K. Tsukamoto, T. Kawamura, T. Takeuchi, T. D. Beard, Jr. and M. J. Kaiser, eds.
Fisheries for Global Welfare and Environment, 5th World Fisheries Congress 2008, pp. 317–334.

Harmful Algal Blooms and Ocean Observing Systems: Needs, Present Status and Future Potential

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Harmful algal blooms (HABs), commonly called "red tides" are increasingly common worldwide. These are caused by the growth and aggregation of microscopic algae, leading to negative impacts of many types, including illness and death in human consumers of contaminated fish and shellfish, as well as mortalities of wild and farmed fish, marine mammals, and other animals. The diversity of HAB species and their impacts, as well as the oceanographic complexity of these phenomena all present significant challenges to those responsible for the management of coastal resources and the protection of public health. A promising development in this regard is the advent of ocean observing systems (OOSs)—arrays of moored and mobile instruments that can collect and transmit data continuously from remote locations to shore-based scientists and managers. The potential benefits from ocean observing systems are many, and improved monitoring and management of HABs are frequently cited as example benefits to justify the investment of resources in these systems. During this era of accelerated instrument development and deployment through national and international OOS programs, it is instructive to examine the needs, present status, and realistic potential of ocean observatories as tools for HAB monitoring and management. HABs represent a biological component of coastal waters that challenges present technologies, in part because of the need for species- or toxin-specific detection capabilities. This paper discusses the observing system capabilities or assets that are needed for effective HAB monitoring and management, highlighting and evaluating new technologies and future capabilities. Examples are given of HABs worldwide, with special emphasis on paralytic shellfish poisoning (PSP) outbreaks in the northeastern United States as a HAB system with representative challenges in observatory design and capabilities.

KEYWORDS HABs; ocean observing systems; monitoring; management

1. Introduction

The Integrated Earth Observation System (GEO 2007) is becoming a reality, as satellites, ocean buoys, weather stations and *in-situ* earth observing instruments are being deployed worldwide and their data assimilated into advanced numerical models to provide the analysis and understanding needed to address critical societal issues such as climate change, drought, fisheries management, or pollution, to name but a few. This reflects the growing recognition that decision making benefits from access to a wide variety of environmental, biological, economic, statistical, and other data, and more importantly, the ability to integrate, analyze and evaluate these types of data within a common framework shared by many countries and end users.

Within this large and visionary context, ocean observing systems (OOSs) are being planned and deployed on a major scale. Oceanography has entered a new era—one in which the traditional ways of obtaining data from the ocean using research vessels are being replaced by arrays of moored and mobile instruments that can collect and transmit data continuously from remote locations to shore-based scientists and managers. Just as networks of meteorological stations and numerical models of atmospheric dynamics revolutionized our understanding of the weather and greatly improved our ability to provide accurate forecasts of weather events, OOSs and their associated numerical models of ocean dynamics have the potential to document long-term patterns and changes in the sea, to detect infrequent events that previously went unobserved, and to make predictions or forecasts about these and other phenomena that directly affect human populations and marine ecosystems. Advances in communications, robotics, computing, platform design, power systems, and sensor technology now make it possible to get broader and deeper views of the oceans over longer periods and to share that information in real time among scientists, policymakers, resource managers, educators, and students.

The potential benefits from OOSs are many, and include the detection and prediction of climate variability, facilitation of safe and efficient marine operations, ensuring national security, preserving and restoring healthy marine ecosystems, mitigating natural hazards, managing living resources, and ensuring public health (NORLC 1999). Under the last three topics, harmful algal blooms (HABs) are frequently cited as phenomena that can be better understood and managed using ocean observatories (e.g., ORION Executive Steering Committee 2005). HABs are highly visible phenomena that affect the general public and coastal resources in many ways, and clear economic and managerial benefits would accrue from advance warning and forecasting capabilities. This potential is based on a need to understand the biological, physical, and chemical factors controlling the dynamics of individual HAB species at appropriate scales, but it is also a recognition that management of HABs can benefit from improved cell and toxin detection capabilities, coupled with modeling and forecasting of bloom transport and landfall (Ramsdell *et al.* 2005). However, HABs represent a biological component of coastal waters that challenges present technologies, in part because of the need for species- or toxin-specific detection capabilities.

In recent years, progress has been made in molecular and immunological cell and toxin detection technologies, opening the door to an era where remote, subsurface, near real-time detection of specific HAB taxa and toxins can be envisioned. Given this potential, a logical conclusion would be that instruments and observatory systems currently being planned or deployed would include some that will detect HAB cells or toxins, and that these systems are being deployed

in areas where HABs are a serious and recurrent problem. This, unfortunately, is generally not the case, as other scientific priorities have been used in observatory siting decisions, and available funds are being used to build and deploy instruments that are well proven for oceanographic measurements, but which do not provide the species-specific data needed for HABs. In effect, HAB-specific observatory instrumentation is not yet ready for operational deployment (Sellner *et al.* 2003; Paul *et al.* 2007). Here the objective is to examine the capabilities that are needed specifically for HAB detection and forecasting in regional monitoring and management programs, and to assess where we are with new technologies, and where we need to go if we are to meet the expectations of those who have funded the OOSs. It is hoped that this review will help to guide research and funding programs, and that it will inject a level of realism that moderates the lofty expectations and claims about future capabilities.

2. Harmful Algal Blooms

Over the last several decades, countries throughout the world have experienced an escalating trend in the incidence of "harmful algal blooms" (HABs; Anderson 1989; Hallegraeff 1993). HAB events are characterized by the proliferation and occasional dominance of particular species of toxic or harmful algae. When toxic algae are filtered from the water as food by shellfish, their toxins accumulate in those shellfish to levels that can be lethal to humans or other consumers. Another type of HAB impact occurs when marine fauna are killed by algal species that release toxins and other compounds into the water. HABs also cause mortalities of wild fish, seabirds, whales, dolphins, and other marine animals. Non-toxic blooms of algae can cause harm, often due to the high biomass that some blooms achieve, and the deposition and decay of that biomass, leading to anoxia.

