Vol. 67: 177–182, 1990 | MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser. | Published October 18

Nitzschia pseudodelicatissima – a source of domoic acid in the Bay of Fundy, eastern Canada

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ABSTRACT: Circumstantial evidence is presented that domoic acid detected in softshell clams *Mya arenaria* and blue mussels *Mytilus edulis* from the southwestern Bay of Fundy, eastern Canada, during July to October 1988 was obtained by feeding on the pennate diatom *Nitzschia pseudodelicatissima*. This microalga was the most abundant organism observed in weekly samples collected at the surface, 10 m depth and bottom from nearly all of the 18 locations sampled during the same period. Phytoplankton net hauls consisting principally of *N. pseudodelicatissima* contained levels of domoic acid up to 3.5 $\mu g\ g^{-1}$ Isolates of 9 dominant phytoplankton species occurring in the southwestern Bay of Fundy during July to October 1988 were cultured and tested for the presence of domoic acid; only *N. pseudodelicatissima* cultures produced the toxin at concentrations of 7.0×10^{-15} to 9.8×10^{-14} g cell^{-1} Since cultures of *N. pseudodelicatissima* were not axenic, the possibility exists that either an intra or extracellular microorganism is acting independently, or in association with the diatom, to produce domoic acid.

INTRODUCTION

During late fall of 1987, an episode of food poisoning – later described as amnesic shellfish poisoning (ASP) – occurred in Canada that resulted in more than 100 illnesses and 3 deaths (Todd 1990). The source of the poisoning was determined to be from cultured blue mussels *Mytilus edulis* harvested from aquaculture operations in eastern Prince Edward Island (PEI), Canada. Toxins from microalgae were accumulated while filter feeding and stored in their tissues. The toxin in the mussels was identified as domoic acid (Wright et al. 1989) and the major source was found to be associated with *Nitzschia pungens* forma *multiseries* (Subba Rao 1988, Bates et al. 1989).

Human symptoms of ASP include gastric (within 24 h) and neurological signs such as dizziness, disorientation and memory loss (within 48 h). Several months later, some of those over 40 yr old that had been affected still showed neurological symptoms and short-term memory loss (Todd 1990).

Although most species of microalgae are harmless, a few can produce toxins that cause harm, and even death, to vertebrate consumers. Prior to 1988, the organism of major concern to the shellfish industry in the Bay of Fundy was *Alexandrium fundyense**, the organism responsible for paralytic shellfish poisoning (PSP) on Canada's Atlantic coast. *A. fundyense* blooms annually in the Bay of Fundy (Martin & White 1988). Shellfish accumulate the toxins while filter feeding and the toxins are stored in their tissues. Many shellfish areas in the Bay of Fundy are closed to harvesting annually during periods when shellfish contain unacceptable levels of PSP toxins. As a result of the ASP outbreak in PEI during November 1987, shellfish from other coastal regions in Canada were monitored for domoic acid in 1988/89. Results from this monitoring showed that extracts of blue mussels and soft-shell clams *Mya arenaria* from some areas in southwestern Bay of Fundy also contained domoic acid (Gilgan et al. 1990, Haya et al. unpubl.).

This paper presents evidence that production of domoic acid in the southwestern Bay of Fundy in 1988 was associated with a species of pennate diatom, *Nitzschia pseudodelicatissima*, not previously reported as a source of toxin production.

MATERIALS AND METHODS

Samples were collected from the surface, 10 m depth and 1 m above bottom at 18 locations off the New Brunswick coast in the southwest Bay of Fundy (Fig. 1). Temperature, salinity and dissolved oxygen were meas-

