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Bloom Dynamics and Physiology of Domoic-Acid-Producing *Pseudo-nitzschia* Species

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1 Introduction

In late November to December 1987, Canadians awoke to headlines such as “Mystery Toxin Taints Mussels”, “Killer Shellfish Algae Found in River”, and “Fatal Mussel Toxin Found”. After an unprecedented 104 h of detective work, the neurotoxin domoic acid (DA) was identified as the contaminating agent in blue mussels (*Mytilus edulis*) from Cardigan Bay, eastern Prince Edward Island (PEI), Canada (Wright *et al.* 1989). This toxin caused at least 107 illnesses, killed at least three elderly people (Perl *et al.* 1990; Todd 1993), and temporarily devastated the molluscan shellfish aquaculture industry (Addison and Stewart 1989; Wessells *et al.* 1995). Symptoms included abdominal cramps, vomiting, and neurologic responses involving disorientation and memory loss that could persist indefinitely. Due to the latter, the term Amnesic Shellfish Poisoning (ASP) was given to this clinical syndrome. Domoic Acid Poisoning (DAP) is also sometimes used for this illness since shellfish are not always a vector.

The identification of DA as a toxin was at first treated with skepticism. This water-soluble tricarboxylic amino acid with a molecular weight of 311 (Fig. 1) was known as a folk medicine to treat intestinal pinworm infestations in young children in Japan (Takemoto and Daigo 1958). However, an order of magnitude greater dose was consumed in the eastern Canadian toxic episode, and those most affected were elderly or infirm (Perl *et al.* 1990). As an analog of glutamate, an excitatory neurotransmitter, DA binds to the kainate type of glutamate receptors, but with a binding capacity three times greater and 20 times more powerful than kainic acid (Teitelbaum *et al.* 1990). In the presence of endogenous glutamate, DA causes massive depolarization of the neurons, with a subsequent increase in cellular Ca^{2+}, leading to neuronal swelling and death. These nerve cells, located in the hippocampus, are associated with memory retention, hence the memory loss characteristic of ASP.

This overview focuses on the distribution and bloom dynamics of pennate diatoms of the genus *Pseudo-nitzschia*, some (but not all) of which produce DA. A review of the physiology of DA production, based on laboratory studies, is given by Bates (this volume). Laboratory research helps to interpret field studies of *Pseudo-nitzschia* blooms, but there is still a paucity of field data. Nevertheless, data sets are becoming available from eastern Canada, western North America, and the Gulf of Mexico. This permits a comparison of commonalities and differences among these events, in order to understand mechanisms of bloom formation, and eventually, to be able to predict their occurrence. Indeed, information gathered to date has enabled monitoring programs that now protect consumers of molluscan shellfish in an increasing number of countries.

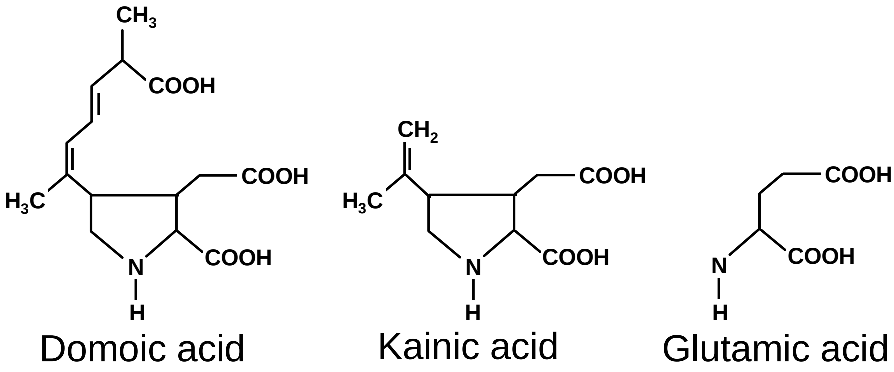

Fig. 1. Structure of domoic acid and its analogues.

1.1 Domoic-Acid-Producing Algae

Domoic acid was first isolated from the rhodophycean macroalga *Chondria armata* in Japan (e.g., Maeda *et al.* 1986), and is named after the Japanese word for this seaweed, "domoi". It was later found in the rhodophytes *Alsidium corallinum*, from the east coast of Sicily (Impellizzeri *et al.* 1975), and *Chondria baileyana,* from southern Nova Scotia and PEI, Canada (Laycock *et al.* 1989). It has since also been found in *Amansia glomerata*, *Digenea simplex*, and *Vidalia obtusiloba*, all belonging to the family Rhodomelaecae (Sato *et al.* 1996). The source of DA in the 1987 episode on PEI was the pennate diatom, *Pseudo-nitzschia multiseries* (Subba Rao *et al.* 1988a; Bates *et al.* 1989). This was the first known instance of a diatom producing a phycotoxin. The nomenclature of the genus *Pseudo-nitzschia*, once in a state of flux, has now been clarified: the DA-producing diatom was originally reported as *Nitzschia pungens* forma *multiseries*, then as *Pseudonitzschia pungens* f. *multiseries*, *Pseudo-nitzschia pungens* f. *multiseries*, and finally as *Pseudo-nitzschia multiseries* (Hasle 1965; 1994; Hasle *et al.* 1996). Based on morphological, physiological, and genetic features, it has been raised to the rank of species, to distinguish it from the nominate form, *P. pungens* f. *pungens*, now named *P. pungens* (Hasle 1995). One of the major distinctions between diatoms of the genera *Nitzschia* and *Pseudo-nitzschia* is that the latter form stepped colonies, i.e., chains of cells with overlapping tips, when seen in girdle view (Hasle 1994).

The 1987 DA episode generated an awareness of this toxin and resulted in its discovery in several other locations and phytoplankton species around the world (Table 1). To date, at least eight species of diatoms have been shown to produce DA in culture (Table 2). Curiously, all are pennate diatoms, and all but one belong to the genus *Pseudo-nitzschia*. It would not be surprising, however, if this toxin were to be found in numerous other microorganisms, including bacteria and other phytoplankton genera. Indeed, the source of DA that contaminated sea scallops on Georges/Browns Bank, Nova Scotia in 1995, cultured blue mussels in Newfoundland in 1994, and razor clams and Dungeness crabs in Oregon, Washington, and British Columbia since 1991, has not been determined. The taxonomic identity of other possible producers, such as *P. turgidula* (Rhodes *et al.* 1996), must be confirmed.

Until recently, it was believed that *P. pungens* was non-toxic, as shown in reports from Atlantic Canada (e.g., Smith *et al.* 1990b; Bates *et al.* 1993b), the Atlantic

Table 1. Locations around the world where species of *Pseudo-nitzschia* have been shown to produce domoic acid.

Geographic Area	Species Name	Reference
Prince Edward Island, Canada	*P. multiseries*	Bates *et al.* 1989; Smith *et al.* 1990a; b
Massachusetts Bay, MA, USA		Villareal *et al.* 1994
Narragansett Bay, RI, USA		Hargraves *et al.* 1993
Galveston Bay, TX, USA		Fryxell *et al.* 1990; Reap 1991; Dickey *et al.* 1992; Villac 1996
Monterey Bay, CA, USA		Villac *et al*. 1993b; Villac 1996
Willapa Bay, WA, USA		Sayce and Horner 1996
Hood Canal, WA, USA		Horner *et al.* 1996
Dutch Wadden Sea		Vrieling *et al.* 1996
Ofunato Bay, Japan		Kotaki *et al.* 1996
Jinhae Bay, Korea		Lee and Baik 1995
Bay of Fundy, NB, Canada	*P. pseudodelicatissima*	Martin *et al.* 1990; 1993
Prince Edward Island, Canada	*P. delicatissima*	Smith *et al.* 1990b
Denmark	*P. seriata*	Lundholm *et al.* 1994
Galicia, Spain	*P. australis*	Míguez *et al.* 1996
Monterey Bay, CA, USA		Buck *et al.* 1992; Fritz *et al.* 1992; Garrison *et al.* 1992; Villac *et al.* 1993b; Walz *et al.* 1994
Ilwaco, WA, USA		Villac *et al.* 1993b
Coos Bay, OR, USA		Villac *et al.* 1993b
New Zealand		Rhodes *et al.* 1996
Marlborough Sd., New Zealand	*P. pungens*	Rhodes *et al.* 1996
Bay of Plenty, New Zealand	*P. fraudulenta*	Rhodes *et al.* 1998

Table 2. Diatoms shown to produce domoic acid in culture, although some clones are non-toxic.

Species Name	Reference
Amphora coffeaeformis	Shimizu *et al.* 1989; Maranda *et al.* 1990
Pseudo-nitzschia multiseries	Subba Rao *et al.* 1988a; 1990; Bates *et al.* 1989; 1991; 1993b; 1995; 1996; Fryxell *et al.* 1990; Reap 1991; Dickey *et al.* 1992; Douglas and Bates 1992; Wohlgeschaffen *et al.* 1992; Douglas *et al.* 1993; Lewis *et al.* 1993; Villac *et al.* 1993a; b; Villareal *et al.* 1994; Wang *et al.* 1993; Hargraves *et al.* 1993; Whyte *et al.* 1995a; b; Pan *et al.* 1996a; b; c; Vrieling *et al*. 1996; Villac 1996
P. pseudodelicatissima	Martin *et al.* 1990; Lundholm et al. 1997
P. delicatissima	Smith *et al.* 1990b; Rhodes *et al.* 1998
P. australis	Garrison *et al.* 1992; Villac *et al.* 1993b; Rhodes *et al.* 1996
P. seriata	Lundholm *et al.* 1994
P. fraudulenta	Rhodes *et al.* 1998
P. pungens	Rhodes *et al.* 1996; G.J. Doucette; R.A. Horner; C.A. Scholin; V.L. Trainer; J.N.C. White, pers. commun.

coast of the USA (Villareal *et al.* 1994; Wang *et al.* 1993), the Gulf of Mexico (Villac *et al.* 1993b), Monterey Bay (Villac *et* al. 1993b), Europe (Lundholm *et al.* 1994; Vrieling *et al*. 1996), and New Zealand (Mackenzie *et al.* 1993). However, there is now a published report of DA production by a *P. pungens* isolate from one location in New Zealand, although isolates from other sites were not toxic (Rhodes *et al.* 1996). Several additional cases of toxic *P. pungens* have now been reported: four clones from Monterey Bay, CA, collected during two separate years (G.J. Doucette and C.A. Scholin, unpubl.); one isolate from Hood Canal, WA, and another from open coastal waters of WA (R.A. Horner and V.L. Trainer, unpubl.). Although the cellular DA levels are low in these examples (generally < 0.1 pg cell^{-1}) relative to *P. multiseries* (cf. Table 3), certain molluscan shellfish may still become contaminated over time.

A similar contradictory finding regarding toxicity was reported for *P. seriata* (Lundholm *et al.* 1994), which was previously shown to be non-toxic in culture (Bates *et al.* 1989). Likewise, there are reports of non-toxic strains of *P. multiseries* (Villareal *et al.* 1994), *P. pseudodelicatissima* (Reap 1991; Villareal *et al.* 1994; Lundholm *et al.* 1994; Hallegraeff 1994; Walz *et al.* 1994), *P. australis* (Villac *et al.* 1993b), and *P. delicatissima* (Villac *et al.* 1993b; Lundholm *et al.* 1994). In contrast to Shimizu *et al*. (1989) and Maranda *et al.* (1990), *Amphora coffeaeformis* (strain CCMP127; Bates *et al.* 1989), and another *A. coffeaeformis* strain isolated from Esquimalt Lagoon on southern Vancouver Island, Canada, failed to produce DA (L.M. Brown; R.F. Addison, unpubl.).

