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INVESTIGATIONS ON THE SOURCE OF DOMOIC ACID RESPONSIBLE FOR THE OUTBREAK OF AMNESIC SHELLFISH POISONING (ASP) IN EASTERN PRINCE EDWARD ISLAND

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September, 1988

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ABSTRACT

An outbreak of shellfish poisoning in Canada during November, 1987, was traced to cultured blue mussels (Mytilus edulis L.) from the Cardigan River region of eastern Prince Edward Island (P.E.I.). The shellfish toxin, identified as domoic acid, has not previously been found in any shellfish and this outbreak represents the first known occurrence of human poisoning from this neurotoxin. In this report we document the occurrence and distribution of toxic mussels in inshore waters of P.E.I. We relate this to the spatial and temporal abundance of macrophytes and phytoplankton and to their level of contamination by domoic acid. Our analysis of the relationship between these data sets has led us to conclude that the pennate diatom, Nitzschia pungens f. multiseries, was the major source of the domoic acid contained in the digestive glands of toxic mussels from the Cardigan River region of eastern P.E.I.

INTRODUCTION

Shellfish toxicity and its association with exceptional blooms of plankton has a long and established history (Taylor and Seliger, 1979; Parker and Tett, 1987). This relationship is particularly well defined in the case of paralytic shellfish poisoning, for example, which is a recurrent phenomenon in some coastal areas of New Brunswick, Canada (White, 1987), as well as along the eastern seaboard of the United States (Anderson and Morel, 1979).

The recent outbreak of shellfish poisoning in Canada during November, 1987, was traced to cultured blue mussels (Mytilus edulis L.) from the Cardigan River region of eastern Prince Edward Island (P.E.I.). Health and Welfare Canada identified 153 cases of acute intoxication and three deaths, following consumption of mussels from that area. The unique aspects of the toxic response in test mice used for bioassays, and the symptomology of hospitalized patients established that the intoxication was not caused by known paralytic or diarrhetic shellfish poisons produced by dinoflagellates.

Symptoms of intoxication in humans included abdominal cramps, vomiting within the first few hours, and neurologic responses involving memory loss and disorientation that could persist indefinitely (Perl et al., 1988; Debonnel et al., 1988). The toxin was identified as domoic acid (Wright et

al., 1988), and the term Amnesic Shellfish Poisoning (ASP) was proposed as an appropriate name for the clinical syndrome resulting from the consumption of molluscs contaminated with this neuroexcitatory amino acid (J. Hockin, personal communication, Health and Welfare Canada). Domoic acid has not previously been found in any shellfish and this outbreak represents the first known occurrence of human poisoning by this neurotoxin.

In response to the severity of the problem, a task force was quickly formed with the participation of staff from Health and Welfare Canada, the Department of Fisheries and Oceans, the Atlantic Research Laboratory (ARL) of the National Research Council of Canada, and the University of Prince Edward Island. The objectives of the ARL team were to: (a) isolate and characterize the toxin in mussels from the affected area in P.E.I.; (b) develop procedures for its quantitative analysis; and (c) identify possible sources of the toxin. Within a week of intense effort, the toxin was characterized as domoic acid (Bird et al., 1988; Wright et al., 1988), and procedures for its routine analysis were soon established (Quilliam et al., 1988a; 1988b; Wright et al., 1988). A review of events relating to the domoic acid problem is given by Couturier (1988).

Knowing the source of the toxin is of critical importance to the Atlantic shellfish industry. If the source was a marine organism, it must have been a very abundant and an

extremely active producer of domoic acid in order to account for the large amount, in excess of 6 kg, of the toxin in 63,000 kg of mussels from the affected area (Bird *et al.*, 1988). The only previously reported sources of domoic acid had been the rhodophycean macroalgae, *Chondria armata* Okamura (Takemoto and Daigo, 1958; 1960) and *Alsidium corallinum* C. Agardh (Impellizzeri *et al.*, 1975). A congener of *C. armata*, *C. baileyana* (Montagne) Harvey is known to occur in the southern Gulf of St. Lawrence (Edelstein *et al.*, 1974), and moreover has been found to contain domoic acid (Bird *et al.*, 1988), suggesting that macroalgae were possible sources in the present instance.

In this report we document the occurrence and distribution of toxic shellfish in inshore waters of P.E.I. We relate this to the spatial and temporal abundance of macrophytes and phytoplankton and to their level of contamination by domoic acid. Our analysis of the relationship between these data sets has led us to conclude that the pennate diatom, *Nitzschia pungens*, was the major source of the domoic acid contained in the digestive glands of toxic mussels from the Cardigan River region of eastern P.E.I.

MATERIALS AND METHODS

Domoic Acid Analysis

Domoic acid concentrations in mussel tissue and in samples of natural blooms of plankton were determined by reversed-phase high-performance liquid chromatography (HPLC) (Quilliam et al., 1988a; 1988b).

Mussel tissue (100 g drained wet weight) was homogenized and then heated and boiled for 5 min (stirring) with distilled water (100 mL). The cooked mixture was centrifuged at 3500 rpm (5 min), the supernatant decanted and the residual pellet washed with water (50 mL) and recentrifuged. The combined supernatants were made up to 250.0 mL.

Plankton tow samples (usually received as a slurry of cells in seawater) were extracted by adding an equal volume of acetonitrile and boiling with stirring for 5 min. After filtration through a 0.45 µm filter (Durapore Millex-HV; Millipore Products Division, MA), filtrates were evaporated to near dryness under vacuum on a rotary evaporator. The residue was dissolved in distilled, deionized water (4 x 0.5 mL) and then subjected to the clean-up. A supernatant was also provided for samples that had been centrifuged in the field. Appreciable amounts of domoic acid were detected in these supernatants if the plankton had been allowed to lyse before being centrifuged. These supernatant samples were analyzed directly by HPLC after filtration, and the quantity

of domoic acid so determined was added to that detected in the centrifugate.

Sample clean-up was performed by placing an aliquot of the crude extract (2.00 mL) on a pre-rinsed ODS (C-18) solid phase extraction cartridge (Supelco, Bellefonte, PA). The sample was then eluted with 3 mL of 10% aqueous acetonitrile, with the eluate being collected in a 5-mL volumetric flask. The contents of the flask were made to volume with 10% aqueous acetonitrile. An aliquot was then passed through a dry 0.22 µm filter and used for HPLC analysis.

Analyses were performed on a Hewlett-Packard model 1090M HPLC equipped with a DR5 solvent delivery system, variable volume (1 to 25 µL) injector and autosampler, built-in HP1040 diode array detector (DAD) and HP79994 data system. Columns (25 cm x 4.6 mm I.D. or 2.1 mm I.D.) packed with 5 µm Vydac 201TP (Separations Group, Hesperia, CA) were used at 40°C. The mobile phase was aqueous acetonitrile with 0.1% v/v trifluoroacetic acid. High resolution separations were performed with the 4.6 mm I.D. column using a 20 µL injection and linear gradient elution from 5% to 25% acetonitrile over 25 min at 1 $mL \cdot min^{-1}$. High speed analyses used the 2.1 mm column with a 5 µL injection volume and isocratic elution with 10% acetonitrile at 0.5 $mL \cdot min^{-1}$. Detection was effected by monitoring absorption at 242 nm with a 10 nm bandwidth. UV spectral acquisition was either triggered by peaks or continuous at 640 msec intervals. Quantification

was accomplished by comparing the areas of peaks from unknowns with those from standard solutions prepared from pure domoic acid (Quilliam et al., 1988b). The detection limit with the 2.1 mm column was 0.3 ng injected. With typical sample preparations, this allowed detection of 0.5 $\mu g \cdot g^{-1}$ (wet weight) in mussel tissue and 1 $\mu g \cdot g^{-1}$ (dry weight) in plankton.

Confirmation of the identity of the domoic acid in mussel tissue and in plankton from P.E.I. was made by matching HPLC retention times, ultraviolet spectra, infrared spectra (Falk, 1988), mass spectra (Thibault et al., 1988), and nuclear magnetic resonance spectra (Wright et al., 1988), as shown in Figs. 1 to 5.