A poorly defined but potentially significant concern relates to sublethal, chronic impacts from toxic HABs that can affect the structure and function of ecosystems. Adult fish can be killed by the millions in a single outbreak, with long- and short-term ecosystem impacts (Okaichi *et al.* 1989; Kim *et al.* 1999). Likewise, larval or juvenile stages of fish or other commercially important species can experience mortalities from algal toxins (White *et al.* 1989). Chronic toxin exposure may have long-term consequences that are critical with respect to the sustainability or recovery of natural populations at higher trophic levels (Ramsdell *et al.* 2005).

2.1. Paralytic shellfish poisoning in the Gulf of Maine

It is useful to view the issues involving HABs and observing systems in the context of a real-world problem—i.e., recurrent HAB outbreaks in an important site that has been well studied and characterized, and that offers representative challenges and constraints to the successful deployment of a HAB observing and forecasting capability. The example selected is the Gulf of Maine in the northeastern United States, the site of widespread outbreaks of PSP caused by the dinoflagellate *Alexandrium fundyense*.

A dominant feature underlying *A. fundyense* regional bloom dynamics is the Maine Coastal Current or MCC (Fig. 1; Lynch *et al.* 1997)—a composite of multiple segments and branch points. The two major transport features in this system are the eastern and western segments of the MCC, hereafter termed the EMCC and WMCC. Conceptual models of *A. fundyense* bloom dynamics have been provided by Anderson *et al.* (2005b) and McGillicuddy *et al.* (2005). Key features in the models are two large cyst "seedbeds"—one in the Bay of Fundy and the other offshore of mid-coast Maine (Fig. 2; Anderson *et al.* 2005b). Cysts germinate from the BOF seedbed, causing

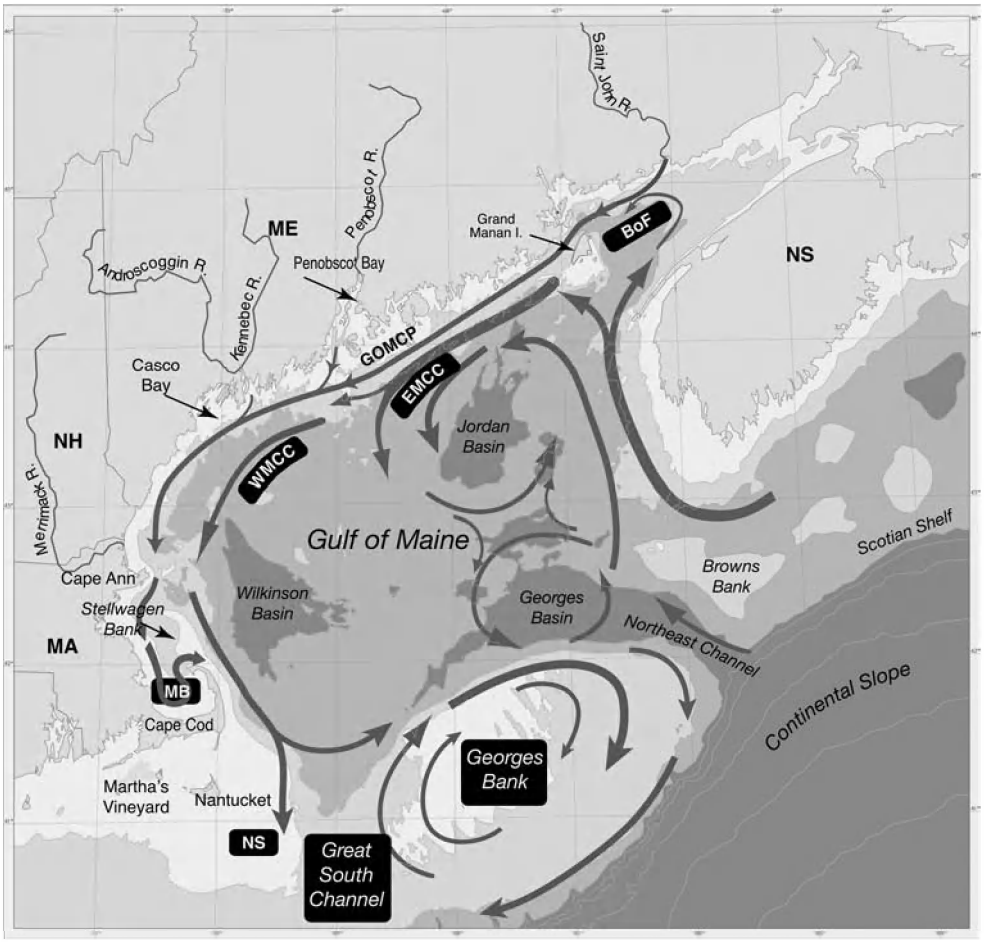

Fig. 1. Gulf of Maine surface circulation, with eastern and western segments of the Maine Coastal Current system identified (EMCC and WMCC). Other abbreviations: GOMCP—Gulf of Maine coastal plume (from Keafer *et al.* 2005); MA—Massachusetts; ME—Maine; NH—New Hampshire; NS—Nova Scotia (Modified from Pettigrew *et al.* 2005).

recurrent coastal blooms that are self-seeding with respect to future outbreaks in that area. The blooms also contribute to populations in the EMCC, as some cells escape the Bay of Fundy and enter the EMCC where they bloom. Some cells travel south and west with the EMCC, while others deposit cysts in the mid-coast Maine seedbed. In subsequent years, these latter cysts (combined with cells from the EMCC) inoculate WMCC blooms that cause toxicity in western portions of the Gulf and possibly offshore waters as well.