These findings suggest a) genetic variability among strains of the same *Pseudo-nitzschia* and *Amphora* species from different geographic locations; b) differences in factors controlling DA production (e.g., nutrients, bacteria); c) an incomplete study of growth conditions conducive for toxin production; d) mis-identification of the organism, given that species of the genus *Pseudo-nitzschia* are difficult to distinguish morphologically; or e) mis-interpretation of analytical results. To avoid the latter, it is essential that the identity of the toxin be confirmed, e.g., by tandem mass spectrometry, in reporting any new producer of DA. The analytical technique used for measuring cellular DA should be sensitive, and its limit of detection should be reported. A rigorous examination of growth conditions (see Bates, this volume) must also be carried out before the ability for toxin production can be ruled out with confidence. Finally, it is essential that the species identity be confirmed by a taxonomic authority. Many countries are beginning to expand their monitoring programs, at great expense, to include *Pseudo-nitzschia* spp. and DA. It is therefore critical to be certain about the taxonomic identity of any new toxigenic species and about the chemical identity DA.

The cosmopolitan distribution of toxigenic *Pseudo-nitzschia* species along our coasts (Hasle 1965; Hasle *et al.* 1996) is cause for concern about the safety of the natural and aquaculture harvest of molluscan shellfish that may accumulate DA (Addison and Stewart 1989). Food chain transfer has been demonstrated, as pelicans (*Pelecanus occidentalis*) and cormorants (*Phalacrocorax penicillatus*), and their food source, anchovies (*Engraulis mordax*), have been affected (Fritz *et al.* 1992; Work *et al.* 1993; Wekell *et al.* 1994; McGinness *et al.* 1995), as have Dungeness crabs (*Cancer magister*) and razor clams (*Siliqua patula*) (Horner *et al.* 1993; Wekell *et al.* 1994). Indeed, the number of countries that report problems with this diatom genus is growing by the year (Table 1). An understanding of factors that control the physiology of DA production by *Pseudo-nitzschia* species (see Bates, this volume) and its bloom dynamics is therefore essential.

One of the more difficult tasks with field populations is to be able to discriminate among the various *Pseudo-nitzschia* species, which are morphologically similar and tend to be confused with one another (Hasle *et al*. 1996). Aside from its ability to produce DA, at one time *P. multiseries* could only be distinguished from the non-toxic *P. pungens* by the number of rows of poroids on the silicon frustule, as seen by electron microscopy (e.g., Villac *et al.* 1993a). The two species can now be discriminated by an immunofluorescence assay (Bates *et al.* 1993a), lectin-binding assays (Fritz 1992; K.E. Pauley, unpubl.), and by differences in nucleic acid sequences (Scholin *et al.* 1994; 1996; Douglas *et al.* 1994; Manhart *et al.* 1995; Miller and Scholin, 1996; Scholin, this volume). Because of the species' morphological similarity, DNA probes developed against *P. australis*, *P. delicatissima*, *P. fraudulenta*, *P. americana*, and *P. heimii* (Scholin *et al.* 1994; 1996) are invaluable for identification purposes. Molecular probes for identifying *P. seriata* are thus far still lacking. An alternative approach for determining if one species is different from or the same as another is by carrying out clonal breeding experiments in culture (see Section 4).

2 Case Histories of *Pseudo-nitzschia* Blooms

Pseudo-nitzschia blooms differ from, e.g., certain dinoflagellate blooms, in that high concentrations (at least 100,000 cells L^{-1}) of the DA-producing species must be present in order for shellfish to become contaminated to a level that closes harvesting (20 µg DA g^{-1} wet weight of tissue). A complicating factor is that the morphologically-similar *P. pungens* is generally non-toxic, thus cell numbers alone will not indicate the certainty of DA intoxication; hence the need for methods to distinguish the species (see above). There are no reports of *Pseudo-nitzschia* blooms causing problems independent of their toxicity. With the exception of the dense bloom of *P. multiseries* in 1987 in PEI, there appears to be nothing unusual about the occurrence of *Pseudo-nitzschia* blooms.

2.1 Prince Edward Island, Canada

The first-reported site of a DA-producing *P. multiseries* bloom was in estuaries of Cardigan Bay, eastern PEI (Fig. 2), where cell concentrations and DA levels in mussels reached record levels in 1987 (Table 3) (Bates *et al*. 1989). Meteorological conditions

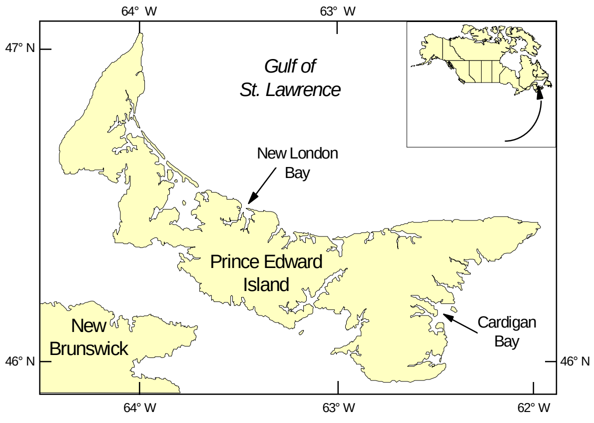

Fig. 2. Location of *P. multiseries* and *P. pungens* blooms in eastern and northern Prince Edward Island, Canada.

Table 3. *Pseudo-nitzschia multiseries* plus *P. pungens* cell concentrations (cells L^{-1}) and domoic acid in mussels (µg g^{-1}) in Cardigan Bay, PEI; harvesting closure occurs at 20 µg g^{-1}.

Year	*Pseudo-nitzschia*	Domoic Acid	Conditions
1987	15,000,000	790	Dry summer, rainy autumn; Calm weather, light S winds
1988	1,200,000	280	Dry summer, early autumn rains; Strong SE gale
1989	460,000	16	Wet summer, no autumn runoff
1990	132,000	0.6	N winds disperse incipient bloom of *P. multiseries*
1991	354,000	not detected	Violent storms, then calm
1992	48,000	not detected	No unusual weather
1993	92,000	not detected	Mostly *P. pungens*
1994	79,000	not detected	Mostly *P. pungens*
1995	14,000	not detected	Mostly *P. pungens*

apparently contributed to the bloom formation: a prolonged dry period in summer, followed by an unusually rainy autumn (Smith 1993). This may have provided nutrients, via river runoff, to drive the bloom. Similar, but less severe meteorological conditions followed in the autumn of 1988 (Smith *et al.* 1990a). The *Pseudo-nitzschia* bloom that year was therefore less intense, but still resulted in closure of mussel harvesting due to elevated levels of DA. A 9-day lag between the peak of DA in the algae and in the mussels provided an early warning of an impending health hazard (Fig. 3A). Early in the bloom, cellular DA appeared to accumulate when the *Pseudo-nitzschia* concentration stopped increasing (Fig. 3B), consistent with laboratory studies linking toxin production to the stationary phase of growth (see Bates, this volume). Increases in cell concentration corresponded with pulses of nitrate from rivers, following rain events. The bloom was likely terminated by the decrease in light and temperature in December, as illustrated in a generalized schematic (Fig. 4).

In 1989, there was no dry summer, nor was there any substantial runoff during the autumn; the *Pseudo-nitzschia* bloom was again smaller than in 1987 and 1988. Cells of *P. pungens* were present in early August, with no detectable DA (Fig. 5) (Smith *et al.* 1990b). This was followed by a small bloom of *P. pungens* plus *P. multiseries* in late August to September, with the appearance of DA. The major autumn bloom then began in mid-October, with *P. multiseries* reaching 100% of the *Pseudo-nitzschia* spp. population in late November, when mussels rapidly accumulated DA. In 1990, non-toxic *P. pungens* bloomed in October, but an incipient *P. multiseries* bloom was dispersed seaward by a series of violent, primarily northerly storms (Smith 1993). Since then, there have been only minor *Pseudo-nitzschia* blooms, composed mainly of *P. pungens*, and no detectable levels of DA in Cardigan Bay mussels (Table 3). In contrast, mussel harvesting was closed due to DA in New London Bay (Fig. 2), in October 1991 and 1994. The dynamics of these latter blooms have not been studied.

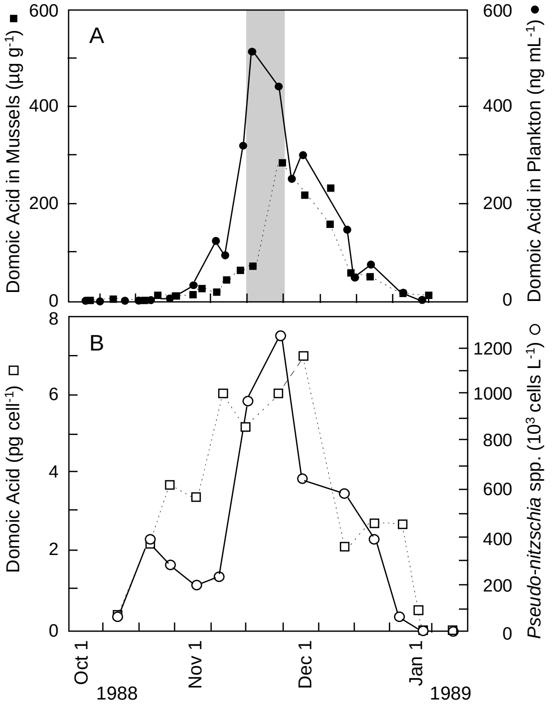

Fig. 3. Cardigan Bay, Prince Edward Island, Canada. A) Domoic acid concentration in mussels and phytoplankton, and time lag (shaded area) between peaks of the two; B) cellular domoic acid and *Pseudo-nitzschia* spp. concentration. Redrawn from Smith *et al*. 1990a.

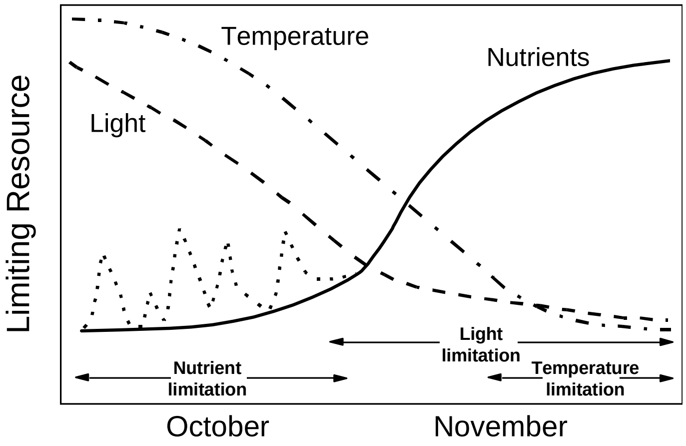

Fig. 4. Schematic of changes in temperature, light and nutrients, and periods of limitation that may affect the decline of *P. multiseries* blooms in Prince Edward Island, Canada. The dotted line represents pulses of nutrient input from rivers or sediments.

