Biodiversity of Harmful Marine Algae

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Glossary

Allelochemical   A bioactive secondary metabolite with no known major function in primary metabolism but with the capacity to affect the growth, survival, or behavior of target organisms. Many allelochemicals act as chemical defense agents, although they may also mediate beneficial interactions with the targets.

Bootstrap support   A method of attempting to estimate confidence levels of inferred relationships in phylogenetic trees. The bootstrap analysis resamples the original data matrix with replacement of the characters and reconstructs the tree. The bootstrap support for any internal branch is the number of times it was recovered during the bootstrapping procedure.

Clade   A group of species that shared a common ancestor (i.e., monophyletic).

Heterokonts   A monophyletic group (also known as *stramenopiles*) that includes all cells with heterodynamic flagella: the mature flagellum bearing small tripartite tubular hairs termed *mastigonemes* and the second being smooth.

Maximum likelihood (ML)   A method of inferring phylogenetic relationships using a prespecified (often user-specified) model of sequence evolution. Given a tree (a particular topology, with branch lengths), the ML process asks the question, ''What is the likelihood that this tree would have given rise to the observed data matrix, given the prespecified model of sequence evolution?''

Microsatellite   Short tandem repeats (e.g., ACn, where $n > 8$) of nucleotide sequences; the tandem units can be dinucleotides, trinucleotides or tetranucleotides. Each individual should have a unique number of repeat units for each microsatellite. When two individuals share the same number of repeat units, then they are more closely related than those whose repeat units differ in number.

Molecular clock   A hypothesis that mutation rates and substitution rates do not vary among lineages in a tree, thus the timing of divergences can be dated from known times of divergence in the fossil record. However, it has been shown that rates of evolution along a lineage can vary. Two different kinds of clocks can be used: a fixed or a relaxed clock, depending on whether the rate of evolution is allowed to vary.

Outgroup   A species or other taxonomic group chosen to establish the root of any phylogeny

Ribotyping   Fingerprinting of genomic DNA restriction fragments that contain all or part of the ribosomal RNA gene to identify and distinguish among strains or species, which are thereby defined as *ribotypes*.

Distribution and Biogeography

Harmful marine algal taxa are globally distributed from tropical to polar latitudes and occupy ecological niches ranging from brackish water, such as the Baltic Sea, to oceanic environments. Nevertheless, high-biomass harmful blooms tend to be predominant in coastal and shelf seas and high-magnitude effects are most often expressed in these areas. Whereas an apparent global increase in the frequency, magnitude, and biogeographical range in harmful algal blooms (HABs) over the last several decades has often been cited and appears to be legitimate in a general sense (Hallegraeff, 1993) (Figure 1), the effects on local and regional biodiversity remain unresolved. Mechanisms invoked for the ''global spreading hypothesis'' (Smayda, 1990) include transport of exogenous species via ship ballast-water transport or transfer of aquaculture stock, changes in ocean currents, and human intervention in coastal zones (e.g., increased eutrophication, coastal engineering, dredging, and sediment disruption). The socioeconomic impact of HABs has clearly increased in recent years, perhaps partly caused by accelerated and modified use of coastal resources, but it is premature to conclude that this accurately represents verifiable changes in biodiversity. Enhanced scientific awareness and refined monitoring programs for HAB taxa and associated toxins have also contributed to the apparent global increase scenario.

Whether or not discovery of previously undetected or undescribed HAB taxa in a new environment represents a true shift in biodiversity or merely reflects inadequate observations of cryptic species is often difficult to determine. There are, however, well-documented cases of apparent range extension of HAB species and associated syndromes, often rather suddenly, into new environments. For example, until recently, the dinoflagellate *Karenia brevis* responsible for neurotoxic shellfish poisoning (NSP) was apparently confined to the Gulf of Mexico and the east coast of Florida. However, in 1987, an unusual northward flow pattern of the Gulf Stream apparently distributed a major bloom of *K. brevis* into North Carolina waters, and since then such blooms are recurrent along the coast of the Carolinas (Tester *et al.*, 1991). Extreme weather events, such as hurricanes, are known to expand the existing distribution of cyst-producing toxic dinoflagellates. As a classic example, a toxigenic population of the dinoflagellate *Alexandrium tamarense* was apparently introduced from coastal Nova Scotia to New England, where it established resident populations as far south as Cape Cod in the aftermath of a hurricane in 1972 (Anderson, 1994).
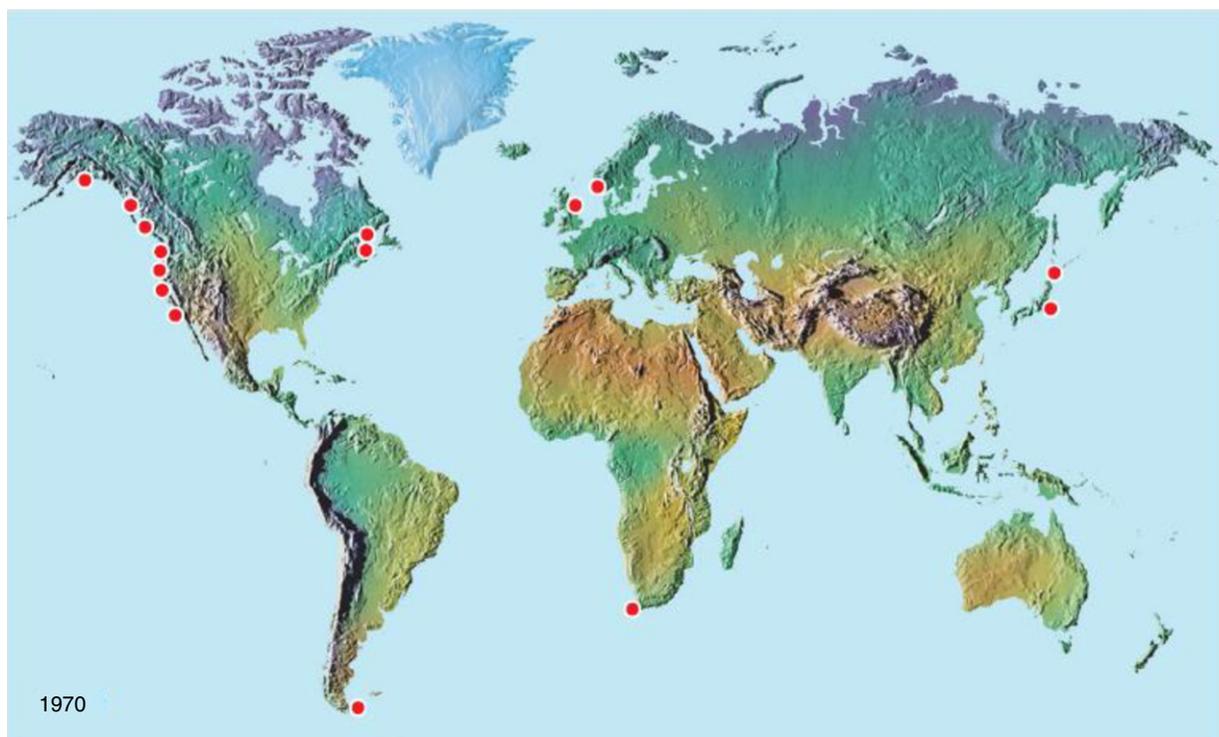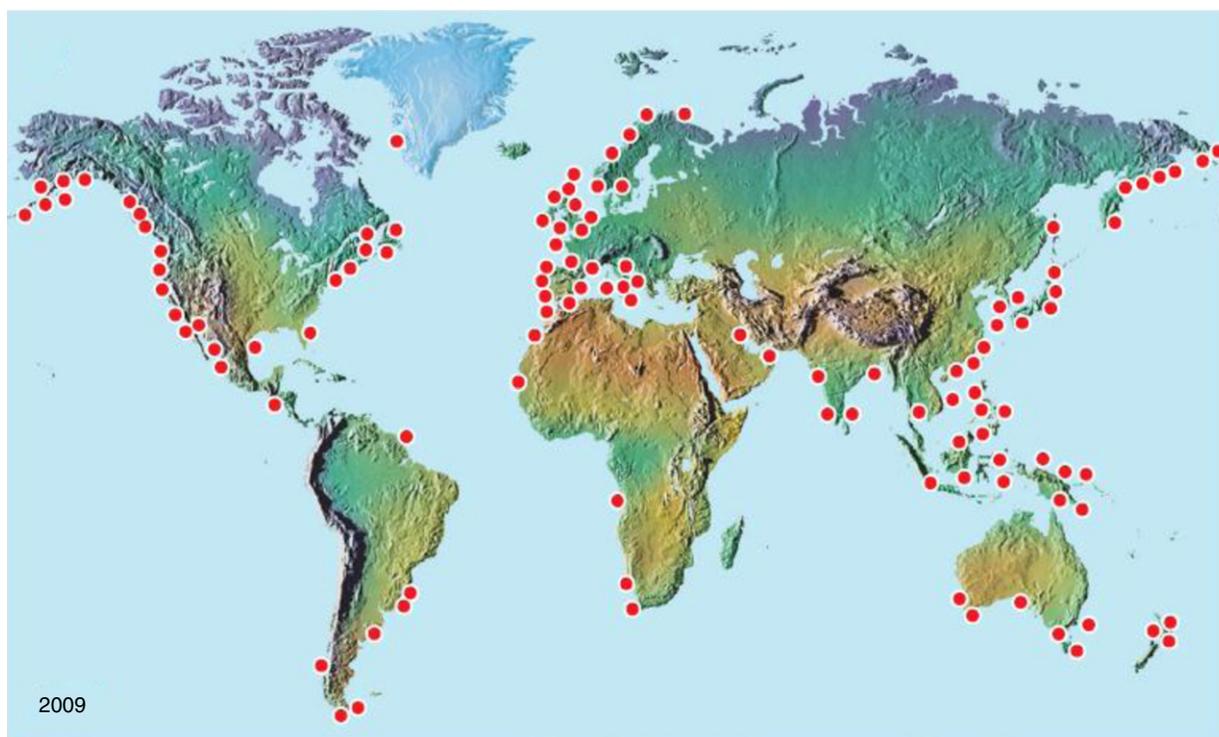
Figure 1   Distribution of events up to (a) 1970 and (b) 2009, respectively, where paralytic shellfish poisoning toxins were detected in shellfish or fish, thereby providing strong circumstantial evidence for a range expansion in the distribution of the causative toxigenic dinoflagellate blooms. Reproduced with permission from US National Office for Harmful Algal Blooms.