Numerical modeling of *A. fundyense* and PSP dynamics in the Gulf of Maine utilizes a hierarchy of physical–biological models. The current *Alexandrium* sub-model formulation follows Stock *et al.* (2005) and McGillicuddy *et al.* (2005) and includes germination, growth, mortality, and nutrient limitation. Each year, germination from cyst seedbeds provides the inoculum for subsequent

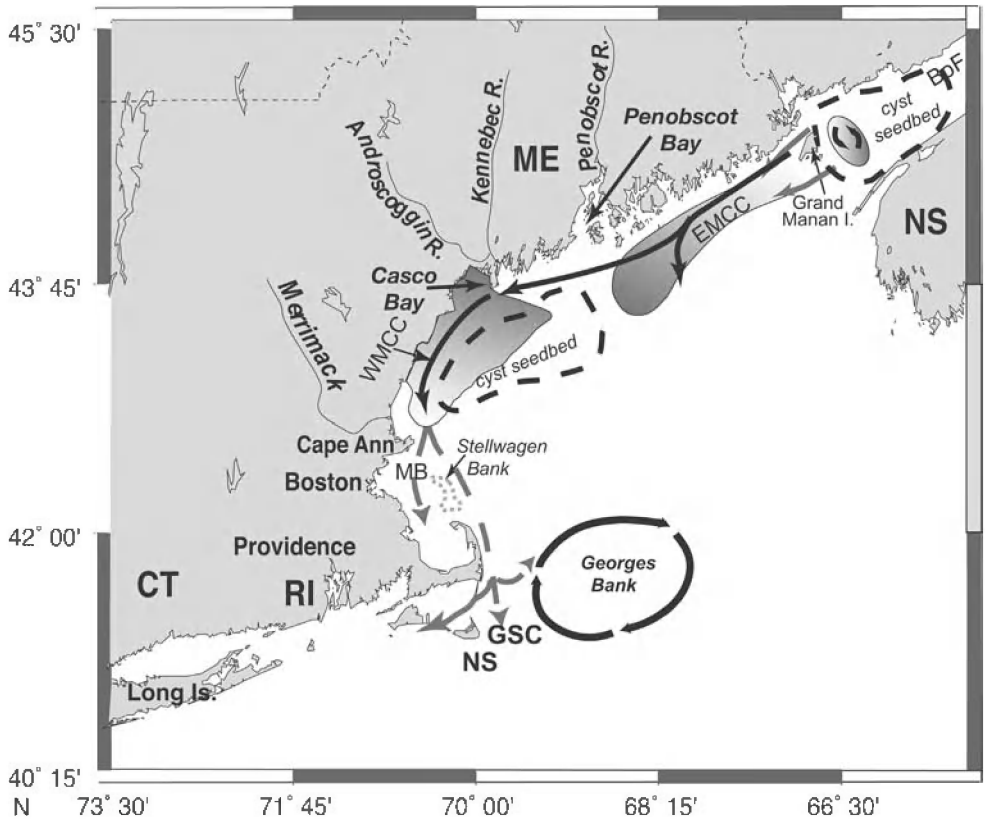

Fig. 2. Conceptual model of *A. fundyense* bloom dynamics and PSP toxicity. Solid black lines denote the eastern and western segments of the Maine Coastal Current system (EMCC and WMCC, respectively). Solid black lines also depict the circulation around Georges Bank. Short, dashed black lines delimit the cyst seedbeds in the BOF and mid-coast Maine. The shaded areas within the Gulf represent portions of the EMCC, WMCC, and BOF where *A. fundyense* blooms tend to occur with the highest color intensity denoting areas with higher cell concentrations. Dashed grey lines show the transport pathways of these water masses and their associated *Alexandrium* cells. Modified from Anderson *et al.* (2005b).

growth in the overlying waters, while wind events influence delivery onto the coast. Currently, realistic nowcasts of bloom development are possible using observed cyst distributions, cyst germination and vegetative cell growth rates, and continuous real time river flow and hydrographic data (He *et al.* in press). Forecasts are also being generated during the bloom season, but only on an experimental basis at present.

PSP outbreaks in the Gulf of Maine are sufficiently well characterized and modeled (McGillicuddy *et al.* 2005; Stock *et al.* 2005; He *et al.* in press) that they could benefit from automated observations through an ocean observatory system. In the sections that follow, the technologies and instrumentation that can be used to achieve this goal will be highlighted, and the challenges to realization of this potential discussed.

3. Observational and Analytical Needs for HAB Monitoring and Management

Anderson *et al.* (2001) highlight the different approaches adopted by countries and commercial enterprises worldwide to monitor and manage HABs in coastal waters. This is typically accomplished through the establishment of programs for toxin and cell detection (and quantitation) in water, aerosols, shellfish, fish, etc., development of bloom forecasting and early warning capabilities as well as medical intervention and therapeutic strategies, and to a growing extent, bloom prevention and mitigation strategies. There are, however, many challenges associated with these activities, due to the complexity and diversity of HAB phenomena. Resource managers and regulatory officials must deal with multiple toxins and multiple toxic algal species, multiple toxic fisheries resources, and large- and small-scale HAB events that occur intermittently. Many new technologies are emerging that can address these management challenges, however, as discussed herein. A comprehensive review on this topic is provided by Sellner *et al.* (2003), and a more general review by Paul *et al.* (2007) in the context of biological sensors that are not HAB-specific.

