8
Domoic Acid

Stéphane La Barre, Stephen S. Bates, and Michael A. Quilliam

Abstract

Domoic acid (DA) was of no special scientific interest until a series of case studies revealed its role as the major marine neurotoxin causing amnesic shellfish poisoning (ASP). The analysis, toxicology, synthesis and degradation of the highly polar amino acid DA and its kainoid congeners are discussed in this chapter. Although DA is structurally simple and ubiquitous in contaminated food samples, it was not simple to prove that it was the causative agent of ASP in humans and of DA poisoning in carnivorous birds and mammals. Furthermore, its detection and the prevention of ASP requires regular monitoring of seafood using rapid and accurate analyses. The main producers of DA are certain seasonally blooming diatoms of the genus *Pseudo-nitzschia*, major components of coastal phytoplankton. Here, details are provided of the species most likely to be involved in food-poisoning episodes, together with a brief account of the molecular mechanisms that underlie DA toxicity, which cause symptoms of acute and chronic neurotoxicity. DA may attain critically toxic levels within two major food chains involving benthic filter-feeders (e.g., mussels) or planktivorous fish (e.g., anchovies). Preventive measures must be complemented by risk assessments of seasonal toxigenic blooms, especially in nutrient-enriched coastal areas. The major chemical and biotic factors that influence diatom bloom formation and toxigenicity are outlined. Genomics of DA production allow the development of novel molecular tools to better understand DA biosynthesis at the gene level, and the evolutionary significance of DA as a metabolite with primary and secondary characteristics.

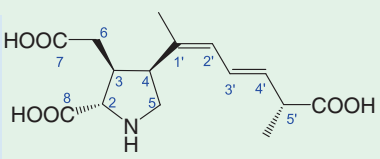
Box 8.1: Domoic Acid

{2*S*-[2α,3β,4β(1*Z*,3*E*,5*R*)]}-2-Carboxy-4-(5-carboxy-1-methyl-1,3-hexadienyl)-3-pyrrolidineacetic acid (IUPAC)

Isolated from the red alga *Chondria armata* from Japan (Takemoto and Daigo, 1958) and *Alsidium corallinum* from the Mediterranean Sea (Impellizzeri *et al.*, 1975). Domoic acid is also produced by at least 14 of the over 38 species of the pennate diatom genus *Pseudo-nitzschia*, as well as some strains the of pennate diatom *Nitzschia navis-varingica* (q.v. Lelong *et al.*, 2012; Trainer *et al.*, 2012)

Elemental formula: $C_{15}H_{21}NO_6$

MW: 311.33
CAS RN: 14277-97-5
BRN: 5768789
Colorless crystal needles, highly water soluble



Commercially available as an analytical and pharmacological tool; cost ca. €200 per milligram.

Used traditionally in Japan as an anthelmintic agent (Daigo, 1959a, 1959b, 1959c) and insecticide (Maeda *et al.*, 1984; Maeda *et al.*, 1987a).

Synonyms: 2α-carboxy-4β-(5-carboxy-1-methyl-1,3β-hexadienyl)-3-pyrrolidineacetic acid, (2*S*,3*S*,4*S*)-2-carboxy-4-[(1*Z*,3*E*,5*R*)-5-carboxy-1-methyl-1,3-hexadienyl]-3-pyrrolidineacetic acid; (3*S*,4*S*)-4-[(2*Z*,4*E*,6*R*)-6-carboxyhepta-2,4-dien-2-yl]-3-(carboxymethyl)-L-proline.

Caution: Toxic if ingested, inhaled, or in skin contact. The use of dust mask type N95 (US), eyeshields and gloves is required within a well-ventilated space. May cause rapid gastrointestinal and neurological disturbances (amnesic shellfish poisoning syndrome) as acute symptoms; causes brain/CNS long-term functional impairments and structural damages on chronic exposure.

Outstanding Marine Molecules: Chemistry, Biology, Analysis, First Edition. Edited by Stéphane La Barre and Jean-Michel Kornprobst.

8.1 Historical Background

The story of this relatively simple molecule is unusual in many respects, its significance having gradually unfolded since its presence in the red alga *Chondria armata* was first discovered in 1958 (Takemoto and Daigo, 1958). Domoic acid (DA, see Box 8.1) (from *domoi*, the vernacular name of *C. armata* in Japan) is the active ingredient of this seaweed, which has been used traditionally on the island of Tokinoshima to treat ringworm infestations, and this may have prompted the initial chemical investigations. DA resembled another molecule, kainic acid, which had been identified a few years earlier (in 1953) from another red alga, *Digenea simplex*, and used as an anthelminthic in Japan since the ninth century to cure infants of roundworm infection (Higa and Kuniyoshi, 2000). *Domoi* was also used for insect control by the inhabitants of Yakushima Island, when it was noticed that flies landing on these algae became intoxicated and died (Maeda *et al.*, 1984). DA was identified as the active ingredient in 1958 (Daigo, 1959a, 1959b, 1959c), and its insecticidal properties, along with those of the isodomoic acids A, B and C (Table 8.1), were further studied (Maeda *et al.*, 1984, 1986, 1987b). Although a total synthesis of the molecule was completed in 1982 (Ohfune and Tomita, 1982), DA was relatively unheard of outside Japan at the time because its neurotoxic effects after oral intake were not apparent at prescribed levels.

The global significance of DA emerged gradually (Trainer, Hickey, and Bates, 2008; Trainer *et al.*, 2012; Lelong *et al.*, 2012). Initially, a single massive seafood intoxication in 1987, originating at Prince Edward Island in eastern Canada, had caused several deaths and severe complications in well over 100 people who had consumed blue mussels (*Mytilus edulis*) (Perl *et al.*, 1990; Teitelbaum *et al.*, 1990; as described in Case study #1). Subsequently, a new term – amnesic shellfish poisoning (ASP) – was coined to account for the disorientation and memory deficiencies observed in many individuals; these were accompanied by gastrointestinal effects, followed some time later by epileptic seizures in at least one patient, and death in four others. Investigations promptly led to DA being designated as the causative agent (Quilliam and Wright, 1989), and the pennate diatom *Nitzschia pungens* forma *multiseries* (now known as *Pseudo-nitzschia multiseries*) as the source of the toxin, after having examined the shellfish flesh and gut contents, and isolating the diatom in culture (Bates *et al.*, 1989; see Case study #1). This was the first time that a biotoxin had been shown to be produced by a diatom, and the monitoring of shellfish beds has been undertaken consistently since then. Safety measures were immediately implemented, which forbade the sale or harvesting of molluskan shellfish when the DA content of the edible flesh exceeded 20 $\mu g\, g^{-1}$ fresh weight (Wekell *et al.*, 2002).

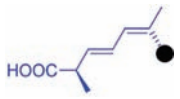
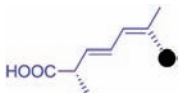
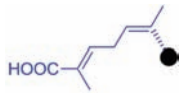
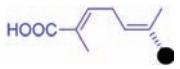
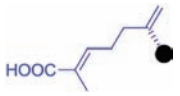
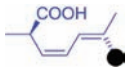
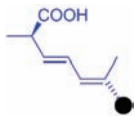
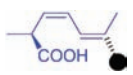
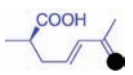
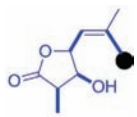
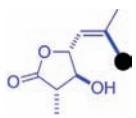
The first verified case of vertebrate animal DA intoxication occurred in 1991, in Monterey Bay, California, when brown pelicans (*Pelecanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*) died after having eaten anchovies contaminated by DA from another producer, *Pseudo-nitzschia australis* (Fritz *et al.*, 1992; Work *et al.*, 1993; as described in Case study #2). High levels of DA contamination were also reported in crabs, razor clams and mussels at many other sites in the USA (Bates, Garrison, and Horner, 1998; Trainer *et al.*, 2012). In 1998, epizootics affecting sea lions (*Zalophus californianus*) were attributed to DA accumulation in planktivorous fish that had consumed toxigenic *P. australis* (Scholin *et al.*, 2000; as described in Case study #3). In addition to the documented acute toxicity syndromes, repeated exposure to sublethal doses of DA was found to be responsible for epileptic seizures observed over the following decade among sea lion populations. With the annual increase in toxigenic blooms along the California coast, DA is now established as a prominent environmental neurotoxin (Trainer, Hickey, and Bates, 2008), with acute and long-term neurological effects on wildlife that feeds on intoxicated fish and invertebrates (Bejarano *et al.*, 2008).

The full environmental significance of recurrent blooms of toxigenic *Pseudo-nitzschia* diatoms has shifted progressively from isolated risk zones in eastern Canada and the Pacific coast of the USA to a worldwide concern, such that DA monitoring has become an emerging necessity in some temperate Asian, European and South American localities. For example, *P. australis*, which originally was described only from the southern hemisphere, was later identified on the west coast of California, and more recently in Europe (Lelong *et al.*, 2012). The global transport of exogenous plankton in ships' ballast water could be held partly responsible for this expansion. Moreover, these problems can be expected to worsen with increased global warming, as this may allow certain toxigenic species to proliferate in new locations. Increased levels of carbon dioxide, which accompany ocean acidification and global warming, will increase *Pseudo-nitzschia* toxicity (Sun *et al.*, 2011; Tatters, Fu, and Hutchins, 2012). The experimental and natural iron enrichment of oceanic waters may also stimulate plankton productivity and reduce carbon dioxide levels, but this correlates positively with the occurrence of toxigenic *Pseudo-nitzschia* blooms (Silver *et al.*, 2010; Trick *et al.*, 2010); the environmental role of DA as a siderophore (metal-capturing molecule) is still debated, however (Lelong *et al.*, 2012).

Blooms of toxic *Pseudo-nitzschia* tend to occur in high-productivity areas, and occasionally in association with waters impacted by urban and farm discharges, which provide abundant nitrogen (e.g., nitrate, ammonium) for growth. Urea can be used as a primary nitrogen source by these diatoms, and this clearly enhances the production of DA in *P. australis* (Howard *et al.*, 2007), though this may not always be the case for other *Pseudo-nitzschia* species (Auro and Cochlan, 2013). The enrichment of coastal waters exacerbates the recurrence of harmful algal bloom (HAB) episodes in North America, and this has now become a major issue (Anderson *et al.*, 2008; Heisler *et al.*, 2008). A newly described DA-producing diatom is *Nitzschia navis-varingica*, isolated from shrimp farms in Viet Nam (Lundholm and Moestrup, 2000). This diatom is found over a large latitudinal range in Asia, where it thrives in brackish waters (Kotaki *et al.*, 2004; Thoha *et al.*, 2012) and has become a major concern to local shrimp farmers.

The full toxicological significance of DA has taken years to investigate, with numerous neurophysiological studies having been carried out in laboratory animals, including vertebrates (fish to mammals) and invertebrates (insects, crustaceans,

Table 8.1 The domoic acid family. From left to right: Domoic acid with its isoforms A–F, its 5′- epimer and its two lactone derivatives, as defined by the side chain at position 4. The black dot represents the common pyrollidineacetic moiety; Original biological source in which the molecule was first identified; Reported bioactivities; Novel syntheses.

	**Domoic acid**	**First isolation** *Chondria armata* (Takemoto and Daigo, 1958); *Alsidium corallium* (Impellizzeri *et al.*, 1975); 14 *Pseudo nitzschia* species (see Lelong *et al.*, 2012), *Mytilus edulis* (Wright *et al.*, 1988)	**Bioactivity** Potent insecticide (Maeda *et al.*, 1987a) Very potent ASP	**Total synthesis** (Ohfune and Tomita, 1982)
	**5′-epi-Domoic acid** (DA diastereoisomer)	**First isolation** *Mytilus edulis* (Walter, Falk, and Wright, 1994)	**Bioactivity** Potent ASP	DA heat-degradation product
	**Isodomoic acid A** (DA geometric isomer)	**First Isolation** *Chondria armata* (Maeda *et al.*, 1986)	**Bioactivity** Potent insecticide (Japanese thesis) Weak ASP	
	**Isodomoic acid B** (DA geometric isomer)	**First isolation** *Chondria armata* (Maeda *et al.*, 1986)	**Bioactivity** Potent insecticide Weak ASP	**Total synthesis** (Lemière *et al.*, 2011)
	**Isodomoic acid C** (DA geometric isomer)	**First isolation** *Chondria armata* (Maeda *et al.*, 1986)	**Bioactivity** Potent insecticide Weak ASP	**Total synthesis** (Clayden, Knowles, and Baldwin, 2005b)
	**Isodomoic acid** D (DA geometric isomer)	**First isolation** *Chondria armata* (Maeda *et al.*, 1985); *Mytilus edulis* (Wright *et al.*, 1990)		
	**Isodomoic acid E** (DA geometric isomer)	**First isolation** *Mytilus edulis* (Wright *et al.*, 1990)		**Total synthesis** (Lemière *et al.*, 2011)
	**Isodomoic acid F** (DA geometric isomer)	**First isolation** *Mytilus edulis* (Wright *et al.*, 1990)		**Total synthesis** (Lemière *et al.*, 2011)
	**Isodomoic acid G** (DA geometric isomer)	**First isolation** *Chondria armata* (Zaman *et al.*, 1997)		**Total synthesis** (Ni *et al.*, 2003, 2009; Denmark, Liu, and Muhuni, 2009, 2011)
	**Isodomoic acid H** (DA geometric isomer)	**First isolation** *Chondria armata* (Zaman *et al.*, 1997)		**Total synthesis** (Ni *et al.*, 2009; Denmark, Liu, and Muhuni, 2009, 2011)
	**Domoilactone A** (DA analog)	**First isolation** *Chondria armata* (Maeda *et al.*, 1987b)		
	**Domoilactone** B (DA analog)	**First isolation** *Chondria armata* (Maeda *et al.*, 1987b)		

mollusks). These studies have been supplemented by post-mortem investigations on the brain and central nervous system (CNS) of humans with a history of ASP. DA intoxication is dose-dependent with regards to acute symptoms, while long-term (chronic) exposure can result in a cumulative impairment of function. As blooms of toxic *Pseudo-nitzschia* tend to occur naturally in high-productivity areas, and occasionally in association with waters impacted by urban discharges, residents are facing a higher risk of chronic intoxication (with onset after up to 20 years) by consuming contaminated seafood on a regular basis, even if the detected levels of DA are deemed acceptable (Lefebvre and Robertson, 2010). Indeed, DA is present in many animal species (in addition to mussels) that are consumed by humans, including recreational fish, anchovies, razor clams, Dungeness crabs, king scallops, squid, and cuttlefish (Trainer *et al.*, 2012).

8.2
Case Studies

8.2.1
Case Study #1: The 1987 Outbreak on Prince Edward Island

Prior to 1987, the only biotoxins of concern to Canada, and much of North America, were those such as saxitoxins produced by dinoflagellates of the genus *Alexandrium* that caused paralytic shellfish poisoning (PSP). This syndrome usually occurred during the summer months, when the stratified water column produced conditions that were conducive to the proliferation of these dinoflagellates. It was therefore a great surprise when very sick individuals exhibiting similar symptoms began arriving at hospitals in New Brunswick and Quebec, starting on 22 November 1987 (a chronology of events is given in Anderson *et al.*, 2001). On 29 November, epidemiologists from Health and Welfare Canada (HWC) determined that all of the patients had consumed blue mussels (*Mytilus edulis*) from eastern Prince Edward Island, eastern Canada. Tests for PSP toxins, trace metal contamination and the usual bacterial or viral agents proved negative, while water samples taken through holes drilled in the ice in early December, in Cardigan Bay, Prince Edward Island, showed an absence of toxic dinoflagellates, but this was not surprising given the time of year.

The deaths of at least four elderly individuals and the sickness of over 100 others (Perl *et al.*, 1990; Teitelbaum *et al.*, 1990) led to an immediate closure for harvesting of all shellfish, including mussels, clams, quahogs and scallops, on 11 December. This was devastating to the aquaculture and wild shellfish industries, especially just prior to the lucrative Christmas season, and consequently the story made national headlines and great pressure was applied to the Canadian government to resolve the problem. Hence, a major effort was mounted to identify the toxin in the contaminated mussels.

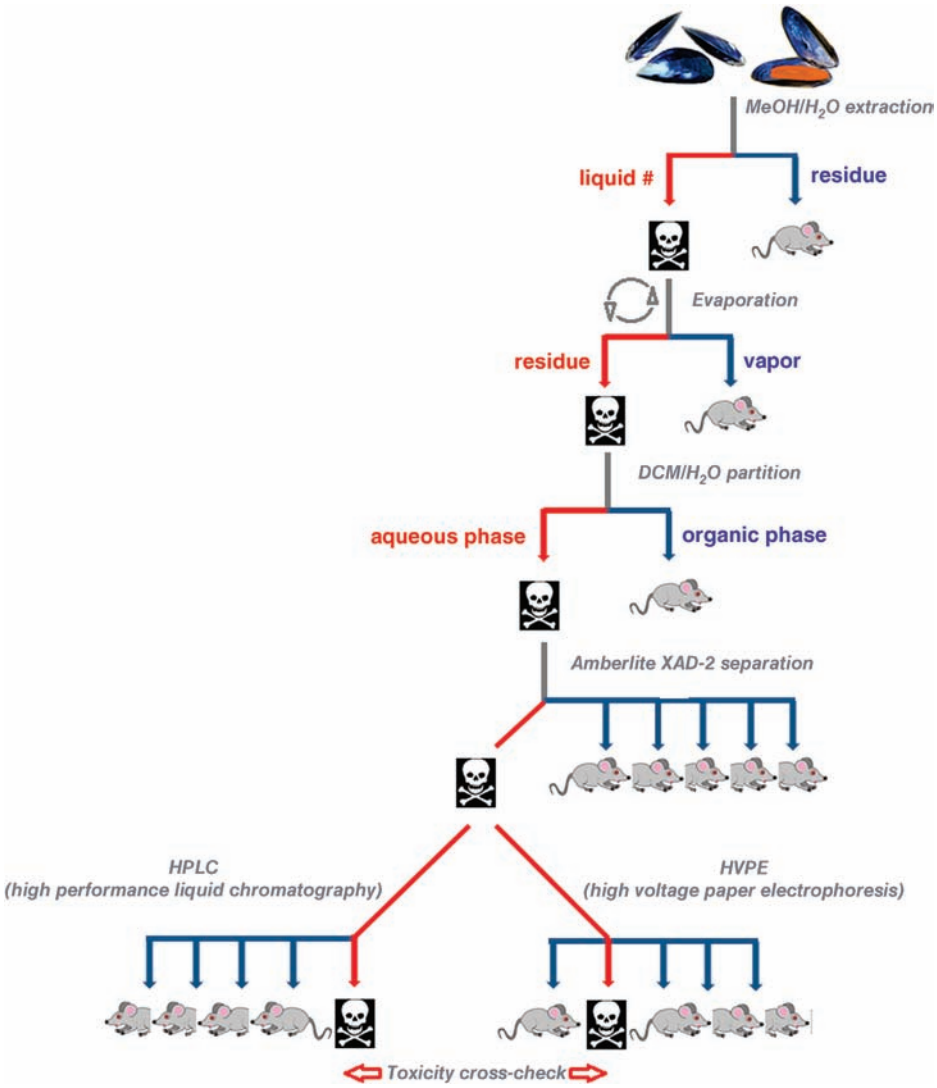


Figure 8.1 Flow chart showing the original extraction and separation procedure used to identify the toxic fraction from mussel flesh in the 1987 intoxication event on Prince Edward Island, Canada. HPLC coupled with diode array detection (DAD) used the 242 nm UV peak of domoic acid for quantification. Active fractions are indicated by red arrows; the final extracts obtained by HPLC and by HVPE (high-voltage paper electrophoresis) were crosschecked to confirm activity. (Adapted with permission from Quilliam and Wright. © (1989) American Chemical Society.)