Phylogenetic and Taxonomic Relationships

The HAB phenomenon is characterized based on a societal definition ("harmfulness") and thus not surprisingly the associated taxa do not fall neatly into tight taxonomic or monophyletic groups. Marine HAB events have been attributed to members of various eukaryotic algal groups, including dinoflagellates (Dinophyta), prymnesiophytes or haptophytes (Haptophyta), diatoms (Bacillariophyta), raphidophytes (Raphidophyta), and pelagophytes (Pelagophyta); certain cyanobacteria from marine and brackish habitats are also included because of their consequences to ecosystems and human health. In the following sections, the authors discuss three major marine groups of harmful algae.

Dinoflagellates

Origin of the Group

The dinoflagellates are monophyletic, with *Oxyrrhis* (pre-dinoflagellate) lying outside the core dinoflagellates, and sister to the perkinsid flagellates that cause malaria (Apicomplexa) with high bootstrap support to form a clade that is sister to the ciliates (e.g., *Paramecium*), again with high bootstrap support (Leander and Keeling, 2004). Collectively, these groups comprise the superphylum or kingdom Alveolata (Adl *et al.*, 2005), defined morphologically by the series of cortical membranes or membrane bags beneath the plasmalemma and tubular cristae in the mitochondria. A less robust sister relationship (<50%) is recovered in most phylogenetic trees with the stramenopiles or heterokont organisms (Leander and Keeling, 2004), which together with the cryptomonads and haptophytes form the chromalveolates.

The evolution and morphological adaptation of the dinoflagellates, leading to a final radiation of the free-living autotrophic forms, has been reviewed (Saunders *et al.*, 1997). In this scheme, the Noctilucales, which undergo an unusual gametic meiosis and lack chloroplasts, are basal to the core dinoflagellates. Myzotosis or peduncle feeding, whereby a tube from a heterotrophic dinoflagellate attaches to a prey cell and the content of the prey cell is sucked into the predator, is proposed to have evolved early in the dinoflagellates. Many dinoflagellates possess a membrane-bound cell wall, known as a *theca*, that is divided into plates of cellulose (*armor*) within membrane vesicles. The tabulation of these plates (number, structure, and orientation) provides a distinctive morphological feature for taxonomic and phylogenetic analysis. Subsequent to the radiation of the core dinoflagellates, there are three alternative evolutionary hypotheses to explain the separation of thecate and nonthecate genera (Bujak and Williams, 1981), *viz.*, the plate increase, the plate reduction, and the plate fragmentation models, with the first and third models placing the naked or nonthecate Gymnodiniales in a derived position. The peridinioid (thecate) taxa are in a different evolutionary position in each of the three models.

New phylogenetic trees derived from molecular data must be evaluated in light of these evolutionary schemes based on morphology, but there are few such trees covering the entire range of dinoflagellates, with most molecular trees focusing only on a group of species or closely related genera. Saldarriaga *et al.* (2004) have produced one of the earliest trees that sampled across most major core dinoflagellates. Using ciliates as an outgroup, the parasitic and atypical taxa diverge in exactly the sequence predicted by their morphological features until the divergence of the core dinoflagellates. After these divergences, clades of nonthecate gymnodinioid dinoflagellates diverge several times, alternating with clades of thecate peridinioid dinoflagellates. Each clade consists of monophyletic well-supported genera, with the exception of *Gymnodinium*, which is paraphyletic. However, relationships between the clades are not supported. There is a final divergence of the Gonyaulacales, but the Prorocentrales are paraphyletic; thus no real support is provided for any of the morphological models. Adding more taxa to the tree has not really improved the situation but has yielded a few new surprises. Figure 2 shows a tree of dinoflagellate phylogeny from a maximum likelihood analysis of the 18S rRNA gene. Divergences from the ciliates to the core dinoflagellates follow a similar pattern as seen in the other trees. The core dinoflagellates diverge simultaneously into four major clades. The first major clade contains a mixture of gymnodinioid and peridinioid taxa with nonthecate *Amphidinium* spp. often occurring as a basal divergence in a peridinioid clade, which suggests that a naked form gave rise many times to a thecate genus. Dinophysiales are a basal divergence, and the Prorocentrales are split into benthic and planktonic clades not too distantly related. Hoppenrath and Leander (2010) used the heat shock protein (HSP) 90 gene and Zhang *et al.* (2007) used three genes to find that the Prorocentrales were a monophyletic lineage. The second major group in the large rRNA tree (Figure 2) is a smaller gymnodinioid clade. The third major clade contains a mixture of gymnodinioid and peridinioid taxa. However, Noctilucales are embedded in this clade, a position also recovered in the HSP gene tree by Hoppenrath and Leander (2010). Gonyaulacales are a final divergence. The fourth major clade consists primarily of naked dinoflagellates with the Suessiales (found as coral endosymbionts) as a final divergence. With a three-gene concatenated dataset, Zhang *et al.* (2007) found *Amphidinium* at the base of the core dinoflagellate lineage. Their major well-supported divergence was between endosymbiotic taxa and free-living taxa, with the Gonyaulacales being monophyletic with one exception.

