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Kevin G. Sellner · Gregory J. Doucette
Gary J. Kirkpatrick

Harmful algal blooms: causes, impacts and detection

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Abstract Blooms of autotrophic algae and some heterotrophic protists are increasingly frequent in coastal waters around the world and are collectively grouped as harmful algal blooms (HABs). Blooms of these organisms are attributed to two primary factors: natural processes such as circulation, upwelling relaxation, and river flow; and, anthropogenic loadings leading to eutrophication. Unfortunately, the latter is commonly assumed to be the primary cause of all blooms, which is not the case in many instances. Moreover, although it is generally acknowledged that occurrences of these phenomena are increasing throughout the world's oceans, the reasons for this apparent increase remain debated and include not only eutrophication but increased observation efforts in coastal zones of the world. There is a rapidly advancing monitoring effort resulting from the perception of increased impacts from these HABs, manifested as expanding routine coastal monitoring programs, rapid development and deployment of new detection methods for individual species, toxins, and toxicities, and expansion of coastal modeling activities towards observational forecasts of bloom landfall and eventually bloom prediction. Together, these many efforts will provide resource managers with the tools

needed to develop effective strategies for the management and mitigation of HABs and their frequently devastating impacts on the coastal environment.

Keywords Harmful algal blooms · Detection · Molecular techniques · Remote sensing · Modeling

Introduction

Large accumulations of phytoplankton, macroalgae and, occasionally, colorless heterotrophic protists are increasingly reported throughout the coastal areas of all continents. Aggregations of these organisms can discolor the water giving rise to red, mahogany, brown, or green tides, can float on the surface in scums, cover beaches with biomass or exudates (foam), and deplete oxygen levels through excessive respiration or decomposition. Alternatively, certain species in harmful algal blooms (HABs) can exert their effects through the synthesis of compounds (e.g., toxins) that can alter cellular process of other organisms from plankton to humans. The most severe, and therefore memorable, effects of HABs include fish, bird, and mammal (including human) mortalities, respiratory or digestive tract problems, memory loss, seizures, lesions and skin irritation, as well as losses of coastal resources such as submerged aquatic vegetation and benthic epi- and in-fauna.

For certain toxin producing species, significant impacts occur at population densities of only several hundred cells per liter. For example, *Dinophysis* need only be present at 100s of cells l⁻¹ to induce diarrhetic symptoms, as they are concentrated by shellfish and then ingested by human consumers. *Pfiesteria piscicida* and *P. shumwayiae* are associated with fish lesions, skin and eye irritation, and short-term neurocognitive disorders [59], and need only reach levels of 250 zoospores l⁻¹ to be of concern. Toxin-producing species are found in other groups besides the dinoflagellates, including raphidophytes, diatoms, cyanobacteria, and several

K. G. Sellner (✉)
Chesapeake Research Consortium,
645 Contees Wharf Road, Edgewater,
MD 21037, USA
E-mail: sellnerk@si.edu
Tel.: +1-410-7981283
Fax: +1-410-7980816

G. J. Doucette
Marine Biotoxins Program,
Center for Coastal Environmental
Health and Biomolecular Research,
NOAA/National Ocean Service,
219 Fort Johnson Road Charleston,
SC 29412, USA

G. J. Kirkpatrick
Mote Marine Laboratory,
1600 Ken Thompson Parkway,
Sarasota, FL 34236, USA

other groups with fewer toxic representatives (e.g., prymnesiophytes). The primary groupings of HAB toxins according to syndrome include paralytic shellfish poisons (PSP), neurotoxic shellfish poisons (NSP), amnesic shellfish poisons (ASP), diarrhetic shellfish poisons (DSP), azaspiracid shellfish poisoning (AZP), ciguatera fish poisoning (CFP), and cyanobacteria toxin poisoning (CTP). Represented in this diverse group are neurotoxins, carcinogens, and a number of other compounds, chemistries (e.g., free radical formation), and symptomologies that affect living resources or humans exposed to the causative organisms or to their toxins following concentration by filter-feeding bivalves or planktivorous fish. Several recent reviews provide a detailed treatment of the range of algal toxins and their effects [29,75,179].

Reasons for the increasing interest in HABs include not only public safety concerns associated with protecting human health, but also adverse effects on living resources of many coastal systems, economic losses attributed to reduced tourism, recreation, or seafood related industries, and costs required to maintain public advisory services and monitoring programs for shellfish toxins, water quality, and plankton composition. A recent study [65] estimated approximately US \$49 million was lost annually to HAB-related impacts in the United States over a 5 year study period (1987–1992). Further, many areas ideal for establishing productive and profitable wild shellfisheries (e.g., Alaska and Georges Bank) remain closed year-round due to persistent toxicity of the resource resulting from repeated toxin exposure and/or an inability to depurate accumulated toxin from the contaminated shellfish. The potential production for an Alaskan shellfishery has been estimated at US \$50 million annually, a considerable economic benefit that cannot be realized. Similarly, the Georges Bank surf clam fishery has been closed since 1989 due to continuing PSP toxicity and a United States roe-on scallop industry for this area has consequently not been developed.

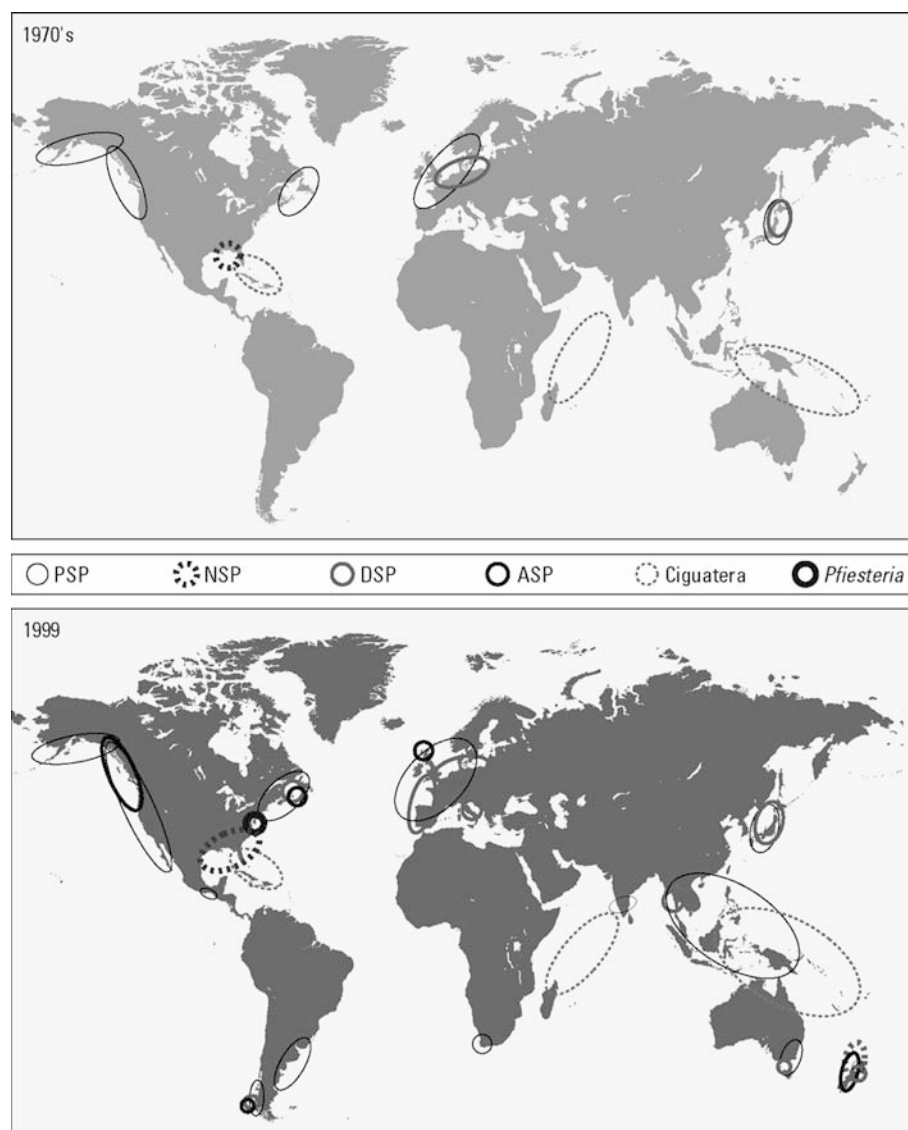
A global response to anthropogenic loadings?

There is no doubt that HABs are occurring in more locations than ever before (Fig. 1) and new sightings are reported regularly. Several researchers have argued that this trend is due to increasing eutrophication throughout the world [152] and there are several classic examples relating HAB frequency to anthropogenic activities. For example, red tides in Tolo Harbor, Hong Kong, showed a remarkable increase from 1976 to 1986 that strongly paralleled a local rise in population density (Fig. 2a); nitrogen (N) and phosphorus (P) also increased 25- and 6-fold, respectively, over the same period [74]. In a similar time series, population and industrial development in the Seto Inland Sea region of Japan [103] for the period 1968–1976 coincided with about a 6-fold increase in the number of HABs each year, to a maximum of 300, while N and P increased more than 30- and 5-fold,

respectively (Fig. 2b). Implementation of better management practices in the early 1970s that reduced chemical oxygen demand (COD) has resulted in a substantial decline in bloom frequency to one-half the maximum number, N concentrations only 13-fold higher than initially (i.e., 4 μM), and P at pre-development levels. In another example, increased nutrient loading to the southern North Sea since World War II has resulted in prolonged spring phytoplankton maxima, with the colonial bloom-forming alga *Phaeocystis* following the initial diatom bloom. This successional shift is attributed to silicon-limitation leading to collapse of the diatom assemblage opening a niche for *Phaeocystis* [134]. With overall elevated nutrient levels and colony formation promoted under nitrate replete conditions [126], this haptophyte, considered a sub-optimal food source for zooplankton grazers, can dominate surface waters.

Elevated nutrient loading has also been proposed as the primary reason for increasing HABs in a number of other systems. Low salinity coastal waters throughout the world are experiencing substantial increases in halotolerant cyanobacteria in response to elevated nutrient loading stemming from human activities. For example, coastal embayments in Brazil are highly enriched and support large *Microcystis aeruginosa* populations [44]. This potentially toxic species is also increasing in headwaters of the Chesapeake Bay [P. Tango, personal communication]. *Pseudo-nitzschia*, the diatom genus responsible for domoic acid production and amnesic shellfish poisoning, has increased dramatically over the last 50 years along the Louisiana coast, strongly correlated with nitrate loading in the Mississippi River (Fig. 3; see [108, 170]). Very recently, it has been argued that precipitation-driven coastal runoff and associated anthropogenic nutrient loads may be responsible for *Pseudo-nitzschia* blooms off central California, and that upwelling might not be as important as is often suggested [73]. Mass introduction of nutrients from North Carolina hog farm waste ponds resulted in an approximate 6-fold increase in *Pfiesteria piscicida* zoospores (from Fig. 12 in [26]), mimicking laboratory observations of zoospore responses to inorganic and organic enrichment. Elevated nutrient inputs into the Northern Adriatic, Aegean, and Black Sea have resulted in increasing frequencies of HAB-related events [23, 98, 148]. Nutrient inputs accompanied by harbor construction have led to longer residence times and recirculating embayment gyres, maintaining HABs for extended periods in small harbors of southern Spain [42]. Cyanobacterial pigments, likely from the summer dominant taxa *Nodularia* and *Aphanizomenon*, dramatically increased in Baltic Sea sediments in the 1960s (Fig. 4), after relatively low levels for the previous 7,000 years [113]. As anthropogenic loading was minimal several thousand years ago, these data suggest cyanobacterial abundance may have increased in response to post World War activities (i.e., industrial expansion and nutrient loading), previously documented as a period for rapid increases in total N and P [77].

