Domoic acid: a fascinating marine toxin

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Abstract

There are indications that toxic algal blooms are increasing because of pollution of coastal waters and worldwide shipping. This mini-review deals with the marine biotoxin domoic acid, also known as amnesic shellfish poison, and its main producing diatom genus Pseudo-nitzschia (Bacillariophyceae). Besides contamination of seafood, these organisms have also been involved in human and marine wildlife mortality. The article aims to give an overview of all biological and environmental factors that should be considered when trying to evaluate a possible increase in toxic blooms of Pseudo-nitzschia spp.. Pseudo-nitzschia blooms characteristically occur in a low light regime, at a time when the temperature is falling and at a wide range of salinities. Laboratory studies have shown that the production of domoic acid, a water-soluble amino acid, is related to silicon, phosphorus, nitrogen and trace metal (mainly iron) availability. Domoic acid has no known function in defence or primary metabolism; a role in excretion of excess photosynthetic energy or as a binding ligand for trace metals is suggested. The variability in domoic acid production by different Pseudo-nitzschia spp., or the presence of toxic and non-toxic strains of the same species, cannot be explained. The conclusion is drawn that an increase in toxic blooms of Pseudo-nitzschia spp. might be possible, especially because of the expected increase in nutrient availability from pollution and desert dust. Global warming may have an influence as well by lengthening the growth period for Pseudo-nitzschia, enlarging their global distribution and increasing the dust load through desertification. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Several intoxication cases have been described in which the diatom genus Pseudo-nitzschia was involved. The first documented case occurred in 1987, when three people died and over a 100 became ill after eating mussels from Prince Edward Island, Canada (Bates et al., 1998). Three victims died 11–24 days after intoxication, by septic shock or pneumonia, while others recovered within 10 days after a period of confusion and nausea, but continued to show evidence of selective short-term memory loss (Todd, 1993). Mussels from stores and restaurants were examined and it was discovered that they contained considerable amounts of the neuroexcitatory toxin domoic acid. The source of domoic acid was traced to a bloom of what was then called Nitzschia pungens, but what is now known as Pseudo-nitzschia multiseries (Bacillariophyceae) (Hasle, 1995), once thought to be an innocent diatom, free of all toxicity (Bates et al., 1989). Toxic blooms of P. multiseries re-occurred along the eastern Canadian coast for the following 3 years, but human intoxication was prevented by an intensive monitoring program (Smith et al., 1990a; Villac et al., 1993).

More cases have been described since. As a result of an expanded monitoring program in eastern Canada, domoic acid, produced by Pseudo-nitzschia pseudodelicatissima, was found in mussels (Mytilus edulis) from the Bay of Fundy in 1989 (Martin et al., 1990). In 1991, the first toxic Pseudo-nitzschia bloom occurred on the Pacific coast of the United States. More than 100 brown pelicans (Pelecanus occidentalis) and Brandt’s cormorants (Phalacrocorax penicillatus) died in Monterey Bay, California, after they had eaten anchovies contaminated by another toxic species, Pseudo-nitzschia australis. High concentrations of domoic acid were also found in crabs, clams and mussels at many other sites...
in the United States (Horner et al., 1997). Several people in Washington State suffered mild symptoms after eating razor clams, but domoic acid poisoning was not confirmed (Bates et al., 1998). During 1991–1994, blooms of *P. australis* were most common along the Pacific coast. Domoic acid occurred in Dungeness crab, anchovies and sardines in 1996 when, again, hundreds of seabirds were killed in Baja, Mexico (Sierra Beltrán et al., 1997). Over 400 California sea lions (*Zalophus californianus*) drifted ashore along the Californian coast in May and June 1998 (Scholin et al., 2000). Only 70 animals were collected alive and cared for at The Marine Mammal Center, Sausalito; 47 of them died with neurotoxic symptoms. Domoic acid was detected in their faeces by high-performance liquid chromatography–ultraviolet and by a receptor-binding assay. Frustules of *P. australis* were present in anchovy guts and faecal material (Lefebvre et al., 1999). High levels of domoic acid were also measured the same year in Dungeness crabs from the coastal areas of Washington State (Wekell et al., 1994). In September 2000, the molluscan harvesting was temporarily closed for the State (Wekell et al., 1994). In September 2000, the molluscan harvesting was temporarily closed for the State (Wekell et al., 1994). In September 2000, the molluscan harvesting was temporarily closed for the State (Wekell et al., 1994). In September 2000, the molluscan harvesting was temporarily closed for the State (Wekell et al., 1994).

