangroves. For Florida (subtropical), we focus on *B. maritima* in four habitats in a mangrove-dominated impoundment along the Indian River Lagoon (IRL) that has a history of intervention for purposes of mosquito control (Rey et al., 1990). For Belize (tropical), we focus on *B. maritima* in disturbed and undisturbed sites on offshore mangrove islands. For both sites, we also present data on the relationships between percent cover of *B. maritima* and the density of mangrove seedlings.

STUDY SITES

Belize

Twin Cays is the focus of our *B. maritima* studies in Belize. Twin Cays (91.5 ha) is an archipelago of peat-based mangrove islands (Figure 1) located near the crest of the barrier reef of central Belize. These islands are located approximately 17 km east of the mainland, and the only source of freshwater is precipitation. Vegetation on Twin Cays is dominated by the mangroves *R. mangle*, *A. germinans*, and *L. racemosa*. The forest structure is heterogeneous and characterized by gradients in hydrology and tree height that include a seaward fringe of *R. mangle* around the periphery of the islands, along tidal creeks, and in perennially flooded ponds (Feller et al., 1999). *Avicennia germinans* and *L. racemosa* primarily occur in habitats that are not water covered at low tide. Vegetation patterns on Twin Cays are complex, and the dynamics have been the focus of many studies (Feller, 1995; Feller and McKee, 1999; Rodriguez and Feller, 2004; Lovelock et al., 2006a). However, none of the previous research has focused on the distribution or ecology of *B. maritima* even though it occurs in almost all habitats except those that do not experience prolonged flooding (D. Whigham, personal observation). Human activities have altered parts of Twin Cays (Rodriguez and Feller, 2004; McKee et al., 2007), and the primary anthropogenic activity has been the clearing

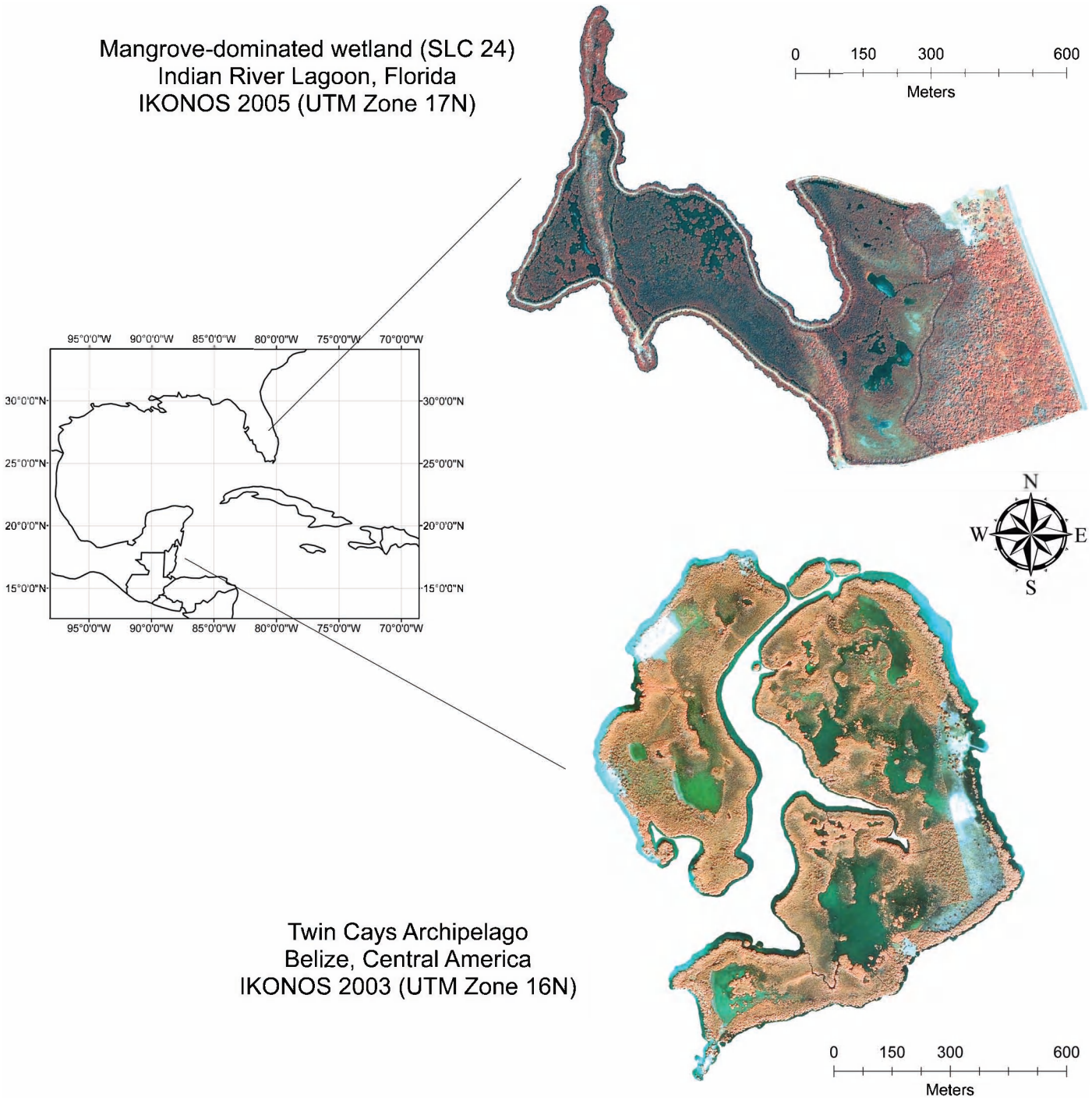

FIGURE 1. Approximate locations of SLC24 and Twin Cays (inset map) and IKONOS images of the two study sites. For SLC 24, the white line that is seen around the impoundment is a dike. Dark areas within the impoundment are dredged from adjacent subtidal habitats. Darker areas on the two large islands are internal tidally influenced ponds that are most often shallowly water covered.

of mangroves with or without the addition of sediments dredged from nearby subtidal habitats. In this study, we compared the distribution of *B. maritima* in disturbed and undisturbed mangrove habitats at Twin Cays (described in further detail below).

Florida

An impounded, mangrove-dominated wetland (SLC 24) in St. Lucie County in the IRL is the focus of the Florida studies (see Figure 1). SLC 24 has been managed in a variety of ways since it was diked in 1970. Rey et al. (1990) describe management activities and patterns of vegetation change in SLC 24 between 1970 and 1987. SLC 24 was hydrologically isolated from the IRL by a dike (Figure 1) until 1985 when a culvert was installed to remove excess water deposited during two tropical storms. Once water levels were lowered, the culvert was sealed, and the impoundment remained isolated until 1987 when the culvert was reopened and other culverts were installed. The cover of all vegetation decreased from 75% to near 30% following construction of the diked impoundment in 1970. Over subsequent years, the cover of herbaceous halophytes, including *B. maritima*, changed in response to variations in the timing and duration of flooding and the establishment and growth of mangroves. Rey et al. (1990) concluded that a steady decline in the cover of herbaceous halophytes after 1984 was primarily caused by shading as the canopies of mangroves developed. Vegetation patterns are also complex in the numerous impoundments that have been established in the IRL, and they have been the focus of several studies focused primarily on nutrient limitation within mangroves (Feller et al., 2003; Lovelock and Feller, 2003; Lovelock et al., 2006b). We sampled *B. maritima* in three mangrove-dominated habitats and areas associated with salt pans where dwarf *A. germinans* (sensu Feller et al., 2003) occurs as scattered individuals or in patches with almost continuous cover. Details of sampling locations and methods are given below.

METHODS

Belize

We sampled *B. maritima* in two disturbed sites and six undisturbed sites on Twin Cays. One disturbed site is a 2 ha area on West Island that was cleared of mangroves and burned in 1991 and covered with material dredged from the adjacent subtidal area in 1995 (Rodriguez and Feller, 2004; McKee et al., 2007). The other disturbed site was clear cut in 2004, but no dredged material was added. In both disturbed sites, we sampled *B. maritima* in 10 randomly located plots (each 1 × 1 m) in which we made visual estimates of its cover, measured its height at five randomly chosen locations in each plot, and identified and counted all mangrove seedlings and saplings. Seedlings of *A. germinans* and *L. racemosa* had cotyledons present. Seedlings of *R. mangle* were individual, with no more than one pair of true leaves. Saplings were defined as individuals less than 50 cm in height with no cotyledons present, or with more than one pair of true leaves in the case of *R. mangle*.

For our undisturbed sites at Twin Cays, we sampled *B. maritima* in three forested habitat types (Fringe, Transition, Interior), which were located at different distances from the ecotone between the mangrove forest and open water. Fringe habitats, which were dominated by trees 4 to 5 m tall, were at the outer boundary between mangroves and open water, either along ponds located in the interior of Twin Cays or along the ocean. *Avicennia germinans* was the dominant tree in the three Fringe habitats adjacent to interior ponds. *Rhizophora mangle* was the dominant tree in the three Fringe habitats adjacent to the ocean. Transition and Interior habitats were all dominated by *A. germinans*. Transition habitats were located approximately 15 m further into the mangrove forest from the Fringe habitats, and Interior habitats were located approximately an additional 15 m beyond the Transition habitats. We sampled 5 randomly located plots (same procedures as described above) in each of the 90 plots (5 plots × 3 habitat types × 6 sites) in undisturbed mangrove.

Florida

We sampled *B. maritima* in SLC 24 in four habitat types (Fringe = *R. mangle*, Dense = *A. germinans*, Sparse = *A. germinans*, Dwarf = *A. germinans*). The Fringe habitats, dominated by *R. mangle* 4 to 6 m tall with scattered *A. germinans*, were located at the boundary between mangroves and open water. The two habitats dominated by taller (3–6 m) *A. germinans* (Dense, Sparse) differed in the size and spatial configuration of the dominant trees. The Dense *A. germinans* habitat had trees that were mostly 4 to 6 m tall and formed a continuous canopy dominated by *A. germinans*. The Sparse *A. germinans* habitat was also dominated by *A. germinans* but the trees were usually shorter (3–5 m) and were more widely spaced, resulting in a more open canopy. The Dwarf *A. germinans* habitat was always adjacent to salt pans that were mostly unvegetated or only had a few scattered dwarf trees (usually less than 1 m tall). We sampled *B. maritima* in one randomly located plot in each of the replicate sites for each habitat

type. In each 1 × 1 m plot, we made the same set of measurements as described above for Belize.

