7 The Ecology of Harmful Diatoms

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7.1 Introduction

Diatoms represent one of the most important groups within marine phytoplankton and are characterized by having a siliceous cell wall (frustule). They contribute up to 45 % of the total primary production in the ocean (Mann 1999), or 20–25 % globally (Werner 1977). Diatoms form the base of the food web in many marine ecosystems and are major players in the biogeochemical cycling of C, N, P, Si, and biologically required trace metals (Sarthou et al. 2005). Their success implies that they have highly efficient and adaptable survival mechanisms and growth strategies. A key to this ecological success may lie in their use of Si to form a frustule, which requires less energy to synthesize relative to organic cell walls (Raven 1983). Diatoms may thus take advantage of available Si, and given favorable light, may grow rapidly and dominate the phytoplankton assemblage, forming a "bloom". As such, they are often classed as opportunistic r-strategists, although some stages of their life cycle may be more like K-strategists (Fryxell and Villac 1999). The success of some diatom species may also be explained by their ability to form long chains, which are difficult or impossible for some grazers to ingest. Their frustules also provide mechanical protection against some classes of grazers because exceptional force is required to disrupt them (Hamm et al. 2003). In addition to this mechanical defense, some diatoms produce unsaturated aldehydes that significantly reduce the reproductive success and hence the population growth of zooplankton and other invertebrates (Ianora et al. 2003). In contrast to other smaller phytoplankton, diatoms require nutrient-rich conditions for growth, as well as turbulence to keep them in suspension. They are therefore often found in coastal regions, where their impacts on humans and marine food webs are more often observed.

Most diatoms are considered benign, but some are known to cause harm either by physical means, by causing oxygen depletion, or by the production of a phycotoxin. The first diatom species found to produce a phycotoxin belongs to the genus *Pseudo-nitzschia*; the remainder of this chapter will focus on the

ecology of this pennate diatom. For a description of harmful non-toxic diatoms, see Hasle and Fryxell (1995), and Fryxell and Hasle (2003); Ochoa et al. (2002) list harmful diatoms from Mexico.

7.2 Toxin-Producing Diatoms, Genus *Pseudo-nitzschia*

In 1987, the pennate diatom *Pseudo-nitzschia multiseries* (then called *Nitzschia pungens* f. *multiseries*) was identified as the source of the neurotoxin domoic acid (DA) that poisoned humans in eastern Canada (reviewed in Bates et al. 1998). Previously, diatoms were not thought to produce phycotoxins. However, since this first event in Canada, other species of *Pseudo-nitzschia*, thus far totaling 11–12 (depending on the inclusion of *P. pseudodelicatissima*), have become problematic in other parts of the world due to the production of DA. These species include (see Bates et al. 1998; Bates 2000; Moestrup 2004): *P. australis*, *P. calliantha* (Lundholm et al. 2003), *P. cuspidata* (Bill et al. 2005), *P. delicatissima*, *P. fraudulenta*, *P. galaxiae* (Cerino et al. 2005), *P. multiseries*, *P. multistriata*, *P. pseudodelicatissima*, *P. pungens*, *P. seriata*, and *P. turgidula*. Interestingly, all toxigenic species are primarily coastal, although some may be found up to 150 km offshore. Hasle (2002) tentatively concluded that most DA-producing *Pseudo-nitzschia* species, with the exception of *P. seriata*, which is restricted to cold waters of the North Atlantic Ocean, are cosmopolites (see Chap. 3).