It is clear that the large interannual variability and general decline in bloom intensity are the most obvious characteristics of the Cardigan Bay blooms. The time series is still too short to unequivocally determine the cause of the decline in intensity since the 1987 outbreak. However, several hypotheses may be advanced: decreases in nutrient loading from rivers; an increase in predation by cultured mussels; cycles in the sexual reproduction of *P. multiseries*; changes in bacterial populations affecting diatom growth; and attack by parasites. For the latter, parasitic oomycetes and/or chytrids have infected *P. multiseries* and *P. pungens* cells in eastern PEI (K.E. Pauley, unpubl.; L.A. Hanic, pers. commun.; Elbrachter and Schnepf, this volume). Their importance in regulating bloom dynamics has yet to be determined, but their presence is suggestive of one cause of the bloom decline since 1987. In support of this, Horner *et al.* (1996) observed a fungal parasite in some *Pseudo-nitzschia* spp. cells during the decline of a bloom in coastal Washington, USA. Hasle *et al.* (1996) reported an unexplained decrease in *P. multiseries* abundance in the Skagerrak between 1991 and 1993, and also raised the possibility of control by parasitic fungi.

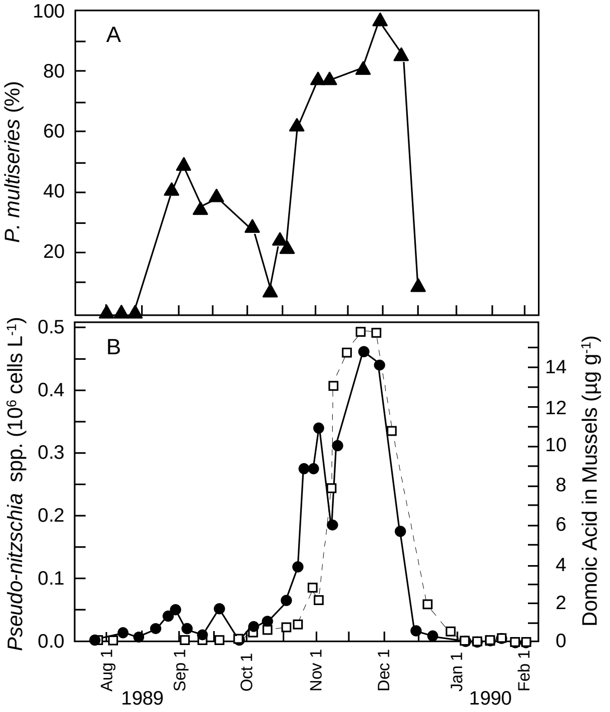

Fig. 5. Cardigan Bay, Prince Edward Island, Canada. A) Percentage of *Pseudo-nitzschia multiseries* in *P. pungens* plus *P. multiseries* populations (determined using scanning electron microscopy); B) concentration of *P. pungens* plus *P. multiseries* cells in the seawater (●), and domoic acid in mussels (0). Redrawn from Smith *et al*. 1990b.

2.2 Bay of Fundy, Eastern Canada

Because of the 1987 ASP event in PEI, monitoring for *Pseudo-nitzschia* and DA was initiated in the southwest Bay of Fundy in 1988. Regions of the Bay of Fundy are commonly closed due to PSP toxins, but no shellfish toxicity had previously been associated with diatom blooms. In late July, 1988, monitoring revealed the presence of DA in blue mussels and soft-shell clams (*Mya arenaria*) in Passamaquoddy Bay, and harvesting was closed in late August; the highest DA concentration was 74 µg g^{-1}. The dominant phytoplankter at the time was *Pseudo-nitzschia pseudodelicatissima*, which produced DA in laboratory culture (Martin *et al.* 1990). It must be noted, however, that strains of *P. pseudodelicatissima* from other parts of the world have failed to produce DA in culture (see Section 1.1). Likewise, no DA was detected in at least two predominantly *P. pseudodelicatissima* blooms in coastal California (Walz *et al.* 1994).

Although *P. pseudodelicatissima* has historically been present in the Bay of Fundy, no toxicity had previously been associated with it. This may be because shellfish harvesting is often prohibited anyway, due to PSP toxins, during the summer months when DA may also be present. Subsequent surveys have shown that this diatom occurs throughout the year, but with a small bloom generally in June, followed by a larger bloom in late August to early September (Fig. 6) (Martin *et al*. 1993). Shellfish harvesting was again closed due to DA from this diatom in September, 1995. The late summer appearance of *P. pseudodelicatissima* links it to elevated water temperatures, although neither its temperature preference, nor the conditions conducive for growth and toxin production, have been studied in culture. Cells are distributed throughout the water column (Fig. 6), consistent with the vigorous tidal mixing in the area, and making them available to contaminate benthic shellfish.

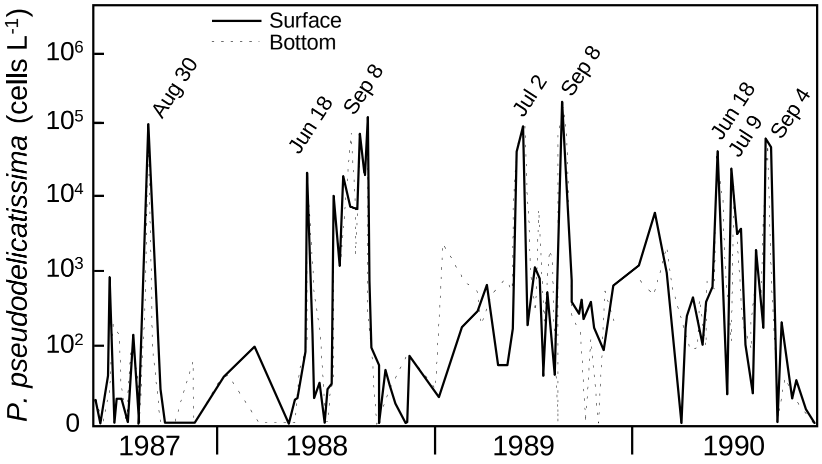

Fig. 6. Concentration of *Pseudo-nitzschia pseudodelicatissima*; Passamaquoddy Bay, southwest Bay of Fundy, Canada. Redrawn from Martin *et al*. 1993.

2.3 Monterey Bay, California, USA

Until 1991, eastern Canada was the only region documented to have problems associated with toxigenic *Pseudo-nitzschia* species. Then, in September, 1991, unusual neurological symptoms and deaths of more than 100 brown pelicans and Brandt's cormorants led to the discovery of DA in Monterey Bay, CA (Work *et al*. 1993). The DA was traced to the birds' food source, northern anchovies, which in turn had fed on the diatom *Pseudo-nitzschia australis* (Buck *et al*. 1992; Fritz *et al*. 1992). This diatom produced DA in culture (Garrison *et al.* 1992; Villac *et al*. 1993b; Bates, this volume).

The Monterey Bay event was significantly different from the Cardigan Bay, PEI outbreak, not only because of the other *Pseudo-nitzschia* species involved, and birds rather than humans were poisoned, but also because the vector was northern anchovies, not shellfish; hence the use of the term DA Poisoning (DAP). The highest concentrations of DA, up to 2,300 µg g^{-1} wet weight, were found in the viscera of the anchovies (Loscutoff 1992), along with the *P. australis* cells; these fish continue to be contaminated with DA (McGinness *et al*. 1995). Further, DA has been found in some grazing zooplankton (Buck *et al*. 1992; Haywood and Silver 1994). With the exception of the seabirds, there are no effects or impacts of DA on the pelagic food web. There is evidence, based on historical accounts of seabird behavior and mortalities, and on archived plankton samples, that massive blooms of *P. australis* have occurred since at least the 1920's (Garrison *et al.* 1992; Villac *et al*. 1993b; Lange *et al*. 1994). It is likely, however, that the species had been erroneously reported as *Nitzschia seriata*, because at the time *P. australis* was believed to be limited to the southern hemisphere.

Pseudo-nitzschia species and DA were monitored in Monterey Bay between 1991 and 1994. At the peak of the 1991 toxic event, DA levels in coastal waters reached >10 µg L^{-1} and abundances of *P. australis* were $>10^6$ cells L^{-1} (Walz *et al*. 1994). Since the massive bloom in 1991, DA has been detected in both autumn and spring plankton assemblages, but with concentrations usually <1 µg L^{-1} and cell densities 1-2 orders of magnitude lower (Fig. 7). In contrast to the eastern Canadian situation, blooms have often been simultaneously comprised of up to four potentially-toxic species, i.e., *P. australis*, *P. multiseries*, *P. pseudodelicatissima*, and *P. pungens.* Based on cell volume dominance by *P. australis*, however, most of the DA measured in plankton assemblages was assumed to have been contributed by this species (Fig. 7) (Walz *et al*. 1994). Production of DA has been demonstrated for California isolates of *P. australis* and *P. multiseries* (Garrison *et al*. 1992; Villac *et al*. 1993b; Villac 1996), and recently for four clones of *P. pungens* (see Section 1.1), but not for *P. pseudodelicatissima* (Villac *et al*. 1993b; Walz *et al*. 1994; Villac 1996).

During 1991-1994, blooms of *P. australis* were most common and persisted the longest during late summer to autumn, when the hydrography is usually characterized by warm sea-surface temperatures, thermal stratification, and low nutrient concentrations (Buck *et al*. 1992; Walz *et al*. 1994), conditions associated with the end

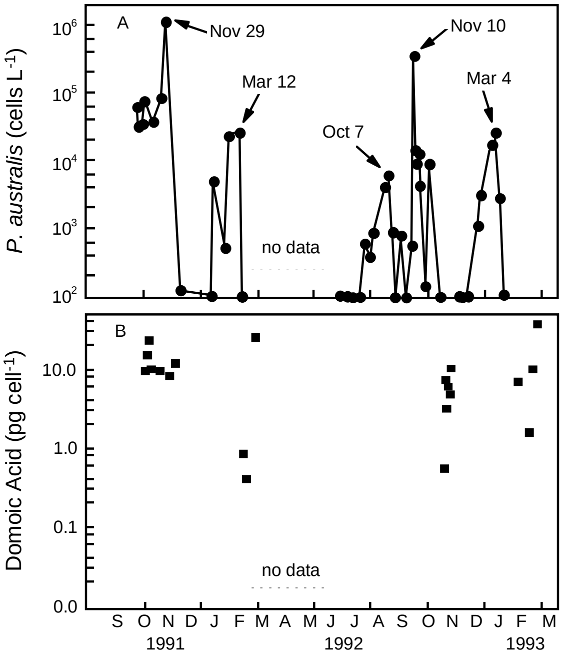

Fig. 7. A) Concentration of *P. australis* in near-surface waters (0 or 5 m); B) cellular domoic acid in *P. australis* calculated from species abundance and domoic acid in net-collected samples, assuming that the domoic acid is apportioned according to biovolume and cell abundance; Monterey Bay, CA, USA. Redrawn from Walz *et al*. 1994.

of the upwelling season. Blooms also occurred during the spring upwelling season, but they were less well-developed and generally shorter in duration (Walz *et al*. 1994). In contrast, the *P. australis* blooms in southern California were most common in the late spring to early summer months, not in the autumn (Lange *et al*. 1994). They were associated with intrusions of cool, high-nutrient waters, probably related to upwelling events. In spite of cell concentrations equivalent to those reached in Monterey Bay, no major toxic episodes were reported. It must be noted, however, that no toxin measurements were made in the phytoplankton and that nearshore bivalves are not good indicators of toxicity in offshore anchovies, and vice versa (Langlois *et al*. 1993).