On 12 December, the National Research Council of Canada (NRC), in Halifax, Nova Scotia, assembled a team of 40 chemists and biologists to tackle the problem. Other scientists from Fisheries and Oceans Canada (DFO) and the Atlantic Veterinary College of the University of Prince Edward Island (Charlottetown), joined in the efforts. A (mouse) bioassay-directed strategy (Figure 8.1) traced the toxicity to a water-soluble fraction of the mussels (Quilliam and Wright, 1989), and chemical methods that included column chromatography, high-voltage paper electrophoresis, HPLC with ultraviolet diode array detection (DAD) and NMR spectrometry were used to analyze the toxic fraction. After an unprecedented 104 h period of detective work, the culprit toxin was identified as DA, an amino acid that had already been isolated in the 1950s from the red seaweed *Chondria armata* (see above). The identification was at first treated with disbelief, because this was the same compound used in Japan to treat children infested with intestinal worms! However, in the case of the Canadian illnesses and deaths, an order of magnitude higher dose of DA was estimated (~290 mg) than was ever given for anthelmintic treatments (~20 mg) (Trainer, Hickey, and Bates, 2008). Furthermore, those affected in 1987 were elderly and had preconditions, such as renal dysfunction and compromised blood–brain barriers, which made them more vulnerable than the children. These findings were later reinforced by several studies that showed an age-dependent DA toxicity in mice and rats (Ramsdell, 2007).

The lessons learned from this 1987 incident were that unexpected biotoxins could be discovered in novel biological sources, and that monitoring efforts must be strengthened. Finally, analytical methods, such as LC-MS/MS (see below), must be used to maintain vigilance against such incidents. As a consequence, several other biotoxins, including spirolide toxins, pectenotoxins, yessotoxins and azaspiracids, which are also found elsewhere in the world, have since been discovered in Canadian waters.

8.2.2 Case Study #2: The 1991 Bird Intoxication Event in California

A bloom of the pennate diatom *Pseudo-nitzschia australis*, which occurred in early September 1991 at Monterey Bay, California, coincided with an episode of mortality in brown pelicans (*Pelicanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*). High levels of DA, the ASP toxin, were recorded in the plankton samples (Fritz *et al.*, 1992; Work *et al.*, 1993). Furthermore, high levels of DA, as well as numerous remnants of *P. australis* frustules, were found in the stomach contents of the affected birds and in the visceral contents of local anchovies, a major food source of the seabirds. This was the first confirmed report of DA poisoning since the 1987 outbreak on Prince Edward Island (see Case study #1), and was also the first evidence of a herbivorous fish acting as a vector for this toxin. Interestingly, currently available data indicate that DA-producing algal blooms do not cause fish kills or neuroexcitotoxic behavior in fish (Lefebvre *et al.*, 2012). A little known fact is that, at the time, a large shipment of the highly toxic anchovies was heading for another country but was recalled a short while later, possibly saving many lives.

Box 8.2: The Birds: A Suspense Story

Generations of thriller lovers have seen Alfred Hitchcock's *The Birds* since its original screening in 1963. The screenplay, written by Evan Hunter, borrowed the title of Daphné Du Maurier's 1952 short story *The Birds*, about angry birds attacking humans and invading small English towns. This was developed into a suspense story which started from trivia and gradually climaxed into full horror. Hitchcock's scenario, however, drew its inspiration from an 18 August 1961 report in a local Californian newspaper, *The Santa Cruz Sentinel*, titled "Seabird invasion hits coastal homes" (http://www.santacruzpl.org/history/articles/183/):

> "A massive flight of sooty shearwaters, fresh from a feat of anchovies, collided with shoreside structures from Pleasure Point to Rio del Mar during the night. Residents, especially in the Pleasure Point and Capitola area were awakened about 3 a.m. today by the rain of birds, slamming against their homes. Dead and stunned seabirds littered the streets and roads in the foggy, early dawn. Startled by the invasion, residents rushed out on their lawns with flashlights, then rushed back inside, as the birds flew toward their light. (. . . /) When the light of day made the area visible, residents found the streets covered with birds. The birds disgorged bits of fish and fish skeletons over the streets and lawns and housetops, leaving an overpowering fishy stench."

It was not until a similar episode in 1991, involving brown pelicans and cormorants in Monterey Bay, California (see Case study #2), that what became "Hitch's secret" was finally resolved. Carefully preserved samples of the gut contents of zooplankton collected in July–August 1961, from Monterey Bay, California, were shown to contain *Pseudo-nitzschia* species (Bargu *et al.*, 2012). In the 1991 incident, high DA concentrations were found in the food regurgitates of the birds, together with *Pseudo-nitzschia* diatom frustules (Fritz *et al.*, 1992; Work *et al.*, 1993). The similarities between events in 1961 and the DA-induced poisoning of 1991, suggested that toxic *Pseudo-nitzschia* were probably responsible for the odd behavior and death of sooty shearwaters in August 1961.

8.2.3 Case Study #3: Massive Sea Lion Mortality in Just a Few Weeks

In the spring of 1998, hundreds of sea lions (*Zalophus californianus*) were found stranded along the central Californian coast; many of these were already dead, while others displayed severe neuropathological symptoms. Investigations showed that this event had coincided with seasonal "flash" blooms of *Pseudo-nitzschia australis*, a newly discovered producer of DA, which causes ASP. The identified trophic link was northern anchovies (*Engraulis mordax*) and Pacific sardines (*Sardinops sagax*) that feed on phytoplankton in the nutrient-rich waters, and accumulate DA to toxic levels during *P. australis* blooms. Indeed, the toxin was found both in extracts of these fish and in the body fluids of dead sea lions (Scholin *et al.*, 2000). An examination of the stomach contents of the fish showed frustules of *P. australis* to be present, and blooms of this species co-occurred during sea lion mortality events, whereas blooms of the nontoxic *P. pseudodelicatissima* corresponded with a fall in DA levels in the flesh of these fish. Furthermore, histopathological sections of the anterior hippocampal region of the sea lion brain (see Section 8.5) revealed DA-induced lesions typically found in other post-mortem autopsies.

As was the case with seabirds, earlier observations of similar but unresolved cases of marine mammal intoxication events could then be linked to deadly blooms of toxigenic diatoms. Thus, other sea lion, fur seal, dolphin and cetacean mortalities reported from Mexican (Baja California peninsula) and Californian coasts could be documented in connection with potentially toxigenic *Pseudo-nitzschia* blooms (e.g., Ochoa *et al.*, 1998).

There is now a growing concern that repeated seasonal blooms of toxigenic *Pseudo-nitzschia* species may expose marine wildlife to sublethal doses of DA that will trigger pathological symptoms years later. Although water-soluble DA is not bioaccumulated (excess DA is rapidly eliminated by kidneys), chronic exposure to DA may cause irreversible damage to the brain and CNS by repeated fixation to glutamate receptors (see Section 8.5). For example, Goldstein *et al.* (2008) have predicted that chronic DA toxicosis will induce epileptic fits that can be qualified as novel symptomatology, in contrast to the acute syndromes described above. Young female sea lions are known to be under high risk due to chronic exposure to DA, with effects on their embryos and subsequent early life stages; this adds to poisoning by industrial discharges of other neurotoxins (e.g., DDT) at localities where human influence has already been highly detrimental (Ramsdell, 2010).

It is now necessary to reassess the health risks to marine wildlife and humans in light of predictive anthropogenetic and biogeoclimatic factors that may synergistically lead to the emergence of high-risk DA-producing diatom blooms.

8.3 Chemistry

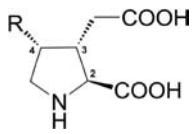
8.3.1 Physico-Chemical Properties

Domoic acid is highly water-soluble (8 mg ml^{-1}) but much less soluble in methanol (0.6 mg ml^{-1}). The pure compound is most often described as colorless crystal needles, with a density of 1.273 g cm^{-3}. DA is a moderately thermostable molecule (it resists cooking conditions), and is photodegradable upon sunlight and UV exposure. Acidic conditions (pH <3) tend to accelerate its degradation. Controlled heat treatment converts DA into its 5′-epimer (without loss of toxicity) and UV-exposed dilute DA solutions photoisomerize reversibly into isodomoic acids D, E and F, and decarboxylate irreversibly (Table 8.1) (Bouillon *et al.*, 2008). The significance, both environmental and socioeconomic, of the degradation processes is developed below.

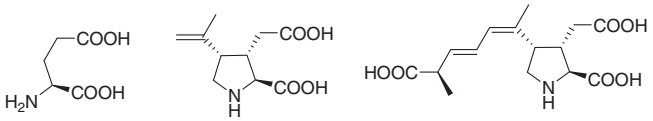
8.3.2 Structure Determination

8.3.2.1 The Kainic Acid Family

DA is a member of the kainoid group of neurologically active nonproteinogenic amino acids, of which kainic acid (KA) is the parent molecule (Lefebvre *et al.*, 2002). With respect to their affinity to target glutamate receptors, kainoids share a 2,3,4-trisubstituted pyrollidine core structure (Laycock, de Freitas, and Wright, 1989).



Structurally, DA is very similar to KA, and both are functional analogs of the amino acid glutamic acid; all three molecules are considered as potentially excitotoxic. In addition to the imino group common to kainates, the distinguishing features of DA are: (i) the three carboxyl groups that account for its exceptional ionization potential and solubility; and (ii) the electron-rich conjugated diene region that is responsible for its biological activity, although this activity varies greatly according to the molecule's configuration.



glutamic acid kainic acid domoic acid

With four chiral centers, and thus a total of 16 possible stereoisomers, each with four geometric isomers, there are 64 theoretical isomer combinations of DA. Of these, several geometric isomers (isodomoic acids A–H), the distereoisomer

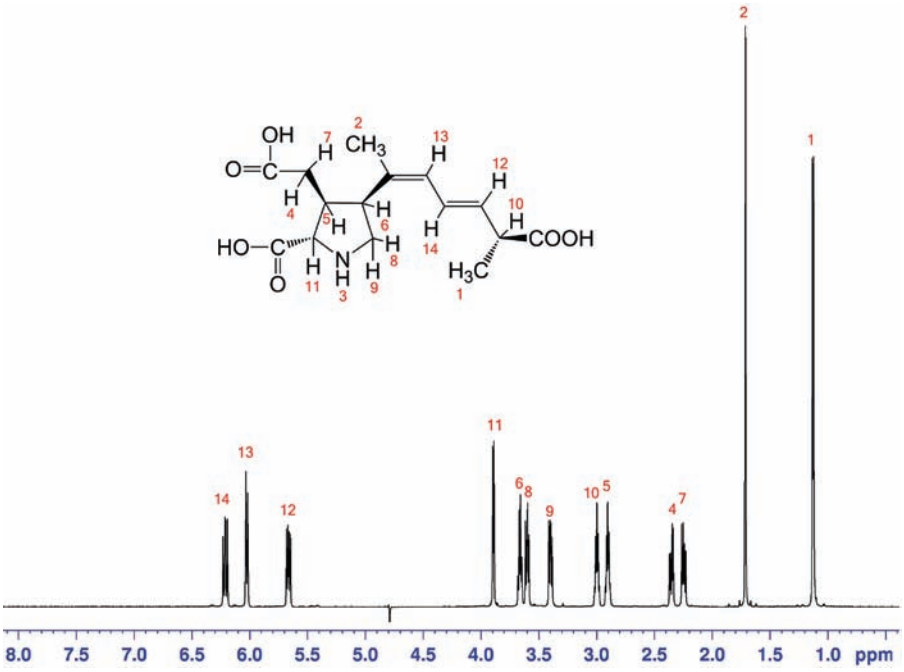


Figure 8.2 Proton NMR of purified domoic acid at neutral pH. The upfield to downfield sequence of signals is reported as proton positions on the DA molecule. (Personal data provided by Dr Michael Quilliam.)

5′-epi-domoic acid and two known lactone derivatives, isodomoilactone A and B, have been described (Table 8.1).

8.3.2.2 Nuclear Magnetic Resonance (NMR) Spectroscopy

The molecular structure of DA isolated from the red alga *Chondria armata* was first proposed based on NMR evidence (Takemoto and Daigo, 1958; Daigo, 1959c; Takemoto *et al.*, 1966), but was later revised following total synthesis (Ohfune and Tomita, 1982). Following the 1987 ASP event on Prince Edward Island (see Case study #1), Canadian chemists and toxicologists examined DA and its congener molecules produced by toxigenic diatoms, as well as those concentrated or transformed along the food chain, and of filter-feeding mollusks in particular. A typical proton one-dimensional NMR spectrum is represented in Figure 8.2, and data from both proton and ^{13}C acquisitions are shown in Table 8.2.

Table 8.2 Proton and ^{13}C NMR spectral details of purified domoic acid at pH 3.4. From left to right: Proton signals (in red), numbered in upfield to downfield order as in Figure 8.2; Proton chemical shifts, in ppm, relative to TSP as zero reference; Proton signal splitting pattern: s (singlet), d (doublet), t (triplet), q (quadruplet), b (broad); Proton (pairs) coupling constants, in Hz; Position of corresponding carbon (in blue) on molecule; ^{13}C chemical shifts, in ppm; ^{13}C signal splitting pattern, same letter code as above, with m (multiplet); Proton type respective to carbon to which attached. (Adapted from Wright *et al.*, 1988 © Canadian Science Publishing.)

up > downfield	H (δ ppm)	H splitting	J (H, H) Hz	Position	^{13}C (δ ppm)	^{13}C splitting	Attached H type
1	1.27	d	(5′, 6′) 7,1	5′-CH_3	18.6	qdd	allylic methyl
2	1.81	s	None	1′-CH_3	23.5	qdd	vinylic methyl
7	2.50	dd	(3, 6b) 9.1	6b	35.4	bt	vicinal to COOH
4	2.76	dd	(3, 6a) 5.8	6a			vicinal to COOH
5	3.05	dddd	(2, 3) 8.1 (3, 4) 8.4 (3, 6a) 5.8 (3, 6b) 9.1	3	44.6	bd	methine
10	3.30	dq	(4′, 5′) 7.8 (5′, 6′) 7.1	5′	44.9	bd	methine
9	3.49	dd	(4′, 5α) 7,3 (5α, 5β) −12.2	5α	49.1	bt	beta to N
8	3.71	dd	(4, 5β) 7.9 (5α, 5β)-−12.2	5β			beta to N
6	3.84	ddd	(4, 5α) 7.3 (4, 5β) 7.9	4	42.7	bd	methine
11	3.98	d	(2, 3) 8.1	2	67.1	bd	methine
12	5.78	dd	(3′-4′) 14.9 (4′-5′) 7.8	4′	135.2	bd	ethylenic H
13	6.13	d	(2′, 3′) 11.1	2′	132.8	bd	ethylenic H
14	6.35	dd	(2′, 3′) 11.1 (3′-4′) 14.9	3′	128.6	dd	ethylenic H
				1′	133.8	bm	none
				7	177.5	dt	none
				2-COOH	174.9	dd	none
				5′-COOH	181.9	m	none

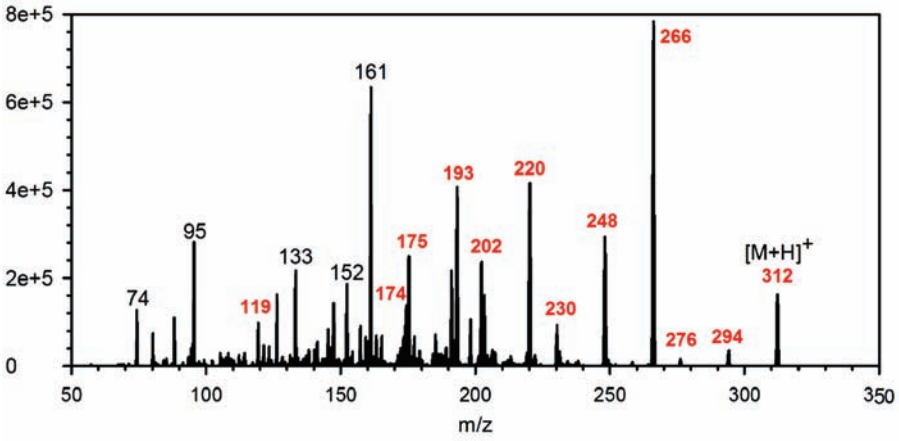


Figure 8.3 Typical product ion mass spectrum of domoic acid in positive ion mode. Peaks in red correspond to species resulting from fragmentation pattern in Figure 8.4. (Personal data provided by Dr Michael Quilliam.)

8.3.2.3 Mass Spectrometry (MS)

A typical product ion mass spectrum of the DA molecule is represented in Figure 8.3. The protonated molecule $[M+H]^+$ appears at m/z 312, with major peaks at m/z 266 and 220 corresponding to successive decarboxylation losses of HCOOH (46). Other peaks correspond to eliminations of H_2O (18), HCN (27) and CO (28) (Figure 8.4). One low-mass ion at m/z 74 can be attributed to the ion $[CO_2H—CH=NH_2]^+$, which is characteristic of protonated amino acids (Thibault *et al.*, 1989). The peaks with red labels in Figure 8.3 correspond to ions in the fragmentation pathway of Figure 8.4.

8.3.2.4 UV spectroscopy (UV)

The UV spectrum as scanned from 200 to 300 nm consists of a single intense band (signal onset at 275 nm) with a maximum at 242 nm (Figure 8.5), corresponding to an α,β-unsaturated carboxylic acid in the side chain. The absorbance peak is pH-sensitive, the 242 nm maximum corresponding to an approximately neutral pH. Falk, Walter, and Wiseman (1989) determined the λ_{max} and ε_{max} profiles of DA at different protonation stages in order to facilitate the interpretation of UV spectra commonly used in the detection of the toxin in seafood samples analyzed using liquid chromatography (see Wright *et al.*, 1988).

