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REVIEW PAPER

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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

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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

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].

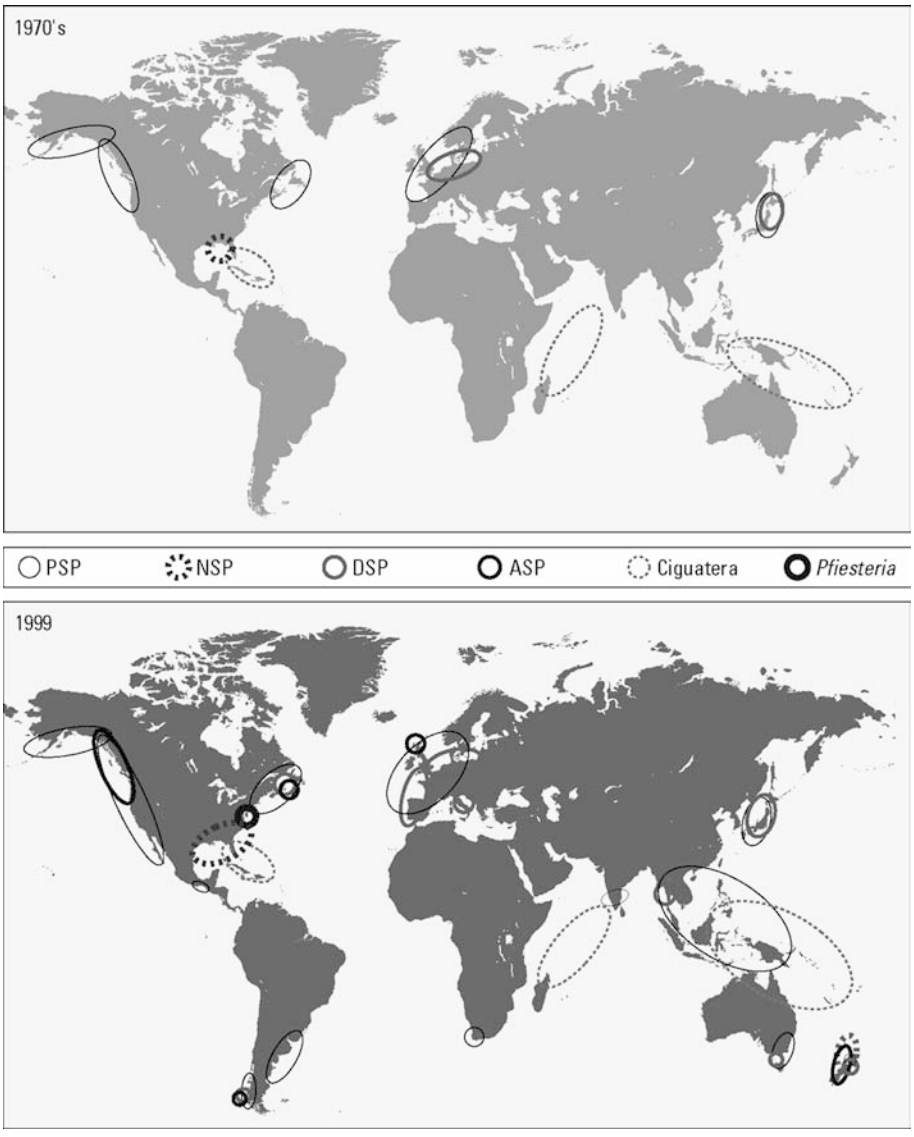

Fig. 1 Global distribution of harmful algal bloom (HAB) toxins and toxicities (from [178])

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,

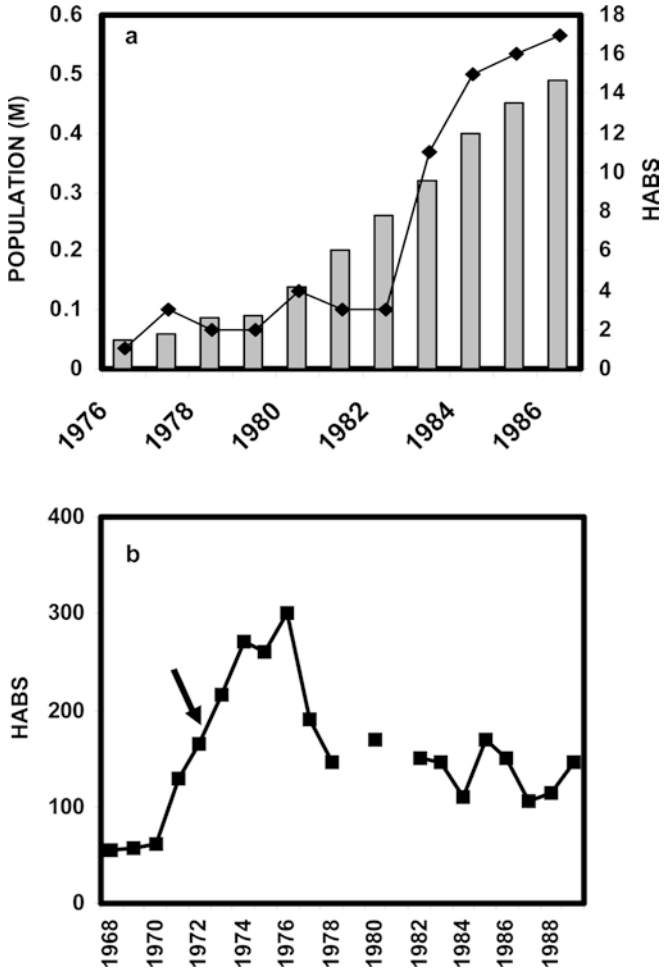

Fig. 2a, b Anthropogenic-induced HAB in coastal systems. **a** Tolo Harbor, Hong Kong [74]; *bars* population, *line* HABs per year. **b** Seto Inland Sea, Japan [103]; *arrow* implementation of waste reduction practices for the coastal discharges

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,

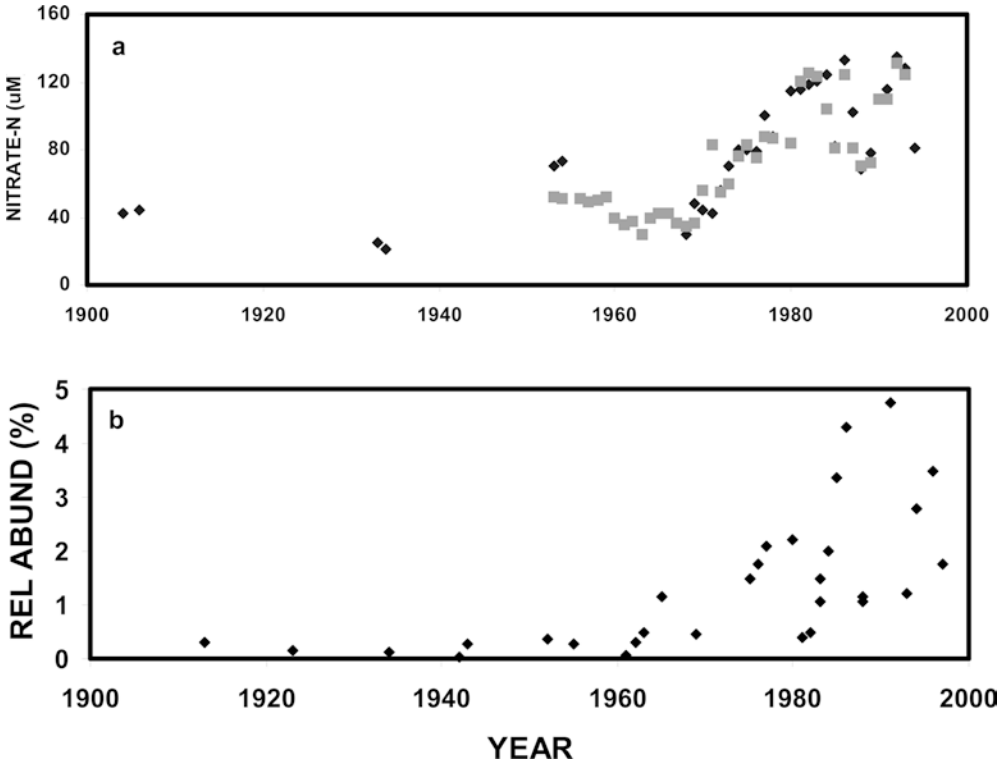

Fig. 3a, b Time series of nutrient and *Pseudo-nitzschia* in the Mississippi delta and shelf. **a** Nitrate-nitrogen increase at two locations in the Mississippi River delta (adapted from [170]); **b** densities of *Pseudo-nitzschia* spp. in surficial sediments of five cores collected on the Mississippi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

