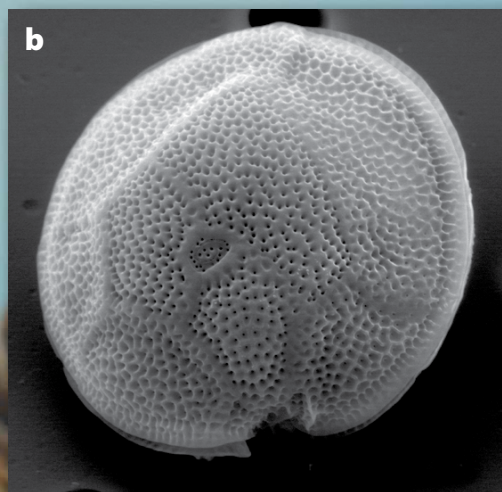# Harmful Algal Blooms in Benthic Systems

Recent Progress and Future Research

By Elisa Berdalet, Patricia A. Tester, Mireille Chinain, Santiago Fraga, Rodolphe Lemée, Wayne Litaker, Antonella Penna, Gires Usup, Magda Vila, and Adriana Zingone

ABSTRACT. Shallow, well-illuminated coastal waters from tropical to temperate latitudes are attractive environments for humans. Beaches and coral reefs have provided lodging and food to coastal communities for centuries. Unfortunately, tropical regions traditionally have been threatened by outbreaks of the toxic benthic dinoflagellate *Gambierdiscus,* which is associated with ciguatera fish poisoning. The ciguatoxins produced by *Gambierdiscus* bioaccumulate in reef fishes and are responsible for the most common algal toxin-related illnesses, globally affecting the greatest number of victims and often with significant long-term health effects. Recently, *Gambierdiscus* has been documented in subtropical and temperate latitudes. Blooms of another benthic and toxic dinoflagellate, *Ostreopsis*, have become more frequent and intense, especially in temperate waters. *Ostreopsis* produces palytoxins and analogues, and some outbreaks have been associated with massive benthic faunal damage and respiratory irritations in humans exposed to aerosols. The increased frequency of harmful events and the biogeographic extension of benthic microalgae incentivized the launch of the Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) Core Research Project on "Benthic Harmful Algal Blooms" in 2010. This article summarizes the main scientific advances and gaps in related knowledge as well as advances the project has made toward managing and mitigating the impacts of benthic HABs on human illnesses and marine resource losses.

INTRODUCTION

"Benthic Harmful Algal Blooms" (BHAB) was the last Core Research Project (CRP) launched by the Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) program. Through discussions concerning the state of knowledge about BHABs at the international Open Science Meeting (OSM) in Honolulu in June 2010, participants identified the main gaps in knowledge of the dynamics of these events and the research priorities that would advance understanding of BHABs, aid in their prediction, and manage their impacts on human health and the environment. Results of these discussions are presented in the GEOHAB report of this meeting (GEOHAB, 2012). The objectives and main questions were organized based on the key research elements identified in the GEOHAB science and implementation plans. A section on toxins and their impacts on human health and ecosystems was also included in this report (GEOHAB, 2012). The document constituted a sound basis for

implementation of the BHAB CRP and was also presented at the First International Conference on *Ostreopsis* Development held in Villefranche-sur-mer, France, in April 2011 (Zingone et al., 2012).

Since the launch of the BHAB CRP, the international community has worked intensively to address the key questions identified at the 2010 OSM. Although GEOHAB concluded in 2014, this article is the first published as a global product of its BHAB CRP. Although not a complete summary, it demonstrates the rapid and relevant progress made in the study of BHABs while addressing the overall goals of the BHAB CRP. We also highlight research activities that require further efforts and will benefit from cooperative international research.

THREATS TO HUMAN HEALTH AND WELL-BEING POSED BY BHABS

Ciguatera fish poisoning (CFP), the most frequent algal toxin-related seafood-borne illness, is caused by ingestion of

marine fish (Figure 1) contaminated with ciguatoxins (CTXs). CTXs are produced by several *Gambierdiscus* species, which grow epiphytically, mainly in macroalgae in coral reefs. It is thought that the toxins enter the food web through herbivorous fish and are subsequently bioaccumulated and biomagnified in upper trophic levels (carnivorous fish) that are consumed by humans; however, elucidation of the exact food web transfer pathway is still needed. CTXs are neurotoxins, and typical CFP intoxication is characterized by different symptoms (gastrointestinal, bradycardia with hypotension, cold allodynia, paresthesia, dysesthesia) that can last from weeks to months (e.g., Friedman et al., in press, and references therein). CFP is estimated to affect globally 25,000 to 50,000 people annually, but the real incidence is not known due to underreporting and misdiagnosis. People consulting a physician would account only for 0.1% or less of affected persons in the Caribbean islands and 20% of the actual cases in the South Pacific (Skinner et al., 2011). Furthermore, medical professionals often lack knowledge of CFP or do not participate in voluntary, or even mandatory, notification programs (e.g., Chateau-Degat et al., 2007).

Although CFP is endemic to tropical and subtropical areas, increased fish trade and tourism has resulted in "imported" cases of CFP in Europe, Canada, and the United States (e.g., Centers for Disease Control and Prevention, 2013; Mattei et al., 2014). Furthermore, CFP has also been reported in previously unaffected areas such as the coast of West Africa, Madeira, and the Canary Islands (Bienfang et al., 2008; Boada et al., 2010). Recent observations suggest expansion of the biogeographical range of *Gambierdiscus* spp. and ciguatoxic fish (see later section on Future Trends of BHABs with Climate Change).

In tropical areas, CFP affects other aspects beyond human health (e.g., Rongo and van Woesik, 2012). Public health system costs increase; productivity is reduced due to illness; valuable fisheries

FACING PAGE. (a) Ciguatera represents a serious impediment to the development of coral reef fish trade in the tropics. *Credit: Institut Louis Malardé* (b) Scanning electron microscopy (SEM) image of *Gambierdiscus balechii* from the Celebes Sea. *Credit: S. Fraga* (c) SEM image of *Ostreopsis* and *Coolia* cells. *Credit: M. Vila* (background photo) Underwater, the mucous carpet containing *Ostreopsis* cells covers the macroalgal community dominated by *Jania* spp. (Cala San Basilio, Gulf of Naples, Italy). *Credit: D. di Cioccio, SZN*
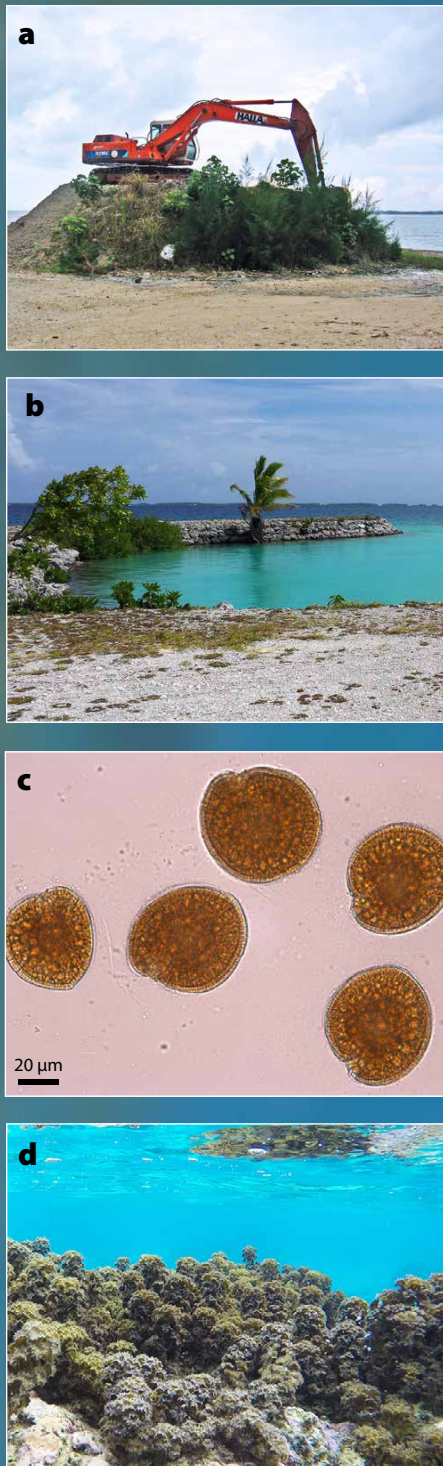
FIGURE 1. Anthropogenic disturbances such as (a) dredging and (b) dock construction are factors likely to favor the proliferation of the (c) ciguatera-causing dinoflagellate *Gambierdiscus* (scale bar: 10 μm) within (d) macro-algal mats colonizing dead coral substrates. Ciguatera represents a serious impediment to the development of coral reef fish trade in the tropics. *Photo credits: Institut Louis Malardé*

resources are underutilized, leading to a loss in tourism revenue; and there are cultural changes from the traditional food supply to new, imported protein sources. In addition, costly monitoring and management programs are required to protect the public.