Origin of the Toxic Species

The toxigenic species are not a monophyletic group and although the various genera containing toxic members are more or less monophyletic, they are distributed among the four different major clades shown in Figure 2. The dinoflagellate toxins are structurally diverse, ranging from linear and macrocyclic polyketides to tetrahydropurine alkaloids, and are derived via divergent pathways. Thus, the various major types of toxins likely evolved more than once among the dinoflagellates, and perhaps even within clades. Alternatively, if toxins have evolved only once, then there have been many multiple losses of toxigenic capability. Clade 1 includes the genera *Dinophysis* and *Prorocentrum* associated with diarrhetic shellfish poisoning, the karlotoxin-producing genus *Karlodinium* sister to *Azadinium*, which contains an azaspiracid toxin–producing

Figure 2   RaxML phylogenetic tree from 1490 small subunit rRNA sequences from dinoflagellates and closest sister groups. The core dinoflagellates have a simultaneous divergence of four major clades. All major clades are collapsed, showing the dominant cell morphology in the clade (either peridinioid (thecate) or gymnodinioid (naked)). Toxic genera are mentioned where they occur throughout the tree.

species and the ichthyotoxic *Pfeisteria*; each of these genera is in a different monophyletic clade within clade 1 and have nontoxic species as their closest sister group. The NSP toxin producers of the genus *Karenia* are found in clade 2. In clade 3, the monophyletic genus *Alexandrium*, containing approximately a dozen species that produce paralytic

shellfish poisoning (PSP) toxins, is sister to the ciguatera toxin–producing genus *Gambierdiscus*, which in turn is sister to the thecate genus *Pyrodinium*, including *P. bahamense* var. *compressum* that also synthesizes PSP toxins. *Gymnodinium catenatum*, a nonthecate PSP toxin producer, is the only toxic species found in clade 4.

Haptophytes

Origin of the Group

Haptophytes are unicellular algae that are important members of the marine phytoplankton involved in many important biochemical cycles. Cells possess two smooth flagella and another organelle, called a *haptonema*, inserted between the flagella. The cells are covered by organic scales, which are calcified in one group, the coccolithophorids. The true sister group of the haptophytes is unknown but is weakly allied with the Cryptophyta (see review in Lane and Durnford, 2010). Haptophytes comprise two clades that correspond to the two classes. In the class Coccolithophyceae are several clades that correspond to order level taxonomy in the group.

Origin of the Toxic Species

Among the haptophytes, only a few planktonic species are responsible for harmful and nuisance events through the production of high biomass, which may be accompanied by apparent toxicity effects on marine fauna – for example, mass fish mortalities. Members of the genera *Chrysochromulina* and *Prymnesium* are associated with ichthyotoxicity in coastal marine, brackish, and even freshwater systems, whereas *Phaeocystis* blooms can produce copious foam that forms unpleasant aggregations on beaches but are not known to be toxigenic. In Figure 3, only the order Prymnesiales is shown because all toxic haptophyte species fall into one clade, namely, the family Prymnesiaceae within this order. This is in contrast to the dinoflagellates for which toxigenic taxa are distributed throughout the phylogenetic tree.

The genus *Chrysochromulina* is paraphyletic and falls into two clades, one of which contains the toxic species as a separate and well-supported subclade. The high bootstrap and morphological support for major clades and subclades in the Prymnesiales justified the recent substantial taxonomic revision (Edvardsen *et al.*, 2011), whereby all species in one of
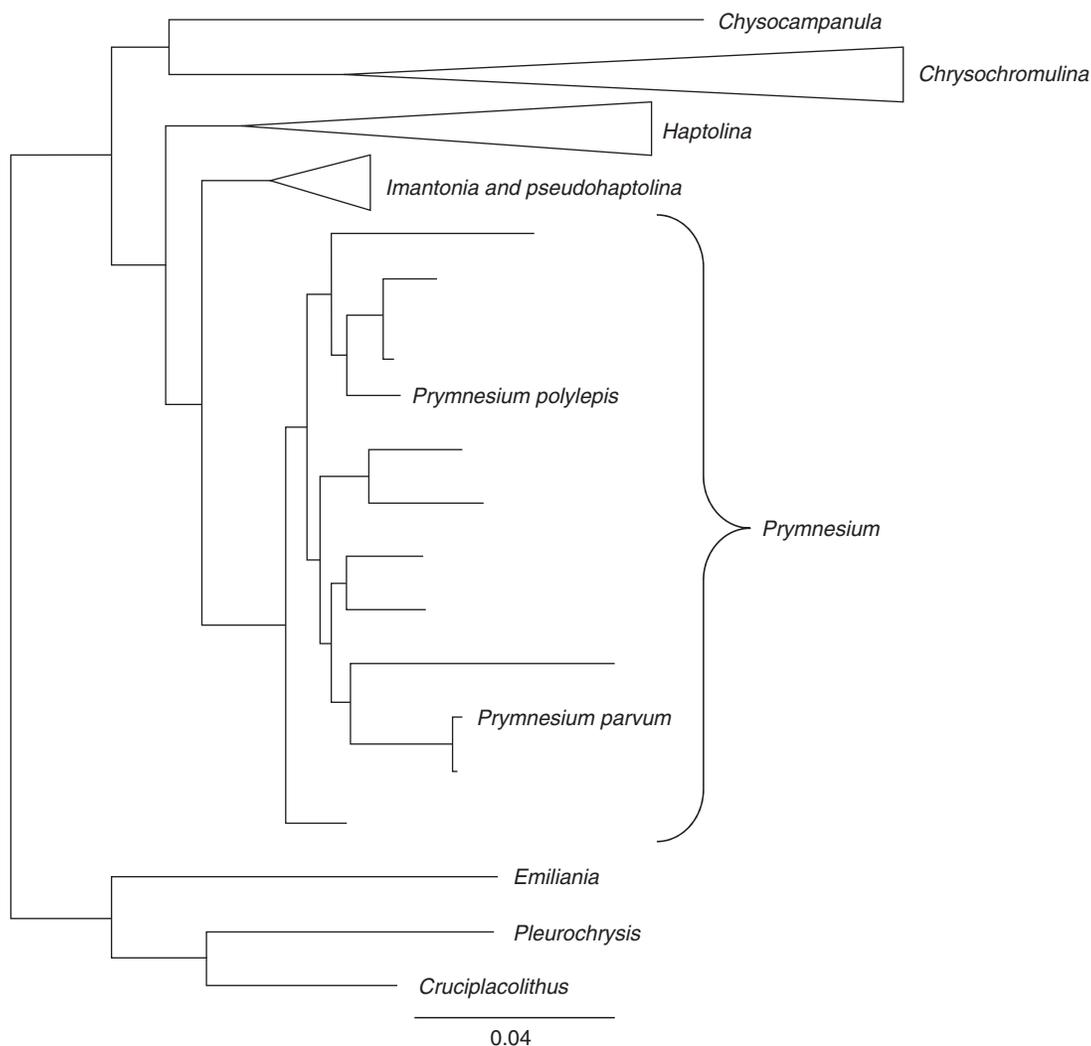
Figure 3   Consensus Bayesian tree based on concatenated nuclear 18S and partial 28S and plastid 16S ribosomal encoding DNA sequences of members of the Prymnesiales. *Cruciplacolithus neohelis*, *Emiliania huxleyi*, and *Pleurochrysis carterae* were used as outgroups. Redrawn from Edvardsen B, Eikrem W, Throndsen J, Saez A, Probert I, and Medlin LK (2011) Ribosomal DNA phylogenies and a morphological revision set the basis for a new taxonomy of the Prymnesiales (Haptophyta). *European Journal of Phycology* 46: 202–228.

the subclades have been transferred into *Prymnesium*. This genus includes the toxic species *Chrysochromulina polylepis* (now as *Prymnesium polylepis*); with *Prymnesium parvum*, these are the two main fish-killing species in the haptophytes.

Heterokonts

Origin of the Group

The Heterokonts are another major eukaryotic radiation, often linked to the alveolate lineage with moderate bootstrap support (Leander and Keeling, 2004). The basal lineages are heterotrophic and photosynthetic lineages are the last major divergence in the group (Cavalier-Smith and Chao, 2006). All toxigenic and otherwise harmful groups of heterokont algae occur within the photosynthetic lineage.