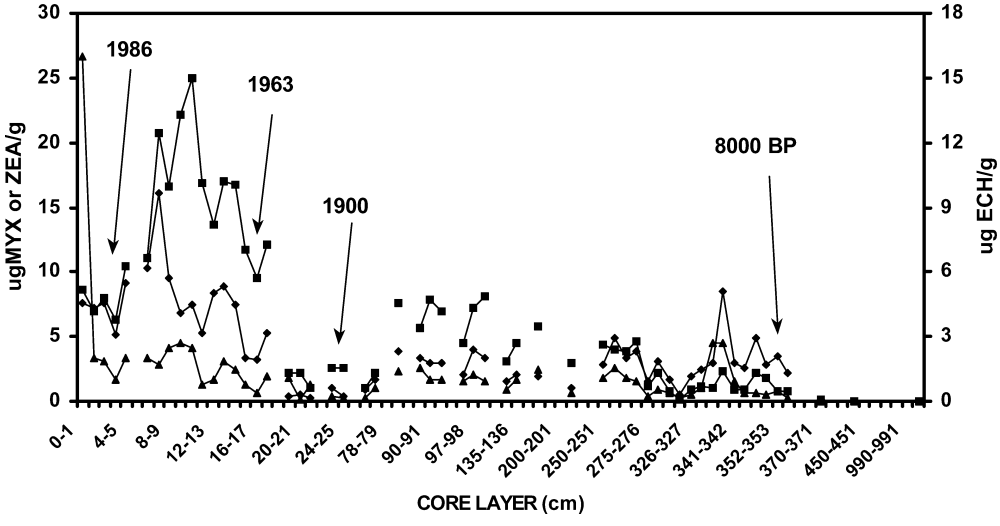

Fig. 4 Historical record of cyanobacteria in the Gotland Deep, Baltic Sea (from [113]). *Filled squares* Myxoxanthophyll, *filled diamonds* zeaxanthin, *filled triangles* echinenone

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

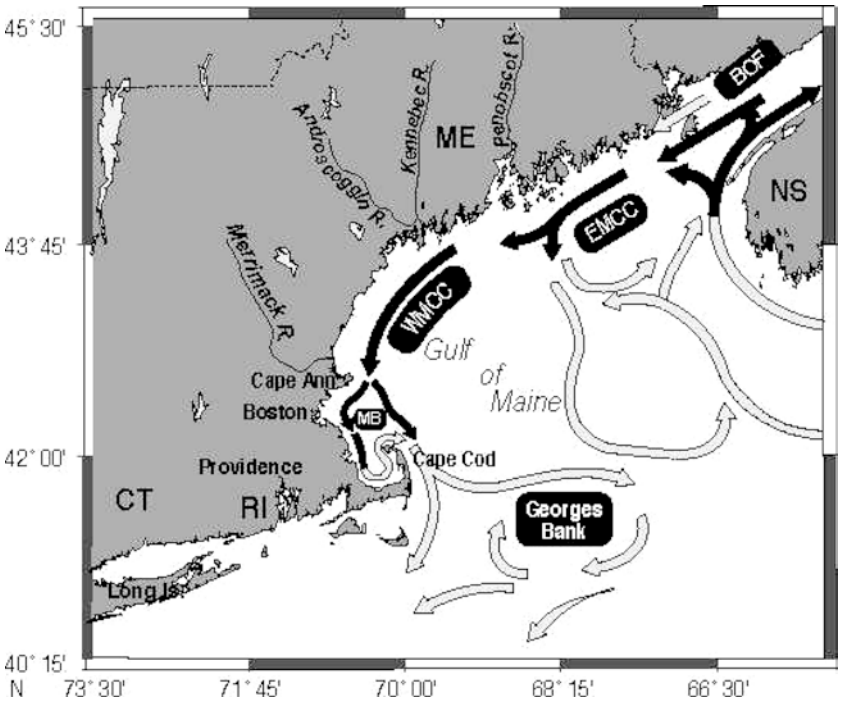

Fig. 5 Circulation in the Gulf of Maine, northeastern United States. *Alexandrium* populations initially entered the basin in 1972 south of Nova Scotia and are now found in the mouth of the Bay of Fundy (*BOF*). Populations are advected south and west in the eastern Maine coastal current (*EMCC*) to (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])

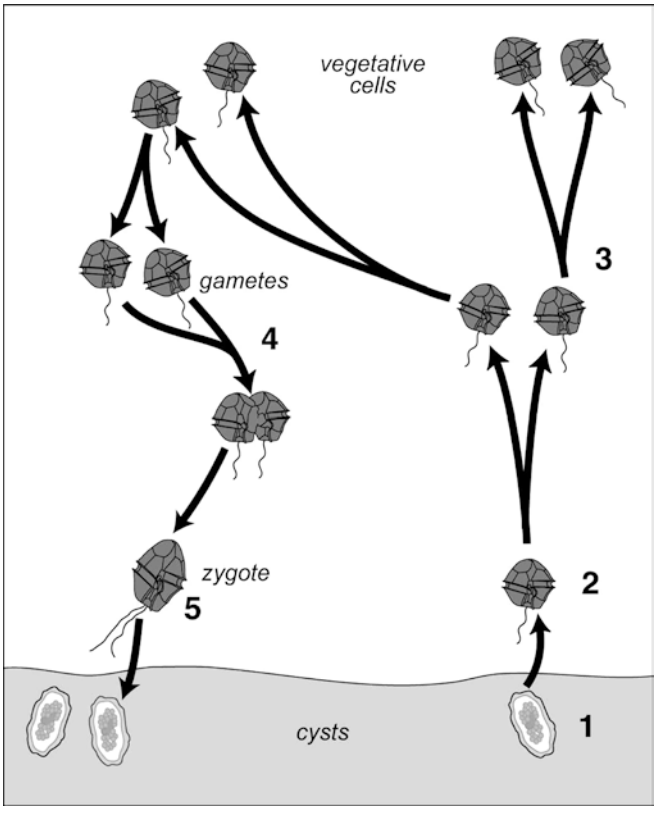

Fig. 6 The life cycle of *Alexandrium*, a dinoflagellate with cyst resting stages (1) that can act as reservoirs for new population growth (adapted from D.M. Anderson, personal communication). The resting stages rupture (excyst) to yield swimming cells (2) which continue to divide to produce a vegetative 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].

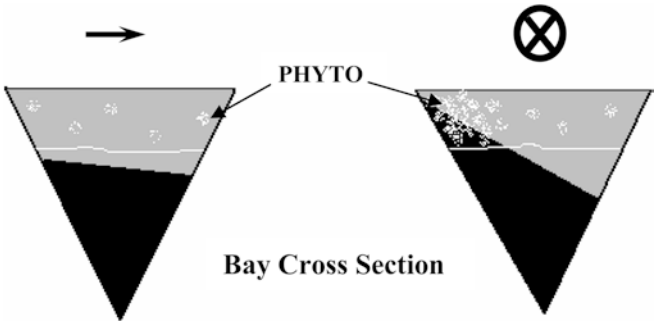

Fig. 7 Cross-bay tilting of the pycnocline (represented as a *line* between gray and black regions) induced by winds shifting from calm to weak westerly winds (→) to strong southerly winds (⊗) along the axis of the Chesapeake Bay. The tilting drives sub-pycnocline remineralized nutrients into the euphotic 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 μg 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 Skaggerak 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,

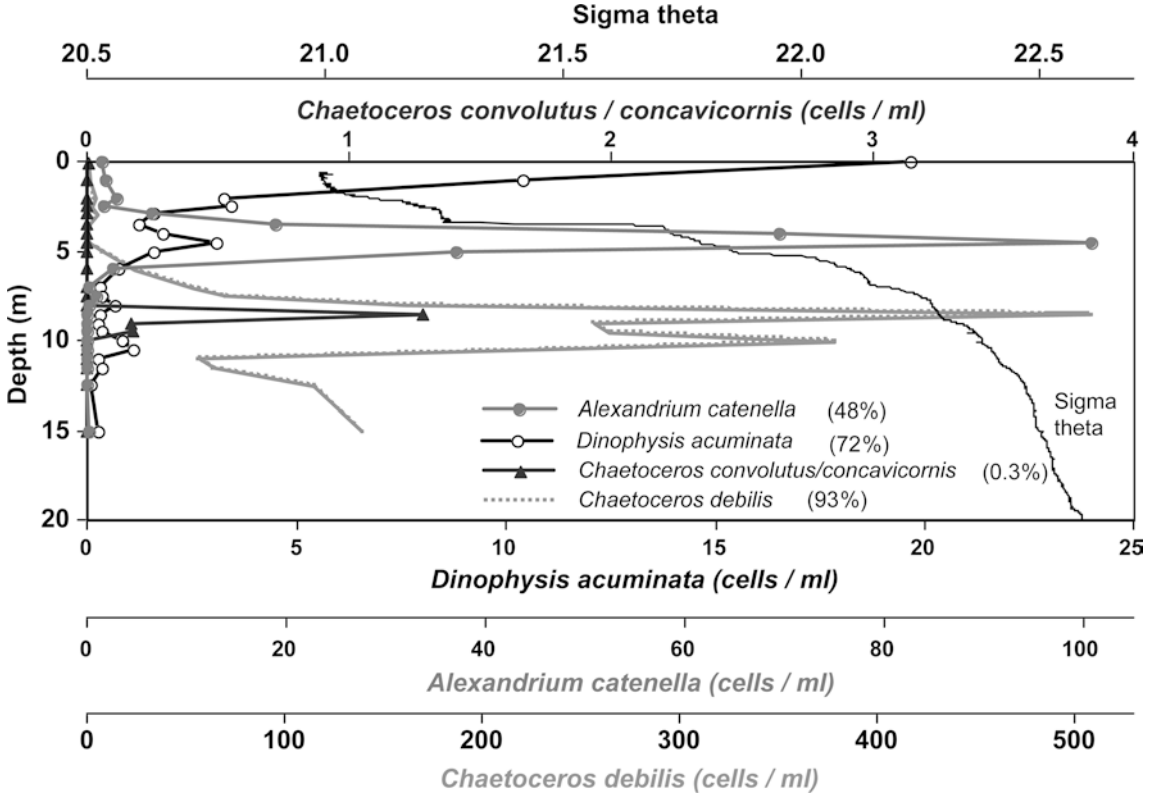

Fig. 8 Thin layers of harmful algae observed in East Sound (Wash.) in August 1997 overlain on vertical density structure. *Alexandrium catenella* and *Dinophysis acuminata* were the dominant net plankton at the depth of their thin layer (making up 72% and 48% of the net plankton, respectively). In contrast, 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 onboard 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