Data Analysis

Because of the different sampling regimes, we made separate statistical comparisons for the Belize and Florida data sets. Based on initial screening of the data (Proc Univariate; SAS Institute, 1990), we determined that none of the data were normally distributed either in their original form or any of the possible transformations. We used the nonparametric PROC NPAR1WAY (SAS Institute, 1990) to make comparisons of *B. maritima* data (percent cover, height) and the number of mangrove seedlings + saplings for the different habitat types at both locations.

RESULTS

Belize

Percent cover of *B. maritima* differed (Figure 2a) significantly (df = 4, chi-square for Kruskal–Wallis test = 27.9272, $P < 0.0001$) among the sites on Twin Cays. Mean percent cover ranged from 50% to 53% for the two disturbed sites and the undisturbed Fringe habitat. Percent cover decreased from the Fringe to the Transition ([mean ± 1 SE] = 35.5% ± 4.7%) and Interior (16.9% ± 2.9%) undisturbed sites. The average height of *B. maritima* also differed significantly between sites (Figure 2b; df = 4, chi-square for Kruskal–Wallis test = 29.0273, $P < 0.0001$). Heights were similar at the two disturbed sites (24.4 ± 2.3 cm = clear-cut + fill; 26.4 ± 1.0 cm = clear-cut). At the undisturbed sites, height was greatest at the Fringe habitat (61.7 ± 18.5 cm) and decreased toward the interior of the mangrove forest (40.4 ± 2.2 = Transition; 34.7 ± 2.0 = Interior).

The number of mangrove saplings + seedlings also differed across sites (Figure 2c), and there were significant differences for all three species and for the total of all species (df = 4, chi-square for Kruskal–Wallis test = 38.9958, $P < 0.0001$; 12.5551, $P > 0.0137$; 11.3187, $P < 0.0232$; 15.5953, $P < 0.0036$ for *R. mangle*, *A. germinans*, *L. racemosa*, and total mangroves, respectively).

The total number of mangrove saplings + seedlings was higher at the clear-cut and filled site (24.6 ± 13.8 m^{-2}) compared to the clear-cut site (2.5 ± 0.8 m^{-2}) and undisturbed mangrove habitats (mean for all three undisturbed sites was 7.3 ± 1.2 m^{-2}). *Avicennia germinans* saplings + seedlings at the clear-cut and filled site were less than 1 m^{-2} (Figure 2c). *Rhizophora mangle* was the most abundant species at the Fringe habitat, whereas *A. germinans* was

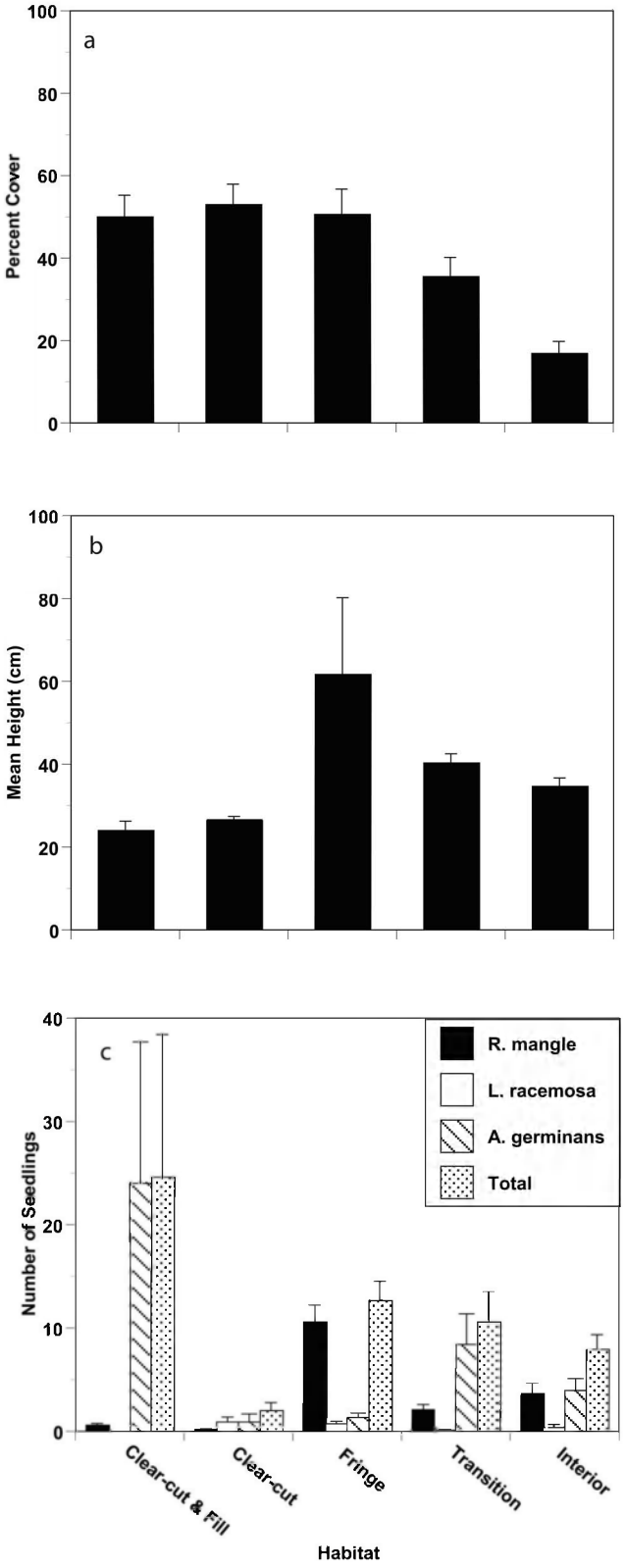

FIGURE 2. Cover (a), height (b), and sapling + seedling (c) data for two disturbed and three undisturbed habitats on Twin Cays (Belize). Values are means ± 1 SE.

the most abundant species at the Transition and Interior habitats.

Florida

Percent cover ($df = 3$, chi-square for Kruskal–Wallis test = 38.9252, $P < 0.0001$) and height ($df = 3$, chi-square for Kruskal–Wallis test = 33.0923, $P < 0.0001$) of *B. maritima* differed between the four habitat types in SLC 24 (Figure 3a). There was no *B. maritima* in the plots that were sampled in the Fringe *R. mangle* habitat, and the cover (2.8% ± 1.2%) was very low in the Dense *A. germinans* habitat. Percent cover was 42.9 ± 8.1 and 27.8 ± 4.6 in the Sparse and Dwarf *A. germinans* habitats, respectively. Height differences (Figure 3b) among the four habitats had the same pattern with the tallest plants occurring in the Sparse *A. germinans* habitat (48.9 ± 4.5) and shortest in the Dense *A. germinans* habitat (13.1 ± 5.4). The total number of saplings + seedlings and the means for each mangrove species also differed significantly (Figure 3c) among the four habitat types ($df = 3$, chi-square for Kruskal–Wallis test = 11.5483, $P < 0.0091$; 12.7678, $P < 0.0052$; 16.4377, $P < 0.0009$; 13.4660, $P < 0.0037$ for *R. mangle*, *A. germinans*, *L. racemosa*, and total mangroves, respectively).

DISCUSSION

The objective of this initial investigation of *Batis maritima* was to quantify aspects of its distribution in a variety of habitats in mangroves at long-term Smithsonian study sites in Belize and Florida. The impetus for the research was the observation that *B. maritima* is widespread in mangroves and, in some habitats, its high abundance and cover suggest that it potentially plays an important role in these systems. There have, however, been few studies that shed light on its possible ecological importance in mangroves. Studies in salt marshes near its northern limit found that it was not a dominant species and did not compete well with other marsh plants (Zedler, 1977). There is some suggestion that *B. maritima* may be a fugitive species because it is common in disturbed sites (Milbrandt and Tinsley, 2006). Pennings and Richards (1998), for example, found that stands of *B. maritima* were associated with areas that were disturbed by wracks of litter in a Georgia salt marsh.

Batis maritima has been described as a species that does not do well in shaded conditions or under conditions of continuous flooding (Rey et al., 1990; Alexander and Dunton, 2002). However, Keer and Zedler (2002) found

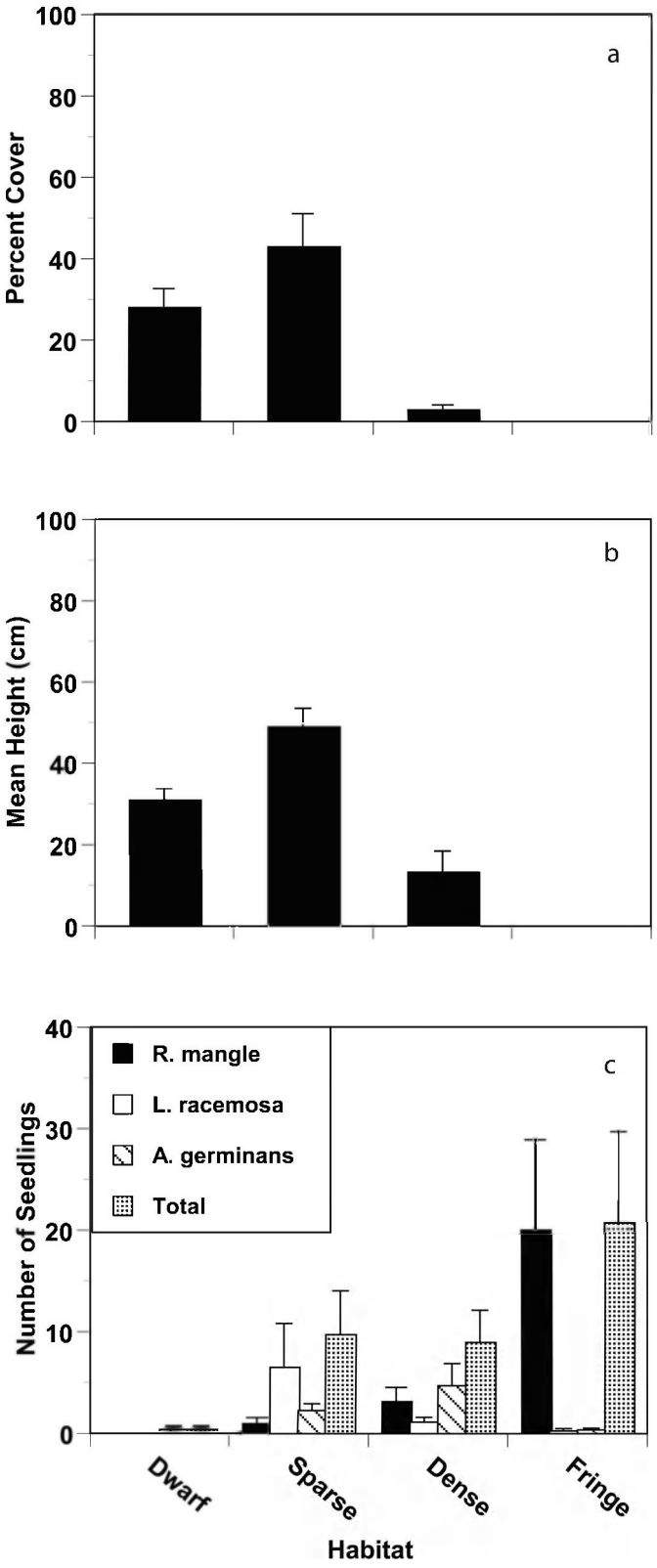

FIGURE 3. Cover (a), height (b), and sapling + seedling (c) data for four habitats in mosquito impoundment SCL 24 in the Indian River Lagoon, Florida. Values are means ± 1 SE.