Shellfish Collection

Shellfish were obtained through the courtesy and cooperation of eastern P.E.I. mussel growers and several private individuals residing in the Cardigan Bay area, and from the Department of Fisheries and Oceans (Charlottetown, P.E.I.). A batch of processed mussels (shucked, steamed and individually quick-frozen), harvested in early November, 1987, was obtained from North Ocean Enterprises Ltd. (Souris, P.E.I.).

Fig. 1. Reversed-phase HPLC-DAD analysis of an aqueous solution of (A) pure domoic acid, and aqueous-methanol extracts of (B) toxic mussels from the Cardigan River; and (C) Cardigan River plankton. Conditions: 25 cm x 2.1 cm I.D. 5 μm Vydac 201TP column at 40°C; 0.5 mL min^{-1} of 10% CH_3CN and 0.1% TFA in water.

FIGURE 1.

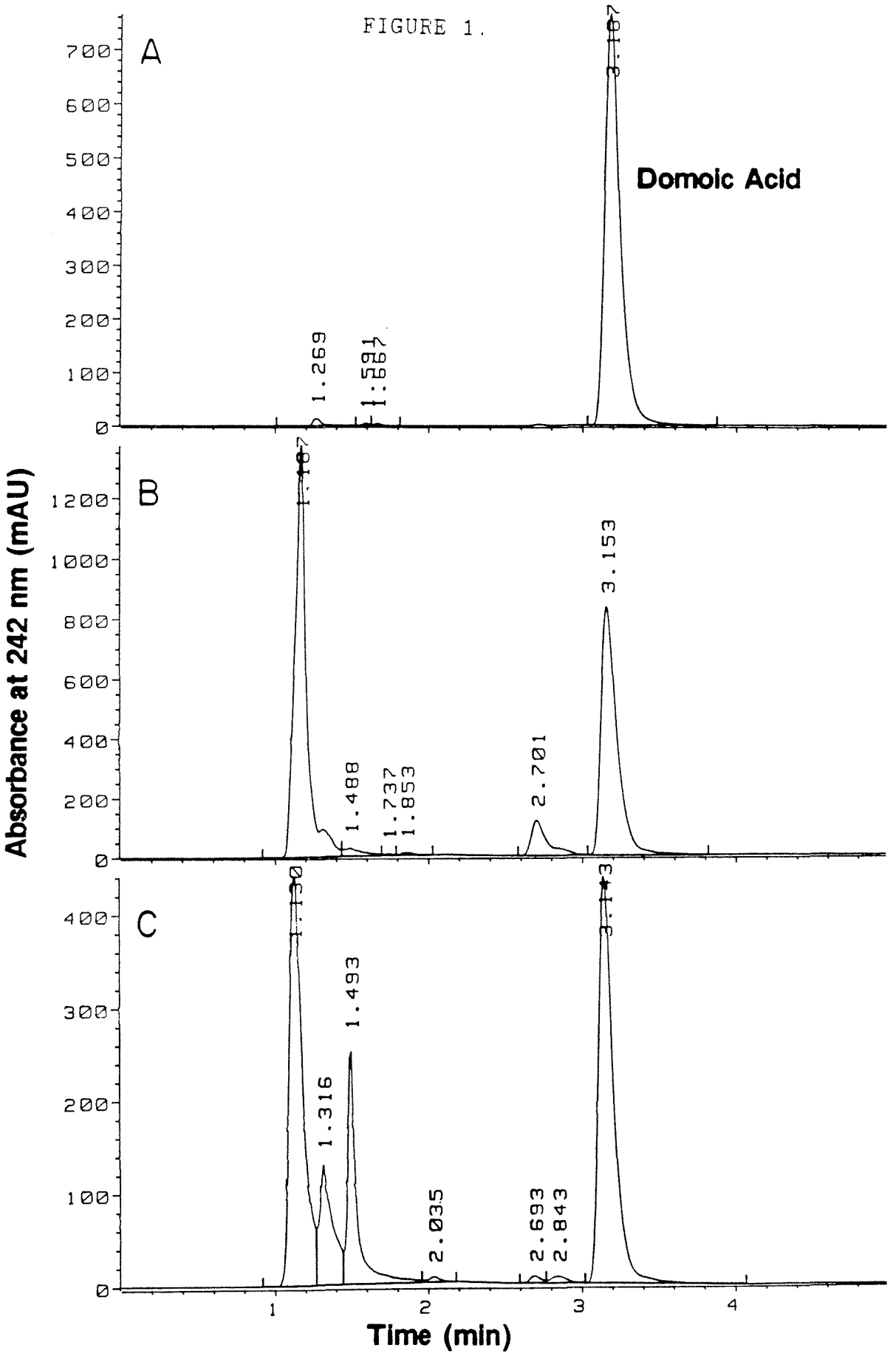

A
B
C
Domoic Acid
1.269
1.591
1.667
3.167
1.488
1.737
1.853
2.701
3.153
1.316
1.493
2.035
2.693
2.843
Absorbance at 242 nm (mAU)
Time (min)

Fig. 2. Ultraviolet absorption spectra of domoic acid, isolated from: (A) Cardigan River plankton, and (B) Cardigan River cultured mussels. These spectra were acquired by DAD during HPLC analysis (see Fig. 1).

FIGURE 2.