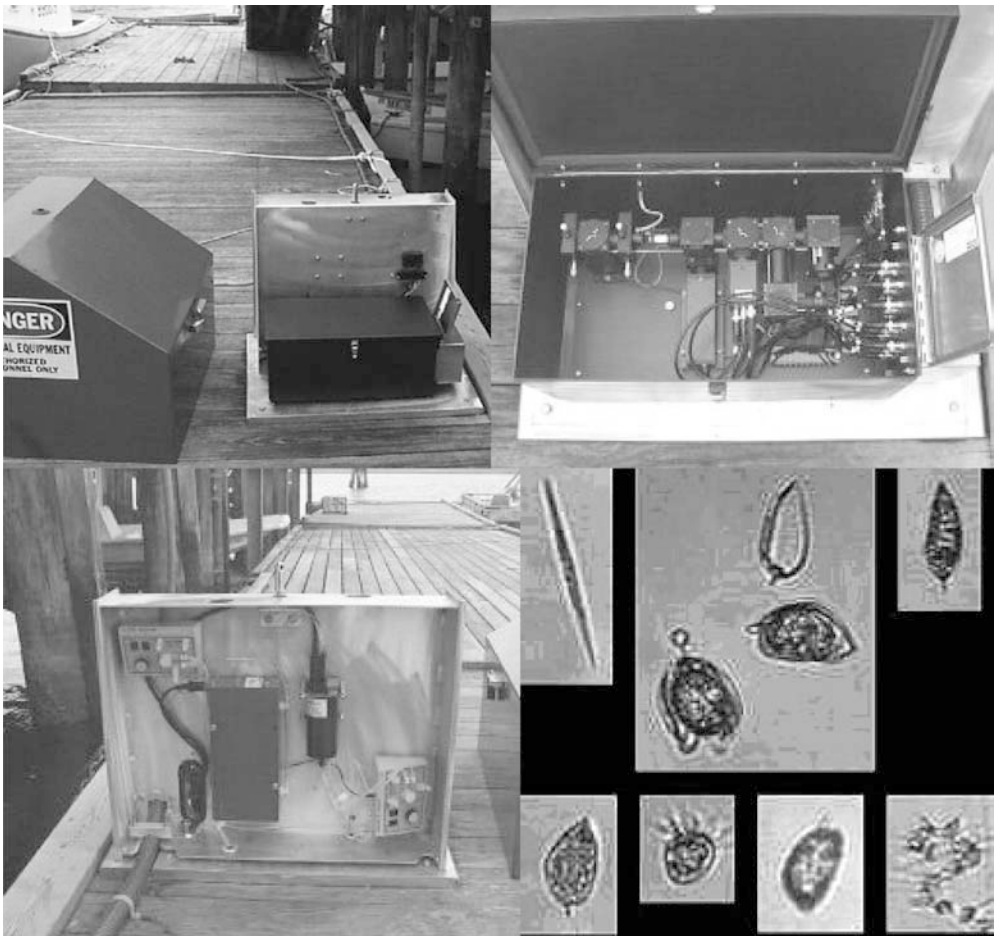

Fig. 9 Docktop flow cytometer and microscope (FLOWCAM) system developed by Fluid Imaging Technologies (FIT) (*top left*). Flow cytometer component (*top right*) and optical sensor component (*bottom left*) are contained in a weatherproof housing that can be equipped with wireless internet access. Sampling 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. Thibaudeau, 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., titered 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 microscropy 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 immunosorbant 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]).

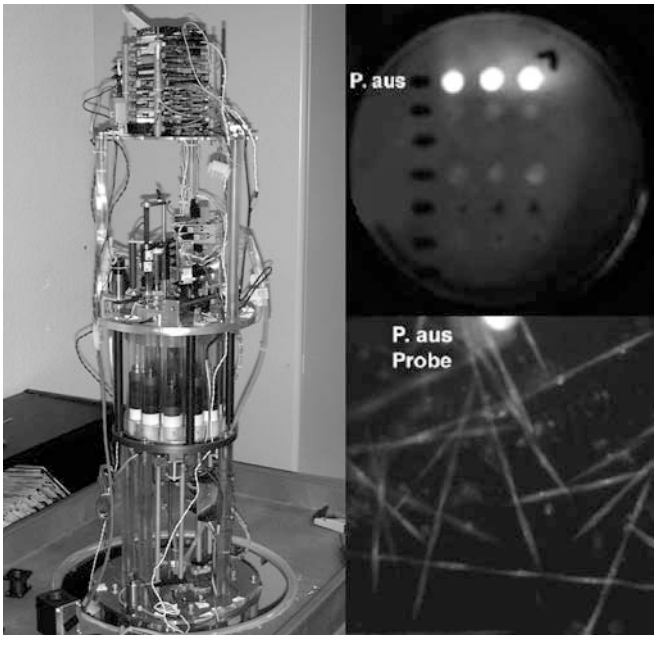

Fig. 10 Environmental sample processor (ESP; *left*) developed by the Monterey Bay Aquarium Research Institute (MBARI) for the automated, in situ conduct of rRNA sandwich hybridization (SH) assays and sample archival capabilities for whole cell (WC) hybridizations. Array photo (*top right*) shows positive 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. austrailis* cells. Photos courtesy of C. Scholin (MBARI)

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

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), azaspiracids (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

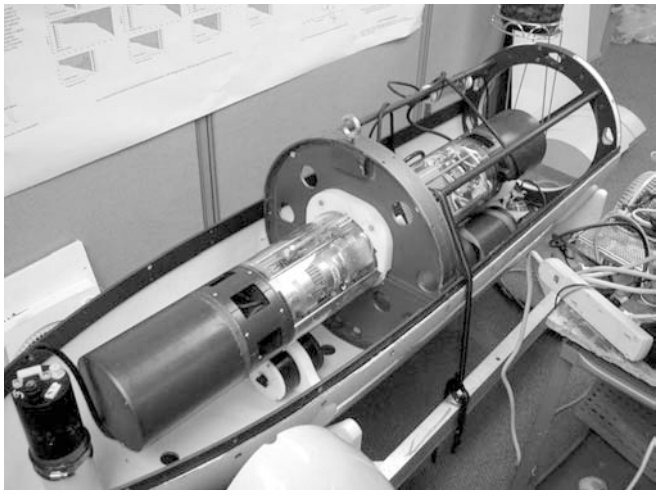

Fig. 12 Underwater quadrupole mass spectrometer contained in an autonomous underwater vehicle. Developed by the Center for Ocean Technology (COT), this first deployable version is aimed at detection and quantification of volatile organic compounds and dissolved gases. Current efforts include development 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 Jellett Biotek (http://www.jellettbiotek.ca/) called the MIST Alert for PSP toxins (to be re-issued as the Jellett Rapid Test for PSP toxins; J. Jellett, 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 Jellett 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.

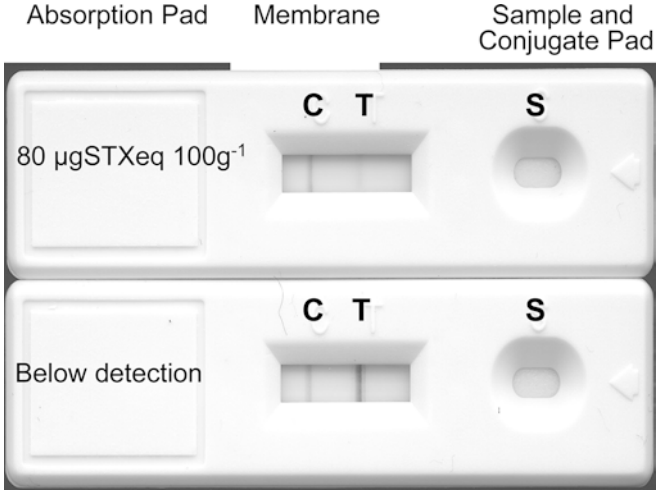

Fig. 13 MIST Alert kit for paralytic shellfish poisons (PSP) toxins (to be re-issued as the Jellett Rapid Test for PSP toxins) developed by Jellett Biotek, showing cassettes with positive (*top*) and negative (*bottom*) test strips. No *T* line indicates that toxin is present in a sample, while a visible *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. Jellett, Jellett 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