Fig. 1 Global distribution of harmful algal bloom (HAB) toxins and toxicities (from [178])



Nodularia was also a primary contributor to the nutrient-rich Peel-Harvey estuary in Australia [85], prior to river diversion and salinity increases that severely limited the growth of this cyanobacterium. Macroalgae respond similarly to these environmental changes. Increasing macroalgae in shallow, highly nutrient-enriched New England estuaries is well documented, leading to losses of submerged aquatic vegetation [64,177]. For oceanic environments, LaPointe [76] summarized the recent literature to conclude that increases in ambient nutrient levels in nutrient-limited reef environments favor frondose macroalgal growth and, in some cases, overgrowth of corals and coral mortality. It should be noted, however, that this view is not universal, as Szmant [161] concluded that overgrowth and coral degradation are likely due to a number of factors besides nutrient enrichment. Whether nutrient-driven or not, increasing macroalgae could also favor growth of attached benthic dinoflagellates responsible for ciguatera poisonings [21].

Increasing inputs of N and P are often associated with declining silicon contributions due to a number of river management strategies such as dam construction [67], leading to lower available silicon for diatom production and greater contributions of non-silicon-requiring species like *Phaeocystis*, dinoflagellates, and prymnesiophytes. These authors have presented an excellent summary of such impacts for the Black and Baltic Seas, while Anderson et al. [13] have also reviewed the effects of altered N/P and P/Si ratios in a number of other systems.

There is increasing discussion on the potential role of aquaculture and mariculture in HAB development. Cultured shellfish and finfish populations produce huge amounts of feces, pseudofeces, and other excretory products rich in N and P important to algal growth. Particulates, either defecated materials or uneaten fish foods (only 30% of added fish food is harvested as fish biomass, see [47]), settle to the bottom and, through remineralization, yield soluble N (either oxidized nitrate,

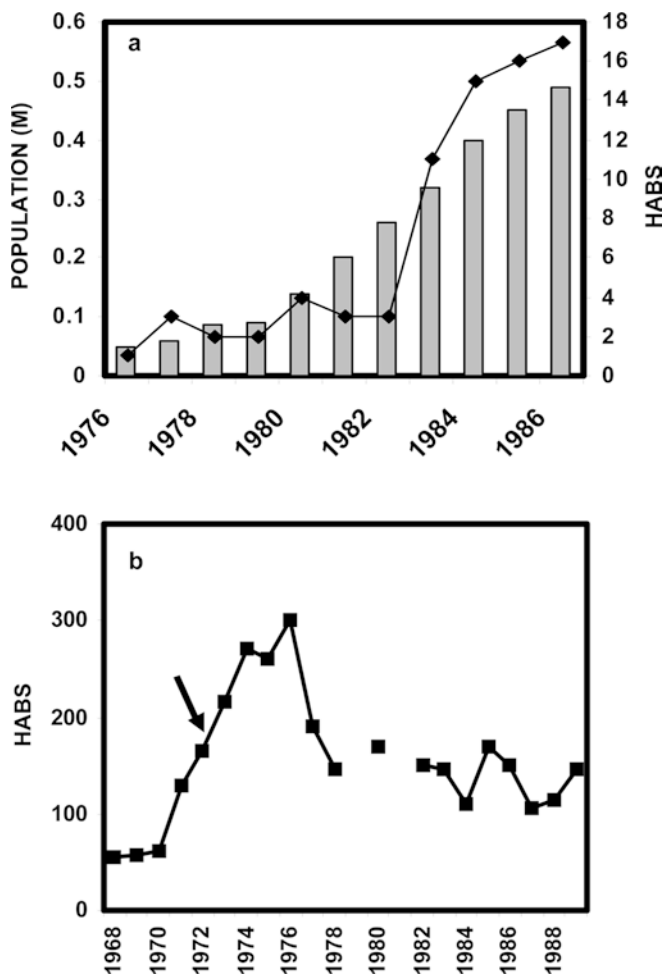


Fig. 2a, b Anthropogenic-induced HAB in coastal systems. **a** Tolo Harbor, Hong Kong [74]; bars population, line HABs per year. **b** Seto Inland Sea, Japan [103]; arrow implementation of waste reduction practices for the coastal discharges

nitrite, or reduced ammonium) and P. If the system is highly flushed, potential utilization by autotrophs in overlying waters is likely limited [121]. However, several shallow, poorly flushed areas with extensive mariculture operations report increasing and unique HABs not observed prior to introduction of the managed fish stocks [188], suggestive of culture operation-induced HAB expansion. Several HABs in Spanish rias have also been attributed to growth resulting from utilization of remineralized detritus settled from bivalve rope cultures [128]; however, the initial nutrient input was from upwelling sources and hence these HAB events represent a combined effect of natural and anthropogenic processes.

These examples suggest that HAB occurrence is strongly associated with human activities in coastal zones. Moreover, those cases outlined above represent only a few of the numerous citations available, and articles by Anderson [5], Smayda [152], Van Dolah [178], and Anderson et al. [13] should be consulted for additional locations and discussions.

Natural processes are equally important

The preceding discussion would lead one to believe that human activities and the associated increase in nutrient loadings are likely the primary reason for HABs occurring in our world's oceans. In fact, this is not the case, and the scientific community has a responsibility to indicate the importance of natural events in bloom formation. Oceanic and estuarine circulation and river flow greatly influence the abundance and distribution of plankton and the combined physical (e.g., currents, upwelling, etc.)-chemical (e.g., salinity, nutrients, etc.) factors of these systems, coupled with unique life cycles and behaviors of some HAB taxa, result in blooms that impact coastal ecosystems and populations. There are numerous examples indicating the importance of these processes.

As discussed above, *Pseudo-nitzschia* spp. off Louisiana are apparently increasing as a direct result of nitrate delivery from the Mississippi River watershed, a phytoplankton response to non-point source introduction of fertilizers. In several other areas with recurrent *Pseudo-nitzschia* and domoic acid-related problems, however, nutrient supply is a natural process, involving: (1) storm-associated forcing as either rain-induced river discharge or wind-induced mixing of deep nitrate pools into surface waters as in Prince Edward Island in 1988 [153] and Puget Sound in 1997 [167]; (2) wind-induced coastal upwelling off California and Washington states, and the Iberian peninsula [2, 36, 143, 168]; (3) physical transport and deposition of healthy phytoplankton populations into the United States Pacific Northwest [169]; and (4) physically-controlled thin layer formation and maintenance as in East Sound, Wash. and Monterey Bay, Calif. [40, 127].

Basin scale circulation, and not anthropogenic forces, also act as effective vectors for distributing bloom taxa, leading to coastal blooms and adverse impacts on living resources, and in some HAB species, unique characteristics of the life cycle combine with regional physics enabling successful proliferation. *Alexandrium* spp. in the Gulf of Maine (Fig. 5) are transported from the Bay of Fundy along the New England coast in two separate coastal currents, the Eastern and Western Maine Coastal Currents, part of the Gulf of Maine circulation [8]. Shellfish intoxication is due primarily to introduction of these populations during downwelling favorable wind conditions, followed by southwesterly alongshore transport. Additionally, introduction of the PSP-producing *Alexandrium* population into the Gulf of Maine in 1972 was driven by meteorology: a hurricane brought coastal Nova Scotian populations south across the Scotian shelf into the northern Gulf of Maine [6].

The life cycle of *Alexandrium* spp. also aids in the successful establishment of bloom populations. This dinoflagellate produces a resting stage—a cyst—during periods of suboptimal growth conditions. The cyst sinks to the bottom and, after an obligate dormancy period,

Fig. 3a, b Time series of nutrient and *Pseudo-nitzschia* in the Mississippi delta and shelf. **a** Nitrate-nitrogen increase at two locations in the Mississippi River delta (adapted from [170]); **b** densities of *Pseudo-nitzschia* spp. in surficial sediments of five cores collected on the Mississippi shelf (adapted from [108])

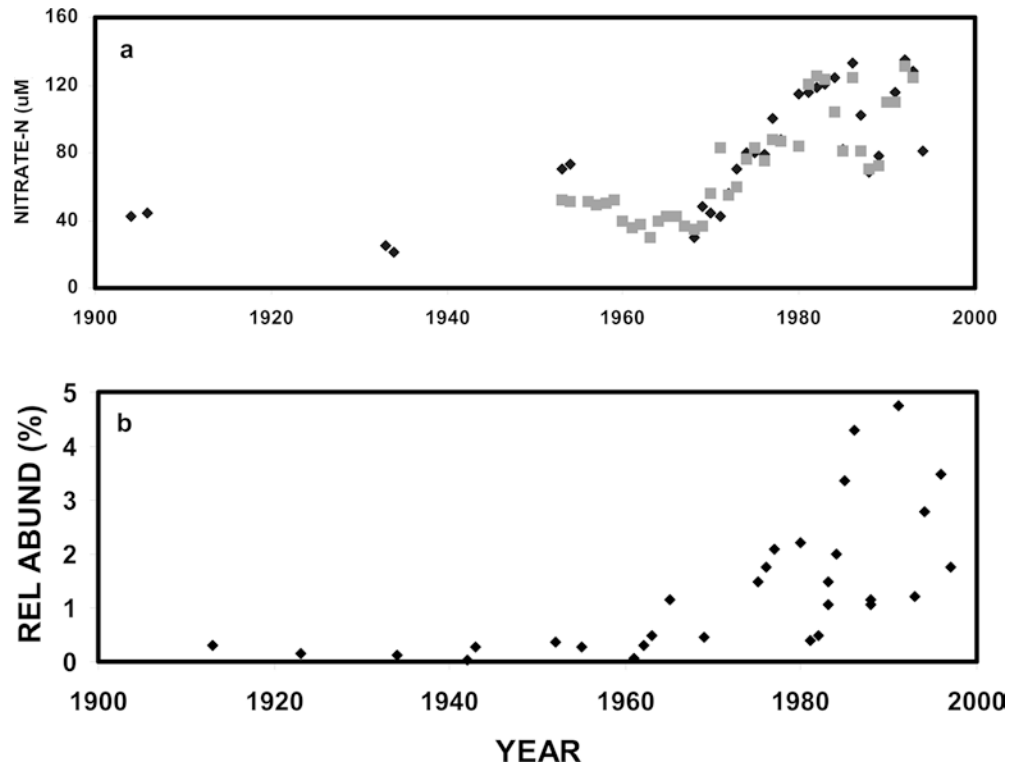
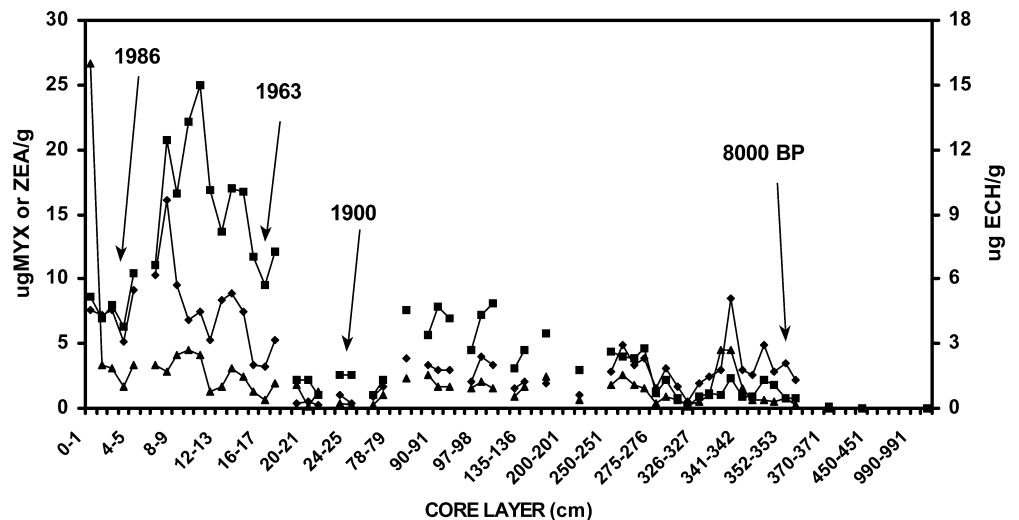


Fig. 4 Historical record of cyanobacteria in the Gotland Deep, Baltic Sea (from [113]). Filled squares Myxoxanthophyll, filled diamonds zeaxanthin, filled triangles echinenone



can excyst (break open) to release vegetative cells that swim to the surface to re-seed bloom populations (Fig. 6). This resting stage therefore provides this dinoflagellate with a unique competitive advantage over populations that cannot persist under poor conditions, and migration into surface circulation cells ensures transport throughout a region. Some other dinoflagellates and flagellates can produce cysts, and resting stages are also documented for some diatoms (spores) and cyanobacteria (akinetes), ensuring re-introduction of vegetative populations into overlying waters of high irradiance for potential growth, accumulation, and blooms.