Similarly, *Ostreopsis* constitutes a threat to human health and well-being (Figure 2). The potent neurotoxin palytoxin (PLTX) has been isolated in several *Ostreopsis* species (Taniyama et al., 2003; Ciminiello et al., 2010). PLTX from *Ostreopsis* was first related, although not confirmed, to occurrences of seafood poisoning (clupeotoxism) in tropical areas (Randall, 2005). In the last 20 years, outbreaks of *Ostreopsis* have been increasing in more temperate waters and are associated with macrofauna mortality (e.g., Shears and Ross, 2009). In the Mediterranean, blooms *O.* cf. *ovata* near certain beaches have been related to acute respiratory irritation (rhinorrhoea, pharyngeal pain, dry cough, nose irritation) and general malaise, headache, fever (≥38ºC), eye irritation, and/or dermatitis (references in Vila et al., 2016). Most of the symptoms disappear within a few hours without specific medication when people move away from the area affected by the *Ostreopsis* bloom, and people exposed to the irritative aerosol rarely require hospitalization. Molecular analyses of aerosols collected at a northwestern Mediterranean beach confirmed the presence of *O.* cf. *ovata* cells (Casabianca et al., 2013); at the same time, ovatoxin-a (OVTX-a, a PLTX-like toxin) and trace levels of OVTX-b, d+e, and putative PLTX were detected (Ciminiello et al., 2014). A recent simultaneous epidemiology and ecology study in an *O.* cf. *ovata* hotspot suggested that the health symptoms seem to occur during short periods within a longer bloom event (Vila et al., 2016). The irritating compounds could be produced during a particular physiology cell stage, and certain wind and water hydrodynamic conditions could facilitate dispersion; however, more in-depth studies are required to prove these connections. Fortunately, until now, no seafood poisoning has been reported in the Mediterranean. However, the detection of PLTX-like compounds in marine macrofauna (Aligizaki et al., 2008; Biré et al., 2015) above the recommended threshold levels established by the European Food Agency (EFSA, 2009) raised concern. Although, to date, the impact on human health has been minor, the potential expansion of the genus to temperate waters indicates more severe impacts are possible (Lemée et al., 2012). For example, it was estimated that in the Provence-Alpes-Côte-d'Azur region of the French Mediterranean coast, an increase in *Ostreopsis* outbreaks could have an economic cost of several hundred thousand to several million euros from lost tourism, depending on the severity of the blooms (Lemée et al., 2012).

Because of all of the various impacts discussed above, there is a need for close collaboration among scientists, managers, and public health authorities to address the challenges posed by BHABs, as laid out by the new program GlobalHAB (http://www.globalhab.info).

IMPROVING TAXONOMY TO IDENTIFY ORGANISMS

Reliable taxonomy of *Gambierdiscus* and *Ostreopsis* species is fundamental for efficient monitoring of their distribution trends and to interpret environmental influences on their population dynamics. For these reasons, great effort has been made to facilitate the identification of benthic taxa by combining morphological characteristics and effective molecular methods, as recently integrated in Hoppenrath et al. (2014).

In the case of *Gambierdiscus*, comparison of ribosomal sequences allowed Litaker et al. (2009) to clarify phylogenetic relationships between *G. australes*, *G. belizeanus*, *G. caribaeus*, *G. carolinianus*, *G. carpenteri*, *G. pacificus*, *G. polynesiensis*, *G. ruetzleri*, *G. toxicus*, *G. yasumotoi*, and two ribotypes (1 and 2) that could represent new species. Screening of samples from divergent locations with these assays has provided new insights into the biogeography of these species (e.g., some are endemic to the tropical Pacific or Caribbean while others are more widely distributed). Since then, six additional

species have been newly described: *G. excentricus* and *G. silvae* from the Canary Islands (Fraga et al., 2011; Fraga and Rodríguez, 2014); *G. scabrosus* from Japanese coastal waters (Nishimura et al., 2014); *G. balechii* discovered in the Celebes Island (Fraga et al., 2016; see opening page photo b); *G. cheloniae* from the Cook Islands (Smith et al., 2016), and *G. lapillus* in Great Barrier Reef waters (Kretzschmar et al., 2017). The *Gambierdiscus* cf. *caribaeus* isolate characterized by Jeong et al. (2012) likely represents a distinct species as well. Recently, globular species of *Gambierdiscus* were transferred to a new genus, *Fukuyoa,* which now includes the former species *G. ruetzleri* and *G. yasumotoi* plus a new species, *F. paulensis* (Gomez et al., 2015).

The taxonomy of the *Ostreopsis* genus requires major revision due to the not-well-understood high variability of the species' morphology and the lack of genetic characterization. Several recent phylogenetic studies (Sato et al., 2011; Penna et al., 2012) supplied an increasing number of molecular clades (i.e., representing groups with a common ancestor). According to Penna et al. (2012), the *Ostreopsis* species would be grouped in four main clades: (1) *Ostreopsis* cf. *lenticularis*/*O.* cf. *labens* (in the Indo-Pacific region), (2) *Ostreopsis* cf. *siamensis* (Atlantic, Pacific, and Mediterranean isolates), (3) *Ostreopsis* cf. *ovata* (species complex including different Atlantic, Mediterranean, and Pacific isolates), and (4) *Ostreopsis* spp. (including new isolates from the Mediterranean Sea and East Atlantic Ocean). Recently, strains isolated from Cyprus and the coast of Lebanon were identified as the new species *Ostreopsis fattorussoi* (Accoroni et al., 2016).

Progress in molecular taxonomy has fostered the development of species-specific polymerase chain reaction (PCR) assays for many *Gambierdiscus* and some *Ostreopsis* species. So far, real-time PCR (qPCR) assays exist for most of the *Gambierdiscus* species (Vandersea et al., 2012; Nishimura et al., 2016) and for *O.* cf. *ovata* (Perini et al. 2011; Casabianca et al., 2013). The availability of such assays applied to different environmental samples (i.e., macroalgae, water, aerosol, and other substrates) will be extremely helpful for timely monitoring of target cells during harmful events.

TOWARD A STANDARDIZED SAMPLING METHOD FOR CELL ABUNDANCES

Interest in benthic HABs is relatively recent, which could explain the lack of a standard quantitative method for estimation of cell abundance to compare data across studies. Finding the ideal sampling procedure for BHABs is not an easy task. The method should be compatible with the structural complexity and intrinsic patchiness of their benthic habitats, the diversity of substrates where the benthic cells attach and proliferate (macroalgae, seagrass, sand, pebbles, rocks, coral, and coral rubble), the biology of the species (which alternate planktonic and benthic stages), and the associated spatial and temporal scales of variability. At the small scale, variability in cell distribution may be quite high, linked to local habitat characteristics (e.g., wave and light exposure, topography, substrate). At the larger scale, there is a need to track possible biogeographic and global changes of BHABs.

Collection of substrate is the most common method for obtaining benthic HAB species for estimation of cell abundance (Yasumoto et al., 1980). Macroalgae, coral, or sand are collected, placed in containers, and vigorously shaken to remove the attached cells. The samples are then sieved to remove detritus and larger organisms before the detached cells are preserved for counting. Cell densities are expressed as cells·g⁻¹ wet weight algae (coral, sand). The destruction of coral and the ephemeral nature of macrophyte substrates is of concern when using this method.

Recently, the use of an artificial substrate has been proposed as a standard method for sampling BHAB species (Tester et al., 2014; Jauzein et al., 2016). A piece of fiberglass screen (e.g., 10.2 cm × 15.2 cm), easy to deploy and nondestructive, allows clean sample collection (Figure 3). The method is based on the observation that benthic HAB species

FIGURE 2. (a) Optical microscopy image of *Ostreopsis* cf. *ovata* cells (scale: 20 µm). (b) Mucilageous aggregates of *O.* cf. *ovata* cells floating at surface during bloom in the summer 2014 off Sant Andreu de Llavaneres beach (Northwest Mediterranean). (c) Underwater, the mucous carpet containing *Ostreopsis* cells covers the macroalgal community. (d) Detail of the cell aggregates released from the benthos in Villefranche-sur-mer (South of France). *Credits: (a, b, c) E. Berdalet and M. Vila (d) C. Jauzein (UPMC-CNRS)*

migrate into the water column and colonize new substrates over short distances (Nakahara et al., 1996). Thus, the benthic cells recruited on the artificial substrate over 24 hours, normalized to the surface area of the screen, constitute a proxy of the overall density of BHAB cells on macrophytes or corals in the surrounding habitat. This method allows comparison of results among groups, independent of the local substrates, but it requires two trips to the sampling site.