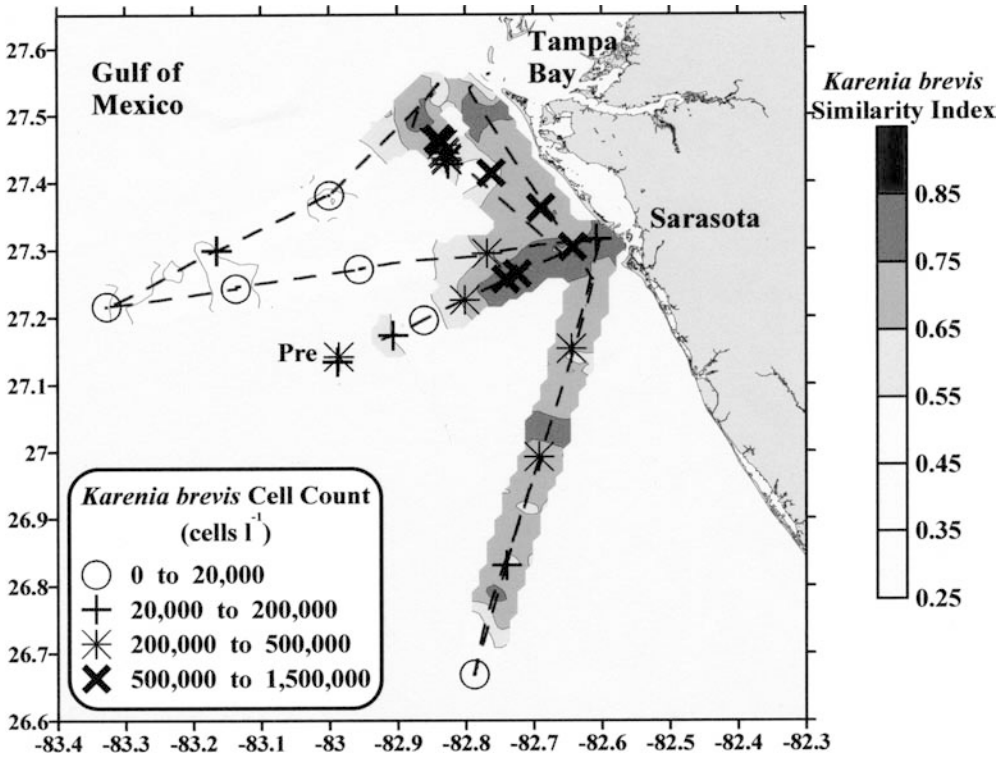

Fig. 14 Contour map of the distribution of the Florida red tide, *Karenia brevis*, based on taxa similarity index determined by the R.V. "Breve Buster" during the 2001 ECOHAB: Florida Process Cruise Leg B. Data were collected on 24 and 25 October 2001. Water was pumped by the ship's seawater system from 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 northeastern 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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References

1. Adachi M, Sako Y, Ishida Y (1996) Identification of the toxic dinoflagellates *Alexandrium catenella* and *A. tamarense* (Dinophyceae) using DNA probes and whole-cell hybridization. J Phycol 32:1049–1052
2. Adams NG, Lesoing M, Trainer VL (2000) Environmental conditions associated with domoic acid in razor clams on the Washington coast. J Shellfish Res 19:1007–1015
3. Aguilera A, López-Rodas V, González-Gil S, Costas E (1995) Use of FITC-labelled lectins to identify dinoflagellate species. In: Lassus P, Arzul G, Erard E, Gentien P, Marcaillou C (eds) Harmful marine algal blooms. Lavoisier, Paris, pp 707–715
4. Amorim A, Moita MT, Oliveira P (2002) Dinoflagellate blooms related to coastal upwelling plumes off Portugal. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 12

5. Anderson DM (1989) Toxic algal blooms and red tides: a global perspective. In: Okaichi T, Anderson DM, Nemoto T (eds) Red tides. Biology, environmental science and toxicology. Elsevier, New York, pp 11–16
6. Anderson DM (1994) Red tides. Sci Am 271:52–58
7. Anderson DM (1995) Identification of harmful algal species using molecular probes: an emerging perspective. In: Lassus P, Arzul G, Erard E, Gentien P, Marcaillou C (eds) Harmful marine algal blooms. Lavoisier, Paris, pp 3–13
8. Anderson DM (1997) Bloom dynamics of toxic *Alexandrium* species in the northeastern U.S. Limnol Oceanogr 42:1009–1022
9. Anderson DM, Kulis D, Cosper EM (1990) Immunofluorescent detection of the brown tide organism *Aureococcus anophagefferens*. In: Cosper EM, Bricelj VM, Carpenter EJ (eds) Novel phytoplankton blooms: causes and impacts of recurrent brown tides and other unusual blooms. Springer, Berlin Heidelberg New York, pp 213–228
10. Anderson DM, Kulis DM, Keafer BA, Sullivan JJ, Hall S, Lee C (1990) Dynamics and physiology of saxitoxin production by the dinoflagellates *Alexandrium* spp. Mar Biol 104:511–524
11. Anderson DM, Keafer BA, Kulis DM, Waters RM, Nuzzi R (1993) An immunofluorescent survey of the brown tide chrysophyte *Aureococcus anophagefferens* along the northeast coast of the United States. J Plankton Res 15:563–580
12. Anderson DM, Kulis DM, Keafer BA, Berdalet E (1999) Detection of the toxic dinoflagellate *Alexandrium fundyense* (Dinophyceae) with oligonucleotide and antibody probes: variability in labeling intensity with physiological condition. J Phycol 35:870–883
13. Anderson DM, Glibert PM, Burkholder JM (2002) Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. Estuaries 25: 704–726
14. Anderson DM, Keafer BA, Kulis DM, Connell L, Scholin CA (2002) Application of molecular probes in studies of *Alexandrium* in the Gulf of Maine: success and problem areas. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 14
15. AOAC (1991) Domoic acid in mussels, liquid chromatographic method, first action (1991). Official methods of analysis. Association of Official Analytical Chemists, Washington, D.C., section 991.26
16. Bargu S, Powell CL, Coale SL, Busman M, Doucette GJ, Silver MJ (2002) Krill: a potential vector for domoic acid in marine food webs. Mar Ecol Prog Ser 237:209–216
17. Bates SS (1998) Ecophysiology and metabolism of ASP toxin production. In: Anderson DM, Cembella AD, Hallegraeff GM (eds) Physiological ecology of harmful algal blooms. NATO ASI series, vol G41, Springer, Berlin Heidelberg New York, pp 405–426
18. Bates SS, Leger C, Keafer BA, Anderson DM (1993) Discrimination between domoic-acid-producing and non-toxic forms of the diatom *Pseudonitzschia pungens* using immunofluorescence. Mar Ecol Prog Ser 100:185–95
19. Beardsley R, Butman B, Geyer R, Smith P (1997) Physical oceanography of the Gulf of Maine: an update. In: Wallace G, Braasch E (eds) Proceedings of the Gulf of Maine ecosystem dynamics scientific symposium and workshop, RARGOM Report 97-1. Hanover, N.H., pp 39–52
20. Belgrano A, Lindahl O, Hernroth B (1999) North Atlantic Oscillation primary productivity and toxic phytoplankton in the Gullmar Fjord, Sweden (1985–1996). Proc R Soc Lond B 266:425–430
21. Bird P, Hewson I, Watkinson A, Dennison W (2000) Effects of increased nutrients on ciguatera associated dinoflagellates of the Great Barrier Reef. Abstract, 9th International Harmful Alagl Blooms Conference, Hobart, Tasmania
22. Bissett WP, Schofield O, Glenn S, Cullen JJ, Miller WL, Plueddemann AJ, Mobley CD (2001) Resolving the impacts and feedbacks of ocean optics on upper ocean ecology. Oceanography 14:30–49
23. Bodenau N (1993) Microbial blooms in the Romanian area of the Black Sea and contemporary eutrophication conditiones. In: Smayda TJ, Shimizu Y (eds) Toxic phytoplankton blooms in the sea., Elsevier, New York, pp 203–209
24. Bolch CJS (2001) PCR protocols for genetic identification of dinoflagellates directly from single cysts and plankton cells. Phycologia 40:162–167
25. Bowers HA, Tengs T, Glasgow HB Jr, Burkholder JM, Rublee PA, Oldach DW (2000) Development of real-time PCR assays for rapid detection of *Pfiesteria piscicida* and related dinoflagellates. Appl Environ Microbiol 66:4641–4648
26. Burkholder JM, Glasgow HB Jr (1997) *Pfiesteria piscicida* and other *Pfiesteria*-like dinoflagellates: behavior, impacts, and environmental controls. Limnol Oceanogr 42:1052–1075
27. Capone DG, Subramaniam A, Montoya JP, Voss M, Humborg C, Johansen AM, Siefert RL, Carpenter EJ (1998) An extensive bloom of the N_2-fixing cyanobacterium *Trichodesmium erythraeum* in the central Arabian Sea. Mar Ecol Prog Ser 172:281–292
28. Carder KL, Steward RG (1985) A remote-sensing reflectance model of a red-tide dinoflagellate off west Florida. Limnol Oceanogr 30:286–298
29. Carmichael WW (2001) Health effects of toxin-producing cyanobacteria: the CyanoHABs. Human Ecol Risk Assess 7:1393–1407
30. Caron DA, Dennett MR, Moran DM, Schaffner RA, Lonsdale DJ, Gobler CJ, Nuzzi R, McLean TI (2003) Development and application of a monoclonal antibody technique for counting *Aureococcus anophagefferens*, an alga causing recurrent brown tides in the mid-Atlantic United States. Appl Environ Microbiol (in press)
31. Carreto JI, Montoya NG, Colleoni ADC, Akselman R (1998) *Alexandrium tamarense* blooms and shellfish toxicity in the Argentine Sea: a retrospective view. In: Reguera B, Blanco J, Fernández ML, Wyatt T (eds) Harmful algae. UNESCO, Vigo, pp 131–134
32. Cembella AD, Doucette GJ, Garthwaite I (2003) In vitro assays for phycotoxins. In: Hallegraeff GM, Anderson DM, Cembella AD (eds) Manual on harmful marine microalgae, 2nd edn. IOC-UNESCO, Paris (in press)
33. Chang FH (1999) *Gymnodinium brevisulcatum* sp. nov. (Gymnodiniales, Dinophyceae), a new species isolated during the 1998 summer toxic bloom in Wellington Harbor, New Zealand. Phycologia 38:377–384
34. Cho ES, Hur HJ, Byun HS, Lee SG, Rhodes LL, Jeong CS, Park JG (2002) Monthly monitoring of domoic acid producer *Pseudo-nitzschia multiseries* (Hasle) Hasle using species-specific DNA probes and WGA lectins and abundance of *Pseudo-nitzschia* species (Bacillariophyceae) from Chinhae Bay, Korea. Bot Mar 45:364–372
35. Coyne KJ, Hutchins DA, Hare CE, Cary SC (2001) Assessing temporal and spatial variability in *Pfiesteria piscicida* distributions using molecular probing techniques. Aquat Microb Ecol 24:275–285
36. Cuadrado MMS, Diaz F, Fdez-Riverola F, Corchado JM, Torres JM (2002) Sea surface temperatures of northwest coast of the Iberian Peninsula using AVHRR data in order to detect *Pseudo-nitzschia* spp. blooms. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 63
37. Cullen JJ, Ciotti AM, Davis RF, Lewis MR (1997) Optical detection and assessment of algal blooms. Limnol Oceanogr 42:1223–1239
38. Della Loggia R, Sosa S, Tubaro A (1999) Methodological improvement of the protein phosphatase inhibition assay for the detection of okadaic acid in mussels. Nat Tox 7:387–392
39. Dixon LK, Steidinger KA (2002) *Karenia brevis* bloom dynamics in the eastern Gulf of Mexico with respect to rainfall and riverine flow. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 73
40. Donaghay PL, Van Holliday D, Rines JEB, Sullivan JM, McManus MA(2003) The importance of finescale structure (thin layers) in controlling the dynamics and biodiversity of