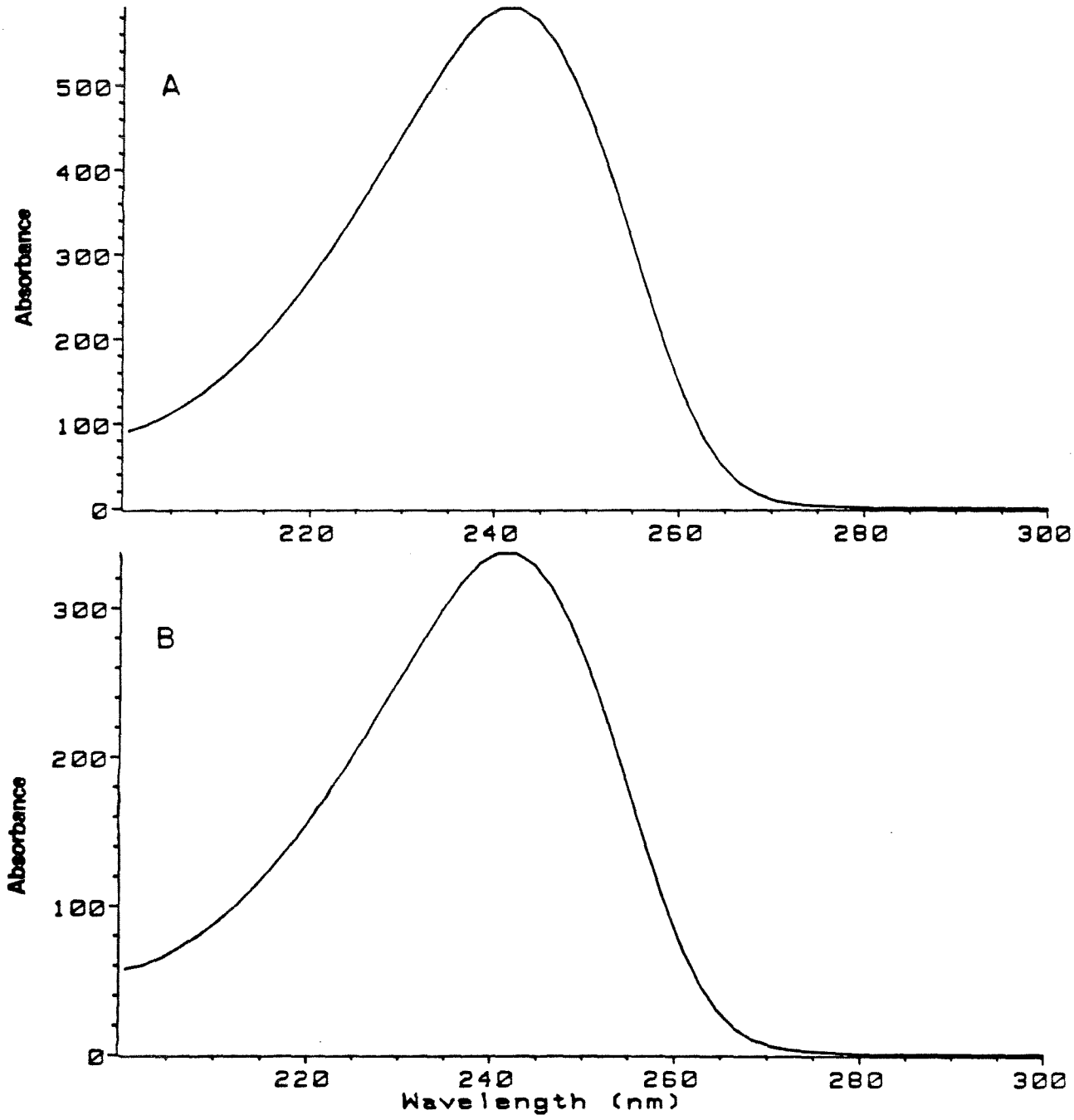

A
B
Absorbance
Absorbance
0
100
200
300
400
500
220
240
260
280
300
Wavelength (nm)

Fig. 3. Infrared spectra of solid films of domoic acid in the fully protonated state, prepared by evaporation on a AgCl plate of a solution in 0.1 N HCl, from:
(A) Cardigan River plankton (20 December, 1987); and
(B) Cardigan River cultured mussels (October, 1987).

FIGURE 3.

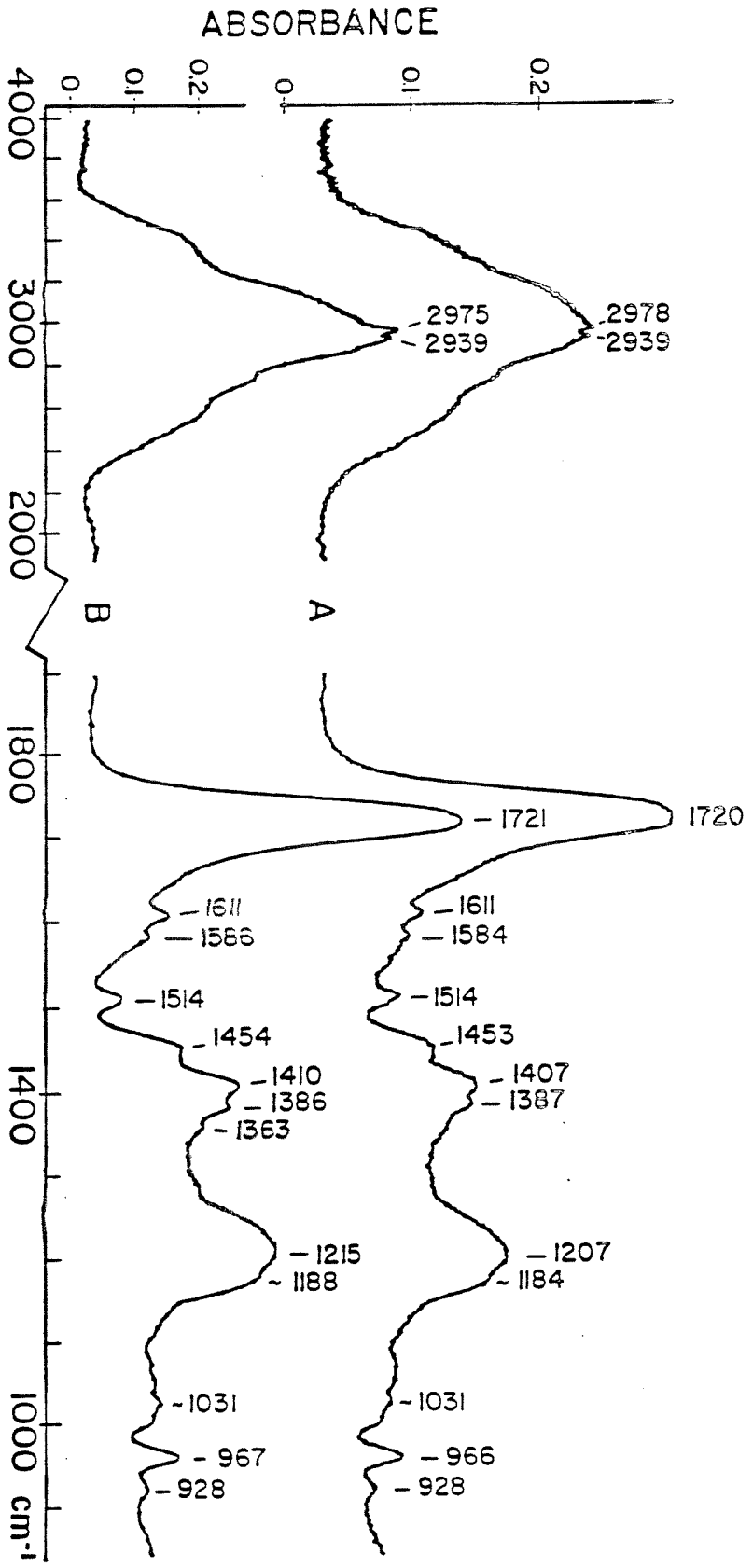

ABSORBANCE
0
0.1
0.2
0
0.1
0.2
4000
3000
2000
1800
1400
1000 cm-1
B
A
2975
2939
2978
2939
1721
1720
1611
1586
1514
1454
1410
1386
1363
1215
1188
1031
967
928
1611
1584
1514
1453
1407
1387
1207
1184
1031
966
928

Fig. 4. Tandem mass spectra of protonated molecules (m/z 312, fast atom bombardment ionization) of domoic acid from: (A) Cardigan River plankton (20 December, 1987); and (B) Cardigan River cultured mussels (October, 1987).

FIGURE 4.