* Formerly referred to as *Gonyaulax excavata*

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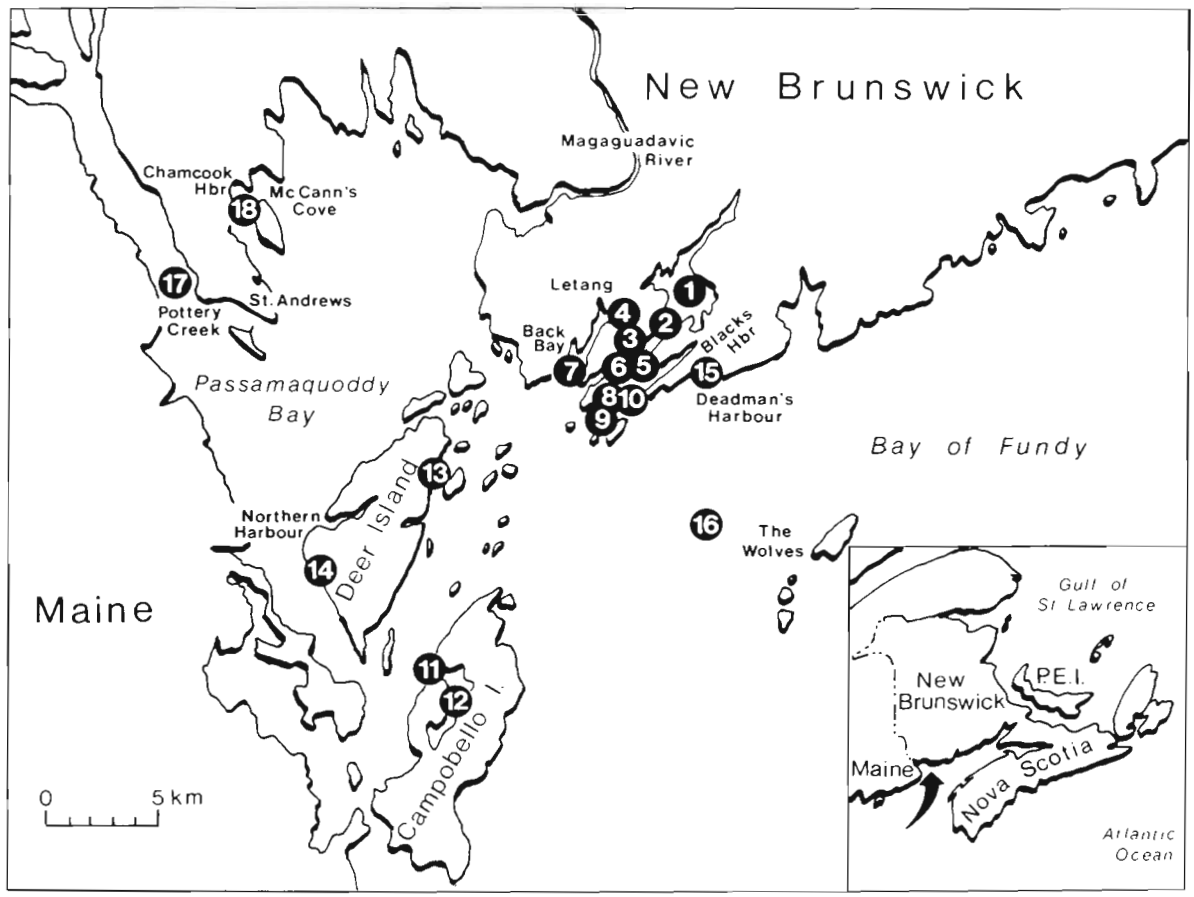

Fig. 1. Sampling locations in the southwestern Bay of Fundy, Canada

ured as described previously (Wildish et al. 1990) and phytoplankton species were identified. Samples were taken weekly from June 1 to October 25, 1988, between 09:00 and 16:00 h, irrespective of the tidal stage. Surface water was collected by bucket; additional samples were collected at 10 m depth and 1 m above bottom by a 1.8 l Niskin bottle equipped with a reversing thermometer.

Phytoplankton samples were immediately preserved in 2.5 % formalin:acetic acid (1:1) or 2.0 % Lugol's Iodine and later 50 ml subsamples were counted with an inverted microscope. All organisms >5 µm were identified; they included diatoms, dinoflagellates, ciliates and smaller zooplankton.

Phytoplankton were identified as described in Wildish et al. (1988, 1990). Identification of the most abundant organism, *Nitzschia pseudodelicatessima* Hasle, was confirmed by M. Poulin (National Museums of Canada, Ottawa, Ontario, Canada) and G. R. Hasle (University of Oslo, Norway). Cells were 55 to 80 µm in length and 1.5 to 2.0 µm in width, symmetrical and tapered toward the outer ends (Fig. 2). Cells were united in chains of 8 to 10 cells by overlapping of the ends.