Development and transport in other large current systems independent of anthropogenic contributions is also characteristic of several other bloom species. The N-fixing cyanobacteria, *Trichodesmium* spp., are carried throughout the tropical and subtropical latitudes in oligotrophic systems with the potential for bloom development in at least one locale, the west Florida shelf, a function of wind-delivered iron-rich dust from the Sahara [186]. The N-fixing cyanobacteria, including *Trichodesmium*, grow well in generally N-limited oceanic waters due to their ability to fix atmospheric nitrogen, N_2 . Populations of this taxon in the eastern Gulf of Mexico may pre-condition Florida shelf waters

Fig. 5 Circulation in the Gulf of Maine, northeastern United States. *Alexandrium* populations initially entered the basin in 1972 south of Nova Scotia and are now found in the mouth of the Bay of Fundy (BOF). Populations are advected south and west in the eastern Maine coastal current (EMCC) to (1) encyst just south of Penobscott Bay and settle to subsequently excyst and seed new populations further south in the western Maine coastal current (WMCC) or river mouths south of Penobscott, (2) occasionally be carried as vegetative populations from the EMCC to the WMCC, or (3) be carried offshore (adapted from [8, 19])

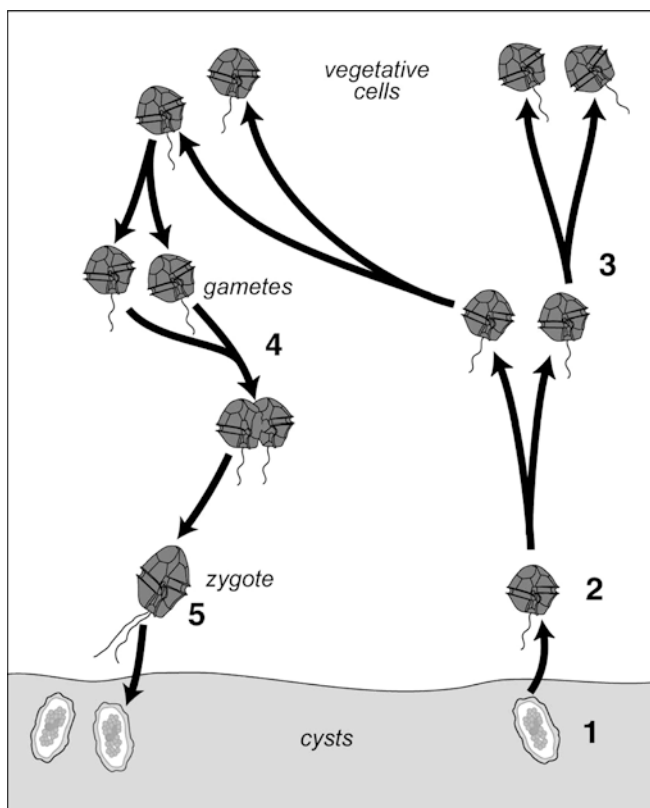
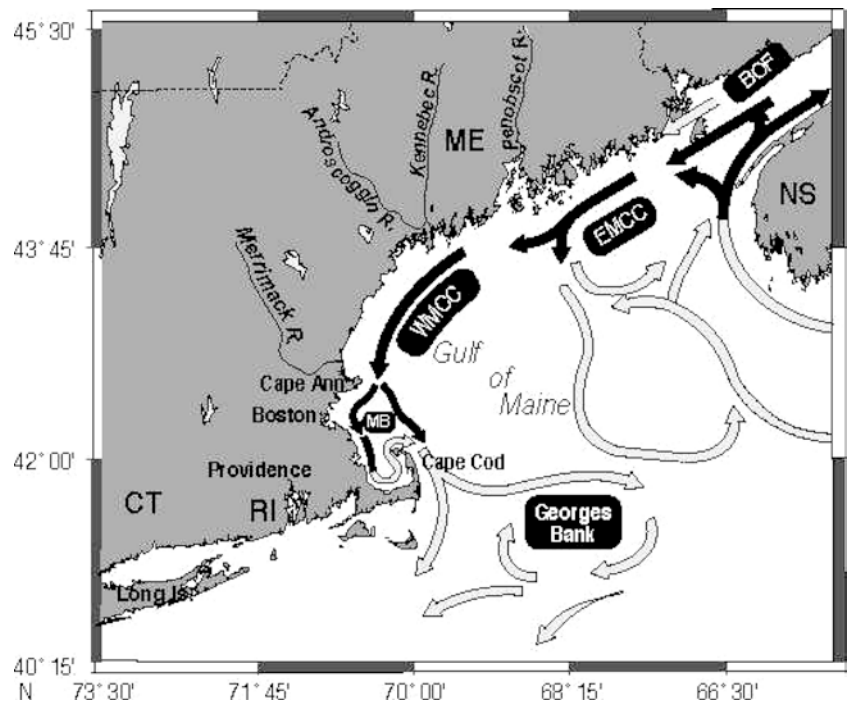


Fig. 6 The life cycle of *Alexandrium*, a dinoflagellate with cyst resting stages (1) that can act as reservoirs for new population growth (adapted from D.M. Anderson, personal communication). The resting stages rupture (excyst) to yield swimming cells (2) which continue to divide to produce a vegetative population (3). As nutrients are depleted, division slows and gametes are formed that fuse to form a zygote and then a cyst (4, 5)

for subsequent blooms of the neurotoxic red tide organism *Karenia brevis*, an almost annual bloom-former along the western coast of Florida. Again, circulation determines impacts locally and afar: in 1987, *K. brevis* originating in the eastern Gulf of Mexico were transported to North Carolina in the Gulf Stream, devastating local estuaries and associated industries with total losses estimated at US \$25 million [163, 165].

Recurrent introductions of nutrients and several harmful taxa into nearshore environments are associated with a number of areas typified by upwelling/downwelling regimes. As noted above for *Pseudo-nitzschia* in California and Washington State, the coasts of France, Spain, and Portugal experience upwelling/downwelling-induced exposures to several toxic algal species, including *Dinophysis*, *Karenia mikimotoi*, *Alexandrium affine*, *Gymnodinium catenatum*, and *Lingulodinium polyedrum* [4, 45, 50, 84, 97, 119]. *K. mikimotoi* is also a common dominant in upwelling centers along the Benguela region of the South African coast [112] and Chang [33] has reported upwelling induced bloom formation and subsequent mass mortalities caused by *Gymnodinium brevisulcatum* (*K. brevisulcatum*). On much smaller scales, wind-induced upwelling in estuaries and coastal bays can promote growth of HAB species: in the Chesapeake Bay, wind-induced tilting of the pycnocline (Fig. 7) and introduction of bottom recycled nutrients resulted in blooms of mixed dinoflagellate species [147] and cyanobacteria in summer stratified waters of the Gulf of Finland [71]. Spring tide-induced destratification also yields algal blooms, as demonstrated by a large bloom of *Cochlodinium* that followed a water column mixing event in the York River estuary, Chesapeake Bay [60].

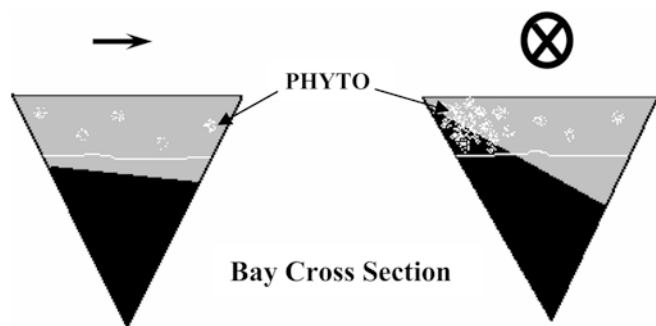


Fig. 7 Cross-bay tilting of the pycnocline (represented as a line between gray and black regions) induced by winds shifting from calm to weak westerly winds (\rightarrow) to strong southerly winds (\otimes) along the axis of the Chesapeake Bay. The tilting drives subpycnocline remineralized nutrients into the euphotic zone (white line 1% light level) leading to phytoplankton blooms (see [147]). Wind-induced upwelling along western coasts of the major continents leads to similar nutrient introduction and elevated surface production, often harmful algal species

Wind-driven flows can also introduce oceanic or shelf populations into coastal embayments, leading to harmful and/or toxic blooms. Advection of shelf populations of *K. mikimotoi*, *Dinophysis acuta*, and *D. acuminata* into Irish embayments has been repeatedly observed [89, 102, 117, 118], many rife with mussel culture, leading to harvesting closures and economic hardship due to contamination of the resource. Moita et al. [97] argue for northerly winds distributing southerly *G. catenatum* populations along the west coast of Portugal in a coastal current, following wind-driven breakdown of a persistent front off Cape South Vicente. In the north (Galicia), southerly winds forced warm, offshore waters into the Ria de Vigo in 1985, leading to a *G. catenatum* bloom [49]. Similar wind-driven flooding of Spanish rias has introduced harmful taxa into these environments, resulting in toxicity of raft mussels. Gentien et al. [53] have constructed a simple model indicating that Bay of Brest *Gymnodinium nagasakiense* populations can be readily advected far to the south by strong northwesterly winds in April, reproducing field observed population distributions.

Hydrological events, such as rain-induced buoyant plume formation or delivery of micronutrients, also favor HAB development. In the Chesapeake Bay Loftus et al. [82] reported increases of dinoflagellate biomass to over $300 \mu\text{g}$ chlorophyll l^{-1} following a heavy rainfall, with the populations aggregating in the thin buoyant lens of fresher water immediately below the surface. Mid-coast, inshore red tides along Florida's west coast might persist longer due to rainfall and river flow from central Florida [39], with no reason given for the expanded durations. Granéli et al. [57] suggested that selenium and cobalt elution from local soils during heavy rains might have been partially responsible for blooms of *Chrysochromulina polylepis* in the Skaggeak and Kattegat.