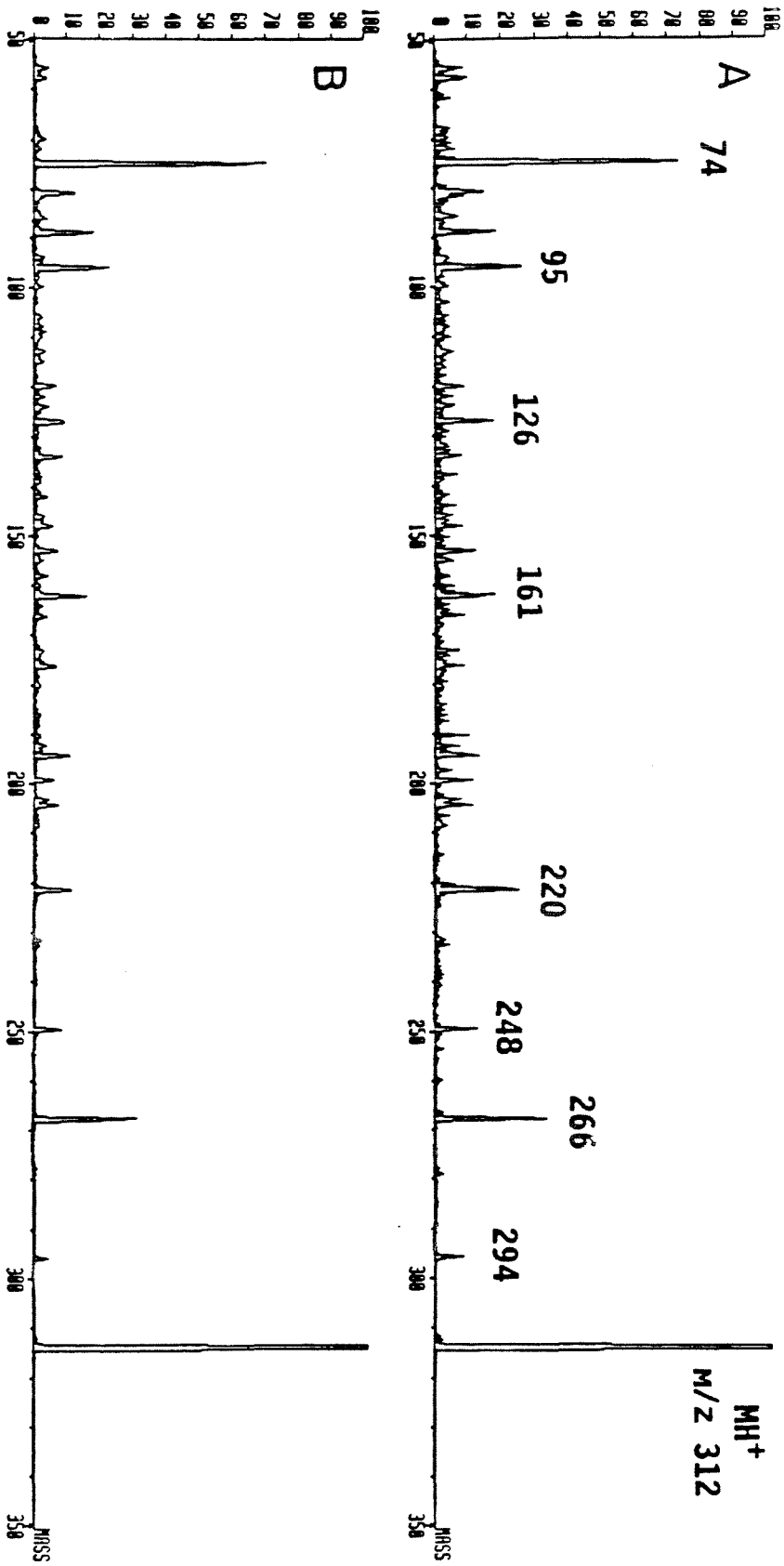
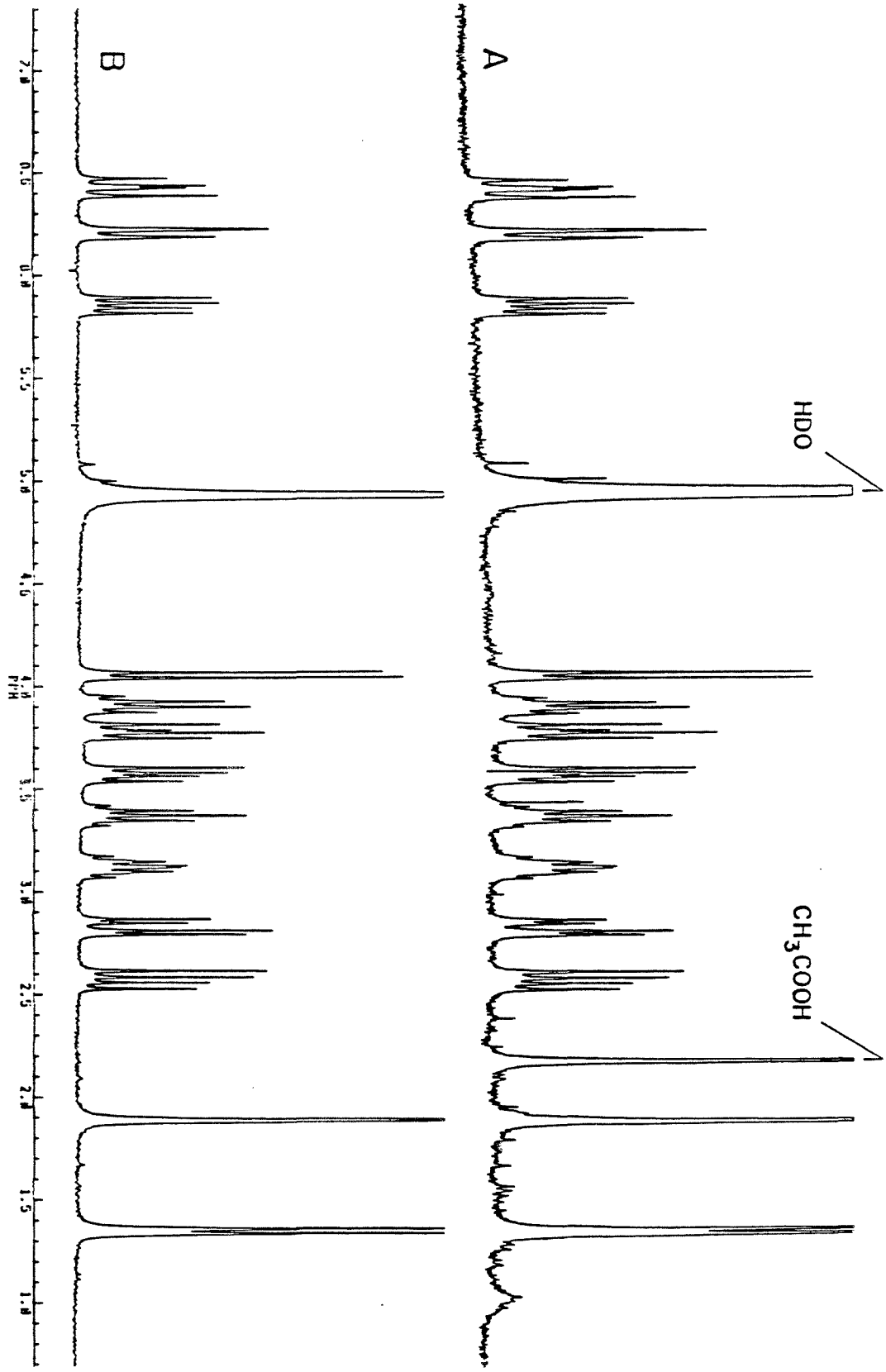


Fig. 5. Proton NMR spectra of domoic acid from: (A) Cardigan River plankton (20 December, 1987); and (B) Cardigan River cultured mussels (October, 1987). Conditions: 300 MHz; solutions in D_2O; pD about 3; reference to TSP dissolved in D_2O, in a concentric tube. TSP: sodium trimethyl silylpropionate-2,2,3,3-d_4.

FIGURE 5.



Macroalgal Investigations

Mussels, and netting used to culture the mussels, were examined for the presence of perennating basal residua of Chondria baileyana (Novaczek et al., 1987). Intact "socks" of live cultured mussels from the Cardigan area were removed from an affected site in early December, 1987, and held at a clean locale for several weeks prior to examination. Shells of these mussels were scanned with the unaided eye for basal discs of perennial algae. Although some epizoic algae may have been acquired at the new site, the original perennial flora from the Cardigan system would have persisted unless undue desiccation or thermal stress (Novaczek et al., 1987) had occurred during transport. Suspect shells were checked with a stereomicroscope; those selected for culture to verify the identity of attached algae were removed from the animal, cleaned of silt with a soft brush, and incubated in sterile seawater at 15°C, 16:8 h light:dark, irradiance of 40-50 μE m^{-2} s^{-1}, and with gentle agitation by compressed air. Sections of nylon netting, 1 m and 2 m from the top of the "sock", were brushed and cultured in the same manner. The seawater was replaced after 48 h with enriched seawater medium SWM-3 (McLachlan, 1977), and cultures were maintained for two weeks, sufficient for bases of C. baileyana to initiate growth of uprights (Novaczek et al., 1987).

One- and two-year-old mussels collected from Boughton River and Tracadie Bay from June through November, 1987, were

frozen for other purposes (A.L. Mallet, C.E.A. Carver, Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS). A subset of these mussels was thawed in seawater and examined under a stereomicroscope for young stages and basal discs of macroalgae.