that it can tolerate prolonged flooding, and it does well in waterlogged conditions when light is not limiting (Zedler, 1980). It also responds positively to increasing salinity (Ibarra-Obando and Poumian-Tapia, 1991) but is eliminated under hypersaline conditions (Zedler et al., 1986; Dunton et al., 2001).

The only long-term study of *B. maritima* in changing environmental conditions occurred in one of our study sites, SCL 24 in Florida. Rey et al. (1990) examined a sequence of aerial photographs taken over a period of time when hydrological conditions varied from years when there was continuously flooding, to years when the impoundment was drained, and to years when there was partial tidal exchange with the IRL. Vegetation almost completely disappeared when the impoundment was continuously flooded. Once the impoundment was opened to limited tidal exchange, herbaceous halophytes increased in abundance and cover and *B. maritima* eventually became the dominant species. Over time, mangroves recruited and eventually dominated the vegetation in most parts of the impoundment. As the abundance and size of mangroves increased, *B. maritima* declined along with other herbaceous halophytes in response to increased shading by mangroves (Rey et al., 1990).

Results of our surveys support several of the earlier studies and suggest that light levels, regular tidal flooding, and soil salinity are three important factors that determine where *B. maritima* occurs and how abundant it is. There have been at least two studies (Pennings and Richards, 1998; Milbrandt and Tinsley, 2006) suggesting that *B. maritima* is a fugitive species that colonizes high-light disturbed sites. In Belize, the highest percent cover was at the two disturbed sites and the Fringe habitat in the undisturbed mangroves (see Figure 2a). Even though the mean cover of *B. maritima* in the undisturbed sites on Twin Cays was lower (34.3% ± 3.1.9%) than the disturbed sites, the highest *B. maritima* cover (80.3% ± 2.6%) of any of the habitats sampled was in the three Fringe habitats that were associated with interior ponds. Edge habitats associated with interior ponds are mostly in full sun and are exposed to tidal flooding, but the flooding is rarely more than a few centimeters deep (D. Whigham, personal observations). The substrates are almost always waterlogged, and the sediments are soft, mostly composed of floc that accumulates on the downwind side of the interior ponds. The highest *B. maritima* cover in SLC 24 in Florida also occurred at sites that had no overhead mangrove canopy or only a discontinuous canopy.

The mean cover of *B. maritima* was least in the shaded habitats in Florida and Belize, supporting the suggestions of López-Portillo and Ezcurra (1989) that low light levels can limit its abundance and distribution. The absence of *B. maritima* in the sample plots at the Fringe habitat associated with SLC 24 and the lower cover in the Fringe habitats closest to the ocean on Twin Cays (20.9% ± 2.9%) also support the suggestions that regular inundation by tidal flooding has a negative effect on the species (Alexander and Dunton, 2002).

The mean height of the *B. maritima* canopy also varied among habitats, and the patterns are most likely the result of variations in light and salinity (Zedler et al., 1986; Dunton et al., 2001). In SLC 24, mean height decreased from the more open Dwarf and Sparse *A. germinans* habitats to the shadier Dense *A. germinans* habitat (Figure 3b). At the undisturbed sites in Belize, mean height decreased from the Fringe to the Interior, most likely in response to decreasing light. The height of the plants was greatest in the Fringe habitat associated with the Edge sites that were closest to the Interior ponds. Mean height at the Fringe habitats associated with the ponds was 85.4 ± 27.1 cm compared to 40.4 ± 2.2 at the more shaded Fringe habitats closest to the ocean. Taller average height associated with the Edge habitats may be the result of higher phosphorus concentrations in the sediments. In a separate fertilization experiment, we found that *B. maritima* responded significantly to the addition of phosphorus at all the undisturbed sites on Twin Cays, but the smallest response was at the Edge habitat associated with interior ponds, suggesting that phosphorus was more available in those sediments (D. Whigham, unpublished data). Compared to the Fringe habitats on Twin Cays and the Sparse *A. germinans* habitat in the SLC 24, mean height decreased toward the sites with no mangrove canopy (Dwarf *A. germinans* habitat in SLC 24 and the two disturbed sites at Twin Cays). Lower mean height at the open sites is likely the result of increased salinity as the Dwarf *A. germinans* site in SLC 24 is hypersaline (i.e., soil salinity as high as 100‰; D. Whigham unpublished data). In addition, soil salinity at the clear-cut and filled site on Twin Cays, while variable during an annual cycle, can be more than 60‰ (McKee et al., 2007).

Mangrove seedlings are widely dispersed, and their occurrence varies spatially in response to light levels and their ability to withstand flooding, salinity, and attacks from herbivores (Ellison and Farnsworth, 1993; Olusegun and Creese, 1997). If *B. maritima* facilitates the establishment of mangrove seedlings, we would expect a positive relationship between percent cover and the number of seedlings + saplings for one or more of the mangrove species. Milbrandt and Tinsley (2006) found that the presence of *B. maritima* had a positive effect on the survival of *A. germinans* seedlings. McKee et al. (2007), however, found that *B. maritima* had no effect on the recruitment and survival

of mangrove seedlings, even though mangrove seedlings benefited by the presence of other herbaceous species (i.e., *Distichlis spicata*, *Sesuvium portulacastrum*) at clear-cut and filled sites sampled in this study. Although there were habitat differences in the number of seedlings + saplings, the presence of seedlings + saplings in 88% of the plots sampled in Belize and 55% of the plots in Florida indicated that mangrove establishment may have been facilitated by *B. maritima*. We found no relationship, however, between the amount of *B. maritima* cover and the density of seedlings + saplings for any of the mangrove species (Figure 4). The potential for *B. maritima* to influence the distribution and growth of mangroves trees and other mangrove plants and animals remains unknown. But, given the abundance of the species across a range of habitats, the potential seems high, especially in areas where it is the dominant species.

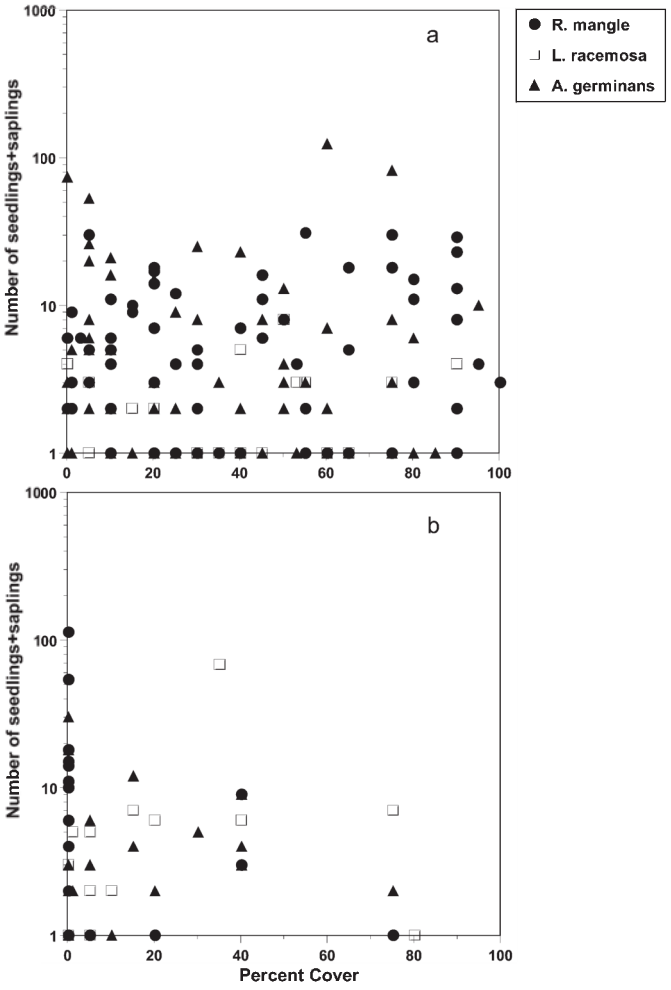

FIGURE 4. Number of mangrove saplings + seedlings (# m^{-2}) plotted against percent cover of *Batis maritima* for 1 × 1 m plots sampled in Belize (a) and Florida (b) study sites.

In summary, *B. maritima* was widespread in most mangrove habitats at both our study locations, and there were significant interhabitat differences in all the variables measured. Mangrove seedlings and saplings were common in areas occupied by *B. maritima*, but we found no evidence that the establishment of mangroves benefited by increasing cover of this common halophytic species. The ubiquitous distribution of *B. maritima* at all the sites sampled, however, indicates that its role in mangrove ecosystems deserves further consideration.