Origin of the Toxic Species

Within the pigmented heterokonts, two major classes or phyla include many toxic species. Among the class Raphidophyceae, some strains of the genera *Chattonella* and *Heterosigma* at high cell concentrations can kill fish, but the exact mode of action and the nature of the "toxins" remains unresolved (Rensel and Whyte, 2003). Other raphidophytes, such as members of the freshwater genus *Gonyostomum*, may secrete copious amounts of mucilage that are detrimental to fish gills but show no evidence for a defined toxin.

Among the Bacillariophyta, harmful effects may be produced by alternative mechanisms, primarily by toxin production or by physical-mechanical damage. In the latter case, some centric diatoms, such as *Chaetoceros* spp., have a life form (cells in a chain with long spiny protuberances called *setae*) that can clog fish gills and thereby kill fish, but they do not produce any toxins. Toxin producers are only found so far among the marine pennate diatoms (**Figure 4**), largely or exclusively belonging to the genus *Pseudo-nitzschia*. This genus contains more than a dozen species known to produce the neurotoxin domoic acid, the causative agent of amnesiac shellfish poisoning. Most of the older-named species were originally placed in the genus *Nitzschia* because the genus *Pseudo-nitzschia* was originally its own genus before it was reduced to a section of the genus *Nitzschia* and then elevated back to genus status by Hasle (1993). From a diversity perspective, the toxigenic and taxonomic status remains confusing because extensive molecular analyses continue to recover cryptic species. As a result, many toxin-producing *Pseudo-nitzschia* have been transferred or promoted from *forma* or varieties of *Pseudo-nitzschia* to species level, and new species have been described, based on morphological characteristics normally at the limit of resolution of the light microscope. Electron microscopy must be used to confirm their identity in field samples as well as to describe them as new taxa. Thus, name changes among the taxa abound. For example, the first pennate diatom known to produce domoic acid was originally reported as *Navia pungens* forma *multiseries*, then as *Pseudo-nitzschia pungens* f. *multiseries*, and finally as *P. multiseries* (Bates *et al.*, 1998). Early reports of domoic acid in marine species of *Nitzschia sensu stricto* and the brackish water species, *Amphora coffaeformis*, require confirmation. Further complications arise from the fact that toxigenicity may be lost in cultures without sexual production and domoic acid production within strains is highly inducible or repressible. Finally, even among clearly defined strains belonging to the same morphospecies or genetic species of *Pseudo-nitzschia*, the capacity for domoic acid production may be highly inconsistent and is often related to the stage in the growth cycle with many strains only becoming toxic as they enter stationary growth.

Population Genetics and Bloom Dynamics

Protein variants known as *isozymes*, with the same enzymatic functional role but differing slightly in $1°$, $2°$, $3°$ or $4°$ structure, were the first molecular markers for species- and population-level studies on marine species, including those of HAB taxa. Small differences in their molecular size or isoelectric point enable separation of isozymes by electrophoresis. Isozymes were the markers of choice for early investigations because they were quick and easy to resolve and detect, and they reflect primary gene products. But the requirement that isozymes must still be functional in the biochemical pathways strongly limits the number of possible mutations and, therefore, the number of alleles and the heterozygosity of this marker. Another disadvantage of this marker is that protein content and enzyme activity and thus the detection of isozymes is strongly influenced by the environment and physiological status of the cells.

The goal of most early molecular studies on microalgae based on isozyme analysis was to resolve species-level issues among species with conflicting or little morphological resolution rather than to study genetic structure within bloom populations. Thus, the early isozyme results were applied to the recognition of cryptic species or the recognition of previously discounted morphological markers for separation of members of a species complex. In the first inter- and intra-specific study of members of the *A. tamarense* and *catenella* species complex, isozyme analyses showed a high degree of enzymatic heterogeneity among isolates from the west coasts of the USA and Canada but indicated that isolates from the same locality were most closely related (Cembella and Taylor, 1986). In contrast, a relative lack of enzymatic heterogeneity was revealed by a similar analysis of *Alexandrium* populations from the east coast of the USA (Hayhome *et al.*, 1989). Isozyme data suggested a common origin for the East Coast populations and supported the dispersal hypothesis along the East Coast of the USA from Canada down to Massachusetts, as related to hydrographic events dissipating a massive red tide that occurred in 1972. Isolates of the dinoflagellate *Gambierdiscus toxicus* from similar geographical regions within the tropics were shown not to be closely related by isozyme comparison, which suggested a multiclonal origin for these populations (Chinain *et al.*, 1997). Among HAB taxa, isozyme studies are largely confined to the dinoflagellates, as there are no published studies for the toxic haptophytes and only very preliminary investigations have been conducted on toxic *Pseudo-nitzschia* spp. (Skov *et al.*, 1997).

As a consequence of the limitations of isozyme analysis to reflect genomic differences at high resolution, alternative marker types were later developed that directly addressed the nucleic acid level and hence are relatively refractory to
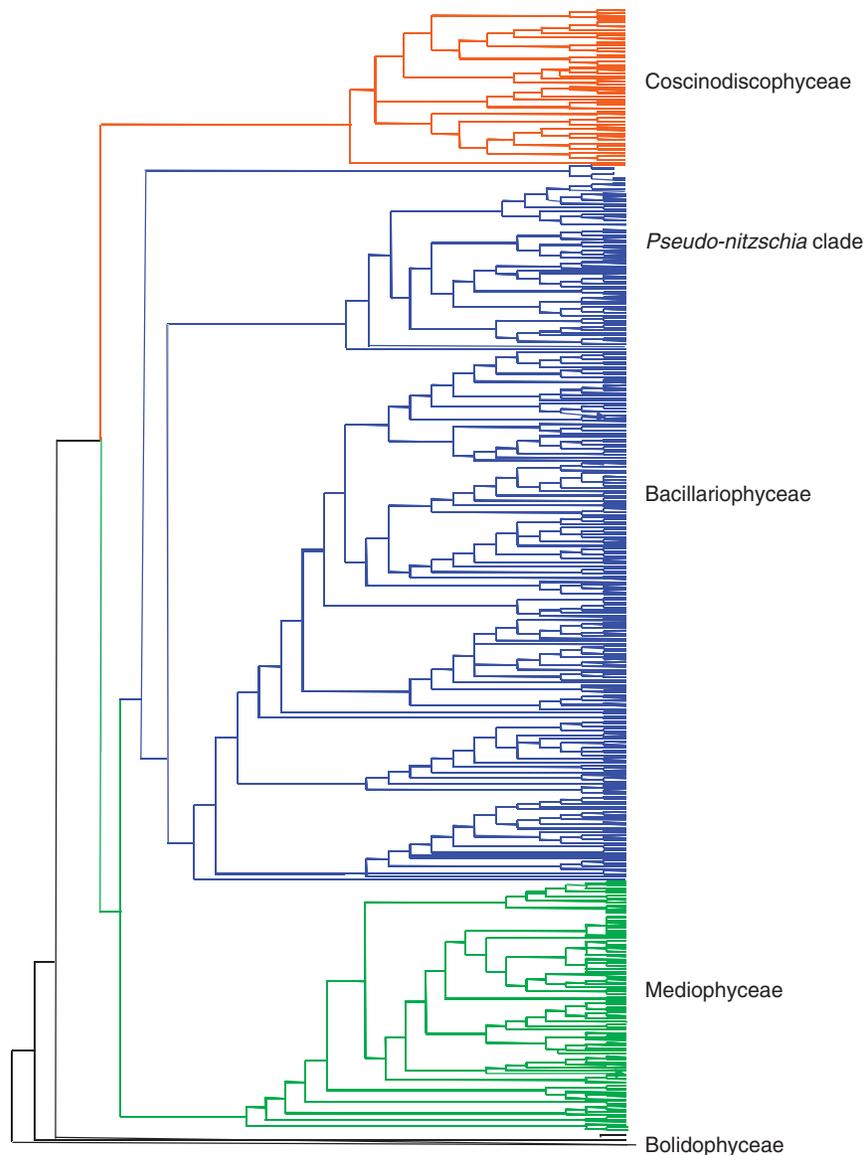
Figure 4   Baysian analysis of 920 diatom taxa rooted with the Bolidomonads. Three monophyletic classes occur, each shown in a different color. The toxic diatom genus *Pseudo-nitzschia* occurs as one of the most derived or advanced pennate diatom genera. Redrawn from Bowler C, Allen AE, Badger JH, *et al.* (2008) The *Phaeodactylum* genome reveals the dynamic nature and multilineage evolutionary history of diatom genomes. *Nature* 456: 239–244.