plankton populations in stratified coastal waters. In: Kerfoot C (ed) Limnol Oceanogr special issue (in press)
41. Eller G, Medlin L (2002) Molecular probes for the rapid detection of toxin marine microalgae. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 83
42. Estrada M, Camp J (2002) Environmental sustainability: the case of nutrients and harmful algal blooms in the Catalan coast (NW Mediterranean). In: Lipiatou E (ed) Thresholds of environmental sustainability: the case of nutrients. Research in Enclosed Seas, vol 11. EUR 20170, pp 57–58
43. Fernandez ML, Cembella AD (1995) Mammalian bioassays. In: Hallegraeff GM, Anderson DM, Cembella AD (eds) Manual on harmful marine microalgae, IOC-UNESCO, Paris, pp 213–228
44. Ferrão-Filho AS, Domingos P, Azevedo SMFO (2002) Influences of *Microcystis aeruginosa* Kützing bloom on zooplankton population in Jacarepaguá Lagoon (Rio de Janeiro, Brazil). Limnologica 32:295–308
45. Figueras FG, Wyatt T, Alvarez-Salgado XA, Jenkinson IR (1995) Advection, diffusion, and patch development in the Rias Baixas. In: Lassus P, Arzul G, Erard E, Gentien P, Marcaillou C (eds) Harmful marine algal blooms, Lavoisier, Paris, pp 579–584
46. Figueiras FG, Alvarez-Salgado XA, Castro CG, Villarino ML (1998) Accumulation of *Gymnodinium catenatum* Graham cells in western Iberian Shelf waters in response to poleward flowing slope currents. In: Reguera B, Blanco J, Fernandez ML, Wyatt T (eds) Harmful algae. UNESCO, Vigo, Spain, pp 114–117
47. Folke C, Kautsky N, Troell M (1997) Salmon farming in context: response to Black et al. J Environ Manage 50:95–103
48. Fraga S, Bakun A (1993) Global climate change and harmful algal blooms: the example of *Gymnodinium catenatum* on the Galician coast. In: Smayda TJ, Shimizu Y (eds) Toxic phytoplankton blooms in the sea. Elsevier, New York, pp 59–65
49. Fraga S, Anderson DM, Bravo I, Reguera B, Steidinger KA, Yentsch CM (1988) Influence of upwelling relaxation in dinoflagellate and shellfish toxicity in Ria de Vigo, Spain. Estuarine Coastal Shelf Sci 27:349–361
50. Fraga S, Reguera B, Bravo I (1990) *Gymnodinium catenatum* bloom formation in the Spanish Rias. In: Granéli E, Sundstrom B, Edler L, Anderson D (eds) Toxic marine phytoplankton. Elsevier, New York, pp 149–154
51. Franks PJS (1997) Spatial patterns in dense algal blooms. Limnol Oceanogr 42:1297–1305
52. Gentien P, Lunven M, Le Haître M, Duvent JL (1995) In situ depth profiling of particle sizes. Deep Sea Res 42:1297–1312
53. Gentien P, Lazure P, Raffin B (1998) Effect of meteorological conditions in spring on the extent of a *Gymnodinium* cf *nagasakiense* bloom. In: Reguera B, Blanco J, Fernández ML, Wyatt T (eds) Harmful algae, UNESCO, Vigo, pp 200–203
54. Glasgow HB, Springer JJ, Allen CI, Burkholder JM (2002) Utilization of lectin binding assays to differentiate toxic and nontoxic isolates of *Pfiesteria* and other HAB dinoflagellates. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 105
55. Godhe A, Otta SK, Rehnstam-Holm A-S, Karunasagar I, Karunasagar I (2001) Polymerase chain reaction in detection of *Gymnodinium mikimotoi* and *Alexandrium minutum* in field samples from southwest India. Mar Biotechnol 3:152–162
56. Gower JFR (1994) Red tide monitoring using AVHRR HRPT imagery from a local receiver. Remote Sensing Environ 48:309–318
57. Granéli E, Paasche E, Maestrini SY (1993) Three years after the *Chrysochromulina polylepis* bloom in Scandinavian waters in 1988: some conclusions of recent research and monitoring. In: Smayda TJ, Shimizu Y (eds) Toxic phytoplankton blooms in the sea. Elsevier, New York, pp 23–32
58. Granéli E, Gisselson L-A, Carlsson P, Pallon J (2002) *Dinophysis* blooms in the deep euphotic zone of the Baltic Sea: do they grow in the dark? Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 113
59. Grattan LM, Oldach D, Morris JG (2001) Human health risks of exposure to *Pfiesteria piscicida*. Bioscience 51:853–857
60. Haas LW, Hastings SJ, Webb KL (1981) Phytoplankton response to a stratification-mixing cycle in the York River estuary during late summer. In: Neilson BJ, Cronin LE (eds) Estuaries and nutrients. Humana Press, Clifton, N.J., pp 619–636
61. Hales S, Weinstein P, Woodward A (1999) Ciguatera (fish poisoning), El Niño, and Pacific sea surface temperatures. Ecosyst Health 5:20–25
62. Hall S (1999) Volunteer phytoplankton program. In: Martin JL, Haya K (eds) Proceedings of the Sixth Canadian Workshop on Harmful Marine Algae. Can Tech Rep Fish Aquat Sci 2261, p 30
63. Hallegraeff GM, Anderson DM, Cembella AD (eds) (2003) Manual on harmful marine microalgae, 2nd edn. Monographs on Oceanographic Methodology, vol 11. IOC-UNESCO, Paris
64. Hauxwell J, Cebrian J, Furlong C, Valiela I (2000) Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. Ecology 82:1007–1022
65. Hoagland P, Anderson DM, Kaoru Y, White AW (2002) The economic effects of harmful algal blooms in the United States: estimates, assessment issues, and information needs. Estuaries 25:819–837
66. Holligan PM (1979) Dinoflagellate blooms associated with tidal fronts around the British Isles. In: Taylor DL, Seliger HH (eds) Toxic dinoflagellate blooms. Elsevier, New York, pp 249–256
67. Humborg C, Conley DJ, Rahm L, Wulff F, Cociasu A, Ittekkot V (2000) Silicon retention in river basins: far-reaching effects on biogeochemistry and aquatic food webs in coastal marine environments. Ambio 29:45–50
68. Johnsen G, Samset O, Granskog L, Sakshaug E (1994) In vivo absorption characteristics in 10 classes of bloom-forming phytoplankton: taxonomic characteristics and responses to photoadaptation by means of discriminant and HPLC analysis. Mar Ecol Prog Ser 105:149–157
69. Kahru M, Horstmann U, Rud O (1994) Satellite detection of increased cyanobacteria blooms in the Baltic Sea: natural fluctuation or ecosystem change? Ambio 23:469–472
70. Kirkpatrick GJ, Millie DF, Moline MA, Schofield OM (2000) Optical discrimination of a phytoplankton species in natural mixed populations. Limnol Oceanogr 45:467–471
71. Kononen K, Kuparinen J, Mäkelä K, Laanemets J, Pavelson J, Nõmmann S (1996) Initiation of cyanobacterial blooms in a frontal region at the entrance to the Gulf of Finland, Baltic Sea. Limnol Oceanogr 41:98–112
72. Kremp A, Anderson DM (2002) The use of lectins to detect life cycle stages in two species of cyst forming dinoflagellates. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 156
73. Kudela RM, Cochlan W, Roberts A (2002) Spatial and temporal patterns of *Pseudo-nitzschia* spp. in central California related to regional oceanography. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 157
74. Lam CWY, Ho KC (1989) Red tides in Tolo Harbor, Hong Kong. In: Okaichi T, Anderson DM, Nemoto T (eds) Red tides. biology, environmental science and toxicology. Elsevier, New York, pp 49–52
75. Landsberg JH (2002) The effects of harmful algal blooms on aquatic organisms. Rev Fish Sci 10:1–113
76. Lapointe BE (1997) Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamaica and southeast Florida. Limnol Oceanogr 42:1119–1131
77. Larsson U, Elmgren R, Wulff E (1985) Eutrophication and the Baltic Sea. Ambio 14:9–14
78. Laycock MV, Jellett JF, Belland ER, Bishop PC, Thériault BL, Russell-Tattrie AL, Quilliam MA, Cembella AD, Richards RC (2001) Mist Alert™: a rapid assay for paralytic shellfish poisoning toxins. In: Hallegraeff GM, Blackburn SI, Bolch CJ, Lewis RJ (eds) Harmful algal blooms 2000. IOC-UNESCO, Paris, pp 254–256