Plankton for toxin analysis was collected during the main part of the bloom at Pottery Creek, Chamcook Harbour and The Wolves (Fig. 1) with a 20 µm Nitex mesh net that was 50 cm in diameter and towed near the surface at a speed of less than 1 knot for 10 min. Contents were kept on ice for 1 to 2 h during the return trip to the laboratory. A subsample was removed for microscopic examination to identify tow contents. The seawater was removed by aspiration and centrifugation at 750 × *g* for 15 min. The phytoplankton was weighed, then resuspended in a minimum amount of distilled water. The slurry was sonicated with a Sonifer™ cell disruptor (Branson) until microscopic examination indicated >90 % of cells were ruptured. The supernatant was passed through a Centrifree™ 10 micropartition system (30 000 MW cut-off; Amicon) by centrifugation at 2500 × *g* for 60 min.

Domoic acid concentrations were determined by the HPLC method of Lawrence et al. (1989) with the following conditions: Whatman Partisil II ODS-10 250 × 4.6 mm C18 column; Altex Model 110B pump delivering 12.5 % acetonitrile in distilled water (pH adjusted to 3 with H_3PO_4) at 1 ml min^{-1}; 20 µl Altex injection loop; UV detection using a Schoeffel SF 770 variable

wavelength detector set at 242 nm. The UV detector response was captured by an Apple IIe computer running Chromatochart™ (Interactive Microware Inc.). The calibration curve (20 µl injection, 0.4–5.5 µg ml^{-1}) was prepared with domoic acid (Diagnostic Chemicals, Charlottetown, PEI), standardized with an instrument calibration solution, DACS-1 (89 µg m^{-1}; National Research Council of Canada, Halifax, NS). Identification of domoic acid was also confirmed by FMOC derivitization and HPLC analysis (R. Pocklington, Dartmouth, NS).

Algal species observed in plankton samples containing domoic acid were isolated into culture. Single cells were taken with a capillary pipet, washed 2 to 3 times and grown in culture tubes initially using f/8-Si and later f/2 (Guillard 1984) at 12 to 14 °C, with an irradiance level of 45 µE m^{-2} s^{-1} from cool-white fluorescent bulbs and a 14 : 10 h light:dark cycle. There was no domoic acid detected in the sterile medium used for growth.

RESULTS

During late July 1988, low levels of domoic acid were detected in soft-shell clams *Mya arenaria* and blue mussels *Mytilus edulis* from the following shellfish areas in Passamaquoddy Bay – Chamcook Harbour, Magaguadavic River, Pottery Creek, Brandy Cove, near St. Andrews and western Deer Island, Canada (Fig. 1) (Gilgan et al. 1990, Haya et al. unpubl.). Domoic acid levels increased slowly and, during late August-early September, reached levels above 20 µg domoic acid g^{-1} of whole tissue, necessitating the closure of such areas to harvesting. Most of these areas remained closed to harvesting until early October.

Analysis of phytoplankton from the southwestern Bay of Fundy region showed that for most of the 18 stations sampled, the pennate diatom *Nitzschia pseudodelicatissima* (Fig. 2) was the predominant organism observed between July 28 and September 27. Cell numbers of *N. pseudodelicatissima* for 1988 are shown in Table 1. During late July, *N. pseudodelicatissima* represented 60 to 90 % of the total density for all species of phytoplankton identified. In early August to mid-September, it was 75 to 99 % of the total; during late September, 99 %. The phytoplankton from all locations sampled tended to follow this pattern except at Deadman's Harbour (Stn 15) where concentrations of *N. pseudodelicatissima* remained considerably lower throughout sampling. At Chamcook Harbour (Stn 18) during 1988, the highest concentration of cells of *N. pseudodelicatissima* at the surface (1.20×10^6 cells l^{-1}) was observed on September 23. Domoic acid levels in