Reports (e.g., Malone 1971; Garrison 1979) suggest that diatom blooms in the central California region are mostly a coastal (i.e., continental shelf) phenomenon, and this is also likely the case with *Pseudo-nitzschia* spp. blooms. Moreover, the well-developed blooms in Monterey Bay and along the continental shelf to the north appear to be associated with a local mesoscale circulation pattern that develops during periods of active upwelling and results in water being retained near the coast (Graham *et al*. 1992). Coastal populations, however, are frequently carried offshore in jets or filaments of coastal upwelling water and then may find their way back onshore in the complexity of eddies and meanders that occur with upwelling episodes followed by relaxations (e.g., Garrison 1981). Although the California Current is a continuous system extending from approximately Vancouver Island to northern Baja California, there are pronounced latitudinal variations in physical and biological dynamics (Horner *et al.* 1997). These variations may account for the differing descriptions of *Pseudo-nitzschia* bloom dynamics reported in southern California (Lange *et al*. 1994), central California, and the Oregon/Washington region.

2.4 Oregon and Washington, USA

The September, 1991 DA episode in Monterey Bay prompted an examination of shellfish to the north. By late October and November, DA was found in razor clams (*Siliqua patula*) and Dungeness crabs (*Cancer magister*) on the Oregon and Washington coasts. Several people in Washington suffered mild symptoms after eating razor clams, but the illnesses were mild and short-lived and DA poisoning was not confirmed. Moreover, neither the source(s) nor pathway(s) of DA to the razor clams and Dungeness crabs have been identified. No phytoplankton samples were available from offshore, and samples collected from ocean beaches since 1991 usually contain few cells of *Pseudo-nitzschia* spp. (Horner and Postel 1993; R. Horner, unpubl.). However, several potentially-toxic *Pseudo-nitzschia* species (i.e., *P. multiseries*, *P. australis*, *P. pungens*, and *P. pseudodelicatissima*) are found in local waters. The 1991 open coast event occurred after a record hot, dry period lasting 45 d followed by rain (Horner and Postel 1993), conditions similar to eastern PEI in 1987. It is possible that similar oceanographic conditions, including unusually warm weather associated with El Niño, and phytoplankton assemblages existed along the US west coast, with the DA source(s) part of these widespread populations (Taylor and Horner 1994). However, levels of DA within razor clams vary considerably along the Washington coast. All clam beaches were not affected to the same extent and there is considerable clam-to-clam variability in DA content on a single beach (J.C. Wekell, pers. commun.).

In coastal embayments, i.e., Willapa Bay, *Pseudo-nitzschia* abundances show some correlation with weather, but usually do not occur until after a salinity near 29 ‰ is reached (Sayce and Horner 1996), suggesting the intrusion of high-salinity ocean water. Regardless of the source of cells, oysters within Willapa Bay have not contained DA.

Pseudo-nitzschia pungens, *P. multiseries*, *P. pseudodelicatissima*, and *P. australis* have been identified in inland marine waters of western Washington, i.e., Puget Sound and Hood Canal, since the summer of 1990. They often persist together in some combination at low concentrations (ca. 1-5 x 10^3 cells L^{-1}) for several months each year and not just in the autumn. When increases (blooms) occur, sometimes after warm dry periods followed by rain, they are usually short-lived and concentrations rarely exceed 10^5 cells L^{-1} (Horner and Postel 1993). An exception was a bloom of *P. pungens*, *P. multiseries* and *P. australis* in Hood Canal in the autumn of 1994 that lasted from mid-October to mid-December with cell concentrations near 5 x 10^5 L^{-1}. Mussels used as the sentinel organism for toxins by the Washington Department of Health contained about 10 µg DA g^{-1}, the first time DA was found at relatively high levels in these inland waters (Horner *et al*. 1996).

The seed stock for *Pseudo-nitzschia* spp. along the open Pacific coasts and coastal embayments of Oregon and Washington appears to be from offshore, although few offshore samples are available to confirm this hypothesis. In contrast with the Gulf of Mexico (see Section 2.5), no *Pseudo-nitzschia* cells have been found at depth or near the bottom in samples collected over the continental shelf and nearshore, but sampling has not been extensive (R. Horner, J. Postel, M. Ross, unpubl.). In inland waters of Puget Sound, *Pseudo-nitzschia* populations apparently originate *in situ* (R. Horner, unpubl.), as is the case in eastern PEI (J.C. Smith, pers. commun.). As noted above, several *Pseudo-nitzschia* species co-exist within one water mass along the US west coast. Within Coos Bay, OR, species shifts are prevalent on short-term, seasonal, and annual time scales (G.A. Fryxell, pers. commun.). During one season in Coos Bay, for example, *P. multiseries* was replaced by *P. australis* in the course of less than three weeks, perhaps reflecting subtle differences in physiological preferences.

2.5 Other Global Locations

Awareness of DA has prompted the search for DA and DA-producing algal species in other parts of the world. Not surprisingly, DA-contaminated Dungeness crabs and several species of molluscan shellfish, including razor clams, were found on the coast of British Columbia (BC), western Canada, since 1992 (Forbes and Chiang 1994). As on the Pacific coast of the USA, the responsible organism(s) has not yet been determined, but *P. multiseries*, *P. australis*, *P. pungens*, *P. pseudodelicatissima*, *P. seriata*, and *Amphora coffeaeformis* are present (Forbes and Denman 1991; Taylor *et al*. 1994; Taylor and Haigh 1996). In Sechelt Inlet, BC, *P. pungens* bloomed in the summer and autumn, similar to eastern PEI, but the autumn abundance of *P. multiseries* was not great (Taylor *et al*. 1994). Abundant populations of *Pseudo-nitzschia* spp. also occur in Barkley Sound, an open coastal embayment on western Vancouver Island (Taylor and Haigh 1996). Populations of *P. pungens* and/or *P. multiseries* (a distinction was not made) persisted from June to September; *P. australis* was present mainly during May and June. Domoic acid appeared in *Mytilus californianus* in September, only after a rise in the concentrations of both *P. multiseries* (and/or *P. pungens*) and *P. delicatissima*. The strong prevailing onshore winds during the summer suggested that populations of *P. australis* and *P. multiseries* (and/or *P. pungens*) were advected from offshore. Large differences in species abundance between the two years of the study indicate that a longer time series is required to ascertain trends. The question of a possible link between *Pseudo-nitzschia* populations along the continental coast could not be answered, but it was suggested that local variations in physical regimes may lead to blooms in disparate locations.

Most of the earlier *Pseudo-nitzschia* blooms and DA-producing species have been reported in colder, northern waters (Table 1). However, because this genus is cosmopolitan (Hasle 1994), it is not surprising to also find blooms in the more southern latitudes. For example, an as yet unconfirmed species of *Pseudo-nitzschia*, along with DA, was found in the stomachs of sardines and mackerel along the southern tip of the Baja Peninsula, Mexico, in early 1996 (Ochoa *et al*. 1996). As in Monterey Bay, this was accompanied by the deaths of more than 100 brown pelicans and other seabirds. Elsewhere, strains of DA-producing *P. multiseries* were isolated from Galveston Bay, TX, northwest Gulf of Mexico (Fryxell *et al*. 1990; Dickey *et al*. 1992). In the northern Gulf of Mexico, annual blooms of *Pseudo-nitzschia* spp. occur during the spring and sometimes in the autumn (Fig. 8) (Dortch *et al*. 1997). Potentially-toxic *P. multiseries* (present in 60% of the samples), *P. pseudodelicatissima* (in 80% of the samples), and *P. delicatissima* are found in the northern Gulf, although no DA contamination of molluscan shellfish has yet been reported. Elsewhere, several *Pseudo-nitzschia* spp., including the dominant *P. fraudulenta*, *P. pungens*, and *P. pseudodelicatissima,* are found in Australian waters, but none have produced DA in culture (Hallegraeff 1994).

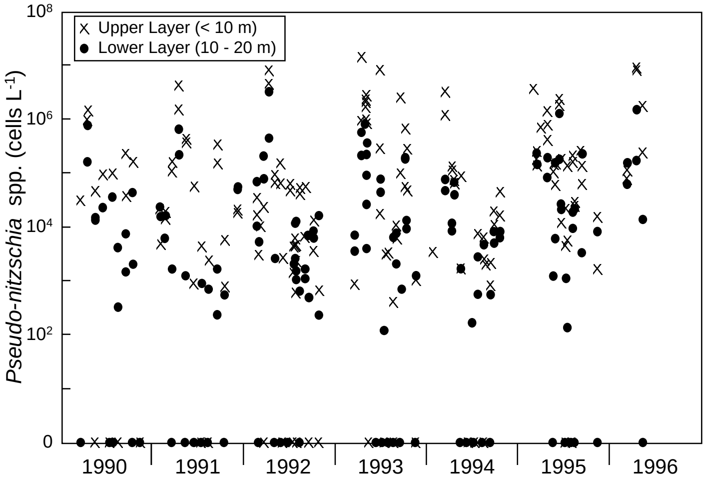

Fig. 8. Temporal variation in *Pseudo-nitzschia* spp. abundance; shelf site, northern Gulf of Mexico. Redrawn from Dortch *et al*. 1997, with updates from Q. Dortch.

3 Factors Influencing *Pseudo-nitzschia* Bloom Dynamics

3.1 Temperature and Irradiance

One of the more curious aspects of the *P. multiseries* blooms in PEI is that they have occurred during the autumn, when both temperature and irradiance levels rapidly decrease (Fig. 4). Furthermore, *P. multiseries* appears to have an affinity for cold waters elsewhere. For example, its population increased during winter and spring in Galveston Bay, TX, following cold fronts (Fryxell *et al.* 1990; Dickey *et al.* 1992; Villac *et al.* 1993a), and during the winter on the Pacific coast of Mexico (Ochoa *et al.* 1996). It must be remembered, however, that winter-spring temperatures in southern latitudes do not necessarily bracket those in the north. In Europe, *P. multiseries* was recorded in the Oslofjord, Norway, during the autumn and early winter at temperatures from 14° to about 2°C (Hasle 1965), and during the autumn and early winter in the Skagerrak (Hasle *et al.* 1996). Blooms of *P. seriata* occurred in the autumn in Galicia, Spain (Míguez *et al*. 1996). In Japan, toxic *P. multiseries* and DA in bivalves were observed from October to February (Kotaki *et al.* 1996). Perhaps these species have unique physiological adaptations that enable them to survive and to outcompete other algae during the cold seasons. Exceptions to this pattern may be the summer blooms of *P. australis* in Monterey Bay (14° - 16°C) when upwelling relaxes (Walz *et al.* 1994), *P. pungens* in British Columbia and Washington (Taylor and Haigh 1996; R. Horner, pers. observ.), and *P. pseudodelicatissima* in the Bay of Fundy (Martin *et al*. 1993).