79. Lin S, Carpenter EJ (1996) An empirical protocol for whole-cell immunofluorescence of marine phytoplankton. J Phycol 32:1083–1094
80. Lindahl O (1993) Hydrodynamical processes: a trigger and source for flagellate blooms along the Skagerrak coasts? In: Smayda TJ, Shimizu Y (eds) Toxic phytoplankton blooms in the sea, Elsevier, New York, pp 775–781
81. Litaker W, Sundseth R, Wojciechowski M, Bonaventura C, Henkens R, Tester P (2000) Electrochemical detection of DNA or RNA from harmful algal bloom species. In: Hallegraeff GM, Blackburn SI, Bolch CJ, Lewis RJ (eds) Harmful algal blooms 2000. IOC-UNESCO, Paris, pp 242–245
82. Loftus ME, Subba Rao DV, Seliger HH (1972) Growth and dissipation of phytoplankton in Chesapeake Bay. I. Response to a large pulse of rainfall. Chesapeake Sci 13:282–299
83. Lohrenz SE, Fahnenstiel GL, Kirkpatrick GJ, Carroll CL, Kelly KA (1999) Microphotometric assessment of spectral absorption and its potential application for characterization of harmful algal species. J Phycol 35:1438–1446
84. Loyer S, Gentien P, Lazure P, Menesguen A (2002) Modelling (3-D) of *Karenia mikimotoi* in the Bay of Biscay, French coast: vertical, spatial, and temporal 6-years validation. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 176
85. Lukatelich RJ, McComb AJ (1986) Nutrient levels and the development of diatom and blue-green algal blooms in a shallow Australian estuary. J Plankton Res 8:597–618
86. Maclean JL (1989) Indo-Pacific red tides, 1985–1988. Mar Pollut Bull 20:304–310
87. Manger RL, Leja LS, Lee SY, Hungerford JM, Hokama Y, Dickey RW, Granade HR, Lewis R, Yasumoto T, Wekell MM (1995) Detection of sodium channel toxins: directed cytotoxicity assays of purified ciguatoxins, brevetoxins, saxitoxins, and seafood extracts. J Assoc Off Anal Chem Int 78:521–527
88. Marquette CA, Coulet PR, Blum LJ (1999) Semi-automated membrane based chemiluminescent immunosensor for flow injection analysis of okadaic acid in mussels. Anal Chim Acta 398:173–182
89. McMahon T, Raine R, Silke J (1998) Oceanographic control of harmful phytoplankton blooms around southwestern Ireland. In: Reguera B, Blanco J, Fernández ML, Wyatt T (eds) Harmful algae. UNESCO, Vigo, pp 128–130
90. Medlin LK, Kerkmann K, Huljic S, Eller G, Lange M (2002) Application of molecular probes for the detection of harmful algae on DNA-microchips. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 193
91. Reference deleted
92. Miller PE, Scholin CA (1998) Identification and enumeration of cultured and wild *Pseudo-nitzschia* (Bacillariophyceae) using species-specific LSU rRNA-targeted fluorescent probes and filter-based whole cell hybridization. J Phycol 34:371–382
93. Miller PE, Scholin CA (2000) On detection of *Pseudo-nitzschia* (Bacillariophyceae) species using whole cell hybridization: sample fixation and stability. J Phycol 36:238–250
94. Millie DF, Schofield OM, Kirkpatrick GJ, Johnsen G, Tester PA, Vinyard BT (1997) Detection of harmful algal blooms using photopigments and absorption signatures: a case study of the Florida red tide, *Gymnodinium breve*. Limnol Oceanogr 42:1240–1251
95. Millie DF, Schofield OME, Kirkpatrick GJ, Johnsen G, Evens TJ (2002) Using absorbance and fluorescence spectra to discriminate microalgae. Euro J Phycol 37:313–322
96. Moisander PH, Rantajärvi E, Huttunen M, Kononen K (1997) Phytoplankton community in relation to salinity fronts at the entrance to the Gulf of Finland, Baltic Sea. Ophelia 46:187–203
97. Moita MY, da Graca Vilarinho M, Palma AS (1998) On the variability of *Gymnodinium catenatum* Graham blooms in Portugese waters. In: Reguera B, Blanco J, Fernández ML, Wyatt T (eds) Harmful algae. UNESCO, Vigo, pp 118–121
98. Moncheva S, Gotsis-Skretas O, Pagou K, Krastev A (2001) Phytoplankton blooms in Black Sea and Mediterranean coastal ecosystems subjected to anthropogenic eutrophication: similarities and differences. Estuarine Coastal Shelf Sci 53:281–295
99. Muller-Karger FE, Walsh JJ, Evans RH, Meyers MB (1991) On the seasonal phytoplankton concentration and sea surface temperature cycles of the Gulf of Mexico as determined by satellites. J Geophys Res 96:12645–12665
100. Naar J, Weidner A, Baden D (2002) Competitive ELISA an accurate, quick and effective tool to monitor brevetoxins in environmental and biological samples. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 210
101. Nagasaki K, Uchida A, Ishida Y (1991) A monoclonal antibody which recognizes the cell surface of red tide alga *Gymnodinium nagasakiense*. Nippon Suisan Gakkaishi 57:1211–1214
102. O'Boyle S, Nolan G, Raine R (2001) Harmful phytoplankton events caused by variability in the Irish Coastal Current along the west of Ireland. In: Hallegraeff GM, Blackburn SI, Bolch CJ, Lewis RJ (eds) Harmful Algal Blooms 2000, IOC-UNESCO, Paris, pp 143–148
103. Okaichi T (1989) Red tide problems in the Seto Inland Sea, Japan. In: Okaichi T, Anderson DM, Nemoto T (eds) Red tides. Biology, environmental science and toxicology. Elsevier, New York, pp 137–144
104. Oshima Y, Blackburn SI, Hallegraeff GM (1993) Comparative study on paralytic shellfish toxin profiles of the dinoflagellate *Gymnodinium catenatum* from three different countries. Mar Biol 116:471–476
105. Ouellette AJA, Boyer GL, Wilhelm SW (2002) Quantitative PCR and sequence analysis for determination of microbial community structure and the detection of toxic *Microcystis* in Lake Erie. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 222
106. Pan Y, Parsons ML, Busman M, Moeller PDR, Dortch Q, Powell CL, Doucette GJ (2001) *Pseudo-nitzschia* sp. cf. *pseudodelicatissima*—a confirmed producer of domoic acid from the northern Gulf of Mexico. Mar Ecol Prog Ser 220:83–92
107. Parsons ML, Scholin CA, Miller PE, Doucette GJ, Powell CL, Fryxell GA, Dortch Q, Soniat T (1999) *Pseudo-nitzschia* species (Bacillariophyceae) in Louisiana coastal waters: molecular probe field trials, genetic variability, and domoic acid analyses. J Phycol 35:1368–1378
108. Parsons ML, Dortch Q, Turner RE (2002) Sedimentological evidence of an increase in *Pseudo-nitzschia* (Bacillariophyceae) abundance in response to coastal eutrophication. Limnol Oceanogr 47:551–558
109. Penna A, Magnani M (1999) Identification of *Alexandrium* (Dinophyceae) species using PCR and rDNA-targeted probes. J Phycol 35:615–621
110. Peperzak L, Sandee B, Scholin C, Miller P, van Nieuwerburgh L (2000) Application and flow cytometric detection of antibody and rRNA probes to *Gymnodinium mikimotoi* (Dinophyceae) and *Pseudo-nitzschia multiseries* (Bacillariophyceae). In: Hallegraeff GM, Blackburn SI, Bolch CJ, Lewis RJ (eds) Harmful algal blooms 2000. IOC-UNESCO, Paris, pp 206–209
111. Peperzak L, Vrieling EG, Sandee B, Rutten T (2000) Immuno flow cytometry in marine phytoplankton research. Sci Mar 64:165–181
112. Pitcher GC, Roesler CS, Nelson G (2002) The importance of surface boundary layer characteristics and advection in the development of red tide in the southern Benguela upwelling system. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 232
113. Poutanen E-L, Nikkilä K (2001) Carotenoid pigments as tracers of cyanobacterial blooms in recent and post-glacial sediments of the Baltic Sea. Ambio 30:179–183
114. Powell CL, Doucette GJ (1999) A receptor binding assay for paralytic shellfish poisoning toxins: recent advances and applications. Nat Tox 7:393–400