IMPROVED AND RELIABLE DETECTION OF TOXINS IN *OSTREOPSIS* AND *GAMBIERDISCUS* CELLS AND THROUGH THE FOOD WEB

Accurate characterization of toxin profiles is essential for determining the interspecific and intraspecific toxicity of BHAB species and isolates. This information could support the use of species identification to determine the environmental risk factors for CFP or any other health risk. Currently, two of the greatest impediments to progress in this area include the lack of (1) commercially available analytical CTX, PLTX-like standard compounds (ovatoxins and ostreocins), and (2) inexpensive and reliable screening tests for these toxins.

In the last several years, major advances have occurred simultaneously in analytical technology (e.g., Caillaud et al., 2010), cell culture techniques, and functional assays such as the mouse neuroblastoma cell line N2A and the receptor-binding assay (RBA). Some preliminary data about species-specific *Gambierdiscus* toxicity have been obtained by a new, rapid extraction procedure, which facilitates the simultaneous detection of the lipid-soluble CTX and the water-soluble maitotoxin (MTX) fractions obtained from *Gambierdiscus* extracts and validated by liquid chromatography mass spectrometry (LC/MS) and functional bioassay (Lewis et al., 2016). Based on the combination of these and other methods, CTX and MTX have been clearly detected in *G. polynesiensis* and *G. scabrosus* in the Pacific, and *G. excentricus* in the Caribbean/Atlantic; these species would likely pose the main concerns for toxin transfer into the marine food webs where detected (Chinain et al., 2010a; Fraga et al., 2011; Nishimura et al., 2014; Rhodes et al., 2014).

Furthermore, Hardison et al. (2016) developed a fast (<3 hours to complete), non-radioactive, cost-effective, fluorescence-based receptor binding assay (RBA$_{(F)}$) for screening fish samples for CTXs. The assay is stable over long periods of time, is compatible with common extraction methods for CTX and most fluorescence plate readers, and it could be incorporated into routine CTX monitoring programs in the CFP endemic areas.

With the latest advances in liquid chromatography/high-resolution multiple-stage mass spectrometry (LC-HRMS/MS) analytical technologies, new PLTX analogues have been described. García-Altares et al. (2015) identified OVTX-g and isobaric PLTX in strains isolated off the south of Catalonia (Northwest Mediterranean) and Tartaglione et al. (2016) four new OVTXs (i, j$_1$, j$_2$ and k) in the *Ostreopsis fattorussoi* from Cyprus. This new species exhibited very low total toxin content (0.06–2.8 pg·cell^{-1}) compared to the values reported in *O.* cf. *ovata* Mediterranean strains (10–75 pg·cell^{-1}; e.g., Séchet et al., 2012; see more references in Accorni and Totti, 2016). Usually, OVTX-a is the dominant form.

Refined analytical methods are necessary to understand the toxin transfer processes through food webs and the impacts on macrofauna. Progress has been slow for CTX (e.g., Mak et al., 2013) and PLTX-like compounds (Brissard et al., 2014; Biré et al., 2015). Ecotoxicology tests are revealing a distinctive sensitivity of model organisms to these toxins (e.g., Giussani et al., 2015). Pathological inflammatory responses in tissues and organs have been observed in mussels (Carella et al., 2015). Natural populations of the Mediterranean sea urchin *Paracentrotus lividus* exposed to *Ostreopsis* suffered reproductive impairments, with their offspring continuing to exhibit developmental anomalies for several months after the bloom abated (Migliaccio et al., 2016).

UNDERSTANDING HABITAT PREFERENCES AND ECOLOGICAL LINKS

BHABs occur in relatively shallow waters, where microalgae attach to different substrates by producing mucopolysaccharide filaments and mucous layers (e.g., Honsell et al., 2013). At the small scale, these habitats are highly variable. Water motion often may be intense, although calm conditions frequently favor the blooms (e.g., Shears and Ross, 2009; Richlen et al.,
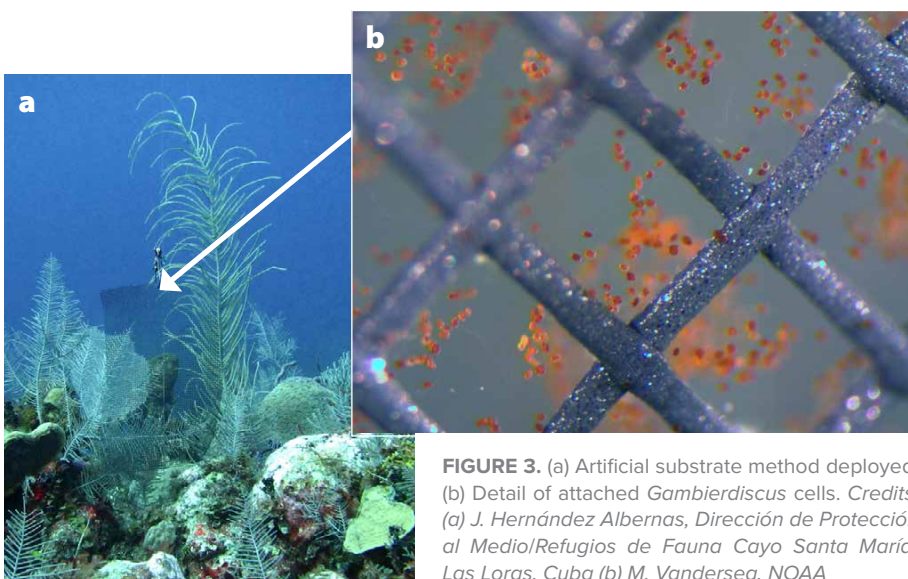

FIGURE 3. (a) Artificial substrate method deployed. (b) Detail of attached *Gambierdiscus* cells. *Credits: (a) J. Hernández Albernas, Dirección de Protección al Medio/Refugios de Fauna Cayo Santa María-Las Loras, Cuba (b) M. Vandersea, NOAA*

2011). Usually, irradiance is high, but the microalgae can also find protection within the mucous matrix or the macroalgae structure. *Gambierdiscus* blooms occur in tropical waters, but the controlling role of temperature is not clear in the case of *Ostreopsis* that thrive along more temperate coasts. Efforts have focused on understanding the physiological adaptations of these organisms to variations in physical, chemical, and biological conditions, which limit or promote their growth and toxicity. So far, the research shows species-specific responses, and only some aspects are summarized here. Overall, we would expect *G. caribaeus, G. carpenteri*, and *G. pacificus* to be more tolerant to variable environmental conditions than other species tested (*G. australes, G. belizeanus, G. carolinianus, F. ruetzleri*, and ribotype 2; Kibler et al., 2012; Xu et al., 2016), but such comparisons among *Ostreopsis* species have not yet been made.

Concerning *Gambierdiscus* species, in the laboratory, optimal growth occurs at temperatures between 21.0°C and 32.5°C and salinities between 20 and 45, with growth rates up to 0.3 d^{-1} (rarely higher) (Kibler et al., 2012; Xu et al., 2016). In the Caribbean, *Gambierdiscus* cell abundances correlate positively with temperature (Tester et al., 2010).

In the case of *Ostreopsis*, optimal growth conditions in the laboratory are found between 22°C and 30°C, but a direct correlation with temperature is not clear. In the northern Mediterranean (Mangialajo et al., 2011), the higher cell abundances mostly occur in mid-summer (end of July) in the northwest basin and in late summer/early fall (September–October) in the Adriatic Sea. However, data from three consecutive years (2007–2009) at 14 sites shows poor correlation between cell densities and seawater temperatures, with maximal abundance periods appearing to be site- and year-specific. In the Northwest Adriatic Sea, the highest abundances of *O.* cf. *ovata* were always recorded when temperature was decreasing from 25°C to 20°C (Accoroni and Totti, 2016). Cohu et al. (2011) suggest the existence of

distinct patterns in bloom timing in the Northwest Mediterranean Sea that correspond to very different hydroclimatic scenarios, and especially, spring weather. Temperature windows (23°C to 27.5°C) allow *Ostreopsis* growth along the French Mediterranean coast where the first epiphytic cells were detected at 13.0°C, and high abundances (>10,000 cells g^{-1} of macroalgae wet weight) began to occur at 18°C (Cohu et al., 2013). Southward, along Spain's Catalan coast, bloom burst usually starts at the end of June (Vila et al., 2016), after a sustained increase in air and water temperatures above a threshold of 20°C and wind intensities below 3 m s^{-1} (on average). Along the Atlantic coast of the Iberian Peninsula, absolute temperature alone cannot explain *Ostreopsis* distribution (David et al., 2012); three continuous months with sea surface temperature above 19.5°C may be necessary for *Ostreopsis* to be present in that area.