It is known that nutrient enrichment in coastal areas with limited mixing with ocean waters increases microbially polluted by toxic as well as non-toxic phytoplankton species (Anderson, 1994; Prescott et al., 1996; Paerl and Whitall, 1999). Evidence shows that the numbers, scale and complexity of blooms of some toxic alga species, e.g. *Alexandrium tamarense*, has increased worldwide (Anderson, 1994), also relative to total phytoplankton populations (Abboud-Abi Saab et al., 2000). This increase is attributed to a greater availability of nitrogen and phosphorus in coastal waters as a result of agricultural run-off, atmospheric deposition, harbour pollution or sewage, and the worldwide shipping of ballast water that unintentionally promotes algal distribution by transporting their cells into new habitats (Anderson, 1994; Prescott et al., 1996; Paerl and Whitall, 1999). No such evidence has yet been found for *Pseudo-nitzschia* spp., but it might be a concern for the future. The aim of this mini-review is to overview the factors that should be considered in such an evaluation, from the organism level up to a global scale.

2. Biology of *Pseudo-nitzschia*

*Pseudo-nitzschia* (Fig. 1) is a cosmopolitan genus of pennate diatoms restricted to marine environments (Hasle et al., 1996). Their typical golden brown colour is due to the pigment fucoxanthin, which enables them to grow early as well as late in the season, when the light availability is low and most species of algae are still unable to grow optimally. They consist of two silica halves or thecae (epi- and hypothece) of crystallised silica (Si(OH)₄) that overlap like a petri dish and are together called a frustule. Intracellular material is connected with the surrounding environment through poroids in the frustule. These poroids are also an important characteristic for distinguishing among species (Hasle et al., 1996). When the diatom reproduces asexually, each daughter cell receives one parent cell theca as an epitheca and then forms its own hypothece, after which cell division is complete (Hasle and Syversten, 1996). Therefore, asexual reproduction in diatoms results in a smaller cell size with each reproduction cycle. When diminished to about 30% of their original size (depending on the species), sexual reproduction occurs to regenerate the large cell size (Prescott et al., 1996; Davidovich and Bates, 1998; Hiltz et al., 2000). Sexual reproduction is facilitated when more individuals are present in the water column, because of the higher chance of meeting. This can occur, for example, during a bloom, and increases cell numbers far more rapid than asexual reproduction (Prescott et al., 1996). The light regime influences gamete production by *P. multiseries* (in culture), and is greatest at a light period corresponding in nature with the day length of fall blooms (Hiltz et al., 2000).

A bloom, or population explosion, occurs in the absence of limiting factors such as grazing zooplankton, fungal infections, lower light availability and low concentrations of nutrients. Warm temperatures, low salinity and a calm sea creates the ideal environment for blooming of most species (Raven et al., 1992; Villac et al., 1993), although not for *Pseudo-nitzschia*. Their blooms typically occur at a time when the temperature is falling and they can out-compete other types of
phytoplankton, e.g. dinoflagellates that do require calm stratified water. At those times, the temperature change causes a turnover of the water column, which breaks down the thermocline and thus brings water of higher nutrient concentration from below to the lighted surface, where the diatoms can take advantage of it. Also, because diatoms tend to sink, they need this more turbulent water to keep them from sinking out of the euphotic zone. The broad salinity range in which they are able to survive, for *Pseudo-nitzschia pungens* between 6 and 30‰ and for *P. multiseries* between 15 and 45‰ (Jackson et al., 1992), lets them grow in an estuarine environment, which can receive wide fluctuations in salinity due to freshwater runoff.