On the west coast of North America, the major DA producers are *P. australis*, *P. multiseries*, and *P. cf. pseudodelicatissima* (e.g., Adams et al. 2000; Stehr et al. 2002); the latter may have been misidentified and may actually be *P. cuspidata* (cf. Lundholm et al. 2003), which is now a confirmed DA producer (Bill et al. 2005). The *Pseudo-nitzschia* species that contaminated molluscan shellfish in the Bay of Fundy, eastern Canada in 1989 and 1995, was reported as *P. pseudodelicatissima* (see Bates et al. 1998). However, Lundholm et al. (2003) re-examined the field material and identified the cells as *P. calliantha* sp. nov. The question of which *Pseudo-nitzschia* species (*P. pseudodelicatissima* or *P. calliantha*) is the source of the toxin in the Bay of Fundy is still under debate (cf. Kaczmarska et al. 2005b). However, high numbers of non-toxic *P. calliantha* were found in bays of Prince Edward Island, eastern Canada in 2001 and 2002 (Bates et al. unpubl.). In 2002, an unusual spring closure of most of the southern Gulf of St. Lawrence was caused by toxic *P. seriata* (Bates et al. 2002). In Europe, the problematic *Pseudo-nitzschia* species are *P. australis*, *P. seriata*, and *P. multiseries*. In New Zealand, *P. australis* is the main source of DA, although other toxigenic species are present (Rhodes et al. 1998).

Note that all of the *Pseudo-nitzschia* species shown to be toxigenic, with the exception of *P. multiseries*, also have strains that do not produce DA at

detectable levels (Bates et al. 1998). Coastal species that have not been shown to produce DA include *P. americana* (Villac et al. 1993), *P. brasiliana* (Lundholm et al. 2002b), and *P.* cf. *subpacifica* (Lundholm et al. 2002a); several other species have not yet been tested. Genetic studies are needed to clarify whether all *Pseudo-nitzschia* species are capable of DA production. It should also be noted that toxin production has been reported for *Nitzschia navis-varingica* (Kotaki et al. 2000, 2004), suggesting that the ability to produce DA may include other genera, as well as other *Pseudo-nitzschia* species thus far not shown to be toxigenic.

7.3 Domoic Acid in the Marine Food Web

Domoic acid has frequent, recurrent impacts on many levels of the food web in certain coastal areas and minimal impacts in others. This may be due to a combination of factors, including the variability of toxin production among *Pseudo-nitzschia* strains, differences in shellfish retention or release of toxin, sensitivity and resistance of exposed organisms to ingested toxins, and composition of the food webs in each region. DA is available to pelagic and benthic organisms that filter feed directly on toxic *Pseudo-nitzschia* cells or on "marine snow" containing flocculated intertwined chains of *Pseudo-nitzschia* (e.g., Trainer et al. 1998), and to fish, birds and mammals that feed on contaminated food at higher trophic levels (see Chap. 22). Molluscan shellfish are the most common vector for DA transfer. However, other vectors continue to be found, implicating DA as an important agent for disrupting marine food webs. DA can be passed up the food web via krill (Bargu et al. 2002, 2003; Lefebvre al. 2002a; Bargu and Silver 2003), copepods (Lincoln et al. 2001; Tester et al. 2001; Maneiro et al. 2005), crabs (e.g., Wekell et al. 1994; Costa et al. 2003), other benthic organisms (Goldberg 2003), cephalopods (Costa et al. 2004, 2005), and fish (Lefebvre et al. 1999, 2001, 2002a, 2002b; Vale and Sampayo 2001; Costa and Garrido 2004; Busse et al. 2006). The latter has led to notable mortalities of marine birds (Sierra-Beltrán et al. 1997) and marine mammals (Lefebvre et al. 1999, 2002b; Scholin et al. 2000; Kreuder et al. 2003). Cellular toxicity may vary greatly, depending on the physiological condition of the *Pseudo-nitzschia* cells (Bates et al. 1998); therefore, it is difficult to predict toxin transfer based solely on cell concentrations.