3.1. Sampling platforms

There are many possible ocean observatory configurations, varying in geographic scale as well as in the manner in which instruments are deployed, powered, and utilized. Instrument packages can be located at specific sites and depths using surface moorings, they can be sequestered on the ocean bottom, rising through the water column to obtain profile data, and they can be mounted on underwater vehicles of various types that travel through the water in a given area on preprogrammed missions. They can also be mounted on surface vessels or simple platforms that house instruments that collect water at depth for automated analysis at the surface. Each of these design features has advantages and disadvantages with respect to HAB monitoring. For example, sampling position in the water column is a critical design constraint, as there can be considerable variability in the vertical distribution of HAB species. Some are often found in subsurface, thin layers that are difficult to detect and sample (e.g., Xie *et al.* 2007), but others are predominantly confined to the surface mixed layer, and thus would be amenable to detection using an instrument package moored within that layer. In the Gulf of Maine, for example, considerable information has been obtained from observations of the surface distribution of *Alexandrium fundyense* (e.g., Townsend *et al.* 2001; Anderson *et al.* 2005a). Thin, subsurface layers of *Alexandrium* have also been detected in some areas of the Gulf (Townsend *et al.* 2001) but the importance of these accumulations remains unknown in the context of shellfish toxicity and general bloom dynamics. A surface-deployed instrument package would therefore be highly informative in that region, but one that could obtain profiles of cell distributions would be even more so. In this latter instance, the value of the vertical profile data must be balanced against the additional costs incurred. The higher resolution of the vertical profile would require more reagents, filters, sensors, or arrays, and the instrument package would therefore need to be serviced more frequently than an instrument that samples at a single depth. This is an important consideration given the cost of supplies as well as personnel and vessel time for the servicing operations.

Horizontal coverage is an equally important factor in HAB monitoring, and again, the Gulf of Maine provides a good example of the issues that need to be considered in instrument siting. As described above, *A. fundyense* is transported to the

south and west in two coastal currents—the EMCC and the WMCC (Fig. 1). Not only are the *Alexandrium* distributions non-uniform within these currents (Townsend *et al.* 2001), but the location of these water masses varies through time as well (Pettigrew *et al.* 2005). With appropriate wind and hydrodynamic forcings, the EMCC and its associated *A. fundyense* populations can be carried offshore into the central Gulf of Maine, and/or delivered toward shore (Luerssen *et al.* 2005; Keafer *et al.* 2005). This variability in alongshore transport has direct implications to the patterns of PSP toxicity in nearshore shellfish (Luerssen *et al.* 2005). A single observatory mooring placed in these coastal waters would therefore not provide accurate cell abundance information. In this instance, multiple instruments arrayed along a cross-shore transect would be an expensive, but much more informative configuration.

These concerns about vertical and horizontal resolution can be addressed with mobile autonomous underwater vehicles (AUVs), but for most HABs, considerable development is needed if these vehicles are to provide species-specific or toxin-specific data. With the exception of *Karenia brevis* and the BreveBuster described by Robbins *et al.* (2006), most HAB species are not amenable to optical detection due to their lack of distinctive pigments or other features that can be distinguished optically. As a result, water samples must be collected, manipulated and complex chemistries performed, and this in turn requires power, space, and robotic capabilities that are not possible in AUVs at present. For most HABs, and for the near future, it therefore appears that AUVs will be used to supply contextual data (e.g., salinity, temperature, turbidity, chlorophyll) that will help in the understanding the patterns of HAB abundance and toxicity that are observed with other instruments. This limitation in HAB detection capabilities will change when miniaturized mass spectrometers are configured to detect HAB toxins dissolved in seawater (see below), or when other features of these cells or toxins are identified that can be readily measured with the relatively simple type of robotics and detectors that can be deployed within power- and space-limited AUVs.

The foregoing discussion highlights one of the major obstacles that has slowed progress in the detection of HAB cells and toxins at ocean observatories—the need to process water samples through filters or other concentrating devices, and to manipulate those samples for extraction and analysis of toxins or the cellular targets needed for species identification and enumeration. Technologies are available for many of these analyses, but they need to be incorporated into an instrument that can be deployed underwater and that can perform the series of robotic functions needed for each analysis.

One instrument that provides these capabilities and that can be configured for use for HAB cell and toxin detection in ocean observing systems is the Environmental Sampling Processor (ESP; Goffredi *et al.* 2006; Scholin *et al.* 1998, in press). The ESP (Fig. 3) autonomously collects discrete water samples from the ocean subsurface, concentrates microorganisms (particulates), and automates application of molecular probes to identify specific microorganisms and their gene products (Scholin *et al.* 2006). The current prototype is the second generation ESP or 2G ESP. It consists of three major components: the core sample processor, analytical modules, and sampling modules. The core ESP is designed to collect and process small- to moderate-sized samples (mLs to several liters) at depths to 50 m. Analytical modules are stand-alone detection systems that can be added to the core ESP to impart different analytical functions downstream of common sample processing operations (e.g., PCR, capillary electrophoresis, competitive immunoassays, etc.).

The ESP currently utilizes DNA probe and protein arrays to detect target molecules

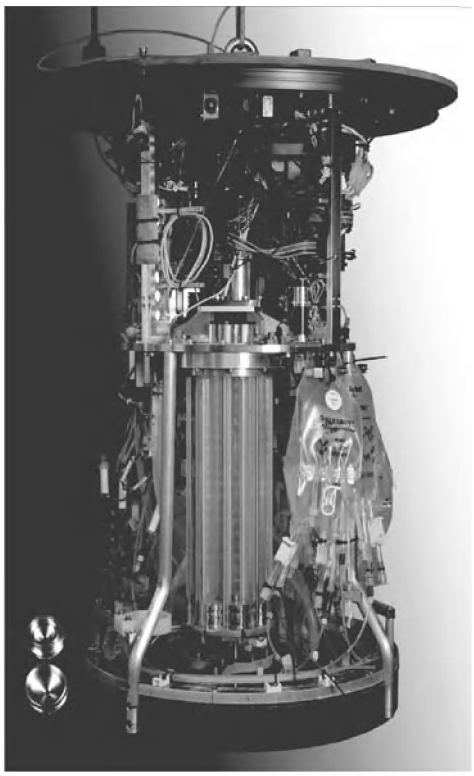

Fig. 3. Second generation ESP instrument (16″d × 30″h). bottom left: puck magnified ~2x relative to those loaded in the carousel. See also: http://www.mbari.org/ESP/esp2G_compare.htm