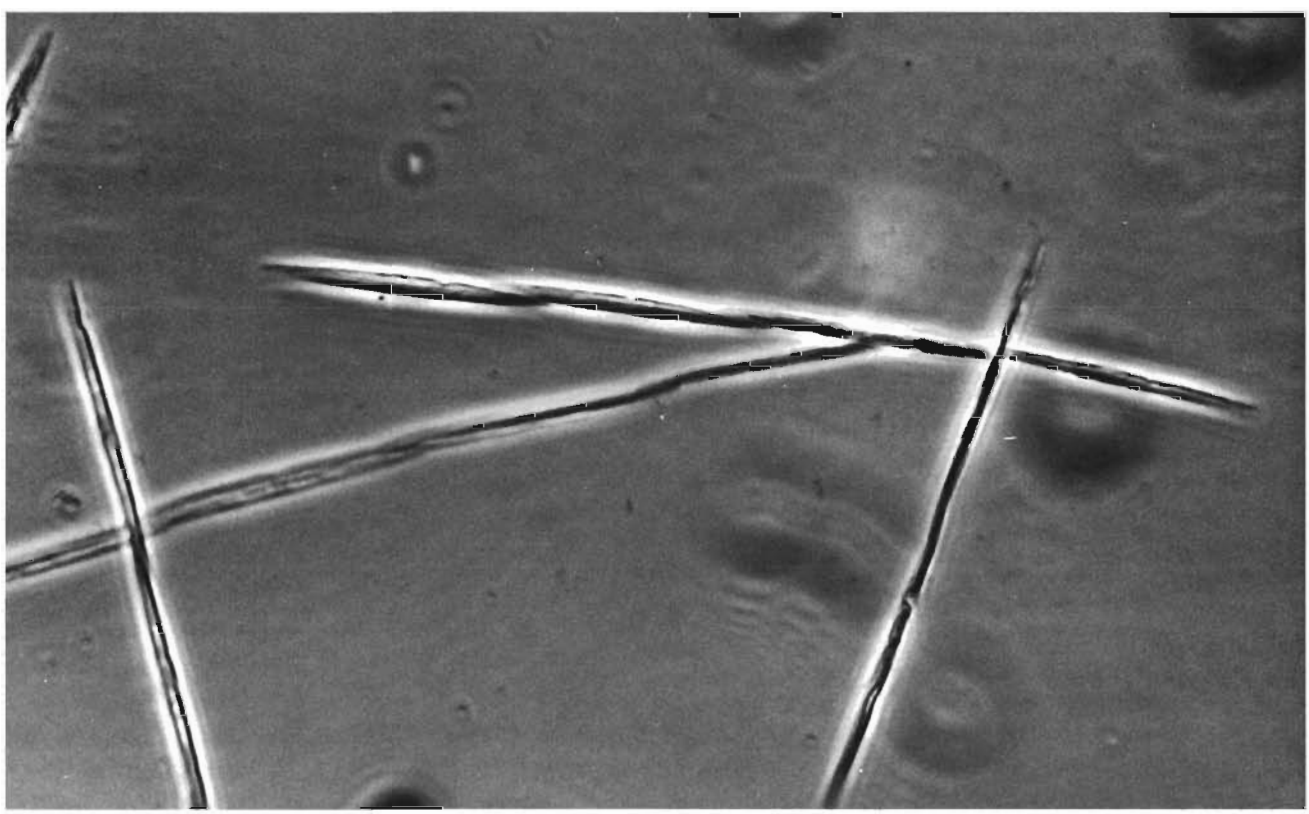

Fig. 2. *Nitzschia pseudodelicatissima*. Chains from culture, × 500

Table 1. *Nitzschia pseudodelicatissima*. Density as number of cells l^{-1} observed in surface samples during 1988

Stn	Jul 12	Jul 19	Jul 28	Aug 2	Aug 9	Aug 16	Aug 30	Sep 6	Sep 13	Sep 21	Sep 27	Oct 4
1	20	20	20	1260	100	920	2820	720	31520	1560	31000	20
2	0	0	100		860	2800	14240	720	48960	3100	110980	40
3	0	20	40	6840	1060	23860	7100	7760	89760	15760	57120	180
4	60	20	40		2340	26120	5200	15600	32640	16680	66920	60
5	0	0	300	4540	1080	29120	55480	2080	62200	14000	78340	80
6	0	20	60	2680	6620	13320	2520	1240	44060	15380	35900	80
7	0	80	2020	18200	2960	6620	93020	52220	551620	173800	138720	80
8	80	240	40	23760	0	6720	19400	4300	88120	39200	159940	20
9	0	140	360	16060	8960	9940	122400	14480	150140	27120	280700	0
10	20	60	160	620160	2480	50600	54520	13380	197480	23100	143620	120
11	0	11940	6400		2140	9540	5960	60380		48560	22840	40
12	60	1900			720	3840	35900	47320		62020	21200	0
13		9780	13700		12340	60380	88120	127300		202360	78340	0
14	0	32240	81800	29540	11720	5580	76700	380260		105640	39160	180
15	0	80	0		1020	5420	660	20	14800	6800	2860	200
16	40	6160	5080	1060	14180	32640	23060	564680	406360	127300	173000	1300
17	60	45700	91400	57120	31000	27740	75080	158300	66920	355780	76700	1340
18									111100	1201160	380620	57120