The role of salinity in *Ostreopsis* growth and bloom development is still unclear. Again, laboratory experiments report species-specific and strain-specific responses to salinity ranges. In the field, maximum cell abundances are found at salinities of 30 to 39, and are very low in river plumes (salinity values 25–35; e.g., Delgado et al., 2006; Blanfuné et al., 2015). However, because low salinities are associated with land runoff and potential nutrient supply, discrimination of the individual roles of these two factors is difficult.

The limited available data do not allow a clear causal relationship between inorganic nutrient supply and *Ostreopsis* sp. blooms to be drawn (GEOHAB, 2012). The nutrients required to reach bloom concentrations are likely acquired directly from the nutrient-rich sediments or directly from the macroalgal surfaces (Litaker et al., 2010, and references therein). Furthermore, it is hypothesized (but not tested) that nutrient inputs that favor the growth of macroalgae (Lapointe et al., 2010) could also promote blooms.

The role of irradiance in *Ostreopsis* bloom dynamics is also difficult to

separate from the preferential depth. The macroalgae host thallus may protect cells from high sun exposure in shallow waters. Furthermore, the available experiments are not conclusive and are often contradictory (reviewed in Accoroni and Totti, 2016). In the case of *Gambierdiscus,* for all strains tested, growth was higher at 110–400 μmol photons m^{-2} s^{-1} than at 55 μmol photons m^{-2} s^{-1} (Kibler et al., 2012; Xu et al., 2016).

As an adaptation to benthic life, in addition to having flattened shapes, both *Gambierdiscus* and *Ostreopsis* produce a mucopolysaccharide matrix to attach to substrates. The internal organelles related to mucus production and extrusion, trichocysts and mucocysts, have been described for *Gambierdiscus* (Durand-Clément and Conté, 1991) and *Ostreopsis* (Honsell et al., 2013; Escalera et al., 2014; Figure 4). However, the exact role of these unique structures (e.g., attachment, defense) is not clear in these dinoflagellates.

Finally, research has been initiated to determine the allelopathic relationships between *Ostreopsis* spp. and microphytobenthos and macroalgal substrates and to characterize the related bacterial assemblages (e.g., Blanfuné et al., 2015; Accoroni and Totti, 2016, and references therein; Vanucci et al., 2016).

INVESTIGATING THE LIFE CYCLE OF BENTHIC DINOFLAGELLATES TO BETTER UNDERSTAND BLOOM DYNAMICS

For many dinoflagellate species, life history is known to play a key role in bloom formation (e.g., Bravo and Figueroa, 2014). We are just beginning to understand the life histories of *Gambierdiscus* and *Ostreopsis*. Bravo et al. (2014) described meiosis and gametogenesis, and the occurrence of division processes in both the sexual and asexual cycle in *Gambierdiscus*. Still, it is unclear at what stage mitosis and meiosis occur. *Ostreopsis* species exhibit high morphological variability that could correspond to different life-cycle stages, although

the exact role of the different forms and stages has not yet been established. Sexual and asexual reproduction, and temporary and resting cysts have been observed in both laboratory and field samples of *Ostreopsis* (Accoroni and Totti, 2016). Big (and dark) cells are associated with less-favorable proliferation conditions (e.g., nutrient limitation, exposure to high hydrodynamism) and/or to the stationary phases of the bloom, while small cell forms that could act as gametes are found in different phases of the blooms.

EXPLORING NEW BHAB EVENTS PRODUCING ORGANISMS

GEOHAB (2012) points out the uncertainties regarding the toxicity of other benthic genera, including other dinoflagellates (*Coolia* spp., *Prorocentrum lima*), diatoms (*Nitzschia navis-varingica*, *Amphora coffeaformis*), and cyanobacteria. In the case of *Coolia monotis*, toxicity was reported in the first study by Holmes et al. (1995). However, none of the strains examined genetically, morphologically, and toxicologically by Penna et al. (2005) were toxic.

Several cyanobacteria species, *Hydro-coleum glutinosum*, *Phormidium laysanense*, *Spirulina weissi*, *Oscillatoria* cf. *bonnemaisonii*, *Anabaena* sp., and *Trichodesmium erythraeum* were associated with atypical CFP incidents in New Caledonia, French Polynesia, and Vanuatu (Laurent et al., 2012) following the ingestion of giant clams and/or fish from lower trophic levels. Toxicological studies conducted on cyanobacterial mats and/or the molluscs collected from contaminated locations using RBA, cell-based assay, and LC-MS/MS confirmatory analysis suggest the presence of complex toxin suites in these organisms, including CTX-like compounds, paralyzing toxins, PLTX, anatoxin-a, and homoanatoxin-a (Méjean et al., 2010; Kerbrat et al., 2011). Recent observations from French Polynesia also highlight similar potential health risks linked to consumption of the gastropod *Tectus niloticus* (Gatti et al., 2015). A new ecotoxicological phenomenon, ciguatera shellfish poisoning (CSP) to describe CTXs in shellfish has been proposed, and monitoring of cyanobacteria concurrently with other toxic microalgae is recommended in order to effectively manage tropical seafood poisoning.

FUTURE TRENDS OF BHABS WITH CLIMATE CHANGE

Benthic HABs seem to be following the trends of other harmful algal bloom species that are increasingly reported in both temperate and tropical regions (GEOHAB, 2012; Kibler et al., 2015). This increase may be due to more effective detection through improved observation and monitoring capacities, but also may be due to anthropogenic forcing and global climate change.

The genus *Gambierdiscus* has a pan-tropical distribution between 35°S and 35°N (see map distribution in GEOHAB, 2012). Recent observations also indicate expansion of *Gambierdiscus* to temperate areas (Jeong et al., 2012). Tester et al. (2010) suggested the possibility of range expansion in certain areas and described positive correlations between water temperature and the abundance of different *Gambierdiscus* species. Also, Gingold et al. (2014) found associations between climate variability and CFP incidence and suggested that, provided that other (complex) socio-environmental factors remain constant, climate change could increase the CFP
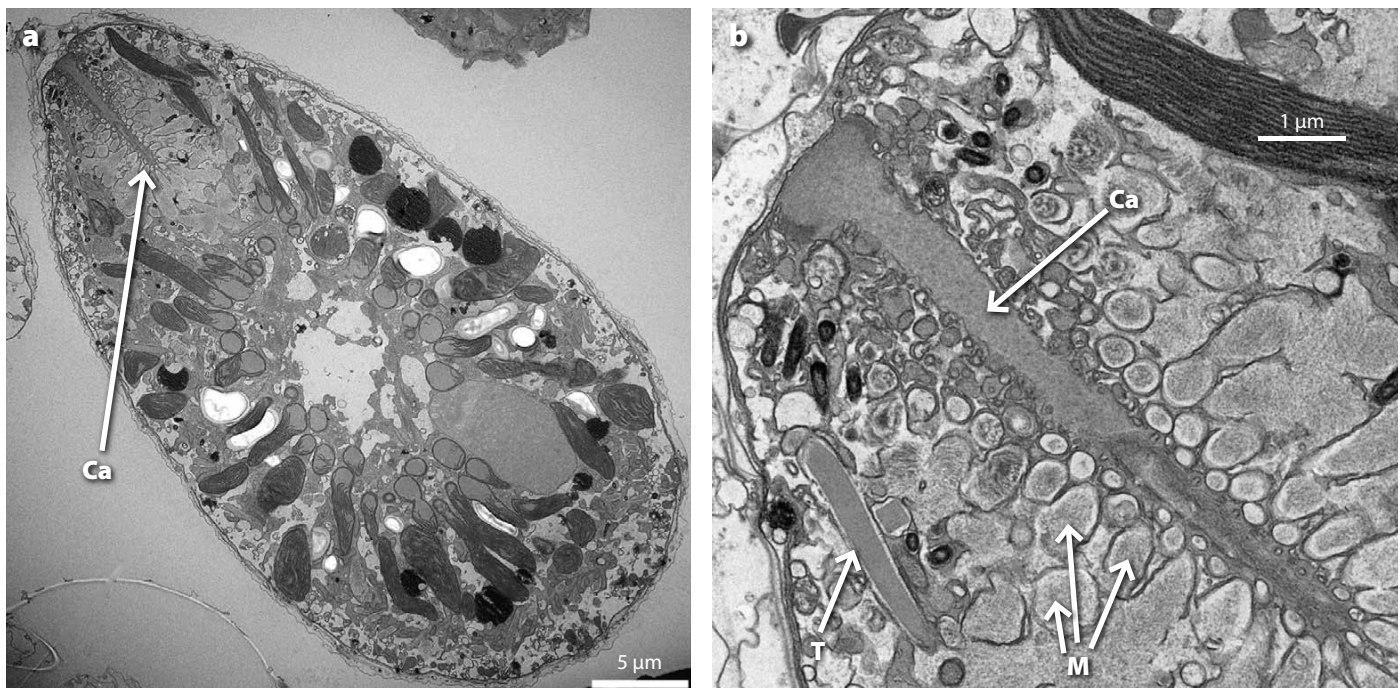

FIGURE 4. Ultrastructure (longitudinal sections) of *Ostreopsis* cf. *ovata*. (a) Whole cell. (b) Detail of the internal canal (Ca) surrounded by mucocysts (M) and trichocysts (T), located in the sulcal area of the cell (upper left corner in a). *From Escalera et al. (2014), with permission*

burden. These findings have implications for disease prediction, surveillance, and public health preparedness with warming ocean temperatures. The situation could be aggravated by other natural (hurricanes, coral bleaching episodes, or *Acanthaster plancii* outbreaks) and anthropogenic (dredging, construction, or sewage discharge in touristic coastal areas) disturbances (Figure 1) that foster the increase of CFP outbreaks in endemic areas (Chinain et al., 2010b; Rongo and van Woesik, 2012).