Plankton Sampling

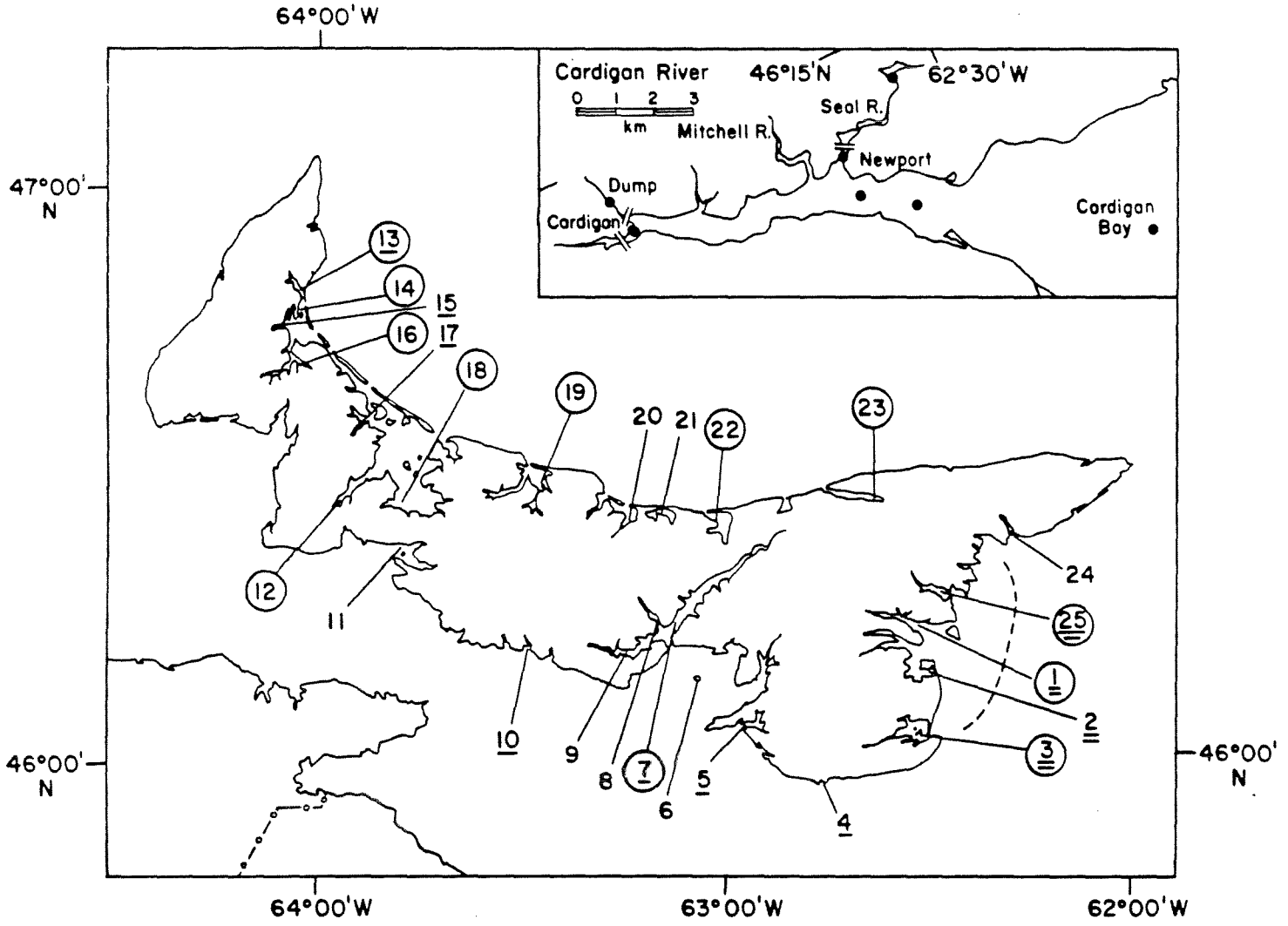
Plankton samples were collected (see acknowledgments) from inshore and estuarine waters of Prince Edward Island (Fig. 6), either by towing or by pumping into a net with a 20-µm mesh size. The sites, timing and method of sampling depended on local weather and ice conditions. Generally, samples were collected within a depth range of 1 to 3 m. A sample collected on December 15, 1987, was derived from a large volume (ca. 10,000 L) of water from the Cardigan River site opposite Newport (Fig. 6). The water sample was pumped into 120-L plastic drums, maintained at 0 to 5°C (not frozen), and immediately transported by truck to ARL for filtration and centrifugation. One batch of 100 L was promptly filtered (Millipore type PH, 0.3-µm pore size, 29 cm diameter), and subsamples of the retentate (suspended in seawater) were sent to P.J. Wangersky (Dalhousie University, Halifax, N.S.) and to J. Martin (Department of Fisheries and Oceans, St. Andrews, N.B.) for examination, isolation and culturing of component phytoplankton. Another 3,000- to 4,000-L portion was centrifuged (Model Z41, New Brunswick Scientific, operated in the clarification mode at a throughput rate of

Fig. 6. Map of Prince Edward Island, showing sites where plankton was collected: 1. Cardigan River; 2. St. Marys Bay; 3. Murray River; 4. Wood Islands; 5. Pinette River; 6. Governor Island; 7. Hillsborough River; 8. North River; 9. West River; 10. Victoria Harbour; 11. Bedeque Bay; 12. Grand River (Malpeque Bay); 13. Kildare River; 14. Alberton; 15. Mill River; 16. Foxley River; 17. Bideford River; 18. Bentick Cove (Malpeque Bay); 19. New London Bay; 20. Rustico Bay; 21. Covehead Bay; 22. Tracadie Bay; 23. St. Peters Bay; 24. Souris River; 25. Boughton River.

Most collections were made during mid-December, 1987, to mid-January, 1988; see Table 2 for specific collection dates of analyzed samples. Underlining signifies trace quantities or low percentages of Nitzschia pungens observed; double underlining, significant to large percentages. Circled numbers indicate samples analyzed for domoic acid. Broken line off the eastern edge of the island represents the seaward extent of N. pungens on 18 December, 1987.

Inset: detail of Cardigan River system, with sampling sites indicated by solid circles (see Table 2).

FIGURE 6.


64°00'W
47°00' N
46°00' N
63°00'W
62°00'W
Cardigan River
46°15'N
62°30'W
0 1 2 3
km
Mitchell R.
Seal R.
Newport
Dump
Cardigan
Cardigan Bay
13
14
15
16
17
18
19
20
21
22
23
24
25
1
2
3
4
5
6
7
8
9
10
11
12

ca. 16 L min^{-1}) within 36 h of collection. The centrifugate was sent to Health and Welfare Canada (Ottawa) for bioassay. The centrifugate from a second 3,000- to 4,000-L batch was examined at ARL.

Plankton Analyses

Abundance of organisms in the plankton, with particular reference to the pennate diatom, Nitzschia pungens Grunow f. multiseries Hasle, the dominant taxon in the plankton bloom, was determined using light microscopy. Up to 15 randomly selected microscope fields were counted at 250X or 400X until a stable percentage abundance of N. pungens was achieved. More fields (up to 50) were counted when the incidence of N. pungens was low, and the higher magnification was used for denser preparations. Only pigmented diatoms were registered as part of the plankton; empty frustules were regarded as detritus. These abundance values were used to estimate relative volumes of N. pungens and other major diatom species.

The identity of diatom species was verified by scanning electron microscopy (SEM). Plankton samples were first acid-cleaned by the method of Boyle and Pickett-Heaps (1984), but with boiling prolonged to 1.5 h to ensure complete removal of all detrital organic matter. The clean diatom frustules were then concentrated on a 5-µm Nuclepore filter, washed with distilled water, sputter-coated with gold, and examined in a JEOL-35 scanning electron microscope. The species

composition of residual planktonic food items in the digestive glands of mussels was examined by light microscopy. The digestive tissue was acid cleaned and examined by SEM, as above, for confirmation of diatom species.