115. Powell CL, Ferdin ME, Busman M, Kvitek RG, Doucette GJ (2002) Development of a protocol for determination of domoic acid in the sand crab (*Emerita analoga*): a possible new indicator species. Toxicon 40:481–488
116. Quilliam MA (1996) Liquid chromatography-mass spectrometry of seafood toxins. In: Barcelo D (ed) Applications of LC-MS in environmental science. Elsevier, Amsterdam, pp 415–444
117. Raine R, Joyce B, Richard J, Pazos Y, Moloney M, Jones K, Patching JW (1993) The development of a bloom of the dinoflagellate *Gyrodinium aureolum* (Hulbert) on the southwest Irish coast. ICES J Mar Sci 50:461–469
118. Raine R, Nolan G, Chamberlain T, McDermott G, Silke J (2002) Monitoring and forecasting harmful phytoplankton events in southwestern Ireland. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 239
119. Reguera B, Bravo I, Marcaillou-leBaut C, Masselin P, Fernandez ML, Miguez A, Martinez A (1993) Monitoring of *Dinophysis* spp. and vertical distribution of okadaic acid on mussel rafts in Ria de Pontevedra (NW Spain). In: Smayda TJ, Shimizu Y (eds) Toxic phytoplankton blooms in the sea. Elsevier, New York, pp 553–558
120. Rehnstam-Holm AS, Godhe A, Anderson DM (2002) Molecular studies of *Dinophysis* (Dinophyceae) species from Sweden and North America. Phycologia 41:348–357
121. Rensel JE, Whyte JNC (2003) Finfish mariculture and harmful algal blooms. In: Hallegraeff GM, Anderson DM, Cembella AD (eds) Manual on harmful marine microalgae. Monographs on oceanographic methodology, vol 11, chapter 25. UNESCO, Paris
122. Rhodes L, Scholin C, Garthwaite I, Haywood A, Thomas A (1998) Domoic acid producing *Pseudo-nitzschia* species deduced by whole cell DNA probe-based and immunochemical assays. In: Reguera B, Blanco J, Fernandez ML, Wyatt T (eds) Harmful algae. Xunta de Galicia & IOC-UNESCO, Paris, pp 274–277
123. Rhodes L, Scholin C, Tyrrell J, Adamson J, Todd K (2000) The integration of DNA probes into New Zealand's routine phytoplankton monitoring programmes. In: Hallegraeff GM, Blackburn SI, Bolch CJ, Lewis RJ (eds) Harmful algal blooms 2000. IOC-UNESCO, Paris, pp 429–432
124. Rhodes L, Mackenzie AL, Kaspar HF, Todd KE (2001) Harmful algae and mariculture in New Zealand. ICES J Mar Sci 58:398–403
125. Rhodes L, Haywood A, Adamson J, Scholin C (2002) DNA probes in whole cell format for the detection of *Karenia* species in New Zealand waters. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 242
126. Riegman R, Noordeloos AAM, Cadée GC (1992) *Phaeocystis* blooms and eutrophication of the continental coastal zones of the North Sea. Mar Biol 112:479–484
127. Rines JEB, Donaghay PL, Dekshenieks MM, Sullivan JM, Twardowski MS (2002) Thin layers and camouflage: hidden *Pseudo-nitzschia* spp. (Bacillariophyceae) populations in a fjord in the San Juan Islands, Washington, USA. Mar Ecol Prog Ser 225:123–137
128. Ríos AF, Fraga F, Figueiras FG, Pérez FF (1995) New and regenerated production in relation to the proliferations of diatoms and dinoflagellates in natural conditions. In: Lassus P, Arzul G, Erard E, Gentien P, Marcaillou C (eds) Harmful marine algal blooms. Lavoisier, Paris, pp 663–668
129. Roelke DL, Kennedy CD, Weidemann AD (1999) Use of discriminant and fourth-derivative analyses with high resolution absorption spectra for phytoplankton research: limitations at varied signal to noise ratio and spectral resolution. Gulf Mexico Sci 17:75–86
130. Roesler CS, McLeroy-Etheridge SL (1998) Remote detection of harmful algal blooms. SPIE Ocean Optics XIV 1:117–128
131. Roesler CS, Perry MJ (1995) In situ phytoplankton absorption, fluorescence emission, and particulate backscattering spectra determined from reflectance. J Geophys Res 100:13279–13294
132. Roesler CS, Perry MJ, Carder KL (1989) Modeling in situ phytoplankton absorption from total absorption spectra in productive inland marine waters. Limnol Oceanogr 34:1510–1523
133. Ross NW, Bates SS (1996) Electro-immunoblotting characterization of *Pseudo-nitzschia multiseries* and *P. pungens* antigens recognized by antibodies directed against whole cells. J Appl Phycol 8:51–58
134. Rousseau V, Leynaert A, Daoud N, Lancelot C (2002) Diatom succession, silicification, and silicic acid availability in Belgian coastal waters (southern North Sea). Mar Ecol Prog Ser 236:61–73
135. Rublee PA, Kempton JW, Schaefer EF, Burkholder JM, Glasgow HB, Oldach D (1999) PCR and FISH detection extends the range of *Pfiesteria piscicida* in estuarine waters. Virginia J Sci 50:325–336
136. Rublee PA, Kempton JW, Schaefer EF, Allen C, Harris J, Oldach DW, Bowers H, Tengs T, Burkholder JM, Glasgow HB (2001) Use of molecular probes to assess geographic distribution of *Pfiesteria* species. Environ Health Perspect 109:765–767
137. Saito K, Drgon T, Robledo JAF, Krupatkina DN, Vasta GR (2002) Development of standard and quantitative-competitive PCR-based diagnostic assays for *Pfiesteria piscicida* targeted to the non-transcribed spacer of rDNA. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 248
138. Sako Y, Adachi M, Ishida Y (1993) Preparation and characterization of monoclonal antibodies to *Alexandrium* species. In: Smayda TJ, Shimizu Y (eds) Toxic phytoplankton blooms in the sea. Elsevier, Amsterdam, pp 87–93
139. Sako Y, Murakami T, Adachi M, Uchida A, Ishida Y, Yamaguchi M, Takeuchi T (1996) Detection of the toxic dinoflagellate *Alexandrium* species by flow cytometry using a monoclonal antibody. In: Yasumoto T, Oshima Y, Fukuyo Y (eds) Harmful and toxic algal blooms. IOC-UNESCO, Sendai, pp 463–466
140. Schmidt W, Drews G, Weckesser J, Fromme I, Borowiak D (1980) Characterization of the lipopolysaccharides from eight strains of the cyanobacterium *Synechococcus*. Arch Microbiol 127:209–215
141. Schofield O, Grzymski J, Bissett WP, Kirkpatrick GJ, Millie DF, Moline M, Roesler CS (1999) Optical monitoring and forecasting systems for harmful algal blooms: possibility or pipe dream? J Phycol 35:1477–1496
142. Scholin CA, Buck KR, Britschgi T, Cangelosi G, Chavez FP (1996) Identification of *Pseudo-nitzschia australis* (Bacillariophyceae) using rRNA-targeted probes in whole cell and sandwich hybridization formats. Phycologia 35:190–197
143. Scholin CA, Miller P, Buck K, Chavez F, Harris P, Haydock P, Howard J, Cangelosi G (1997) Detection and quantification of *Pseudo-nitzschia australis* in cultured and natural populations using LSU rRNA-targeted probes. Limnol Oceanogr 42:1265–1272
144. Scholin CA, Marin III R, Miller PE, Doucette GJ, Powell CL, Haydock P, Howard J, Ray J (1999) DNA probes and a receptor-binding assay for detection of *Pseudo-nitzschia* (Bacillariophyceae) species and domoic acid activity in cultured and natural samples. J Phycol 35:1356–1367
145. Scholin CA, Gulland F, Doucette GJ, Benson S, Busman M, Chavez FP, Cordaro J, DeLong R, DeVogelaere A, Harvey J, Haulena M, Lefebrve K, Lipscomb T, Loscutoff S, Lowenstine LJ, Marin R III, Miller PE, McLellan WA, Moeller PDR, Powell CL, Rowles T, Silvagni P, Silver M, Spraker T, Trainer V, VanDolah FM (2000) Mortality of sea lions along the central California coast linked to a toxic diatom bloom. Nature 403:80–84
146. Scholin C, Marin R III, Massion E, Jensen S, Cline D, Roman B, Doucette G (2002) Remote detection of HAB Species using the Environmental Sample Processor (ESP): progress and future directions. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 254