Table 2. Plankton tows collected from the southwestern Bay of Fundy and analyzed for domoic acid

Sampling stn	Date (1988)	Dominant organism (density > 50 % of total)	Domoic acid (μg g^{-1} wet wt)
15	Aug 3	*Mesodinium rubrum*	ND
15	Aug 4	*M. rubrum*	ND
09	Sep 20	*Nitzschia pseudodelicatissima*	0.9
17	Aug 8	*N. pseudodelicatissima*	Trace
17	Sep 20	*N. pseudodelicatissima*	0.8
18	Sep 23	*N. pseudodelicatissima*	2.6
18	Sep 30	*N. pseudodelicatissima*	3.5
18	Oct 7	*Scrippsiella trochoidea*	ND

ND = not determined

blue mussels reached the highest levels detected for the region (74 μg g^{-1}) at Chamcook Harbour on September 22 (Gilgan et al. 1990).

When domoic acid was detected in shellfish and plankton tows, cells of organisms found in the water were isolated and cultured. The organisms cultured were the following diatoms: *Chaetoceros simplex, Nitzschia closterium, N. pseudodelicatissima, N. pungens, Pleurosigma strigosum, Rhizosolenia alata, R. delicatula* and a small, unidentified pennate diatom. The dinoflagellate *Scrippsiella trochoidea* and bacteria from the seawater were also cultured. Only cultures containing *N. pseudodelicatissima* produced domoic acid in the laboratory. *N. pseudodelicatissima* cells from cultures were extracted between 30 and 75 d after initial inoculations and yielded from 7.0×10^{-15} to 9.8×10^{-14} g domoic acid $cell^{-1}$ Total numbers of cultured cells harvested varied from 3.7×10^5 to 7.5×10^7 cells total.

From plankton tow samples which were chemically analyzed (Table 2), detectable levels of domoic acid were found only in those where *Nitzschia pseudodelicatissima* was the dominant species observed. When other species dominated the plankton and *N. pseudodelicatissima* was either undetectable or present in low numbers, no domoic acid was detected.

DISCUSSION

Our results provide strong evidence that *Nitzschia pseudodelicatissima* played an important role in the production of domoic acid in the southwestern Bay of Fundy during 1988. It was the dominant species observed in the phytoplankton for the 2 mo period from July 28 to September 27 and, at times, made up more than 99 % of the species observed for some areas. It therefore provided a major food source to shellfish in many shellfish-producing beds in the southwestern Bay of Fundy waters for an extended period of time.

Microscopic examination of gut contents from soft-shell clams and blue mussels from affected areas revealed the presence of *N. pseudodelicatissima* frustules. Once ingested, the cells may have released domoic acid which was subsequently stored in bivalve tissues in a manner similar to the accumulation of paralytic shellfish toxins.

Our sampling of *Nitzschia pseudodelicatissima* populations during 1988 indicated that the cells were dispersed throughout the water column (Wildish et al. 1990) with high concentrations observed at all depths, making the organism available for feeding on a continuous basis. This is consistent with physical oceanographic work reported by Trites & Garrett (1983), indicating that within the Passamaquoddy region there is vigorous tidal mixing, resulting in weak stratification. Daborn (1986) suggested that this intense vertical mixing can also influence the whole of the shallow estuarine water column resulting in high primary productivity from nutrient recirculation. Nutrients were not analyzed at our sampling locations during 1988. However, experiments conducted in the laboratory indicate that the nutrient supply must have been maintained in ideal proportions and at sufficient levels in order to promote the growth of *N.pseudodelicatissima* as well as to enable it to flourish in the water column for such an extended period of time. Our phytoplankton surveys from other years indicate that the phytoplankton behaved differently in 1988. During 1987 and 1989, the *N. pseudodelicatissima* bloom lasted for a much shorter period and at lower densities (Wildish et al. 1988, 1990). Although the benthic and zooplanktonic (or other potential grazer) populations were not measured during 1988, they may have been poorer than in most years. This may be a possible explanation for *N. pseudodelicatissima* blooming so densely that millions of cells were observed in southwestern Bay of Fundy waters. There may also have been an increased nutrient supply during the summer of 1988 so that a bloom was stimulated.