environmental variation and shifts in physiological status. Diversity below the species level is now most robustly measured by DNA- or RNA-fingerprinting methods of which microsatellites are the most computationally intensive. Recently, there has been a rapid increase in studies using DNA microsatellite markers to estimate gene flow and resolve dispersal mechanisms in natural populations as evidenced by the development of microsatellite markers for many species, with particular focus on harmful species (see references in Table 1). The primary limiting factor to developing microsatellites and performing ecological analyses on large spatial and temporal scales is the need to make single-cell isolations from planktonic populations and to maintain them in culture for analysis. Amplification of microsatellites from single

dinoflagellate cells (Henrichs *et al.*, 2008) represents a way to overcome culture and sampling bias for planktonic organisms.

Cryptic speciation in several harmful algal species has been studied in more detail using sequence analysis of rapidly evolving genomic regions, such as the internal transcribed spacer (ITS) and the hypervariable D1/D2 region of the large subunit (LSU) rRNA gene. Within the *Alexandrium tamarense–fundyense–catenella* species complex, perhaps the most intensively studied HAB group, isolates were shown to be related by geographic origin rather than by morphological affinities (Scholin *et al.*, 1995), with the rRNA gene data essentially confirming the early isozyme analyses. *Alexandrium* isolates from within the species complex will interbreed more successfully if they have similar isozyme patterns from two

Table 1 Summary of microsatellite studies on harmful dinoflagellates

Species	Source
Alexandrium tamarense/fundyense/catenella NA	Nagai, *et al.* (2004, 2007a), Alpermann, *et al.* (2009)
Alexandrium tamarense/fundyense/catenella TA	Nagai, *et al.* (2006b), Nishitani, *et al.* (2007)
Alexandrium minutum	Nagai, *et al.* (2006a)
Cochlodinium polykrikoides	Nagai, *et al.* (2009)
Heterocapsa circularisquama	Nagai, *et al.* (2007b)
Heterosigma akashiwo	Nagai, *et al.* (2006c)
Karenia brevis	Renshaw, *et al.* (2006)
Lingulodinium polyedrum	Frommlet and Iglesias-Rodríguez (2008)
Akashiwo sanguinea	Cho, *et al.* (2009)

All species listed are known to produce an identifiable toxin, except as indicated in bold.

different locations than will isolates from the same locations but with different isozyme patterns (Sako *et al.*, 1990). Currently, the geographic clade names have been replaced with numbers because their present-day known distribution exceeds the original areas (Lilly *et al.*, 2007). Thus, the North American clade is now termed Group I with primarily a Pacific distribution but also found in northern Europe, the Mediterranean clade of Group II is still endemic to the Mediterranean; the Western European clade equals Group III with one Japanese relative, the temperate Asian clade equals Group IV with relict populations in the Mediterranean, and the tropical Asian clade equals Group V known only from Australia.

The worldwide biogeographic dispersal of ancestral populations of the *A. tamarense* species complex from the Pacific into the Atlantic has also been hypothesized from rRNA gene data. Using a molecular clock, John *et al.* (2003) proposed a scenario for the biogeographic history of this complex from a single global ancestor. The later divergences of the nontoxic Western European clade from the toxic temperate Asian (TA) could be dated to the closing of the Tethys Sea, which likely explains the presence of relict cells of this clade in the Mediterranean. The separation of the nontoxic Mediterranean clade from the toxic North American clade was dated to the rising of the Isthmus of Panama. With time, the Mediterranean clade went extinct in the western tropical Atlantic waters, but it thrives today in the Mediterranean, where it is now endemic.

Microsatellites have clarified the origin of the TA clade of the *Alexandrium tamarense–fundyense–catenella* species complex. Lilly *et al.* (2002), comparing Mediterranean and Asian strains with LSU rRNA sequence data, concluded that the presence of TA strains in the Mediterranean was a clear case of ballast-water introduction. Penna *et al.* (2005), using ITS sequence data, also concluded that it was likely the result of ballast-water introduction. Masseret *et al.* (2009), using microsatellites, showed the Mediterranean populations to be clearly distinct and distant from the Asian strains but could not offer any reasonable explanation for their occurrence in the Mediterranean. However, referring back to the historical biogeography of the species complex by John *et al.* (2003), the closing of the Tethys Sea, which is the vicariant event separating the West European from the TA strains, the presence of the TA genotypes in the Mediterranean is likely a relict population from when the two groups were once joined. Their notable presence in Mediterranean French lagoons is likely caused by a change in environmental conditions that yield

dramatic blooms. The first hierarchical study of genetic diversity has been conducted on populations of *A. tamarense* (NA clade) off the Orkney Islands, UK (Alpermann *et al.*, 2009, 2010). On a global scale, the Scottish east coast populations found near the Orkney Islands are more closely related to Japanese Pacific isolates than to populations on the east coast of North America, which suggests that the Orkney populations were introduced to that region by cells transported directly across the Arctic Ocean. This interpretation is in contrast to the hypothesis put forward by Medlin *et al.* (1998) that these populations entered from the Pacific and moved along coastal pathways of the eastern side of North America until they reached the Gulf Stream, which carried them across to the Scottish east coast. Moving to the diversity of local populations, at one site off the Orkney Islands, four populations were discovered that could interbreed, and these populations were proposed to represent different year classes that had hatched from local cyst beds (Alpermann *et al.*, 2010).

Karenia brevis has been shown to be a single population in the Gulf of Mexico (Renshaw *et al.*, 2006 and unpubl.) based on microsatellite analysis, although molecular probes have also supported the recognition that the Florida red tides previously assumed to be monospecific *K. brevis* blooms are often multispecific (Steidinger *et al.*, 2008). In *Alexandrium minutum*, several microsatellites have been found to occur on the same chromosome (Nagai *et al.*, 2006b). *Alexandrium minutum* from the Atlantic Ocean and the Mediterranean Sea can be segregated into four genetic populations that more or less correspond to four regional seas in the Mediterranean and the relatedness of these four populations can be explained by circulation patterns in the Mediterranean (Casabianca *et al.*, 2012).

Among HAB taxa, most microsatellites have been developed for dinoflagellates but also for a few raphidophytes, such as *Chattonella* spp. (Demura *et al.*, 2007) and *Heterosigma akashiwo* (Nagai *et al.*, 2006b). Microsatellites for the raphidophytes were found to be very heterozygous but have not yet been applied for ecological interpretations at the population level.