The *Ostreopsis* genus is present in tropical and temperate coastal waters of Australia, the Indian Ocean, eastern Asia, the Caribbean Sea, and Brazil, and, in the last decade, in colder waters of the North Pacific coasts of Japan and Russia, New Zealand, and Portugal, and the Atlantic coasts of France and Spain (e.g., GEOHAB, 2012; Accoroni and Totti, 2016). In the last 20 years there have been recurrent blooms of *Ostreopsis* cf. *ovata* in some locations in the northern and southern Mediterranean (e.g., Mangialajo et al., 2011; Illoul et al., 2012).

It is still unclear whether global warming will expand and intensify *Ostreopsis* blooms. As noted earlier, neither the physiological studies in the laboratory nor the field observations clarify whether blooms are modulated by this environmental factor. In addition, variations in sea temperature could have a greater impact on hydrography (e.g., wave intensity and currents), which may have indirect and hardly predictable consequences for the abundance and distribution of benthic species.

The potential effects of ocean acidification on BHAB events require investigation. The limited available data suggest that direct effects are unlikely. At Ischia Island (Mediterranean Sea), a site with volcanic CO_2 emissions, *Ostreopsis* cf. *ovata* was recorded at bloom concentrations at pH values similar or even lower than those predicted for the 2100 coastal ocean (Di Cioccio et al., 2014). However, ocean acidification could indirectly affect BHABs, for example, through the demise of coral reefs and their replacement by macroalgal-dominated systems that provide expanded habitats for BHABs.

CONCLUSIONS AND NEXT STEPS

This article summarizes many advances in BHAB research since the launch of the "Benthic Harmful Algal Blooms" CRP in the GEOHAB program in 2010, and notes the gaps that still need to be addressed. Progress may continue by focusing on two elements of GEOHAB: the comparative research approach and modeling. The comparative approach can contribute to understanding similarities and differences concerning CFP incidences and *Gambierdiscus* dynamics in the main affected areas (i.e., the Pacific and the Caribbean). Comparing the dynamics of *Ostreopsis* and *Gambierdiscus* can also shed light on poorly known ecological aspects related to benthic life and facilitate the design of effective management strategies in each case. Modeling BHAB dynamics will be useful, and is currently mainly limited by scarce parametrization of many biological and physical processes that occur at small scales in complex benthic ecosystems. Furthermore, long time series of BHAB events and health impacts are needed to assess the effects of climate change and put BHAB data into perspective.

Progress in benthic HAB research has greatly benefited from the international and multidisciplinary coordination fostered by GEOHAB. These collaborations will continue under the umbrella of the new program GlobalHAB, whose goal is to improve understanding, prediction, management, and mitigation of HABs in aquatic ecosystems (http://www.globalhab.info). 🄯

REFERENCES
Accoroni, S, T. Romagnoli, A. Penna, S. Capelacci, P. Ciminiello, C. Dell'Aversano, L. Tartaglione, M. Abboud-Abi Saab, V. Giussani, V. Asnaghi, and M. Chiantore. 2016. *Ostreopsis fatturussoi* sp. nov. (Dinophyceae), a new benthic toxic *Ostreopsis* species from the eastern Mediterranean Sea. *Journal of Phycology* 52:1,064–1,084, https://doi.org/10.1111/jpy.12464.

Accoroni, S., and C. Toti. 2016. The toxic benthic dinoflagellates of the genus *Ostreopsis* in temperate areas: A review. *Advances in Oceanography and Limnology* 7:1–15, https://doi.org/10.4081/aiol.2016.5591.

Aligizaki, K., P. Katikou, G. Nikolaidis, and A. Panou. 2008. First episode of shellfish contamination by palytoxin-like compounds from *Ostreopsis* species (Aegean Sea, Greece). *Toxicon* 51:418–427, https://doi.org/10.1016/j.toxicon.2007.10.016.

Bienfang, P., B. Oben, S. DeFelice, P. Moeller, K. Huncik, P. Oben, R. Tooen, T. Daly-Engel, and B. Bowen. 2008. Ciguatera: The detection of neurotoxins in carnivorous reef fish from the coast of Cameroon, West Africa. *African Journal of Marine Science* 30:533–540, https://doi.org/10.2989/AJMS.2008.30.3.8.642.

Biré, R., S. Trotereau, R. Lemée, D. Oregioni, C. Delpont, S. Krys, and T. Guérin. 2015. Hunt for palytoxins in a wide variety of marine organisms harvested in 2010 on the French Mediterranean coast. *Marine Drugs* 13:5,425–5,446, https://doi.org/10.3390/md13085425.

Blanfuné, A., C.F. Boudouresque, H. Grossel, and T. Thierry. 2015. Distribution and abundance of *Ostreopsis* spp. and associated species (Dinophyceae) in the northwestern Mediterranean: The region and the macroalgal substrate matter. *Environmental Science and Pollution Research* 22:12,332–12,346, https://doi.org/10.1007/s11356-015-4525-4.

Boada, L.D., M. Zumbado, O.P. Luzardo, M. Almeida-González, S.M. Plakas, H.R. Granade, A. Abraham, E.L.E. Jester, and R.W. Dickey. 2010. Ciguatera fish poisoning on the West Africa Coast: An emerging risk in the Canary Islands (Spain). *Toxicon* 56:1,516–1,519, https://doi.org/10.1016/j.toxicon.2010.07.021.

Bravo, I., and R. Figueroa. 2014. Towards an ecological understanding of dinoflagellate cyst functions. *Microorganisms* 2:11–32, https://doi.org/10.3390/microorganisms2010011.

Bravo, I., R.I. Figueroa, and S. Fraga. 2014. Cellular and nuclear morphological variability within a single species of the toxigenic dinoflagellate genus *Gambierdiscus*: Relationship to life-cycle processes. *Harmful Algae* 40:1–8, https://doi.org/10.1016/j.hal.2014.09.009.

Brissard, C., C. Herrenknecht, V. Séchet, F. Hervé, F. Pisapia, J. Harcouet, R. Lémée, N. Chomérat, P. Hess, and Z. Amzil. 2014. Complex toxin profile of French Mediterranean *Ostreopsis* cf. *ovata* strains, seafood accumulation and ovatoxins prepurification. *Marine Drugs* 12:2,851–2,876, https://doi.org/10.3390/md12052851.

Caillaud, A., P. De la Iglesia, H.T. Darius, S. Pauillac, K. Aligizaki, S. Fraga, M. Chinain, and J. Diogène. 2010. Update on methodologies available for ciguatoxin determination: Perspectives to confront the onset of ciguatera fish poisoning in Europe. *Marine Drugs* 8:1,838–1,907, https://doi.org/10.3390/md8061838.

Carella, F., A. Sardo, O. Mangoni, D. Di Cioccio, G. Urciuolo, G. De Vico, and A. Zingone. 2015. Quantitative histopathology of the Mediterranean mussel (*Mytilus galloprovincialis* L.) exposed to the harmful dinoflagellate *Ostreopsis* cf. *ovata*. *Journal of Invertebrate Pathology* 127:130–140, https://doi.org/10.1016/j.jip.2015.03.001.

Casabianca, S., A. Casabianca, P. Riobó, J.M. Franco, M. Vila, and A. Penna. 2013. Quantification of the toxic dinoflagellate *Ostreopsis* spp. by qPCR assay in marine aerosol. *Environmental Science and Technology* 47:3,788–3,795, https://doi.org/10.1021/es305018s.

Centers for Disease Control and Prevention. 2013. Ciguatera fish poisoning: New York City, 2010–2011. *Morbidity and Mortality Weekly Report* 62:61–65, https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6204a1.htm.