Nitzschia pseudodelicatissima has been present in the Bay of Fundy for a considerable time. Monthly records collected by Gran & Braarud (1934) indicate that it bloomed regularly from March to September in the 1930's, with the highest concentration observed in 1931 (1.0×10^3 cells l^{-1}). However, they caution that since they used the centrifugation (instead of sedimentation) method for phytoplankton analysis, it may yield only 10 % of the actual numbers of minute forms such as *Nitzschia* sp. *N. pseudodelicatissima* has been observed annually since 1976 from our samplings in the area, although little attention was given to it due to the lack of knowledge that it was associated with a human toxin in the Bay of Fundy. It also appears that this organism is cosmopolitan in its existence and not confined to Bay of Fundy waters (Hasle 1965). The question, therefore, arises as to whether domoic acid is associated with *N. pseudodelicatissima* in waters other than the Bay of Fundy or whether conditions and the strain are unique to this particular part of the world.

It must be emphasized that the *Nitzschia pseudodelicatissima* cultures used for detection of domoic acid in the lab were unialgal, but not axenic. The possibility exists, therefore, that either an intracellular or extracellular microorganism may be responsible for domoic acid production or that a symbiotic relationship may be required with the algal cells in order to produce domoic acid. Further studies are required in order to establish an axenic culture of *N. pseudodelicatissima*.

One of the organisms isolated from eastern PEI during 1988 that has been shown to produce domoic acid in culture is *Amphora coffaeiformis* Ag. (Maranda et al. 1990). Another organism thought to be the major source of domoic acid in eastern PEI during the 1987 toxic mussel problem was a different species of *Nitzschia* – *N. pungens* forma *multiseries* (Subba Rao 1988, Bates et al. 1989). Another form of *N. pungens* is also found in Passamaquoddy Bay waters. However, when this organism was observed during our studies, it did not bloom very densely and generally made up < 1 % of the total phytoplankton population at any particular sampling time. During 1988, a *N. pungens* isolate from Stn 17 was cultured and analyzed for domoic acid. No domoic acid was detected in the culture (detection limit = 0.2 μg ml^{-1}).

The production rate of domoic acid by *Nitzschia pungens* forma *multiseries* as determined by Bates et al. (1989) was between 1 and 6×10^{-12} g $cell^{-1}$. The rate that we have determined for *N. pseudodelicatissima* was considerably lower: 7.0×10^{-15} to 9.8×10^{-14} g $cell^{-1}$. However, the *N. pseudodelicatissima* cells are considerably smaller in size and volume. *N. pseudodelicatissima* are 55 to 80 μm long and 1.5 to 2.0 μm wide, whereas *N. pungens* forma *multiseries* are 80 to 140 μm long and 4.5 to 6 μm wide. Assuming cylindrical shape, *N. pseudodelicatissima* would be ca 18 times less in volume than *N. pungens*; hence toxin production is more comparable on a cytoplasmic basis, although still 3.5 times less than in the former species. This may also explain why, although *Nitzschia* numbers reached similar densities (10^6 cells l^{-1}) in both PEI and the Bay of Fundy, domoic acid levels in blue mussels from eastern PEI were in excess of 300 μg g^{-1}, whereas at Stn 18 (Fig. 1) in Passamaquoddy Bay, the highest levels of domoic acid detected in blue mussels was 74 μg g^{-1} (Gilgan et al. 1990).

We conclude that the pennate diatom *Nitzschia pseudodelicatissima* was the major source of domoic acid occurring in shellfish and seawater in the southwestern Bay of Fundy during 1988. Since this diatom

has been found throughout the world, it is essential to conduct more extensive studies in other localities. Domoic acid may be present in other regions of the world, whether it is produced by another algal species, fungus or bacteria. Until further studies of domoic acid production by axenic and non-axenic cultures are completed, many questions remain unanswered that need to be resolved to better understand the production and fate of domoic acid.

Acknowledgements. We thank M. Poulin and G. R. Hasle for confirmation of the identity of *Nitzschia pseudodelicatissima;* M. LeGresley and J. Power, for phytoplankton analyses; Capt. F. Guptill, M. Ringuette and A. J. Wilson, for collection of samples; W McMullon and F. Cunningham, for graphic services; B. Best for typing the manuscript; R. Pocklington for confirmation of domoic acid.

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This article was submitted to the editor

Manuscript first received: May 9, 1990
Revised version accepted: July 30, 1990