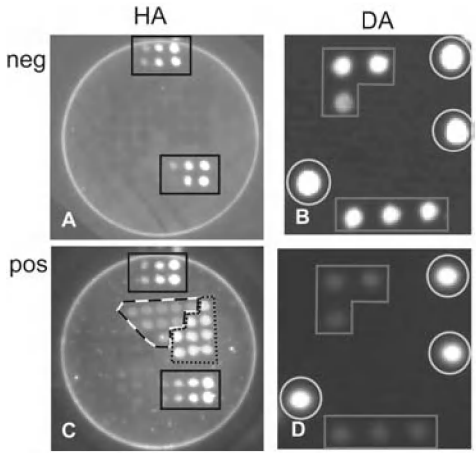

Fig. 4. *In situ* detection of *Pseudo-nitzschia* spp. and domoic acid (DA) in Monterey Bay, March 2006 using the ESP. **A**) DNA array with probes for *Pseudo-nitzschia australis*, *P. multiseries/pseudodelicatisima*, *Alexandrium catenella*, and *Heterosigma akashiwo* (HA array). Only control spots are outlined as targeted species are below detection level. **B**) Protein array for DA, corresponds to (A). Circled spots are IgG controls confirming consistency of detection chemistry. Spots in red rectangles are DA conjugate. Maximum intensity in DA conjugate spots indicates DA levels are below detection limit, whereas decreasing spot intensity indicates presence of DA (competitive ELISA). **C**) HAB array from a later time indicates the presence of *P. australis* (spots bounded by dashed lines) and *P. multiseries/pseudodelicatissima* (spots bounded by dotted lines), both of which can produce DA. **D**) DA array that corresponds to (C); circled spots are IgG controls; spots in rectangles are DA conjugate; the DA conjugate spots are considerably dimmed, indicating the presence of this toxin (from Doucette *et al.* and Greenfield *et al.*, in prep.).

indicative of species and the substances they produce. As described below, within the core ESP, DNA probe arrays specific for target HAB species capture ribosomal RNA (rRNA) from a crude sample homogenate using a quantitative sandwich hybridization assay (SHA; Fig. 4A). A quantitative PCR module is under development as an alternative approach to cell enumeration. Protein arrays utilize a competitive ELISA (enzyme-linked immunosorbent assay) technique for detecting target substances such as domoic acid (e.g., Fig. 4b). The ESP can also archive samples for laboratory analyses after the instrument is recovered, including fluorescent *in situ* hybridization (FISH), various nucleic acid analyses (cloning, sequencing) and algal toxin measurement (e.g., Greenfield *et al.* 2006).

Sample manipulations are carried out in reaction chambers called "pucks" that are loaded into and removed from various stations by robotic mechanisms. The automated

process from collection of a live sample to broadcast of an imaged DNA or protein probe array takes ~2 hours and can occur subsurface. The instrument can perform ~30–40 of those operations before servicing is required. The current limitation is the number of pucks stored in the carousel. There are, however, design options for increasing the number of sampling/analytical events and decreasing power consumption.

The instrument can be bundled with contextual sensors such as a CTD, fluorometer, transmissometer, and nutrient analyzer. Data from the external sensors along with results of the probe assays are uploaded periodically from the deployed instrument to a shore station for analysis and interpretation. Two-way communication allows for rescheduling of mission sampling profiles if desired. Further details on the instrument's design and operation are described elsewhere (Greenfield *et al.* 2006; Scholin *et al.* 2006; Roman *et al.* 2007; Paul *et al.* 2007; see also http://www.mbari.org/esp).

As promising as this instrument is, it is not yet commercially available, and thus testing has been predominantly through research grants to the developers and collaborators. To date, the ESP has been deployed multiple times in surface waters for periods of several weeks to a month, during which time it has successfully automated application of three classes of DNA probe arrays (HABs, bacteria/archaea, invertebrate larvae) and the domoic acid assay (Goffredi *et al.* 2006; Greenfield *et al.* 2006; Paul *et al.* 2007; Jones *et al.* in press; C. Scholin, G. Doucette, unpub. data). Further details on the instrument's design and operation are described in these citations and at http://www.mbari.org/esp.

At the present time, the only other advanced robotic instrument capable of *in situ* water collection, processing, and sophisticated molecular and biochemical analysis is the Autonomous Microbial Genosensor (AMG), designed to detect specific microbial targets in coastal or oceanic waters (Paul *et al.* 2007). The current prototype of the AMG collects water samples, filters cells and extracts RNA, and performs amplification autonomously. A second generation AMG will incorporate microfluidic liquid processing, array technology, and intensified light detection.

3.2. Toxin detection

Of paramount importance to many HAB monitoring programs are methods to detect and quantify the toxins produced by HAB species, which include a broad spectrum of compounds ranging in size, potency, and solubility. In all cases, the marine HAB toxins that cause the human poisoning syndromes consist of families or groups of structurally related compounds, with individual derivatives exhibiting potencies that can significantly differ from other congeners (Van Dolah 2000). During food web transfer, HAB toxins can also be metabolized or biotransformed into structurally different compounds. The broad chemical and structural diversity of algal toxins and their derivatives and metabolites, coupled with differences in their potency account for many of the challenges associated with their detection in ocean observatory programs.