At first glance, the outstanding feature of DA is its unusual ionization properties due to the presence of the aforementioned carboxyl and amino groups, which leads to five potential charge states of the molecule. At physiological pH, the prevailing form of DA is deprotonated at all three carboxyl groups and protonated at the amino group, leading to a net charge of −2. This has

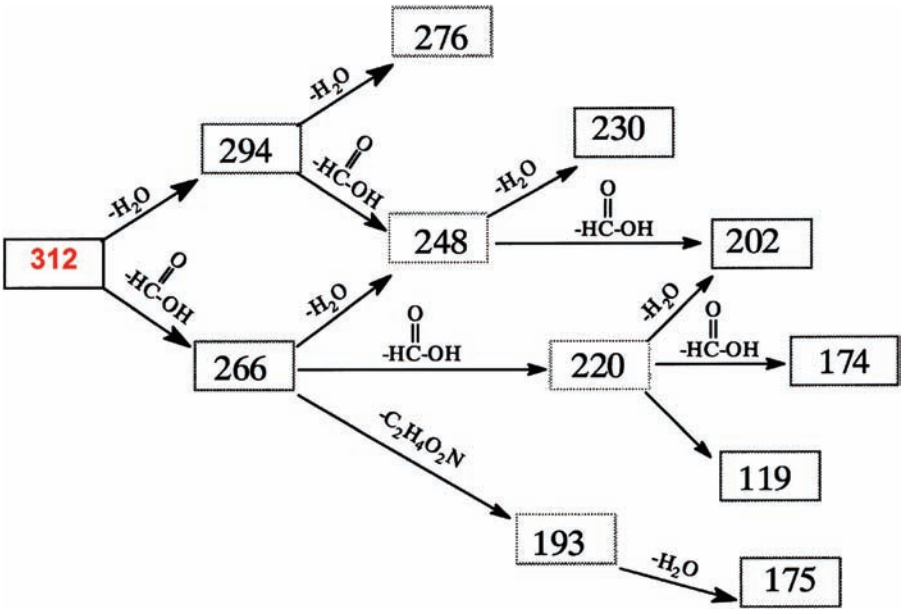


Figure 8.4 Characteristic fragmentation pathway of domoic acid in positive ion mode. The peaks correspond to red labeled species in Figure 8.3. (After Thibault *et al.*, 1989.)

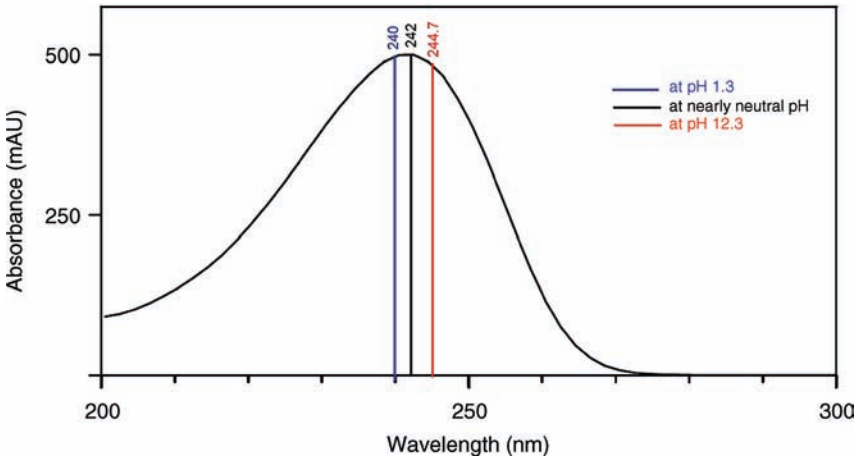


Figure 8.5 Ultraviolet spectrum of domoic acid using diode-array spectrometer (UV-DAD). There is a small shift in the λ_{max} (wavelength for maximum absorbance) according to the pH of the solution. (Personal data provided by Dr Michael Quilliam.)

important implications for the neurotoxicity of DA when administered to animals (Ramsdell, 2007).

DA bears a hexadienoic C4 side chain, which confers its unique toxigenic character with respect to its kainate/glutamate analogs. The presence of two conjugated double bonds in the C4 side chain, and the geometry of these moieties, are directly related to the interaction of DA at the glutamate receptor and to the toxicity of the molecule (Swanson and Sakai, 2009). The presence of these stable dienes enables DA to absorb UV light, which at neutral pH gives an emission maximum of 242 nm, and this is used in one method to quantify DA by liquid chromatography (Quilliam *et al.*, 1989a).

8.3.3 Extraction, Separation, Purification, and Detection of DA

8.3.3.1 Extraction and Cleanup

Most of the extraction procedures use mollusk flesh as the starting material, whether to obtain the pure compound or to perform routine toxin identification and quantitation from samples. Aqueous methanol (1 : 1) or hot water may be used as solvent under homogenizing conditions to achieve an efficient extraction of DA from tissue samples (Quilliam *et al.*, 1989a; Quilliam, Xie, and Hardstaff, 1995). In preparative isolation procedures, pigments and other molecules of medium polarities may be removed from crude extracts by evaporating the methanol and partitioning the extract with a nonmiscible solvent, such as dichloromethane. A selective sample cleanup of aqueous methanol extracts, based on solid-phase extraction (SPE) with a strong anion-exchange column, is used widely as a cleanup for analytical purposes (Quilliam, Xie, and Hardstaff, 1995; He *et al.*, 2010).

8.3.3.2 Separation and Purification

The high polarity of DA is due to the presence of three carboxyl groups and one imino group with pK_a values of 1.85, 4.47, 4.75, and 10.60, respectively. Thus, DA may exist in five different charge states, from −3 to +1 depending on the pH, and this can affect its retention in different chromatographic systems. For example, an acidic mobile phase is required for reversed-phase chromatography in order to suppress ionization of the carboxyl groups which, in their anionic form, will lead to a poor retention as well as an adverse interaction with residual silanol functions (Quilliam *et al.*, 1989a; Quilliam, Xie, and Hardstaff, 1995). Both, capillary electrophoresis (CE) and capillary electrochromatography have proved to be useful alternatives to HPLC for DA monitoring (Zhao, Thibault, and Quilliam, 1997; He *et al.*, 2010); in this case, a basic running buffer is used to ensure that DA is in an anionic form for maximum electrophoretic mobility.

8.3.3.3 Detection, Quantification, and Monitoring in Food Samples

The method of choice for detecting and quantifying DA in samples is liquid chromatography combined with UV spectrophotometry (using a diode array detector; DAD) at a fixed wavelength of 242 nm to detect DA specifically, or with full spectrum scanning (e.g., between 220 and 360 nm) for a better confirmation of identity. Reversed-phase thin-layer chromatography with DA visualized by a ninhydrin spray has been proposed for those laboratories lacking HPLC equipment (Quilliam, Thomas, and Wright, 1998). LC-MS has proven to be a very sensitive and selective method for DA (Quilliam *et al.*, 1989b; Quilliam, Xie, and Hardstaff, 1995). Routine monitoring in shellfish tissue requires the detection of DA at concentrations well below safe limits for human consumption, and protocols must be simple to operate and reliable regarding quantitation. This subject is reviewed by Quilliam (2003).

When quantifying DA in complex extracts, careful attention is required regarding the selection of mobile and stationary phases, and also to the column temperature, in order to achieve an adequate separation of DA from the many other natural compounds with chromophores present in the sample. One such example is tryptophan and its oxidation products, which can have similar retention times to that of DA (Quilliam *et al.*, 1989a). If the instrument used is fitted with a DAD, the compounds can be easily distinguished by their full UV spectra.

A strong anion-exchange SPE cleanup prior to HPLC provides a much higher degree of selectivity (Quilliam, Xie, and Hardstaff, 1995). This cleanup is quite remarkable; when aqueous methanol extracts are loaded, only very strongly acidic compounds such as DA will be retained, while weaker amino acids such as tryptophan are washed away; the DA can then be eluted with acidic water (pH 3) or with 1 M NaCl in water. The limit of detection (LOD) for DA when using this method is 20 ng g^{-1}, after sample cleanup. Mafra *et al.* (2009) developed a high-sensitivity HPLC-UV method for detecting trace levels of DA in seawater and phytoplankton cells, using a large-volume injection with an ion-pairing agent and gradient elution; a LOD for DA of 42 pg ml^{-1} was achieved in this way. As a variant to classical reversed-phased HPLC columns packed with fine particles, Regueiro *et al.* (2011) proposed a simple and efficient method for the routine determination of DA from shellfish samples. Using graphitized nonporous carbon as a dispersive solid-phase extraction (dSPE) cleanup sorbent for the dispersive extraction of the shellfish flesh homogenates (concentrated at 50 mg ml^{-1}), the authors were able to separate the centrifuged supernatant using a monolithic silica stationary phase (high flow rate and high exchange surface). This helped to reduce the backpressure buildup and decrease the runtime versus efficiency of the HPLC separation process. With acetonitrile/water gradient as the mobile phase under acidic conditions, it was possible to separate the sample and detect DA and its congeners (UV at 242 nm) in about 3 min, which was three- to fourfold faster than with conventional HPLC procedures, without reducing the performance of the column between runs.

A very selective and sensitive detection of DA can be achieved by using electrospray ionization (ESI) LC-MS, especially in the selected reaction monitoring mode where transitions from the $[M + H]^+$ ion to specific product ions (as in Figures 8.3 and 8.4) are measured. Triple quadrupole or ion-trap MS systems can be used to produce such data (Quilliam *et al.*, 1989b; Furey *et al.*, 2001; Mafra *et al.*, 2009), using reversed-phase HPLC columns eluted with an acidified acetonitrile–water gradient from 5 : 95 to 40 : 60. Mafra *et al.* (2009) demonstrated the suitability of the large-volume injection method with MS detection for the trace level detection of DA in seawater and phytoplankton cells; in this case, an LOD for DA of 15 pg ml^{-1} could be achieved.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS is suitable for the routine detection of higher-level DA samples (Paz, Riobó, and Franco, 2011). Although the DA signal is hampered by matrix noise and limited observable fragmentation patterns (193, 248, 266), the technique has been used in the efficient diagnosis of DA-related toxicosis of sea lion samples (Neely *et al.*, 2012).

Other protocols aimed at detecting DA diluted in seawater and in phytoplankton samples include derivatization followed by HPLC with fluorescence detection (reviewed by Riobó, López, and Franco, 2011). 9-Fluorenylmethylchloroformate (FMOC-Cl) is known to react rapidly with DA, and its application to seawater samples gave an LOD for DA of 15 pg ml^{-1} (Pocklington *et al.*, 1990). Other derivatizing reagents that have been used successfully on phytoplankton and seawater samples after preconcentration with SPE include 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) and 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F).

8.3.3.4 Immunological Method

Recently, enzyme-linked immunosorbent assays (ELISAs) have been widely used for toxin detection because they are relatively cheap, rapid, and simple in operation. Garthwaite *et al.* (1998) developed an antibody to detect DA in shellfish and seawater extracts via an indirect competitive enzyme-linked immunosorbent assay (cELISA) with a LOD for DA of <0.01 ng ml^{-1}. In such methods, the generation of an antibody is conditioned to attaching DA to a larger carrier (protein). Baco *et al.* (2010) showed that a C-4 linkage of the protein to the DA ring generated antibodies with a greater specificity than when carboxy or imino-linkages were employed.

Antibody-based DA detection methods are also useful indicators of chronic exposure and increased neurologic sensitivity in both marine mammal and human populations. Lefebvre *et al.* (2012) exposed zebrafish (*Danio rerio*) to chronic sub-level doses of DA and identified a specific antibody biomarker to this toxin, which was subsequently used to detect DA in sea lion populations that are particularly exposed.

8.3.4 Domoic Acid and Related Molecules

A number of DA isomers have been isolated from various sources (Table 8.1). Structurally, isodomoic acids A–H differ from DA in the position and the configuration of side-chain double bonds, and hence are constitutional isomers of DA. A number of the isoforms of DA have been found in the red alga *Chondria armata* and the blue mussel *Mytilus edulis*. The 5′-epimer (Table 8.1) in cooked shellfish is a heat-transformation product that has retained the activity of its precursor. Two relatively inactive lactones are also found as transformation derivatives of DA. Some of the geometric isomers are active as insecticides or as anthelmintic agents, but lose most of their neurotoxicity when evaluated against vertebrates as test organisms.

8.3.5 Synthesis

The kainoid amino acids are highly valuable experimentally to neuroscience and medicinal chemistry (Denmark, Liu, and Muhuni, 2009), as they display a range of bioactivities, some with a very high potency. Most, however, can only be obtained from natural sources in submilligram quantities, which emphasizes the need for their synthesis on demand. This is certainly the case for KA, which is still quite expensive, and for several of its analogs. The total synthesis of DA was completed in 1982 (Ohfune and Tomita, 1982), in view of the growing interest in its biological activities, and the stereochemistry of the active

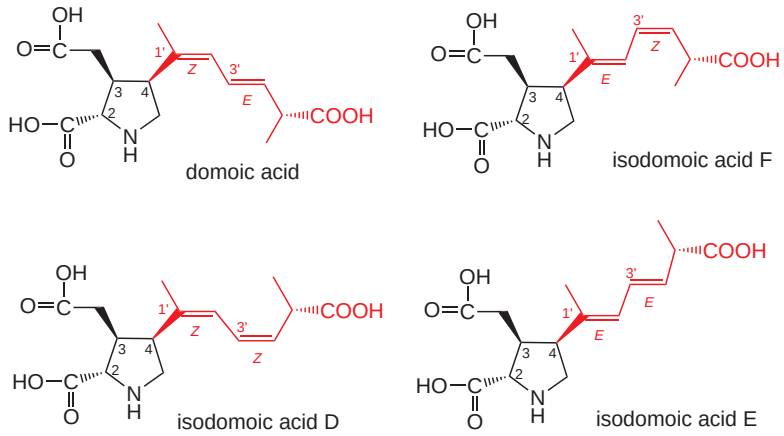


Figure 8.6 Side chain (in red) of domoic acid with diene at 1′-2′ and 3′-4′ positions. The C1′ alkene with *Z*-configuration is essential for its activity. The *Z*-, *E*-configuration at top left confers maximum excitotoxic activity.

configuration later confirmed in 1992, using X-ray crystallography (Nomoto *et al.*, 1992).

The more complex C-4 side chain of DA leads to an increased excitotoxicity compared to KA (Hashimoto, Ohfune, and Shirahama, 1995), due to the strength of ligand binding to the kainate receptor. Such binding is directly dependent on C4 stereochemistry, C4 substituents and molecular conformation (Clayden, Read, and Hebditch, 2005a). With regards to the C4 substituents, a C1′ alkene with *Z*-configuration is more active than the *E*-configuration; *sp2* substituents (dienes) are up to 1000-fold more active than *sp3* (saturated) substituents (Figure 8.6). Not surprisingly, the intriguing and diverse biological activities, together with the various stereochemical orientations and positioning of sites of unsaturation of the C4 substituents of members of the DA family of compounds, have allowed new strategies for developing stereochemically controlled synthetic analogs (Ni *et al.*, 2003, 2009; ElDouhaibi *et al.*, 2011; Denmark, Liu, and Muhuni, 2009, 2011).

8.3.6 Biosynthesis

8.3.6.1 Labeled Precursor Investigations

In *Pseudo-nitzschia multiseries*, DA is hypothesized to be formed through an enzymatic reaction of two precursors (Douglas *et al.*, 1992; Ramsey *et al.*, 1998; Pan, Bates, and Cembella, 1998). One of these is an activated citric acid derivative from the Krebs tricarboxylic acid cycle, and the other is isopentenyl pyrophosphate (also known as geranyl pyrophosphate) from the methylerythritol phosphate (MEP) pathway in the plastid, but possibly also originating from the 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) pathway in the cytosol (Figure 8.7).

Further isotopic labeling experiments were undertaken in order to facilitate identification of the enzymatic pathways leading to formation of the final DA molecule, and of the genes that would enable studies of how environmental factors might regulate its biosynthesis at the molecular level. Two mechanisms could account for the cyclization of the imine ring from the above precursors: (i) direct displacement of the pyrophosphate (PPi) of geranyl diphosphate (precursor A) by the nucleophilic amino nitrogen of the glutamate (precursor B); and (ii) precursor A may be dephosphorylated and later oxidized to the aldehyde prior to condensation with the amino nitrogen of glutamic acid (precursor B) to form an imine. The use of deuterium (^{2}H or D) labeling of precursor A (geranyl

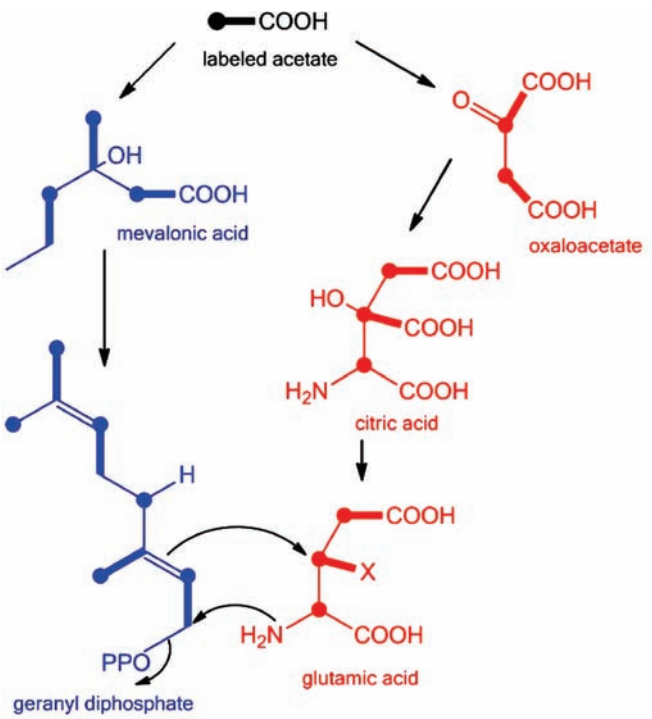


Figure 8.7 Suggested domoic acid biosynthetic pathway as evidenced from ^{13}C-labeled acetate incorporation experiments. Red-labeled acetates are incorporated through the activated TCA cycle (glutamic acid, in red as precursor B); blue labels are from the MEP pathway (geranyl diphosphate as precursor A). (Adapted from Kalaitzis *et al.*, 2010 with kind permission by Elsevier, from the hypothesis of Douglas *et al.*, 1992.)