Pseudo-nitzschia multiseries, the only species studied thus far, has a marked temperature dependence for growth, photosynthesis (P_{max} and I), and DA production (Lewis *et al.* 1993; Pan *et al.* 1993; Bates, this volume). The Q_{10} for growth was 1.8 to 2.8 between 5° - 15°C, and 2.1 between 10° - 20°C, which is within the range of other algae. Optimal growth and photosynthesis occurred in the temperature range of 15° - 25°C, well above the ambient autumn to winter temperatures (13° to -1°C) at which *P. multiseries* typically blooms. The broad range of temperature tolerance (5° to 25°C) was confirmed for *P. multiseries* isolated from northern Nova Scotia, Canada (Seguel 1991); the upper tolerance limit was not determined. It is evident that factors other than temperature must have initiated bloom development. These studies suggest that *P. multiseries* has no particular physiological adaptations that would enable it to outcompete other species at low temperatures. The broad temperature optima are consistent with its world-wide distribution at temperatures ranging from 2° to 28°C (Hasle 1965; Hasle *et al.* 1996), and may also indicate genetically-different strains (see Section 4). Indeed, *P. multiseries* was found under the ice in January, in eastern PEI at temperatures of -1.5°C (Bates *et al.* 1989), and during the summer in Galveston Bay, TX, at temperatures up to 30°C (Reap 1991; Dickey *et al.* 1992).

One consistent finding, perhaps related to temperature, is that there is a species succession from the usually non-toxic *P. pungens* to the toxic *P. multiseries* in several locations in North America and elsewhere. During the 1989 field season in northern and eastern PEI, *P. pungens* appeared in early August when no DA was detected in the mussels (Smith *et al.* 1990b). This was followed by a decline in the proportion of *P. pungens* and an increase in the proportion of *P. multiseries* to nearly 100% in late November, when the mussels quickly became toxic (Fig. 5). The two species did co-occur, although at varying proportions, during September to October. Seasonality between the two species is also found in Galveston Bay, TX (Fryxell *et al.* 1990; Reap 1991; Dickey *et al.* 1992). Although *P. multiseries* is resident all year, it dominates *P. pungens* from winter through spring, decreasing in abundance during the summer and into the autumn. It was especially abundant during cold "blue norther" storms in February (Reap 1991). In Sechelt Inlet, British Columbia, Canada, the summer populations of *Pseudo-nitzschia* spp. were predominantly *P. pungens* (Taylor *et al.* 1994). This species was also found in the autumn, as was *P. multiseries*, but at a lower concentration. Occurrences of *P. multiseries* and *P. pungens* usually coincided in Monterey Bay, CA, during the autumn, but with *P. pungens* being numerically less abundant (Walz *et al.* 1994). In the Skagerrak (Hasle *et al.* 1996), there was no evidence of a seasonal succession as in North America. If there is a temperature dependence to its seasonal succession, however, this cannot be supported by other physiological measurements. For example, when grown at 18°C, the only growth temperature studied, *P. pungens* gave a maximal nitrate reductase activity at a lower temperature (15°C) than did *P. multiseries* (23°C) (L. Rivard, unpubl.). It may be that the degree of water column mixing, rather than temperature, is important. Vigorous mixing may be required for *P. multiseries*, while the summer appearance of *P. pungens* was associated with more stratified waters (Fryxell *et al*. 1990; Taylor *et al*. 1994).

The other characteristic peculiar to the autumn *P. multiseries* blooms in eastern PEI is that they occur when irradiance levels are rapidly decreasing, both in duration and intensity (Fig. 4). In spite of this property, relatively few laboratory, and no field studies have been devoted to the effects of irradiance on growth and DA production. Photosynthesis vs irradiance (P-I) characteristics of *P. multiseries* (Pan *et al.* 1991; 1996d) do not indicate any extraordinary adaptations for growth at low irradiance levels. Cellular chlorophyll *a* levels and I^B (the initial slope of the P-I curve, a measure of the efficiency of photon absorption) in stationary-phase cultures do increase at low growth irradiance, but other phytoplankton also show this type of photoadaptation. The irradiance level necessary to saturate photosynthesis (I_m = 200 - 600 µmol photons m^{-2} s^{-1}) is substantially greater than that reported above for saturation of growth. Another study (Bates and Léger, unpubl.) gives a lower range (I_m = 100 - 200 µmol photons m^{-2} s^{-1}). Again, these irradiance levels are generally greater than those found in the growth habitat of *P. multiseries* in the autumn (e.g., 200 µmol photons m^{-2} s^{-1} at 2 m; Pan *et al*. 1996d). No P-I values are reported for monospecific blooms of *P. multiseries* in the field. Laboratory experiments, however, demonstrate that *P. multiseries* can outcompete other phytoplankton species at low irradiance levels when grown with a relatively short light:dark cycle of 8:16 h (Sommer 1994).

3.2 Nutrients

Based on laboratory studies, nutrient conditions conducive for DA production include limitation by silicate or phosphate, and an excess of nitrogen (see Bates, this volume). This is sometimes, but not always, seen in natural situations due to their complexity and our inability to quantify nutrient fluxes adequately. A low silicate concentration (0.62 µM) was found during the original 1987 Cardigan Bay bloom (Subba Rao *et al.* 1988b), consistent with optimum conditions for DA production. Nitrate and ammonium values, however, were also low (0.89 µM and 0.75 µM, respectively), contrary to the N requirement for DA production. The highest DA values did occur several days later, when nitrate increased and phosphate fell to a low concentration (0.04 µM), suggesting that limiting P concentrations may also have triggered DA production. The following autumn, a major *P. multiseries* bloom was preceded by a prolonged, intense bloom of the diatom *Skeletonema costatum* (Smith *et al.* 1990a). This, plus the presence of the *P. multiseries* bloom itself, may have depleted the silicate and phosphate concentrations in the water column, creating conditions conducive for toxin production. Much could be learned from following the seasonal succession of these diatoms. The 1988 blooms of *P. multiseries* in Cardigan Bay, PEI, were associated with pulses of nitrate, either from river runoff after rain events or resuspended sediments after wind events (Smith *et al.* 1990a). Increases in cellular DA appeared to roughly coincide with periods of nitrate influx, and preceded an increase in cell number (Fig. 3B).

In the northern Gulf of Mexico, *Pseudo-nitzschia* spp. abundance was negatively related to nitrate, ammonium, and silicate concentrations (Dortch *et al*. 1997). Walz *et al.* (1994) observed the highest concentrations of DA in a *P. australis*-dominated bloom in Monterey Bay, CA, in late summer and autumn, during post-upwelling conditions when nitrate concentrations were low (0.2 - 3.0 µM). Regenerated nutrients may have supported growth and DA production under these low-nitrogen conditions.

The riverine or sediment input of nutrients could lead to an enrichment of N and P relative to Si. This may occur in Cardigan Bay, PEI, as well as in the northern Gulf of Mexico, where spring blooms of *Pseudo-nitzschia* spp. are associated with peaks in outflow from the Mississippi River (Dortch *et al*. 1997). The substantial increase in N inputs relative to Si due to eutrophication have decreased the Si/N ratio by a factor of four, thus favoring *Pseudo-nitzschia* blooms and the possibility of DA production in that part of the Gulf of Mexico. This problem is compounded by an apparent ability of *P. multiseries* to grow and outcompete other phytoplankton at low concentrations of silicate relative to nitrate (Sommer 1994). Indeed, Smayda (1990) hypothesized that altered nutrient ratios as a result of changing patterns of anthropogenic inputs of N and P into the sea may favor the growth of certain harmful algal species.

Coastal environments where toxigenic blooms occur are particularly rich in ammonium and organic forms of N. Only minimal work, however, has been carried out to study growth and DA production in relation to these forms of N. Growth rates of *P. multiseries* with nitrite, glutamine and urea are similar to those with nitrate (Hillebrand and Sommer 1996); unfortunately, DA production was not measured. Ammonium prevented the growth of *P. multiseries* at a concentration of 880 µM and decreased the stationary phase cell number at 220 - 440 µM, as well as enhanced DA production (Bates *et al.* 1993b). A similar growth-inhibiting effect of ammonium, but starting at 20 µM, was found by Hillebrand and Sommer (1996). These deleterious concentrations of ammonium are lower than those found for most other diatoms. This is curious, given that *P. multiseries* grows in waters where ammonium levels may be high due to remineralization, agricultural runoff, and excretion from wild and cultured mussels.

3.3 Salinity

One commonality of *Pseudo-nitzschia* spp. is that they are coastal or estuarine. Not surprisingly, therefore, an isolate of *P. multiseries* from Pomquet Harbour, Nova Scotia, Canada, showed a broad salinity range (30 - 45 ‰) for optimum growth (Fig. 9); cells did not grow below 9 ‰ (Seguel 1991; Jackson *et al.* 1992). Interestingly, the salinity optimum is higher than the range found in estuaries (5 - 29 ‰). The salinity range for optimum growth of *P. multiseries* is higher than that of *P. pungens*, but this does not explain their seasonal succession (see Section 3.1). Isolates of *P. multiseries* from Galveston Bay, TX, grew successfully at salinities of 13 - 34 ‰ (Reap 1991), and a mixture of *Pseudo-nitzschia* spp. was found over a broad salinity range (0 - 36 ‰) near the Mississippi River (Dortch *et al*. 1997), also showing that they are very halotolerant.

The physical regime of low salinity associated with peak abundances of *P. australis* in Monterey Bay, CA, led Buck *et al.* (1992) to conclude that this species has different environmental constraints compared to those of *P. multiseries* from eastern Canada. An examination of their salinity plots, however, reveals that while peaks of *P. australis* coincide with periods of lower salinity, the range shown (33.2 - 33.8 ‰) is, in fact, higher than that encountered in eastern Canadian embayments.

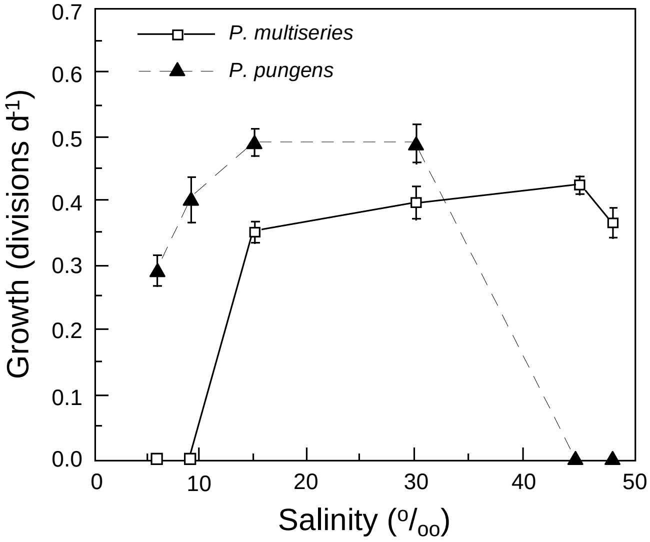

Fig. 9. Growth of *Pseudo-nitzschia multiseries* and *P. pungens*, isolated from northern Nova Scotia, Canada, as a function of salinity. Redrawn from Jackson *et al*. 1992.

Thus far, none of the chemical or physiological characteristics, including maximum division rates (Table 4), is necessarily unique to this genus. This leads one to conclude that *Pseudo-nitzschia* species will bloom when there are a sufficient number of such cells initially present, and the conditions generally bracket the cells' rather wide physiological tolerances.

Table 4. Division rates (d^{-1}) of *Pseudo-nitzschia multiseries* in culture at different growth temperatures (°C) and irradiance levels (μmol photons m^{-2} s^{-1}).

