Trainer, V.L., B. Hickey, and S.S. Bates. 2008. Toxic diatoms. In: P.J. Walsh, S.L. Smith, L.E. Fleming, H. Solo-Gabriele, and W.H. Gerwick [eds.], Oceans and human health: risks and remedies from the sea. Elsevier Science Publishers, New York, p. 219-237.

CHAPTER

12

Toxic Diatoms

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INTRODUCTION

A tragic event in 1987 changed forever the way diatoms are viewed. Most diatoms are beneficial to the oceans' ecosystems and ultimately to human health. However, a unique food poisoning in eastern Canada led to the illness of more than 100 people and the death of four elderly individuals. The potent neurotoxin domoic acid (DA) was found to be the culprit, and its source was the marine diatom *Pseudo-nitzschia multiseries*. This event resulted in intense scientific interest in this genus, because it was the first time that a diatom had been shown to produce a toxin. The clinical syndrome was subsequently called amnesic shellfish poisoning (ASP) because of one of its reported symptoms, memory loss. Here, we introduce the general biology of diatoms and focus specifically on toxic diatoms of the genus *Pseudo-nitzschia*. We also discuss DA and its mode of action, describe oceanographic factors that lead to the growth of this toxic diatom, and include specific examples of *Pseudo-nitzschia* blooms and related physiology.

GENERAL BIOLOGY OF DIATOMS

Diatoms are microscopic single-celled plants that live in freshwater and marine ecosystems, from the poles to the tropics. They are found in sea ice and snow; some are even blown into the air. Benthic diatoms live in or on the sediments and on the surface of other plants (epiphytic), animals (epizootic), or rocks (epilithic). Those that live in the water column, together with the dinoflagellates (Chapter 13), other flagellates, and blue-green algae (cyanobacteria) (Chapter 15), among others, make up the phytoplankton (Greek *phyton* = "plant," *planktos* = "wanderer"), also called *micro-algae*. Diatoms are the most abundant of the phytoplankton based on biomass and numbers of species; there are estimated to be greater than 50,000 species in the aquatic ecosystem, and up to 18,000 in the marine environment (Fryxell and Hasle, 2003).

Some diatoms are found as single cells; others form long "chains" by linking to the adjacent cell, either by abutting end to end or by joining their protruding spines or setae. Like all plants, diatoms contain chlorophyll and other pigments that capture the energy of sunlight to convert carbon dioxide and water molecules into carbohydrates via photosynthesis. Their survival also requires nutrients such as nitrogen, phosphorus, silicon, and trace metals. Diatoms are a major source of the world's oxygen, producing amounts equivalent to all the tropical rain forests (Field *et al.*, 1998). They are also responsible for 40% to 45% of the total production of organic carbon compounds in the oceans (Mann, 1999), or 20% to 25% for the entire Earth. As such, they are a significant source of food for other organisms within the marine food web and play an important role in the biogeochemical cycling of elements (Sarthou *et al.*, 2005). Because of their large cell size and rapid sinking rate, they also play an important role as part of the "biological pump" that removes carbon dioxide from the atmosphere and places it at depth, thus helping to moderate the increasing levels of carbon dioxide associated with climate change.

The name *diatom* comes from the Greek word *diatomos* (*dia* = "through" + *temnein* = "to cut"), meaning "cut in two." This is because a diatom cell is composed of two overlapping halves (thecae) that fit together like a petri dish: the upper half is called the "epitheca" and the lower half the "hypotheca" (Fig. 12-1). The theca is composed of an upper (epi-) or lower (hypo-) valve, plus girdle bands that encircle the middle of the cell to hold the two halves together. Each valve possesses a slit, called a "raphe," that runs along the whole length of the valve and is reinforced on the interior

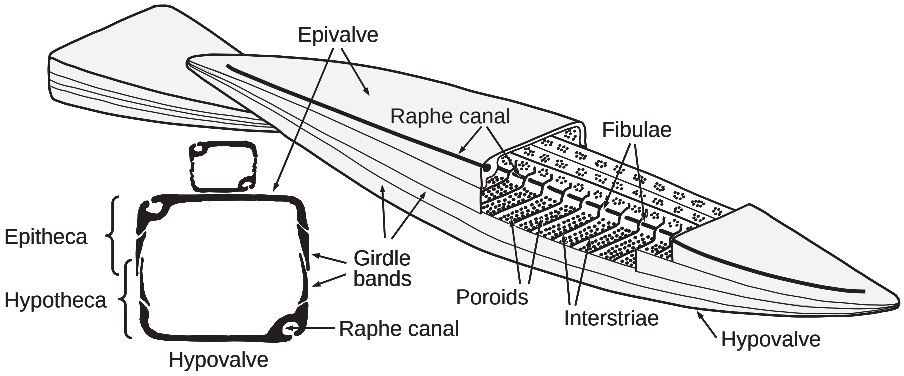

FIGURE 12-1. Silica frustules of *Pseudo-nitzschia multiseries* shown diagrammatically as one cell overlapping part of another, with cell length much reduced for illustration purposes. Raphe is shown on edge of upper valve (epivalve) and opposite corner of lower valve (hypovalve). Section of epitheca removed to show raphe canal, interstriae, three to four rows of poroids in the stria areas, and several poroids on the inner side of girdle bands that encircle the frustule. Diagram at left shows cross-section of two overlapping cells, with raphe canal in diagonally opposite position. Redrawn from MacPhee *et al.* (1992).

side with bridges, called "fibulae." The valves are ornamented with striae (strips containing rows of small poroids), alternating with narrower riblike strips called "interstriae." The entire diatom shell is called the "frustule" and is composed of silica (SiO_2 = glass) that is covered by an organic membrane through which nutrients pass for cell growth. Based on how the silica ribs on the valve radiate, diatoms (division Heterokontophyta, class Bacillariophyceae) are placed into two orders: radially symmetric (round) cells are called centric diatoms and longitudinally symmetric (long, narrow) cells are pennate (from the Latin *penna*, meaning "feather") diatoms. *Pseudo-nitzschia* species are pennate diatoms.

Centric diatoms have flagellated gametes but are otherwise swept passively by currents. Pennate diatoms are capable of limited motility. With *Pseudo-nitzschia*, single cells, or an entire chain of cells, can be seen gliding across a solid surface (such as a microscope slide), sometimes stopping and then reversing direction. The role of this motility in *Pseudo-nitzschia* is uncertain because these cells are found mostly suspended in the water column. Motility is facilitated by a raphe, the longitudinal slit that runs along an edge of the valve face (Fig. 12-1). Mucus is secreted through the raphe and attaches to particles or substrata; as the diatoms move, they leave a trail of this sticky substance. The details of what causes the motive force are still somewhat mysterious, but filaments of the protein actin are thought to change shape, thus moving the cell along.

Diatoms encounter a serious problem because their frustule is composed of solid silica and the hypotheca is slightly smaller than the epitheca. During vegetative cell division, the daughter cell formed on the side of the hypotheca is thus slightly smaller than the parent cell. As cell division continues, the average cell size of the population therefore decreases. Eventually, the cells can become so small that they are no longer able to divide, and they die. This occurs in clonal cultures (i.e., started with one cell) of *Pseudo-nitzschia* and other pennate diatoms. However, cells of many sizes, including large cells, are found in the ocean; most diatoms are able to restore their large size via sexual reproduction. In centric diatoms, the same cell can produce both small flagellated male gametes plus large nonmotile female gametes (i.e., they are homothallic and oogamous).

In pennate diatoms, clones of opposite mating type are needed (i.e., they are heterothalic), and they produce nonflagellated gametes of the same size (i.e., they are isogamous). Thus, mating will not occur in a clonal culture; a clone of opposite mating type must be added. In both types of diatoms, the gametes fuse, and an oval (in centrics) or elongated (in pennates) structure, called an auxospore, is produced. A large initial cell is formed within the fully expanded auxospore and is eventually released, thus restoring the large cell size of the diatom cell. The sexual reproduction of *Pseudo-nitzschia* species can affect bloom dynamics (population growth) and perhaps even toxicity (e.g., Davidovich and Bates, 1998). For a more detailed description of the evolution, morphology, sexuality, systematics, taxonomy, and biology of diatoms, see Round *et al.* (1990) and Hasle and Syvertsen (1997).

Because they are abundant, geographically widespread, live in a wide variety of environments, and are generally well preserved, modern and fossilized diatoms have many uses by human society (Stoermer and Smol, 1999). Fossilized diatom frustules form diatomaceous earth, also called diatomite, which is commonly used as a filter medium in swimming pools and aquaria, a mild abrasive in metal polishes and toothpaste, cat litter, insulating material, chromatographic separation material such as silica gel, a mechanical insecticide (it works by cutting, and thus dehydrating the insects), an absorbent for toxic spills, and in the manufacture of dynamite, among other uses. Fossilized diatoms are also used in paleolimnology and paleoceanography to reconstruct past climate changes. Modern diatoms are used as an indicator of eutrophication (i.e., the enrichment of water by nutrients, especially nitrogen and phosphorus, causing an accelerated growth of algae and leading to undesirable disturbances in the balance of organisms and in water quality) and acidification (i.e., the negative consequences of acid rain). Forensic investigators look for diatom frustules in the lungs to determine if a person died from drowning; they can also tell if the drowning occurred in a marine or freshwater environment (Pollanen, 1997). Nanotechnologists are studying how diatoms lay down their intricate frustules in order to apply this to building smaller silicon computer chips (Bradbury, 2004).

Diatoms can be grown in mass culture in order to extract natural products for biotechnological applications: total lipids for biodiesel fuel, amino acids for cosmetics, antibiotics and antiproliferative agents for the medical field, and silicon derived from frustules for use in nanotechnology (Lebeau and Robert, 2003). The diatom, *Nitzschia laevis*, is grown on a semi-industrial scale for the production of eicosapentaenoic acid (EPA), a polyunsaturated omega-3 fatty acid widely recognized for its beneficial effects on human health (see Chapter 10). Mass cultures containing up to 200 species of marine diatoms are grown for the production of natural supplements, called phytonutrients, reported to boost human health. Thus, diatoms are an essential source of food for aquatic organisms, of products for human needs, and of oxygen for all living creatures.