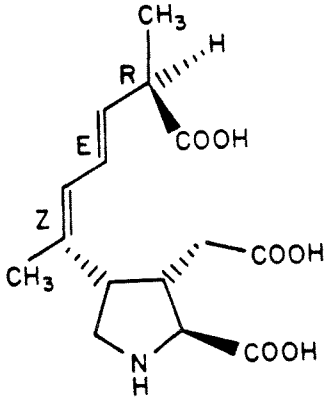
The number of N. pungens cells present in the plankton tow samples (cells g^{-1} dry weight of plankton sample) that were analyzed for domoic acid was determined as follows. About 1 mg of dried sample residue was weighed to ±5 µg accuracy, and acid-cleaned as above. The clean residue, consisting of dissociated and fragmented diatom valves, was suspended in a known volume (usually 3-mL) of distilled water, and 10- or 20-µL aliquots were sampled for counting on the light microscope. Separate aliquots were taken for examination by SEM to assist in taxonomic identification and enumeration of the diatom valves. Four size-classes (by length) of N. pungens fragments were used: <25%, 25-50%, 50-75%, and 75-100%. Forty random fields were counted at 400X to ensure recognition of N. pungens. This covered about 0.8% of the area occupied by the sample on the slide and overcame the non-random distribution of particles in that additional counts did not significantly change the calculated cell density (t-test, $P > 0.1$). Counts in each of these size-class fragments were converted to number of valves by multiplying the mean fractional length of the respective size-classes (i.e., 0.125, 0.375, 0.625, 0.875) by the total number of fragments in each size-class. Cell number was

obtained by dividing the total for all classes by two. These cell number values permit calculation of the number of cells per unit dry weight of plankton sample, and the concentration of domoic acid per cell of N. pungens present in the plankton sample, assuming that all of the domoic acid in the sample was derived from N. pungens. The carbon content of the plankton was determined with a Perkin-Elmer Model 240C Elemental Analyzer on duplicate 1-3 mg subsamples of material that was used for domoic acid extraction.

RESULTS

Confirmation of the Identity of Domoic Acid

Results confirming the identity of domoic acid are presented in Figs. 1 to 5, documenting HPLC retention times and infrared, mass, and proton nuclear magnetic resonance spectra on extracts from mussel tissue and plankton from the affected area. These spectra are essentially identical and strongly suggest a common source for the domoic acid found in both the mussels and the plankton.



DOMOIC ACID

Temporal and Spatial Distribution of Toxic Shellfish

Results on domoic acid levels in mussels and other shellfish harvested prior to, during and following the outbreak of mussel poisoning are documented in Table 1. The distribution of mussels containing domoic acid shows that measurable levels of contamination were confined to the Cardigan Bay region. For example, domoic acid was observed

TABLE 1. Domoic acid levels in shellfish[1] harvested from eastern Prince Edward Island.

LOCATION	DATE HARVESTED	SHELLFISH TYPE	DFO #	DOMOIC ACID (µg/g wet wt)
Cardigan River (South side opp. M0242)	Fall, 1986	Guahogs (canned)	-	ND[2]
Cardigan River (ISI lease[3])	May, 1987 (last week)	Mussels	12-034	ND
Cardigan River (North side 0.8 Km E of Newport wharf)	June, 1987 (first week)	Bar clams (bottled)	9-042	ND
Cardigan River (North side between Maitland Pt. and DeGros Marsh)	July, 1987 (last week)	Bar clams (bottled)	8-042	ND
Cardigan River (mouth Mitchell R.)	August, 1987 (first week)	Clams[4]	11-034	ND
Cardigan River (North side, above M0117A)	August, 1987 (second week)	Clams	10-042	ND
Cardigan River (0.7 km E of Cardigan causeway, N side of river)	September, 1987 (first week)	Clams	--	ND
Cardigan River (0.4 Km E of #10-042, above)	October, 1987 (last week)	Clams	11-042	ND
Cardigan River (South side opp. M0242)	Last week Oct. first week Nov. 1987	Mussels (pickled)	-	170
Murray River (M0145, M0146, M0130, M0139)	2-Nov-88	Mussels[5]	1-034	ND
Cardigan River (85% M0177C) and Murray River (15% M0145)	3-Nov-88	Mussels[5]	2-034 1-009	628 575

TABLE 1 (Continued)

LOCATION	DATE HARVESTED	SHELLFISH TYPE	DFO #	DOMOIC ACID (µg/g wet wt)
Cardigan River (M0117C)	6-Nov-88	Mussels[b]	2-009	820
			36-020	753
		whole tissue	36-020	765
		digestive gland		1535
		meat		423
Cardigan River (M0117B)	11-Nov-88	Mussels[b]	3-034	737
			3-009	670
			4-009	719
			36-020	871
Cardigan River (92% M0117B) and Murray River (8% M0151)	11-Nov-88	Mussels[b]	4-034	788
			5-009	867
Cardigan River (ISI lease[c])	16-Nov-87	Mussels[d]	13-348	
		whole tissue		283
		digestive gland		807
		meat		50
Cardigan River (mouth Mitchell R.)	7-Dec-87	Clams	12-341	38
St. Marys Bay	7-Dec-87	Mussels	10-341	58
Boughton River (Annandale)	7-Dec-87	Mussels	5-341	22
Boughton Island	9-Dec-87	Mussels	0-117C	189
Boughton River	10-Dec-87	Mussels	8-344	39
Cardigan River (Mitchell R., Lease M0121)	10-Dec-87	Mussels[d]	3-352	
		digestive gland		280
Cardigan River (Lease M0121)	14-Dec-88	Mussels	12-348	160
Hillsborough River	14-Dec-87	Mussels (fresh)	11-348	ND
Seal River (below bridge)	5-Jan-88	Clams	17-005	6

TABLE 1 (Continued)

LOCATION	DATE HARVESTED	SHELLFISH TYPE	DFO #	DOMOIC ACID (µg/g wet wt)
Cardigan River (mouth of Mitchell R.)	5-Jan-88	Clams	18-005	6
Cardigan River (Lease M0121)	11-Jan-88	Mussels	8-011	30
Cardigan River (Lease M0121)	18-Jan-88	Mussels (fresh)	5-018	20
Tracadie Bay	18-Jan-88	Mussels	9-018	ND
Seal River (20 m from bridge)	19-Jan-88	Clams	30-020	3
Cardigan River (West side of Cardigan causeway)	19-Jan-88	Clams	32-020	1
Cardigan River (Lease M0121)	25-Jan-88	Mussels	5-025	20
Tracadie Bay	25-Jan-88	Mussels	2-025	ND
Cardigan River (Lease M0121)	1-Feb-88	Mussels	5-032	20
Cardigan River	1-Mar-88	Mussels	3-028	4
Cardigan River	1-Mar-88	Mussels	3-033	ND
Cardigan River	1-Mar-88	Mussels	3-035	ND
Cardigan River	2-Mar-88	Mussels	3-048	ND
Cardigan River	2-Mar-88	Mussels	3-050	ND
Cardigan River	2-Mar-88	Mussels	3-051	7
Cardigan River	3-Mar-88	Mussels	3-060	ND
Cardigan River	3-Mar-88	Mussels	3-063	ND
Cardigan River	3-Mar-88	Mussels	3-064	ND
Buctouche Harbour	4-Mar-88	Mussels	4-003	ND

TABLE 1 (Continued)

LOCATION	DATE HARVESTED	SHELLFISH TYPE	DFO #	DOMOIC ACID (µg/g wet wt)
Cardigan	7-Mar-88	Mussels	3-091	ND
Cardigan	7-Mar-88	Mussels	3-092	ND
Cardigan	7-Mar-88	Mussels	3-096	ND
Buctouche Harbour	8-Mar-88	Mussels	4-005	ND
Buctouche Harbour	14-Mar-88	Mussels	4-009	ND
Tracadie Bay	24-Mar-88	Mussels	R-1556	ND
Tracadie Bay	24-Mar-88	Mussels	R-1557	ND
Cardigan Bay	24-Mar-88	Mussels	R-1558	ND

[1]All shellfish samples were obtained frozen unless indicated otherwise.

[2]ND = Domoic acid Not Detected (<1 µg g^{-1}).

[3]Island Shellfish Inc. lease.

[4]All clam samples were obtained shucked, steamed and frozen.