ACKNOWLEDGMENTS

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Sponge Community Dynamics on Caribbean Mangrove Roots: Significance of Species Idiosyncrasies

Janie L. Wulff

ABSTRACT. Descriptions of the rich sponge faunas inhabiting mangrove roots at various Caribbean sites are unanimous in pointing out the heterogeneity of species distribution and abundance patterns at all scales, from different portions of a single root to geographic subregions. Abiotic factors have often been implicated by correlation, but ecological interactions, and the life history and morphological characteristics of the sponge species, may also play key roles. Published studies vary widely in methods used, hampering direct comparisons of results, and raising the possibility that conclusions might be influenced by methods. I have been exploring the processes underlying distribution and abundance patterns by applying identical methods to studying community composition and dynamics at two sites in Belize (Twin Cays) and one site in Panama (Bocas del Toro). Established communities on roots have been fully censused, by volume and numbers of individuals, yearly for three years (i.e., four censuses). Community composition, when evaluated in terms of total volume of component species, is very similar at these three sites, although abiotic factors differ and geographic distances between sites range from 330 m to 1,200 km. The nine species found on censused roots at all three sites constituted a total of 89%, 84%, and 73%, respectively, of the total sponge volume at these sites. In general, species exhibited similar patterns of growth, size decrease, and mortality at all sites where they were found, suggesting that these are species-level characteristics. Numbers of individuals and volume provide very different assessments of the relative importance of different species in these communities. Community change over time appeared to be substantial, when measured in terms of shifts in total numbers of individuals or total sponge volume. However, taking into account dynamics of individual species provides a very different view, as most large changes in numbers or volume were not community wide but tended to reflect life history characteristics typical of early successional stage species or idiosyncratic responses of one or a few species to particular environmental circumstances.

INTRODUCTION

Organisms that live in habitats consisting of discrete patches within an uninhabitable matrix have fascinated biologists who are simultaneously attracted to community ecology and to life history evolution. A rich set of conceptual frameworks has developed to explain the dynamics of community assembly and development within each patch in the context of interconnections among patches. Theories of, for example, island biogeography (MacArthur and Wilson, 1967), multiple stable points (Sutherland, 1974), competitive networks (Jackson and

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Buss, 1975), the intermediate disturbance hypothesis (e.g., Connell, 1978), and meta-communities (e.g., Mouquet and Loreau, 2002) have helped us understand community dynamics in patchy habitats ranging from oceanic islands and tropical mountaintops to badger mounds, holes in mussel beds, and ponds.

Prop roots of Caribbean red mangrove (*Rhizophora mangle*) trees are easily accessible, experimentally tractable, and extraordinarily colorful examples of inherently patchy communities. The sessile inhabitants that cover the root surfaces colonize in the form of water-borne propagules, and most of them are thereafter confined to the root on which they landed. Although post-recruitment interactions with neighbors and consumers may have relatively deterministic outcomes, a stochastic element, contributed by the uncertainty that any particular species will land on a particular root, is ever present. Throughout the wider Caribbean region sponges are prominent members of the prop root communities, and their abundance and diversity of species, color, and forms have inspired time-series monitoring and experimental manipulations as well as comparative faunal studies (e.g., Sutherland, 1980; Ellison and Farnsworth, 1992; Bingham and Young, 1995; Rützler, 1995; Farnsworth and Ellison, 1996; Rützler and Feller, 1996; Rützler et al., 2000; Diaz et al., 2004; Diaz, 2005; Wulff, 2000, 2004, 2005; Engel and Pawlik, 2005). Specific conclusions relating to how dynamic these sponge communities are, and what processes drive the dynamics and influence distribution and abundance patterns, have differed widely among studies, but a consistent theme is that the resulting distribution and abundance patterns are highly heterogeneous on scales ranging from within individual roots to between geographic subregions.

Sutherland (1980) complemented repeated monitoring of natural communities on prop roots with a study of community development on flat settlement panels suspended among the roots in Venezuela. He concluded that these sponge-dominated communities are relatively stable over time, and that high diversity could be maintained by a trade-off between competitive ability and colonization efficiency, combined with the continued addition of fresh roots that provide refuges for inferior competitors. Farnsworth and Ellison (1996) surveyed prop root communities of mangroves in a variety of abiotic settings in Belize, focusing on spatial scales of distribution patterns. They were able to identify scales of heterogeneity that included backs versus fronts of individual roots, leeward versus windward shores, and coastal versus island mangal. At the 11 sites where they sampled twice, their data corroborated Sutherland's (1980) conclusions that community change is minimal. Bingham and Young (1995) concluded very differently, from their work in the Florida Keys, that dynamics of sponges on mangrove roots can be extreme, influenced by perturbations from physical disturbance, predators, and asexual recruitment. They attributed differences in community dynamics between sites in the Florida Keys and Venezuela to differences in seasonality (subtropical versus tropical) and abiotic stressors, and suggested that the differences between their study and Sutherland's (1980) study could be explained by equilibrium versus non-equilibrium situations, with the Venezuelan mangrove communities primarily structured by competitive interactions.

Disentangling the effects of biogeography and different suites of abiotic factors by making direct comparisons among studies is hampered by the wide variety of approaches that have been applied. Published studies differ with respect to units of study, time course and frequency of monitoring, and metrics for evaluating abundance. To control for technique, I used identical methods to evaluate sponge community composition and dynamics for three years on mangrove prop roots at three sites in Belize and Panama. Following the fates of individual sponges was a priority, because my chief interest was in how the morphological and life history strategies of the different sponge species constrain or enhance their ability to coexist on the prop roots. Rather than focusing on community-level metrics, such as species diversity or primary space occupancy, I recorded survival and changes in volume of the same individuals over time and attempted to identify the causes of size decrease, fragmentation, or mortality. Two sites near each other in Belize differed in abiotic conditions, and a site in Panama provided a geographic comparison. My goals included (1) assessing the similarity of species composition among sites differing in abiotic factors and geographic distance, (2) comparing community dynamics among sites, with respect to both numbers of individuals and volume, and (3) exploring the possibility that each mangrove sponge species adheres to a characteristic approach for maintaining its representation in this community, regardless of the specific abiotic context and other species present.

METHODS

Three sites characterized by well-developed mangrove prop root epiphytic communities were chosen for yearly censuses. The three sites were chosen primarily because experiments had been established at each several years before, and so regular visits were already required for moni-

toring. Top priorities in initial site choice had been easy access and sufficient sponge individuals for experimental manipulations; species composition was secondary. The two Belize sites, both at Twin Cays, near the Smithsonian Institution's Carrie Bow Cay research station (map and further site descriptions in Rützler et al., 2004; Diaz et al., 2004), allow comparison of a main channel versus a tidal creek near each other (330 m). The Panama site, directly across the channel from the Smithsonian Tropical Research Institute marine laboratory on Isla Colon in Bocas del Toro (map coordinates and description of the overall area are found in Diaz, 2005), adds a geographic comparison (1,200 km distant) between two main channel sites. The submerged portions of the prop roots (i.e., the portion on which sponges could grow) were from 24 to 143 cm long, with the majority between 40 and 80 cm in length.

At each site, mangrove roots or root clusters were chosen that appeared to be healthy (i.e., no signs of rot or incipient breakage) and on which it was possible to identify and measure all sponges on all sides of each root. Root clusters were added to the initial census at each site until species accumulation curves had leveled off for sponges, and at least 163 sponge individuals (the number of sponges in the first census at the first site) were included: a total of 10 clusters, 1 to 5 roots each (24 roots initially) at Hidden Creek; 13 clusters, 1 to 4 roots each (37 roots initially), at Sponge Haven; and 15 clusters, 1 to 3 roots each (42 roots initially), at the Bocas del Toro site. Roots were labeled with small plastic tags, coded by color and shape, on narrow (1 mm) beaded nylon cable ties. Full censuses were made at approximately 1 year intervals, for a total of 3 years (i.e., four censuses at each site, except for Bocas del Toro, where the 2nd year census was skipped), beginning in March 2004 at both Belize sites and in June 2003 at the Panama site. At each census, every root or root cluster was drawn and root lengths measured. Every sponge was drawn to scale, in place on the root drawings, and sufficient dimensions measured to accurately estimate volume by conglomerations of appropriate geometric solids. In this way, every sponge could be followed for survival, growth, decrease in size, and fragmentation. New recruits were added to the root maps as they were discovered (recruitment data will be reported in a separate publication), and notes were made on interactions between neighboring sponges and other sessile organisms, as well as damage caused by physical disturbance, predation, and disease. Some roots at each site were lost by breakage during the 3 years. To be able to interpret the time-series data clearly, only roots for which at least some portion persisted throughout the study were included in the time-series data analysis, and the only roots added to the study were those that branched directly off subtidal portions of the originally censused roots.

RESULTS

Species Composition and Relative Abundance, by Volume and Number of Individuals

A total of 21 sponge species were represented by at least 0.1% of the total sponge volume on censused roots at one or more sites (Table 1). These species represent the demosponge orders Poecilosclerida (8 species), Haplosclerida (6 species), Halichondrida (4 species), and Dictyoceratida (3 species), in a variety of colors, and with growth forms ranging from thinly encrusting to irregularly branching to clusters of volcanoes (Figure 1). Of these most abundant 21 species, 9 were found on censused roots at all three sites, and another 6 were found on censused roots at two of the three sites (Figure 2). Many of the sponge species are relatively rare, and so were present at a site but not on a censused root. Adding three cases in which species were found at a second or third site on roots directly adjacent to at least one censused root increases the number of species shared by all three sites to 10, with an additional 7 species shared by two of the three sites. Geographic distance was not a strong predictor of the percent of species shared. Sponge Haven and Hidden Creek, only 330 m apart, shared 74% (14/19) of their most common species, and Sponge Haven and Bocas del Toro, 1,200 km apart, shared 60% (12/20) of their most common species (comparisons not significantly different by the G test: $0.1 < P < 0.5$). The Hidden Creek and Bocas sites, geographically distant from each other and also differing in abiotic factors, shared 55% (11/20) of their most common species (comparison with the proportion of species shared by Hidden Creek–Sponge Haven by the G test: $0.05 < P < 0.1$).