Because diatom cells are made of silica, they are generally denser than seawater. Therefore, they require special oceanographic conditions, such as mixing and upwelling (i.e., the wind-driven movement of dense, cool, and usually nutrient-rich water toward the ocean surface to replace the warmer, usually nutrient-depleted surface water) that allow them to grow within the sunlit upper layer of the ocean. Currents may also benefit the cells by transporting them to areas that may be more conducive to cell growth. The cells may then proliferate rapidly, causing a “bloom.” Most diatom blooms are beneficial. However, when the concentration of some diatom species reaches bloom proportions, their pigments may color the water. Such blooms are popularly known as “red tides,” which can also be composed of dinoflagellates or other types of phytoplankton. Scientists now prefer the term “harmful algal bloom” (HAB), because these blooms do not always color the water red (they may be other colors or even colorless), and they are certainly not tides (Glibert *et al.*, 2005a).

Some HABs contain diatom species that can harm other marine organisms by physical means (e.g., they have spines or setae that can damage the gills of finfish) or by removing oxygen from the water because of decomposition when they die. Such diatoms have no direct impact on human health and are discussed elsewhere (Fryxell and Hasle, 2003). However, a few (<20) diatom species cause harm by producing toxins that affect molluscan shellfish, marine mammals, birds, and even humans. *Pseudo-nitzschia* was the first diatom genus known to produce a compound that is toxic to humans and other animals. This naturally occurring toxin is called domoic acid (DA), and the syndrome of DA poisoning in humans is called ASP. The remainder of this chapter will focus on toxic diatoms of the genus *Pseudo-nitzschia*: their production of DA, the effects of this toxin on human health, and the importance of oceanography in triggering, maintaining, and dissipating *Pseudo-nitzschia* blooms.

THE DISCOVERY OF AMNESIC SHELLFISH POISONING

Molluscan shellfish, such as mussels, clams, oysters, and scallops, feed directly on phytoplankton cells suspended in the water column; some individuals filter up to 200 liters of seawater per day. Most food items are beneficial. However, a small portion of marine phytoplankton, only about 100 out of 5000 species (or about 2% of all species), produces algal toxins (phycotoxins) that can be deadly. These phycotoxins become concentrated in the digestive tract of molluscan shellfish as a result of their filter feeding and are thus easily passed on to humans, or other animals, who consume them. Although most shellfish are not affected adversely by the phycotoxins, humans can become seriously sick or even die as a result of consuming these contaminated shellfish. Fortunately, most countries around the world have public health agencies that monitor for the presence of phycotoxins in molluscan shellfish, or the numbers of toxic algal cells in the seawater (see below). When levels of a phycotoxin within the shellfish reach the regulatory limit, harvesting is prohibited until the toxin decreases to acceptable levels after the toxic bloom has dissipated. This has prevented human poisonings, except in cases where individuals illegally harvest shellfish from an area closed to harvesting.

Before 1987, the group of toxin-producing phytoplankton of concern to human health included mostly dinoflagellates; different species of these flagellated cells produce toxins causing paralytic shellfish poisoning (PSP; saxitoxins), diarrhetic shellfish poisoning (DSP; okadaic acid and dinophysis toxins), neurotoxic shellfish poisoning (NSP; brevetoxins) (Chapter 13), and ciguatera fish poisoning (CFP; ciguatoxin) (Chapter 14) (Landsberg *et al.*, 2005). Then, in late autumn, 1987, an outbreak of an unusual human poisoning in Canada was traced to blue mussels (*Mytilus edulis*) that originated at aquaculture sites in bays of eastern Prince Edward Island (Bates *et al.*, 1989). The known shellfish poisoning toxins were quickly ruled out; it was suspected that something new was occurring.

Within a span of only a few days in late November and early December, more than 250 individuals who had consumed these mussels were admitted to hospitals in Quebec and New Brunswick. They had varying degrees of an acute illness characterized by gastrointestinal problems and unusual nervous system abnormalities (Perl *et al.*, 1990). A case was defined as the occurrence of gastrointestinal symptoms within 24 hours, or of neurological symptoms within 48 hours, of consuming the mussels. Of those 107 patients who met this strict case definition, most showed symptoms of vomiting (76%), followed by abdominal cramps (50%), diarrhea (42%), headache—often incapacitating (43%)—and loss of short-term memory (25%). Nineteen patients remained in hospital; 12 required intensive care because of seizures, profuse respiratory secretions, or unstable blood pressure. Approximately equal numbers of males and females were affected (47 and 60 patients, respectively); 46% were 40 to 59 years of age, and 36% were 60 or older. Three elderly male patients (ages 71, 82, and 84) died in hospital, and a fourth (84 years of age) died 3 months after eating the mussels. A closer evaluation of 14 of the more severely affected patients detailed the degree of confusion and disorientation that occurred within 1.5 to 48 hours after consuming the contaminated mussels (Teitelbaum *et al.*, 1990). Most of these patients had difficulty remembering ongoing events occurring since consuming the toxic mussels (i.e., they had a predominantly anterograde amnesia or short-term memory loss). However, some seriously affected patients also had difficulty remembering events that happened several years before the mussel-induced intoxication (i.e., they had retrograde amnesia). Because of the memory problems, the term *ASP* was later given to this clinical syndrome. However, *DA poisoning (DAP)* is sometimes used because shellfish are not always the vector. For a critical review of studies linking HAB illnesses with neuropsychological impairments, see Friedman and Levin (2005).

Identification of Domoic Acid, Its Structure and Mechanism of Action

During these hospitalization episodes, an intensive search began in the mussels for the causative toxin. A bioassay-directed strategy was taken, whereby extracts of both toxic and control mussels were injected into mice to determine which fraction elicited a toxic response (Quilliam and Wright, 1989). Various chromatographic techniques were used to characterize and eventually identify the toxic compound. After an unprecedented 4-day round-the-clock investigation, the molecule in the toxic fraction was pinpointed as a known compound, DA. This was at first met with disbelief, as the literature showed that DA had been used as a treatment in Japan to remove intestinal worms in children. Tests performed in the mid-1950s on the antihelmintic (vermifuge) properties of DA found that a single dose of 20 mg could be given to children and adults without harmful effects. However, in the 1987 event, the two persons with the most severe neurological symptoms ingested 290 mg (i.e., an order of magnitude greater than that given to the Japanese children); those who were unaffected ingested from 15 to 20 mg per person (Perl *et al.*, 1990). As well, those affected in 1987 were elderly and had preconditions that made them more vulnerable (see below). Assuming an average body weight of 50 to 70 kg, DA would have no effect at 0.2 to 0.3 mg/kg, mild effects at 0.9 to 2.0 mg/kg and serious effects from 1.9 to 4.2 mg/kg (Tasker, 2002).

DA was originally isolated from a species of red macroalga (*Chondria armata*); its name comes from "domoi" (or "doumoi"), a local name for that seaweed in Japan (see Bates *et al.*, 1998). It has since been isolated from five other species of red macroalgae. Japanese scientists observed that flies that were attracted to and contacted *C. armata* drying on the seashore died shortly afterward. DA was later shown to have insecticidal properties and was 14 times more potent than DDT when administered into the abdomen of the American cockroach. Another red macroalga, *Digenea simplex*, isolated in southern Japan, was found to contain kainic acid (Fig. 12-2). Both of these "toxins" had been used as a vermifuge for Japanese children.

DA (also called "domoate" in the neurophysiological literature) is a low-molecular-weight (311 daltons), water-soluble, heat-stable, secondary amino acid (Fig. 12-2). It is a member of the kainoid class of organic compounds that includes kainic acid, the marine toxin described earlier. Both domoic and kainic acids contain a domain that is structurally identical to the amino acid, glutamate (glutamic acid), a compound important for proper functioning of the nervous system (Fig. 12-2). Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system; it is responsible for many of the functions within the brain, including cell-to-cell communication and hippocampal long-term potentiation, a process important in learning and memory. As a neurotransmitter, it takes part in the transmission of a nerve impulse from one neuron to another. Simply put, glutamate binds onto specific receptor sites (i.e., NMDA, AMPA, and kainate sites, named after the molecules that stimulate them) on the membrane of the nerve fiber (dendrite). This causes the receptor molecule to undergo a change in its conformation, or shape. In turn, this opens up a microscopic channel (like a gate) in the membrane, which allows the influx of sodium or calcium into the axon (Fig. 12-3). As a result, the neuron is triggered, sending an impulse down its axon fiber via Na^+ and K^+ mediated action potentials (see Chapter 28).

Glutamate is released by the presynaptic neuron into the synaptic cleft (the narrow space between the two abutting nerve cells) and subsequently binds to the receptor sites on the dendrite of the postsynaptic neuron. This causes the next

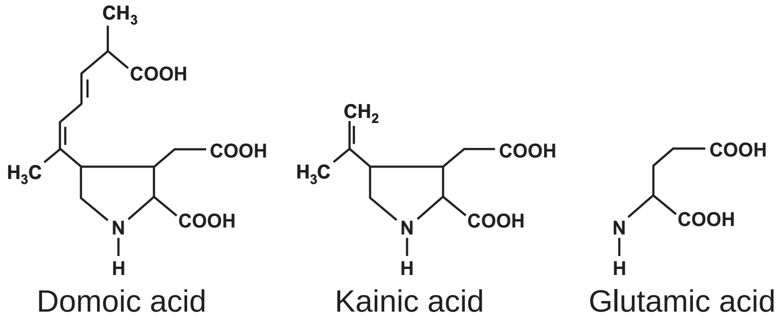

FIGURE 12-2. Structure of domoic acid and its analogues. From Bates *et al.* (1998).

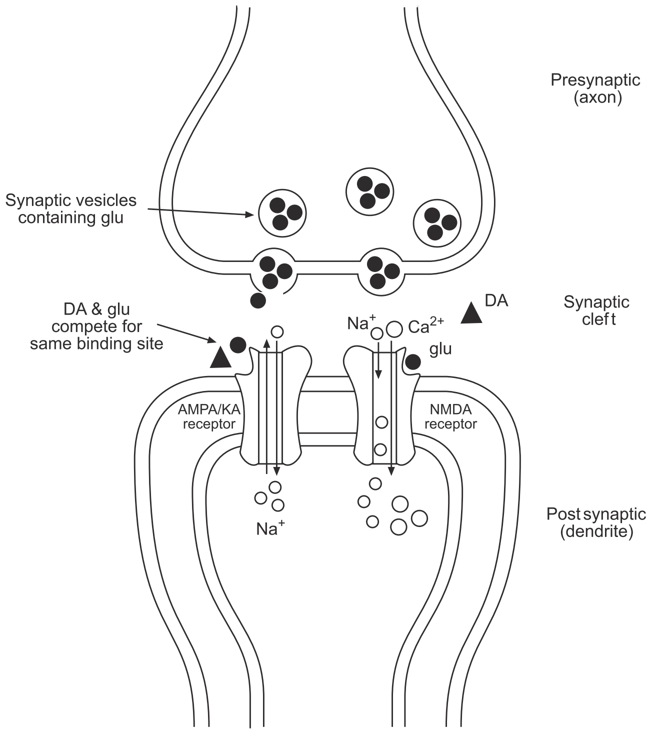

FIGURE 12-3. Diagram of a nerve showing its axon and dendrite. Glutamate (glu) contained in synaptic vesicles is released from the nerve terminal into the synaptic cleft. In this example, DA and glu are competing for the same binding site on the AMPA or kainate (KA) receptor. The activation of AMPA/kainate receptors upon DA binding leads to nerve depolarization and coactivation of NMDA receptors by glutamate binding. Calcium enters through activated NMDA receptors. The sum of this prolonged activation results in ion disturbances and swelling, eventually resulting in DA-induced nerve cell death.

neuron to fire, sending its message down the line. The neuronal firing is controlled because the glutamate is rapidly reabsorbed back into the neuron bulb or is inactivated by special enzymes; this removal closes the sodium and calcium channels. However, cell damage and cell death can occur when excessive amounts of glutamate are released from neuronal cells and cannot be removed.