It is interesting that examples of toxigenic *Pseudo-nitzschia* blooms in which DA is found at several levels of the food web appear primarily in upwelling regions, i.e., off the west coasts of the USA, Spain, Portugal, and in Chile. These regions are conducive to blooms of several toxic *Pseudo-nitzschia* species, but especially of *P. australis*, which can contain high levels of DA because of its large cell size (e.g., Cusack et al. 2002). Recently, the presence of DA in phytoplankton and planktivorous fish (pilchard) samples associated

with a wildlife mortality event off the Namibian coast, also an upwelling area, was confirmed by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) (DC Louw, B Currie, GJ Doucette pers. comm.). In contrast, molluscan shellfish continue to be the primary vector on the Canadian east coast, and DA has so far not been found at any other trophic level; no resulting mortalities of sea birds or marine mammals have been observed. This is curious because comparable links in the food web (e.g., herring, seals) are present. An exception may be in the Bay of Fundy, where LC-MS/MS has confirmed the presence of DA in North Atlantic right whales; the vector is still being sought (GJ Doucette, RM Rolland pers. comm.).

7.4 Physiological Ecology of *Pseudo-nitzschia* spp.

Laboratory studies with cultured isolates of *Pseudo-nitzschia* in natural and artificial seawater media have given us clues about environmental factors that may control cell growth and DA production (see Chap. 18). These physiological studies (up to about 1997) have been reviewed by Bates (1998). Briefly, DA production is non-detectable or minimal during exponential growth in batch culture, and increases during the stationary phase as cell division slows and then ceases due to stress. Pan et al. (1998) argued that the preferential need for cellular energy (ATP) limited DA biosynthesis during exponential growth when metabolic energy is used for primary metabolism. During the stationary phase, photosynthesis continues to produce ATP, which hence becomes increasingly available for DA biosynthesis. Early studies with *P. multiseries* consistently demonstrated that DA production was correlated with Si or P limitation, both in batch and in chemostat cultures. This same pattern has also been shown for *P. australis* (Cusack et al. 2002) and *P. seriata* (Bates et al. 2002; Fehling et al. 2004). An exception is *P.* cf. *pseudodelicatissima* (which may be identified as either *P. pseudodelicatissima* or *P. cuspidata*) (Lundholm et al. 2003) from the Gulf of Mexico. The highest DA production rates were during the early exponential phase, with no net production during the stationary phase (Pan et al. 2001). Other *Pseudo-nitzschia* species require study to determine if there are truly different patterns of DA production.

Recent laboratory studies with *P. multiseries* and *P. australis* have revealed that DA production is also associated with stress due to limitation by Fe and/or Cu, as well as to excess Cu (see Chap. 16). The presence of three carboxyl groups in the chemical structure of DA suggests that it could chelate trace metals (Bates et al. 2001), as was demonstrated by Rue and Bruland (2001). Fe- and Cu-stressed *P. multiseries* and *P. australis* cells produce increasing amounts of dissolved and particulate DA during the exponential phase (Rue and Bruland 2001; Maldonado et al. 2002; Wells et al. 2005). In

addition, dissolved DA reduces Cu toxicity in cultured *P. multiseries* and *P. australis* (Maldonado et al. 2002; Ladizinsky 2003), and high Cu concentrations increase DA production by *P. australis* during stationary phase (Rhodes et al. 2004). Cu chelation by DA may play a role in a Cu-reliant high-affinity Fe acquisition system, which would potentially provide toxigenic *Pseudo-nitzschia* species with a competitive advantage in areas where Fe is limiting (Wells et al. 2005). Both laboratory and field evidence indicate that dissolved DA enhances the rate of Fe uptake (Maldonado et al. 2002; Wells et al. 2005). Given this potential role of DA, it is surprising that none of the open ocean *Pseudo-nitzschia* species (i.e., *P. granii*, *P.* cf. *fraudulenta*, *P.* cf. *heimii*, *P.* cf. *inflatula*, *P. turgidula*) isolated from "high-nutrient, low-chlorophyll" Fe-limited waters of the NE subarctic Pacific produced detectable amounts of DA when Si-starved (Marchetti 2005); different conditions may be required to stimulate DA production in oceanic *Pseudo-nitzschia* species. In contrast to the above results showing increased DA production in Fe-stressed cells, Bates et al. (2001) found decreased DA production during the stationary phase when *P. multiseries* was grown in artificial seawater with decreasing amounts of added Fe. Differences in initial nutrient levels and in the time required to acclimate to low Fe stress may help to explain these disparities.