Chateau-Degat, M.-L., E. Dewailly, N. Cerf, N.L. Nguyen, M.-O. Huin-Blondey, B. Hubert, F. Laudon, and R. Chansin. 2007. Temporal trends and epidemiological aspects of ciguatera in French Polynesia: A 10-year analysis. *Tropical Medicine and International Health* 12:485–492, https://doi.org/10.1111/j.1365-3156.2006.01798.x.

Chinain, M., H.T. Darius, A. Ung, P. Cruchet, Z. Wang, D. Ponton, D. Laurent, and S. Pauillac. 2010a. Growth and toxin production in the ciguatera-causing dinoflagellate *Gambierdiscus polynesiensis* (Dinophyceae) in culture. *Toxicon* 56:739–750, https://doi.org/10.1016/j.toxicon.2009.06.013.

Chinain, M., H.T. Darius, A. Ung, M. Tchou Fouc, T. Revel, P. Cruchet, S. Pauillac, and D. Laurent. 2010b. Ciguatera risk management in French Polynesia: The case study of Raivavae Island (Australes Archipelago). *Toxicon* 56: 674–690, https://doi.org/10.1016/j.toxicon.2009.05.032.

Ciminiello, P., C. Dell'Aversano, E. Dello Iacovo, E. Fattorusso, M. Forino, L. Grauso, L. Tartaglione, F. Guerrini, and R. Pistocchi. 2010. Complex palytoxin-like profile of *Ostreopsis ovata*: Identification of four new ovatoxins by high-resolution liquid chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry* 24:2,735–2,744, https://doi.org/10.1002/rcm.4696.

Ciminiello, P., C. Dell'Aversano, E. Dello Iacovo, E. Fattorusso, M. Forino, L. Tartaglione, G. Benedettini, M. Onorari, F. Serena, C. Battocchi, and others. 2014. First finding of *Ostreopsis* cf. *ovata* toxins in marine aerosols. *Environmental Science and Technology* 48:3,532–3,540, https://doi.org/10.1021/es405617d.

Cohu, S., L. Mangialajo, T. Thibaut, A. Blanfuné, S. Marro, and R. Lemé. 2013. Proliferation of the toxic dinoflagellate *Ostreopsis* cf. *ovata* in relation to depth, biotic substrate and environmental factors in the North West Mediterranean Sea. *Harmful Algae* 24:32–44, https://doi.org/10.1016/j.hal.2013.01.002.

Cohu, S., T. Thibaut, L. Mangialajo, J.-P. Labat, O. Passafiume, A. Blanfuné, N. Simon, J.-L. Cottalorda, and R. Lemée. 2011. Occurrence of the toxic dinoflagellate *Ostreopsis* cf. *ovata* in relation with environmental factors in Monaco (NW Mediterranean). *Marine Pollution Bulletin* 62:2,681–2,691, https://doi.org/10.1016/j.marpolbul.2011.09.022.

David, H., U. Ganzedo, A. Laza-Martínez, and E. Orive. 2012. Relationships between the presence of (Dinophyceae) in the Atlantic coast of the Iberian Peninsula and sea-surface temperature. *Cryptogamie, Algologie* 33:199–207, https://doi.org/10.7872/crya.v33.iss2.2011.199.

Delgado, G., C.H. Lechuga-Deveze, G. Popowski, L. Troccoli, and C.A. Salinas. 2006. Epiphytic dinoflagellates associated with ciguatera in the northwestern coast of Cuba. *Revista de Biología Tropical* 54:299–310, https://doi.org/10.15517/rbt.v54i2.13870.

Di Cioccio, D., M.C. Buia, and A. Zingone. 2014. Ocean acidification will not deliver us from *Ostreopsis*. Pp. 85–88 in *Harmful Algae 2012. Proceedings of the 15th International Conference on Harmful Algae. International Society for the Study of Harmful Algae, Changwon, Korea.* H.G. Kim, B. Reguera, G. Hallegraeff, C.K. Lee, M.S. Han, and J.K. Choi, eds.

Durand-Clément, M., and A. Couté. 1991. Tabulation and ultrastructure of theca and trichocysts of *Gambierdiscus toxicus* (Dinophyceae, Peridinales) in culture. *Cryptogamie, Algologie* 12:137–156.

EFSA (European Food Safety Authority). 2009. Scientific opinion on marine biotoxins in shellfish—palytoxin group: Panel on contaminants in the food chain. *European Food Safety Authority Journal* 7:1,393–1,430, https://www.efsa.europa.eu/it/efsajournal/pub/1393.

Escalera, L., G. Benvenuto, E. Scalco, A. Zingone, and M. Montresor. 2014. Ultrastructural features of the benthic dinoflagellate *Ostreopsis* cf. *ovata* (Dinophyceae). *Protist* 165:260–274, https://doi.org/10.1016/j.protis.2014.03.001.

Fraga, S., and F. Rodríguez. 2014. Genus *Gambierdiscus* in the Canary Islands (NE Atlantic Ocean) with description of *Gambierdiscus silvae* sp. nov., a new potentially toxic epiphytic benthic dinoflagellate. *Protist* 165:839–853, https://doi.org/10.1016/j.protis.2014.09.003.

Fraga, S., F. Rodríguez, A. Caillaud, J. Diogène, N. Raho, and M. Zapata. 2011. *Gambierdiscus excentricus* sp. nov. (Dinophyceae), a benthic toxic dinoflagellate from the Canary Islands (NE Atlantic Ocean). *Harmful Algae* 11:10–22, https://doi.org/10.1016/j.hal.2011.06.013.

Fraga, S., F. Rodríguez, P. Riobó, and I. Bravo. 2016. *Gambierdiscus balechii* sp. nov. (Dinophyceae), a new benthic toxic dinoflagellate from the Celebes Sea (SW Pacific Ocean). *Harmful Algae* 58:93–105, https://doi.org/10.1016/j.hal.2016.06.004.

Friedman, M.A., M. Fernandez, L. Backer, R. Dickey, J. Bernstein, K. Schrank, S. Kibler, W. Stephan, M.O. Gribble, P. Bienfang, and others. In press. An updated review of ciguatera fish poisoning: Clinical, epidemiological, environmental, and public health management. *Marine Drugs*.

García-Altares, M., L. Tartaglione, C. Dell'Aversano, O. Carnicer, P. de la Iglesia, M. Forino, J. Diogène, and P. Ciminiello. 2015. The novel ovatoxin-g and isobaric palytoxin (so far referred to as putative palytoxin) from Ostreopsis cf. ovata (NW Mediterranean Sea): Structural insights by LC-high resolution MSⁿ. *Analytical and Bioanalytical Chemistry* 407:1,191–1,204, https://doi.org/10.1007/s00216-014-8338-y.

Gatti, C.M., H.T. Darius, M. Chinain, and D. Lonati. 2015. First report of a mass-poisoning outbreak following the consumption of *Tectus niloticus* (Gastropod) in French Polynesia: A novel pathway of Ciguatera shellfish poisoning? *Harmful Algae News* 50:19–20, http://www.e-pages.dk/ku/1086.

GEOHAB. 2012. *Global Ecology and Oceanography of Harmful Algal Blooms: Core Research Project—Harmful Algal Blooms in Benthic Systems.* E. Berdalet, P. Tester, and A. Zingone, eds, IOC of UNESCO and SCOR, Paris, France, and Newark, Delaware, 64 pp.

Gingold, D.B., M.J. Strickland, and J.J. Hess. 2014. Ciguatera fish poisoning and climate change: Analysis of the national poison center data in the United States, 2001–2011. *Environmental Health Perspectives* 122:580–586, https://doi.org/10.1289/ehp.1307196.

Giussani, V., F. Sbrana, V. Asnaghi, M. Vassalli, M. Faimali, S. Casabianca, A. Penna, P. Ciminiello, C. Dell'Aversano, L. Tartaglione, and others. 2015. Active role of the mucilage in the toxicity mechanism of the harmful benthic dinoflagellate *Ostreopsis* cf. *ovata. Harmful Algae* 44:46–53, https://doi.org/10.1016/j.hal.2015.02.006.

Gomez, F., D. Qui, R.M. Lopes, and S. Lin. 2015 *Fukuyoa paulensis* gen. et sp. nov., a new genus for the globular species of the dinoflagellate *Gambierdiscus* (Dinophyceae). *PLoS ONE*, https://doi.org/10.1371/journal.pone.0119676.

Hardison, D.R., W.C. Holland, J.R. McCall, A.J. Bourdelais, D.G. Baden, H.T. Darius, M. Chinain, P.A. Tester, D. Shea, H.A. Flores Quintana, and others. 2016. Fluorescent receptor binding assay for detecting ciguatoxin in fish. *PLoS ONE*, https://doi.org/10.1371/journal.pone.0153348.