[5]Shucked, steamed, and individually quick frozen by North Ocean Enterprises Ltd.

[6]Mussels held in wet storage for 10 d depuration prior to analysis.

in extracts of mussels only from Cardigan River and closely adjacent areas, such as Boughton River to the north and St. Marys Bay to the south.

Domoic acid was undetectable in samples of various shellfish, including mussels, harvested during the summer and early autumn of 1987. The earliest appearance of domoic acid was from mussels harvested from the Cardigan River in late October. Analysis of fractionated tissue revealed that, in all cases, the toxin was localized in the digestive glands (Wright et al., 1988). The domoic acid concentration in extracts of the digestive gland tissue reached a peak of 1535 µg domoic acid g^{-1} wet weight in early November (Table 1). The domoic acid content then declined fairly rapidly in mussels harvested from mid-November, 1987, to mid-January, 1988, and the affected area was officially cleared for harvesting mussels, clams and quahogs on April 5, 1988.

Distribution of Domoic Acid in Mussel Digestive Glands

Transmission electron micrographs of mussel digestive glands (taken by C.M. Morrison, Department of Fisheries and Oceans, Halifax), and scanning electron micrographs revealed the presence of large numbers of lysosomes in toxic mussels. This could be attributed, in part, to the apparent healthy nutritional state of the mussels. However, it also suggested the possibility that the toxin might be sequestered in these organelles (M. Moore, personal communication, Department of Fisheries and Oceans, Halifax). To test this hypothesis,

control and toxic mussel digestive glands were fractionated (Moore, 1985) for the preparation of mussel lysosomes. The results of this step-gradient ultra-centrifugation of the tissue extract indicated that 30% of the domoic acid was found in the cell stroma, debris and nuclei centrifuged down in the first spin. Subsequently, ca. 60% was found in the particulate-free soluble fraction and only 5% in the primary fraction. None was found in the secondary and tertiary lysosome fractions. This indicated that the greater amount of the domoic acid existed free in the cytoplasm, although it cannot be ruled out that some of this free domoic acid may have been released from lysosomes damaged and ruptured during the fractionation process.

Macroalgae Associated with Toxic Mussels

The algal flora on mussels from toxic areas did not differ noticeably from that on mussels from an unaffected area (Tracadie Bay). Basal discs of Chondria baileyana were not seen on any of the shells examined, and cultures of fresh Cardigan shells failed to disclose the presence of perennating basal cells or minute young stages. Culture netting was similarly negative for C. baileyana. All shells were remarkably clean of algae, even those collected during summer, the period of rapid growth for many macrophytic algae. In both fresh and frozen samples, identifiable macrophytes were small in stature, even those belonging to

intrinsically larger species (e.g. Giffordia sandriana (Zanardini) Hamel, Polysiphonia harveyi Bailey).

Species Composition of the Plankton

From mid-December, 1987 to mid-January, 1988, the plankton of the Cardigan River consisted almost entirely of the pennate diatom, Nitzschia pungens Grunow forma multiseries Hasle (Table 2; Fig. 7). Nitzschia pungens occurred in chains of up to 38 cells (Fig. 8A), rarely singly, and was distinguished from 16 other chain-forming species of Nitzschia by its bilateral symmetry, narrow (3-5 µm) width, acute apices, lack of a central nodulus, and possession of 9-15 costae per 10 µm (Fig. 8B). As forma multiseries, it differed from the nominate form in having 3-4 rows of poroids between the costae, and spacing of poroids at 4-6 per 1 µm (Fig. 8C; Hasle, 1965). Samples and SEM photomicrographs of N. pungens were sent to M. Poulin (Division of Botany, National Museum of Natural Sciences, Ottawa, Ontario K1A 0M8), G.A. Fryxell (Department of Oceanography, Texas A&M University, College Station TX 77843, U.S.A.), G.R. Hasle (Department of Biology, University of Oslo, P.O. Box 1069, N-0316 Oslo, Norway), and D.G. Mann (Department of Botany, University of Edinburgh, The King's Buildings, Mayfield Rd., Edinburgh EH9 3JH, U.K.), who confirmed the identity.

After mid-January, the relative abundance of N. pungens declined in the central part of the Cardigan estuary (Seal River), and several species of the centric diatom

TABLE 2. Percent abundance of *Nitzschia pungens*, and other major constituents in the plankton of some inshore waters, Prince Edward Island, mid-December, 1987 to mid-March, 1988; refer to Fig. 6.

LOCATION	DATE	*N. pungens* % ABUNDANCE	OTHER COMMON OR DOMINANT TAXA
Cardigan Region			
Cardigan River*	15-Dec-87	>99	
Cardigan River*	16-Dec-87	>99	
Cardigan River*	20-Dec-87	100	
10-Km offshore*	20-Dec-87	>99	
Cardigan River*	1-Jan-88	>99	
Cardigan Bay*	1-Jan-88	98	
Seal River*	18-Jan-88	77	*Rhizosolenia*
Seal River*	23-Jan-88	62	*Rhizosolenia*
Seal River*	24-Jan-88	43	*Rhizosolenia*
Seal River*	27-Jan-88	26	*Rhizosolenia*
Upper Seal River	13-Feb-88	0	*Licmophora*
Seal River*	14-Feb-88	12	*Rhizosolenia*
dump site	14-Feb-88	0	*Fragilaria*
Seal River	26-Feb-88	<1	*Rhizosolenia*, other centric diatoms, *Fragilaria*
Seal River	9-Mar-88	<1	*Rhizosolenia*, other centric diatoms
bridge	14-Mar-88	<1	*Rhizosolenia*, *Thalassiosira*
Murray River*	31-Dec-87	69	*Rhizosolenia*, *Thalassiosira*, *Thalassionema*
Boughton River*	2-Jan-88	93	
Hillsborough River	6-Jan-88	0	*Rhizosolenia*, *Thalassiosira*
St. Peters Bay	11-Jan-88	0	Oligotrichida (tintinnid loricae), Crustacea
Tracadie Bay	12-Jan-88	0	*Biddulphia*, Oligotrichida
New London Bay	13-Jan-88	0	Crustacea, Oligotrichida
Alberton	15-Jan-88	0	Crustacea
Bentick Cove (Malpeque Bay)	15-Jan-88	0	Crustacea, Oligotrichida
Foxley River	16-Jan-88	0	Crustacea, Oligotrichida
Grand River (Malpeque Bay)	17-Jan-88	0	Crustacea, Oligotrichida
Kildare River	18-Jan-88	<1	mostly detritus; no clearly dominant taxa

*Samples contained domoic acid.