Temperature	Light Intensity	Division Rate	Reference
10	45	0.24	Bates *et al.* 1991
10	145	0.44	Bates *et al.* 1991
10-12	105*	0.56	Pan *et al.* 1991
10-12	1100*	0.74	Pan *et al.* 1991
15	70-80	1.07	Reap 1991
15	100	0.30	Jackson *et al.* 1992
5	180	0.25	Lewis *et al.* 1993
20	180	0.65	Lewis *et al.* 1993
0	350-440	0.21	Pan *et al.* 1993
15-25	350-40	1.20	Pan *et al.* 1993
10-12	1100	0.32	Subba Rao *et al.* 1995
10	53*	0.21	Pan *et al.* 1996d
10	250*	0.21	Pan *et al.* 1996d
10	410*	0.85	Pan *et al.* 1996d
10	815*	0.55	Pan *et al.* 1996d
10	1100*	0.80	Pan *et al.* 1996d

*Continuous irradiance *P. australis*: 0.56 d^{-1} at 15°C (Garrison *et al.* 1992)

4. General Biology of *Pseudo-nitzschia* Species

Because they are diatoms, *Pseudo-nitzschia* species decrease in cell size as a result of normal vegetative division. However, until recently it was not known why they did not undergo sexual reproduction in culture to revert to the original, large cell dimensions. It has now been demonstrated that *P. multiseries*, *P. pungens*, and *P. pseudodelicatissima* are dioecious, so that sexual reproduction can be induced by mixing "male" and "female" clones of the appropriate minimal cell size (Davidovich and Bates 1998). The following has been observed: pairing of parent cells (allogamy); production of four morphologically isogamous and non-flagellated gametes per gametangial pair; fusion of gametes to form zygotes, revealing physiological anisogamy; enlargement of auxospores; and formation of long initial cells. These observations of allogamous reproduction are consistent with those reported for other pennate diatoms, and differ significantly from those in the report of Subba Rao *et al*. (1991), which we regard as erroneous for reasons also given in Fryxell *et al.* (1991) and Rosowski *et al.* (1992).

An understanding of the life history of *P. multiseries* may provide some insight into the reasons for the yearly variability in bloom intensity and toxicity of this species in eastern PEI, Canada. Knowledge of the minimal cell size suitable for sexual reproduction and of the rate of decrease in cell size allows one to predict when sexual reproduction can take place. A dense bloom in a given year would favor sexual reproduction by increasing the chance for the cells to pair while in the water column. This would lead to an increase in the proportion of large cells, hypothesized to be more toxic and to have a faster division rate, ultimately allowing the species to persist into the following seasons. It likely takes several years for cells to decrease to the suitable size, thus resulting in a long-term periodicity in bloom intensity and toxicity. Measurements of cell size-frequency distributions in the field over a period of several years would increase our understanding of *Pseudo-nitzschia* spp. bloom dynamics.

Another unknown is why *P. multiseries* cells bloom only during the autumn in eastern Canada. Few, if any, cells are present in the water column during the spring and early summer. To where, and in what form, do these cells disappear? There is evidence that the autumn blooms originate within the bays and estuaries in eastern PEI, not from the immediate coastal waters (J.C. Smith, pers. commun.). In this case, the cells may overwinter in the sediments, perhaps as "resting cells" that are morphologically indistinguishable from vegetative cells, but with condensed chloroplasts. Sedimentation of *Pseudo-nitzschia* cells can be significant (Fryxell *et al*. 1990; Villac 1996; Dortch *et al*. 1997), so the sediments may be one repository for dormant cells. Surprisingly, however, there is thus far no evidence of an abundance of *Pseudo-nitzschia* cells in the sediments, at least in PEI in late autumn (S. Bates, pers. observ.), or in the California Bight (C. Lange, unpubl.). Cells of *P. pungens* were nearly always found in the upper 20 m off the northwestern WA coast in July, 1996 (R. Horner, pers. observ.). Thus, it is difficult to generalize about *Pseudo-nitzschia* cells sinking to the sediments at all locations. Because of their tendency to sink out of the euphotic zone, one might think that *Pseudo-nitzschia* species would have a requirement for a well-mixed water column. This is the case in the turbulent bays of PEI. Similarly, in the northern Gulf of Mexico, the abundance of a mixture of *Pseudo-nitzschia* species was negatively related to delta σ_t (difference in σ_t from surface to bottom), an approximation of water column stability (Dortch *et al*. 1997). However, *P. multiseries* (and/or *P. pungens*) was found under stratified conditions in Sechelt Inlet, British Columbia (Taylor *et al*. 1994).

When interpreting laboratory experiments in relation to field studies, it is important to consider that *Pseudo-nitzschia* spp. cells tend to become deformed after relatively short periods in culture (Subba Rao and Wohlgeschaffen 1990; Subba Rao *et al.* 1991; Reap 1991; Garrison *et al.* 1992; Hillebrand and Sommer 1996), perhaps when they decrease to <50% of their original cell length (Villac 1996). These variants are described variously as being “beaked”, “sickle-shaped”, or “lobed“ (Subba Rao and Wohlgeschaffen 1990). Teratologous *P. multiseries* cells were reported at low frequency (<1%) in eastern PEI (Subba Rao and Wohlgeschaffen 1990), southern Nova Scotia, Canada (Y. Pan, pers. commun.), and in Monterey Bay, USA (C.A. Scholin, pers. commun.). However, others have not seen this type of cell in nature (J.C. Smith; K.E. Pauley; P.E. Hargraves, pers. commun.; R. Horner, unpubl.). *Pseudo-nitzschia pungens* cells with 1-3 “swellings” were described from eutrophic coastal waters of Japan (Takano and Kikuchi 1985). Once they appear, deformities are passed onto succeeding generations because they become part of the frustule “template”. Cultures of *Pseudo-nitzschia* spp. may also form stacked colonies (similar to *Fragilariopsis* spp.), rather than “stepped’ chains; thus, the chain morphology may not be a good generic character. The deformities may occur during stationary phase, perhaps as a result of extracellular autoinhibiting metabolites or of Si limitation. Villac (1996) hypothesized that the dense nucleus, located at the mid-section of the long cell, may produce an undulation of the valve mantle in Si-limited cells. Deformities are not part of the sexual life cycle as once proposed by Subba Rao *et al.* (1991). Their existence, however, reminds us that if the morphology of cells can change under unnatural culture conditions, other physiological and chemical characteristics may also be aberrant.

5 Conclusions

- Toxigenic *Pseudo-nitzschia* species are cosmopolitan in coastal waters. However, different species or mixtures of species generally dominate in different regions of the world (*P. multiseries* in eastern PEI, Canada; *P. pseudodelicatissima* in the Bay of Fundy, Canada; co-existence of *P. multiseries*, *P. australis*, and *P. pseudodelicatissima* along the west coast of North America; *P. multiseries* and *P. delicatissima* in Holland; *P. seriata* in Denmark; *P. australis* in Spain; *P. australis* and *P. delicatissima* in New Zealand).
- Thus far, the same *Pseudo-nitzschia* species may be toxic in one part of the world but not in another (*P. pseudodelicatissima* in the Bay of Fundy, Canada; *P. delicatissima* originally in eastern Canada, but now also in Denmark and New Zealand; *P. seriata* in Denmark). Although still rare, there is also the enigma of toxic strains of *P. pungens* (so far only in the Pacific Ocean) and non-toxic strains of *P. multiseries.* The most sensitive analytical techniques must be used to confirm the presence or absence of toxicity.
- The life cycle of *Pseudo-nitzschia* species in relation to bloom dynamics is not understood, nor is the location of the seed beds (sediments, water column) and overwintering populations.
- Blooms of *P. multiseries* generally occur during the colder seasons (autumn to spring); blooms of *P. pungens*, *P. pseudodelicatissima*, and *P. australis* tend to occur during the warmer seasons. No unusual physiological adaptations to irradiance or temperature have yet been found to account for their presence at those times of the year.
- A better understanding is required of the effects of mesoscale features and short-term events on bloom dynamics, and of triggers of DA production in natural populations.

6 Acknowledgments

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7 References

Addison, R.F., Stewart, J.E. (1989). Domoic acid and the eastern Canadian molluscan shellfish industry. *Aquaculture* 77: 263-269.

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., 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., de Freitas, A.S.W., Milley, J.E., Pocklington, R., Quilliam, M.A., Smith, J.C., 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., Léger, C., Keafer, B.A., Anderson, D.M. (1993a). Discrimination between domoic-acid-producing and nontoxic forms of the diatom *Pseudonitzschia pungens* using immunofluorescence. *Mar. Ecol. Prog. Ser.* 100: 185-195.

Bates, S.S., Worms, J., Smith, J.C. (1993b). Effects of ammonium and nitrate on domoic acid production by *Nitzschia pungens* in batch culture. *Can. J. Fish. Aquat. Sci.* 50: 1248-1254.

Bates, S.S., Douglas, D.J., Doucette, G.J., Léger, C. (1995). Enhancement of domoic acid production by reintroducing bacteria to axenic cultures of the diatom *Pseudo-nitzschia multiseries*. *Nat. Toxins* 3: 428-435.

Bates, S.S., Léger, C., Smith, K.M. (1996). Domoic acid production by the diatom *Pseudo-nitzschia multiseries* as a function of division rate in silicate-limited chemostat culture. In: Yasumoto, T., Oshima, Y, Fukuyo, Y. (eds.) *Harmful and Toxic Algal Blooms*, Intergov. Oceanogr. Comm., UNESCO, Paris, pp. 163-166.

Buck, K.R., Uttal-Cooke, L., Pilskaln, C.H., Roelke, D.L., Villac, M.C., Fryxell, G.A., Cifuentes, L., Chavez, F.P. (1992). Autecology of the diatom *Pseudonitzschia australis* Frenguelli, a domoic acid producer, from Monterey Bay, California. *Mar. Ecol. Prog. Ser.* 84: 293-302.

Davidovich, N.A., Bates, S.S. 1998. Sexual reproduction in the pennate diatoms *Pseudo-nitzschia multiseries* and *P. pseudodelicatissima* (Bacillariophyceae). *J. Phycol.* 34: 126-137.

Dickey, R.W., Fryxell, G.A., Grande, H.R., Roelke, D. (1992). Detection of the marine toxins okadaic acid and domoic acid in shellfish and phytoplankton in the Gulf of Mexico. *Toxicon* 30: 355-359.

Dortch, Q, Robichaux, R., Pool, S., Milsted, D, Mire, G., Rabalais, N.N., Soniat, T.M., Fryxell, G.A., Turner, R.E., Parsons, M.L. (1997). Abundance and vertical flux of *Pseudo-nitzschia* in the northern Gulf of Mexico. *Mar. Ecol. Prog. Ser.* 146:249-264.

Douglas, D.J., Bates, S.S., Bourque, L.A., Selvin, R.C. (1993). Domoic acid production by axenic and non-axenic cultures of the pennate diatom *Nitzschia pungens* f.

multiseries. In: Smayda, T.J., Shimizu, Y. (eds.) *Toxic Phytoplankton Blooms in the Sea*, Elsevier, Amsterdam, pp. 595-600.