Holmes, M.J., R.J. Lewis, A. Jones, and A.W. Hoy. 1995. Cooliatoxin, the first toxin from *Coolia monotis* (Dinophyceae). *Natural Toxins* 3:355–362, https://doi.org/10.1002/nt.2620030506.

Honsell, G., A. Bonifacio, M. DeBortoli, A. Penna, C. Battocchi, P. Ciminiello, C. Dell'Aversano, E. Fattorusso, S. Sosa, T. Yasumoto, and A. Tubaro. 2013. New insights on cytological and metabolic features of *Ostreopsis* cf. *ovata* Fukuyo (Dinophyceae): A multidisciplinary approach. *PLoS ONE*, https://doi.org/10.1371/journal.pone.0057291.

Hoppenrath, M., S.A. Murray, N. Chomérat, and T. Horiguchi. 2014. Marine benthic dinoflagellates: Unveiling their worldwide biodiversity. Kleine Senckenberg-Reihe 54, Schweizerbart'sche Verlagsbuchhandlung, Germany, 276 pp.

Illoul, H., F. Rodríguez, M. Vila, N. Adjas, A. Aït Younes, M. Bournissa, A. Koroghli, N. Marouf, S. Rabia, and F.L.K. Ameur. 2012. The genus *Ostreopsis* along the Algerian coastal waters (SW Mediterranean Sea) associated with a human respiratory intoxication episode. *Cryptogamie, Algologie* 33:209–216, https://doi.org/10.7872/crya.v33.iss2.2011.209.

Jauzein, C., A. Fricke, L. Mangialajo, and R. Lemee. 2016. Sampling of *Ostreopsis* cf. *ovata* using artificial substrates: Optimization of methods for the monitoring of benthic harmful algal blooms. *Marine Pollution Bulletin,* https://doi.org/10.1016/j.marpolbul.2016.03.047

Jeong, H.J., A.S. Lim, S.H. Jang, W.H. Yih, N.S. Kang, S.Y. Lee, Y.D. Yoo, and H.S. Kim. 2012. First report of the epiphytic dinoflagellate *Gambierdiscus caribaeus* in the temperate waters off Jeju Island, Korea: Morphology and molecular characterization. *Eukaryotic Microbiology* 59(6):637–650, https://doi.org/10.1111/j.1550-7408.2012.00645.x.

Kerbrat, A.S., Z. Amzil, R. Pawlowiez, S. Golubic, M. Sibat, H.T. Darius, M. Chinain, and D. Laurent. 2011. First evidence of palytoxin and 42-hydroxy-palytoxin in the marine cyanobacterium *Trichodesmium. Marine Drugs* 9:543–560, https://doi.org/10.3390/md9040543.

Kibler, S.R., R.W. Litaker, W.C. Holland, M.W. Vandersea, and P.A. Tester. 2012. Growth of eight *Gambierdiscus* (Dionphyceae) species: Effects of temperature, salinity and irradiance. *Harmful Algae* 19:1–14, https://doi.org/10.1016/j.hal.2012.04.007.

Kibler, S.R., P.A. Tester, K.E. Kunkel, S.K. Moore, and R.W. Litaker 2015. Effects of ocean warming on growth and distribution of dinoflagellates associated with ciguatera fish poisoning in the Caribbean. *Ecological Modeling* 316:194–210, https://doi.org/10.1016/j.ecolmodel.2015.08.020.

Kretzschmar, A.L.V., A. Verma, T. Harwood, M. Hoppenrath, and S. Murray. 2017. Characterization of *Gambierdiscus lapillus* sp. nov. (Gonyaulacales, Dinophyceae): A new toxic dinoflagellate from the Great Barrier Reef (Australia). *Journal of Phycology*, https://doi.org/10.1111/jpy.12496.

Lapointe, B.E., R. Langton, B.J. Bedford, A.C. Potts, O. Day, and C. Hu. 2010. Land-based nutrient enrichment of the Buccoo Reef Complex and fringing coral reefs of Tobago, West Indies. *Marine Pollution Bulletin* 60:334–343, https://doi.org/10.1016/j.marpolbul.2009.10.020.

Laurent, D., A.S. Kerbrat, H.T. Darius. F. Rossi, B. Yeeting, M. Haddad, S. Golubic, S. Pauillac, and M. Chinain. 2012. Ciguatera shellfish poisoning (CSP), a new ecotoxicological phenomenon: From cyanobacteria to humans via giant clams. Pp. 1–44 in *Food Chain: New Research*. M.A. Jensen and D.W. Muller, eds, Nova Publishers New York, NY.

Lemée R., L. Mangialajo, S. Cohu, Z. Amzil, A. Blanfuné, N. Chomérat, N. Ganzin, S. Gasparin, H. Grossel, L. Guidi-Guivar, and others. 2012. Interactions between scientists, managers and policy makers in the framework of the French MediOs Project on *Ostreopsis* (2008–2010). *Cryptogamie, Algologie* 33:137–142, https://doi.org/10.7872/crya.v33.iss2.2011.137.

Lewis, R.J., M. Inserra, I. Vetter, W.C. Holland, D.R. Hardison, P.A. Tester, and R.W. Litaker. 2016. Rapid extraction and identification of maitotoxin and ciguatoxin-like toxins from Caribbean and Pacific *Gambierdiscus* using a new functional bioassay. *PLoS ONE*, https://doi.org/10.1371/journal.pone.0160006.

Litaker, R.W., M.W. Vandersea, M.A. Faust, S.R. Kibler, M. Chinain, M.J. Holmes, W.C. Holland, and P.A. Tester. 2009. Taxonomy of *Gambierdiscus* including four new species, *Gambierdiscus caribaeus*, *Gambierdiscus carolinianus*, *Gambierdiscus carpenteri* and *Gambierdiscus ruetzleri* (Gonyaulacales, Dinophyceae). *Phycologia* 48:344–390, https://doi.org/10.2216/07-15.1.

Litaker, R.W., M.W. Vandersea, M.A. Faust, S.R. Kibler, A.W. Nau, W.C. Holland, M. Chinain, M.J. Holmes, and P.A. Tester. 2010. Global

distribution of ciguatera causing dinoflagellates in the genus *Gambierdiscus*. *Toxicon* 56:711–730, https://doi.org/10.1016/j.toxicon.2010.05.017.

Mak, Y.L., T.-C. Wai, M.B. Murphy, W.H. Chan, J.J. Wu, J.C. Lam, L.L. Chan, and P.K. Lam. 2013. Pacific ciguatoxins in food web components of coral reef systems in the Republic of Kiribati. *Environmental Science & Technology* 47:14,070–14,079, https://doi.org/10.1021/es403175d.

Mangialajo, L., N. Ganzin, S. Accoroni, V. Asnaghi, A. Blanfuné, M. Cabrini, R. Cattaneo-Vietti, F. Chavanon, M. Chiantore, S. Cohu, and others. 2011. Trends in *Ostreopsis* proliferation along the Northern Mediterranean coasts. *Toxicon* 57:408–420, https://doi.org/10.1016/j.toxicon.2010.11.019.

Mattei, C., I. Vetter, A. Eisenblätter, B. Krock, M. Ebbecke, H. Desel, and K. Zimmermann. 2014. Ciguatera fish poisoning: A first epidemic in Germany highlights an increasing risk for European countries. *Toxicon* 91:76–83, https://doi.org/10.1016/j.toxicon.2014.10.016.

Méjean, A., C. Peyraud-Thomas, A.S. Kerbrat, S. Golubic, S. Pauillac, M. Chinain, and D. Laurent. 2010. First identification of the neurotoxin homoanatoxin-a from mats of *Hydrocoleum lyngbyaceum* (marine cyanobacterium) possibly linked to giant clam poisoning in New Caledonia. *Toxicon* 56:829–835, https://doi.org/10.1016/j.toxicon.2009.10.029.

Migliaccio, O., I. Castellano, D. Di Cioccio, G. Tedeschi, A. Negri, P. Cirino, G. Romano, A. Zingone and A. Palumbo. 2016. Subtle reproductive impairment through nitric oxide-mediated mechanisms in sea urchins from an area affected by harmful algal blooms. *Scientific Reports* 6:26086, https://doi.org/10.1038/srep26086.

Nakahara, H., T. Sakami T.M. Chinain, and Y. Ishida. 1996. The role of macroalgae in epiphytism of the toxic dinoflagellate *Gambierdiscus toxicus* (Dinophyceae). *Phycological Research* 44:113–117, https://doi.org/10.1111/j.1440-1835.1996.tb00385.x.