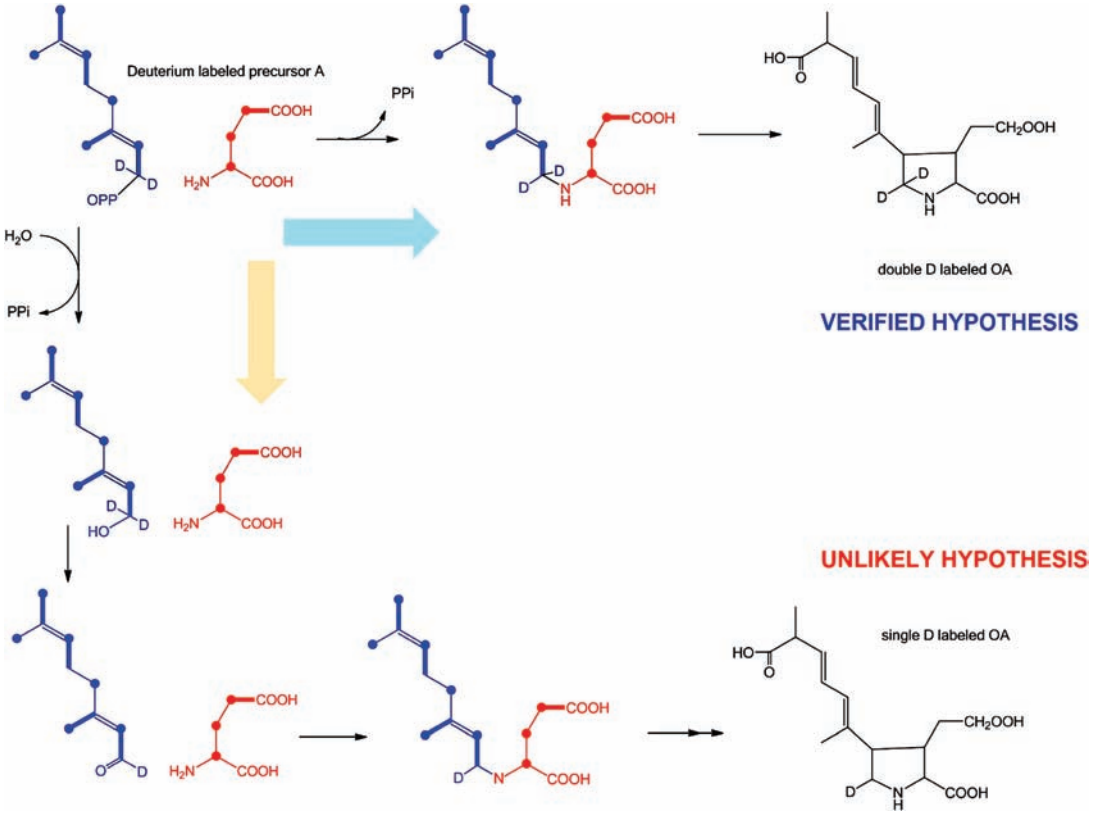


Figure 8.8 Possible alternatives for the condensation reaction using deuterium-labeled geranyl diphosphate precursor. In the "short" pathway (blue arrow), the amino nitrogen may serve as a nucleophile to displace the pyrophosphate on the geranyl diphosphate (precursor A). In the alternative "long" pathway (pink arrow), precursor A may be dephosphorylated and later oxidized to the aldehyde prior to condensation with an amino nitrogen to form an imine. The presence of the two deuterium labels in the end products provides evidence that the short pathway is the correct one.

diphosphate, in blue in Figure 8.8; Savage *et al.*, 2012) showed that direct displacement of PPi by the amino nitrogen acting as a nucleophile was the likely condensation mechanism in *P. australis* labeling experiments, as both deuterium labels were present in the end product (only one label would be expected in the longer pathway).

8.3.6.2 Regulation of DA Production

The results of laboratory studies have confirmed that the production of DA is related to the availability of silicon, phosphorus, nitrogen and trace metals (mainly iron) (Lelong *et al.*, 2012; Trainer *et al.*, 2012), all of which can be limiting for diatom growth (Morel and Price, 2003). The situation in the natural environment, however, is more complex, as detailed in Section 8.7. For example, open-ocean strains of *Pseudo-nitzschia* are less prone to producing the toxin than are coastal conspecifics (cf. Marchetti *et al.*, 2008). These differences may be attributable to:

- Environmental parameters, such as nutrient availability and ratios, and physical parameters. For example, cellular Si : C ratios decrease with increasing CO_2 concentration, whereas DA production is stimulated (Tatters, Fu, and Hutchins, 2012). Phosphorus limitation under increasing pCO_2 (lower pH) also stimulates DA production (Sun *et al.*, 2011). Salinity, temperature and light each have a positive influence on DA production, but in association with other parameters that modulate its production under limiting conditions.
- Different bacteria associated with *Pseudo-nitzschia* cells can affect the DA-producing ability in cultivated versus wild strains (cf. Kaczmarska *et al.*, 2005).
- Variability of responses between ecotypes. Preliminary genome investigations have revealed the presence of an unusually large number of transposons or "jumping genes," which may strengthen the capacity of new ecotypes to emerge and respond to environmental fluctuations (see Armbrust, 2009). There may also be inherent differences in the genomes between coastal and oceanic *Pseudo-nitzschia* species.

There is, therefore, a need for a complete, annotated genome of toxigenic *Pseudo-nitzschia* species, as a complement to comparative metatranscriptomic studies, in order to unravel the complex interaction network regulating DA biosynthesis, and this project is under way (see below; Parker, Maumus, and Armbrust, 2013). Furthermore, directed mutagenesis studies and subsequent genome comparisons may allow the identification of genes responsible for the toxigenic phenotypes.

8.3.7 Degradation

8.3.7.1 Photodegradation

Natural DA removal and degradation are both chemically and biologically driven processes (for a review, see Lelong *et al.*, 2012). Being extremely polar, DA is easily lost from *Pseudo-nitzschia* cells into the water column (Bates *et al.*, 1991), where it is readily diluted and undergoes natural photodegradation in the presence of sunlight. DA is not associated with particulate or colloid matter in high proportions (Lail *et al.*, 2007), but the presence of transient and highly reactive oxygenated species (e.g., hydrogen peroxide, singlet oxygen, hydroxyl radicals and superoxide ions due to the degradation of surface colloids) may be an important factor for DA degradation in the water column. Furthermore, DA is able to bind metal cations (Fe^{3+}, Cu^{2+}) and become more susceptible to photolysis as a complex, but not in the absence of light. This iron-chelating capacity of DA may: (i) initially facilitate the rapid formation of blooms of *Pseudo-nitzschia* (i.e., a siderophore-like function); and (ii) subsequently facilitate the natural recycling of this molecule during the decay phase of the bloom.

The proportion of DA in the extractible pool of shellfish ASP toxins is 90%, compared to 5%, 2% and 1%, respectively, for the DA isomers D, E, and F. The proportion of DA can be decreased to 35%, however, after a 9- to 12-min exposure to UV-A light (253.7 nm), during which time the proportions of isomers D, E and F increase to 15%, 34% and 16%, respectively (Clayden, Read, and Hebditch, 2005a). This demonstrates that the UV-A treatment of DA can reduce its toxicity.

8.3.7.2 Photo-oxidative Degradation

Where local concentrations can be problematic (e.g., in desalination plants), advanced oxidation processes can be used to photo-oxidize DA (Lelong *et al.*, 2012). Both the amine group of the pyrollidine ring and the conjugated diene of the side chain are susceptible to reacting with singlet oxygen $^1O_2$ (Parekh, 2012).

8.3.7.3 Bacterial and Enzymatic Degradation

The bacterial degradation of DA and isomers is suspected to occur in the digestive tracts of certain contaminated filter-feeding mollusks, and this may also occur in sediments from high-risk areas. Blue mussels (*Mytilus edulis*) and soft-shell clams (*Mya arenaria*), when supplemented with bacterial growth factors, show an increase in bacterial biomass coupled with the biodegradation of DA, suggesting the presence of potentially DA-degrading bacterial strains. This is in contrast to sea scallops (*Placopecten magellanicus*), which sequester DA for longer periods (Stewart *et al.*, 1998), and do not seem to rely on DA-degrading bacterial strains in a lengthy depuration process. More recently, Hagström *et al.* (2007) showed that the sampling of bacteria from sediment and pseudo-feces of *Mytilus edulis* from contaminated areas yielded actively DA-biodegrading strains/enzymes. This was in contrast to "inactive" copepod fecal pellets, which suggests that copepods lack both the bacteria and the enzymes capable of degrading DA. As an important link of the marine food chains, copepods may thus act as bioaccumulating vectors of DA to higher trophic levels.

The identification of specific DA-degrading bacterial strains is not a simple task, and is further complicated by major taxonomic revisions in critical taxa, such as *Pseudoalteromonas* (Ivanova, Flavier, and Christen, 2004), which are commonly engaged in functional relationships with eukaryotic hosts. Donovan *et al.* (2009) have identified *Pseudoalteromonas* strains isolated from PSP-contaminated mussels as potential toxin-degrading agents, which could encourage similar additional investigations on ASP toxins.

8.4 DA-Producing Organisms

Domoic acid, as well as most of its isomers and kainic acid, are of algal origin, either from red algae (Rhodophyta) and/or two genera of pennate diatoms. Other DA derivatives are transformation products isolated from bivalves or degradation products resulting from heat or UV exposure (Table 8.1).

8.4.1 Red Algae

As noted above, DA was first discovered in the rhodophyte *Chondria armata* (Figure 8.9) as the principal anthelmintic medicinal ingredient (Takemoto and Daigo, 1958). This red macrophyte also produces isodomoic acids A, B, C (Maeda *et al.*, 1986), D (Maeda *et al.*, 1985), G, H (Zaman *et al.*, 1997), and two analogs, domoilactones A and B (Maeda *et al.*,

Figure 8.9 The red seaweed *Chondria armata*, a producer of domoic acid. (From *Common Seaweeds of China* (1984) (ed. C.K. Tseng); reproduced with permission from Kugler Publications.)

1987b) (Table 8.1). *Chondria baileyana* (Laycock, de Freitas, and Wright, 1989), *Alsidium corallinum* (Impellizzeri *et al.*, 1975), *Amansia glomerata*, *Digenea simplex* and *Vidalia obtusiloba*, all of which belong to the order Ceramiales, also synthesize DA, often concurrently with KA (Sato *et al.*, 1996).

8.4.2 Diatoms

The other major sources of DA, and of several of its isomers, are pennate diatoms of the genera *Pseudo-nitzschia* and *Nitzschia*. Of the 38 described species of *Pseudo-nitzschia*, 14 have been reported to be toxigenic, although several other species have not yet been tested (Lelong *et al.*, 2012; Lim *et al.*, 2012). *Pseudo-nitzschia multiseries* (originally described as *Nitzschia pungens* forma *multiseries*) was documented as the DA source of the Prince Edward Island 1987 toxicity episode reported above.

Although *Pseudo-nitzschia multiseries* has been reported to contain >3% (dry weight) DA (Laycock, de Freitas, and Wright, 1989), some strains of reputedly toxigenic species may not always test positive for DA (Lelong *et al.*, 2012). The same is true for certain strains of *Nitzschia navis-varingica*, the other DA-producing species (e.g., Romero *et al.*, 2012). Thus, investigations of the environmental and epigenetic factors that regulate DA production are necessary, in addition to classical taxonomy, for characterizing *Pseudo-nitzschia* and *Nitzschia* blooms as potentially harmful.

Following the 1987 event, other DA-producing species were documented in connection with: (i) the mortalities of marine birds and mammals; (ii) the contamination of finfish consumed by humans or other marine organisms; (iii) shallow-water benthic shrimp (by toxic *Nitzschia navis-varingica*), crabs and soft-bodied worms; and (iv) deeper benthic food webs caused by the rapid transport of particulate DA to depths in excess of 800 m (Sekula-Wood *et al.*, 2009). Low, chronic doses of DA have yet-to-be-understood negative impacts on human and marine populations, and therefore DA has broad ramifications not only for human health but also for ecosystem health. A detailed account of which *Pseudo-nitzschia* species was involved with each toxic episode (Lelong *et al.*, 2012), and maps showing the location of each species and where the harm had occurred (Trainer, Hickey, and Bates, 2008; Trainer *et al.*, 2012) are available elsewhere.

Most *Pseudo-nitzschia* species form chains with overlapping tips (Figure 8.10), which distinguishes them from *Nitzschia* species that are solitary cells. These chains are of variable length in a natural situation (up to hundreds of cells), but in culture they tend to be only a few cells long, and can become single-celled when they are depleted of nutrients for growth.

Diatoms secrete a cell wall, a *frustule*, which is made from amorphous silica (hydrated silicon dioxide). The biogenic source of the silica is mostly silicic acid $Si(OH)_4$, the availability of which is critical for successful diatom bloom formation. Indeed, diatoms are regarded as important silicon sinks and

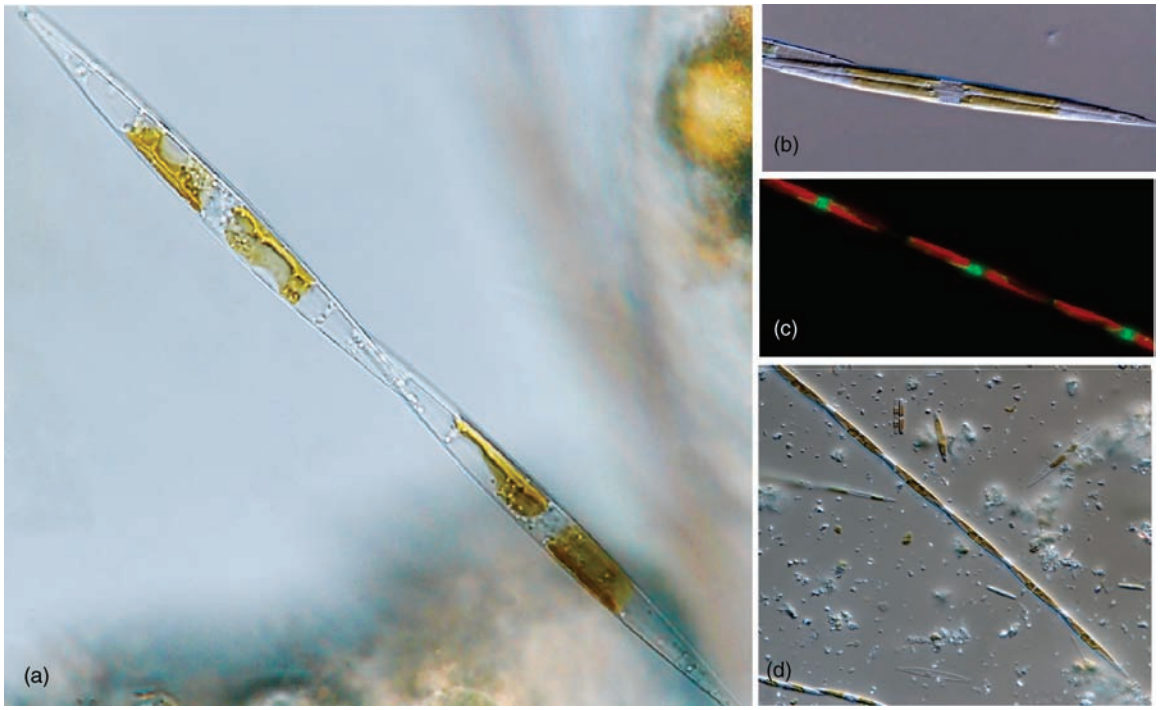


Figure 8.10 Live toxigenic *Pseudo-nitzschia* species under light microscopy. (a) *P. australis* chain, side (girdle) view, showing the overlapping see-through frustules revealing the plastid pigments; sampled during a toxic bloom (Cabrillo Beach, CA, USA; 9 March 2011); (b) *P. multiseries* cultivated strain from eastern Canada, showing a cell in the early stage of division; (c) *P. australis* under epifluorescence microcopy. The nucleus appears bright green and chlorophyll appears red; (d) *P. australis* chain, top (valve) view. Panels (c) and (d) are taken from the same source as panel (a). (All images reproduced with kind permission of Karie Holtermann.)

primary producers of organic carbon in the oceans. Photosynthesis removes volatile carbon, which was originally in the form of carbon dioxide in the atmosphere. When diatoms die, they sink to the oceans' depths where the carbon remains, in some cases long-term in the form of fossil fuel. Diatoms thus not only mediate a major greenhouse gas but also produce almost half of the world's oxygen.

Diatom frustules are made up of two halves (hence the name "diatom," which is Greek meaning "cut in two"), that are referred to as valves. The larger valve fits over the rim of the smaller one, somewhat like the lid of a Petri dish, and the two are held together by girdle bands. The general shape of the long *Pseudo-nitzschia* frustule (whether the sides of the frustule are symmetrical, straight or curved), the cell width (whether $>3\,\mu m$ or $<3\,\mu m$) and the degree of cell overlap within a chain can easily be determined with light microscopy, and are used to group the cells into several possible species. However, a more definitive identification requires examining the highly intricate patterns formed during the silicification process. For example, the number of structural ridges (fibulae), striae and pore arrangements within each valve and at the inter-valve girdle band region (Figure 8.11) are unique to each *Pseudo-nitzschia* species (see Trainer, Hickey, and Bates, 2008). These features are better examined using either scanning electron microscopy (SEM) or transmission electron microscopy (TEM).

For the most definitive species confirmation, morphometric information is backed up by breeding studies and molecular identification (Lelong *et al.*, 2012). Breeding studies confirm the ability of the suspected species to mate with the same, known species in culture. Taxonomists use molecular tools to characterize cryptic species (morphologically identical, but genetically different) or pseudo-cryptic species (minor morphological differences, and genetically different), or novel species (see Lelong *et al.*, 2012; Trainer *et al.*, 2012). The development of DNA amplification tools (PCR and microarrays) and automated ribosomal intergenic spacer analysis (ARISA) will help to resolve any apparently conflicting species designations. The most recent example of this is the newly described species *Pseudo-nitzschia circumpora* from Malaysian Borneo (Lim *et al.*, 2012). The approach used by these, and other, authors was to study the nucleotide sequences of the nuclear-encoded ribosomal DNA (rDNA) to establish genetic species-specificity, in addition to classical morphotyping. Moreover, a better understanding of the local driving forces in modulating the formation of HABs and favoring the production of toxins is possible using "molecular toolboxes" (Kudela *et al.*, 2010). It is hoped that this systems biology approach will greatly benefit the predictions of HAB events by helping to understand the critical parameters that underlie their formation.

8.5 Molecular Basis of DA Acute and Chronic Poisoning

8.5.1 The Kainoids' Mode of Action

The mode of biological action is linked to the structural similarity between the various members of the kainoid family to

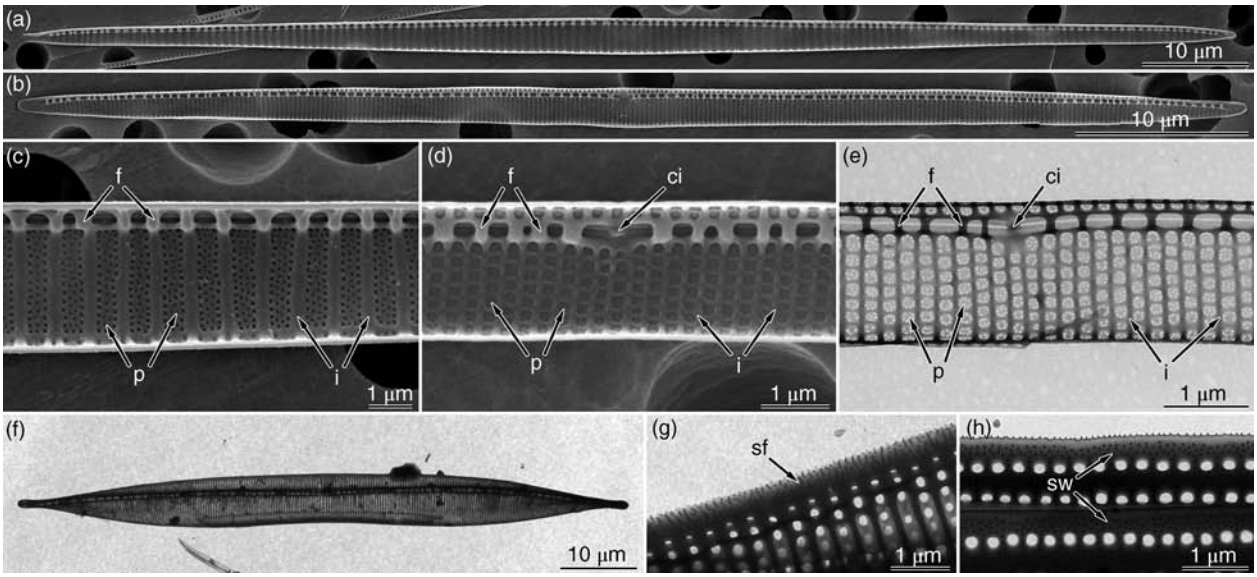


Figure 8.11 Scanning electron microcopy (SEM) and transmission electron microscopy (TEM) images of frustules of domoic-acid-producing diatoms, showing essential morphological features used to differentiate between species. Inner view of valve of (a) *Pseudo-nitzschia multiseries* (SEM) and (b) *P. calliantha* (SEM), showing the general shape of the cells. Detail of central part of valve of (c) *P. multiseries* (SEM); (d) *P. calliantha* (SEM); and (e) *P. calliantha* (TEM), showing fibulae (**f**), interstriae (**i**) and central interspace (**ci**). Note the absence of **ci** in *P. multiseries* (a, c). The TEM (e) shows the characteristic division of the poroids of *P. calliantha* into flower-like sectors; (f) View of valve of *Nitzschia navis-varingica* (TEM). Details of *Nitzschia navis-varingica* frustule (TEM), showing (g) silica pattern (**sf**) of vermiform ridges on the margin of the valve; and (h) girdle band, with silica warts (**sw**). (Images (a) to (e) courtesy of James Ehrman, Digital Microscopy Facility, Mount Allison University [www.mta.ca/dmf]). Specimens provided by Dr Stephen Bates, Fisheries and Oceans Canada. Images (f) to (h) courtesy of Dr Nina Lundholm, The Natural History Museum of Denmark, University of Copenhagen [snm.ku.dk/english]).)