147. Sellner KG, Brownlee DC (1990) Dinoflagellate-microzooplankton interactions in Chesapeake Bay. In: Granéli E, Sundström B, Edler L, Anderson DM (eds) Toxic marine phytoplankton. Elsevier, New York, pp 221–226
148. Sellner KG, Fonda-Umani S (1999) Dinoflagellate blooms and mucilage production. In: Malone TC, Malej A, Harding LW Jr, Smodlaka N, Turner RE (eds) Ecosystems at the land-sea margin: drainage basin to coastal sea. Coastal Estuarine Studies 55, American Geophysical Union, Washington, D.C., pp 173–206
149. Sellner KG, Lacouture RV, Cibik SJ, Brindley A, Brownlee SG (1991) Importance of a winter dinoflagellate-microflagellate bloom in the Patuxent River estuary. Estuarine Coastal Shelf Sci 32:27–42
150. Reference deleted
151. Reference deleted
152. Smayda TJ (1990) Novel and nuisance phytoplankton blooms in the sea: Evidence for a global epidemic. In: Granéli E, Sundström B, Edler L, Anderson DM (eds) Toxic marine phytoplankton, Elsevier, New York, pp 29–40
153. Smith JC, Cormier R, Worms J, Bird CJ, Quilliam MA, Pocklington R, Angus R, Hanic L (1990) Toxic blooms of the domoic acid containing diatom *Nitzschia pungens* in the Cardigan River, Prince Edward Island. In: Granéli E, Sundström B, Edler L, Anderson DM (eds) Toxic marine phytoplankton. Elsevier, New York, pp 227–232
154. Sournia A (1978) Phytoplankton manual. Monographs on oceanographic methodology, vol 6. UNESCO, Paris, pp 337
155. Steidinger KA, Haddad KD (1981) Biologic and hydrographic aspects of red tides. Bioscience 31:814–819
156. Stock CA, McGillicuddy DJ Jr, Signell RP, Anderson DM (2002) A quantitative modeling study of the initiation and development of *Alexandrium fundyense* blooms in the western Gulf of Maine. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 270
157. Stumpf RP, Tyler MA (1988) Satellite detection of bloom and pigment distributions in estuaries. Remote Sensing Environ 24:385–404
158. Stumpf RP, Culver ME, Tester PA, Tomlinson M, Kirkpatrick GJ, Pederson BA, Truby E, Ransibrahmanakul V, Soracco M (2003) Monitoring *Karenia brevis* blooms in the Gulf of Mexico using satellite ocean color imagery and other data. Harmful Algae 2:147–160
159. Suarez-Isla BA, Sierralta J, Compagnon D, Fonseca M, Loyola H (1997) Real life strategies for red tide management in Chile: systematic application of receptor-based radioassays for SPS and ASP toxins in international seafood safety programs. Abstract, 8th International Conference on Harmful Algae, Vigo, Spain
160. Suzuki MT, Taylor LT, DeLong EF (2000) Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5′-nuclease assays. Appl Environ Microbiol 66:4605–4614
161. Szmant AM (2002) Nutrient enrichment on coral reefs: is it a major cause of coral reef decline? Estuaries 25:743–766
162. Taylor FJR, Horner RA (1994) Red tides and other problems with harmful algal blooms in Pacific Northwest coastal waters. In: Review of the Marine Environment and Biota of Strait of Georgia, Puget Sound and Juan de Fuca Strait. Can J Fish Aquat Sci Tech Rep 1948, BN Typographics West, Victoria, B.C., pp 175–186
163. Tester PA, Fowler PK (1990) Brevetoxin contamination of *Mercenaria mercenaria* and *Crassostrea virginica*: A management issue. In: Granéli E, Sundström B, Edler L, Anderson DM (eds) Toxic marine phytoplankton. Elsevier, New York, pp 499–503
164. Tester PA, Stumpf RP (1998) Phytoplankton blooms and remote sensing: what is the potential for early warning. J Shellfish Res 17:1469–1471
165. Tester PA, Stumpf RP, Vukovich FM, Fowler PK, Turner JT (1991) An expatriate red tide bloom: transport, distribution and persistence. Limnol Oceanogr 36:1053–1061
166. Towers NR, Garthwaite I (2001) Biological assay and detection methods for marine "shellfish" toxins. In: Massaro E (ed) Neurotoxicology handbook, vol 1., Humana Press, London, pp 269–291
167. Trainer VL, Adams NG, Bill BD, Anulacion BF, Wekell JC (1998) Concentration and dispersal of a *Pseudo-nitzschia* bloom in Penn Cove, Washington, USA. Nat Toxins 6:113–126
168. Trainer VL, Adams NG, Bill BD, Stehr CM, Wekell JC, Moeller P, Busman M, Woodruff D (2000) Domoic acid production near California coastal upwelling zones, June (1998). Limnol Oceanogr 45:1818–1833
169. Trainer VL, Hickey BM, Horner RA (2002) Biological and physical dynamics of domoic acid production off the Washington coast. Limnol Oceanogr 47:1438–1446
170. Turner RE, Rabalais NN (1991) Changes in the Mississippi River this century: implications for coastal food webs. Bioscience 41:140–147
171. Tyler MA (1984) Dye tracing of a subsurface chlorophyll maximum of a red-tide dinoflagellate to surface frontal regions. Mar Biol 78:285–300
172. Tyler MA, Heinbokel JF (1985) Cycles of red water and encystment of *Gymnodinium pseudopalustre* in the Chesapeake Bay: effects of hydrography and grazing. In: Anderson DM, White AW, Baden DG (eds) Toxic dinoflagellates. Elsevier, New York, pp 213–218
173. Tyler MA, Seliger HH (1978) Annual subsurface transport of a red tide dinoflagellate to its bloom area: water circulation patterns and organism distributions in the Chesapeake Bay. Limnol Oceanogr 23:227–246
174. Tyler MA, Coats DW, Anderson DM (1982) Encystment in a dynamic environment: deposition of dinoflagellate cysts by a frontal convergence. Mar Ecol Prog Ser 7:163–178
175. Tyrrell JV, Bergquist PR, Bergquist PL, Scholin CA (2001) Detection and enumeration of *Heterosigma akashiwo* and *Fibrocapsa japonica* (Raphidophyceae) using rRNA-targeted oligonucleotide probes. Phycologia 40:457–467
176. Uchida A, Nagasaki K, Hiroishi S, Ishida Y (1989) The application of monoclonal antibodies to an identification of *Chattonella marina* and *Chattonella antiqua*. Nippon Suisan Gakkaishi 55:721–725
177. Valiela I, McClelland J, Hauxwell J, Behr PJ, Hersh D, Foreman K (1997) Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. Limnol Oceanogr 42:1105–1118
178. Van Dolah FM (2000) Marine algal toxins: origins, health effects, and their increased occurrence. Environ Health Perspect 108S:133–141
179. Van Dolah FM (2000) Diversity of marine and freshwater algal toxins. In: Botana LM (ed) Seafood and freshwater toxins: pharmacology, physiology, and detection. Dekker, New York, pp 19–43
180. Van Dolah FM, Ramsdell JS (2001) Review and assessment of in vitro detection methods for algal toxins. J Assoc Off Anal Chem Int 84:1617–1625
181. Van Dolah FM, Finley EL, Haynes BL, Doucette GJ, Moeller PD, Ramsdell JS (1994) Development of rapid and sensitive high throughput assays for marine phycotoxins. Nat Toxins 2:189–196
182. Vrieling EG, Anderson DM (1996) Immunofluorescence in phytoplankton research—application and potential. J Phycol 32:1–16
183. Vrieling EG, Peperzak L, Gieskes WWC, Veenhuis M (1994) Detection of the ichthyotoxic dinoflagellate *Gyrodinium* cf. *aureolum* and morphologically related *Gymnodinium* species using monoclonal antibodies: a specific immunological tool. Mar Ecol Prog Ser 103:165–174
184. Vrieling EG, Gieskes WWC, Rademaker RWM, Vriezekolk G, Peperzak L, Veenhuis M (1995) Flow cytometric identification of the ichthyotoxic dinoflagellate *Gyrodinium aureolum* in the central North Sea. In: Lassus P, Arzul G, Erard E, Gentien P, Marcaillou C (eds) Harmful marine algal blooms. Lavoisier, Paris, pp 743–748

185. Vrieling EG, Vriezekolk G, Gieskes WWC, Veenhuis M, Harder W (1996) Immuno-flow cytometric identification and enumeration of the ichthyotoxic dinolfagellate *Gyrodinium aureolum* Hulburt in artificially mixed algal populations. J Plankton Res 18:1503–1512
186. Walsh JJ, Steidinger KA (2001) Saharan dust and Florida red tides: the cyanophyte connection. J Geophys Res 106:11597–11612
187. Walsh JJ, Dieterle DA, Milroy SP, Jolliff JK, Darrow BP, Lenes JM, Weisberg RH, He R (2002) Three-dimensional biophysical models of Florida red tides. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 295
188. Yuzao Q, Ning X, Yan W, Songhui L, Jufang C, Zhaohui W (2002) *Phaeocystis globosa*, its taxonomy and blooms in China. Abstract, 10th International Conference on Harmful Algae, St. Pete Beach, Fla., p 237
189. Zhang H, Lin S (2002) Detection and quantification of *Pfiesteria piscicida* by using the mitochondrial cytochrome *b* gene. Appl Environ Microbiol 68:989–994