Traditionally, biotoxin monitoring programs have relied on measurements of toxins in shellfish samples collected weekly or bi-weekly from key locations in areas affected by HABs (e.g., Shumway *et al.* 1988). This procedure works well and provides appropriate public health protection if the stations are well sited, and the toxin assays are run at relatively frequent intervals. Toxin measurement methods can be grouped into three main types: chemical, *in vitro*, and *in vivo* assays (Hallegraeff *et al.* 2003). The latter (bioassays) have had a long history in HAB toxin detection, but are obviously not amenable to automation and high-throughput analysis in ocean observatories, so the

only options in that context are measurements of toxin in seawater using either chemical analyses or *in vitro* assays. This immediately introduces some concerns, as considerable work will be needed to relate measurements of toxins dissolved in seawater, or in particulate form in that water, to the risk to human consumers of shellfish or fish. In the Gulf of Maine example, where measurements of toxicity in shellfish tissues are used for regulatory decisions, measurements of *Alexandrium* toxins in the water column at ocean observatories will likely be used as supplementary information in assessing risk, both current and future. It will be many years before sufficient data are accumulated to allow such measurements to be used for regulatory purposes for nearshore shellfish.

Chemical methods for toxin analysis include high performance liquid chromatography (HPLC), and mass spectrometry coupled to liquid chromatographic separation (Quilliam 1996). Of these two alternatives, only mass spectrometry shows the potential for use in ocean observatories, and there the challenges remain significant due to the diversity, size, and solubility of the toxins, as well as the matrices in which they occur (e.g., particulate versus dissolved). Another constraint is the need to perform spectrometry in a vacuum and underwater, which poses significant engineering challenges. Progress has been good, however. For example, a small, modular mass spectrometer has been developed and mounted in an AUV (Wenner *et al.* 2004). That system consists of an *in-situ* membrane-introduction linear-quadrupole mass spectrometer capable of detecting dissolved gases and volatile organic compounds at sub parts-per-billion concentrations. This instrument is still under development and has not been configured for HAB toxins, but future designs may permit the analysis of HAB toxins that occur dissolved in seawater (e.g., brevetoxins, domoic acid, okadaic acid). Analysis of toxins in particulate form will require a different approach, such as Laser Desorption Mass Spectrometry (LDMS), which is widely employed in analytical laboratories due to its simplicity of operation and rapid analysis times. One benefit of LDMS is that many different types of materials can be vaporized and ionized by a tightly focused laser beam (Cotter 1997). This can avoid sample purification or preparative techniques, which is critical to deployment of such technologies in a moored or mobile configuration in an OOS, as it will greatly reduce sampling and handling requirements, and thus power drain, space needs, and reagent needs as well. LDMS has been used for the detection of bacterial spores, vegetative cells, viruses, and toxins in aerosol environments (Fenselau and Demirev 2001), and efforts are underway to apply this method to HAB cells and dissolved toxins in seawater (A. Place, pers. comm.).

Another important and rapidly developing group of HAB toxin detection methods comprises the *in vitro* assays. One subgroup—the functional assays—relies on detection of a toxin's biochemical activity while the other—structural assays—depends on recognition of chemical structure at the molecular level (reviewed in Cembella *et al.* 2003; Van Dolah and Ramsdell 2001). A variety of functional assays have been developed for the detection of HAB toxins, including cytotoxicity assays (e.g., Manger *et al.* 1995), enzyme inhibition assays (e.g., Della Loggia *et al.* 1999), and receptor binding assays (e.g., Van Dolah *et al.* 1994). Nevertheless, retention of the biological activity of a cell line or a receptor preparation outside the laboratory remains a significant, and thus far, insurmountable obstacle to *in situ* use of these assays (Sellner *et al.* 2003).

In contrast, structural assays show considerable promise for automated deployment in an observatory system. These assays rely on the structural or conformational interaction of a toxin with a recognition factor such as an antibody. Antibody-based assays have

been developed for a variety of HAB toxins and many of these tests are now commercially available (Laycock *et al.* 2001; Cembella *et al.* 2003). In many ways, the procedures used for toxin immunoassays are similar to those used for HAB cell detection using oligonucleotide probes (described below), and thus these technologies have the potential to be combined in a single instrument that can detect HAB cells and toxins simultaneously. Doucette and co-workers (unpub. data) are developing an immunoassay-based method for detection of domoic acid in robotic fashion on board the ESP, described above. This analysis utilizes a known quantity of domoic acid–antibody conjugate immobilized on membranes as replicate spots. These membranes are then exposed to a simple extract of filtered plankton, and a competitive ELISA performed. The resulting arrays can be imaged (Fig. 4B) and the data sent to shore electronically. This is an example of a toxin-detection technology that can be automated and performed *in situ* in a moored instrument.

Other investigators are developing alternative immunosensors that also have the potential for *in situ* deployment, though most have only been configured for laboratory-based, bench-top formats at present. One novel immunoassay utilizes surface plasmon resonance (SPR) in a portable system developed for rapid field quantification of toxin levels in both shellfish and seawater. (Stevens *et al.* 2007). The SPR assay had a limit of detection of 3 ppb domoic acid and a quantifiable range from 4 to 60 ppb. Comparison of analyses with standard HPLC protocols gave an excellent correlation. This same technology should also function for detection of domoic acid (and other algal toxins for which antibodies are available) in concentrated algal extracts or high dissolved levels in seawater. With refinement of the extraction protocols and generation of higher affinity monoclonal antibodies, detection of much lower levels of toxin should be possible, leading to eventual application of automated SPR biosensors on moorings.

Another novel and potentially useful approach for *in situ* observations is a competitive immunoassay using screen-printed electrodes (SPEs; Kreuzer *et al.* 2002; Micheli *et al.* 2004). Excellent sensitivity and accuracy has been achieved with HAB toxins such as okadaic acid, brevetoxin, and domoic acid. For all toxins investigated, results compared favorably with other toxin analysis techniques. The advantages of speed of analysis, simplicity of design, *in situ* measurement capability, stability (storage up to four weeks prior to use), and disposability make SPE immunosensors good candidates for observatory instrumentation. Adaptation of this and other immunoassay technologies to robotic systems and deployment in remote locations is thus possible, but will require further development effort.