Studies (see Bates 1998; Kotaki et al. 2000; Chap. 19) have also shown that bacteria play an important role in enhancing DA production by *P. multiseries*; there is still no conclusive proof of autonomous production of DA by bacteria. These bacteria may be attached (Kaczmarska et al. 2005a) or free-living. Certain bacteria may provoke *Pseudo-nitzschia* to produce DA. One hypothesis (Osada and Stewart 1997) is that some bacteria (e.g., *Alteromonas* spp.) produce chelating agents (e.g., gluconic acid) that remove essential trace metals from use by the *P. multiseries* cells. To counter this, the diatom may produce its own chelator, i.e., DA. This hypothesis links the potential role of DA as a chelator with the observed stimulation of DA production by bacteria. Ultimately, field studies are required to tie together the various findings of laboratory studies.

Understanding the influence of other biotic and abiotic factors is necessary to help predict blooms and their toxicity. Photoperiod differentially affects the growth of *P. delicatissima* and *P. seriata* and the toxicity of *P. seriata* (Fehling et al. 2005), as well as the sexual reproduction of *P. multiseries* (Hiltz et al. 2000). The timing of sexual reproduction is important, as the cells cannot increase in number while undergoing gametogenesis, and cell toxicity may change with the sexual stage (Davidovich and Bates 1998; Bates et al. 1998). Elevated pH, as can be found during intense blooms, also enhances DA production by *P. multiseries* when growth rates decrease with increasing pH (Lundholm et al. 2004). *Pseudo-nitzschia* species are euryhaline (see also Bates et al. 1998), able to grow in culture from ca. 6–45 PSU and observed at salinities from 1 to ~35 PSU in Louisiana-Texas coastal waters (Thessen et al. 2005); on the other hand, these authors also found that *P. delicatissima*, *P.* cf. *pseudodelicatissima* and *P. multi-*

series have distinct salinity preferences for growth. The form of N may influence DA production. Nitrate- or ammonium-grown *P. australis* cultures in exponential growth produce equivalent amounts of DA, whereas DA production is enhanced in cultures growing on urea as their sole N source, while their growth rate is reduced (Cochlan et al. 2005). Regarding biotic factors, it is curious that *P. multiseries* lacks allelopathic effects (Lundholm et al. 2005; see also Bates 1998; Chap. 15), given that almost monospecific blooms may last for extended periods. Parasitic fungi and viruses may also play an important role in *Pseudo-nitzschia* bloom dynamics (see Bates et al. 1998).

An understanding of the hydrographic environments in which *Pseudo-nitzschia* spp. thrive will aid in bloom prediction (see Chap. 10). One approach is to study retentive zones where phytoplankton, including HAB species, accumulate because of unique chemical, biological and physical characteristics. Field surveys have shown that toxigenic *Pseudo-nitzschia* spp. are found at certain seasonally retentive sites, e.g., the Juan de Fuca eddy region (Washington State), Heceta Bank (Oregon), and Point Conception (California) (Trainer et al. 2001). Toxic cells can be reliably found during summer months in the Juan de Fuca eddy region, a "natural laboratory" where ecological studies can be carried out with field populations to determine environmental factors that enhance or diminish DA-producing capabilities (Trainer et al. 2002; Marchetti et al. 2004).

Because *Pseudo-nitzschia* spp. are planktonic, their movement depends greatly on the surrounding ocean physics (e.g., Horner et al. 2000). Topographical features (e.g., canyons, shallow shelves, sills) influence both nutrient flux and phytoplankton placement in retentive regions. The coupling of physical and biological processes has concentrated *Pseudo-nitzschia* cells into layers from several meters (Ryan et al. 2005) to less than a meter (Rines et al. 2002) thick; these may be missed by normal sampling techniques. Apparently healthy cells in deep layers may be transported long distances, thus providing an inoculum to distant surface waters, resulting in an unexpected bloom. Another form of hidden flora is *P. pseudodelicatissima* cells intermingled within colonies of the diatom *Chaetoceros socialis* (Rines et al. 2002). This close association suggests a chemically mediated interaction, and may provide a competitive advantage by offering a microenvironment different from that of the water column.