Census data from all years at each site were added together for an average relative representation of species, with respect to both volume and number of individuals (Figure 3). At all three sites the most abundant species by volume, *Tedania ignis*, accounted for about half (49%–57%) of the total sponge volume. The nine species found on censused roots at all three sites contributed a total of 89%, 84%, and 73% of the total volume at, respectively, Hidden Creek (HC), Sponge Haven (SH), and Bocas del Toro (BT). Similarity of species representation at these sites is also borne out by Morisita's index of community similarity (using volume as abundance measure),

TABLE 1. Sponge species on censused roots at Hidden Creek and Sponge Haven, both at Twin Cays, Belize; and at Isla Colon, Bocas del Toro, Panama. A total of 21 sponge species were represented by at least 0.1% of the total sponge volume on censused roots at one or more sites. Species that rank in the top half of the species on censused roots at a site, with respect to volume, are indicated by "XX", and those that rank in the bottom half are indicated by "X". Species that occurred on censused roots at one site but were only seen on a root or roots directly adjacent to at least one censused root at another site are indicated by "x". A dash (–) indicates species was not found at a site.

	Location		
Sponge taxon	Hidden Creek	Sponge Haven	Bocas del Toro
Order Dictyoceratida			
Dysidea etheria de Laubenfels, 1936	X	x	X
Spongia tubulifera Lamarck, 1814, and *S. obscura* Hyatt, 1877	XX	XX	XX
Order Halichondrida			
Amorphinopsis sp.	XX	–	–
Halichondria magnicomulosa Hechtel, 1965	XX	XX	XX
Halichondria sp.	–	XX	–
Scopalina ruetzleri (Wiedenmayer, 1977)	X	x	–
Order Haplosclerida			
Chalinula molitba (de Laubenfels, 1949)	XX	–	X
Haliclona curacaoensis (van Soest, 1980)	X	X	–
Haliclona implexiformis (Hechtel, 1965)	XX	X	X
Haliclona manglaris Alcolado, 1984	X	X	X
Haliclona sp. a	X	x	–
Haliclona sp. b	–	–	XX
Order Poecilosclerida			
Biemna caribea Pulitzer-Finali, 1986	XX	X	X
Clathria campecheae Hooper, 1996	X	X	–
Clathria schoenus (de Laubenfels, 1936)	–	–	XX
Clathria venosa (Alcolado, 1984)	X	X	X
Lissodendoryx isodictyalis (Carter, 1882)	XX	XX	X
Mycale microsigmatosa Arndt, 1927	–	XX	XX
Tedania ignis (Duchassaing and Michelotti, 1864)	XX	XX	XX
Tedania klausi Wulff, 2006	–	XX	XX

with similarities of HC–SH = 0.977, SH–BT = 0.971, and HC–BT = 0.957. These index values are strongly influenced by the similar dominance of *T. ignis* at all three sites, but other species were also consistently either relatively abundant or rare at all sites. When sponge species at each site are divided into those that rank in the top half by volume versus those that rank in the bottom half, pairwise comparisons between sites (see data in Table 1) yield 23 site pairs in which a species was ranked in either the top half or bottom half at both sites and only 7 pairs in which a species was ranked in the top half at one site and in the bottom half at the other site (significantly different from an even distribution by the *G* test at $P < 0.005$).

At each site species were present on censused roots that were represented by volumes of less than 0.1% of the total. Among these species were *Scopalina ruetzleri* (Wiedenmayer, 1977) at Hidden Creek, *Clathrina coriacea* (Montagu, 1818) at Hidden Creek and Sponge Haven, and *Mycale magnirhaphidifera* (van Soest, 1984) at Sponge Haven; and *Hyrtios violaceus* (Duchassaing and Michelotti, 1864), *Haliclona vansoesti* (de Weerdt, de Kluijver, and Gomez, 1999), *Haliclona caerulea* (Hechtel, 1965), and *Tethya actinia* (de Laubenfels, 1950), all at Bocas del Toro, as well as several as yet unidentified species.

In general, number of individuals and total volume provide very different views of the relative importance of the species in these communities (see Figure 3). This discrepancy is strikingly illustrated by the high representation by numbers of individuals of *Haliclona manglaris* (15.2%, 27.9%, and 50.1% at HC, SH, and BT, respectively), which also consistently contributed minimal volume (0.06%, 0.08%, and 0.37%). By contrast, *Tedania ignis*, which contributed half the volume at each site, contributed only 8.4% to 20.4% of the individuals.

FIGURE 1. Photographs of some of the most common sponge species inhabiting mangrove prop roots at Hidden Creek and Sponge Haven, Twin Cays, Belize, and across the channel from the STRI Bocas del Toro Marine Station, Isla Colon, Panama. Top row, from left to right: *Clathria venosa*, *Haliclona curacaoensis*, *Haliclona manglaris* (turquoise) and *Haliclona* sp. b (purple), *Dysidea etheria* (ethereal blue). Second row: *Chalinula molitba*, *Mycale microsigmatosa*, *Biemna caribea*, *Tedania ignis* (three individuals). Third row: *Lissodendoryx isodictyalis*, *Tedania klausi*, *Haliclona* sp. a. Bottom row, from left: *Halichondria magniconulosa*, *Haliclona implexiformis* (purple). Authors of species are given in Table 1.

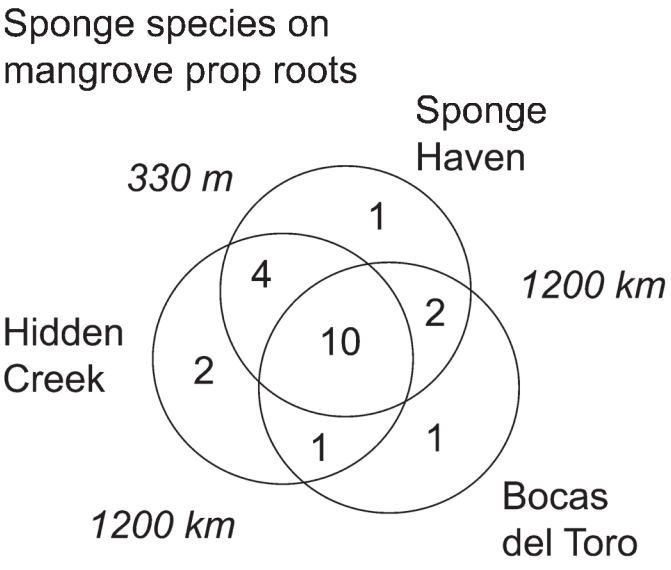

FIGURE 2. Diagram showing sponge species shared among two sites in Belize (Hidden Creek and Sponge Haven) and one site in Panama (Bocas del Toro). Only the 21 species that each constituted at least 0.1% of the total sponge volume on censused roots at a minimum of one of the three sites are included.

Mean density of numbers of sponge individuals per unit length of subtidal prop root was similar for all three sites (15, 11, and 15 individuals per meter length of root for Hidden Creek, Sponge Haven, and Bocas del Toro, respectively). Reflecting a relative preponderance of small individuals at the Bocas del Toro site, sponge density measured as volume per unit length of subtidal prop root was only 5.7 cm^3/cm at Bocas, compared with 20.8 cm^3/cm at Hidden Creek and 15.7 cm^3/cm at Sponge Haven. Although variation in root diameter renders root length imprecise as a measure of substratum area monitored, length was deemed a better measure than number of roots because of the sixfold variation in root lengths (i.e., from 24 to 143 cm).

Sponge Community Dynamics Compared Within and Between Sites, by Volume and Number of Individuals

During the three years of monitoring, the largest difference between the highest and lowest total sponge volume was 12%, 35%, and 27% at, respectively, Hidden Creek, Sponge Haven, and Bocas del Toro; and the largest difference between the highest and lowest number of sponge individuals was 50%, 34%, and 39%, respectively (Figure 4). Based on these total abundance values, community-wide change appears to be substantial. However, comparison over time of abundance of individual species, with respect to both volume and numbers of sponge individuals, sheds light on the components of change and provides a very different picture. In many cases, large overall changes in total volume or numbers in the course of a particular year reflect changes in just one or a few species. For example, the drop in total sponge volume between 2005 and 2006 at Sponge Haven (see Figure 4) was mostly caused by losses from *Halichondria* sp., *Halichondria magniconulosa* (almost to the point of elimination),

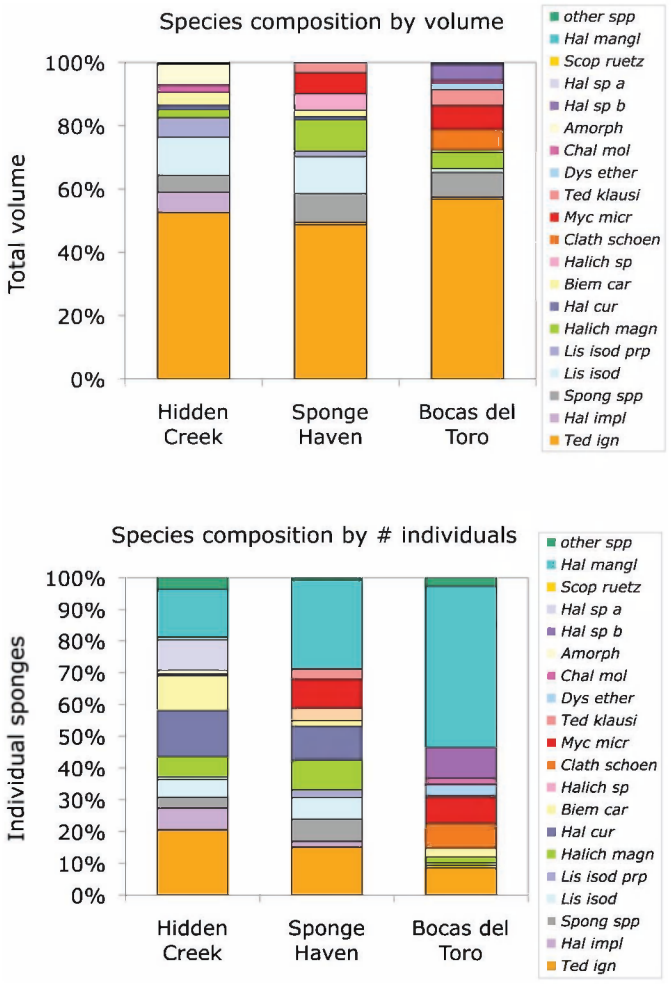

FIGURE 3. Sponge species assemblage composition, with respect to total volume contributed by each species, and with respect to the total number of individuals of each species, on mangrove prop roots at three Caribbean sites. These average relative abundances were calculated by adding together the volume or numbers of individuals for all four yearly census dates (three dates in the case of Bocas del Toro). See Table 1 for complete spelling of species names.