Other large-scale meteorological events can lead to bloom formation. El Niño driven lower-than-normal sea

temperatures of New Zealand's northeast coast have been linked to recurrent spring *Mesodinium/Noctiluca* blooms giving way to summer raphidophyte and dinoflagellate blooms, particularly *K. mikimotoi* in the latter group [124]. This succession contrasts with normal years of spring diatoms, summer dinoflagellates, and diatoms once more in the fall. PSP and CFP increases in the Indo-Pacific have also been linked to El Niño events [61, 86]. North Atlantic Oscillations (NAO) have also been implicated as drivers for upwelling-induced blooms along Spain's coast, generated by increased alongshore winds developing as a result of greater temperature differences between the land and sea [48]. In the Galician coast of northwest Spain, this effect should lead to increased abundance of *G. catenatum*, a strong vertical migrator capable of utilizing deeper remineralized nutrients from the decomposition of post-bloom sedimented materials. Belgrano et al. [20] have correlated primary productivity, chlorophyll *a*, and three *Dinophysis* species in a Swedish fjord with the positive phase of the NAO index (milder, warmer winters, and higher salinities) in the 1980s and suggest that summer blooms of *C. polylepis* and *G. aureolum* in 1988 were partially attributable to this decadal phenomenon.

Aggregation at density discontinuities may also be important in bloom formation and maintenance. Franks [51] has described in detail the actual process of cell accumulation at frontal systems. Several HABs in frontal regions, maintained on the downwelling sides of fronts through active migration, are well documented. Holligan [66] described *K. mikimotoi* accumulations at the Ushant front in the western English Channel. Frontal accumulations of *Nodularia* and *Aphanizomenon* have also been described at the mouth of the Gulf of Finland [71, 96]. In the Chesapeake Bay and its tributaries, at least four species are associated with such surface fronts, including *Prorocentrum minimum*, *Gyrodinium uncatenum*, *Heterocapsa rotundata*, and *Gymnodinium pseudopalustre* [149, 171, 172, 174]. The importance of frontal accumulations in shellfish toxicity is exemplified in the Iberian Peninsula, where *G. catenatum* accumulates both at a downwelling front between the poleward slope current comprised of naked dinoflagellates, and through migration at the convergence [46]. The accumulated cells are trapped close to shore and enter the rias to intoxicate mussel rafts. In Argentina, *Alexandrium tamarense* accumulates in coastal fronts associated with subantarctic waters off Patagonia and are subsequently transported inshore during wind reversals [31], thereafter causing serious PSP toxin contamination of shellfish in the region.

As noted above for *Pseudo-nitzschia*, thin layers of many phytoplankton species including several harmful taxa, may be previously unrecognized recurrent features of coastal systems. These layers are unique and can be, but need not be, associated with the pycnocline or nutricline. Aside from *Pseudo-nitzschia*, *Dinophysis* and *Alexandrium* have now been observed in these narrow but horizontally wide features, along the West coast

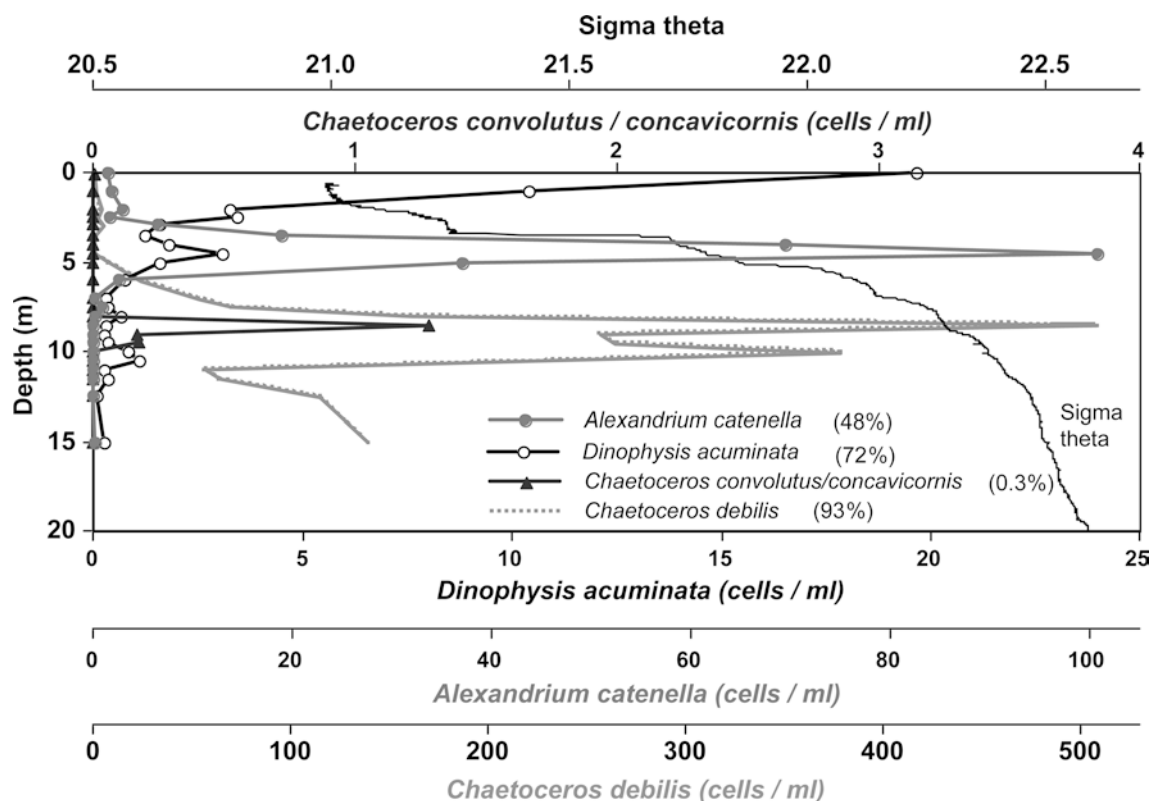
of the United States (Fig. 8) and in Swedish waters [40, 58, 127]. The thin layers have some integrity and appear to remain intact for some time and distance. In East Sound, Wash., a thin layer of the diatom *Pseudo-nitzschia* persisted as an intense feature along the entire length of the 12 km fjord for 3 days between wind events, only to reappear after passage of the winds [127]. Further, a 7 day time series of finescale hourly profiles in Monterey Bay, Calif. indicated that *Pseudo-nitzschia* could form thin layers in open coastal waters that have similar intensity, thickness, and persistence to those observed in East Sound [40]. Although the *Pseudo-nitzschia* layers in both systems showed little sign of sinking (e.g., they were associated with a relative narrow density range throughout the period), the depth at which the *Pseudo-nitzschia* layer occurred varied by more than 10 m in response to internal waves in Monterey Bay and by subduction by the inflow of lighter waters in East Sound. These vertical shifts in depth were large enough to radically change light availability and the potential for contact with the benthos. It is interesting to speculate on the possible aperiodic role of these layers in seeding

inshore areas and suspension feeders with vegetative cells, cysts/spores, and toxin, providing episodic and undetectable seeding for events that have no apparent seed population, perhaps explaining domoic acid poisonings in razor clams and Dungeness crabs in Oregon and Washington coasts (observed by Taylor and Horner [162]) in October–November 1991.

There is considerable evidence for sub-surface maxima of several taxa occasionally contributing to HABs and adverse effects (e.g., shellfish toxicity) upon delivery to inshore areas. Offshore *Dinophysis* populations in discrete layers can serve as “seed” for surface blooms/intoxications in Spain and Sweden, entering into shallow depths through either upwelling or other wind driven events (e.g., [80]). A pycnocline-associated *K. mikimotoi* population off the Bay of Biscay, France resides there year-round [84], using remineralised ammonium for growth in situ. This may be the same population reported at the seasonal thermocline in the western English Channel, leading to eventual surface blooms at the Ushant front [66]. During winter-early spring in the Chesapeake Bay, the dinoflagellate *Prorocentrum minimum* is carried northwards in thin layer aggregations just below the pycnocline, resurfacing through occasional destratification events, shoaling at the flanks, and in association with mixing events at the northern extreme of the deep trough of the bay, to form the annual spring maximum of this species [173].

The findings discussed above argue strongly for the dual role of natural processes and anthropogenic forcing in HAB formation. Bloom events driven by circulation,

Fig. 8 Thin layers of harmful algae observed in East Sound (Wash.) in August 1997 overlain on vertical density structure. *Alexandrium catenella* and *Dinophysis acuminata* were the dominant net plankton at the depth of their thin layer (making up 72% and 48% of the net plankton, respectively). In contrast, the thin layer of *Chaetoceros convolutus/concavicornis* occurred at the same depth as the much more abundant *Chaetoceros debilis* (making up only 0.3% the net plankton). While the concentrations of *Chaetoceros convolutus/concavicornis* are low, they are just below the two cells ml^{-1} level reported to cause problems in fish [40]



meteorology, or natural nutrient loading (e.g., upwelled nutrients, river discharge from relatively sparsely inhabited regions) will likely occur regardless of human intervention. In contrast, HAB species that are strongly influenced by factors derived from human activities that impact land, water, or air are potentially manageable, providing the political will is present to commit the resources needed to manage loads associated with watershed and coastal development. In either case, the impact of HABs on coastal communities is significant and has resulted in efforts to pro-actively reduce the environmental and public health threat from these events by enhancing our ability to detect blooms, toxins, and toxicities.

HAB detection: current and future possibilities

The preceding sections provide an overview of the many possible causes and effects of HABs in the coastal zone. As is the case with any natural- or anthropogenic-driven phenomenon that represents a potential hazard to the health of humans, wildlife, or ecosystems, effective management and mitigation strategies are essential for reducing the hazards associated with HAB events. While no single approach can address all possible impacts, timely detection of harmful algal species and the toxins they produce represents a critical component of most HAB management plans. Such information, if made available early in the process of HAB initiation/development, can provide coastal resource managers, fishermen, aquaculture operators, and public health officials with the data needed to recommend or take actions for minimizing the effects of HABs. Moreover, organism and toxin detection capabilities are also critical tools for researchers studying HAB population and toxin dynamics, and developing models needed to forecast and predict these events. The following section describes some of the current and future approaches to detecting HAB species as well as their toxins.

Detection of HAB species

The classical approach for detecting and enumerating phytoplankton species, including those referred to as harmful and/or toxic, is direct observation by light microscopy of live or preserved material (see [154] and chapters therein). Although this technique provides important visual confirmation of the presence of a species in a water sample and generates reasonably accurate estimates of cell abundance, it is generally considered to be tedious and time-consuming while requiring an appropriate level of experience/expertise in phytoplankton identification. Light microscopy is therefore of limited use when real-time or near-real-time detection is the objective. Nonetheless, several volunteer phytoplankton monitoring programs have incorporated the use of portable field microscopes and training focused

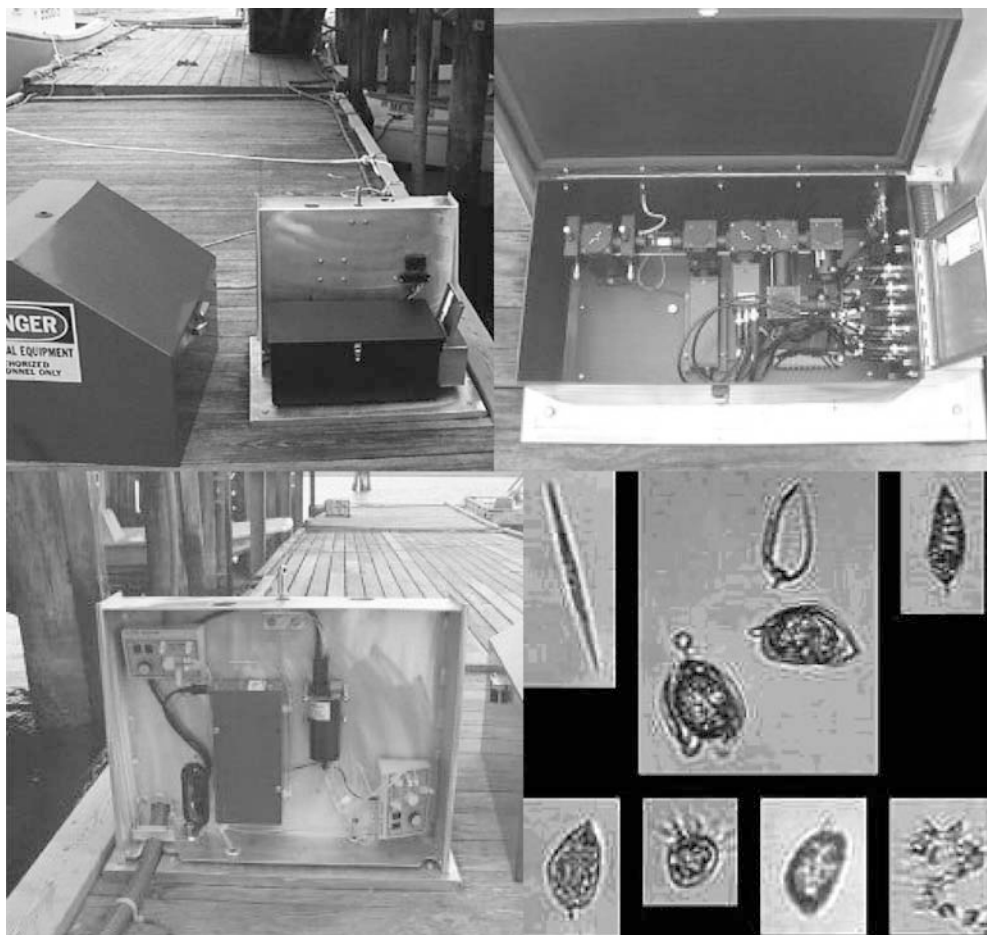
exclusively on the recognition of potential HAB species in order to assist coastal managers in the early detection of possible bloom events in certain areas (e.g., [62]).