Blaylock (1994) provided a layperson's explanation of this phenomenon, as well as a description of the toxicity of monosodium glutamate (MSG; the flavor-enhancing food additive, which is simply a glutamate molecule with a sodium ionically bound), and how this relates to the neurophysiology of DA toxicity. Blaylock uses a "key" and "lock" analogy: because the glutamate and DA molecules ("keys") are structurally similar, they both fit into the same receptor (the "lock") on the neuron surface. The five-sided ring structure of DA makes it less flexible than glutamate, which causes it to bind more tightly, resulting in a 30 to 100 times more powerful effect per molecule than seen with glutamate. Whereas glutamate, at low concentration, is rapidly removed, DA is not. Thus, DA affects the brain in a way similar to glutamate neurotoxicity; the neuron becomes over stimulated, meaning that calcium continues to flood into the cell, ATP energy reserves become depleted in an attempt to pump out the excess calcium, and then the neuron begins to swell with water, causing it to burst (as in hepatic encephalopathy, see Chapter 29). DA binds with high affinity (i.e., at low nanomolar concentrations) to kainate receptors that contain either of the protein subunits designated as "GluR5" and "GluR6." These subunits are both highly expressed in the hippocampus, a part of the brain associated with processing and laying down new memories; this explains the memory deficits shown by the patients poisoned with DA (hence the name amnesiac shellfish poisoning or "ASP"). Doble (2000) provided a review of the pharmacology of DA, and Ramsdell (2007) reviewed the molecular and integrative basis of DA toxicity.

Organismal Susceptibility to Domoic Acid

It is remarkable that the major brain regions affected by DA and the resulting behavioral effects of ASP are consistent among different mammalian species (Jeffery *et al.*, 2004). For this reason, experimental organisms (such as mice, rats, rabbits, or monkeys) can be used experimentally to understand the mechanisms of toxicity in humans. There are, however, some species differences in the potency of DA. For example, mice are less sensitive than rats, which in turn are less sensitive than monkeys and humans. It is notable that molluscan shellfish (including oysters, clams and most mussels) appear to be resistant to even huge doses of DA (1000 µg/g or mg/kg = ppm), in contrast to humans who appear to be sensitive to DA at much lower doses (as discussed previously). It has been speculated that some shellfish may contain binding proteins that sequester toxins away from their nerves or that their receptors are altered by mutation at the site of toxin binding (Trainer and Bill, 2004).

The effects of long-term exposure of humans to low concentrations of DA in shellfish are poorly understood. However, there is concern that populations that subsist heavily on shellfish may be at increased risk of chronic, long-term exposure. An epidemiological study of DA exposure in a Native American tribe in Washington State has suggested that infants born in years when DA levels in coastal razor clams were above the regulatory limit of 20 µg/g had lower mental development indices than infants born in other years (Grattan *et al.*, 2003). Further studies must be carried out to assess effects of long-term exposure to DA in humans.

Exposure of DA in mice and rats has indicated an age dependence of neurotoxicity (Ramsdell, 2007). Neonatal rats appear to be up to 40 times more susceptible to DA exposure than adults, presumably because of insufficient clearance of toxin in the poorly developed renal system and to incomplete development of the blood-brain barrier. This is significant because at ecologically relevant levels, DA can be transferred to the neonate via breast milk. However, the amount of toxin transferred appears to be well below symptomatic levels.

Older humans, especially males, may be particularly sensitive to the effects of DA, as evidenced by the four males over the age of 70 who died in the 1987 intoxication event in Canada (Perl *et al.*, 1990; Teitelbaum *et al.*, 1990). Dose-responses for seizures upon DA exposure are about three times greater in aged rats compared to young rats. The mechanism for increased sensitivity to domoic and kainic acids in elderly humans has not been precisely determined. However, decreased renal clearance and changes in excitatory and inhibitory pathways are known to accompany aging. In addition, the loss of a tolerance to DA that diminishes with age appears to be related to a reduction in constitutive GTPase activity in the rat hippocampus.

Identification of *Pseudo-nitzschia* Species

The challenge in the 1987 DA episode was to identify the source of the DA that contaminated the mussels in Cardigan Bay (eastern Prince Edward Island). Rapidly, the known sources of DA (i.e., several species of red macroalgae) were ruled out, as there were not enough of these seaweeds to account for the estimated 1000 kg of DA produced in Cardigan Bay (Bates *et al.*, 1989). Examination of the seawater revealed an almost monospecific bloom of a chain-forming diatom, *Pseudo-nitzschia multiseries* (then called *Nitzschia pungens* forma *multiseries*), reaching concentrations as high as 15 × 10^6 cells per liter. Furthermore, the digestive tract of the mussels was engorged with identifiable fragments of this same diatom. Finally, cells from Cardigan Bay were isolated into culture and were confirmed to produce DA.

The initial research focused on the unambiguous identification *Pseudo-nitzschia*. As pennate diatoms, the cells have a "lanceolate" shape (i.e., they are long and narrow, gradually tapering toward the ends). What distinguishes them from all other pennate diatoms is that the tips overlap slightly so that the cells form chains ("stepped" colonies, Figs. 12-4 and 12-5a). If left undisturbed (e.g., in a petri dish or in calm ocean waters), chains composed of dozens of cells can be formed. Because the cells narrow toward their tips, each cell that attaches to the next one near the tip is at a slight angle to it; the result is that the chains can form long spirals. When some *Pseudo-nitzschia* species cease growing, however, the chains may fall apart into single cells. Hasle and Syvertsen (1997) and Fryxell and Hasle (2003) provided a guide to the identification and distribution of marine diatoms, including *Pseudo-nitzschia* species.

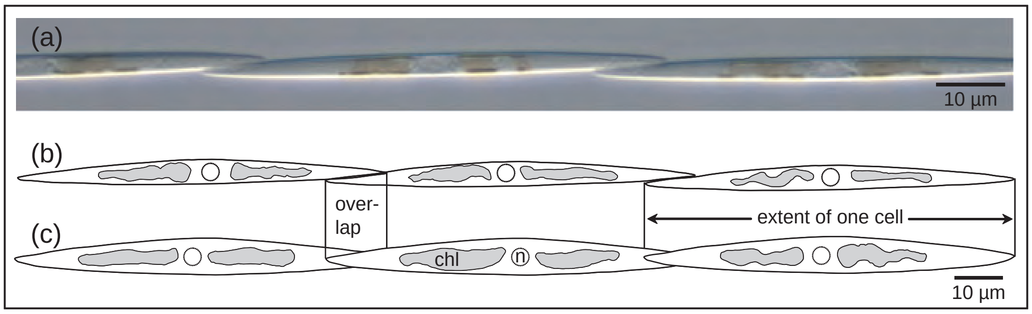

FIGURE 12-4. Chain of *Pseudo-nitzschia* cells. (a) light microscope image of *P. multiseries*, showing girdle (side) view (photo courtesy of Karie Holtermann, University of Washington). (b) Drawing of girdle view. (c) Drawing of valve (top) view. The general shape of the cells and the degree of cell overlap are characteristics used for identifying the species. chl = chloroplast; n = nucleus. Redrawn from Fehling (2004).

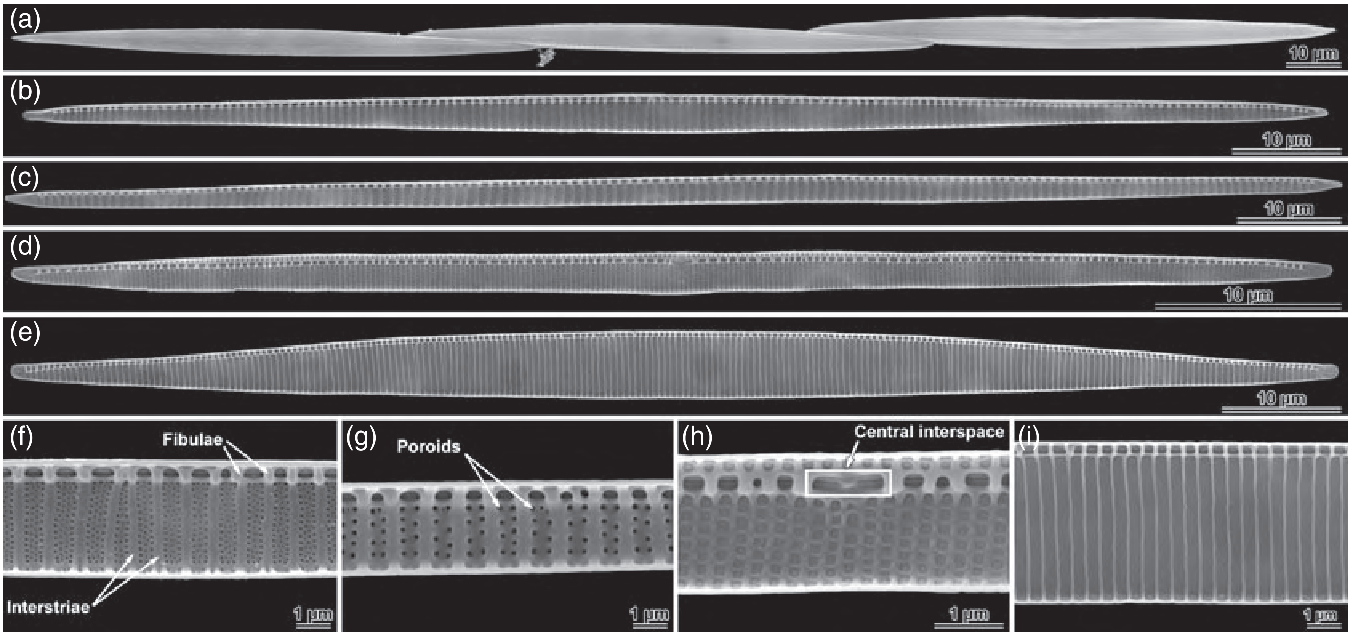

FIGURE 12-5. (a) Scanning electron micrograph (SEM) of ethanol-dehydrated/freeze-dried *Pseudo-nitzschia pungens* in girdle view, showing a chain of cells. (b–e) SEMs of acid-cleaned *Pseudo-nitzschia* species at low magnification, showing the entire cell; and (f–i) at high magnification, showing the central part of the cell. (b, f) *P. multiseries*; (c, g) *P. pungens*; (d, h) *P. calliantha*; and (e, i) *P. seriata*. Notice differences in width of the cells, size, and shape of poroids (but too small to be seen in *P. seriata* image i), number of interstriae and fibulae per unit distance, and the presence (h) or absence (f, g, i) of a central interspace (the large oval structure); all of these characteristics are used to distinguish among species. SEMs from James Ehrman, Digital Microscopy Facility, Mount Allison University, Canada.