Douglas, D.J., Landry, D., Douglas, S.E. (1994). Genetic relatedness of toxic and nontoxic isolates of the marine pennate diatom *Pseudonitzschia* (Bacillariophyceae): phylogenetic analysis of 18S rRNA sequences. *Nat. Toxins* 2: 166-174.

Forbes, J.R., Chiang, R. (1994). Geographical and temporal variability of domoic acid in samples collected for seafood inspection in British Columbia, 1992-1994. In: Forbes, J.R. (ed.) *Proceedings of the Fourth Canadian Workshop on Harmful Marine Algae. Can. Tech. Rep. Fish. Aquat. Sci*. 2016, pp. 11-12 (abstract).

Forbes, J.R., Denman, K.L. (1991). Distribution of *Nitzschia pungens* in coastal waters of British Columbia. *Can. J. Fish. Aquat. Sci*. 48: 960-967.

Fritz, L. (1992). The use of cellular probes in studying marine phytoplankton. *Korean J. Phycol.* 7: 319-324.

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

Fryxell, G.A., Reap, M.E., Valencic, D.L. (1990). *Nitzschia pungens* Grunow f. *multiseries* Hasle: observations of a known neurotoxic diatom. *Beih. Nova Hedwigia* 100: 171-188.

Fryxell, G.A., Garza, S.A., Roelke, D.L. (1991). Auxospore formation in an Antarctic clone of *Nitzschia subcurvata* Hasle. *Diatom Res.* 6: 235-245.

Garrison, D.L. (1979). Monterey Bay Phytoplankton. I. Seasonal cycles of phytoplankton assemblages. *J. Plankton Res*. 1: 241-256.

Garrison, D.L. (1981). Monterey Bay phytoplankton. II. Resting spore cycles in coastal diatom populations. *J. Plankton Res*. 3: 137-156.

Garrison, D.L., Conrad, S.M., Eilers, P.P., Waldron, E.M. (1992). Confirmation of domoic acid production by *Pseudonitzschia australis* (Bacillariophyceae) cultures. *J. Phycol.* 28: 604-607.

Graham, W.H., Field, J.G., Potts, D.C. (1992). Persistent upwelling shadows and their influence on zooplankton distributions. *Mar. Biol.* 114: 561-570.

Hallegraeff, G.M. (1994). Species of the diatom genus *Pseudonitzschia* in Australian waters. *Bot. Mar.* 37: 397-411.

Hargraves, P.E., Zhang, J., Wang, R., Shimizu, Y. (1993). Growth characteristics of the diatoms *Pseudonitzschia pungens* and *P. fraudulenta* exposed to ultraviolet radiation. *Hydrobiol*. 269: 207-212.

Hasle, G.R. (1965). *Nitzschia* and *Fragilariopsis* species studied in the light and electron microscopes. II. The group *Pseudonitzschia*. *Skr. Norske Vidensk-Akad. I. Mat.-Nat. Kl. Ny Serie* 18: 1-45.

Hasle, G.R. (1994). *Pseudo-nitzschia* as a genus distinct from *Nitzschia* (Bacillariophyceae). *J. Phycol*. 30: 1036-1039.

Hasle, G.R. (1995). *Pseudo-nitzschia pungens* and *P. multiseries* (Bacillariophyceae): nomenclatural history, morphology, and distribution. *J. Phycol.* 31: 428-435.

Hasle, G.R., Lange, C.B., Syvertsen, E.E. (1996). A review of *Pseudo-nitzschia*, with special reference to the Skagerrak, North Atlantic, and adjacent waters. *Helgol. Meeresunters.* 50: 131-175.

Haywood, G.J., Silver, M.W. (1994). The concentration of domoic acid by zooplankton. *EOS* 75: 89 (abstract).

Hillebrand, H., Sommer, U. (1996). Nitrogenous nutrition of the potentially toxic diatom *Pseudonitzschia pungens* f. *multiseries* Hasle. *J. Plankton Res.* 18: 295-301.

Horner, R.A., Postel, J.R. 1993. Toxic diatoms in western Washington waters (U.S. west coast). *Hydrobiol*. 269: 197-205.

Horner, R.A., Kusske, M.B., Moynihan, B.P., Skinner, R.N., Wekell, J.C. (1993). Retention of domoic acid by Pacific razor clams, *Siliqua patula* (Dixon, 1789): preliminary study. *J. Shellfish Res.* 12 451-456.

Horner, R.A., Hanson, L., Hatfield, C.L., Newton, J.A. (1996). Domoic acid in Hood Canal, Washington, USA. In: Yasumoto, T., Oshima, Y, Fukuyo, Y. (eds.) *Harmful and Toxic Algal Blooms*, Intergov. Oceanogr. Comm., UNESCO, Paris, pp. 127-129.

Horner, R.A., Garrison, D.L., Plumley, F.G. (1997). Harmful algal blooms and red tide problems on the U.S. west coast. *Limnol. Oceanogr*. 42: 1076-1088.

Impellizzeri, G., Mangiafico, S., Oriente, G., Piattelli, M., Sciuto, S., Fattorusso, E., Magno, S., Santacroce, C., Sica, D. (1975). Amino acids and low-molecular-weight carbohydrates of some marine red algae. *Phytochem.* 14: 1549-1557.

Jackson, A.E., Ayer, S.W., Laycock, M.V. (1992). The effect of salinity on growth and amino acid composition in the marine diatom *Nitzschia pungens*. *Can. J. Bot.* 70: 2198-2201.

Kotaki, Y., Koike, K., Ogata, T., Sato, S., Fukuyo, Y., Kodama, M. (1996). Domoic acid production by an isolate of *Pseudonitzschia multiseries*, a possible cause for the toxin detected in bivalves in Ofunato Bay, Japan. In: Yasumoto, T., Oshima, Y, Fukuyo, Y. (eds.) *Harmful and Toxic Algal Blooms*, Intergov. Oceanogr. Comm., UNESCO, Paris, pp. 151-154.

Lange, C.B., Reid, F.M.H., Vernet, M. (1994). Temporal distribution of the potentially toxic diatom *Pseudonitzschia australis* at a coastal site in Southern California. *Mar. Ecol. Prog. Ser.* 104: 309-312.

Langlois, G.W., Kizer, L.W., Hansgen, K.H., Howell, R., Loscutoff, S.M. (1993). A note on domoic acid in California coastal molluscs and crabs. *J. Shellfish Res*. 12: 467-468.

Laycock, M.V., de Freitas, A.S.W., Wright, J.L.C. (1989). Glutamate agonists from marine algae. *J. Appl. Phycol.* 1: 113-122.

Lee, J.H., Baik, J.H. (1995). Domoic acid-producing *Pseudonitzschia pungens* f. *multiseries* from Jinhae Bay, Korea: morphology, growth and population dynamics. *Proceed. 7th Internat. Conf. Toxic Phytoplankton*, Sendai, Japan p. 96 (abstract).

Lewis, N.I., Bates, S.S., McLachlan, J.L., Smith, J.C. (1993). Temperature effects on growth, domoic acid production, and morphology of the diatom *Nitzschia pungens* f. *multiseries*. In: Smayda, T.J., Shimizu, Y. (eds.) *Toxic Phytoplankton Blooms in the Sea*, Elsevier, Amsterdam, pp. 601-606.

Loscutoff, S. (1992). The west coast experience - overview. In: *Proceedings Domoic Acid Workshop, February 6-8, 1992, San Pedro, California.* U.S. FDA.

Lundholm, N., Skov, J., Pocklington, R., Moestrup, Ø. (1994). Domoic acid, the toxic amino acid responsible for amnesic shellfish poisoning, now in *Pseudonitzschia seriata* (Bacillariophyceae) in Europe. *Phycologia* 33: 475-478.

Lundholm, N., Skov, J., Pocklington, R., Moestrup, Ø. (1997). Studies on the marine planktonic diatom *Pseudo-nitzschia.* 2. Autecology of *P. pseudodelicatissima* (Hasle) Hasle based on isolates from Danish coastal waters. *Phycologia* 36: 381-388.

Maeda, M., Kodama, T., Tanaka, T., Yoshizumi, H., Takemoto, T., Nomoto, K., 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.

Malone, T.C. (1971). The relative importance of nannoplankton and netplankton as primary producers in the California Current System. *Fish. Bull*. 69: 799-820.

Manhart, J.R., Fryxell, G.A., Villac, C., Segura, L.Y. (1995). *Pseudo-nitzschia pungens* and *P. multiseries* (Bacillariophyceae): nuclear ribosomal DNAs and species differences. *J. Phycol.* 31: 421-427.

Maranda, L., Wang, R., Musauda, K., Shimizu, Y. (1990). Investigation of the source of domoic acid in mussels. In: Granéli, E., Sundström, B., Edler, L., Anderson, D.M. (eds.) *Toxic Marine Phytoplankton*, Elsevier, New York, pp. 300-304.

Martin, J.L., Haya, K., Burridge, L.E., Wildish, D.J. (1990). *Nitzschia pseudodelicatissima* - a source of domoic acid in the Bay of Fundy, eastern Canada. *Mar. Ecol. Prog. Ser.* 67: 177-182.

Martin, J.L., Haya, K., Wildish, D.J. (1993). Distribution and domoic acid content of *Nitzschia pseudodelicatissima* in the Bay of Fundy. In: Smayda, T.J., Shimizu, Y. (eds.) *Toxic Phytoplankton Blooms in the Sea*, Elsevier, Amsterdam, pp. 613-618.

McGinness, K.L., Fryxell, G.A., McEachran, J.D. (1995). *Pseudonitzschia* species found in digestive tracts of northern anchovies (*Engraulis mordax*). *Can. J. Zool.* 73: 642-647.

Míguez, Á., Fernández, M.L., Fraga, S. (1996). First detection of domoic acid in Galicia (NW of Spain). In: Yasumoto, T., Oshima, Y, Fukuyo, Y. (eds.) *Harmful and Toxic Algal Blooms*, Intergov. Oceanogr. Comm., UNESCO, Paris, pp. 143-145.

Miller, P.E., Scholin, C.A. (1996). Identification of cultured *Pseudo-nitzschia* (Bacillariophyceae) using species-specific LSU rRNA-targeted fluorescent probes. *J. Phycol*. 32: 646-655.

Ochoa, J.L., Sierra-Beltrán, A., Cruz-Villacorta, A., Sánchez-Paz, A., Núñez-Vázquez, E. (1996). Recent observations of HABs and toxic episodes - Mexico. *Harmful Algae News* No. 14, pp. 4.

Pan, Y., Subba Rao, D.V., Warnock, R.E. (1991). Photosynthesis and growth of *Nitzschia pungens* f. *multiseries* Hasle, a neurotoxin producing diatom. *J. Exp. Mar. Biol. Ecol.* 154: 77-96.

Pan, Y., Subba Rao, D.V., Mann, K.H., Li, W.K.W., Warnock, R.E. (1993). Temperature dependence of growth and carbon assimilation in *Nitzschia pungens* f. *multiseries*, the causative diatom of domoic acid poisoning. In: Smayda, T.J., Shimizu, Y. (eds.) *Toxic Phytoplankton Blooms in the Sea*, Elsevier, Amsterdam, pp. 619-624.

Pan, Y., Subba Rao, D.V., Mann, K.H., Brown, R.G., Pocklington, R. (1996a). Effects of silicate limitation on production of domoic acid, a neurotoxin, by the diatom *Pseudo-nitzschia multiseries*. I. Batch culture studies. *Mar. Ecol. Prog. Ser.* 131: 225-233.