### 3. Toxin production

*Pseudo-nitzschia* spp. are among several other marine algae that can produce domoic acid. Domoic acid was first isolated in Japan from the macro-algae species *Chondria armata* in 1958, and was consequently called after the Japanese word for seaweed, which is 'domoi' (Villac et al., 1993). Its identification in 1987 as a neurotoxin was first treated with scepticism, because this water-soluble amino acid was known as a folk medicine in Japan to treat intestinal pinworm infestations when used in very small doses. Production of domoic acid by algae seems to be a genetic property for a secondary metabolite with no known function in defence or primary metabolism (Bates, 1998). Large, new cells, resulting from sexual reproduction produce substantially more domoic acid than smaller, older cells that have undergone many generations of vegetative division (Bates et al., 1999). However, the role that sexual reproduction may play in the long-term variability and toxicity of blooms is still a matter of speculation (Davidovich and Bates, 1998; Bates et al., 1999; Hiltz et al., 2000).

Bates (1998) summarized the literature on the relationship between silicon and domoic acid production. Diatoms require silicon not only for frustule formation, but also for DNA synthesis. Cell division and silicon uptake occur during daylight periods. The slowing of cell division near the end of the light period because of an initial depletion of silicon was found to trigger domoic acid synthesis. Domoic acid may simply serve as a way of eliminating excess photosynthetic energy when cells are no longer able to grow optimally. This is consistent with the hypothesis that it is synthesized during periods when photophosphorylated high-energy intermediates such as ATP and NADPH are not used for primary metabolism (Pan et al., 1998) and the fact that most domoic acid is excreted (Bates et al., 1991). That domoic acid is also a neurotoxin may or may not be favourable to the diatom (Bates, 1998).

Production of domoic acid is also known to be triggered by a shortage of phosphorus, although the mechanism is presently unknown (Pan et al., 1998). Nitrogen is an essential element in both cell growth as in the production of domoic acid, as with all amino acids (Bates, 1998). Availability of iron controls the ratios at which nutrients are used (Hutchins et al., 1998), e.g. the Si:N ratio, and affects the rate of cell division of *Pseudo-nitzschia*. Iron depletion results in more silicified, smaller diatoms. Iron addition causes extensive blooms of large diatoms at relatively low silicon levels (Hutchins and Bruland, 1998; Hutchins et al., 1998). Besides a role in the nutrient physiology of *Pseudo-nitzschia*, iron may also be an important factor in their toxin production. Although still under investigation, there is evidence that domoic acid might function as a binding ligand for trace metals, increasing the bioavailability of Fe, and decreasing the toxicity of Cu, when released into the water column (Maldonado et al., 2000; Rue and Bruland, 2000). As phytoplankton are normally able to take up only the ‘free’, unbound trace metal ion, the ligand keeps any ‘excess’ trace metal (at a potentially toxic concentration) sequestered in a form that is not directly bioavailable. However, as the phytoplankton cells take up the free ion, more of that metal becomes bioavailable, as it dissociates from the ligand. According to this statement, these authors found that Fe or Cu stress stimulated the production of domoic acid by *P. multiseries* during the exponential phase. On the other hand, Bates et al. (2000) found that cultures grown with no added Fe produce less domoic acid than Fe-replete cultures during both the exponential and stationary phases. Temperature stress does not boost the rate of domoic acid production (Bates, 1998).

Thus far there is no evidence of intracellular bacteria in *P. multiseries* (MacPhee et al., 1992) nor that bacteria may be responsible for the production of domoic acid. However, extracellular bacteria do enhance domoic acid production, although presumably via a mechanism other than that observed during physiological stress (Bates, 1998). The bacterial population in *Pseudo-nitzschia* cultures fluctuates widely in composition and concentration (Bates, 1998).