From the point of view of human health, it is particularly important to be able to distinguish among the approximately 30 species of *Pseudo-nitzschia*. Of those species, at least 12, thus far, have been shown to produce DA in laboratory culture (Table 12-1). However, not all strains (i.e., organisms of the same species having minor genetic or morphological differences but not considered separate species) produce toxin at detectable levels, while others produce only small amounts of DA. To be able to demonstrate DA production by a species, a single cell (or a chain of cells) must be isolated using a capillary pipette and placed into a seawater medium that contains added nutrients, trace metals, and vitamins to enable their growth. Cells are then harvested at different times from the culture flask: first when they are dividing exponentially and then later when they run out of nutrients and stop dividing during the stationary phase; most species of *Pseudo-nitzschia* produce DA primarily when their growth is slowed (Bates, 1998). They are then analyzed for DA content using any of several available analytical techniques (Quilliam, 2003) or *in vivo* assays (Cembella *et al.*, 2003). This approach assures that the species in question is the source of the toxin; information is also gained about the growth conditions that promoted toxin production.

In general, *Pseudo-nitzschia* species cannot be identified definitively using only light microscopy because of the need to see the fine structure of the frustule. *Pseudo-nitzschia* cells in field samples can initially be divided into those that are wider than 3 µm (the "*seriata* group") and narrower than 3 µm (the "*delicatissima* group") using light microscopy (Hasle and Syvertsen, 1997). The degree of cell overlap can also be characteristic (Fig. 12-4). In some waters, it is possible to divide cells into three groups: (1) *multiseries/pungens*, (2) *australis/fraudulenta/heimii*, and (3) *pseudodelicatissima/delicatissima*. This is because the symmetrically wide, long shape of *P. multiseries/pungens* group can be distinguished by light microscopy from that of the asymmetrically wide, shorter *P. australis/fraudulenta/heimii* group and the much smaller *pseudodelicatissima/delicatissima* group (Trainer and Suddleson, 2005).

However, a definitive identification can only be made by examining the cell with a transmission electron microscope (TEM), or a scanning electron microscope (SEM). The cells are first cleaned with concentrated acids in order to remove the organic cell content as well as the outer organic layer. This exposes the silicon valves, which have intricate structural elements, including poroids, fibulae, interstriae, and, if present, a central interspace (Fig. 12-5). The number and spacing of these genetically fixed ornaments, as well as the shape, length and width of the cell, are used to identify each species (Hasle and Syvertsen, 1997).

Although morphological characteristics are effective for identifying and enumerating *Pseudo-nitzschia* species, the

TABLE 12-1. Species of *Pseudo-nitzschia* proven to produce domoic acid (DA).

			Cell Size (μm)		
Species	**Year**	**DA (pg DA/cell)**	**Length**	**Width**	**Comments**
*P. australis**	1992	0.021–37	100	7.1	Some New Zealand and California isolates below DA detection level
*P. calliantha**	1990	0.007–0.221	71	1.6	Previous records may have been reported as *P. pseudodelicatissima*
*P. cuspidata**	2005	1.21×10^{-4}–0.029	48	1.8	Previous records may have been reported as *P. pseudodelicatissima*; the name of the species is in a state of flux; only two reports of toxicity, from Washington State; limited number of isolates tested for toxigenicity
*P. delicatissima**	1990	0.0002–0.12	56	1.8	Toxicity reported only for some isolates from Prince Edward Island, Washington State, and New Zealand; most isolates below DA detection level
*P. fraudulenta**	1998	0.03	90	5.9	Toxicity reported only for two isolates from New Zealand
*P. galaxiae**	2005	7.8×10^{-7}–3.6×10^{-4}	35	1.5	Only one report of toxicity, from the Gulf of Naples
P. multiseries	1988	0.1–67	99	4.2	All isolates shown to be toxic
*P. multistriata**	2002	0.001–0.697	52	3.3	Only one report of toxicity, from the Gulf of Naples
*P. pseudodelicatissima**	1990	0.007–0.221	74	1.4	May be confused with *P. calliantha* and *P. cuspidata*
*P. pungens**	1996	0.0018–0.47	110	3.6	Only reports of toxicity are for some isolates from Washington State and New Zealand; most isolates below DA detection level
*P. seriata**	1994	0.16–33.6	120	6.4	Toxicity reported only for isolates from the north Atlantic
*P. turgidula**	1996	0.033	55	3.0	Toxicity only reported for some isolates from New Zealand; identification uncertain

Shown are the year first reported, the range in DA concentration found in culture, and mean cell size. An asterisk (*) indicates that DA is below the limit of detection in certain laboratory isolates of that species. References are found in Bates (1998), Lundholm *et al.* (2003), and Bates and Trainer (2006).

SEM and TEM procedures are time consuming and costly. Therefore, a suite of other techniques has been developed in an attempt to expedite the process (Scholin *et al.*, 2003). These are based on designing molecular probes targeting ribosomal RNA sequences that are presumably unique to each species. However, the challenge has been that a probe developed for a *Pseudo-nitzschia* strain from one geographic region is not always able to detect the same species in another part of the world. Thus, it is sometimes necessary to tailor the probes for the diatom strains at the location studied.

Taxonomy of *Pseudo-nitzschia* Species

The taxonomy (i.e., the assigning of scientific names) of phytoplankton sometimes undergoes changes as new information about an organism's morphology or molecular characteristics is discovered. This has certainly been the case with the diatoms associated with DA production, and it can be a source of confusion. The first DA-producing diatom discovered was originally known as *Nitzschia pungens* forma *multiseries* (Bates *et al.*, 1989). However, based on morphological and molecular evidence, it became apparent that taxonomic revision was necessary. As a result, the species with overlapping cell ends were separated from the other species in the genus *Nitzschia* and moved to the genus *Pseudo-nitzschia* (first described in 1900 for cells that produced chains with overlapping ends) (Bates, 2000). Thus, all DA-producing diatom species, except for *Amphora coffeaeformis* (whose toxin-producing ability is disputed) and the newly discovered *Nitzschia navis-varingica*, are now in the genus, *Pseudo-nitzschia* (Bates, 2000). Nevertheless, because of advances in molecular techniques, we are now undergoing a period when some species of *Pseudo-nitzschia* are being reclassified, as some species are split and new species are being discovered (e.g., Lundholm *et al.*, 2003).

Geographic Distribution of Toxigenic *Pseudo-nitzschia* Species

Soon after the 1987 poisoning event in Canada, other species of *Pseudo-nitzschia* became recognized as toxin-producers in other parts of the world. Most *Pseudo-nitzschia* species proven to be capable of producing DA (i.e., toxigenic) in laboratory culture (Table 12-1) are thought to be cosmopolitan, although some are restricted to certain latitudes (Hasle, 2002). Figure 12-6 shows the locations around the world where toxigenic species of *Pseudo-nitzschia* have been identified. Although many locations are shown, only a few have actually reported problems with DA in marine shellfish, finfish (summarized in Table 12-2), birds, or mammals, as indicated by the shading in Figure 12-6. This