An alternative approach to detecting phytoplankton cells also based on their morphological/optical properties and relying largely on the principles of flow cytometry was developed recently. The instrument, referred to as the flow cytometer and microscope (FLOWCAM, Fig. 9; <http://www.fluidimaging.com>), generates data for 12 different intrinsic characteristics (e.g., size, chlorophyll and phycoerythrin content; note that this approach does not involve labeling of the cells in any way), as well as producing a photographic image for each cell or particle that passes through it. An on-board image processor can be “trained” to recognize certain cell types, such as those representing potentially harmful species, and stored images can be accessed at any time following acquisition in order to confirm identifications. The FLOWCAM is a portable unit that can analyze particles ranging in size from 10 to 1,000 μm (which accounts for the majority of harmful algal species), accept either discrete samples or a continuous flow of up to 10 ml min^{-1} , and generate abundance data in terms of numbers per liter for selected cell types. The instrument’s ability to operate in continuous (i.e., pumped) sampling mode for extended periods on AC power in a weatherproof enclosure suggests a strong potential to monitor for the presence of harmful taxa synoptically at multiple shore-based monitoring sites. A submersible version of the FLOWCAM that can be moored temporarily or permanently has recently become commercially available and should further enhance the potential HAB monitoring capabilities of this instrument.

Particle size distributions, ranging from particles 0.7 μm to fish, can be determined with other methodologies as well. Gentien et al. [52] have developed a particle size analyzer for particles from 0.7 to 400 μm based on diffraction; in a profiler mode, vertical distributions of HAB species in the Baltic and off the French coast have been determined. Acoustic profilers are also available [e.g., Tracor acoustic profiling system (TAPS)], permitting characterization of macrozooplankton and larger organisms that may graze or alternatively avoid accumulations of harmful algae.

The most rapidly growing area of HAB species detection involves the targeting of specific molecules, such as chemical moieties located on the cell surface and various components of an organism’s genome. These classes of molecules lend themselves well to detection by antibody or oligonucleotide probes, respectively, using methods derived from previously developed biomedical applications. In the case of cell-surface targets, the most common approach has employed conventional protocols for the immunization and subsequent boosting of a host animal (e.g., rabbit, mouse) with chemically-fixed, whole cells of a given algal species to produce either polyclonal or monoclonal antibodies (see review by Vrieling and Anderson [182]). The antibodies generated

Fig. 9 Docktop flow cytometer and microscope (FLOWCAM) system developed by Fluid Imaging Technologies (FIT) (*top left*). Flow cytometer component (*top right*) and optical sensor component (*bottom left*) are contained in a weatherproof housing that can be equipped with wireless internet access. Sampling of up to 10 ml min^{-1} can be pre-programmed or triggered based on real-time fluorescence/scatter signal and images of each processed particle (*bottom right*) can be obtained. Photos courtesy of C. Sieracki and W. Thiabaudau, FIT



are then screened for reactivity against the target species as well as a range of closely- and distantly-related phytoplankton taxa to confirm the specificity of the recognition. Since the immunogen presented to the host animal is an uncharacterized mixture of cell surface antigens displayed by intact cells, rather than a single, purified compound, the resulting antibodies are produced against one (monoclonal) or more (polyclonal) unidentified constituents present on the cell surface at the time of harvesting and fixation. Such constituents may include polysaccharides [133], proteins [101], and lipopolysaccharides [140], or combinations thereof. Because the composition of cell surface antigens will vary with an alga's physiological status, screening of the antibody should also include testing against the target species grown under different culture conditions to confirm similar labeling across a range of cell metabolic states (e.g., [14, 110]). Once an antibody has been characterized in the laboratory (e.g., titrated and confirmed to be specific for the target species, limited by the cultures available for testing cross-reactivity), field applications can be developed.

Similarly, lectin-cell surface polysaccharide binding has been used to detect several harmful taxa and various cell morphologies associated with different stages in the life cycles of some dinoflagellates [3, 54, 72]. The lectin, a

non-immunogenic carbohydrate-binding protein, is generally a natural plant product specifically recognizing a monosaccharide or simple oligosaccharide and, when labeled with a fluorescent reporter (e.g., fluorescein isothiocyanate), permits discrimination of specific taxa based on surface carbohydrate composition. Although lectins are inexpensive and readily available, there are no reports of lectin-based detection of HAB species currently being used in the field.

There are two strategies currently being employed for the detection of harmful algal species with antibodies and lectins, involving either epifluorescence microscopy or flow cytometry. In both cases, species-specific antibodies recognizing cell surface antigens are applied to intact cells in conjunction with a fluorophore-based reporting system, yielding a fluorescent signal from target cells labeled with an antibody that can be detected with appropriate instrumentation. The use of fluorescence to detect antibody-antigen reactions is collectively referred to as immunofluorescence and the application of techniques based on this approach for phytoplankton research has been critically reviewed by Vrieling and Anderson [182]. It should be noted that antibodies directed against intracellular molecules [e.g., tubulin, Rubisco, PCNA (proliferating cell nuclear antigen)] in phytoplankton, including harmful species, have been

produced and applied using immunofluorescence-based detection [79]. Although such antibodies generally recognize specific, well-characterized proteins and several of these antigens can be visualized within the same sample, their use is aimed more at studies of phytoplankton ecology/physiology rather than species identification. Moreover, development of field applications has favored antibodies targeting cell surface antigens, a trend that likely reflects their ease of preparation and the lack of requirement for the permeabilization of cells that would be needed to expose intracellular antigens to an antibody.

A number of researchers have developed antibodies against cell surface antigens specific for a wide range of harmful taxa (reviewed by Anderson [7]). Examples of algal groups investigated using antibodies include dinoflagellates (e.g., *Alexandrium* spp. [1, 138]; *Gymnodinium* spp. [101, 110]; *Gyrodinium* spp. [183, 185]), diatoms (e.g., *Pseudo-nitzschia* spp. [18, 110]), raphidophytes (e.g., *Chattonella* spp. [176]), and pelagophytes (e.g., *Aureococcus anophagefferens* [9]). From a HAB monitoring perspective, both microscopic and flow cytometric immunofluorescence-based approaches have been applied or evaluated. In the case of the small (ca. 2 μm diameter), relatively nondescript brown tide organism, *A. anophagefferens*, Anderson et al. [11] reported that cells labeled with a species-specific polyclonal antibody could be detected at concentrations as low as 10–20 cells·ml⁻¹ using epifluorescence microscopy. This method was used to map the distribution of *A. anophagefferens* throughout the coastal waters of the northeast United States in order to identify areas with a potential for brown tide outbreaks. The epifluorescence technique and, most recently, a high throughput (96-well plate format), enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody directed to a cell surface antigen [30] have been employed by brown tide monitoring programs conducted in this region. Interestingly, ELISA-based methods for cell detection have yet to see widespread use, but can be expected to grow in popularity given their potential to greatly enhance the speed of analysis and sample throughput while reducing variability between samples. One notable caveat is the elimination of a visual confirmation of labeled cell morphology that is possible with epifluorescence microscopy-based methods.

Several studies have explored the potential of immunofluorescence-based, flow cytometric methods for the detection of natural HAB populations (see review by Peperzak et al. [111]). One of the first such studies was reported by Vrieling et al. [183], who found that antibody-labeled cells of the ichthyotoxic dinoflagellate, *Gyrodinium aureolum*, collected from the North Sea could be identified via flow cytometry, yet quantification as compared to light microscope counts was poor due to loss of cells during sample processing. Other researchers attempting to quantify *Alexandrium* spp. in field samples have also reported problems with quantification resulting from cell loss [139]. Thus, while the technique of

immuno-flow cytometry shows promise as an automated means of detecting antibody-labeled HAB species, issues related to the loss of cells during staining, and thus poor quantification of cell concentrations must still be addressed and have apparently precluded incorporation of this approach into routine HAB monitoring efforts.

In addition to cell surface antigens, the other class of target molecules that has been employed for highly specific detection of HAB taxa is the nucleic acids. In particular, components of the ribosomal RNA genes (rDNA) and their transcriptional products, the corresponding ribosomal RNA (rRNA) molecules possess several characteristics that make these cellular constituents highly amenable to such applications. Genes coding for rRNA are present in all living organisms and thus large, public domain sequence databases are available (e.g., <http://rdp.cme.msu.edu/html/>) to facilitate robust comparisons between newly vs. previously sequenced taxa. Ribosomal gene sequences contain regions that range from highly conserved to highly variable, which allows for the identification of target areas that can distinguish taxa at various levels, including strains, species, genera, and increasingly broad phylogenetic groupings. Moreover, the ribosomes, located in the cytoplasm and comprised largely of rRNA, represent easily accessible, generally abundant targets for the oligonucleotide probes used to bind these molecules. However, as noted above for phytoplankton cell surface antigens, rRNA levels can vary as a function of algal physiological status (e.g., Anderson et al. [12]). It is thus also imperative that labeling intensities of target species be compared under a range of both favorable and unfavorable conditions in the laboratory prior to the development of field applications.

For detecting harmful algal species, the small (18S) and large (24S) subunit rRNA molecules have been most frequently used as the target of oligonucleotide probes—pieces of synthetic DNA that recognize a given target sequence within the rRNA molecule. In all cases, even though a probe is designed to be specific for one or more algal taxa based on the available sequence data, its binding must be empirically verified as the target region may be inaccessible due to folding of the rRNA molecule upon itself. There are two primary approaches for the use of oligonucleotide probes in the detection of HAB species. The first is referred to as either whole cell hybridization (WC) or fluorescence in-situ hybridization (FISH), in which the probe penetrates into chemically fixed, intact cells, hybridizes or binds to its target sequence on the rRNA molecules, and is then visualized via a fluorescent reporter either attached directly to the probe or applied during a secondary labeling step. Similar to immunofluorescence methods described above, algal cells labeled using FISH protocols can be examined directly by epifluorescence microscopy or analyzed using automated methods such as flow cytometry. Also analogous to algal cell surface antigens, the abundance of ribosomes within a cell, and thus labeling intensity, generally varies in proportion to

growth rate. The extent to which fluctuations in ribosome levels under different growth conditions affect the labeling of target cells must therefore be investigated experimentally to aid in interpretation of data from natural populations (e.g., [14, 111]).