Almost all dinoflagellates are nominally haploid in the vegetative stage, with exceptions found among the Noctilucales. A number of HAB species, particularly among the thecate dinoflagellates, produce a resistant benthic resting stage (or cyst) within their life history as an overwintering or survival strategy (**Figure 5**). Differentiation of haploid gametes
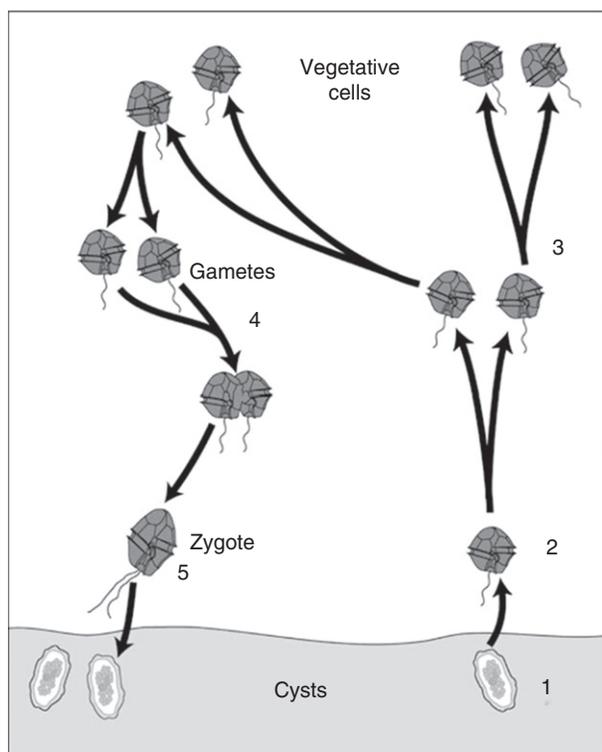
Figure 5 Schematic representation of the life history of *Alexandrium* species, typical of dinoflagellates for which the motile vegetative cells are haploid – that is, they have one set of chromosomes. Excystment from the benthic resting cyst (1) leads to formation of a motile planomeiocyte stage (2) that subsequently divides to form vegetative cells (3). Some vegetative cells become gametes, initiating the sexual phase whereby the gametes fuse (4) to yield a motile planozygote with double the number of chromosomes (5). The planozygote can transform into a resting cyst (hypnozygote) to complete the cycle or for some species can undergo a reduction division to yield a vegetative cell. Modified from Sellner KG, Doucette GJ, and Kirkpatrick GJ (2003) Harmful algal blooms: Causes, impacts and detection. *Journal of Industrial Microbiology and Biotechnology* 30: 383–406, with permission from Springer.

followed by fusion to form diploid planozygotes leads ultimately to nonmotile sexual resting cysts (or hypnozygotes) and thereby provides the opportunity for both genetic recombination and the maintenance of functional diversity within and among populations. The timing of excystment and survival success of hatched cysts as a contribution to the vegetative population or "bloom" is therefore a key determinant of the population genetic structure. This was demonstrated in a conceptual population model for the dinoflagellate genus *Alexandrium* (Alpermann *et al.*, 2009) based on the analysis of multiple isolates from a geographical population using microsatellites and amplified-fragment length polymorphism (AFLP). Clonal selection and shifts in genotype frequencies caused by variations in selective constraints of the environmental regimes is the most likely explanation for observed population genetic substructures revealed by these genetic markers. The interplay between genetic structure and population diversity, as modulated by mating compatibility within contemporaneous populations

and environmental factors affecting survival, is also supported by observation of two genetically distinct subpopulations of *A. fundyense* (Group I) in the northeastern USA, comprising either early bloom or late-bloom genotypes (Erdner *et al.*, 2011).

Apparent biogeographical distinctiveness among clades may arise via reproductive barriers based on limited sexual compatibility. Brosnahan *et al.* (2010) mated strains of Group I and Group III ribotype of the dinoflagellate *A. tamarense*, which yielded true sexual resting cysts, but the subsequently germinated cells did not survive. Such reproductive barriers are indicative that these ribotypes constitute different biological species and also suggests that such barriers may limit successful invasions of foreign ribotypes and range extension via lethal hybridizations. This interpretation is consistent with the mating compatibility model developed earlier for *A. tamarense* (as *Alexandrium excavatum*) by Destombe and Cembella (1990) based on crossing multiple clonal strains and monitoring the presence of fusing gametes, cyst formation, and subsequent germination success. This model postulates a complex spectrum of mating compatibility and affinities rather than two defined parental mating types, even within a geographical population.

Chemical Ecology in Species Interactions Affecting Diversity

As members of pelagic or benthic communities, HAB taxa are subject to species interactions that may alternatively promote or suppress the population growth rate and biomass yield. Population growth may be limited not only by abiotic factors (light, temperature, nutrients, advection) but also by species interactions, such as grazing, parasitism, viral and bacterial attack, and resource competition. Although it is often assumed that HAB taxa achieve high biomass and monospecific dominance via high intrinsic growth rates, this is not always the case. For example, many dinoflagellates, including most harmful taxa, are comparatively slow growing and poor nutrient competitors with respect to contemporaneous diatoms (Smayda, 1997).

In addition to the selective advantage of swimming behavior and vertical migration capabilities of marine flagellates, many HAB flagellates possess the ability to produce toxins or other allelochemicals that may affect species interactions (Cembella, 2003). Among the eukaryotic flagellates, these toxins include linear and polycyclic polyethers and tetrahydropurines, such as saxitoxin and analogues. Within the diatom genus *Pseudo-nitzschia*, some strains of a few species also produce the neurotoxic secondary amino acid known as domoic acid and its derivatives.

The widespread although not ubiquitous distribution of potent toxins and other allelochemicals among phylogenetically diverse groups of HAB taxa suggests that these secondary metabolites have a defined functional role and provide a selective advantage, perhaps related to competition, predator–prey interactions, or other forms of chemical communication (Legrand *et al.*, 2003). The high potency of these secondary metabolites against mammals and cultured cells led to the assumption that the compounds are

components of a "watery arms race" (Smetacek, 2001) against competitors and predators in marine ecosystems. This molecular target idea is further supported by evidence that the toxic profiles are typically stable and highly polymorphic within a clone but subject to high variation within and among species and populations (Cembella, 2003). The chemical defense hypothesis has been intensively investigated over the past three decades in studies with a wide variety of toxigenic taxa producing various toxin spectra, and in combinations with putative predators and competitors. Indeed, in a review of interactions between toxic phytoplankton and metazoan grazers, Turner *et al.* (1998) reported that in some cases exposure of grazers to toxigenic prey caused predator avoidance behavior, incapacitation, or mortality, as would be predicted from the chemical defense hypothesis. In other cases, the grazers appeared not to be negatively affected by the presence of toxins in the prey, but the responses were always highly species- and strain-specific. With respect to microzooplankton grazer interactions, such as dinoflagellates, ciliates, and other protists against toxic HAB taxa, the evidence does not typically support a chemical defense function for the known toxins (Ianora *et al.*, 2011).

This lack of general confirmation of expected toxicity responses should not be interpreted to imply that the toxins play no role in species interactions. In fact, recent studies provide evidence of co-evolutionary mechanisms in chemically mediated species interactions among predators and competitors that could lead to shifts in diversity and dominant genotypes in natural bloom populations. Waterborne cues of predatory copepods can induce a shift up in cell toxin content and changes in gene expression profiles in potential prey, such as *Alexandrium* spp. (Wohlrab *et al.*, 2010; Yang *et al.*, 2011). This functional genomic approach indicated that regulation of serine–threonine kinase signaling pathways has a major influence in directing the copepod cues into different intracellular cascades and networks in toxic prey dinoflagellates (Wohlrab *et al.*, 2010), and may serve as a survival mechanism for dinoflagellates with the capacity for toxin production.

In addition to the known toxins of high potency against mammals, many marine HAB flagellates can synthesize and release allelochemical substances (often poorly chemically characterized) that are capable of immobilizing or lysing cells of potential predators and competitors (Ma *et al.*, 2011). Although the mode of action is unknown or poorly characterized, these allelochemicals may act as "toxins" of high adaptive significance in an ecological sense because of their high lytic activity in natural populations.

The taxonomic and phylogenetic distribution of the known mammalian toxins and apparently unrelated allelochemicals is particularly intriguing from a diversity perspective. For example, the saxitoxin analogues associated with PSP are produced not only by certain species and strains of the related gonyaulacoid dinoflagellates *Alexandrium* spp. and *P. bahamense* but also by the distantly related gymnodinioid dinoflagellate *G. catenatum*. The same toxin group then reappears in members of several cyanobacterial genera, primarily from fresh and brackish water; in the complete operon fully sequenced from *Alexandrium*, however, only three of the eight genes are of cyanobacterial origin (Hackett *et al.*, in press).

Clearly, most of the genes are of a eukaryotic and independent origin.