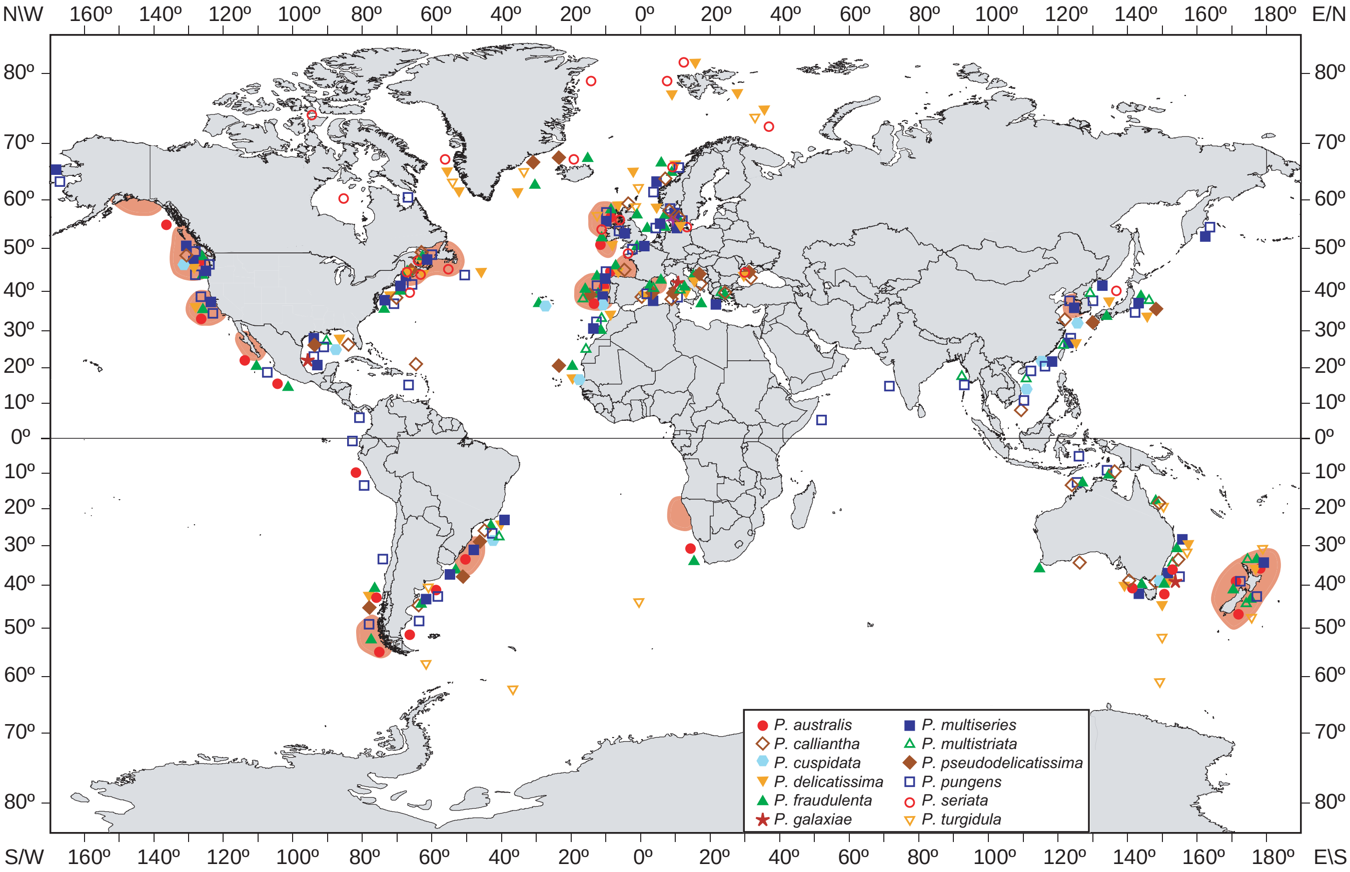

FIGURE 12-6. Worldwide recorded distribution of *Pseudo-nitzschia* species that have been shown to produce domoic acid in laboratory cultures; some strains do not always produce the toxin at detectable levels. Those regions that have experienced domoic acid contamination in marine zooplankton, fish, birds, molluscan shellfish, or mammals are shaded in red. Redrawn and updated from Fehling (2004).

may be because (1) strains of some toxigenic *Pseudo-nitzschia* species may not produce DA at detectable levels; (2) conditions may not be appropriate to trigger the pathway for DA production; (3) the cellular concentration of DA may be low (Table 12-1); (4) the relative abundance of the species may not be great enough, even if it does produce high amounts of DA, for the toxin to accumulate in the animals; and (5) shellfish retain or depurate (eliminate) DA at different rates. In some locations (e.g., coastal Mexico, Namibia, Newfoundland, Alaska), the *Pseudo-nitzschia* species that are the source of the DA contamination have not yet been identified with certainty.

Interestingly, toxigenic species are found mostly in coastal waters, thriving on high nutrient concentrations. Coastal locations are those most prone to high nutrient inputs, from terrestrial sources (Glibert *et al.*, 2005b) or upwelling events, and this could stimulate HABs. However, *Pseudo-nitzschia* species from midocean waters (e.g., Station P, eastern north Pacific) have also been shown to produce low but detectable levels of DA, as measured by the enzyme-linked immunosorbent assay (ELISA). To date, most ASP and DAP events have occurred in temperate regions, although most of the toxic or potentially toxic species can also be found in tropical or subtropical regions, and some of them have been involved in HAB events (Fig. 12-6). Although *Pseudo-nitzschia* species, including known toxigenic species, are found in both Arctic and Antarctic waters, there has been no testing for DA in these areas.

The presence of toxigenic *Pseudo-nitzschia* species in coastal waters around the world often overlaps with those areas that provide food, via aquaculture activities and wild harvests, for human consumption. To address this concern, many countries have established programs to monitor for the presence of phycotoxins in the flesh of molluscan shellfish and finfish destined for human consumption (Andersen *et al.*, 2003). In some countries, this is supplemented with counts of toxic phytoplankton in order to provide an early warning of impending toxicity and to direct further sampling of the food product (Todd, 2003). Trainer and Suddleson (2005) provide an example of such a program for DA events on the U.S. Pacific Northwest coast. The number of areas demonstrated to be affected by DA appears to be on the

TABLE 12-2. Chronology of events, showing the detection of domoic acid (DA) in various marine animals used for human consumption and the physical oceanographic regime in which the event occurred (WBS = Western Boundary System; EBS = Eastern Boundary System).

Location	**Year**	**Affected Species**		***Pseudo-nitzschia* Species Implicated**	**Oceanographic Regime**
		Common Name	**Scientific Name**		
Prince Edward Island, Canada	1987	Blue mussel	*Mytilus edulis*	*P. multiseries*	WBS, Shallow bay
Bay of Fundy, Canada	1988	Soft-shell clam Blue mussel Horse mussel Sea scallop	*Mya arenaria* *Mytilus edulis* *Volsella modiolus* *Placopecten magellanicus*	*P. pseudodelicatissima* or *P. calliantha*	WBS, Estuary
Washington and Oregon coasts, United States	1991	Razor clam Dungeness crab	*Siliqua patula* *Cancer magister*	*P. australis*	EBS, Upwelling
Monterey Bay, California, United States	1991	Northern anchovy	*Engraulis mordax*	Not directly linked	EBS, Upwelling, Bay
Pacific coast of the United States	1991 to 1993	Blue crab Rock crab Stone crab Spiny lobster	*Cancer spidus* *Cancer pagurus* *Menippe adina* *Palinurus elephas*	Not directly linked	EBS, Upwelling
Coastal New Zealand	1993 to 1997	Maori scallop Greenshell mussel Pacific oyster New Zealand cockle Chilean oyster Tuata surf clam	*Pecten novaezealandiae* *Perna canaliculus* *Crassostrea gigans* *Austrovenus stutchburyi* *Tiostrea chilensis* *Paphies subtriangulata*	*P. australis, P. pungens*	WBS, Upwelling
Galicia, NW Spain	1994	Mediterranean mussel	*Mytilus galloprovincialis*	*P. australis*	EBS, Upwelling
Georges, German and Browns Banks, Gulf of Maine	1995	Sea scallop	*Placopecten magellanicus*	*P. seriata* (likely)	WBS, Banks
Baja California peninsula, Mexico	1995	Pacific mackerel	*Scomber japonicus*	*Pseudo-nitzschia* spp.	EBS, Upwelling
Offshore Portugal	1996	Blue mussel Common cockle Peppery furrow shell clam Pullet carpet shell European oyster Razor clam Clam	*Mytilus edulis* *Cerastoderma edule* *Scrobicularia plana* *Venerupis pullastra* *Ostrea edulis* *Ensis* spp. *Ruditapes decussata*	*P. australis* (likely)	EBS, Upwelling
Chinhae Bay, South Korea	1998	Various shellfish	Not specified	*P. multiseries*	WBS, Shallow bay
Washington and Oregon coasts, United States	1991 to 2005	Razor clam	*Siliqua patula*	*P. pseudodelicatissima, P. australis*	EBS, Upwelling
Central coast, California, United States	1998	Northern anchovy	*Engraulis mordax*	*P. australis*	EBS, Upwelling
Offshore Scotland	1999 to 2000	King scallop	*Pecten maximus*	*P. australis, P. seriata*	EBS, Tidal, Downwelling
Offshore Ireland	1999	King scallop	*Pecten maximus*	*P. australis*	EBS, Tidal, Downwelling
Western Brittany, France	1999	Wedge shell clam	*Donax trunculus*	*P. multiseries*	EBS, Upwelling
Monterey Bay, California, United States	2000	Pacific mackerel Albacore tuna Northern anchovy Pacific sardine Market squid	*Scomber japonicus* *Thunnus alalunga* *Engraulis mordax* *Sardinops sagax* *Loligo opalescens*	*P. australis*	EBS, Upwelling

(*continued*)

TABLE 12-2. (*continued*)

Location	Year	Affected Species		***Pseudo-nitzschia*** **Species Implicated**	**Oceanographic Regime**
		Common Name	**Scientific Name**		
Offshore Portugal	2000 to 2001	European sardine European anchovy Blue mussel Pacific sardine Common cockle Pullet carpet shell Clam Oyster Razor clam	*Sardina pilchardus* *Engraulis enchrasicolus* *Mytilus edulis* *Sardinops sagax* *Cerastoderma edule* *Venerupis pullastra* *Ruditapes decussate* *Crassostrea japonica* *Ensis* spp., *Solen* spp.	Not determined	EBS, Upwelling
Southern Gulf of St. Lawrence, Canada	2002	Blue mussel	*Mytilus edulis*	*P. seriata*	WBS, Deep estuary
Offshore Portugal	2002	Swimming crab	*Polybius henslowii*	Not directly linked	EBS, Upwelling
Offshore Portugal	2003	Common octopus Common cuttlefish	*Octopus vulgaris* *Sepia officinalis*	Not directly linked	EBS, Upwelling
Puget Sound, Washington, United States	2003	Blue mussel	*Mytilus edulis*	*P. australis*	Deep estuary
Southern California, United States	2003 to 2004	Red crab Pacific mackerel Jack mackerel Pacific sanddab Longspine combfish	*Pleuroncodes planipes* *Scomber japonicus* *Trachurus symmetricus* *Citharichthys sordidus* *Zaniolepis latipinnis*	*P. australis* and *P. multiseries*	EBS, Upwelling
Monterey Bay, California, United States	2003 to 2004	Rex sole Dover sole English sole Curlfin turbot	*Errex zachirus* *Microstomus pacificus* *Pleuronectes vetulus* *Pleuronectes decurrens*	*P. australis* (likely)	EBS, Upwelling
Santa Cruz wharf, California, United States	2004	White croaker Staghorn sculpin	*Genyonemus lineatus* *Gymnocanthus tricuspis*	*P. australis* (likely)	EBS, Upwelling

The *Pseudo-nitzschia* species implicated may have been fed on either directly or indirectly by the animals. The table does not include marine zooplankton, birds, and mammals that have also been affected by DA. References are found in Bates *et al.* (1998) and Bates and Trainer (2006).

increase since the original 1987 ASP outbreak in eastern Canada. This is probably because toxigenic *Pseudo-nitzschia* species are ubiquitous, and more events are being detected as more countries establish regulatory programs to monitor for the presence of DA in food products from the sea.