*Pseudo-nitzschia* spp. strains that produce domoic acid in one region may produce none or less in another region due to differences in genetics and/or bacterial composition (Bates, 1998). Examples of *Pseudo-nitzschia* species that are non-toxic in some parts of the world but are toxic in others are *P. pseudodelicatissima* in the Bay of Fundy, Canada and *P. seriata* in Denmark.
4. Environmental fate and toxicity

Domoic acid can enter the marine food chain via uptake by molluscan shellfish such as mussels that filter their food out of the water. This water can contain both diatoms themselves and the toxin, which is released to the water column (although there is no evidence yet that the toxin can be taken up directly). The toxin accumulates in the digestive gland and certain tissues of shellfish, and it appears to have no effect on the animals. Domoic acid may be metabolized by bacteria (e.g. of the genera *Alteromonas* and *Pseudomonas*) present in tissue of blue mussels (*M. edulis*) (Stewart, et al., 1998). Scallops are reported not to contain these elimination bacteria (Stewart, et al., 1998). Anchovies can also contain domoic acid in their guts, by feeding on toxic *Pseudo-nitzschia* spp.; this toxin affects their behaviour and survival (Lefebvre et al., 2001). Effects are also seen in seals (Scholin et al., 2000). In humans, consumption of contaminated seafood mostly affected the elderly or infirm (Todd, 1993). The sea otter is the only marine mammal to date to have died from domoic acid poisoning, due to the transfer of the toxin from mussels to its predators (O'Shea, 1999).

The mechanism of domoic acid toxicity is explained by its structural similarity with the excitatory neurotransmitter glutamic acid and its analogues (Fig. 2), but with a much stronger receptor affinity. Domoic acid is three times more potent than its analogue kainic acid and up to 100 times more potent than glutamic acid itself (Todd, 1993). After exposure, domoic acid binds predominately to N-methyl-D-aspartate (NMDA) receptors in the central nervous system (Berman and Murray, 1997), causing depolarisation of the neurons. Subsequently, the intercellular calcium concentration increases, resulting in sustained activation of calcium-sensitive enzymes, eventually leading to depletion of energy, neuronal swelling and cell death. The affected neurones are mainly located in the hippocampus, explaining the most striking effect of domoic acid poisoning, which is short-term memory loss, observed in 25% of the affected persons in the 1987 contaminated mussel event (Todd, 1993). Other symptoms are confusion, nausea, vomiting, gastro-enteritis, cramps and diarrhoea, all within 24 h. Neurological complaints, including ataxia, headaches, disorientation, difficulty in breathing and coma, develop 48 h after consumption. Permanent brain damage can also be caused by domoic acid intoxication. Effects of chronic low level ingestion are unknown (Villac et al., 1993).

Novelli et al. (1992) demonstrated that domoic acid from mussels is more neurotoxic than the chemically pure compound. This increase is due to domoic acid potentiation, caused by high concentrations of glutamic and aspartic acids present in mussel tissue. This neurotoxic synergism occurs through a reduction in the voltage-dependent Mg$^{2+}$ block at the receptor associated channel, following activation of non-NMDA receptors, in addition to the NMDA receptor activation by domoic acid itself.

5. Distribution of *Pseudo-nitzschia* spp.

Early records, partially based on native lore, suggest that marine biotoxins have been present along European and American coasts for hundreds of years. *Pseudo-nitzschia* abruptly occurred in high densities in the Skagerrak as early as 1912 (Hasle et al., 1996). Re-examination of archived samples linked *Pseudo-nitzschia* to high mortality of pelicans in California in the 1970s (Buck et al., 1992). However, information prior to the governmental and research-oriented monitoring that was initiated after the intoxication event of 1987 in Canadian waters (Smith et al., 1990b; Horner et al., 1997), in Monterey Bay (Scholin et al., 2000), and somewhat later in Europe (Dahlstrup et al., 2000; Monteros et al., 2000), is sparse. This intensive monitoring has since continued to document the presence of *Pseudo-nitzschia*.

Cases of domoic acid poisoning have not been reported for European waters thus far, although *Pseudo-nitzschia* species occur in the Skagerrak, North Atlantic and adjacent waters, as monitored by Hasle et al. (1996) from 1979 until 1993. Some European species of *Pseudo-nitzschia* produce small amounts of domoic acid (Lundholm et al., 1994; Vrielings et al., 1996). In Australia (Ajani et al., 2000), New Zealand, Korea, Japan (Kotaki et al., 1996) and Vietnam (Kotaki et al., 2000), *Pseudo-nitzschia* species (including *P. australis* and *P. pseudodelicatissima*) have not bloomed to high concentrations, so that domoic acid has not become harmful to human health. However, on the west coast of the United States (California, Oregon, Washington), cer-
tain Pseudo-nitzschia spp. cause major problems almost every year, related in part to upwelling of deep ocean water in these regions. This process supplies an extra quantity of nutrients (nitrate, nitrite, silicate and trace metals) in the top 300 m of the ocean where light penetrates (Prescott et al., 1996), and allows for higher cell densities. Inshore regions of the California upwelling region are also influenced by riverine iron input (Hutchins et al., 1998).