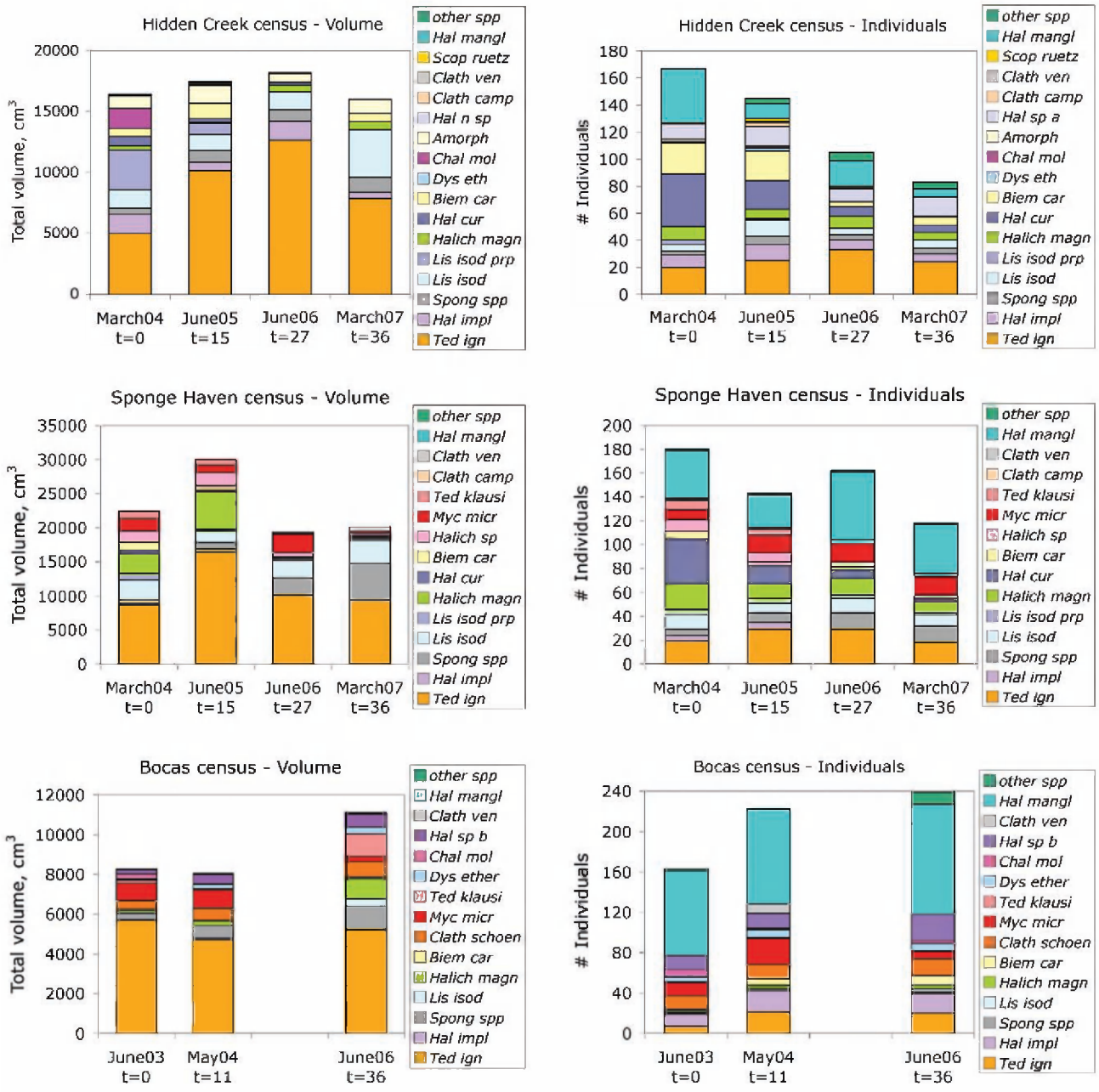

FIGURE 4. Community dynamics for sponges on mangrove prop roots at three Caribbean sites. Relative abundance of the species is represented by both total volume and total numbers of individuals. See Table 1 for complete spelling of species names.

and *T. ignis*. For the second and third species these losses primarily consisted of size decreases and fragmentations of sponges that were subsequently able to regenerate; and thus for both of these species, abrupt and dramatic changes in volume between the second and third censuses are not reflected in tandem changes in numbers of individuals (e.g., *H. magniconulosa* was represented by 12 individuals of total volume 5,518 cm^3 in 2005, 14 individuals of total volume 187 cm^3 in 2006, and 9 individuals of total volume 225 cm^3 in 2007). Large differences in maximum size achieved by sponges of different species further promote asynchronous changes in overall volume and numbers of individuals. For example, during this same year in which total sponge volume at Sponge Haven decreased by 35%, the number of individual sponges there increased by 13%, largely the result of a doubling of the number of *Haliclona manglaris* individuals. Yet each *H. manglaris* individual is so small that, even in the aggregate, they scarcely register in the overall volume tally (0.2% for the June 2006 census; see Figure 4).

Similarly, progressive loss of individuals of *Biemna caribea* and *Haliclona curacaoensis* at Hidden Creek resulted in decreases in total numbers of individuals by more than half in the course of three years (Figure 5). If these species are removed from the "Hidden Creek census – Individuals" graph in Figure 4 (along with the very small bodied *H. manglaris*), the community can be seen to otherwise remain very similar throughout the three years with respect to relative representation of the component species by numbers of individuals. During this same time period, the total volume of all sponges at this site remained very similar, although there were large volume changes for individual species (see Figure 4). The Sponge Haven data show the same pattern of progressive loss of *H. curacaoensis* (see Figure 5) and also *B. caribea*, although the latter species was not as abundant to begin with at this site.

Not all changes in abundance of particular species were abrupt or negative. Volume of *Spongia* spp. steadily increased at all sites (see Figure 5), with little increase in numbers, reflecting high survival of the individuals that were present at the first census. Illustrating a third pattern of dynamics, the volume of *T. ignis* fluctuated at all three sites, but at the end of the three years the total volume of this species at each site was similar to what it was at the start of the study (Figure 5).

Portions of many roots were lost during the three years of the study, but new roots sprouting from subtidal portions of censused roots nearly balanced the losses during some time periods. Thus the total length of prop roots

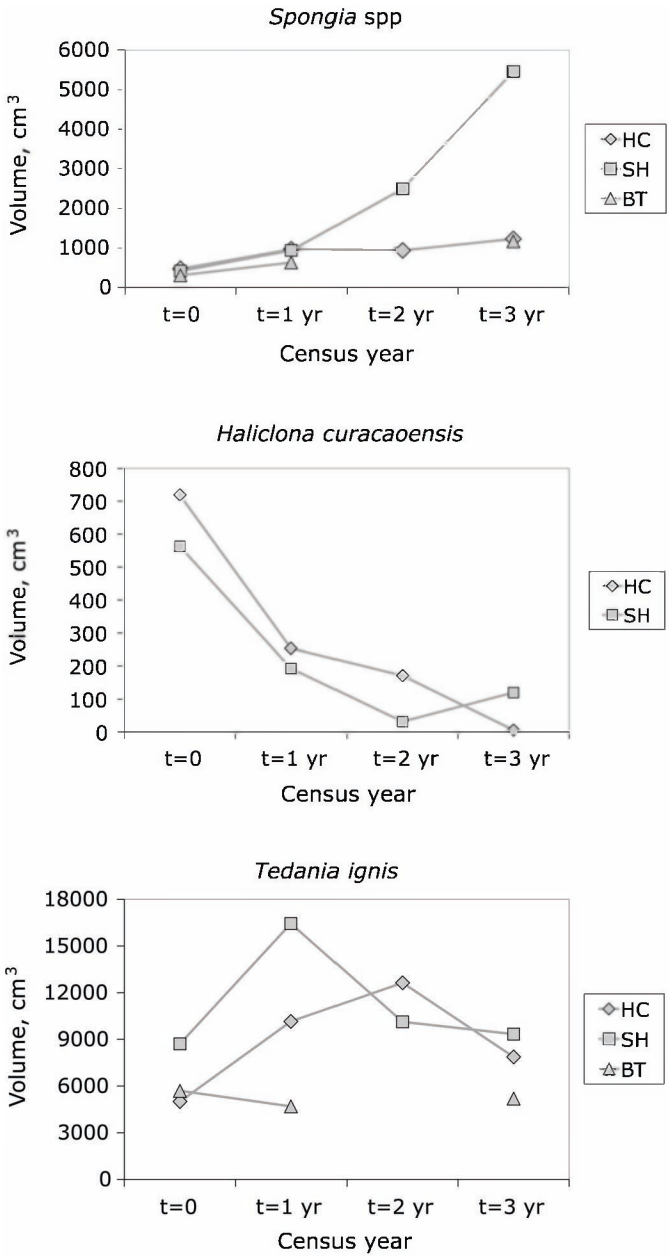

FIGURE 5. Representative population dynamics graphs for three sponge species inhabiting mangrove prop roots at sites where yearly censuses were made (HC = Hidden Creek; SH = Sponge Haven; BC = Bocas del Toro). Total volume of *Spongia* spp. consistently increased between monitoring periods at all three sites; volume of *Haliclona curacaoensis* decreased between monitoring periods at both sites in which it was found; *Tedania ignis* total volume fluctuated over time but ended up very similar to what it had been at all three sites at the start of the study three years earlier.

included in the census was very similar for the first three censuses at Hidden Creek, and for the first two censuses at Bocas del Toro and Sponge Haven, and then, after a decrease, also for the final two censuses at Sponge Haven. Total length (in cm) for the four censuses at Hidden Creek was 968, 896, 995, and 562; for Sponge Haven, 1,847, 1,787, 1,162, and 1,179; and for Bocas del Toro, 1,483, 1,583, and (after two years) 1,203. Substratum available was not necessarily related to sponge abundance with respect to either numbers of individuals or total volume (compare abundance measures reported in Figure 4 with total root lengths censused); for example, sponge volume and total root length were inversely associated over the three years at the Bocas del Toro site.