OCEANOGRAPHY AND TOXIC DIATOM BLOOMS

Toxic blooms may arise under several different oceanographic settings, and the challenge is to tease out which controlling factors are most important. In spite of intense research on the biological and chemical influences on the bloom formation of HABs, the details of bloom initiation and termination and the species composition of a bloom remain elusive. Why does one species of diatom (e.g., toxic *Pseudo-nitzschia multiseries*) begin to grow and become dominant at a particular location and time? The given species must, of course, be present, but then certain biological factors (such as grazing by zooplankton and filter-feeding molluscs, infection by fungi and viruses, and inherent physiological properties) must also exert an important influence. In the case of *Pseudo-nitzschia* species, there is evidence that they are more lightly silicified than other coastal diatoms (Marchetti *et al.*, 2004) and may therefore have a competitive growth advantage at low silicon concentrations. Some work has indicated that toxigenic *Pseudo-nitzschia* species may also have unique capabilities to acquire trace metals such as iron and copper (e.g., Wells *et al.*, 2005), thereby giving them a competitive growth advantage over other phytoplankton.

Ocean circulation and seawater properties exert an additional important influence in the development of toxic diatom blooms; the details are largely unknown, but this is a subject of intense research. Even though a particular physical cause may be invoked for a specific bloom, relationships have been difficult to generalize with any certainty. On the other hand, research has been successful at determining the role of ocean circulation in transporting HABs along the continental shelf (e.g., Adams *et al.*, 2006; Anderson *et al.*, 2006). This circulation can spread the toxin over a greater area; it may also move a bloom onshore to beaches (e.g., Trainer *et al.*, 2002), or export it off the shelf. In areas with certain types of bottom topography (such as banks and submarine canyons), circulation likely plays a role in retaining blooms in a region, thus allowing diatom densities and toxins to increase to dangerous levels (Anderson *et al.*, 2006). Water properties such as stratification may play a role in bloom development by inhibiting turbulence. Because diatoms tend to sink, stratification may allow them to be retained in layers exposed to higher light levels by preventing their sinking across the pycnocline. Macronutrients (such as nitrate and silicate) and micronutrients (such as iron and copper) play an important role in bloom development and the control of DA production.

The Major Coastal Current Systems

Blooms of toxic diatoms have been observed somewhat more frequently worldwide on eastern continental boundaries (where the ocean is bounded by the land on its eastern side) than on western boundaries (Fig. 12-6). Differences between these types of current systems, especially as they might pertain to HABs, are discussed briefly next.

Eastern boundary systems (EBS) support four out of the five most productive upwelling systems on the planet (Hill *et al.*, 1998). For example, the Peruvian/Chilean system produces 15% of all fish landed worldwide. The most productive areas on eastern boundaries are generally those in mid- to lower-, often semi-arid latitudes, where winds blow persistently toward the equator, causing upwelling of deeper, nutrient-rich water masses near the coast. The near-continuous addition of nutrients fuels dense phytoplankton blooms in these regions. Continental shelves on eastern boundaries are generally narrower (~10 to 100 km) than those on western boundaries (~10 to 200 km) (Fig. 12-7). The narrow width results in much greater movement and exchange of suspended material (such as phytoplankton) between the inner shelf (roughly bottom depths shallower than 30 m) and the outer shelf and slope than occurs in a western boundary system (Boicourt *et al.*, 1998; Loder *et al.*, 1998).

The preceding characteristics may be one reason for the more frequent occurrence of toxic diatom impacts on coastal beaches on eastern than western boundaries, especially along U.S. and Canadian coastlines. In general, in EBS upwelling systems (Fig. 12-7, panels at left), currents flow toward the equator at speeds of 10 to 20 km/day over the upper depths of the continental shelf and slope during the spring to summer seasons. At more poleward latitudes, the mean flow reverses to poleward in the fall and winter, reflecting the reversal in along-coast wind direction. Seaward of the continental slope to ~1000 km offshore, flow is generally equatorward throughout the year; this broad, sluggish portion of the "eastern boundary current" (e.g., the California Current) constitutes the eastern limb of the whole ocean basin circulation.

Variability in water properties and currents in an upwelling EBS are dominated by changes in the along-coast component of wind stress (Hickey, 1998). Seasonal variation is generally much greater than day-to-day variability, although the latter may be more critical for moving near-surface phytoplankton onto coastal beaches or offshore. This onshore-offshore movement occurs because the wind dragging on the sea surface generates near-surface currents that move to the right of the wind stress in the northern hemisphere (i.e., on the U.S. West Coast, offshore when winds are blowing toward the south, and onshore when winds are blowing toward the north) (Fig. 12-7, lower-left panel). Currents at deeper depths respond to the near-surface flow, moving in the opposite direction to compensate for the loss of mass in the upper layers (near-bottom dashed arrows in Fig. 12-7); upwelling of deeper, colder, nutrient-rich waters thus occurs to close the loop. Seasonally varying wind stress results in upwelling of nutrient-rich deeper water from the continental slope onto the shelf throughout the spring to fall period. At some locations, upwelling occurs all season long. Episodic wind events infuse nutrients into the coastal zone at several-day intervals, resulting in the renewal of existing phytoplankton blooms or the development of new blooms over the inner to midshelf.

Another important feature of an EBS, with possible relevance to HABs, is the existence of poleward-flowing currents beneath the equatorward surface currents over the continental slope (Fig. 12-7, lower-left panel). This flow, denoted an "undercurrent," is generally concentrated just below the shelf break (~150 to 300 m depths) and has a relatively narrow (~10 to 30 km) high-speed (10 to 20 km/day) core. The undercurrent can be continuous for hundreds or even thousands of kilometers along the coast (Hill *et al.*, 1998). Much of the water that is seasonally upwelled comes from the undercurrent. Also, the undercurrent is thought to play an important role in the poleward transport of larvae or phytoplankton. The existence of an undercurrent may provide a mechanism for phytoplankton, such as toxigenic diatoms, to spread along a coast counter to the prevailing surface currents. We note that evidence for this spreading mechanism has not been documented.

In shallow regions with modest stratification, coastal currents tend to follow the orientation of isobaths (i.e., contour lines mapping areas of identical depth). However, in regions where isobaths change direction abruptly, such as when the continental shelf narrows near a coastal cape, coastal cur-

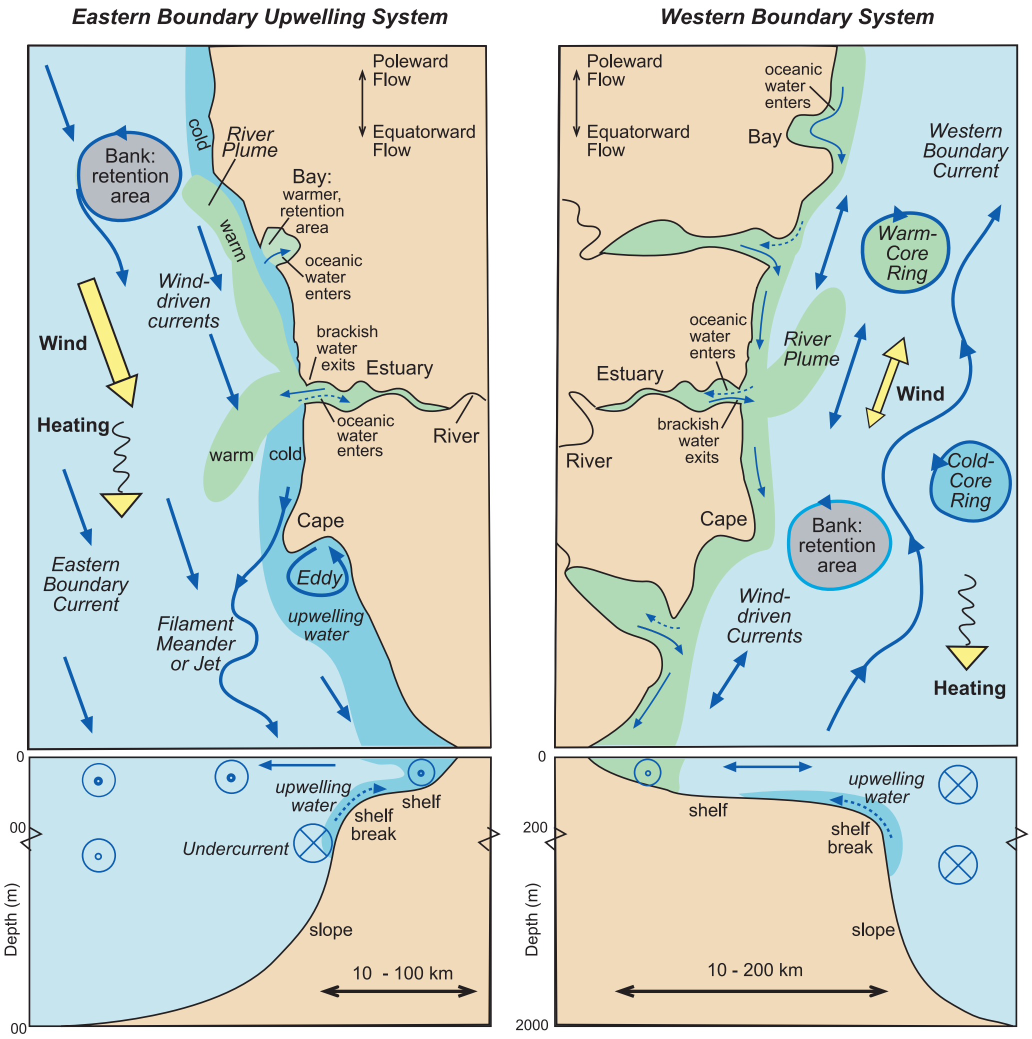

FIGURE 12-7. Schematic depicting major oceanographic features in Eastern (left) and Western (right) Boundary Current Systems (EBS and WBS, respectively) in the northern hemisphere. Upper panels show plan view, lower panels show depth versus distance offshore. The left panels are applicable to the U.S. West Coast; the right panels to the U.S. East Coast. For the U.S. West Coast, the EBS is the California Current; the WBS on the East Coast is the Gulf Stream. Symbols indicate currents (blue arrows; solid for near surface, dashed for beneath the surface), wind (yellow arrows), and heat flux (yellow arrows with tail). Shallow banks (which generally have more retentive circulation patterns) are shown in gray. Upwelled water is shown in dark blue; river plumes, bays, and estuaries are generally warmer (light green). Filaments, meanders, eddies, and jets that are frequently associated with features on an EBS coast, such as capes, are depicted, as well as rings (eddies) and meanders related to the WBS. Flow direction on the lower panels is indicated with a circle with a dot in the center (coming toward you) or a circle with a cross (away from you).

rents may be forced off the shelf by the rapid change in the direction of ocean bottom isobaths. This happens frequently in an EBS, such as the U.S. West Coast (Fig. 12-7, upper-left panel), so that the region offshore of the shelf is dominated by meandering filaments (thin features that stream from a site of origin), jets and eddies, many of which originate on the shelf near the coast (Hill, 1998; Hickey, 1998). The implication for HABs in general, and for toxigenic diatoms in particular, is that cells would be moved offshore and would therefore be less likely to reach the coast in regions where such features occur.