Box 8.3: Glutamate Receptors of the Central Nervous System

Ionotropic receptors (iGluRs) are ligand-gated transmembrane ion channels, and can be classified pharmacologically into subtypes according to their sensitivity to α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), KA, and NMDA (*N*-methyl-D-aspartate). Similarities between the iGluR subtypes are reflected by their amino acid sequence homologies. AMPA receptors (GluR1-4 subtypes) evoke excitatory postsynaptic potentials and mediate fast (<10 ms) synaptic transmission. In contrast, KA receptors (GluR5-6 and KA1/2 subtypes) and NMDA receptors (NR1-3 subtypes) mediate slower synaptic transmission (10–100 ms) and exert effects on plasticity, that is, age-dependent and environmentally induced structural and functional central nervous system adaptive remodeling. For further details, see Traynelis *et al.* (2010).

Metabotropic glutamate receptors (mGluRs) are coupled to G-proteins, and influence a variety of intracellular second messenger systems that modulate neuronal excitability, synaptic plasticity (subtypes of mGluRs are also found in glial cells) and neurodegeneration. As a result, mGluRs are involved in physiological and pathophysiological processes, including development, learning and memory, pain, ischemia, stroke, epileptic seizures, schizophrenia, as well as chronic neurodegenerative diseases.

glutamic acid, and to aspartic acid. This results in an attraction to similar types of receptor in the brain.

8.5.1.1 Glutamate Receptors

Glutamate (Glu) is the major excitatory neurotransmitter in the mammalian CNS, which includes the brain and the spinal cord. In the brain, Glu is released into the synaptic cleft by most neurons as they emit signals to other neurons. Glu exerts its actions by binding to several classes of glutamate receptor (GluR), which in turn leads to a complex network of downstream signaling events. GluRs are characterized as either ionotropic or metabotropic (see Box 8.3).

In addition to its immediate impact as an excitatory amino acid, Glu has a pivotal role in long-term neuronal potentiation, as a proposed molecular substrate for learning and memory.

Both, AMPA and NMDA synaptic Glu receptors are involved, as they control ion trafficking across specific channels. AMPA controls sodium cation (Na^+) postsynaptic transfer, leading to a local depolarization of the postsynaptic dendrite, eventually triggering the nerve influx release downstream. This occurs when Glu fixes itself onto AMPA receptors.

NMDA receptors regulate calcium cation (Ca^{2+}) flux across the calcium channels, which are blocked by magnesium cations (Mg^{2+}) at the resting potential. A membrane potential depolarization caused by Glu fixation on the AMPA receptor expels the Mg^{2+} divalent cations, allowing Ca^{2+} influx and the intracellular molecular cascade and nerve impulse conduction downstream (as illustrated in Figure 8.14). When DA molecules are present, they compete with Glu because of their similar structure (Figure 8.12). However, DA binds more tightly and therefore prevents Glu from binding (Figure 8.13); this leads to a continuous influx of Ca^{2+} that results in neuronal swelling and eventual death (Figure 8.14). Because these neurons are located in the hippocampus part of the brain, short- and long-term memory are impaired, hence the syndrome is termed ASP (see below).

Glutamate also has the potential to be involved in the pathogenesis of many CNS diseases that are caused by its excessive

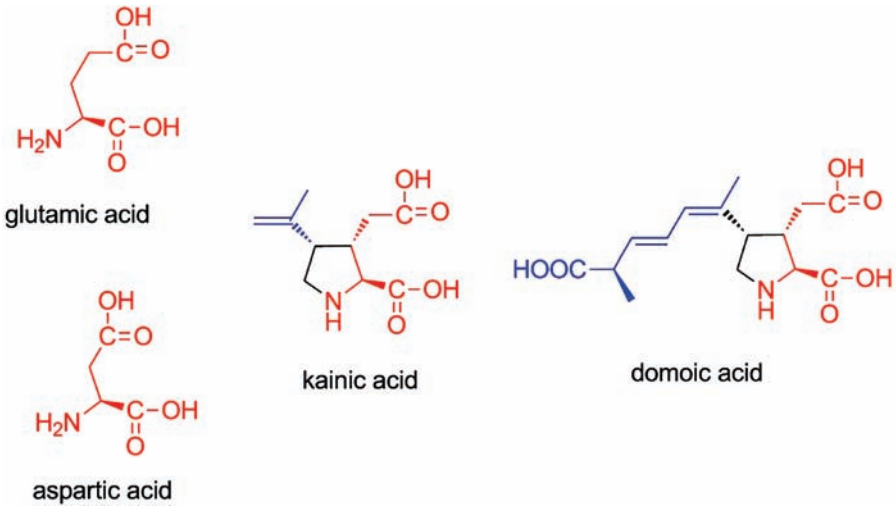


Figure 8.12 Structural resemblance of kainic acid and domoic acid with glutamic acid (template in red) and to aspartic acid. Binding to the kainate receptors (Glu receptors) is strongly influenced by the C4 stereochemistry, C4 substituent and molecular conformation (in blue).

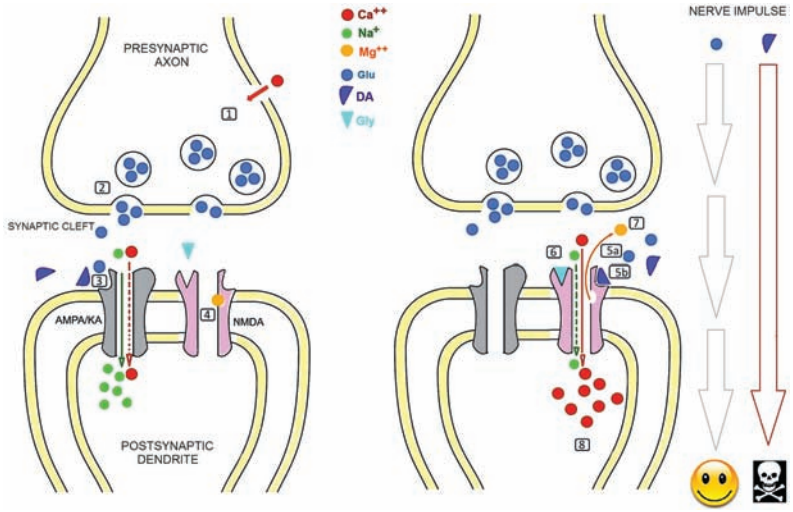


Figure 8.13 Ionic fluxes across the synaptic cleft between two neurons. Left: AMPA and KA receptors. (1) An influx of Ca^{2+} (red dots), increased as a result of DA excitation, mobilizes vesicles containing Glu (blue dots) to the membrane surface; (2) Glu is released into the synaptic cleft by exocytosis; (3) Glu can bind loosely to AMPA and KA sites (in gray) and be later recaptured by endocytosis into the presynaptic axon. DA molecules (purple mounds), if present, compete with Glu, but bind very tightly and prevent Glu binding; (4) AMPA and KA ion channels open and allow preferentially Na^{+} ions inside the postsynaptic dendrite, but also Ca^{2+} ions, resulting in dendrite membrane depolarization and nerve influx transmission (transient if Glu is bound, permanent if DA is bound), as soon as a resting potential is reached. Inactive NMDA receptors have Mg^{2+} ions that block their ion channel at rest. Right: The postsynaptic membrane depolarization and (5a) the fixation of Glu on the NMDA receptor, together with (6) docking of the glycine (Gly, blue triangles) cofactor, lead to (7) the expulsion of Mg^{2+} ions, which opens the specific calcium channel, (8) allowing Ca^{2+} (preferentially to Na^{+}) ions to flow inside the postsynaptic dendrite into a transient, gated regulated manner. However, when DA is present (5b), it binds tightly to NDMA receptors with much greater affinity than does Glu, thus leading to the continuous passage of Ca^{2+}. The postsynaptic intracellular accumulation of Ca^{2+} causes the nerve cell to swell with water and eventually to burst.

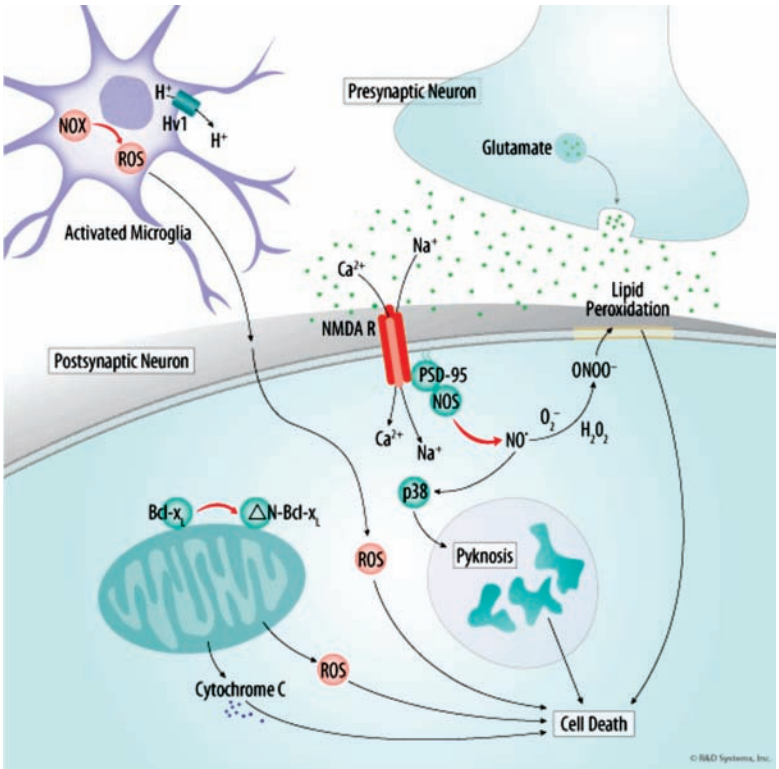


Figure 8.14 Induced neuronal cell death. Abnormally high Ca^{2+} concentrations in the postsynaptic dendrite stimulates the production of reactive oxygen (ROS) and nitrogen (RNS) species, leading to: (i) protease activation and cytoskeletal damages; (ii) lipid peroxidation and disruption of membrane integrity; (iii) microglial activation and production of cytotoxic compounds; (iv) mitochondrial dysfunction; and (v) pyknosis (chromatin condensation). For further details, see Pulido (2008). (Reproduced with permission from R&D Systems; www.rndsystems.com.)

release or reduced uptake or alteration of receptor function (Platt, 2007). In strokes and ensuing head trauma, as well as other excitotoxic conditions, neurons release massive amounts of Glu onto nearby neurons, which then become overexcited and undergo apoptosis after being overloaded with calcium.

Other conditions, such as epilepsy, AIDs, Huntington's disease, Parkinson's disease and Alzheimer's disease, are known to involve Glu-related dysfunctions, and this has been the subject of many neuropathological investigations (e.g., Arundine and Tymianski, 2003). The injection of kainoids gives rise to symptoms that mimic those observed in epilepsy and Huntington's disease, and leads to specific neuronal death in a manner that closely models dementia. The suspicion that regular exposure to monosodium glutamate (MSG), used as a taste enhancer in oriental food, may lead to physiologically excessive Glu levels and hence to chronic poisoning (i.e., beyond the famous "Chinese Restaurant Syndrome") remains a subject of debate between specialists (see Box 8.4). This family of amino acids therefore offers possibilities for developing useful tools in the battle against these debilitating diseases (see Clayden, Read, and Hebditch, 2005a).

The potency of neuroexcitation depends on the strength of binding at the kainate receptor, one of three types of the ionotropic (ion channel) class of glutamate receptors. Binding is influenced strongly by C4 stereochemistry, the C4 substituent, and molecular conformation. The nature of the C4 substituent is particularly critical, with the *Z*-configuration of a C10 alkene more active than the *E*-configuration. Compounds bearing *sp2* substituents have an activity which is >1000-fold higher than that of a compound with an analogous saturated substituent.

The ability of DA to bind to GluRs has been utilized to detect low levels of DA (limit of detection, 0.31 ng ml^{-1}), by using the receptor-binding assay (see Box 8.5).

Box 8.4: MSG: Friend or Foe? The Chinese Restaurant Syndrome, and Beyond

Fermented food has always been used along with spices to increase food palatability or to enhance existing flavors, the aim being to "prepare" the taste buds for a gastronomic experience. The Romans used *garum* as a food condiment; this was in fact a sauce made from fish fermented in brine, and the resulting protein lysates contained free sodium glutamate. The Japanese traditionally used *kombu* (kelp) in soups to bring out the coveted *umami* flavor, which is made perceptible by the high MSG content of the broth stock. As Glu is not normally found in its free state in unprocessed foods, the taste buds are sensitive to the presence of its sodium salt, MSG. This prompted the Asian food industry to produce it as an additive that could be used liberally in oriental cuisine to enhance the flavor of dishes.

The "Chinese Restaurant Syndrome" refers to a series of symptoms typically felt after ingesting an oriental meal to which MSG has been added. The term was coined in 1968, by a medical doctor who described the prominent symptoms as " . . . numbness at the back of the neck, gradually radiating to both arms and the back, general weakness and palpitations" . . . (Ho Man Kwok, 1968).

Studies in animals have revealed that the blood–brain barrier is efficient at preventing neurotoxic effects by MSG, although pregnant women are cautioned because the placental barrier was unable to prevent fetal exposure in experiments conducted in rats. Also, repeated "spikes" in glutamic acid from ingested MSG may lead to chronic excitoxicity with long-term neurodegenerative effects, especially in infants and young children. This is because free glutamic acid is not readily absorbed during the digestion process, and so its salt form, MSG, can act as a "Trojan horse" that crosses the gastrointestinal barrier to elevate glutamate levels to toxic levels at target receptors.

However, no conclusive scientific investigation has yet shown MSG to be more harmful than other food additives, and it will therefore continue to be used by those who are not oversensitive to its immediate effects and enjoy the *umami* taste.

Box 8.5: The Glutamate-Binding Assay

The assay is based on the binding affinity of neurotransmitters and/or related toxins to nerve receptors. In initial assays, nerve receptors were isolated from various animal models, until it became possible to clone receptors from one (model) species and have them reproduced in cells of a different (carrier) species. Thus, GluRs can now be obtained cheaply and reliably for DA determination.

GluRs normally bind glutamic acid, but the latter can be displaced by KA and by DA, which in turn can displace both KA and glutamic acid in competition experiments.

In the DA-binding assay, KA is labeled with tritium (^3H) and allowed to bind to GluRs in cultivated cells. Then, radioactivity counts (counts per minute) are used to measure the amounts of bound KA. The introduction of known amounts of unlabeled DA, which displaces the labeled KA, is reflected by a corresponding decrease in counts per minute (i.e., of the receptor-bound KA), from which a standard curve can be established. By following the same procedure, very low concentrations of DA can be estimated from unknown samples (seawater, shellfish extracts or phytoplankton cells) by comparing the profiles against those of a standard curve (Baugh *et al.*, 2004).

8.5.2 Short- and Long-term Neurological Problems Associated with DA

In addition to postmortem examinations on wildlife impacted by ASP, numerous studies have been carried out on animal models, originally to test for the presence of DA in seafood (e.g., the mouse scratching test; see below), and later to fully understand the wide range of symptoms observed in humans and in wildlife that have ingested DA.

8.5.2.1 Mammal Studies

A synoptic review is provided by Jeffery *et al.* (2004). With regards to DA excretion, active transporters seem to be involved in elimination via the kidneys of rats after intraperitoneal (i.p.) injection. In contrast, DA permeation across the blood–brain barrier of rats after intravenous (i.v.) injection does not seem to be mediated by an active transporter. Initial experiments on acute toxicity after i.p. injection of contaminated shellfish extracts (serial dilutions) into mice revealed a steep dose–response curve, with a lethal dose $(LD)_{50}$ in the range of 2.4 to 3.6 mg kg^{-1}. Symptoms always included scratching, tremors and seizures at both lethal and sublethal doses of extract. In subsequent experiments, these symptoms were interpreted as indicative of neurotoxicity. For example, Tasker, Connell, and Strain (1991) established a dose-dependent behavioral scale by i.p. injections of calculated doses of contaminated mussel extracts into mice. Responses to increased doses included: sedation, rigidity, scratching and head weaving, loss of postural control, tremors, convulsions and death. Iverson *et al.* (1989) and others determined dose-related lesions in various regions of the limbic system of rat and mouse brains, and in particular the hippocampus region of the brain (Figure 8.15). This region converts objective versions of events from short-term to long-term memory, and is therefore affected by DA intoxication (hence the syndrome name, ASP). Chronic exposure leads to seizures and cognitive dysfunctions, because of the high concentration of iGluRs in the hippocampus and other brain parts that provide a substrate for DA attachment. This results in the selective and cumulative cellular and structural damage observed with DA toxicity.

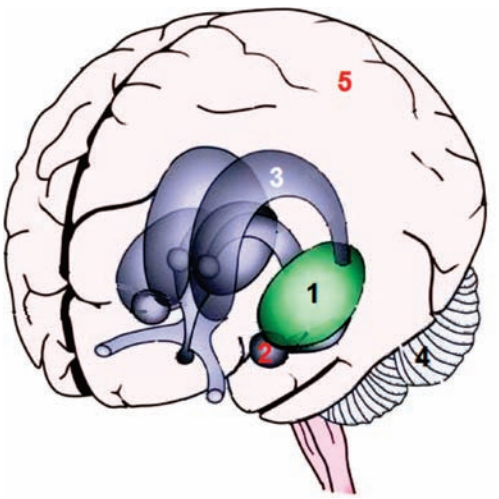


Figure 8.15 The limbic system in the human brain. 1 = hippocampus; 2 = amygdalia; 3 = cingular gyrus; 4 = cerebellum; 5 = cortex.