3.3. Cell detection

Two approaches have been followed to improve on traditional light microscope counts of HAB species in field programs. One utilizes optical characters that are unique to the target organism. The only success in this regard is for *Karenia brevis*, the Florida red tide organism, which produces a pigment called gyroxanthin-diester. This carotenoid is found in other fucoxanthin-containing dinoflagellate species as well, but in some areas, such as the Gulf of Mexico, it is sufficiently unique to be a useful biomarker for *K. brevis* and other toxic or potentially toxic *Karenia* species (Kirkpatrick *et al.* 2000; Richardson and Pinckney 2004). Instruments have been developed that can quantify this pigment in water samples, and these have been mounted on board research vessels (Kirkpatrick *et al.* 2003) and inside an AUV called the BreveBuster (Robbins *et al.* 2006). This approach thus has great potential for monitoring of those HAB species that have this unique pigment, but for the vast majority of other species, alternative approaches to cell detection are needed.

The second approach involves the development of species- or strain-specific "probes" which can be used to label HAB cells of interest so they can then be detected visually, electronically, or chemically. Progress has been rapid and probes and assays of multiple types are already available for many of the HAB species. The most promising of these approaches in the context of ocean observing systems are short pieces of synthetic DNA (probes or primers) that bind to complementary portions of those molecules in the target HAB species. These targets can be visualized and/or quantified using a variety of techniques such as whole-cell fluorescent *in situ* hybridization (FISH; Anderson *et al.* 2005b; Hosoi-Tanabe and Sako 2005), sandwich hybridization assays (SHA; Scholin *et al.* 1996; Diercks *et al.* 2008), and a variety of PCR-based assays (e.g., Penna and Magnani, 1999; Guillou *et al.* 2002). Of these, the FISH technique is not amenable to *in situ* use in observatory systems, so future applications will likely utilize either the SHA or quantitative PCR (qPCR).

The SHA involves chemical lysis of the algal cells to release ribosomal RNA target molecules that are then "captured" by a probe immobilized on a surface, and visualized using a colorimetric, fluorometric, or chemiluminescent reporting system linked to a second ("signal") probe in solution. The SHA allows for rapid, high throughput sample analysis and has been effectively automated in a variety of formats, including in the ESP (Fig. 4A; Scholin *et al.* 2006; Greenfield *et al.* 2006). One advantage of this approach is that it utilizes a crude plankton lysate for analysis—i.e., no RNA purification is needed. Another is its sensitivity—with detection limits of a few thousand cells/L of *Pseudo-nitzschia* species, and 100 cells/L with *Alexandrium* species, given the present pumping and filtering specifications (C. Scholin, pers. comm.). With higher volume sample concentration, currently under development, detection limits can drop to levels of a few cells/mL, sufficient to identify the earliest stages of blooms. For example, STMicroelectronics offers the In-Check® platform, a microfluidic chip that combines PCR amplification and probe array detection functions. Integrated devices like this could find application in an ocean observatory setting.

Another rapidly emerging approach to the detection of HAB species is qPCR (e.g., Bowers *et al.* 2000, 2006; Galluzzi *et al.* 2004; Coyne *et al.* 2005). With respect to *in situ* applications in robotic or moored systems, it is of note that quantitative PCR procedures require extraction and purification of nucleic acids from samples, an enzymatic reaction mixture, and application of one or more thermocycling protocols. Sample handling and processing are thus important considerations for *in situ* measurements. Nevertheless, portable instruments suitable for field applications are being developed and show promise for inclusion in ocean observatory-based HAB monitoring and research programs in the near future.

One example of the manner these constraints are being addressed is the portable sensor technology developed for detection and enumeration of the HAB species *K. brevis* using nucleic acid sequence-based amplification (NASBA; Casper *et al.* 2004). NASBA is an isothermal method for the amplification of RNA, so this simplifies the power and manipulation requirements of the assay which otherwise would require multiple heating and cooling steps. To address the problems with extraction of RNA from water samples, a simple procedure was developed that requires no special equipment or training, and that performs as well as expensive, commercial kits (Casper *et al.* 2007). Further progress was made with the development of a handheld sensor that provides real-time fluorescence plotting of the amplification. Results using the handheld NASBA analyzer compare favorably to

laboratory-based technologies. This extraction protocol and detection sensor are now being incorporated into an autonomous platform called the AMG, described above.

3.4. Modeling and forecasting

The value of data from ocean observatories is greatly enhanced by numerical modeling techniques that can lead to forecasts of HAB transport and dynamics. A region in which HAB-specific instruments are deployed should therefore take steps to develop and validate numerical models that incorporate local HAB species into hydrodynamic models of the region (physical-biological models). The ultimate goal is to obtain data on HAB cells and toxins through instruments in an observatory system, assimilate these data and contextual meteorological and oceanographic observations into the models, and provide continually updated forecasts of bloom behavior. The first step in this process is the formulation of conceptual models that explain in words and simple diagrams how HABs occur in a given area. Examples of conceptual models developed for *Alexandrium* blooms in the Gulf of Maine are given in Anderson *et al.* (2005b) and McGillicuddy *et al.* (2005). If a verbal description of a model can be formulated that is consistent with observations and data over an extended interval of time, it is much easier to formulate a numerical model that captures the same dynamics (McGillicuddy *et al.* 2005). For many areas of the world, there are significant challenges to achieving this goal, as neither conceptual models nor numerical models exist for regional HAB problems.

A significant constraint to numerical model development is the need to identify initial conditions for the biological fields (i.e., the HAB species' distribution). In the Gulf of Maine example, cyst maps in bottom sediments are used as the initial condition, with germination of those cysts producing the vegetative cells that ultimately grow and form the bloom (McGillicuddy *et al.* 2005; He *et al.* in press). In other HAB systems, and in particular those without cyst populations, cell concentrations measured by instruments in an observing system may well provide the initial conditions for subsequent model runs and forecasts of bloom dynamics.