Pan, Y., Subba Rao, D.V., Mann, K.H., Brown, R.G., Pocklington, R. (1996b). Effects of silicate limitation on production of domoic acid, a neurotoxin, by the diatom *Pseudo-nitzschia multiseries*. II. Continuous culture studies. *Mar. Ecol. Prog. Ser.* 131: 235-243.

Pan, Y., Subba Rao, D.V., Mann, K.H. (1996c). Changes in domoic acid production and cellular chemical composition of the toxigenic diatom *Pseudo-nitzschia multiseries* under phosphate limitation. *J. Phycol.* 32: 371-381.

Pan, Y., Subba Rao, D.V., Mann, K.H. (1996d). Acclimation to low light intensity in photosynthesis and growth of *Pseudo-nitzschia multiseries* Hasle, a neurotoxigenic diatom. *J. Plankton Res.* 18: 1427-1438.

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

Reap, M.E. (1991). *Nitzschia pungens* Grunow f. *multiseries* Hasle: growth phases and toxicity of clonal cultures isolated from Galveston Bay, Texas. M.Sc. Thesis, Texas A&M Univ., College Station, TX, 78 p.

Rhodes, L., White, D., Syhre, M., Atkinson, M. (1996). *Pseudo-nitzschia* species isolated from New Zealand coastal waters: domoic acid production *in vitro* and links with shellfish toxicity. In: Yasumoto, T., Oshima, Y, Fukuyo, Y. (eds.) *Harmful and Toxic Algal Blooms*, Intergov. Oceanogr. Comm., UNESCO, Paris, pp. 155-158.

Rhodes, L., Scholin, C., Garthwaite, I., Haywood, A., Thomas, A. (1998). Domoic acid producing *Pseudo-nitzschia* species educed by whole cell DNA probe-based and immunochemical assays. In: Reguera, B. Blanco, J., Fernández, M.L., Wyatt, T. (eds.). *Harmful microalgae*. Xunta de Galicia and the IOC of UNESCO, Paris, pp. 274-277.

Rosowski, J.R., Johnson, L.M., Mann, D.G. (1992). On the report of gametogenesis, oogamy, and uniflagellated sperm in the pennate diatom *Nitzschia pungens* (1991. J. Phycol. 27: 21-26). *J. Phycol*. 28: 570-574.

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

Sayce, K., Horner, R.A. (1996). *Pseudo-nitzschia* spp. in Willapa Bay, Washington, 1992 and 1993. In: Yasumoto, T., Oshima, Y, Fukuyo, Y. (eds.) *Harmful and Toxic Algal Blooms*, Intergov. Oceanogr. Comm., UNESCO, Paris, pp. 131-134.

Scholin, C.A., Villac, M.C., Buck, K. R., Krupp, K.M., Powers, D.A., Fryxell, G.A., Chavez, F.P. (1994). Ribosomal DNA sequences discriminate among toxic and non-toxic *Pseudonitzschia* species. *Nat. Toxins* 2: 152-165.

Scholin, C.A., Buck, K.R., Britschgi, T., Cangelosi, G., Chavez, F.P. (1996). Identification of *Pseudo-nitzschia australis* (Bacillariophyceae) using rRNA-targeted probes in whole cell and sandwich hybridization formats. *Phycologia* 35: 190-197.

Seguel, M.R. (1991). Interactive effects of temperature-light and temperature-salinity on growth of five phytoplanktonic species isolated from a shallow-water embayment of Nova Scotia. M.Sc. Thesis, Acadia Univ., Wolfville, Nova Scotia, Canada, 218 p.

Shimizu, Y., Gupta, S., Masuda, K., Maranda, L., Walker, C.K., Wang, R. (1989). Dinoflagellate and other microalgal toxins: chemistry and biochemistry. *Pure Appl. Chem.* 61: 513-516.

Smayda, T.J. (1990). Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic. In: Granéli, E., Sundström, B., Edler, L., Anderson, D.M. (eds.) *Toxic Marine Phytoplankton*, Elsevier, New York, pp. 29-40.

Smith, J.C. (1993). Toxicity and *Pseudonitzschia pungens* in Prince Edward Island, 1987-1992. *Harmful Algae News* No. 6, pp. 1 and 8.

Smith, J.C., Cormier, R., Worms, J., Bird, C.J., Quilliam, M.A., Pocklington, R., Angus, R., Hanic, L. (1990a). Toxic blooms of the domoic acid containing diatom *Nitzschia pungens* in the Cardigan River, Prince Edward Island. In: Granéli, E., Sundström, B., Edler, L., Anderson, D.M. (eds.) *Toxic Marine Phytoplankton*, Elsevier, New York, pp. 227-232.

Smith, J.C., Odense, P., Angus, R., Bates, S.S., Bird, C.J., Cormier, P., de Freitas, A.S.W., Léger, C., O'Neil, D., Pauley, P., Worms, J. (1990b). Variation in domoic acid levels in *Nitzschia* species: implications for monitoring programs. *Bull. Aquacult. Assoc. Can.* 90-4: 27-31.

Sommer, U. (1994). Are marine diatoms favoured by high Si:N ratios*? Mar. Ecol. Prog. Ser.* 115: 309-315.

Subba Rao, D.V., Quilliam, M.A., Pocklington, R. (1988a). Domoic acid - a neurotoxic amino acid produced by the marine diatom *Nitzschia pungens* in culture. *Can. J. Fish. Aquat. Sci.* 45: 2076-2079.

Subba Rao, D.V., Dickie, P.M., P. Vass. (1988b). Toxic phytoplankton blooms in the eastern Canadian Atlantic embayments. *Comm. Meeting Internat. Cons. Explor. Sea*, C.M. ICES 1988/L:28 1-16.

Subba Rao, D.V., Wohlgeschaffen, G. (1990). Morphological variants of *Nitzschia pungens* Grunow f. *multiseries* Hasle. *Bot. Mar.* 33: 545-550.

Subba Rao, D.V., Partensky, F., Wohlgeschaffen, G., Li, W.K.W. (1991). Flow cytometry and microscopy of gametogenesis in *Nitzschia pungens*, a toxic, bloom-forming, marine diatom. *J. Phycol.* 27: 21-26.

Subba Rao, D.V., Pan, Y., Smith, S.J. (1995). Allelopathy between *Rhizosolenia alata* (Brightwell) and the toxigenic *Pseudonitzschia pungens* f. *multiseries* (Hasle). In: Lassus, P., Arzul, G., Erard, E., Gentien, P., Marcaillou-Le Baut, C. (eds.) *Harmful Marine Algal Blooms*, Lavoisier, Paris, pp. 681-686.

Takano, H., Kikuchi, K. (1985). Anomalous cells of *Nitzschia pungens* Grunow found in eutrophic marine waters. *Diatom* 1: 18-20.

Takemoto, T., Daigo, K. (1958). Constituents of *Chondria armata*. *Chem. Pharmaceutical Bull.* 6: 578-580.

Taylor, F.J.R., Haigh, R. (1996). Spatial and temporal distributions of microplankton during the summers of 1992-1993 in Barkley Sound, British Columbia, with emphasis on harmful species. *Can. J. Fish. Aquat. Sci*. 53: 2310-2322.

Taylor, F.J.R., Horner, R.A. (1994). Red tides and other problems with harmful algal blooms in the Pacific northwest coastal waters. In: Wilson, R.C.H., Beamish, R.J., Aitkens, F., Bell J. (eds.). *Review of the Marine Environment and Biota of Strait of Georgia, Puget Sound and Juan de Fuca Strait. Can. Tech. Rep. Fish. Aquat. Sci.* 1948, pp. 175-185.

Taylor, F.J.R., Haigh, R., Sutherland, T.F. (1994). Phytoplankton ecology of Sechelt Inlet, a fjord system on the British Columbia coast. II. Potentially harmful species. *Mar. Ecol. Prog. Ser.* 103: 151-164.

Teitelbaum, J.S., Zatorre, R.J., Carpenter, S., Gendron, D., Evans, A.C., Gjedde, A., Cashman, N.R. (1990). Neurologic sequelae of domoic acid intoxication due to the ingestion of contaminated mussels. *New England J. Med.* 322: 1781-1787.

Todd, E.C.D. (1993). Domoic acid and amnesic shellfish poisoning - a review. *J. Food Protection* 56: 69-83.

Villac, M.C. 1996. Synecology of the genus *Pseudo-nitzschia* H. Peragallo from Monterey Bay, California, U.S.A. Ph.D. Thesis, Texas A&M Univ., 258 p.

Villac, M.C., Roelke, D.L., Villareal, T.A., Fryxell, G.A. (1993a). Comparison of two domoic acid-producing diatoms: a review. *Hydrobiol.* 269: 213-224.

Villac, M.C., Roelke, D.L., Chavez, F.P., Cifuentes, L.A., Fryxell, G.A. (1993b). *Pseudonitzschia australis* Frenguelli and related species from the west coast of the U.S.A.: occurrence and domoic acid production. *J. Shellfish Res.* 12: 457-465.

Villareal, T.A., Roelke, D.L., Fryxell, G.A. (1994). Occurrence of the toxic diatom *Nitzschia pungens* f. *multiseries* in Massachusetts Bay, Massachusetts, U.S.A. *Mar. Environ. Res.* 37: 417-423.

Vrieling. E.G., Koeman, R.P.T., Scholin, C.A., Scheerman, P., Peperzak, L., Veenhuis, M., Gieskes, W.W.C. (1996). Identification of a domoic acid-producing *Pseudo-nitzschia* species (Bacillariophyceae) in the Dutch Wadden Sea with electron microscopy and molecular probes. *European J. Phycol.* 31: 333-340.

Walz, P.M., Garrison, D.L., Graham, W.M., Cattey, M.A., Tjeerdema, R.S., Silver, M.W. (1994). Domoic acid-producing diatom blooms in Monterey Bay, California: 1991-1993. *Nat. Toxins* 2: 271-279.

Wang, R., Maranda, L., Hargraves, P.E., Shimizu, Y. (1993). Chemical variation of *Nitzschia pungens* as demonstrated by the co-occurrence of domoic acid and bacillariolides. In: Smayda, T.J., Shimizu, Y. (eds.) *Toxic Phytoplankton Blooms in the Sea*, Elsevier, Amsterdam, pp. 607-612.

Wekell, J.C., Gauglitz, E.J. Jr., Barnett, H.J., Hatfield, C.L., Eklund, M. (1994). The occurrence of domoic acid in razor clams (*Siliqua patula*), Dungeness crab (*Cancer magister*), and anchovies (*Engraulis mordax*). *J. Shellfish Res.* 13: 587-593.

Wessells, C.R., Miller, C.J., Brooks, P.M. (1995). Toxic algae contamination and demand for shellfish: a case study of demand for mussels in Montreal. *Mar. Resources Economics* 10: 143-159.

Work, T.M., Barr, B., Beale, A.M., Fritz, L., Quilliam, M.A., 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., McCulloch, A.W., McInnes, A.G., Odense, P., Pathak, V.P., Quilliam, M.A., Ragan, M.A., Sim, P.G., Thibault, P., Walter, J.A., Gilgan, M., Richard, D.J.A., Dewar, D. (1989). Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from eastern Prince Edward Island. *Can. J. Chem.* 67: 481-490.