7.5 Molecular Tools for Studying *Pseudo-nitzschia*

Molecular techniques are currently being applied to *Pseudo-nitzschia* spp. for identification and quantification (see Bates et al. 1998); elucidating phylogenetic relationships (Lundholm and Moestrup 2002; Lundholm et al. 2002a, 2002b, 2003; Orsini et al. 2002, 2004); discriminating among populations of the

same species (Orsini et al. 2004; Evans and Hayes 2004; Evans et al. 2004, 2005); and for gene discovery and expression profiling (Boissonneault 2004).

Studies of mating compatibility among strains of presumably the same species of *Pseudo-nitzschia* are augmenting information gained by genetic and classical morphology studies (Davidovich and Bates 1998; Evans et al. 2004; Amato et al. 2005). Such research is just beginning to confirm the existence of cryptic intraspecific diversity within *Pseudo-nitzschia* species. These studies may also help to explain the existence of the great physiological variability, including toxin production (e.g., Kudela et al. 2004), among different *Pseudo-nitzschia* strains of the same species. The investigation of intraspecific genetic diversity (e.g., Evans et al. 2004) will help us to understand how individual cells within a population can respond differentially to changing environmental conditions.

Identifying and characterizing genes that are related to DA biosynthesis will be valuable for further understanding *Pseudo-nitzschia* physiology and ecology. Two approaches are being taken to elucidate these genes. Boissonneault (2004) designed a cDNA microarray to screen for genes whose expression patterns were correlated with DA production in *P. multiseries*. Expression analysis of 5,372 cDNAs revealed 12 transcripts that were up-regulated during toxin production in stationary phase; among them were several that may be directly involved in DA metabolism. This study demonstrates the potential of applying cDNA microarray technology to investigate the control of toxin production in *P. multiseries*. It has also currently generated sequence data for 2,552 cDNAs, providing a database of actively expressed gene tags that may be used as markers or for further characterizing specific biological functions within *Pseudo-nitzschia*.

A second approach is using subtraction hybridization techniques on Si-replete and deplete cultures of *P. australis* to identify genes that are involved in DA production (B. Jenkins, E. Ostlund, V. Armbrust pers. comm.). Sequence analysis of the subtracted libraries has revealed genes implicated in amino acid transport and biosynthesis. The data provided by both of these described approaches, the completion of the first diatom genome project using *Thalassiosira pseudonana* (Armbrust et al. 2004), and the recent start of the whole genome sequencing of *Pseudo-Nitzschia* multiseries (carried out by E.V. Armbrust, B. Jenkins, and S. Bates), will greatly assist in characterizing *Pseudo-nitzschia* genes involved in cell growth and physiology, including DA production.

7.6 Conclusions and Directions for Future Research

Field sites where toxic diatoms can reliably be found provide natural laboratories where researchers can determine the role of environmental factors in

influencing toxin production through manipulation of healthy populations of cells. These sites will enhance our understanding of why certain coastlines are plagued by recurring toxigenic blooms, whereas others are not. The establishment of long-term monitoring programs in coastal regions is essential to our understanding of the survival mechanisms of toxic and harmful algae amidst the larger complex of phytoplankton species. It has been suggested that the increased incidence and intensity of HABs may be linked to regime shifts, manifested as changes in strength of the North Pacific and North Atlantic pressure systems (Hayes et al. 2001). Global factors also affect the plankton; e.g., high sea surface temperatures increase photosynthesis and metabolism and may contribute to the growth of tropical and temperate species in higher northern and southern latitudes. Only through the collection of comprehensive data sets that address specifically the mechanisms of survival of these highly successful diatoms, will such questions be answered. Further understanding of the factors that control growth and DA production (e.g., Fe limitation, bacteria) will be assisted by the current development of molecular tools.

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