Most of the polyether and tetrahydropurine toxin derivatives produced among HAB dinoflagellates are constitutive metabolites; if present within a strain, the toxin composition is a rather stable phenotypic trait. In contrast, production of the neurotoxin domoic acid by some strains of the diatom *Pseudo-nitzschia* is highly inducible or repressible, depending on nutritional status and other extrinsic factors (Bates, 1998). Among HAB dinoflagellates, the toxin composition is typically represented by a suite of closely related derivatives and is fixed genetically within a clonal strain (Anderson *et al.*, 1990). This led to attempts to apply toxin profiling as a molecular fingerprint or phenotypic marker at both the species and population levels (Cembella, 1998 and references therein). The composition of saxitoxin analogues varies widely within and among *Alexandrium* species, but toxin profiles are generally distinguishable from those of other toxic dinoflagellate genera (*Pyrodinium* and *Gymnodinium*) and cyanobacteria. Within the *A. tamarense* species, complex toxin profiles are generally too diverse and variable for species discrimination, but this chemotaxonomic marker has been successfully applied to differentiate among geographical populations within a species based on cultured isolates (Cembella *et al.*, 1987; Anderson *et al.*, 1994). Comparison of PSP toxin composition of field populations of *A. tamarense* from different sampling sites in eastern Canada (Cembella and Destombe, 1996) showed that geographical populations from the Bay of Fundy and the St Lawrence estuary were more homogeneous in toxin composition than those from eastern Nova Scotia. Such population-level differences in toxin composition can be interpreted as an indication that the St. Lawrence populations are well mixed genetically and perhaps originate from the same cyst beds, whereas populations from eastern Nova Scotia are more reproductively isolated.

Even if the toxin profiles are genetically determined, the interpretation of biodiversity patterns and variation at the species and population levels based on toxin composition of HAB taxa is nevertheless subject to critical limitations. If based on one or a few cultured isolates, then the toxin phenotypic range is not determined and the profiles represent only those of survivor genotypes. Alternatively, if based on directly sampling of mixed field populations, the toxin profile will reflect the average of the multiple genotypes, reflecting their relative abundance and cellular toxin composition and concentration. Indeed, in an integrated study of clonal variation in toxin composition, allelochemical activity, and genetic markers (microsatellites and AFLP) among a high number of isolates (88 clones) of *A. tamarense* from a geographical population on the east coast of Scotland, Alpermann *et al.* (2010) found high heterogeneity in all phenotypic and genotypic characteristics within the population. Despite hierarchical grouping according to toxin profiles, there was no clear linkage to the molecular markers AFLP and microsatellites or to the allelochemical activity measured as lytic effects. Although similar comparative data are not yet available for other toxigenic HAB taxa, there are preliminary indications from biogeographical studies of toxin polymorphisms (Gribble *et al.*, 2005) that such phenotypic heterogeneity and cryptic diversity within and among populations are common features.

Diversity and Global Change Processes

With respect to general biodiversity of plankton, climate-mediated shifts in ocean regimes are expected to lead to (1) range expansion of warm-water species toward higher latitudes and niche displacement of cold-water species; (2) temporal shifts in the frequency and abundance of particular taxa, including the timing of seasonal succession processes; and (3) alteration of food-web interactions, including grazing and prey selectivity. The extent to which these changes will affect the diversity and biogeographical distribution of HAB taxa (reviewed by Dale et al., 2006; Moore et al., 2008; Hallegraeff, 2010) will be a function of their respective ability to adapt to and colonize such emerging niches provided via changes in nutrient concentration and ratios, degree of stratification, and abiotic factors, such as temperature, pH and salinity regime, etc.

A few recent studies have attempted to establish causal links between exceptional or unprecedented algal blooms and climate shifts and anomalies (e.g., Belgrano et al., 1999; Skjoldal and Dundas, 1991; Cloern et al., 2005; Moore et al., 2008), but conclusive evidence remains lacking. Although scenarios for predicted biodiversity shifts are often directly coupled to climate change and variability (e.g., temperature rise, oceanic carbon flux), these issues must be considered in a broader context to include synergistic or compensating interactions at the local and regional scale. For example, nutrient enrichment of coastal waters may interact with increasing temperatures and CO_2 levels to affect the frequency and abundance of sympatrically distributed HAB species, such as the raphidophyte Heterosigma akashiwo and the dinoflagellate Prorocentrum minimum (Fu et al., 2008) but specific outcomes are difficult to predict in nature. As an unintended consequence on diversity, eutrophication abatement strategies may lead to a decrease in the frequency and magnitude of ichthyotoxic blooms of rapidly growing high-biomass producing species to be replaced by blooms of highly toxigenic species causing shellfish toxicity even at relatively low cell concentrations. This is apparently the case for the biodiversity regime shift in the Seto Inland Sea of Japan, with decreasing fish kills linked to blooms of raphidophytes over the last several decades but increasing incidences of shellfish toxicity associated with toxic dinoflagellates (Imai et al., 2006). Such complex interactions may also account for the dramatic increase in ciguatera fish poisoning over the past century in the South Pacific, in particular in French Polynesia (Hallegraeff, 2003). The causative factors are unclear but may include reef damage by military and recreational development, increases in typhoon frequency and intensity, and climate-related effects on coral erosion and destruction via photobleaching and acidification. In any case, the primary causative organism Gambierdiscus toxicus has now apparently spread from the Great Barrier Reef into southeast Australian sea-grass beds in association with a strengthening of the East Australian Current (Hallegraeff, 2010). Within the Mediterranean region, Gambierdiscus populations are also expanding their distributional range (Aligizaki et al., 2008), although this cannot be unequivocally linked to climate-mediated processes.

Conceptual models and hypothetical scenarios of the effects of global change on the diversity and biogeographical distribution of HAB species are based primarily on extrapolations of variation observed over the last several decades (short timescale) or derived from the fossil record. Global change processes affecting HAB diversity are often interpreted within the context of climate variation and oscillations (atmospheric CO_2 levels, sea surface temperature, etc.), but direct anthropogenically mediated modification of coastal ecosystems via coastal engineering, introduction of invasive species and eutrophication are also expected to induce biodiversity shifts on a local and regional scale. Recent decadal scale observations, however, do tend to indicate shifts in abundance and distribution of plankton species, such as those documented from the Hardy Continuous Plankton Recorder (CPR) data from the North Sea and adjacent waters since the 1940s (Reid et al., 2003). Most multidecadal sets are inadequate with respect to revealing frequency and abundance shifts of HAB plankton. The CPR transects were originally designed to focus primarily on zooplankton, and although they have proven useful for studying coccolithophorid distributions in recent years, many of the cryptic HAB taxa, particularly small-celled species, are not well represented or have been previously overlooked in the surveys.

Nevertheless, there are a few documented diversity changes in the range and abundance of particular HAB taxa, related either to interannual variation in oceanic regime or to catastrophic events such as hurricanes. Based on decadal scale analysis, there is circumstantial evidence of a coincidence between the magnitude of blooms of the toxigenic dinoflagellate P. bahamense in the southern Pacific and the quasi-periodic climate phenomenon known as El Niño Southern Oscillation (ENSO) (Azanza and Taylor, 2001). Exceptionally high-magnitude PSP episodes caused by blooms of the A. tamarense/catenella along the Pacific Northwest coast of the USA were also temporally correlated with ENSO events in seven of nine cases between 1941 and 1984 (Erickson and Nishitani, 1985). A putative link between ENSO events and a massive offshore bloom of the toxigenic diatom Pseudo-nitzschia in summer 1998 along the coast of California was associated with higher than normal water temperatures in the eastern Pacific during the summer and fall of 1997 followed by a transition from El Niño to cooler La Niña conditions (Bargu et al., 2010). Although the evidence is not conclusive in any of these cases, climate-mediated changes on oceanic regimes, such as ENSO and North Atlantic oscillation (Belgrano et al., 1999), may prove to be a major factor in determining biodiversity shifts and periodicity in the appearance and abundance of HAB taxa, particularly in coastal zones.