Although the unique frustule shape and type of colony formation consisting of overlapping cells readily identifies a diatom as Pseudo-nitzschia, differentiation at the species level requires a detailed examination by electron microscopy (Villac et al., 1993; Hasle et al., 1996). Monitoring for domoic acid-producing species is thereby complicated by the variety and similar morphology of the species complex. P. multiseries is the dominating toxic species on Prince Edward Island, Canada; P. pseudodelicatissima in the Bay of Fundy, Canada; P. australis and P. pseudodelicatissima often co-occur together on the United States west coast; P. multiseries and P. delicatissima are dominating in Dutch waters; P. seriata in Denmark; P. australis in Spain; and P. australis and P. delicatissima in New Zealand (Bates et al., 1998).

Measures other than monitoring have been taken to successfully protect the shellfish industry from toxic blooms. On Prince Edward Island, the mussel harvest can be delayed until the bloom has dissipated in the darkness of ice cover (Villac et al., 1993) and some research is focused on isolating the bacteria able to break down domoic acid in already contaminated mussels (Stewart et al., 1998).

6. Concerns for the future

The increase in toxic algal blooms observed for other species around the world does not seem to apply to Pseudo-nitzschia, as stated by Bates et al. (1998). On the Canadian east coast, Pseudo-nitzschia concentrations in the water and therefore toxin levels in mussels have even slowly decreased since 1987, although intense blooms could re-occur any time as shown by the presence of domoic acid-contaminated mussels in September 2000. An increased demand for seafood (thereby increasing the aquaculture of mussels), the increasing use of coastal waters, and expanded monitoring programmes have caused increased publicity. However, several aspects, e.g. unrecognised events and unreported illnesses, make evaluation challenging. It is more difficult to draw conclusions about toxic Pseudo-nitzschia blooms in other parts of the world; they have not been studied long enough to show trends.

When atmospheric deposition and/or eutrophic water from river inflow, upwelling or anthropogenic pollution supply extra nutrients in a period when nutrients are limiting in a natural system, and when light and temperature are still intense enough for algal growth, then a Pseudo-nitzschia bloom can occur (Bates et al., 1998). Although diatoms, with a clear need for silica, can be disadvantaged because of a change in N:P:Si ratio due to nitrogen and phosphorus inputs from pollution, silica supplied by, for example, upwelling can meet the needs. Another important silica input, which has so far not been taken into consideration, is the worldwide distribution of desert dust. The mass of African dust transported in the atmosphere is about one billion tonnes per year (Moulin et al., 1997). Iron in the minerals composing this desert dust will also be a vital micronutrient in oceanic regions that are deficient in iron (Duce and Tindale, 1991). Run-off of toxic substances such as insecticides can affect zooplankton and result in high phytoplankton levels at a lower nutrient loading than in the absence of toxicants (Jak, 1998). Besides a direct role of human activity, climate-induced changes in the environment could affect the distribution of toxic Pseudo-nitzschia spp. by lengthening the growth period later into winter (Harvell et al., 1999), by enlarging the geographical distribution and by increasing the dust load by desertification (Moulin et al., 1997). However, a bloom of Pseudo-nitzschia spp. cells does not necessary imply that domoic acid production will occur. Large cell numbers, present during a bloom, could eventually deplete the silicon or phosphorus, so their growth slows or stops and (if there is still enough nitrogen in the water left) domoic acid will be produced (Smith et al., 1990b). However, there is no conclusive explanation found so far for the geographical variation in domoic acid production by different Pseudo-nitzschia spp., nor for the presence of toxic and non-toxic strains of the same species.

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