The whole cell hybridization approach has been developed and applied extensively for the detection of many harmful algae, including dinoflagellates (e.g., *Alexandrium* spp. [1]; *Dinophysis* spp. [120]; *Karenia* spp. (C. Mikulski, personal communication, [91]); *Pfiesteria* spp. [135]), diatoms (e.g., *Pseudo-nitzschia* spp. [92, 93, 110, 143]), and raphidophytes (e.g., *Heterosigma akashiwo* [175]; *Fibrocapsa japonica* [175]) (see Fig. 10 for an example). Perhaps the best example of the use of the WC technique to monitor harmful algal species has been reported by workers in New Zealand [123, 124, 125]. In this case, WC-formatted probes for *Alexandrium* spp. and *Pseudo-nitzschia* spp. have been integrated into the country's two-tiered biotoxin monitoring programs for industry and public health. Probes for additional HAB species present in New Zealand's coastal waters, including the dinoflagellates *Karenia* spp. and the raphidophytes *Heterosigma* and *Fibrocapsa*, are also being tested in the WC format to assess their suitability for inclusion in the country's phytoplankton monitoring programs [123, 125]. The probe results for toxic algal species represent the first tier, which provides risk assessment information for decision making by shellfish

harvesters, while the second tier involves testing of shellfish for biotoxin contamination. The laboratory responsible for conducting the WC assays (Cawthron Institute; http://www.cawthron.org.nz/phytoplankton_lab.htm) is approved by International Accreditation New Zealand (recognized under ISO-IEC Guide 25). The use of WC-formatted probes for routine phytoplankton monitoring is also being explored by other countries with severe problems related to HABs (e.g., [34]).

A recently developed technology compatible with the detection of FISH-labeled microbial cells is laser scanning solid phase cytometry. This approach involves the filtration and labeling of cells with fluorescently-tagged rRNA probes followed by scanning of cells on filter membranes (rather than in solution as for flow cytometry) using laser excitation. Protocols are currently being developed for use of this semi-automated method in the detection of HAB species such as *Alexandrium minutum* and *Pseudo-nitzschia* spp., ultimately within the context of routine HAB monitoring programs [41].

The second approach to applying oligonucleotide probes for harmful algal species detection is the sandwich hybridization (SH) method, which involves chemical lysis of the algal cells to release rRNA target molecules, followed by binding of the target by a species-specific "capture" probe immobilized to a solid support (e.g., bead, membrane, etc.), and then hybridization of a "signal" probe to another region of the rRNA. The latter is responsible for visualizing the captured rRNA using a colorimetric, fluorometric, or chemiluminescent reporting system and this reaction chemistry can be configured in a variety of ways (see Fig. 10 for example). While the SH method precludes the direct microscopic observation of labeled target cells, this technique allows for rapid, high throughput sample analysis and has been effectively automated in a variety of formats. Initial application of SH assays for the detection of harmful taxa was reported by Scholin et al. [142, 143] for toxic diatoms of the genus *Pseudo-nitzschia*. In this case, the capture probes were covalently linked to nylon beads and the signal probe-based reporting system produced a colored reaction on the surface of the beads that was proportional to the number of target cells. These researchers have since developed an automated laboratory method that employs a robotic sample processor and has evolved from a system using the nylon beads described above embedded in so-called "plastic analytical cards", which suffered from a number of limitations, to a 96-well plate-based format with the capture probes immobilized on polystyrene prongs that are moved by a robotic arm through a pre-programmed series of wells containing the sample lysate and assay reagents [144]. Note that preparation of the sample by chemically lysing the algal cells following their capture on a filter is performed manually.

This rapid, high throughput SH sample processing technology, including pre-packaged reagents specific for the colorimetric-based detection of a number of

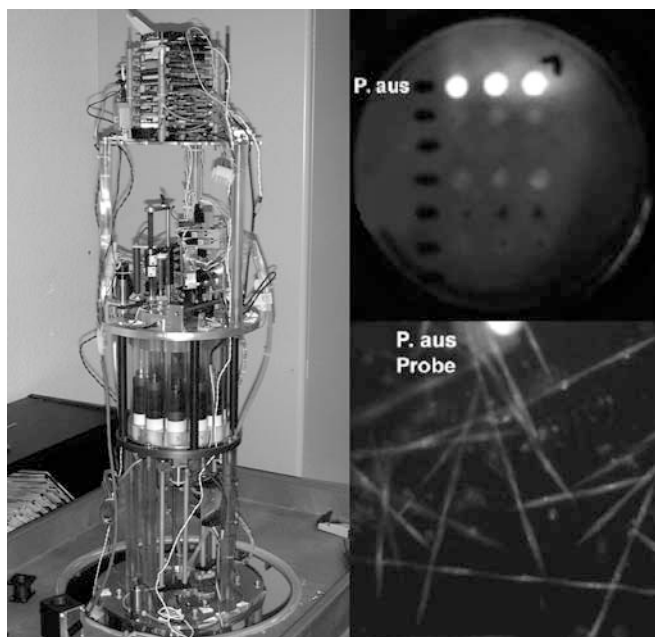


Fig. 10 Environmental sample processor (ESP; left) developed by the Monterey Bay Aquarium Research Institute (MBARI) for the automated, in situ conduct of rRNA sandwich hybridization (SH) assays and sample archival capabilities for whole cell (WC) hybridizations. Array photo (top right) shows positive SH response across triplicate channels for the toxic diatom, *Pseudo-nitzschia australis*, while WC image (bottom right) shows corresponding sample treated with *P. australis* probe and demonstrating presence of *P. australis* cells. Photos courtesy of C. Scholin (MBARI)

individual HAB species (*Pseudo-nitzschia* spp., *Alexandrium* spp., and several raphidophytes), is now commercially available (Fig. 11; Saigene, Seattle, Wash.; <http://www.saigene.com/Technology/ahab.htm>). Detection of these same three groups of harmful algae by automated SH assays is being tested in field trials by New Zealand researchers, with the aim of incorporating this technique into their existing phytoplankton monitoring programs [123]. Other investigators are currently evaluating this method for use in studies of natural HAB populations, such as the PSP-producing dinoflagellates *Alexandrium* spp. (e.g., [13]) and the domoic acid producing diatoms *Pseudo-nitzschia* spp. (e.g., [107, 144]) (Fig. 10).

In addition to colorimetric-based reporting systems such as that just described, the binding of an oligonucleotide probe to its rRNA target in solution (i.e., following algal cell lysis) can be detected through the use of electrochemical methods. This type of reporting system is the centerpiece for a new generation of devices currently being developed and aimed at establishing portable, field-based detection capabilities for harmful algal species (e.g., [81, 90]). As in the case of the automated laboratory SH processor outlined above, the species detected can be changed simply by employing a different suite of taxon-specific probes. Sample preparation [e.g., chemical-based cell lysis, polymerase chain reaction (PCR) amplification, etc.] does, however, remain a manual process. Nonetheless, the benefits of portable detection units to HAB monitoring programs, especially within the aquaculture industry and for use on board vessels, are clear and their use will likely become more commonplace in the future.

As noted above, use of both antibody and nucleic acid probes for HAB species detection evolved from existing biomedical applications. Similarly, the relatively new and rapidly advancing field of array-based detection

is now being adopted for HAB species. One such approach involving the use of membrane-based arrays is aimed at establishing the capability for real-time, in situ detection of harmful algal species (and their toxins; see below) on moored platforms [146]. In this case, the array consists of nanoliter volumes of rRNA capture probe spotted and stabilized onto a membrane. An SH assay, including harvesting and filtration of the algal cells, production of a cell lysate, application of the rRNA-containing lysate to an array, and detection of bound target molecules via a signal probe/chemiluminescent-based reporting system, is completely automated and performed autonomously on board a dedicated instrument called the environmental sample processor (ESP; Fig. 10). The ESP is deployed as part of a stationary mooring and signals generated by the SH assay, as well as ancillary data from the mooring (e.g., salinity, temperature, in vivo fluorescence, etc.), are transmitted in real time to land- or ship-based laboratories for additional processing and data manipulation. Archival capabilities, permitting samples to be tested by WC hybridization and toxin assay methods upon retrieving the instrument and returning to the laboratory, have also been designed into the ESP. Successful trial deployments of the ESP have been conducted in both Monterey Bay, Calif. and in Casco Bay, Me. (C. Scholin et al. personal communication) using arrays configured for both *Pseudo-nitzschia* spp. and *Alexandrium* spp., allowing simultaneous detection of either group. One can envision the concurrent deployment of multiple ESP units configured for detection of several HAB species providing an advanced, synoptic view of bloom development and dissipation within a region as a supplement to existing monitoring programs.

Finally, a rapidly emerging approach to the detection of HAB species targeting the unique genetic signatures of these organisms is the application of PCR-based methods, which have been used widely for the detection of other microbes such as bacteria and viruses (e.g., [160]). Several studies have used taxon-specific PCR primers to amplify selected regions of target genes (e.g., the rRNA gene cluster) of HAB species from a standard genomic DNA preparation, followed by detection of the resulting amplicon by various techniques, including gel electrophoresis and staining/blotting protocols (e.g., *Alexandrium* spp. [109], *Gymnodinium* [55], *Pfiesteria* spp. [136]) and fluorescent fragment detection (e.g., *Pfiesteria* [35]). Such PCR-based methods can be highly sensitive and have been successfully employed to amplify and detect single vegetative cells and cysts of harmful species [24]. More recently developed techniques, such as quantitative competitive PCR, real-time PCR, and time step PCR, have been quickly adopted by the research community for detecting certain HAB species (e.g., *Pfiesteria* [25, 137, 189], *Microcystis* [105]). While these methods have the potential to yield quantitative information on algal cell concentrations, this capability has yet to be realized for harmful taxa occurring in natural samples;



Fig. 11 Universal processor manufactured by Saigene for the automated performance of sandwich hybridization assays to detect HAB species. Samples are loaded into the first row of wells and a prong strip attached to each of the processor arms carries the probes and rRNA target through the assay reagents to yield a colorimetric signal for positive samples in the last row of wells. Photo courtesy of R. Gordon, Saigene

nevertheless, such approaches are reported to be capable of rapid, highly specific and sensitive detection [25]. Of particular note are recent developments in real-time PCR that have yielded portable instrumentation suitable for use in the field (e.g., Cepheid, Sunnyvale, Calif.; <http://www.cepheid.com>) that will likely be incorporated into HAB monitoring efforts and research programs in the near future.

HAB toxin detection

The toxins produced by harmful algal species include a broad spectrum of compounds, ranging in size from several hundred to over 1,000 Da and varying in solubility from highly water-soluble to fat-soluble. The major classes of generally well-characterized toxins include the saxitoxins (PSP), brevetoxins (NSP), domoic acid (ASP), okadaic acid/dinophysistoxins (DSP), azaspiric acids (AZP), ciguatoxins (CFP), and microcystins/anatoxins/cylindrospermopsin (CTP). In most cases, a toxin class consists of a family or group of structurally and functionally related compounds, with individual toxin derivatives exhibiting an intrinsic toxic potency that can differ from that of their congeners by over three orders of magnitude (e.g., PSP toxins [104]). HAB toxins occur not only in the algal species producing them, but also in a variety of other organisms throughout aquatic or marine food webs as a result of trophic transfer processes (e.g., Scholin et al. [145]). In the latter case, a toxin can be metabolized or biotransformed into a structurally different compound that may be of either higher or lower toxicity than the original toxin molecule. The broad chemical and structural diversity of algal toxins, coupled with differences in intrinsic potency and their susceptibility to biotransformation, account for many of the challenges associated with the detection of these compounds.