As previously mentioned, rising sea surface temperatures associated with global warming may be expected to favor a range expansion toward higher latitudes, but this will also bring a disproportionate increase in the abundance of marine flagellates, which tend to thrive in warmer stratified water regimes. Rising sea surface temperatures have already been associated with increases in dinoflagellates in the North Atlantic, the North Sea, and the Baltic Sea, as well as to seasonal shifts in the timing of dinoflagellates in the plankton successional cycle (Dale et al., 2006). Global warming may also tend to favor differentially blooms of cyanobacteria in marine and freshwater ecosystems, including those of harmful and toxigenic species (reviewed by Paerl and Huisman, 2008 and 2009). Nevertheless, these apparent shifts in group diversity and

dominance cannot yet be unequivocally linked to climate-mediated differential effects on particular HAB taxa. Dale *et al.* (2006) argue convincingly that because most HAB taxa are coastal or estuarine and broadly eurythermal, modest water temperature increases of a few degrees associated with global warming are unlikely to be the major determinant of their dominance and persistence.

Climate change is expected to lead to disruptive changes in wind velocity and seasonal shifts in ocean stratification parameters, such as upwelling and downwelling of coastal and shelf seawaters. Many HABs, particularly of dinoflagellates, are associated with postupwelling relaxation events in areas, such as the California coast and within the Benguela Current system off southwest Africa (Pitcher and Boyd, 1996). Wind-driven effects on HAB dynamics and diversity are linked not only to stratification but also to upwelling of macronutrients to surface waters (Kudela *et al.*, 2010). These combined wind effects tend to favor the development of blooms of toxigenic chain-forming dinoflagellates, such as those of *G. catenatum* off the Iberian coast (Fraga and Bakun, 1990) and the *catenella* morphotype of the *A. tamarense* species complex along the west coast of North America (Langlois, 2001). In the case of the fish-killing raphidophyte *Chattonella marina* in Hiroshima Bay, Japan, blooms were apparently enhanced subsequent to typhoon-induced runoff of terrestrial nutrients, but such blooms are also likely associated with excystment triggers and the capacity for vertical migration as adaptive strategies (Hallegraeff, 2010).

With few exceptions, HAB events are typically confined to coastal and shelf seas, therefore a rise in sea level and decreased salinity associated with shifts in precipitation patterns and melting of polar ice sheets may increase the potential for blooms of species favored by low salinity and highly stratified water columns. Examples of such species would include the ichthyotoxic haptophytes, such as *Pr. parvum* and *Chryso-chromulina* (now *Pr.*) *polylepis* and perhaps coccolithophorids in fjord systems. Ocean acidification linked to increasing atmospheric carbon dioxide has been predicted to have a deleterious effect on calcifying organisms, including cocco-lithophorids (Riebesell *et al.*, 2000). However, there is wide variation in strain response and also evidence that in complex assemblages as found in nature and not in a culture vessel increased CO_2 will actually increase calcification (Beaufort *et al.*, 2011). In principle, any change in climate could open new niches for other groups – for example, noncalcareous dinoflagellates – but it is difficult to predict whether or not harmful taxa would be particularly advantaged.

Among the thecate dinoflagellates, the benthic cyst stages (following sexual reproduction) of some species are subject to fossilization. The fossil morphotypes can be related to the vegetative stages of extant species, thereby yielding a potential paleo-oceanographic time series of the distributional shifts in the taxon. The toxigenic dinoflagellate *P. bahamense* provides a particularly instructive example of climate-mediated shifts. This species is presently restricted to tropical and subtropical coastal waters of the Atlantic, the Caribbean, and the Indo-West Pacific. Distribution of *P. bahamense* is strongly associated with mangrove-fringed areas (MacLean, 1977; Azanza and Taylor, 2001), therefore it would be expected that occurrence of this species may be reduced in distribution and even disappear from areas subject to mangrove destruction. The fossil cyst record of this species (as *Polysphaeridium zoharyii*), extending to the substantially warmer Eocene period 50 million years ago, indicates a much wider range of distribution, including the Portuguese coast, than indicated in recent records. Although today absent from the nontropical Australian coast, approximately 100,000 years ago this species ranged as far south as Sydney Harbour (McMinn, 1989).

Given the diverse and polyphyletic evolutionary history of HAB species and the consequent lack of an apparent common ecophysiological adaptive strategy, it is difficult to construct general predictive scenarios of "winners and losers" in response to global change. Autecology of individual species and even intrapopulation responses to ecophysiological shifts must be more thoroughly investigated. Group-specific predictions based on known responses to increased temperature, enhanced surface stratification, intensification or weakening of nutrient up-welling, stimulation of photosynthesis by elevated CO_2, changes in land runoff and nutrient availability, and decreased ocean pH are clearly inadequate. Genetic diversity has been investigated in only a handful of species, so it is also impossible to predict whether or not these species have sufficient genetic diversity to withstand major climate shifts and not becoming globally or locally extinct. Certainly climate change will fragment populations and affect gene flow among them.

The Way Forward

The lack of a scientifically based definition of what constitutes a HAB taxon coupled with the multiplicity of strategies and mechanisms whereby the harmful effects are expressed suggest that it is unrealistic to expect a "unified field theory" to encompass all HAB dynamics and evolution in the context of biodiversity. Until recently, ecophysiological and genetic studies on HAB taxa have followed the autecological model in which one or a few cultured isolates are taken to be representative of the response and diversity in natural populations. The fallacy of this approach was shown by Wood and Leatham (1992), who documented in their review significant variation in response among all strains tested within a given microalgal species for a wide variety of responses to environmental stressors. Thus, this acceptance of only a few isolates or strains as representative of a species has seriously hampered our understanding of the relationship between genotypic and phenotypic variation in the microalgae, including HAB taxa, and how this interaction provides the basis for adaptation and ecosystem resilience.

Advanced studies of the genetic composition and gene expression potential of various HAB taxa – the functional ecogenomic approach – can now provide insights into the capacity for survival and adaptation at both the species and population level. High-throughput sequencing (e.g., next-generation 454-pyrosequencing) coupled with bioinformatics platforms offer the capacity for sequencing and annotation of entire genomes of a wide spectrum of eukaryotic microalgae. For HAB species, several such initiatives are underway or essentially completed, for example, for the occasionally toxigenic diatom *Pseudo-nitzschia pungens* (Armbrust, 2009), and more are in the pipeline. Although the enormous genome size

(from 3000 to 215,000 MB) and complexity of the dino-flagellates remains a challenge for whole genome sequencing, *Heterocapsa circularisquama* is presently being sequenced. This dinoflagellate was chosen because the genome of the virus that kills it has been sequenced. Alternatively, limited genomic investigations based on expressed sequence tags have already contributed critical insights into the genome of several HAB species (Yang *et al.*, 2010; Jaeckisch *et al.*, 2011), including the diversity of genes associated putatively with bloom dynamics and toxin biosynthesis. Attempts to model HAB dynamics must take into account the inherent cryptic diversity within and among populations, particularly with respect to toxigen-esis and growth regulatory genes. New molecular probe tech-nologies offer the ability to detect and identify genotypic variation within HAB species and populations, in some cases at the individual cell level. When implemented on deployable sensor platforms, this opens the possibility for near-real-time assessment of shifts in biodiversity and gene frequency and expression in natural HAB populations, even over seasonal and annual bloom cycles.

Acknowledgments

Appendix

List of Courses

1. Ecology and Evolution of Marine Protists
2. Marine Phytoplankton
3. Marine Chemical Ecology
4. Taxonomy and Phylogeny of Microalgae
5. Evolution of Eukaryotes

See also: Biodiversity, Evolution and. Census of Marine Life. Defining, Measuring, and Partitioning Species Diversity. Diversity, Molecular Level. Diversity, Organism Level. El Niño and Biodiversity. Evolution in Response to Climate Change. Global Species Richness. Landscape Ecology and Population Dynamics. Loss of Biodiversity, Overview. Marine Ecosystems. Microbial Biodiversity. Pelagic Ecosystems. Population Genetics. Species Assemblages, Macroecology, and Global Change

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