When compared to adult LD_{50} values, the lower LD_{50} score for neonatal rats suggests that they may be much more sensitive to DA-induced toxicity. Furthermore, respiratory failure, hemorrhage, neuronal degeneration and compulsive movement were also observed, and attributed to DA-induced spinal cord damage rather than to brain lesions in these young animals (Wang *et al.*, 2000). These laboratory observations confirmed the greater vulnerability to DA intoxication observed in young sea lions, especially when pregnant (see Case study #3). Limited studies in primates have revealed that emesis (vomiting) and diarrhea observed initially may represent natural mechanisms for avoiding ingestion and uptake into the blood, respectively, of intoxicated seafood. This phenomenon was also observed in patients affected during the 1987 ASP episode (see Case study #1). Interestingly, mice and rats are unable to vomit because of the particular anatomy of their stomach, and because they lack the neurophysiological mechanisms for doing so.

8.5.3 Cures Against ASP

To date, there is no cure against DA intoxication once the molecule has become bound to the target receptors. However, recent studies have shown that administration of the flavonol troxerutin to DA-treated mice reverses the memory impairment. Troxerutin was thus recommended as a potential candidate for the prevention and therapeutic treatment of cognitive deficits resulting from brain damage due to DA and other excitotoxins (Lu *et al.*, 2013). Another more recent candidate for the prevention and therapy of cognitive deficits caused by DA intoxication is ursolic acid, a natural triterpenoid compound (Wu *et al.*, 2013).

Otherwise, shellfish harvesters can take preventive measures when the monitoring programs indicate that toxigenic *Pseudo-nitzschia* species or DA levels are increasing in high-risk areas. For example, they can translocate shellfish from open-water farms to depuration areas that are free from toxigenic diatoms. In any case, shellfish harvesting is prohibited when DA levels reach the action limit. Pregnant women and infants (who are more vulnerable) must especially be aware to avoid consuming certain shellfish during these times.

8.6 Understanding and Predicting Toxigenic Diatom Blooms (Macroscopic Scale)

The probability of the occurrence and importance of natural blooms of toxigenic pennate diatoms is site-dependent. Most toxic *Pseudo-nitzschia* species are coastal and occur worldwide, with DA contents usually between 1 and 100 pg per cell, depending on the species (Lelong *et al.*, 2012; Trainer *et al.*, 2012). Nevertheless, open-ocean blooms of toxigenic diatoms

have recently been reported under high-nitrate and low-chlorophyll regimes (Silver *et al.*, 2010; Trick *et al.*, 2010). Upwelling regions (e.g., Pacific coast of North America) or embayments (Atlantic coast of North America) are favored if nutrient availability, exposure to sunlight and climatic conditions are conducive to massive proliferations of phytoplankton. These, in turn, provide food for filter-feeding benthic organisms (mostly bivalves, some crustaceans and soft-bodied invertebrates), or to anchovies and sardines. Toxin accumulation in these organisms and repeated exposures will eventually affect their predators, for example, seabirds, marine mammals and humans (Figure 8.16). Indeed, DA has been detected in the flesh or feces of a wide range of planktivorous and carnivorous fish and mammals, including whales (Lefebvre *et al.*, 2002). This indicates the possibility of generalized chronic exposure of predators to this toxin. Interestingly, sharks seem to be resilient to the DA-induced pathological symptoms (Schaffer *et al.*, 2006).

A portion of the biomass of diatom blooms sinks to the ocean bottom (Sekula-Wood *et al.*, 2009) and contributes to the cycling of organic and inorganic elements because of the highly efficient turnover. In coastal regions, sinking diatoms from a collapsing bloom may sequester DA that is concentrated further by benthic organisms feeding on debris or sediments. This provides an additional route for intoxication along marine food chains.

As DA is readily soluble in seawater, much of the evidence for elucidating toxic episodes involving top consumers (birds, mammals) must rely on examining digested meals or regurgitates for the presence of diatom frustules; these can be identified to species, even from fragments (see case studies, above). Together with presence of DA from tissues of dead animals, and using techniques similar to those employed in routine DA determination in seafood samples (see below), the analysis of debris often provides an unambiguous resolution of ASP scenarios.

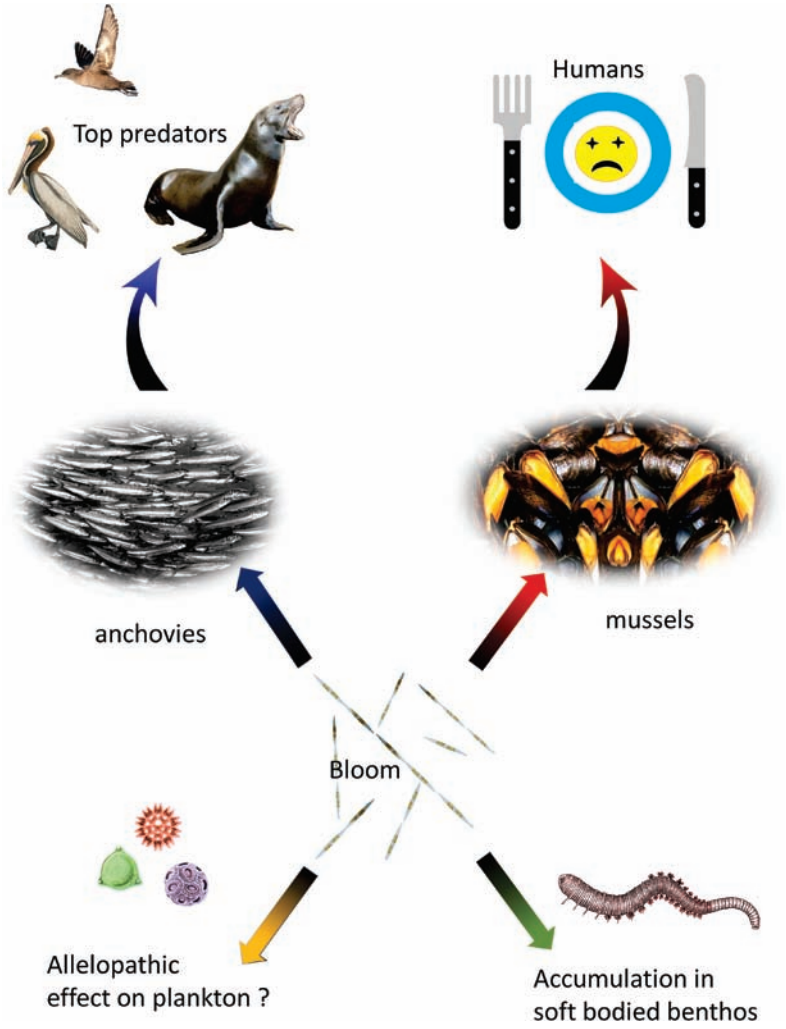


Figure 8.16 Representation of the possible fate and effects of domoic acid generated by a bloom of toxigenic pennate diatoms. Clockwise from top left: Seabirds and mammals are affected by feeding massively on planktivorous fish (anchovies and sardines); humans are mostly affected by occasional consumption of mollusks that concentrate the toxin to very high levels; some of the biomass from decaying blooms accumulates in the sediments, allowing the domoic acid to be concentrated in detritus feeders, such as lugworms; much of the domoic acid from dying diatoms is released into the water column, where it has yet unknown effects on other planktonic organisms, before it is degraded by sunlight or bacteria. Some contaminated fish may also be eaten by humans.

8.7 Natural Factors that Enhance Bloom Formation and/or DA Production

In open-water situations, the interactions between nutrient elements (Si, P, N, Fe), abiotic factors (salinity, temperature, pH) and associated bacteria are far more complex than in the laboratory under controlled conditions. Studies must therefore take into account the complexity of diatom bloom formation, calling for multiscaled investigations.

8.7.1 Silicon

Diatoms control the silicon cycle in the oceans, with a very efficient turnover rate before final deposition as sediments. Silicon bioavailability as silicic acid is an essential component of diatom growth and bloom formation dynamics. Vegetative division allows a rapid cell growth in raphid diatoms (whose frustules contain a slit) such as toxigenic *Pseudo-nitzschia* and *Nitzschia*. Silicic acid is biomanipulated in the diatom membranes by a number of highly specialized polymers that craft genetically controlled hydrated glass ($SiO_2{\cdot}nH_2O$) frustules adorned with processes of intricate motifs and with complex pore arrangements (see Armbrust, 2009 for a review). Whole-genome expression studies on model diatoms have allowed the identification of genes responsible for silicon bioprocesses (Mock *et al.*, 2008). Silicon limitation triggers DA production (Bates *et al.*, 1991).

8.7.2 Phosphorus

DA biosynthesis is particularly ATP-consuming (Pan, Bates, and Cembella, 1998), yet can occur when phosphorus becomes limiting to growth (Bates, Garrison, and Horner, 1998). Phosphorus depletion in combination with elevated CO_2 partial pressure is correlated with an even greater increase in DA biosynthesis (Sun *et al.*, 2011). This adds to the list of negative effects on humans and wildlife caused by the predicted gradual increase in ocean acidification.

8.7.3 Nitrogen

The release of urea into coastal urban areas of California, due to urban waste discharges and the use of fertilizers, is responsible for the maintenance of toxigenic diatom blooms (Howard *et al.*, 2007). Organic and inorganic nitrogen sources (nitrate, ammonium, urea) can boost toxic *P. cuspidata* growth under saturating light flux, but decrease it under a lower light intensity, although toxicity is increased (Auro and Cochlan, 2013). Thus, the control of toxicity is a complex interplay between the nitrogen source and light availability.

Diatoms are unique in possessing a complete urea cycle in their metabolism, possibly in relation to the fluctuating availability of, for example, nickel, a cofactor in urease that would limit the assimilation of this important nitrogen source in open-ocean waters (Price and Morel, 1991; Armbrust, 2009).

8.7.4 Iron

Modern oceans have become iron-depleted since the rise of oxygenic cyanobacteria, the relative insolubility of Fe^{3+} ions making diatoms and other protists dependent on iron-capturing or concentrating strategies. Raphid diatoms such as DA producers have adapted to iron-poor conditions by producing ferritin, an iron-storage molecule that also protects it from oxidative stress. Open-ocean centric diatoms, in contrast, have developed a capacity to use copper ions instead of iron in their electron-transport proteins. The use of ferritin, unique in the Stramenopiles (which include brown algae and plant parasites), is probably inherited by lateral gene transfer from another organism (Marchetti *et al.*, 2009).

8.7.5 The Role of Bacteria in the Biosynthesis of DA by Toxigenic Diatoms

The hitherto unsuspected importance of bacteria in the biosynthesis of many marine natural products has become a major issue since the discovery of quorum-sensing molecules. With the development of systems biology, a general picture is emerging, in which microbes fully participate in the functional aspects of what is known as the "holobiont," that is, a "host" organism and its biodiverse natural microflora (La Barre, 2011). Originally described for sessile diploblastic invertebrates, the holobiont concept is now applied to all living systems in which a eukaryotic host engages in functional relationships with a dedicated/generalist microflora, from protists to humans (Rosenberg and Zilber-Rosenberg, 2011). The exact role of the bacteria is not easy to ascertain, for several reasons: (i) most cannot be cultivated in the laboratory; (ii) quorum sensing can only be reached under specific conditions, presumably afforded by the host organism; and (iii) it is difficult to ascertain who does what regarding the biosynthesis of specific compounds.

Diatoms are known to interact actively with bacteria (Guannel, Horner-Devine, and Rocap, 2011; Amin, Parker, and Armbrust, 2012). In the case of DA, it has been shown unambiguously that the presence of bacteria enhances DA production by *P. multiseries* (Bates *et al.*, 1995). Furthermore, direct contact is necessary for this enhancement, since mere bacterial extracts do not induce DA synthesis (Kobayashi, Takata, and Kodama, 2009). To date, there is no evidence that bacteria can produce DA autonomously. Further efforts are required to elucidate the mechanisms of enhancement, for example, bacterial production of siderophores such as iron that enhance DA production (Wells *et al.*, 2005), of precursors of DA biosynthesis, or of gluconic

acid/gluconolactone, a powerful sequestering agent that may induce *Pseudo-nitzschia* to produce a counter-chelating agent, DA (Osada and Stewart, 1997; Stewart *et al.*, 1997).

8.8 Functional Genomics of Diatoms

8.8.1 The Key to the Evolutionary Success of Diatoms

The *Pseudo-nitzschia multiseries* draft genome is 219 megabases (Mb), with a combination of genes and metabolic pathways unique to the diatom lineage (Armbrust, 2009; Parker, Maumus, and Armbrust, 2013) and inherited from different sources. As with other Stramenopiles, the diatom genome includes plastid elements from cyanobacteria (primary endosymbiosis) and red algae (secondary endosymbiosis).

In addition, genetic elements found in the diatom genome indicate that their precursors might have been infested with Chlamydiae (endosymbiotic/parasitic bacteria), but also with green algae, at a very early stage (before the secondary endosymbiosis). The fusion–acquisition process may originally be a "mitigation" solution, in which the symbiont engulfed by the host is not merely digested as food, but is also exploited by means of targeting transporter proteins from the host to the membranes of the transient symbiont. This would allow the host to obtain energy and nutrients from the fully "protected" symbiont (see Keeling, 2013), thus creating a gene transfer ratchet that would eventually lead to the integration of organelles into the host cell. The integration of these various elements confers a dual plant–animal character to diatom metabolism.

8.8.2 Genomics of DA Biosynthesis and Regulation Networks

8.8.2.1 Genomic Aspects

Global molecular approaches are currently under way in order to determine the genetics of DA biosynthesis and to better understand the underlying factors that modulate its production in DA-producing *P. multiseries* (Boissonneault *et al.*, 2013; Parker, Maumus, and Armbrust, 2013). In the diatom genome, of the 170 or more red algal genes encoded, plastid functions such as photosynthesis, fatty acid biosynthesis, isoprenoids and amino acids have been identified. Yet, only one-third of the known *Pseudo-nitzschia* and only one *Nitzschia* species (see Lelong *et al.*, 2012; Trainer *et al.*, 2012) are known to produce DA. The biosynthetic pathways of the building blocks to DA are well known (MacIntyre *et al.*, 2011) and are common to many photosynthetic organisms; however, the exact molecular mechanisms of the condensation reaction leading to closure of the pyrollidine ring (see above) and the functional genomics (environmental regulation of the corresponding enzymes) underlying it are still under investigation.

8.8.2.2 Transcriptomics of DA-Producing Diatoms

Ongoing whole-genome sequencing of *Pseudo-nitzschia multiseries* has recently revealed that over 60% of the 219 Mb draft genome is composed of repeated sequences, the majority of which are transposable elements (TEs) or so-called "jumping genes," which can be stress-activated. This TE load is much larger than estimates from other sequenced diatoms and microalgae (Micaela Parker, personal communication; http://genome.jgi.doe.gov/Psemu1/Psemu1.home.html).

8.9 Conclusions

No other marine molecule has puzzled and concerned humans as much as DA, with hundreds of scientific publications having been devoted to its pathogenicity, physiology and chemistry, as well as to its transfer through various food chains, where it can remain unchanged or be transformed, but eventually degraded. The taxonomy of its producing organisms, which are restricted to several species of seaweeds and pennate diatoms, as well as their biogeography, ecology and worldwide distribution, have been intensely investigated in these times of rapid global changes. Synthetic chemists have studied ways to optimally produce DA in its bioactive configuration, which sells for approximately €200 per milligram as a pharmacological tool in neurophysiology and as an analytical standard. Chemical ecologists remain unsure about the function of this molecule: whether it is a waste product, osmoprotectant, metal chelator, allelopathic substance, microbial signaling function, pheromone, or antagonist to nutrient enrichment or microbial overload. Molecular approaches, including deciphering of the *Pseudo-nitzschia* whole genome, will eventually unravel the mystery of its production and regulation.

Diatoms have been around for a long time (since at least the early Jurassic period), and have played a prominent role in shaping the biogeochemistry of planet Earth. Some scientists believe in their climate-regulating capability, while their adaptability is remarkable as they can thrive in coastal eutrophic to oceanic oligotrophic (nutrient poor) waters, where nutrient recycling can be optimal. As in other natural ecosystems, biodiversity and efficiency are the result of complex and autoregulating interactions, tropical coral reefs being a prime example. Anthropogenic enrichment with pollutants (intoxicants) and nutrients (organic wastes, resulting in microbial proliferation) interfere with food chain functioning and cause stress to benthic, pelagic and planktonic marine life. Could DA production be a response of some pennate diatoms to such pressures?

Note added in proof

The following five new species of *Pseudo-nitzschia* have been identified (all nontoxic), bringing the total to 43 species: *P. abrensis, P. plurisecta* (Orive *et al.*, 2013), *P. batesiana, P. fukuyoi*, and *P. lundholmiae* (Lim *et al.*, 2013).

Acknowledgments

The authors thank Carolyn Bird for finding the image of *Chondria armata*, and Karie Holtermann, Nina Lundholm and James Ehrman for images of *Pseudo-nitzschia* and *Nitzschia navis-varingica*. They also thank Micaela Parker for providing preliminary information about the draft genome of *Pseudo-nitzschia multiseries*. Sheila Crain is also thanked for the acquisition of NMR spectra.

References

Amin, S.A., Parker, M.S., and Armbrust, E.V. (2012) Interactions between diatoms and bacteria. *Microbiol. Mol. Biol. Rev.*, **76**, 667–684.

Anderson, D.M., Burkholder, J.M., Cochlan, W. P., Glibert, P.M., Gobler, G.J., Heil, C.A., Kudela, R.M., Parsons, M.L., Rensel, J., Townsend, D.W., Trainer, V.L., and Vargo, G. A. (2008) Harmful algal blooms and eutrophication: examining linkages from selected coastal regions of the Unites States. *Harmful Algae*, **8**, 39–53.

Anderson, D.M., Andersen, P., Bricelj, V.M., Cullen, J.J., and Rensel, J.E. (2001) *Monitoring and Management Strategies for Harmful Algal Blooms in Coastal Waters*, APEC #201-MR-01.1, Asia Pacific Economic Program, Singapore, and Intergovernmental Oceanographic Commission Technical Series No. 59, Paris.

Armbrust, E.V. (2009) The life of diatoms in the world's oceans. *Nature*, **459**, 185–192.

Arundine, M. and Tymianski, M. (2003) Molecular mechanisms of calcium-dependent neurodegeneration in excitotoxicity. *Cell Calcium*, **34**, 325–337.

Auro, M.E. and Cochlan, W.P. (2013) Nitrogen utilization and toxin production by two diatoms of the *Pseudo-nitzschia pseudodelicatissima* complex: *P. cuspidata* and *P. fryxelliana*. *J. Phycol.*, **49**, 156–169.