Methods used for detecting algal toxins can be grouped into three main areas, including chemical analyses and *in vitro* and *in vivo* assays and all of these have been recently reviewed [32, 63]. While *in vivo* mammalian bioassays exist for several of the major toxin groups [43], this approach shows no potential for high throughput, automated, or *in situ* application and is thus outside the scope of this review. In the case of chemical analyses, the literature is replete with high performance liquid chromatography (HPLC)-based methods employing either UV- (including individual or scanned ranges of wavelengths) or fluorescence-based detection of either the native toxin molecule or a chemically derivatized form of the toxin (see chapters in [63]). Versions of such methods are currently used for regulatory purposes (e.g., domoic acid [15]) as well as investigations of toxin production in both the laboratory (e.g., Anderson et al. [10]) and field (e.g., [153]). More recently, mass spectrometers have been employed as detectors coupled to liquid chromatographic separation methods for the identification of HAB toxins [116]. Mass

spectrometers yield a highly specific, mass-based detection and, if operated in tandem mode (i.e., LC-MS/MS) such that the toxin molecule is fragmented to produce a series of diagnostic daughter ions, this approach can provide valuable confirmation of a toxin's presence in natural samples (e.g., [16]). The latter is especially critical when dealing with harmful species not previously known to be toxic in a given region (e.g., Pan et al. [106]).

Many advances in HPLC instrumentation and column technology have reduced the time of analysis and the use of programmed autosamplers has automated the sample injection process; however, HPLC analyses remain sequential and thus do not permit the concurrent injection of multiple samples on a single instrument required to truly achieve high throughput testing. In the case of mass spectrometry, technological innovations have resulted in the development of small, modular instruments, and researchers at the Center for Ocean Technology (<http://cot.marine.usf.edu/>) have successfully incorporated these mass spectrometers (Fig. 12) into autonomous underwater vehicles (AUVs). While prototype testing of these systems is still underway, the potential for adapting existing methods for certain algal toxins, especially those known to occur dissolved in seawater (e.g., brevetoxin, domoic acid), suggests that future designs could be configured for the *in situ* detection of toxins on board AUVs. Such instruments would provide mobile, real-time detection capabilities for HAB toxins that would greatly benefit both monitoring and research programs.

The second group of HAB toxin detection methods comprises *in vitro* assays and can be divided broadly into functional- and structural-based approaches. The

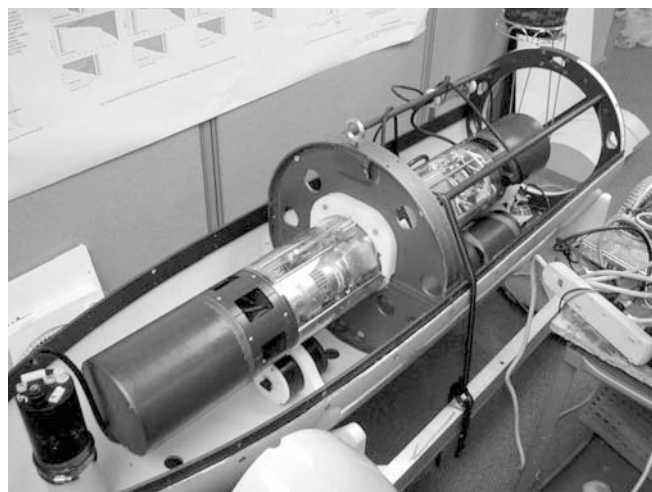


Fig. 12 Underwater quadrupole mass spectrometer contained in an autonomous underwater vehicle. Developed by the Center for Ocean Technology (COT), this first deployable version is aimed at detection and quantification of volatile organic compounds and dissolved gases. Current efforts include development of capabilities for tracing both anthropogenic and natural chemicals (e.g., HAB toxins) using networks of autonomous underwater vehicles (AUVs). Photo courtesy of D. Fries, COT

former rely on detection of a toxin's biochemical activity while the latter depend on recognition of chemical structure at the molecular level. These two categories of *in vitro* assays for HAB toxins have been the subject of several recent reviews [32, 166, 180]. There are advantages and disadvantages to assays based on either the functional or structural approach. Given that functional assays for a toxin (e.g., receptor binding assays) are based on binding by its biological receptor, and that the affinity of this interaction is proportional to the toxin's intrinsic potency, the assay response reflects the integrated or net toxicity of all congeners present in a sample that are bound by the same class of receptor. The same is true for toxins that have been modified structurally (e.g., biotransformations), providing receptor recognition remains unaltered. Nonetheless, such assays cannot be used to identify a toxin(s), only to detect and measure a particular toxic activity. Structural assays (e.g., immunoassays) require the conformational interaction of a toxin with a recognition factor and are thus susceptible to any changes to the toxin molecule that would interfere with this interaction. In the absence of structural modifications, these assays generally display a high degree of specificity for the toxin class they were designed for, yet detection of multiple toxin congeners depends on the degree to which the assay recognition factor (e.g., antibody, in the case of ELISAs) cross-reacts with these various chemical derivatives. Both functional and structural *in vitro* assays are susceptible to non-specific binding of non-target material, which must be accounted for in the assay design and implementation.

Among the various functional assays developed for the detection of HAB toxins, including cytotoxicity assays (e.g., Manger et al. [87]), enzyme inhibition assays (e.g., Della Loggia et al. [38]), and receptor binding assays (e.g., Van Dolah et al. [181]), there are cases in which these tests have been incorporated into existing HAB toxin monitoring programs (e.g., Suarez-Isla et al. [159]). Yet, some of the same features that make such assays useful for estimating toxic activity and protecting the public from consuming contaminated seafood, are actually impediments to formatting these methods for *in situ* toxin detection in HAB species. In particular, retaining the biological activity of a cell line or a receptor preparation (required for toxin recognition) under adverse conditions outside the laboratory remains an obstacle to the development of *in situ* functional assays that has yet to be overcome. Receptor assays for both domoic acid [115] and the saxitoxins [114] conducted in the laboratory have, however, been used to test archival samples collected on board the *in situ* ESP platform in conjunction with material processed for SH and WC assays for the associated HAB species (see above). Such integrated detection of both HAB species and toxins is essential for the accurate assessment of HAB-related risks and studies of HAB dynamics, due to the potentially wide fluctuations in algal toxicity as a function of physiological status [17]. Nonetheless, toxin

measurements on archival samples must ultimately be replaced by determinations performed on board *in situ* platforms such as the ESP in order to achieve real-time or near real-time resolution of HAB development and toxicity.

In comparison to the functional approach, structure-based assays such as immunoassays are collectively robust techniques that lend themselves well to use in the field and likely (in the future) to deployment on *in situ* platforms. Antibody-based assays (e.g., ELISAs) have been developed for a variety of HAB toxins and many of these tests are now commercially available (see [32, 100]). While most ELISA testing is currently performed in the laboratory, generally in a high throughput 96-well plate format, a product distributed by Jellet Biotech (<http://www.jelletbiotech.ca/>) called the MIST Alert for PSP toxins (to be re-issued as the Jellet Rapid Test for PSP toxins; J. Jellet, personal communication) tests single samples on a lateral flow immunochromatographic platform similar to that used for home pregnancy test kits (Fig. 13). The MIST Alert system produces qualitative (i.e., positive/negative) results in less than 20 min and has undergone extensive testing against the AOAC mouse bioassay, presently the regulatory standard for PSP toxin testing [78]. In addition, this product has also been evaluated successfully for use with plankton samples (MS submitted, [151]), as has another version of the MIST Alert for domoic acid (to be re-issued as the Jellet Rapid Test for ASP toxins) (MS submitted, [150]). The portability of this system makes it suitable for the rapid detection of certain algal toxins in field settings, although the sample throughput and quantification capabilities are limited.

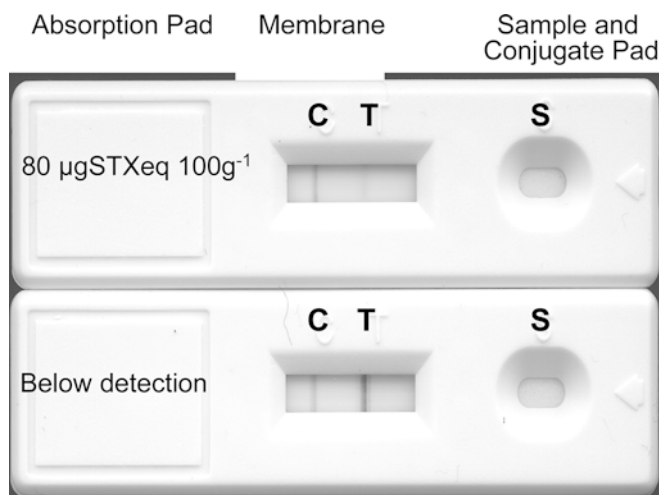


Fig. 13 MIST Alert kit for paralytic shellfish poisons (PSP) toxins (to be re-issued as the Jellet Rapid Test for PSP toxins) developed by Jellet Biotech, showing cassettes with positive (*top*) and negative (*bottom*) test strips. No *T* line indicates that toxin is present in a sample, while a visible *T* line indicates a toxin level below detection limit. The *C* line is a control line showing that the reagents have been sufficiently activated to provide a valid test result. The regulatory limit for PSP toxins is 80 µg STX equivalents 100 g⁻¹. Photo courtesy of J. Jellet, Jellet Rapid Testing

In terms of progress toward the goal of developing in situ sensors for HAB toxins employing in vitro-type assays, there are (to the authors' knowledge) no such systems currently in place. The most promising strategies for in situ toxin detection (in addition to those noted above involving mass spectrometry) appear to be those based on structural recognition of toxin molecules, such as antibody-based tests. In fact, the configuration of toxin immunoassays is often analogous to assays for HAB species detection using oligonucleotide probes (see above) which have already been formatted for in situ applications. Work is now underway to develop an immunoassay-based method for detection of domoic acid on board the ESP platform described above (G.J. Doucette et al. unpublished data) and other investigators are pursuing alternative approaches (e.g., toxin biosensors; [88]) that may also be deployed for remote detection of HAB toxins in the future.

In concluding this section it must be emphasized that, in addition to detection methods for HAB species and toxins, parallel development of technologies for the collection and concentration of potentially dilute analytes such as algal cells and their toxins (especially the latter), as well as the automation of sample preparation protocols, are critical. As discussed above, wide fluctuations in the abundance of harmful algal species and their toxin levels over a variety of temporal/spatial scales are well documented and can pose a challenge to obtaining sufficient material for analysis. Thus, in order to fully realize the potential for in situ detection of HAB species and toxins to address a range of applications, it is critical to engage managers, researchers/engineers, and industry in dialogue to identify the information needs (type, frequency, etc.), the most appropriate technologies (organism/toxin detection, sample collection/processing, etc.) to obtain these data, and the most efficient means to manufacture and bring robust, reliable products to market.

Optical Detection

Remote sensing for the detection of surface pigment, reflectance, or temperature signatures has been utilized for HAB detection for the last 30 years. The large spatial scale and high frequency of observations provided by remote sensing makes it appealing as a means of detecting and assessing HAB features [28, 37, 164]. Steidinger and Haddad [155] demonstrated the utility of a satellite for detecting HABs using imagery from the coastal zone color scanner (CZCS), and the interaction of some hydrographic features and algal blooms can be synoptically assessed for near-surface waters using ocean color sensors [99]. The advanced very high resolution radiometer (AVHRR) has been used to find blooms of phytoplankton that scatter light or that occur in highly turbid water [27, 56, 69, 157]. The sea-viewing, wide-field-of-view sensor (SeaWiFS) currently collects global chlorophyll concentration data on nearly a daily basis.