Variation among Sponge Species in Individual Persistence

Because individual sponges were mapped and measured, their fates from one census to the next could be recorded as (a) increased in size, (b) fragmented, (c) decreased in size, or (d) disappeared. To characterize each species at each site independently of environmental circumstances during a particular time interval, data from all 1 year intervals between censuses (and one 2 year interval in the case of the Bocas del Toro site) were added together in Figure 6. Three patterns emerge from these graphs. First, fragmentation and size decrease are important aspects of persistence for many of these species. The only species represented entirely by individuals that increased in size or vanished (i.e., none decreased in size or fragmented) between censuses were *Spongia* spp. and *Amorphinopsis* sp. Second, at each site variation among species in the degree to which individuals persisted was clear. Yearly rates of loss ranged from 0% (e.g., *Spongia* spp.) to 100% (e.g., *Chalinula molitba*). Third, many species exhibited the same characteristics at each site where they occurred. For example, a set of species characterized by at least 40% of the individuals increasing in size from one yearly census to the next were evident at each site: *Tedania ignis*, *Haliclona implexiformis*, *Spongia* spp., *Lissodendoryx isodictyalis*, and *Halichondria magniconulosa*. The only exceptions were *Haliclona implexiformis* in Bocas del Toro and *Halichondria magniconulosa* at Sponge Haven. The reason for the *H. implexiformis* difference at the Bocas site was not obvious, but individuals of this species were always very small there. At Sponge Haven, both *H. magniconulosa* and *T. ignis* suffered high rates of size decrease and fragmentation between the second and third censuses. These

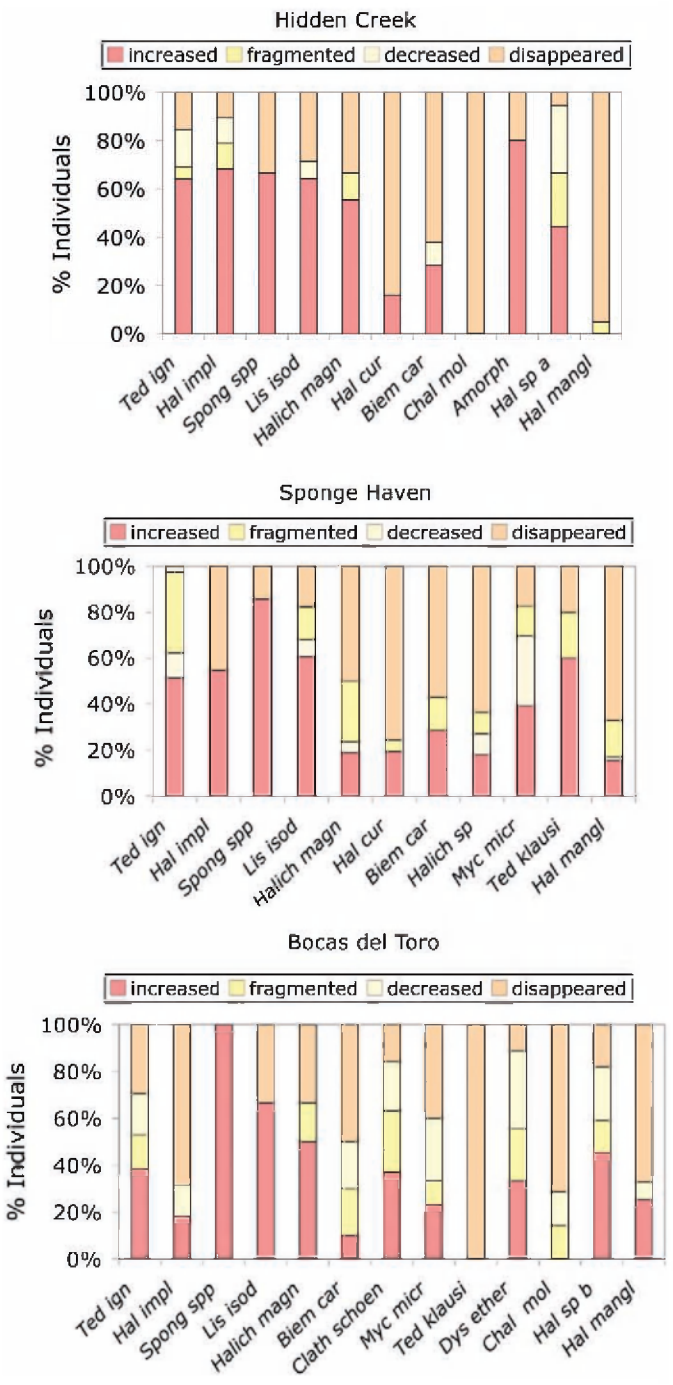

FIGURE 6. Proportions of sponge individuals of each species that increased in volume, fragmented, decreased in volume, or disappeared entirely between yearly census dates at three Caribbean mangrove sites. Although the fate of each individual was recorded in a single category at the time of observation, the distinction between "fragmented" and "decreased" can be fuzzy, as it depends on whether or not fragments generated survive until the time of observation. See Table 1 for complete spelling of species names.

departures from their usual pattern (i.e., most individuals increasing in size during a year) coincided with the unusual and fleeting presence of a couple of French angelfish just before the third census. French angelfish have been demonstrated to readily consume these two species (Wulff, 2005) and *Halichondria* sp. (in preparation), which also decreased in volume during the same time interval. Species that only occurred at two sites also exhibited similar characteristics with respect to persistence at both sites. For example, no *Chalinula molitba* individuals increased in size from year to year at either Hidden Creek or Bocas del Toro, and *Haliclona curacaoensis*, *H. manglaris*, and *Biemna caribea* had consistently low survival at each site. In contrast, *Tedania klausi* survived well at Sponge Haven, but suffered an extreme decline on censused roots at the Bocas del Toro site, coinciding with observed high losses of only this species throughout the site to disease (Wulff, 2006).

DISCUSSION

Community Composition

These three sites that differ in abiotic factors and in geographic distance from each other have very similar species compositions, not only with respect to species present but also with respect to their relative abundance. The 9 species that are shared by all three sites constituted 89%, 84%, and 73% of the total sponge volume at, respectively, Hidden Creek, Sponge Haven, and Bocas del Toro. The ubiquity and consistent local dominance of the fire sponge *Tedania ignis* contributes heavily to similarity among these sites. Previous studies also concur that *T. ignis* is a signature species for this ecosystem throughout the wider Caribbean region, and it was the sole species, of 23, that was recorded in all eight prop root faunal surveys compiled by Wulff (2000) and in four of five of the studies compared by Diaz et al. (2004). Not only does it occur at most sites, it tends to be among the most abundant species by any metric. At five sites in Belize (including Hidden Creek and Sponge Haven), Diaz et al. (2004) recorded *T. ignis* as present on 11% to 34% of the roots, in the top 3 species ranked by frequency of occurrence. By measuring area covered from photographs, Bingham and Young (1995) estimated that 16.7% of the root area at their Florida Keys site was covered by *T. ignis*. Using line transects, along which the length of root covered by each sponge was measured, Sutherland (1980) estimated 5%–12% coverage by *T. ignis* in Bahía de Buche, Venezuela. Evaluating abundance by volume boosts the proportional representation of this species because of its massive growth form, and thus in this study *T. ignis* constituted from 49% to 57% of the total sponge volume on censused roots. This species not only holds a large proportion of the primary substratum space, but it also participates in a mutualism with the mangroves, enhancing the persistence and health of the entire ecosystem by protecting the roots from attacks by boring isopods (Ellison and Farnsworth, 1990; Ellison et al., 1996).

Tedania ignis is not the only species that is both nearly ubiquitous and locally abundant, although it stands out as the most extreme. Consistently *Lissodendoryx isodictyalis*, *Halichondria magniconulosa*, *Spongia* spp., *Haliclona implexiformis*, *Haliclona manglaris*, and *Dysidea etheria* appear on faunal lists and, where authors indicate relative abundance, by whatever metric, they rank highly (Wulff, 2000; Diaz et al., 2004).

Although the three sites in this study are similar with respect to these widespread typical mangrove root sponge species, there are two types of differences among the sites: (1) a few species that are abundant at one site but do not occur elsewhere (e.g., *Amorphinopsis* sp. at Hidden Creek), and (2) many rare species that appear to differ among sites. The virtual lack of overlap of these rarer species on species lists from different sites does not necessarily indicate constrained distribution but may simply reflect their rareness. Diaz et al. (2004) discuss this sampling issue with the highly diverse Caribbean mangrove root sponge fauna and illustrate it well with their data. Diaz et al. (2004) also point out the great degree to which community composition can vary along a particular mangrove fringe. The three sites in the present study are known to share additional sponge species if entire contiguous stretches of mangrove are included (Rützler et al., 2000). For example, at Sponge Haven, *Clathria schoenus* is not found near the censused roots but appears on roots at this site that are further toward the mouth of the main channel.

Sponge species composition differences among sites characterized by very different abiotic circumstances have been well documented, and some sites are sufficiently extreme in abiotic factors that sponges are scarcely present (Farnsworth and Ellison, 1996) or succumbed to unfavorable conditions while being studied (Pawlik et al., 2007). At least some of the differences in species composition between Hidden Creek and Sponge Haven, only 330 m apart, have already been ascribed to less hospitable abiotic factors in the narrow, tidal Hidden Creek. Transplants of 5 species that are conspicuous at Sponge Haven thrived initially in Hidden Creek, but nearly all (61/63) died over the course of one year (Wulff, 2004), possibly implicating episodically wide fluctuations in temperature and salinity.

The similarity between the three sites in this study is especially interesting, considering that they were chosen for accessibility and overall sponge abundance, rather than for species composition, and that they have demonstrated differences in abiotic factors and span a geographic distance of 1,200 km.

Methods for Studying Sponges on Mangrove Prop Roots Can Influence Evaluations of Community Similarity between Sites and Community Stability over Time

Methods of studying composition and dynamics of sponge communities on mangrove roots have varied with respect to metrics for evaluating abundance, sampling unit, choice of which units to sample, time interval of sampling, and materials, size, and shape of recruitment surfaces. This variety reflects the many different questions posed by researchers, and the difficulty of quantifying sponges; but methods may also influence conclusions.