Major physical disturbances to an EBS generally arrive from outside the system (i.e., they travel in the ocean along the coastal boundary from “remote” locations). In particular, the greatest disturbances are caused by El Niño phenomena (see Chapter 1), whose origin is along the equator (Hill, 1998). El Niño is associated with warmer waters and enhanced poleward flow. In the northern hemisphere, nutrients and phytoplankton concentrations are reduced during an El Niño event, and more southern types of plankton and fish are carried poleward in the enhanced poleward undercurrent. Water properties in an EBS can also be impacted by changes in the amount of water entering the system from poleward locations (e.g., enhanced amounts of subarctic water in the Pacific Ocean). In contrast to an El Niño, these intrusions bring enhanced nutrients and result in higher standing stocks of phytoplankton.

Western boundary systems (WBSs) are usually (but not always) characterized by much wider shelves than an EBS (>100 km) (Fig. 12-7) (Loder *et al.*, 1998). This is particularly true for the northwestern and southwestern Atlantic region. Moreover, the mechanisms that control water properties and currents on the shelves often differ from those on eastern boundaries. For example, on the U.S. East Coast, the inner shelf is dominated by the effects of brackish water exiting from the myriad of rivers and estuaries that generally occur on western boundaries at midlatitudes, where rainfall is not inhibited by mountains as on the west coast. On the U.S. East Coast, an equatorward current occurs on the inner continental shelf, driven by freshwater that extends from Greenland to the mid-U.S. East Coast, a distance of more than 2000 km. On the outer continental shelf and slope, currents are poleward on the U.S. East Coast because of the Gulf Stream, and fluctuations are dominated by rings and eddies or currents associated with the meandering the Gulf Stream. The western boundary current (WBC) constitutes the western limb of basin scale circulation. Unlike the sluggish and broad eastern boundary current (such as the California Current), the WBC is narrow and swift.

Because of the wide shelf and fronts (i.e., regions where density changes rapidly within a few kilometers) associated with the freshwater-driven current on the inner shelf, the inner shelf and outer shelf/slope regions in a WBS are frequently somewhat isolated from each other (Boicourt *et al.*, 1998). Along-coast winds can move surface waters onshore and offshore, just as in an EBS, as well as alongshore. However, the winds are generally weaker and less persistently in an upwelling-favorable direction in a WBS (e.g., to the north on the U.S. East Coast) than in an EBS in the spring/summer growing season.

Therefore, upwelling of deep nutrients by along-coast winds, which dominates and controls water properties (including nutrients) on many eastern boundaries, is rarely important on western boundaries. Rather, the dominant source of nutrients on western boundary inner shelves is freshwater runoff that passes through estuaries to the ocean. Much of the nutrient supply is of anthropogenic origin (i.e., resulting from human activities) on many western boundaries. Farther offshore, nutrients are supplied from the continental slope via interactions with the WBC and its meanders and frontal eddies. Tidal mixing can also contribute to the nutrient supply. Major disruptions in a WBS are usually caused by local atmospheric disturbances such as hurricanes and tropical storms, rather than by large-scale oceanic features such as El Niño as on eastern boundaries.

Topographic Features with Importance to HABs

Smaller-scale (~10 to 50 km) features (such as capes, banks and submarine canyons) can locally modify the flow patterns described earlier. These features can also affect stratification in ways that may accelerate phytoplankton growth, perhaps contribute to the onset of toxicity, or influence HAB transport. In regions with equatorward flow over a submarine canyon, upwelling of nutrient-rich water may be enhanced (Hickey, 1997). As well, a retentive circulation pattern forms over the canyon. However, the circulation is modified by local stratification, so that this eddy-like feature is frequently confined to depths below the euphotic zone (i.e., the upper water layer exposed to sufficient sunlight for photosynthesis to occur), where it would not impact local phytoplankton blooms directly.

Eddy-like current patterns form downstream of coastal capes (such as Cape Blanco on the U.S. West Coast) and over banks (such as offshore of the Strait of Juan de Fuca, a feature that separates the western U.S. and western Canada)—for example, Heceta Bank (offshore of central Oregon) and Georges Bank (offshore of the central U.S. East Coast). These features tend to be retentive (i.e., they retain phytoplankton for longer periods than at an open, straight coastline). Because of the longer time spent in that location, phytoplankton may accumulate to high densities. They may also draw down nutrients and thereby experience stress, a contributor to toxin production (e.g., Anderson *et al.*, 2006). Several examples from U.S. coastal regions (given in Table 12-2) are described in more detail in the following text.

A map showing DA levels along the U.S. West Coast during the summer of 1998 shows high DA levels only near features that are known to be retentive: the Juan de Fuca eddy, Heceta Bank, the Farrallon Islands, Monterey Bay, and the Santa Barbara Channel (Trainer *et al.*, 2001; Hickey and Banas, 2003; Fig. 12-8). The Juan de Fuca eddy region has now been shown to sustain toxic *Pseudo-nitzschia* blooms in the summer of almost every year. Blooms appear to be ejected from the eddy under upwelling-favorable wind conditions (Mac-Fadyen *et al.*, 2005). They travel equatorward in the coastal currents on the middle to outer continental shelf and slope. However, brief reversals in wind conditions can transport these toxic patches to the coast, where they toxify razor clams and other shellfish (Trainer *et al.*, 2002).

In Monterey Bay (California), another retentive coastal region off the West Coast of the United States. (Fig. 12-8), toxic *Pseudo-nitzschia* blooms were first identified in 1991, when Brandt's cormorants and brown pelicans were found dying, diving into windows, and otherwise exhibiting behavior indicating that their nervous systems had been impacted (Bates *et al.*, 1998). High levels of DA were found in the sardines and anchovies (phytoplankton-feeding fish) on

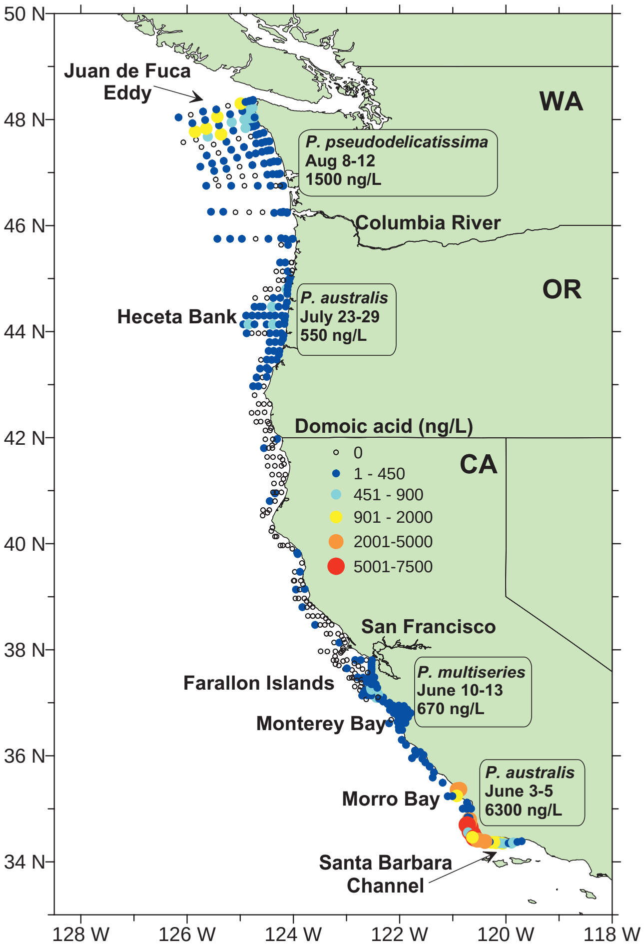

FIGURE 12-8. Particulate domoic acid (ng/L) in surface seawater during cruises off the U.S. West Coast in 1998. The timing and approximate locations of each cruise, as well as the species of *Pseudo-nitzschia* responsible for maximum toxin levels measured during each cruise, are shown in the boxes. Redrawn from Trainer *et al.* (2001); see also Hickey and Banas (2003).

which these birds had fed. A bloom of *Pseudo-nitzschia australis*, identified for the first time as a species producing high levels of DA, was observed in Monterey Bay during this event. However, scientists believe that such marine bird poisonings had also occurred in prior years. In August 1961, in the small central Californian town of Capitola near Santa Cruz, seabirds collided with inanimate objects and attacked police cars and people. This is thought to have inspired Alfred Hitchcock's 1963 movie *The Birds*, based on Daphne du Maurier's short story. Scientists have speculated that this strange behavior was because the birds had consumed anchovies contaminated with DA, much like the 1991 event.

In May and June 1998, more than 400 California sea lions were found dead, and many others, as well as sea otters, displayed signs of neurological dysfunction on beaches along the central California coast, including Monterey Bay and Morro Bay (Scholin *et al.*, 2000). The poisoning behavior in these marine mammals (including scratching, tremors, and seizures) was remarkably similar to that seen in mice used for DA bioassays. DA was found in their tissues, and some contained *P. australis* frustules in their gut. In addition, several affected sea lions showed histological lesions evidenced by nerve cell death in the CA3 region of the hippocampus and dentate gyrus. As in the case of the seabirds, these sea lions had fed upon sardines and anchovies that, in turn, had fed upon toxic *P. australis* cells.

Estuaries and Bays

Coastal estuaries and shallow bays are sometimes sites for toxic diatom blooms. The two environments have significant differences (Fig. 12-7). In particular, an estuary is fed by a river, or several rivers providing fresh water, so that its waters are brackish. Surface and deeper currents generally move in opposite directions in an estuary, with less saline waters flowing out of the estuary at the surface and more saline waters into the estuary at or near the bottom (Hickey and Banas, 2003), sometimes resulting in a density-based stratification of the two layers (see below). In contrast, waters in a bay are usually entirely of oceanic origin and circulation is likely more dominated by currents flowing in the same direction at all depths. Note that the designation "bay" in a name does not necessarily mean that the body of water is a "bay" in the physical sense defined here. Even within these two categories, large differences occur. Estuaries may be deep (e.g., the Strait of Juan de Fuca, Puget Sound or the Gulf of St. Lawrence) or shallow (e.g., Chesapeake Bay or Cardigan Bay).