Nishimura, T., H. Hariganeya, W. Tawong, H. Sakanari, J. Yamaguchi, and M. Adachi. 2016. Quantitative PCR assay for detection and enumeration of ciguatera-causing dinoflagellate *Gambierdiscus* spp. (Gonyaulacales) in coastal areas of Japan. *Harmful Algae* 52:11–22, https://doi.org/10.1016/j.hal.2015.11.018.

Nishimura, T., S. Sato, W. Tawong, H. Sakanari, H. Yamaguchi, and M. Adachi. 2014. Morphology of *Gambierdiscus scabrosus* sp. nov. (Gonyaulacales): A new epiphytic toxic dinoflagellate from coastal areas of Japan. *Journal of Phycology* 50:506–514, https://doi.org/10.1111/jpy.12175.

Penna, A., S. Fraga, C. Battocchi, S. Casabianca, F. Perini, S. Capellacci, A. Casabianca, P. Riobo, M.G. Giacobbe, C. Totti, and others. 2012. Genetic diversity of the genus *Ostreopsis* Schmidt: Phylogeographical considerations and molecular methodology applications for field detection in the Mediterranean Sea. *Cryptogamie, Algologie* 33:153–163, https://doi.org/10.7872/crya.v33.iss2.2011.153.

Penna, A., M. Vila, S. Fraga, M.G. Giacobbe, F. Andreoni, P. Riobó, and C. Vernesi. 2005. Characterization of *Ostreopsis* and *Coolia* (Dinophyceae) isolates in the Western Mediterranean Sea based on morphology, toxicity and internal transcribed spacer 5.8S rDNA sequences. *Journal of Phycology* 41:212–225, https://doi.org/10.1111/j.1529-8817.2005.04011.x.

Perini, F., A. Casabianca, C. Battocchi, S. Accorni, C. Totti, and A. Penna. 2011. New approach using the real-time PCR method for estimation of the toxic marine dinoflagellate *Ostreopsis* cf. o*vata* in marine environment. *PLoS ONE*, 6(3):e17699, https://doi.org/10.1371/journal.pone.0017699.

Randall, J.E. 2005. Review of clupeotoxism, an often fatal illness from the consumption of clupeoid fishes. *Pacific Science* 59:73–77.

Rhodes, L., T. Harwood, K. Smith, P. Argyle, and R. Munday. 2014. Production of ciguatoxin and maitotoxin by strains of *Gambierdiscus australes*,

G. pacificus and *G. polynesiensis* (Dinophyceae) isolated from Rarotonga, Cook Islands. *Harmful Algae* 39:185–190, https://doi.org/10.1016/j.hal.2014.07.018.

Richlen, M.L., and P.S. Lobel. 2011. Effects of depth, habitat, and water motion on the abundance and distribution of ciguatera dinoflagellates at Johnston Atoll, Pacific Ocean. *Marine Ecology Progress Series* 421:51–66, https://doi.org/10.3354/meps08854.

Rongo, T., and R. van Woesik. 2012. Socioeconomic consequences of ciguatera poisoning in Rarotonga, southern Cook Islands. *Harmful Algae* 20:92–100, https://doi.org/10.1016/j.hal.2012.08.003.

Sato, S., T. Nishimura, K. Uehara, H. Sakanari, W. Tawong, N. Hariganeya, K. Smith, L. Rhodes, T. Yasumoto, Y. Taira, and others. 2011. Phylogeography of *Ostreopsis* along west Pacific coast, with special reference to a novel clade from Japan. *PLoS ONE* 6(12):e27983, https://doi.org/10.1371/journal.pone.0027983.

Séchet, V., M. Sibat, N. Chomérat, E. Nézan, H. Grossel, J.-B. Lehebel-Peron, T. Jauffrais, N. Ganzin, F. Marco-Miralles, R. Lemée, and others. 2012. *Ostreopsis* cf. *ovata* in the French Mediterranean coast: Molecular characterisation and toxin profile. *Cryptogamie, Algologie* 33:89–98, https://doi.org/10.7872/crya.v33.iss2.2011.089.

Shears, N.T., and P.M. Ross. 2009. Blooms of benthic dinoflagellates of the genus *Ostreopsis*: An increasing and ecologically important phenomenon on temperate reefs in New Zealand and worldwide. *Harmful Algae* 8:916–925, https://doi.org/10.1016/j.hal.2009.05.003.

Skinner, M.P., T.D. Brewer, R. Johnstone, L.E. Fleming, and R.J. Lewis. 2011. Ciguatera fish poisoning in the Pacific Islands (1998 to 2008). *PLoS Neglected Tropical Diseases* 5(12):e1416, https://doi.org/10.1371/journal.pntd.0001416.

Smith, K.F., L. Rhodes, A. Verma, B.G. Curley, D.T. Harwood, G.S. Kohli, D. Solomona, T. Rongo, R. Munday, and S.A. Murray. 2016. A new *Gambierdiscus* species (Dinophyceae) from Rarotonga, Cook Islands: *Gambierdiscus cheloniae* sp. nov. *Harmful Algae* 60:45–56, https://doi.org/10.1016/j.hal.2016.10.006.

Taniyama, S., O. Arakawa, M. Terada, S. Nishio, T. Takatani, Y. Mahmud, and T. Noguci. 2003. *Ostreopsis* sp., a possible origin of palytoxin (PTX) in parrotfish *Scaris ovifrons*. *Toxicon* 42:37–33.

Tartaglione, L., A. Mazzeo, C. Dell'Aversano, M. Forino, V. Giussani, S. Capellacci, A. Penna, V. Asnaghi, M. Faimali, M.-C. Chiantore, and others. 2016. Chemical, molecular, and eco-toxicological investigation of *Ostreopsis* sp. from Cyprus Island: Structural insights into four new ovatoxins by LC-HRMS/MS. *Analytical and Bioanalytical Chemistry* 408:915–932, https://doi.org/10.1007/s00216-015-9183-3.

Tester, P.A., S.R. Kibler, W.C. Holland, G. Usup, M.W. Vandersea, C.P. Leaw, P.T. Lim, J. Larsen, N. Mohammad-Noor, M.A. Faust, and R.W. Litaker. 2014. Sampling harmful benthic dinoflagellates: Comparison of artificial and natural substrate methods. *Harmful Algae* 39:8–25, https://doi.org/10.1016/j.hal.2014.06.009.

Tester, P.A., A.W. Nau, R.L. Feldman, S.R. Kibler, and R.W. Litaker. 2010. Ciguatera fish poisoning and sea surface temperatures in the Caribbean Sea. *Toxicon* 56:698–710, https://doi.org/10.1016/j.toxicon.2010.02.026.

Vandersea, M.W., S.R. Kibler, W.C. Holland, P.A. Tester, T.F. Schultz, M.A. Faust, M.J. Holmes, M. Chinain, and R.W. Litaker. 2012. Development of semi-quantitative PCR assays for the detection and enumeration of *Gambierdiscus* species (Gonyaulacales, Dinophyceae). *Journal of Phycology* 48:902–915, https://doi.org/10.1111/j.1529-8817.2012.01146.x.

Vanucci, S., F. Guidi, R. Pistocchi, and R.A. Long. 2016. Phylogenetic structure of bacterial assemblages co-occurring with *Ostreopsis* cf. *ovata* bloom. *Harmful Algae* 55:259–271, https://doi.org/10.1016/j.hal.2016.04.003.

Vila, M., R. Abós-Herràndiz, J. Isern-Fontanet, J. Àlvarez, and E. Berdalet. 2016. Establishing the link between *Ostreopsis* cf. *ovata* blooms and human health impacts using ecology and epidemiology. *Scientia Marina* 80S1:107–115, https://doi.org/10.3989/scimar.04395.08A.

Xu, Y., M.L. Richlen, J.D. Liefer, A. Robertson, D. Kulis, T.B. Smith, M.L. Parsons, and D.M. Anderson. 2016. Influence of environmental variables on *Gambierdiscus* spp. (Dinophyceae) growth and distribution. *PLoS ONE* 11(4):e0153197, https://doi.org/10.1371/journal.pone.0153197.

Yasumoto, T., A. Inoue, T. Ochi, K. Fujimoto, Y. Oshima, Y. Fukuyo, R. Adachi, and R. Bagnis. 1980. Environmental studies on a toxic dinoflagellate responsible for ciguatera. *Bulletin of the Japanese Society of Scientific Fisheries* 46:1,397–1,404, https://doi.org/10.2331/suisan.46.1397.

Zingone, A., E. Berdalet, P. Bienfang, H. Enevoldsen, J. Evans, R. Kudela, and P. Tester. 2012. Harmful algae in benthic systems: A GEOHAB core research program. *Cryptogamie, Algologie* 33:225–230, https://doi.org/10.7872/crya.v33.iss2.2011.225.

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