Methods for evaluating abundance have included analysis of photographs for percent cover, line transects down roots with distance covered by each species recorded, point counts through acetate sheets, and presence/absence on each root, as well as the total numbers of individuals and volume of each individual. The advantages and disadvantages of evaluating sponge abundance with respect to volume, area covered, or numbers of individuals have been previously compared in the context of coral reefs (Rützler, 1978; Wulff, 2000, 2001). Choice of metric is influenced by expediency in the field, and also by whether functional roles, life histories, species diversity, or some other aspect of these communities is the central focus of a study. One advantage to measuring sponge abundance by volume is that growth rates can then be calculated if the same sponge individuals are followed over time. As well, functional roles related to trophic interactions, such as filtering food particles from the water column and provision of food to spongivores, probably scale with volume. Unfortunately, sponge volume is time consuming to measure nondestructively in the field, decreasing the number of individuals that can be monitored.

Area can be a problematic measure of sponge abundance, as the amount of sponge tissue under a particular point can range over orders of magnitude. At these three sites, for example, sponges on prop roots varied in thickness 150 fold, from 0.1 to 15 cm. Evaluating mangrove sponge abundance in terms of area is further complicated by the prevalence of epizooism, which results in points falling simultaneously over more than one sponge species. At least one functional role of sponges in mangroves may be related to substratum area covered: protection of mangrove roots from boring isopods (Ellison and Farnsworth, 1990, 1992).

Numbers of individuals are difficult to interpret in the contexts of sponge population dynamics and functional roles, as numbers can increase either by recruitment or fragmentation, and individual size can vary over many orders of magnitude. The lack of concordance between population dynamics of individual species measured in terms of numbers of sponges versus total volume on the same roots (see Figure 4) underscores how divergent conclusions can be when different metrics are chosen for evaluating sponge abundance. Evaluating abundance using two or more metrics at the same site can strengthen understanding of processes underlying the dynamics. For example, data indicating a small increase in numbers of individuals of *Halichondria magniconulosa* at Sponge Haven allowed the coincident large decrease in volume to be interpreted as extensive partial mortality and some fragmentation, rather than heavy losses of individuals.

An abundance measure that lends itself well to biodiversity surveys in this inherently fragmented habitat is presence/absence of a species on each root. Diaz et al. (2004) evaluated relative abundance of sponge species at Hidden Creek and Sponge Haven by prevalence on roots. Specific ranks of the species by prevalence were different from ranks assigned by volume in this study, but the match between the 10 most abundant species with respect to percent of prop roots inhabited (Diaz et al. 2004) and the 10 most abundant species with respect to volume (this study) is 80% at Hidden Creek and 60% at Sponge Haven. Resolution of systematic challenges may increase the match; for example, a second species of *Tedania* was only formally identified (Wulff, 2006) at Sponge Haven after the study by Diaz et al. (2004) was published.

Evaluating abundance by presence/absence can also address an important community assembly issue: the probability that the community on a root will include a particular species. Sutherland (1980) pointed out the great importance of habitat division into small discrete patches by explicitly comparing the course of community development on prop roots versus on the 20 × 122 cm asbestos panels he deployed for evaluating recruitment. The larger area of the panels increased the probability that the competitively dominant, but inefficiently recruiting, *Tedania ignis* recruited onto every physically separated substratum patch. Once settled on a panel, this species was able to continue its growth in every direction, and each panel became quickly dominated by it. Each root had much less

surface area, providing a smaller target for settling larvae of competitively dominant species. As predicted, if the roots are therefore more reliable refuges for competitively inferior species, the species composition on the roots was far more heterogeneous (Sutherland, 1980).

For ranking species by relative abundance, the greatest discrepancies between abundance measures (i.e., volume, area, number of individuals, and percent of roots) emerge when applied to thinly encrusting species, as their volume can be trivial even when they cover large areas (e.g., see Wulff, 2001, for an explicit comparison in a coral reef sponge community). The possibility that encrusting species may be relatively ephemeral because they are easily overgrown is supported by a comparison between the pattern of recruitment of the thinly encrusting species *Clathria campecheae* onto initially bare polyvinyl chloride (PVC) pipes at Hidden Creek (Wulff, 2004) and its abundance in the established community on prop roots. This species was described from coral reefs and had not been reported from mangroves, and yet it distinguished itself by occurring on more pipes (7/8) than any other species at 20 months after they were suspended among the mangrove roots. Once the possibility of its occurrence on mangrove roots was raised, it was discovered at a very low level on prop roots at Hidden Creek and Sponge Haven.

This finding raises the question of how the successional stage of communities on censused roots might influence the evaluation of similarity of assemblages between sites and over time. Sutherland (1980) labeled 116 roots that had not yet entered the water, in addition to 260 roots that had already been colonized below the water surface. Sponge species that specialize on colonizing fresh roots would have therefore been included in his assessment of the total fauna. Because I followed roots with already established sponge faunas, and only added new roots that sprouted from subtidal portions of previously included roots (i.e., new roots that could be colonized by sponge growth from already censused portions), the earliest successional stages were not included in my assessment of community dynamics. *Clathria campecheae*, mentioned above, was not the only species that was disproportionately well represented on PVC pipes deployed for recruitment at Hidden Creek 20 months earlier. *Haliclona curacaoensis*, *Biemna caribea*, and *Haliclona manglaris* were also conspicuous with respect to numbers of individuals, percent of pipes colonized, and (for *H. curacaoensis* and *B. caribea*) volume, in this relatively early stage of community development on initially bare pipes (Wulff, 2004: fig. 3). The pattern of loss of these species from one census to the next (see Figures 4–6) is consistent with the possibility that these are early succession species that are progressively lost from roots as sponge species that are superior competitors accumulate over time. These data support Sutherland's (1980) suggestion that the mangrove root inhabitants illustrate a trade-off between colonization rate and ability to persist in the community, and raise the possibility that stability of these communities, if measured as change over time, will depend on the successional stage on the monitored roots. The earlier in succession the assemblage on a root is, the more likely that subsequent censuses will reveal changes in species composition. Apparent instability will be further magnified if percent cover is the metric chosen for abundance, as thinly encrusting species that are efficient recruiters, but may be eliminated as superior competitors recruit, will initially have very high abundances with respect to area covered.

Observational units in previous studies have ranged from camera framer-length segments of roots to root clusters. Bingham and Young (1995) monitored 21 cm long root segments at 1 and 2 month intervals. Their analysis revealed how changes in abundance appear at different monitoring intervals, providing insight into the complex and rapidly changing dynamics of these communities at particular locations on roots. Their spatial position-focused analysis is complementary to the individual organism-focused analysis in the present study. Because the position of sponge individuals can shift along the prop roots as they increase and decrease in size, it is possible for them to move into a particular root segment without a recruitment event and to move out of a root segment while still persisting on the root. Thus a sponge assemblage within a root segment may appear less stable than the assemblage on that entire root. Differences in conclusions of Sutherland and Bingham and Young were attributed by the latter authors to greater influence of physical disturbance and seasonality on a subtropical site (Florida Keys) relative to a tropical site (Venezuela), but it is possible that difference in choice of observational unit might have also influenced evaluations of stability.

The balance between numbers of individuals monitored, frequency of monitoring, and method of evaluating abundance must be struck with the ultimate aim in mind. Following individual sponges over time and evaluating their size with respect to volume were essential to the aims of this study, which were to understand the life history and morphological strategies employed by each species. Inevitably the number of individuals and roots that could be followed in such detail suffered, as did the frequency of

monitoring. Some compensation for these failings is made by the detail of the time-series drawings of entire roots. Detailed maps of the location of each sponge and comments about its shape and size at each census allowed fragmentation, size decrease, and addition of new recruits to be unambiguously distinguished, even when the causes of size change were not obvious. It is likely that new sponges recruited and vanished, and resident sponges changed in size in multiple ways, during the year-long intervals between censuses, and so my data only indicate the net result of months of unmonitored dynamics.

Significance of Ecological Characteristics of Sponge Species

Proportions of sponges that increased, decreased, fragmented, or disappeared were similar for given species among sites, suggesting that these may be species-level characteristics. With a few exceptions, the set of species that reliably exhibited 40% to 100% of individuals increased between censuses were the same at all three sites (*Tedania ignis*, *Haliclona implexiformis*, *Lissodendoryx isodictyalis*, *Spongia* spp., *Halichondria magniconulosa*), and constituted large proportions of the total sponge volume (85%, 82%, and 72% at HC, SH, and BT, respectively) at each site. Numbers of individuals of these species found on eight initially bare PVC pipes 20 months after they were suspended among prop roots at Hidden Creek, ranged from 0 (*T. ignis*, *H. implexiformis*, and *Spongia* spp.) to 7 (*L. isodictyalis*) (Wulff, 2004). By contrast, the set of species for which only 0% to 30% of the individuals increased in size between censuses (i.e., *B. caribea*, *H. curacaoensis*, *H. manglaris*, *Clathria campecheae*) were each represented on the recruitment pipes by 11 to 14 individuals (Wulff, 2006). These patterns hint at the possibility of integrated sets of ecological characteristics that help to maintain all these species in the mangrove prop root system. Population dynamics of at least some of the typical mangrove root sponge species may be tied to their each being most suited to a particular time period in community development.

Overall community change, measured by total biomass, species diversity, numbers of individuals, and space occupied, can be functionally of great importance on an ecosystem level. However, an exclusive focus on these community-level metrics can obscure the components of community change—that is, changes in the component species—and therefore hamper our understanding of underlying processes. Consideration of the characteristics of individual species, such as their probability of persisting from year to year; their efficiency at recruiting; susceptibility to particular biotic mortality sources such as predators, competitors, or pathogens; and the frequency with which they fragment or suffer partial mortality, may explain much of the community dynamics. Combining these new data on persistence with previously reported recruitment data (Wulff, 2004) indicates that some of the heterogeneity in space and time among mangrove prop root communities may be the result of the community on each root progressing independently through a successional sequence that is mediated, at least in part, by an inverse relationship between ability to hold space on mangrove roots and recruitment into the community that was first suggested by Sutherland (1980). Adding to these life cycle-mediated patterns the observed idiosyncratic responses of particular species at a particular site, such as *Tedania klausi* succumbing to disease at the Bocas del Toro site or *Halichondria magniconulosa* targeted for consumption by a pair of French angelfish at Sponge Haven, allows community dynamics to be understood as the result of a complex set of interactions among individual sponges representing species that are characterized by specific physiological tolerances and morphological and life history traits.

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