From the point of view of HABs, estuaries in an EBS and a WBS may have even greater differences. On eastern boundaries, estuaries tend to be rapidly flushed by tidal currents. This means that phytoplankton (including HABs) and nutrients in EBS estuaries tend to be imported from coastal waters. On western boundaries, estuaries tend to have indigenous ecosystems; therefore, nutrients are supplied by runoff from land, and ocean intrusions and tidal flushing are much reduced (Hickey and Banas, 2003).

Bays and estuaries do have one feature in common that derives from their small size and relatively retentive nature: they tend to be warmer than the adjacent ocean in the spring-summer growing season. This leads to enhanced stratification, and in some cases, weaker turbulence. Shallow bays are ideal locations for the aquaculture of molluscan shellfish because they are sheltered and, if well flushed, have a constant source of phytoplankton needed as food for the shellfish. However, they are also vulnerable to HABs, which often occur at these same locations. Several examples of toxic diatom blooms in estuaries and bays are described next (also, Table 12-2); in these examples, Cardigan Bay and Penn Cove are "estuaries," and East Sound is a "bay."

Bays and estuaries within the Gulf of St. Lawrence, a large estuary in eastern Canada, have experienced periodic blooms of toxic *Pseudo-nitzschia*, resulting in closures of molluscan shellfish harvesting. The original bloom in the fall of 1987 occurred in Cardigan Bay, eastern Prince Edward Island (PEI), and it recurred there during the fall for the next 2 years (Bates *et al.*, 1998). In subsequent years (i.e., 1991, 1992, 1994, 2000, and 2001), the toxic blooms occurred in three different bays on northern PEI. Aquacultured blue mussels (*Mytilus edulis*) were the primary vector for the DA, reaching 790 µg DA/g in the whole animal in the 1987 incident, considerably above the 20 µg DA/g limit. In each case, the blooms were composed of DA-producing *P. multiseries*. These blooms likely originated within the bays, as evidenced by lower cell concentrations outside the bays.

In the southern Gulf of St. Lawrence, the mussel and oyster aquaculture industries were caught off guard in 2002 by high levels of DA that forced the closure of shellfish harvesting for the first time during the spring (March to May). This event involved a different species of *Pseudo-nitzschia*: *P. seriata*, a cold-water diatom that is found only at northern latitudes of the Atlantic Ocean (Hasle, 2002). In contrast to the *P. multiseries* blooms, these originated offshore; they were then drawn into the bays by the tides. Because the source organism bloomed throughout the Gulf of St. Lawrence at the same time, it caused a wide shellfish bed closure for most of the southern Gulf.

Record levels of DA in October 2005 resulted in the closure of an important commercial mussel fishery in Penn Cove (Washington State), considered an “estuary” because of its proximity to the Skagit River. DA levels in Dungeness crabs measured 28 µg/g, just below the closure level of 30 µg/g in this commercial and recreational fishery. Studies in Penn Cove (Trainer *et al.*, 2007) have documented blooms of *Pseudo-nitzschia* after periods of strong freshwater discharge from the nearby Skagit River. Stratification was facilitated by weak winds, sunshine, and a freshwater lens at the mouth of the cove. It has been suggested that because *Pseudo-nitzschia* cells have an efficient nutrient uptake capability, they can survive in macro- and micronutrient depleted environments (Wells *et al.*, 2005) such as stratified systems, where freshwater is layered over denser, saltier water, and there are no sustained inputs of nutrients.

Pseudo-nitzschia cells have been found to be a major component of chlorophyll maxima or “thin layers” found at depth within some bays (e.g., in East Sound, Orcas Island in Puget Sound, considered a “bay” because of the lack of freshwater input) on the West Coast of the United States (Rines *et al.*, 2002). During the 1999 bloom observed in East Sound, sustained winds forced a less saline, lighter plume of water into the Sound, displacing the surface bloom of *P. fraudulenta* to a greater depth, where it formed a thin layer. Although high numbers of *P. fraudulenta* were found within the thin layer in East Sound (>1 million cells per L), this species is not always toxic, so shellfish were not toxic at this site.

Plumes from Rivers

A brackish plume develops in the ocean where each river and estuary empties onto the coast (Hill, 1998). These plumes affect both the local current patterns and the local ecosystem, with greater effects from larger rivers such as the Columbia or the Mississippi. Plumes have associated large vertical stratification, and hence decreased turbulence. Thus, they are particularly susceptible to heating, and also to development of high phytoplankton densities, especially if the nutrient source is sustained by upward mixing of nutrient-rich water in deeper layers or by other processes. Some river plumes on western boundaries, such as the Mississippi, support massive phytoplankton blooms off the coast, a result of the terrigenous (i.e., derived from the land) nutrient loading. Sinking of material from these blooms can result in hypoxic or anoxic (low or no oxygen, respectively) conditions on the ocean floor.

Currents may be accelerated within a plume relative to a region with no plume. Hence, plumes can provide rapid along-coast transport to new regions, facilitating the spread of toxic or toxigenic phytoplankton. Evidence has shown the interaction of the Columbia River plume and toxic *Pseudo-nitzschia* in Washington State coastal waters (Adams *et al.*, 2006). Plumes may even transport blooms in directions opposite to the ambient local currents. If a plume hugs the coast, as it frequently does on both eastern and western boundaries, its seaward front may provide a barrier to onshore transport of toxic blooms that develop offshore of the plume. Hickey *et al.* (2005) hypothesized that the plume from the Columbia River frequently prevents toxigenic *Pseudo-nitzschia* blooms in the Juan de Fuca eddy from reaching the central and southern Washington coast.

Impacts of Offshore HABs

Not all molluscan shellfish are harvested along coastlines; there are also deep-water harvests of wild bivalve shellfish. One such fishery is on Georges and nearby banks, retentive features in the Gulf of Maine. Sea scallops (*Placopecten magellanicus*) are harvested as a “roe-on” product (i.e., sold with roe still attached to the adductor muscle). In May 1995, extremely high levels of DA were found in these sea scallops. The most toxic sample showed the following tissue distribution: digestive gland (3400 µg/g wet weight), roe (55 µg/g), gills plus mantle (19 µg/g), and adductor muscle (0.62 µg/g). This event essentially shut down the lucrative wild harvest of “roe-on” scallops for that year; no contaminated product reached the market. As in other cases of DA in scallops, the adductor muscle had DA levels well below the safety guideline.

SUMMARY AND CONCLUSIONS

In summary, HABs composed of diatoms of the genus *Pseudo-nitzschia* can produce the neurotoxin DA and lead to the risk of ASP in many different coastal environments. It is important that medical professionals be educated about the symptoms of ASP. Although there is presently no antidote to ASP other than supportive care (e.g., respiratory support and control of convulsions), it is essential that a rapid diagnosis be made. Ultimately, early warning of HAB events will minimize exposure of humans to this toxin. However, researchers and coastal managers face a great challenge when designing early warning systems to protect humans from the neurotoxic effects of eating shellfish contaminated with DA while also sustaining the harvest of shellfish on our coasts. The forecasting of these HABs must be a dynamic process that satisfies the unique biological, chemical, and physical characteristics of each coastal area. For example, an offshore HAB in the Juan de Fuca eddy will be detected using very different sensors than a HAB in a bay or estuary such as East Sound or the Gulf of St. Lawrence. To this end, remote, automated, and sensitive ocean observing systems, such as the Environmental Sample Processor, ocean gliders, and sensor arrays, are gradually being used in coastal regions to detect toxins, toxic cells, and the environmental conditions associated with HAB development and transport (Babin *et al.*, 2005).

Acknowledgments

This publication was supported by a grant to the West Coast Center for Oceans and Human Health (WCCOHH) to V.L.T. and B.M.H. as part of the National Oceanic and Atmospheric Administration (NOAA) Oceans and Human Health Initiative, WCCOHH publication no. 15. The WCCOHH is part of the National Marine Fisheries Service's Northwest Fisheries Science Center, Seattle, WA. Support was also provided to V.L.T and B.M.H. from the Ecology and Oceanography in the Pacific Northwest (ECOHAB PNW) program (publications no. 12 for ECOHAB PNW and 232 for the national ECOHAB program) by grants from NOAA (NA17OP2789) and NSF (OCE 0234587) and to B.M.H. by the Pacific Northwest Center for Human Health and Ocean Sciences (NIH/National Institute of Environmental Health Sciences [NIEHS] [P50 ES012762] and NSF [OCE-0434087]).

We thank Rita Horner, Nina Lundholm, Andy Tasker, and Cynthia Brown for reviewing an earlier version of this manuscript. We thank Maureen Auro and William Cochlan for providing unpublished data about *P. cuspidata* toxin production, Johanna Fehling for permission to update her map on *Pseudo-nitzschia* distribution, and Luiz Mafra (Brazil) and Martha Ferrario (Argentina) for information about the distribution of *Pseudo-nitzschia* species in their waters. We thank Sheryl Day for creating Fig. 12-3.

The statements, findings, conclusions, and recommendations are those of the authors and do not necessarily reflect the views of NOAA, the Department of Commerce, or the National Science Foundation.

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STUDY QUESTIONS

1. What potential impacts would there be on the ecosystem and on human health if Arctic and Antarctic species of *Pseudo-nitzschia* were found to be high producers of domoic acid?
2. In what ways would knowledge of the molecular biology of *Pseudo-nitzschia* species help to reduce the impact of these toxic species on human health?
3. What is the relationship between the incidence of toxic blooms of *Pseudo-nitzschia* species and (a) eutrophication; (b) aquaculture activities?
4. What measures can be taken to mitigate the harmful effects of toxic *Pseudo-nitzschia* blooms?
5. Describe what is known about sexual reproduction in pennate diatoms. Why is sex necessary for these diatoms?
6. If you were asked to develop an ocean observing system for *Pseudo-nitzschia* blooms that might impact the U.S. West Coast, where would you place arrays of sensors in the ocean (consider both offshore and coastal sites)? What sensors would you place on your moorings to give the most optimal early warning of HAB events?
7. Why are storms thought to bring toxic cells from offshore regions toward the coast in eastern boundary systems? Describe a sequence of events during the summer months that might result in closures of shellfish beds in an eastern boundary system.
8. How might a river plume affect the development and movement of toxic blooms in the coastal ocean?
9. Why are coastal bays more susceptible to toxic diatom blooms than other coastal regions?
10. How can currents below the euphotic layer have any relation to surface diatom blooms in the coastal ocean?

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