The United States NOAA CoastWatch program now acquires and processes SeaWiFS imagery for HAB monitoring utilizing patterns of chlorophyll anomalies [158].

Utilizing spectral reflectance (ocean color) and an ocean color inversion model [131], the phytoplankton community composition associated with HAB events has been estimated [130]. Additionally, the model is sensitive to optical variations within algal groups related to cell-specific pigment variations making it possible to assess algal physiology at the same time.

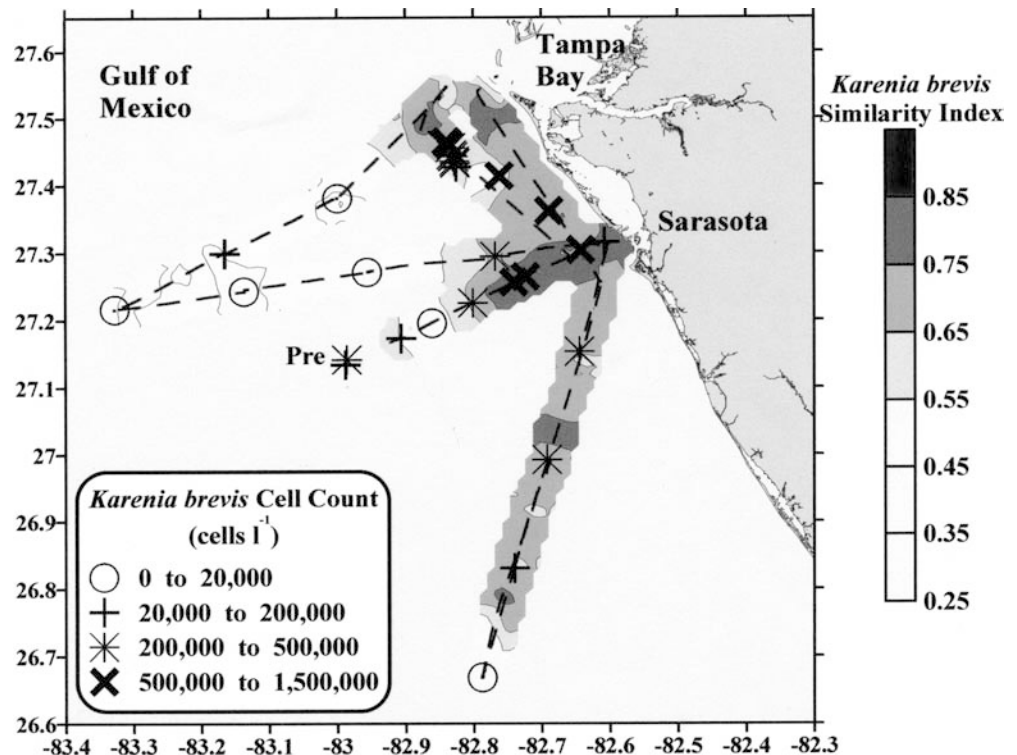
Light absorbance spectra of HABs are maximal in the blue (and to a lesser extent, the red) portion of the visible spectrum. Absorbance attributable to accessory pigments is difficult to discern and quantify because chlorophyll *a* dominates the signal and there is spectral variance imparted by variation in how pigments are packaged [68, 94, 95, 132]. The success of absorbance-based optical techniques to discriminate among distinct taxonomic groups depends upon the ability of the approaches to differentiate subtle absorbance characteristics of accessory pigments. A step forward for use of this approach was the development of microphotometric measurements of single cell absorbance spectra; these provided end member spectra for the numerical decomposition of mixed-species cultures [83]. Modeled contributions assigned to either species displayed trends consistent with the actual proportions contributed to the spectrum by each algal culture. The utility of this approach for identification of algal taxa depends on the capabilities for acquiring high-resolution microphotometric data with low signal-to-noise ratios.

Using particulate absorbance spectra from a diverse range of phytoplankton, noxious bloom-forming dinoflagellates have been delineated from other algae through discriminant analysis [68, 94, 129]. Millie et al. [94] combined fourth-derivative analysis of particulate absorbance spectra with a similarity algorithm to discriminate spectra of the Florida (USA) red-tide dinoflagellate *Karenia brevis* (Davis) within hypothetical mixed culture assemblages. When applied in the eastern Gulf of Mexico, a significant, linear relationship existed between the derivative spectrum-based similarity index and the fraction of chlorophyll biomass contributed by *K. brevis* [70]. An automated, shipboard HAB detector, incorporating the aforementioned derivative spectrum-based similarity index, has refined in situ acquisition of the required hyperspectral absorbance data for unattended, in situ detection of *K. brevis* (Fig. 14). This approach is being adapted to provide detection and mapping of *K. brevis* utilizing AUVs.

Platforms and arrays

Sampling the marine environment is difficult due to large spatial and temporal variations in chemistry, biology, and physics. Classically, off-pier or shipboard sampling has yielded single point determinations in

Fig. 14 Contour map of the distribution of the Florida red tide, *Karenia brevis*, based on taxa similarity index determined by the R.V. "Breve Buster" during the 2001 ECOHAB: Florida Process Cruise Leg B. Data were collected on 24 and 25 October 2001. Water was pumped by the ship's seawater system from 2 m below the surface. Cell counts, by microscope enumeration, were conducted on board the ship. Cell counts labeled *Pre* were collected prior to the beginning of data collection by the Breve Buster, included to highlight the presence of an offshore patch of *K. brevis* detected by the instrument. The dashed line is composed of small black dots indicating where data were collected by the Breve Buster



time and space and, therefore, limited harmful alga-specific sampling except for the rarer, obvious HAB event. With development of the suite of identification methods for species and toxins described above, there has been an increasing commitment to transforming these techniques to in-water capabilities that could be packaged with a suite of platforms and sampling systems permitting simultaneous detection of environment, species, and impact.

Moorings of numerous instrument types, suspended from fixed structures, floats, or buoys, are now routine in oceanography and limnology. These arrays often include autonomous technologies for measuring currents (ADCPs), temperature, conductivity, and depth sensors (CTD systems), fluorescence (pigments and colored dissolved organic matter), optical properties, seston/turbidity, and several nutrient species (nitrate, ammonium, phosphate, iron) with data stored internally or transmitted to shore through wireless communications. Some acoustic procedures are also on-line (e.g., TAPS), permitting estimations of size distributions of organisms in a particular water parcel. These standard packages, fixed in location, have now been refined to permit vertical and spatial sampling, either through tethered sampling over depth or the release of active or passive samplers from the fixed mooring. The ESP described above is one such moored HAB sensor array that, when combined with the above technologies, provides oceanographic conditions accompanying the HAB distributions passing the package.

Another option is moving the package through the water, rather than sampling the water advected past a

fixed mooring location. Towed sensor packages are most routine for this type of gear, and generally include CTD, fluorescence, and occasionally taxa-specific sampling capacities like sippers for small seston and dissolved samples, nets for zooplankton, and recording devices (video, cameras, FLOWCAMs, etc.) for grabbing pictures of suspended material. Hydrowire-deployed or free-falling, nearly neutrally buoyant sensor packages have also been developed, permitting fine scale vertical resolution of a number of water column parameters.

As alluded to above, a promising approach is attaching sensor packages to robotic undersea vehicles (RUVs) or AUVs. These tethered or far-ranging platforms often permit sampling at scales and locations not readily accessible with routine shipboard or moored sampling. The in-water sampling historically completed by field oceanographers is now replaced by large area sensor detection, managed by electronics engineers and equipment maintenance specialists, with oceanographers receiving telemetered data for rapid assimilation and interpretation. Further, aerial and satellite sampling with appropriate sensor packages for salinity, temperature, wave heights, and some pigments ensures rapid data accumulation over much greater spatial scales than previously possible; the repeated overflight schedules eases some temporal limitations and increases sample number, providing greater sample density than otherwise attainable.

As sensors for HAB species and toxins move from the laboratory bench to miniature sensor systems, and wet chemistries are replaced by chip-based arrays, deployment of multi-purpose platforms for ecosystem

assessment and HAB detection will become routine in most monitoring programs.

Modeling and forecasting

As described above, advances in optical instrumentation may provide rapid spatial coverage for HABs and, coupled with data-assimilative modeling, may provide the components necessary for building an automated HAB detection and forecasting system. The desired information must be isolated and extracted from the measured bulk optical signals of the observed water mass. A multi-platform optical approach utilizing remote sensing and in situ moored technologies [37, 141] is promising a capability to provide the near real-time observations over ecologically relevant spatial and temporal scales. Data-assimilation methods fuse these observational networks to optically based models, providing a capability for detection and forecasting [22].

Recent advances in HAB research have extended well beyond the detection methods described above. Models and forecasts of HABs are rapidly advancing so that there are now well-developed biological models linked with general circulation models to recreate distributions of HABs in several environments. This is generally a difficult task, as the growth of often sparse harmful species into sizable portions of a mixed phytoplankton assemblage implies an intimated quantifiable understanding of all processes impacting growth rate of the harmful taxon and its competitive neighbors.

There are several general circulation models for the Gulf of Maine that have been linked to population-specific models for the PSP-producing dinoflagellate *Alexandrium*, with the most advanced housed at the Woods Hole Oceanographic Institution [156]. By coupling currents inherent to the basin with river flows, meteorological forcing, excystment, and net growth estimations, populations of *Alexandrium* are formed and transported in the western portion of the Gulf of Maine. A comparison of model results and field observations yields good correlations, expanding prospects for development of early-warning capabilities for expected intoxication of shellfish populations inshore and in the offshore Georges Bank region.

Other efforts are also under development in several other geographic areas with different HAB taxa. Off Florida, Walsh and colleagues [187] have been developing a three dimensional biophysical model for *Karenia brevis*, using an approach similar to that in the north-eastern United States, tying required net growth rates to regional circulation to ensure bloom densities are estimated for the western Florida shelf. In contrast to the Gulf of Maine-*Alexandrium* complex, however, nutrients are quite dilute and there is no known cyst (resting stage) for *K. brevis* to ensure reinoculation of surface waters with vegetative, reproductive populations. This model is still in development, so practical application is some time in the future.

A simple model has been devised for the Gulf of Mexico for projecting or forecasting *K. brevis* trajectories and therefore possible landfall sites in the region. A *K. brevis* algorithm has been identified for the eastern Gulf of Mexico, which permits location of the red-tide organism in SeaWiFS images from the area. Using predicted wind fields, surface *K. brevis* populations are forecast over several days and broadcast as bulletins to a user community responsible for monitoring and safeguarding public resources in the Gulf [158]. Although restricted to surface detectable populations, the forecasts have been remarkably successful, resulting in continued refinement and wider application around the Gulf of Mexico.

Other promising applications are near. Coastal upwelling-wind driven HAB models are well-developed for France, Spain, Portugal, and South Africa and are actively used for research in these areas. Transformation and application as tools for coastal monitoring still remain to be completed. Similarly, wind-induced intrusions of HAB-rich coastal waters have been modeled in southwestern Ireland [118] and successes of recently deployed inexpensive thermistors in Irish bays indicate that these models may soon be refined as general meteorologically- and tidally-forced HAB models with easily traced temperatures as surrogates for HAB intrusions. Further, the Gulf of Maine modeling approach for cross-shelf transport and circulation is being applied to the west coast of Ireland, in the hope that models developed in one environment might be applied to other systems with less investment than required to develop a completely new model. This cross-system transfer of models is integral to future international cooperation, as in programs like GEOHAB (Global Ecology and Oceanography of Harmful Algal Blooms).

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