Baco, E., Vellutini, L., Pillot, J.-P., Felpion, F.-X., Schmitter, J.-M., Benneteau, B., and Gegueil, M. (2010) Synthesis of novel haptens and conjugates for antibody production against kainoid family. *Synlett*, **13**, 1943–1946.

Bargu, S., Silver, M.W., Ohman, M.D., Benitez-Nelson, C.R., and Garrison, D.L. (2012) Mystery behind Hitchcock's birds. *Nat. Geosci.*, **5**, 2–3.

Bates, S.S., de Freitas, A.S.W., Milley, J.E., Pocklington, R., Quilliam, M.A., Smith, J.C., and Worms, J. (1991) Controls on domoic acid production by the diatom *Nitzschia pungens* f. *multiseries* in culture: nutrients and irradiance. *Can. J. Fish. Aquat. Sci.*, **48**, 1136–1144.

Bates, S.S., Douglas, D.J., Doucette, G.J., and Léger, C. (1995) Enhancement of domoic acid production by reintroducing bacteria to axenic cultures of the diatom *Pseudo-nitzschia multiseries*. *Nat. Toxins*, **3**, 429–435.

Bates, S.S., Bird, C.J., de Freitas, A.S.W., Foxall, R., Gilgan, M., Hanic, L.A., Johnson, G.R., McCulloch, A.W., Odense, P., Pocklington, R., Quilliam, M.A., Sim, P.G., Smith, J.C., Subba Rao, D.V., Todd, E.C.D., Walter, J.A., and Wright, J.L.C. (1989) Pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island, Canada. *Can. J. Fish. Aquat. Sci.*, **46**, 1203–1215.

Bates, S.S., Garrison, D.L., and Horner, R.A. (1998) Bloom dynamics and physiology of domoic-acid-producing *Pseudo-nitzschia* species, in *Physiological Ecology of Harmful Algal Blooms* (eds D.M. Anderson, A.D. Cembella, and G.M. Hallegraeff), Springer-Verlag, Heidelberg, pp. 267–292.

Baugh, K.A., Spencer, S.G., Wekell, J.C.W., and Trainer, V.L. (2004) An alternative method for domoic acid determination in seawater particulates: a receptor-binding assay using glutamate dehydrogenase, in *Harmful Algae 2002* (eds K.A. Steidinger, J.H. Landsberg, C. R. Tomas, and G.A. Vargo), Florida Fish and Wildlife Commission, Florida Institute of Technology and Intergovernmental Oceanographic Commission of UNESCO, pp. 228–230.

Bejarano, A.C., Van Dolah, F.M., Gulland, F.M., Rowles, T.K., and Schwacke, L.H. (2008) Production and toxicity of the marine biotoxin domoic acid and its effects on wildlife: a review. *Hum. Ecol. Risk Assess.*, **14**, 544–567.

Boissonneault, K.R., Henningsen, B.M., Bates, S.S., Robertson, D.L., Milton, S., Pelletier, J., Hogan, D.A., and Housman, D.E. (2013) Gene expression studies for the analysis of domoic acid production in the marine diatom *Pseudo-nitzschia* multiseries. *BMC Mol. Biol.*, **14**, 25.

Bouillon, R.-C., Kieber, R.J., Skrabal, S.A., and Wright, J.L.C. (2008) Photochemistry and identification of photodegradation products of the marine toxin domoic acid. *Mar. Chem.*, **110** (1-2), 18–27.

Clayden, J., Read, B., and Hebditch, K.R. (2005a) Chemistry of domoic acid, isodomoic acids, and their analogues. *Tetrahedron*, **61**, 5713–5724.

Clayden, J., Knowles, F.E., and Baldwin, I.R. (2005b) The synthesis of (−)-isodomoic acid C. *J. Am. Chem. Soc.*, **127**, 2412–2413.

Daigo, K. (1959a) Studies on the constituents of *Chondria armata*. I. Detection of the anthelminthical constituents. *J. Pharmacol. Soc. Jpn*, **79**, 350–353.

Daigo, K. (1959b) Studies on the constituents of *Chondria armata*. II. Isolation of an antihelminthical constituent. *J. Pharmacol. Soc. Jpn*, **79**, 353–356.

Daigo, K. (1959c) Studies on the constituents of *Chondria armata*. III. Constitution of domoic acid. *J. Pharmacol. Soc. Jpn*, **79**, 356–360.

Denmark, S.E., Liu, J.H.-C., and Muhuni, J.M. (2009) Total syntheses of isodomoic acids G and H. *J. Am. Chem. Soc.*, **131**, 14188–14189.

Denmark, S.E., Liu, J.H.-C., and Muhuni, J.M. (2011) Stereocontrolled total syntheses of isodomoic acids G and H via a unified strategy. *J. Am. Chem. Soc.*, **131**, 14188–14189.

Donovan, C., Garduño, R.A., Kalmokoff, M., Ku, J.C., Quilliam, M.A., and Gill, T.A. (2009) *Pseudoalteromonas* bacteria are capable of degrading paralytic shellfish toxins. *Appl. Environ. Microbiol.*, **75**, 6919–6923.

Douglas, D.J., Ramsey, U.P., Walter, J.A., and Wright, J.L.C. (1992) Biosynthesis of the neurotoxin domoic acid by the marine diatom *Nitzschia pungens* forma *multiseries*, determined with [^{13}C]-labelled precursors and nuclear magnetic resonance. *J. Chem. Soc. Chem. Commun.*, **1992**, 714–716.

ElDouhaibi, A.S., Kassab, R.M., Song, M., and Montgomery, J. (2011) Nickel-catalyzed cyclizations of enoates and chiral allenes: an approach to domoic acid. *Chemistry*, **17**, 6336–6329.

Falk, M., Walter, J.A., and Wiseman, P.W. (1989) Ultraviolet spectrum of domoic acid. *Can. J. Chem.*, **67**, 1421–1425.

Fritz, L., Quilliam, M.A., Wright, J.L.C., Beale, A., and Work, T.M. (1992) An outbreak of domoic acid poisoning attributed to the pennate diatom *Pseudonitzschia australis*. *J. Phycol.*, **28**, 439–442.

Furey, A., Lehane, M., Gillman, M., Fernandez-Puente, P., and James, K.J. (2001) Determination of domoic acid in shellfish by liquid chromatography with electrospray ionization and multiple tandem mass spectrometry. *J. Chromatogr. A*, **938**, 167–174.

Garthwaite, I., Ross, K.M., Miles, C.O., Hansen, R.P., Foster, D., Wilkins, A.L., and Towers, N. R. (1998) Polyclonal antibodies to domoic acid and their use in immunoassays for domoic acid in seawater and shellfish. *Nat. Toxins*, **6**, 93–104.

Goldstein, T., Mazt, J.A.K., Zabka, T.S., Langlois, G., Colegrove, K.M., Silver, M., Bargu, S., Van Dolah, F., Leighfield, T., Conrad, P.A., Barakos, J., Williams, D.C., Dennison, S., Haulena, M., and Gulland, F.M.D. (2008) Novel symptomatology and changing epidemiology of domoic acid toxicosis in California sea lions (*Zalophus californianus*): an increasing risk to marine mammal health. *Proc. R. Soc. Lond. B. Biol. Sci.*, **275**, 267–276.

Guannel, M.L., Horner-Devine, M.C., and Rocap, G. (2011) Bacterial community composition differs with species and

toxigenicity of the diatom *Pseudo-nitzschia*. *Aquat. Microb. Ecol.*, **64**, 117–133.
Hagström, J.A., Granéli, E., Maneiro, I., Barreiro, A., Petermann, A., and Svensen, C. (2007) Release and degradation of amnesic shellfish poison from decaying *Pseudo-nitzschia multiseries* in presence of bacteria and organic matter. *Harmful Algae*, **6**, 175–188.
Hashimoto, K., Ohfune, Y., and Shirahama, H. (1995) Synthesis of conformationally restricted analogs of kainic acid. Is the conformation of the C4-substituent of kainoid important to its neuroexcitatory activity? *Tetrahedron Lett.*, **36**, 6235–6238.
He, Y., Fekete, A., Chen, G., Harir, M., Zhang, L., Tong, P., and Schmitt-Kopplin, P. (2010) Analytical approaches for an important shellfish poisoning agent: domoic acid. *J. Agric. Food Chem.*, **58**, 11525–11533.
Heisler, J., Glibert, P.M., Burkholder, J.M., Anderson, D.M., Cochlan, W., Dennison, W. C., Dortch, Q., Gobler, C.J., Heil, C.A., Humphries, E., Lewitus, A., Magnien, R., Marshall, H.G., Sellner, K., Stockwell, D.A., Stoecker, D.K., and Suddleson, M. (2008) Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algae*, **8**, 3–13.
Higa, T. and Kuniyoshi, M. (2000) Toxins associated with medicinal and edible seaweeds. *J. Toxicol. Toxin. Rev.*, **19**, 119–137.
Ho Man Kwok, R. (1968) Chinese restaurant syndrome. *N. Engl. J. Med.*, **18**, 796.
Howard, M.D.A., Cochlan, W.P., Ladizinski, N., and Kudela, R.M. (2007) Nitrogenous preference of toxigenic *Pseudo-nitzschia australis* (Bacillariophyceae) from field and laboratory experiments. *Harmful Algae*, **6**, 206–217.
Impellizzeri, G., Mangiafico, S., Oriente, G., Piattelli, M., Sciuto, S., Fattorusso, E., Magno, S., Santacroce, C., and Sica, D. (1975) Amino acids and low-molecular-weight carbohydrates of some marine red algae. *Phytochemistry*, **14**, 1549–1557.
Ivanova, I., Flavier, S., and Christen, R. (2004) Phylogenetic relationships among marine *Alteromonas*-like proteobacteria: emended description of the family Alteromonadaceae and proposal of Pseudoalteromonadaceae fam. nov., Colwelliaceae fam. nov., Shewanellaceae fam. nov., Moritellaceae fam. nov., Ferrimonadaceae fam. nov., Idiomarinaceae fam. nov. and Psychromonadaceae fam. nov. *Int. J. Syst. Evol. Microbiol.*, **54**, 1773–1788.
Iverson, F., Truelove, J., Nera, E., Tryphonas, L., Campbell, J., and Lok, E. (1989) Domoic acid poisoning and mussel-associated intoxication: preliminary investigations into the response of mice and rats to toxic mussel extract. *Food Chem. Toxicol.*, **27**, 377–384.
Jeffery, B., Barlow, T., Moizer, K., Paul, S., and Boyle, C. (2004) Amnesic shellfish poison. *Food Chem. Toxicol.*, **42**, 545–557.
Kaczmarska, I., Ehrman, J.M., Bates, S.S., Green, D.H., Léger, C., and Harris, J. (2005) Diversity and distribution of epibiotic bacteria on *Pseudo-nitzschia multiseries* (Bacillariophyceae) in culture, and comparison with those on diatoms in native seawater. *Harmful Algae*, **4**, 725–741.
Keeling, P.J. (2013) The number, speed, and impact of plastid endosymbioses in eukaryotic evolution. *Annu. Rev. Plant Biol.*, **64**, 583–607.
Kalaitzis, J.A., Chau, R., Kohli, G.S., Murray, S. A., and Neilan, B.A. (2010) Biosynthesis of toxic naturally-occurring seafood contaminants. *Toxicon*, **56**, 244–258.
Kobayashi, K., Takata, Y., and Kodama, M. (2009) Direct contact between *Pseudo-nitzschia multiseries* and bacteria is necessary for the diatom to produce a high level of domoic acid. *Fish. Sci.*, **75**, 771–776.
Kotaki, Y., Lundholm, N., Onodera, H., Kobayashi, K., Bajarias, F.A., Furio, E.F., Iwataki, M., Fukuyo, Y., and Kodama, M. (2004) Wide distribution of *Nitzschia navis-varingica*, a new domoic acid-producing benthic diatom found in Vietnam. *Fish. Sci.*, **70**, 28–32.
Kudela, R.M., Howard, M.D.A., Jenkins, B.D., Miller, P.E., and Smith, G.J. (2010) Using the molecular toolbox to compare harmful algal blooms in upwelling systems. *Prog. Oceanogr.*, **85**, 108–121.
La Barre, S. (2011) Coral reef biodiversity in the face of climatic changes, *Biodiversity Loss in a Changing Planet* (eds O. Grillo and G. Venora), Biodiversity Series, vol. **4** pp. 77–122. (Published: November 16 2011 under CC BY 30 license 318 pages), http://www.intechopen.com/books/biodiversity-loss-in-a-changing-planet/coral-reef-biodiversity-in-the-face-of-climatic-changes.
Lail, E., Skrabal, S.A., Kieber, R.J., Bouillon, R.-C., and Wright, J.L.C. (2007) The role of particles on the biogeochemical cycling of domoic acid and its isomers in natural water matrices. *Harmful Algae*, **6**, 651–657.
Laycock, M.V., de Freitas, A.S.W., and Wright, J. L.C. (1989) Glutamate agonists from marine algae. *J. Appl. Phycol.*, **1**, 113–122.
Lefebvre, K.A., Bargu, S., Kieckhefer, T., and Silver, M.W. (2002) From sanddabs to blue whales: the pervasiveness of domoic acid. *Toxicon*, **40**, 971–977.
Lefebvre, K.A. and Robertson, A. (2010) Domoic acid and human exposure risks: a review. *Toxicon*, **56**, 218–230.
Lefebvre, K.A., Frame, E.R., Gulland, F., Hansen, J.D., Kendrick, P.S., Beyer, R.P., Bammler, T.K., Farin, F.M., Hiolski, E.M., Smith, D.R., and Marcinek, D.J. (2012) A novel antibody-based biomarker for chronic algal toxin exposure and subacute toxicity. *PLoS ONE*, **7**, e36213.
Lelong, A., Hégaret, H., Soudant, P., and Bates, S.S. (2012) *Pseudo-nitzschia* (Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: revisiting previous paradigms. *Phycologia*, **51**, 168–216.
Lemière, G., Sedehizadeh, S., Toueg, J., Fleary-Roberts, N., and Clayden, J. (2011) A general synthetic approach to the amnesic shellfish toxins: total synthesis of (−)-isodomoic acid B, (−)-isodomoic acid E and (−)-isodomoic acid F. *Chem. Commun.*, **47**, 3745–3747.
Lim, H.C., Leaw, C.P., Su, S.N.P., Teng, S.T., Usup, G., Noor, N.M., Lundholm, N., Kotaki, Y., and Lim, T. (2012) Morphology and molecular characterization of *Pseudo-nitzschia* (Bacillariophyceae) from Malaysian Borneo, including the new species of *Pseudo-nitzschia circumpora* sp. nov. *J. Phycol.*, **48**, 1232–1247.
Lim, H.G., Teng, S.T., Leaw, C.P., and Lim, P.T. (2013) Three novel species in the *Pseudo-nitzschia pseudodelicatissima* complex: *P. batesiana* sp. nov., *P. lundholmiae* sp. nov. and *P. fukuyoi* sp. nov. (Bacillariophyceae), from the Strait of Malacca, Malaysia. *J. Phycol.*, **4**, 902–916.
Lu, J., Wu, D.-M., Zheng, Y.-L., Hu, B., Cheng, W., Zhang, Z.-F., and Li, M.-Q. (2013) Troxerutin counteracts domoic acid-induced memory deficits in mice by inhibiting CAAT/enhancer binding protein β-mediated inflammatory response and oxidative stress. *J. Immunol.*, **190**, 3466–3479.
Lundholm, N. and Moestrup, Ø. (2000) Morphology of the marine diatom *Nitzschia navis-varingica* sp. nov., another producer of the neurotoxin domoic acid. *J. Phycol.*, **36**, 1162–1174.
MacIntyre, H.L., Stutes, A.L., Smith, W.L., Dorsey, C.P., Abraham, A., and Dickey, R.W. (2011) Environmental correlates of community composition and toxicity during a bloom of *Pseudo-nitzschia* spp. in the northern Gulf of Mexico. *J. Plankton Res.*, **33**, 273–295.
Maeda, M., Kodama, T., Tanaka, T., Ohfune, Y., Nomoto, K., Nishimura, K., and Fujita, T. (1984) Insecticidal and neuromuscular activities of domoic acid and its related compounds. *J. Pestic. Sci.*, **9**, 27–32.
Maeda, M., Kodama, T., Tanaka, T., Yoshizumi, H., Takemoto, T., Nomoto, K., and Fujita, T. (1985) Structures of insecticidal substances isolated from a red alga, *Chondria armata* [in Japanese]. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu*, **27**, 616–623.
Maeda, M., Kodama, T., Tanaka, T., Yoshizumi, H., Takemoto, T., Nomoto, K., and Fujita, T. (1986) Structures of isodomoic acids A, B and C, novel insecticidal amino acids from the red alga *Chondria armata*. *Chem. Pharm. Bull.*, **34**, 4892–4895.
Maeda, M., Kodama, T., Saito, M., Tanaka, T., Yoshizumi, H., Nomoto, K., and Fujita, T. (1987a) Neuromuscular action of insecticidal domoic acid on the American cockroach. *Pestic. Biochem. Physiol.*, **28**, 85–92.
Maeda, M.T., Kodama, T., Tanaka, T., Yoshizumi, H., Takemoto, T., Nomoto, K., and Fujita, T. (1987b) Structures of domoilactone A and B, novel amino acids from the red alga *Chondria armata*. *Tetrahedron Lett.*, **28**, 633–636.
Mafra, L.Jr, Léger, C., Bates, S.S., and Quilliam, M.A. (2009) Analysis of trace levels of domoic

acid in seawater and plankton by liquid chromatography without derivatization, using UV or mass spectrometry detection. *J. Chromatogr. A*, **1216**, 6003–6011.

Marchetti, A., Lundholm, N., Kotaki, Y., Hubbard, K., Harrison, P.J., and Armbrust, E. V. (2008) Identification and assessment of domoic acid production in oceanic *Pseudo-nitzschia* (Bacillariophyceae) from iron-limited waters in the northeast subarctic Pacific. *J. Phycol.*, **44**, 650–661.

Marchetti, A., Parker, M.S., Moccia, L.P., Lin, E. O., Arrieta, A.L., Ribalet, F., Murphy, M.E.P., Maldonado, M.T., and Armbrust, E.V. (2009) Ferritin is used for iron storage in bloom-forming marine pennate diatoms. *Nature*, **457**, 467–470.

Mock, T., Samanta, M.P., Iverson, V., Berthiaume, C., Robison, M., Holtermann, K., Durkin, C., Bondurant, S.S., Richmond, K., Rodesch, M., Kallas, T., Huttli, E.L., Cerrina, F., Sussman, M.R., and Armbrust, E. V. (2008) Whole-genome expression profiling of the marine diatom *Thalassiosira pseudonana* identifies genes involved in silicon bioprocesses. *Proc. Natl Acad. Sci. USA*, **105**, 1579–1584.

Morel, F.M.M. and Price, N.M. (2003) The biogeochemical cycles of trace elements in the oceans. *Science*, **300**, 944–947.