In addition to forecasting, numerical models can help in the identification of key locations at which instruments capable of detecting HAB cells and toxins can be deployed. Figure 5 demonstrates one of the challenges associated with siting decisions for observatory instruments. Given the conceptual model described earlier for *A. fundyense* in the Gulf of Maine (Fig. 2) it is possible to identify key locations in the different transport pathways that would facilitate HAB detection and forecasting in this large region. With a hypothetical set of 9 moored instrument packages, scientists would place instruments at key locations and branch points, with horizontal variability in the coastal currents and *Alexandrium* distributions addressed through cross-shore arrays of instruments at each of the key locations (Fig. 5A). However, shellfish managers would choose to locate the same number of instruments along a line just offshore or upstream of important shellfish growing areas (Fig. 5B), providing alongshore resolution, but none in the cross-shore direction. In this instance, the managers opt for early warning through direct cell detection rather than through numerical model forecasting. Resolution of the difference in viewpoints depicted in Fig. 5 would require a demonstration that a scientifically or hydrographically based mooring configuration, coupled with a numerical model, can provide early warning information suitable for management purposes.

Another justification for model development in an area where an ocean observatory is planned is that the model can be used to identify the locations where data on HAB

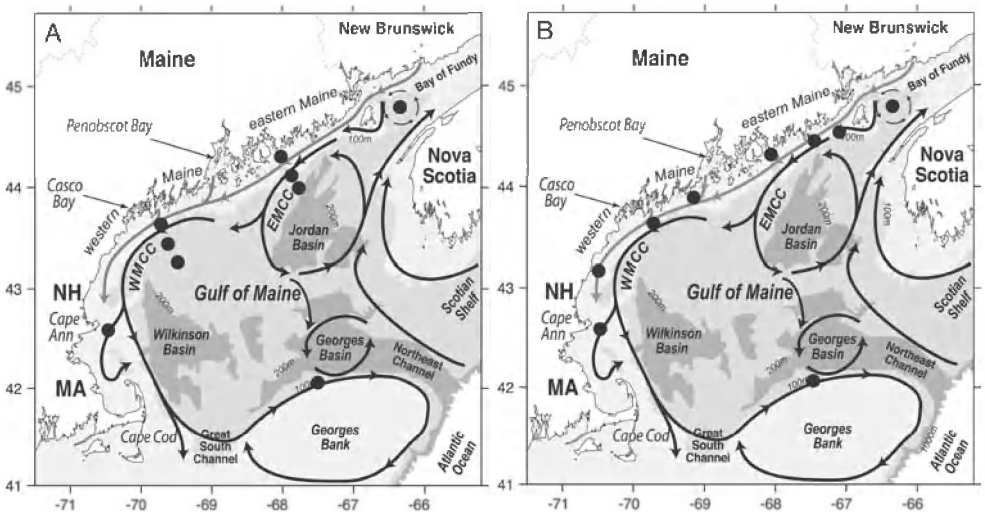

Fig. 5. Map showing possible locations of ESPs or other instruments capable of detecting *Alexandrium fundyense* cells and/or toxins in the Gulf of Maine. **A**) Configuration suggested by scientists to capture horizontal variability in cell distributions and water masses along key transport pathways. **B**) Configuration suggested by managers, who simply want early warning at major shellfish growing areas.

species abundance or toxicity would be the most useful. Termed observing system simulation experiments (OSSEs), these numerical analyses have been used in dynamic meteorology (e.g., Charney *et al.* 1969) and are becoming an important tool in the planning of oceanographic sampling systems (e.g., Robinson *et al.* 1998; McGillicuddy *et al.* 2001). OSSEs can help to optimize the number and location of instruments needed to provide a necessary level of coverage. The ultimate goal is to have an array of instruments located in strategic spots to capture the information that can then be assimilated into numerical models through time, greatly increasing their accuracy and utility. One cannot over-emphasize the importance of proper instrument siting or validated numerical models if the true potential of ocean observing systems is to be realized in HAB research and monitoring programs.

4. Summary

Improvements in HAB monitoring and forecasting are frequently cited as justifications for the deployment of ocean observing systems, yet few of the observatory systems being deployed worldwide have any HAB components. This is largely because the technologies needed to achieve HAB cell or toxin detection are still under development. Only a small number of HAB species can be detected using optical measurements, either *in situ* or remotely from space, and therefore instruments that can detect the vast majority of HAB species need to have capabilities for sample collection, concentration, and manipulation. The chemistries and procedures for cell identification and enumeration using molecular probe assays of various types are well established, so the challenge now is to incorporate these assays into autonomous instruments that are capable of

sample collection and processing while moored or deployed within an AUV. Several instruments have been developed that have this capability in moored configurations (i.e. the ESP and AMG) and others will surely be developed in the future given the need for this type of capability in many other types of environmental monitoring.

In a similar manner, methods for toxin detection have been developed that can be incorporated into moored instrument packages, typically based on structural assays such as immunoassays. Here again, incorporation of these methods into instruments capable of the appropriate processing has only just begun. Development is rapid on all of these fronts, but will be greatly accelerated if research and development funding is targeted to technologies for species-specific detection of HAB cells and metabolites.

All too often these days, ocean observatory resources are dedicated to existing, proven technologies that are ready for deployment. If the development of HAB-specific instrumentation lags too far behind the pace of observatory infrastructure construction, observatory assets will be located in areas or at sites that are not optimal for HAB detection or will not have the appropriate power or mooring capabilities, and thus the goals of improved HAB monitoring and management through ocean observatories will not be realized.

Acknowledgements

This effort was supported in part by the following grants to D.M. Anderson: NOAA Cooperative Agreement NA17RJ1223; NOAA ECOHAB grant NA06NOS4780245; NOAA grant (through University of new Hampshire) NA05NOS4191149; NIEHS Grant 1 P50 ES012742; and NSF Grants OCE-0430724 and OCE-0402707.

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