Neely, B.A., Soper, J.L., Greic, D.J., Carlin, K.P., Favre, E.G., Gulland, F.M.D., Almeida, J.S., and Janech, M.J. (2012) Serum profiling by MALDI-TOF mass spectrometry as a diagnostic tool for domoic acid toxicosis in California sea lions. *Proteome Sci.*, **10**, 18. see http://www.proteomesci.com/content/10/1/18/abstract.

Ni, Y., Kande, K.D., Amarasinghe, K.K., Ksebati, B., and Montgomery, J. (2003) First total synthesis and stereochemical definition of isodomoic acid G. *Org. Lett.*, **5**, 3771–3773.

Ni, Y., Kassab, R.M., Chevliakov, M.V., and Montgomery, J. (2009) Total syntheses of isodomoic acids G and H: an exercise in tetrasubstituted alkene synthesis. *J. Am. Chem. Soc.*, **131**, 17714–17718.

Nomoto, K., Takemoto, T., Maeda, M., Yasuko, I., Doi, M., Inoue, M., and Ishida, T. (1992) Conformational feature of neuroactive domoic acid: X-ray structural comparison with isodomoic acid A and alpha-kainic acid. *Biochem. Biophys. Res. Commun.*, **187**, 325–331.

Ochoa, J.I., Sierra-Beltrán, A.P., Oláiz-Fernández, G., and Del Villar Ponce, L.M. (1998) Should mollusc toxicity in Mexico be considered a public health issue? *J. Shellfish Res.*, **17**, 1671–1673.

Ohfune, Y. and Tomita, M. (1982) Total synthesis of (−)-domoic acid. A revision of the original structure. *J. Am. Chem. Soc.*, **104**, 3511–3513.

Orive, E., Pérez-Aicua, L., David, H., García-Etxebarria, K., Laza-Martínez, A., Seoane, S., and Miguel, I. (2013) The genus *Pseudo-nitzschia* (Bacillariophyceae) in a temperate estuary with description of two new species: *Pseudo-nitzschia plurisecta* sp. nov. and *Pseudo-nitzschia abrensis* sp. nov. *J. Phycol.*, **49**, 1192–1206.

Osada, M. and Stewart, J.E. (1997) Gluconic acid/gluconolactone: physiological influences on domoic acid production by bacteria associated with *Pseudo-nitzschia multiseries*. *Aquat. Microb. Ecol.*, **12**, 203–209.

Parekh, P.K. (2012) *The photooxidation of domoic acid*. Master's thesis, Florida International University, FIU Electronic Theses and Dissertations, p. 112, http://digitalcommons.fiu.edu/etd/770 (accessed 5 June 2013).

Pan, Y., Bates, S.S., and Cembella, A.D. (1998) Environmental stress and domoic acid production by *Pseudo-nitzschia*: a physiological perspective. *Nat. Toxins*, **6**, 127–135.

Parker, M.S., Maumus, F., Armbrust, E.V., and The *Pseudo-nitzschia* Genome Consortium (2013) *Pseudo-nitzschia multiseries CLN-47 genome sequence, Assembly v1* (October 2011). US Department of Energy Joint Genome Institute, http://genome.jgi.doe.gov/Psemu1/Psemu1.home.html (accessed 5 June 2013).

Paz, B., Riobó, P., and Franco, J.-M. (2011) Preliminary study for rapid determination of phycotoxins in microalgae whole cells using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.*, **25**, 3627–3639.

Perl, T.M., Bédard, L., Kosatsky, T.J., Hockin, C., Todd, E., and Remis, R.S. (1990) An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. *N. Engl. J. Med.*, **322**, 1775–1780.

Platt, S.R. (2007) The role of glutamate in central nervous system health and disease – A review. *Vet. J.*, **173**, 278–286.

Pocklington, R., Milley, J.E., Bates, S.S., Bird, C. J., de Freitas, A.S.W., and Quilliam, M.A. (1990) Trace determination of domoic acid in seawater and phytoplankton by high-performance liquid chromatography of the fluorenylmethoxycarbonyl (FMOC) derivative. *Int. J. Environ. Anal. Chem.*, **38**, 351–368.

Price, N.M. and Morel, F.M.M. (1991) Colimitation of phytoplankton growth by nickel and nitrogen. *Limnol. Oceanogr.*, **36**, 1071–1077.

Pulido, O. (2008) Domoic acid toxicologic pathology: a review. *Mar. Drugs*, **6**, 180–219.

Quilliam, M.A. (2003) Chemical methods for domoic acid, the amnesic shellfish poisoning (ASP) toxin, in *Manual on Harmful Marine Microalgae* (eds G.M. Hallegraeff, D.M. Anderson, and A.D. Cembella), Monographs on Oceanographic Methodology, vol. **11**, Intergovernmental Oceanographic Commission (UNESCO), Paris, pp. 247–266.

Quilliam, M.A., Sim, P.G., McCulloch, A.W., and McInnes, A.G. (1989a) High-performance liquid chromatography of domoic acid, a marine neurotoxin, with application to shellfish and plankton. *Int. J. Environ. Anal. Chem.*, **36**, 139–154.

Quilliam, M.A., Thomson, B.A., Scott, G.J., and Siu, K.W.M. (1989b) Ion-spray mass spectrometry of marine neurotoxins. *Rapid Commun. Mass Spectrom.*, **3**, 145–150.

Quilliam, M.A., Thomas, K., and Wright, J.L.C. (1998) Analysis of domoic acid in shellfish by thin-layer chromatography. *Nat. Toxins*, **6**, 147–152.

Quilliam, M.A. and Wright, J.L.C. (1989) The amnesic shellfish poisoning mystery. *Anal. Chem.*, **61**, 1053A–1060A.

Quilliam, M.A., Xie, M., and Hardstaff, W.R. (1995) Rapid extraction and cleanup procedure for the liquid chromatographic determination of domoic acid in unsalted seafood. *J. AOAC Int.*, **78**, 543–554.

Ramsdell, J.S. (2007) The molecular and integrative basis to domoic acid toxicity, in *Phycotoxins: Chemistry and Biochemistry* (ed. L. Botana), Blackwell Publishing Professional, Cambridge, MA, pp. 223–250.

Ramsdell, J.S. (2010) Neurological disease rises from ocean to bring model for human epilepsy to life. *Toxins*, **2**, 1646–1675.

Ramsey, U.P., Douglas, D.J., Walter, J.A., and Wright, J.L.C. (1998) Biosynthesis of domoic acid by the diatom *Pseudo-nitzschia multiseries*. *Nat. Toxins*, **6**, 137–146.

Regueiro, J., Álvarez, G., Mauriz, A., and Blanco, J. (2011) High throughput analysis of amnesic shellfish poisoning toxins in bivalve molluscs by dispersive solid-phase extraction and high-performance liquid chromatography using a monolithic column. *Food Chem.*, **127**, 1884–1891.

Riobó, P., López, E., and Franco, J.M. (2011) Chemical methods for detecting phycotoxins: LC and LC/MS/MS, in *New Trends in Marine and Freshwater Toxins: Food and Safety Concerns* (eds A.G. Cabado and J.M. Vieites), ANFACO-CECOPESCA, Spanish National Association of Sea Food, Producers, Vigo, Spain, pp. 215–266.

Romero, M.L.J., Kotaki, Y., Relox, J.Jr, Lundholm, N., Takata, Y., Kodama, M., and Fukuyo, Y. (2012) Two new ASP toxin production types in strains of *Nitzschia navis-varingica* from the Philippines. *Coastal Mar. Sci.*, **35**, 67–69.

Rosenberg, E. and Zilber-Rosenberg, I. (2011) Symbiosis and development: the hologenome concept. *Birth Defects Res., Part C*, **93**, 56–66.

Sato, M., Nakano, T., Takeuchi, M., Kanno, N., Nagahisa, E., and Sato, Y. (1996) Distribution of neuroexcitatory amino acids in marine algae. *Phytochemistry*, **42**, 1595–1597.

Savage, T.J., Smith, G.J., Clark, A.T., and Saucedo, P. N. (2012) Condensation of the isoprenoid and amino precursors in the biosynthesis of domoic acid. *Toxicon*, **59**, 25–33.

Schaffer, P., Reeves, C., Casper, D.R., and Davis, C.R. (2006) Absence of neurotoxic effects in leopard sharks, *Triakis semifasciata*, following domoic acid exposure. *Toxicon*, **47**, 747–752.

Sekula-Wood, E., Schnetzer, A., Benitez-Nelson, C.R., Anderson, C., Berelson, W.M., Brzezinski, M.A., Burns, J.M., Caron, D.A., Cetinic, I., Ferry, J.L., Fitzpatrick, E., Jones, BH., Miller, P.E., Morton, S.L., Schaffner, R. A., Siegel, D.A., and Thunel, R. (2009) Rapid

downward transport of the neurotoxin domoic acid in coastal waters. *Nat. Geosci.*, **2**, 272–275.
Scholin, C.A., Gulland, F., Doucette, G.J., Benson, S., Busman, M., Chavez, F.P., Cordaro, J., DeLong, R., De Vogelaere, A., Harvez, J., Haulena, M., Lefebvre, K., Lipscomb, T., Loscutoff, S., Lowenstine, L.J., Marin, R. III, Miller, P.E., McLellan, W.A., Moeller, P.D.R., Powell, C.L., Rowles, T., Silvagni, P., Silver, M., Spraker, T., Trainer, V., and Van Dolah, F.M. (2000) Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature*, **403**, 80–84.
Silver, M.W., Bargu, S., Coale, S.L., Benitez-Nelson, C.R., Garcia, A.C., Roberts, K.J., Sekula-Wood, E., Bruland, K.W., and Coale, K. H. (2010) Toxic diatoms and domoic acid in natural and iron enriched waters of the oceanic Pacific. *Proc. Natl Acad. Sci. USA*, **107**, 20762–20767.
Stewart, J.E., Marks, L.J., Wood, C.R., Risser, S. M., and Gray, S. (1997) Symbiotic relations between bacteria and the domoic acid producing diatom *Pseudo-nitzschia multiseries* and the capacity of these bacteria for gluconic acid/gluconolactone formation. *Aquat. Microb. Ecol.*, **12**, 211–221.
Stewart, J.E., Marks, L.J., Gilgan, M.W., Pfeiffer, E., and Zwicker, B.M. (1998) Microbial utilization of the neurotoxin domoic acid: blue mussels (*Mytilus edulis*) and soft shell clams (*Mya arena*) as sources of microorganisms. *Can. J. Microbiol.*, **44**, 456–464.
Sun, J., Hutchins, D.A., Feng, Y., Seubert, E.L., Caron, D.A., and Fu, F.-X. (2011) Effects of changing pCO_2 and phosphate availability on domoic acid production and physiology of the marine harmful bloom diatom *Pseudo-nitzschia multiseries*. *Limnol. Oceanogr.*, **53**, 829–840.
Swanson, G.T. and Sakai, R. (2009) Ligands for ionotropic glutamate receptors. *Prog. Mol. Subcell. Biol.*, **46**, 123–157.
Takemoto, T. and Daigo, K. (1958) Constituents of *Chondria armata*. *Chem. Pharm. Bull.*, **6**, 578–580.
Takemoto, T., Daigo, K., Kondo, Y., and Kondo, K. (1966) Studies on the constituents of *Chondria armata*. VIII. On the structure of domoic acid. *Yakuzaku Zasshi*, **86**, 874–877.
Tasker, R.A., Connell, B.J., and Strain, S.M. (1991) Pharmacology of systemically administered domoic acid in mice. *Can. J. Physiol. Pharmacol.*, **69**, 378–382.
Tatters, A.O., Fu, F.-X., and Hutchins, D.A. (2012) High CO_2 and silicate limitation synergistically increase the toxicity of *Pseudo-nitzschia fraudulenta*. *PLoS ONE*, **7**, e32116.
Teitelbaum, J.S., Zatorre, R.J., Carpenter, S., Gendron, D., Evans, A.C., Gjedde, A., and Cashman, N.R. (1990) Neurologic sequelae of domoic acid intoxication due to the ingestion of contaminated mussels. *N. Engl. J. Med.*, **322**, 1781–1787.
Thibault, P., Quilliam, M.A., Jamieson, W.D., and Boyd, R.K. (1989) Mass spectrometry of domoic acid, a marine neurotoxin. *Biomed. Environ. Mass Spectrom.*, **18**, 373–386.
Thoha, H., Kotaki, Y., Panggabean, L., Lundholm, N., Ogawa, H., Lim, P.T., Takata, Y., Kodama, M., and Fukuyo, Y. (2012) Screening of diatoms that produce ASP toxins in Southernmost Asian waters. *Coastal Mar. Sci.*, **35**, 34–38.
Trainer, V.L., Bates, S.S., Lundholm, N., Thessen, A.E., Cochlan, W.P., Adams, N.G., and Trick, C.G. (2012) *Pseudo-nitzschia* physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. *Harmful Algae*, **14**, 271–300.
Trainer, V.L., Hickey, B.M., and Bates, S.S. (2008) Toxic diatoms, in *Oceans and Human Health: Risks and Remedies from the Seas* (eds P.J. Walsh, S.L. Smith, L.E. Fleming, H.M. Solo-Gabriele, and W.H. Gerwick), Elsevier Science Publishers, New York, pp. 219–237.
Traynelis, S.F., Wollmuth, L.P., McBain, C.J., Menniti, F.S., Vance, K.M., Ogden, K.K., Hansen, K.B., Yuan, H., Myers, S.J., and Dingledine, R. (2010) Glutamate receptor ion channels: structure, regulation and function. *Pharmacol. Rev.*, **62**, 405–496.
Trick, C.G., Bill, B.D., Cochlan, W.P., Wells, M. L., Trainer, V.L., and Pickell, L.D. (2010) Iron enrichment stimulates toxic diatom production in high-nitrate, low-chlorophyll areas. *Proc. Natl Acad. Sci. USA*, **107**, 5887–5892.
Walter, J.A., Falk, M., and Wright, J.L.C. (1994) Chemistry of the shellfish toxin domoic acid: characterization of related compounds. *Can. J. Chem.*, **72**, 430–436.
Wang, G.J., Schmued, L.C., Andrews, A.M., Scallet, A.C., Slikker, W.Jr, and Binienda, Z. (2000) Systemic administration of domoic acid induced spinal cord lesions in neonatal rats. *J. Spinal Cord Med.*, **23**, 31–39.
Wekell, J.C., Trainer, V.L., Ayres, D., and Simons, D. (2002) A study of spatial variability of domoic acid in razor clams: recommendations for resources management on the Washington coast. *Harmful Algae*, **1**, 35–43.
Wells, M.L., Trick, C.G., Cochlan, W.P., Hughes, M.P., and Trainer, V.L. (2005) Domoic acid: the synergy of iron, copper, and the toxicity of diatoms. *Limnol. Oceanogr.*, **50**, 1908–1917.
Work, T.M., Barr, B., Beale, A.M., Fritz, L., Quilliam, M.A., and Wright, J.L.C. (1993) Epidemiology of domoic acid poisoning in brown pelicans (*Pelecanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*) in California. *J. Zoo Wildlife Medicine*, **24**, 54–62.
Wright, J.L.C., Boyd, R.K., de Freitas, A.S.W., Falk, M., Foxall, R.A., Jamieson, W.D., Laycock, M.V., McCullogh, A.W., McInnes, A. G., Odense, P., Pathak, V.P., Quilliam, M.A., Ragan, M.A., Sim, P.G., Thibault, P., and Walter, J.A. (1988) Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from eastern Prince Edward Island. *Can. J. Chem.*, **67**, 481–490.
Wright, J.L.C., Falk, M., McInnes, A.G., and Walter, J.A. (1990) Identification of isodomoic acid D and two new geometrical isomers of domoic acid in toxic mussels. *Can. J. Chem.*, **68**, 22–25.
Wu, D.-M., Lu, J., Zhang, Y.-Q., Zheng, Y.-L., Hu, B., Cheng, W., Zhang, Z.-F., and Li, M.-Q. (2013) Ursolic acid improves domoic acid-induced cognitive deficits in mice. *Toxicol. Appl. Pharmacol.*, **271**, 127–136.
Zaman, L., Arakawa, O., Shimosu, A., Onoue, Y., Nishio, S., Shida, Y., and Nogushi, T. (1997) Two new isomers of domoic acid from a red alga, *Chondria armata*. *Toxicon*, **35**, 205–212.
Zhao, J.-Y., Thibault, P., and Quilliam, M.A. (1997) Analysis of domoic acid and isomers in seafood by capillary electrophoresis. *Electrophoresis*, **18**, 268–276.

About the Authors

Stéphane La Barre is a senior research scientist at the French Centre National de la Recherche Scientifique. He obtained his MSc Degree from Auckland University, New Zealand, and his PhD at James Cook University, Townsville, Australia, before entering CNRS in 1984. He spent two years (1990–1991) as a research scholar at University of California San Diego, working on synthetic peptides with the late Murray Goodman, and on marine natural products with late John Faulkner. His multidisciplinary career includes marine chemical ecology, natural products chemistry of terrestrial and marine organisms and polymer chemistry. He is currently the coordinator of the research cluster BioChiMar (Marine Biodiversity and Chemodiversity), and is promoting research on new analytical tools to evaluate and predict environmental changes on coral reefs diversity, both biological and chemical.

Stephen S. Bates retired from Fisheries and Oceans Canada (DFO) in 2008, but is continuing to write articles and carry out

collaborations as a Scientist Emeritus. He has an MSc in marine biology from the City College of New York (1974) and a PhD in biological oceanography from Dalhousie University, Halifax, Nova Scotia, Canada (1979). His research has centered on the physiological ecology of marine phytoplankton, with special interests in methods for assessing their growth and physiological condition. He has held post-doctoral positions at the University of Quebec, the Bedford Institute of Oceanography (Dartmouth, NS), and the Institute for Marine Biosciences (NRC, Halifax, NS). In 1989, he was hired as a Research Scientist with DFO, in Moncton, New Brunswick, with special funds as a direct result of the 1987 domoic acid "mussel crisis" in Prince Edward Island. His main area of research has been the physiological ecology of pennate diatoms of the genus *Pseudo-nitzschia*, including factors that control their growth and production of the neurotoxin domoic acid. He has written nine review articles, 35 primary articles, 30 secondary articles, and has presented invited lectures on this topic around the world. His participation in ICES working groups and attendance at international conferences, as well as collaborations with scientists internationally, has kept him in contact with the latest advances in the field of harmful algal blooms.

Michael A. Quilliam is Group Leader of Biotoxin Metrology in the National Research Council Canada and Adjunct Professor of Chemistry in Dalhousie University (Halifax, Canada). His PhD in Chemistry was awarded in 1977 (University of Manitoba, Canada). This was followed by post-doctoral research at Université de Montréal and a one-year appointment with the Ontario Ministry of the Environment (Toronto). He then served as Associate Professor of Analytical Chemistry in the Department of Chemistry at McMaster University (Hamilton) from 1978 to 1987. In 1987, he joined the National Research Council of Canada. His research has been multidisciplinary and has encompassed a wide range of topics: detection of toxic substances and their metabolites, including DNA–carcinogen adducts; structure determination of marine and freshwater biotoxins; development of analytical methods, including chemical derivatization and liquid chromatography with mass spectrometry (LC-MS); and the production of certified reference materials.