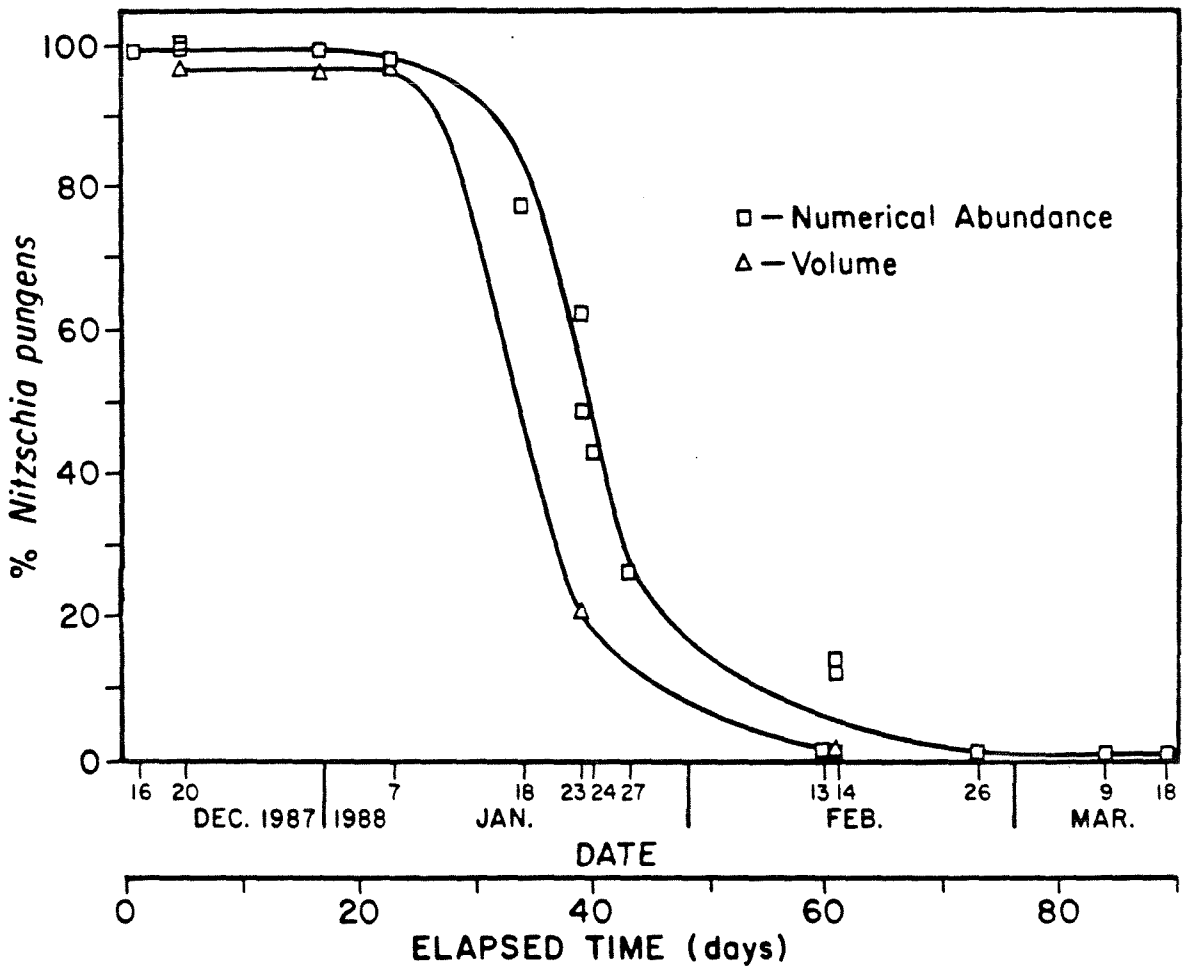


Fig. 7. Nitzschia pungens f. multiseries as a constituent of the phytoplankton in the Cardigan River system.

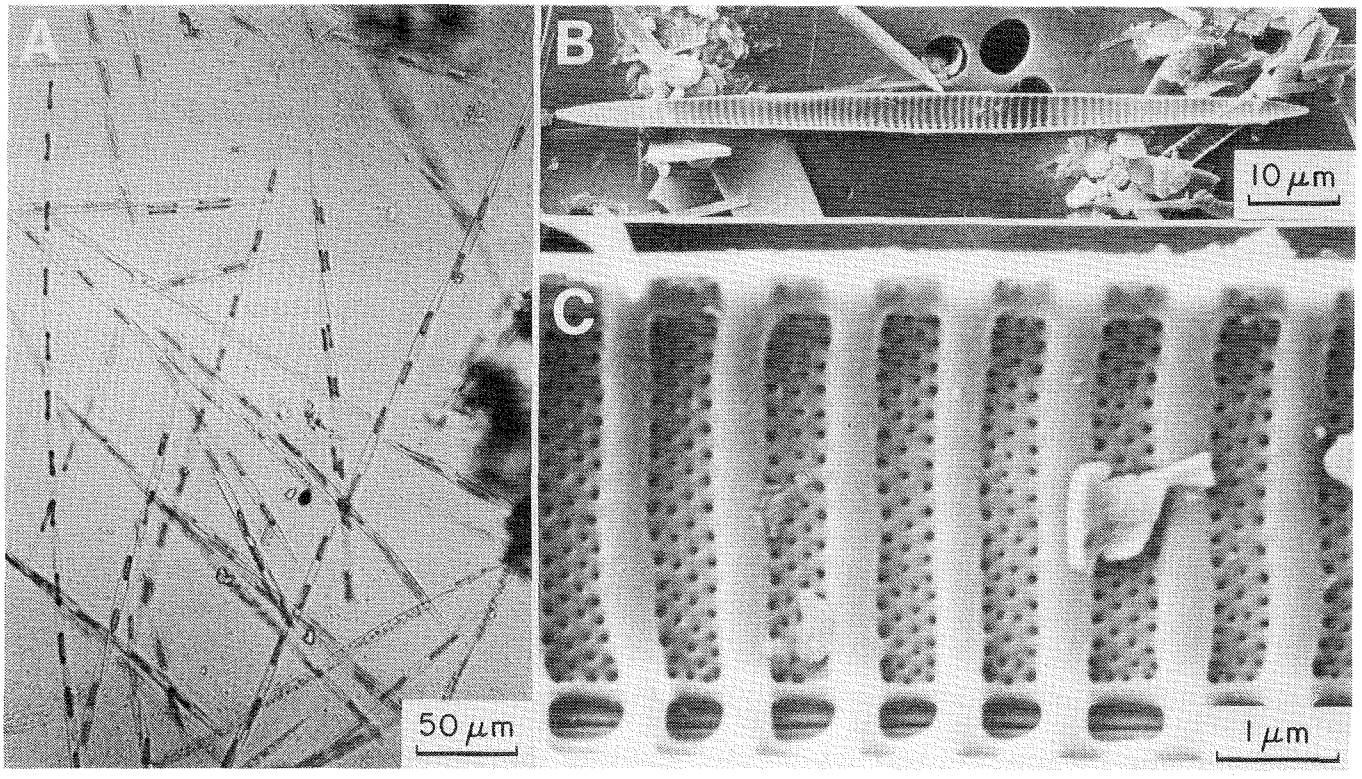


Fig. 8. Nitzschia pungens f. multiseries from the Cardigan River: (A) light photomicrograph showing chains of N. pungens; (B) scanning electron micrograph of a single valve showing the costae; and (C) same valve at higher magnification, showing the rows of poroids between the costae.

Rhizosolenia Brightwell became increasingly common. By the end of February, N. pungens was practically absent, while Rhizosolenia remained dominant, accompanied by minor amounts of other centric diatoms and Fragilaria Lyngbye. A virtually monospecific, very low-density population of N. pungens was also observed in Boughton River in early January, and the Murray River plankton contained a smaller but still significant percentage of this diatom at about the same time (Table 2). Brief descriptions of the plankton in the Cardigan River and other nearshore waters of P.E.I. are given in Appendix A.

During the sampling period, the number of cells of N. pungens per litre in the Cardigan system declined from ca. 8 X 10^6 in mid-December, to 2 X 10^6 in mid-January, to 1 - 5 X 10^4 in mid-February. On December 20, 1987, the bloom extended ca. 10 km seaward from the mouth of the Cardigan River (Fig. 6), from Howe Point (46°18'N) in the north to Cody Point (46°05'N) in the south, at which limits low (5,450 cells L^{-1}) to trace (55 cells L^{-1}) concentrations of the diatom occurred. The decrease in bloom intensity of N. pungens, concomitant with the decline in its relative abundance, was associated with a marked decrease in the concentration of total planktonic biomass. We emphasize, however, that the relative abundance of N. pungens reported here should not be construed as an accurate indicator of relative biomass, especially after mid-January when the

plankton became increasingly dominated by diatoms much larger than N. pungens.

Domoic Acid in the Plankton

Summaries of plankton sample analyses are presented in Tables 2 and 3. Domoic acid was detected only in samples from a contiguous 30-km coastal region of eastern P.E.I. drained by the Cardigan, Murray and Boughton river systems (Fig. 6). The domoic acid concentration was highest (3,000 to 12,000 μg domoic acid g^{-1} dry weight) in samples collected from the Cardigan River in December, 1987, and early January, 1988, and declined thereafter (Table 3). Much lower levels (90 to 1,560 μg domoic acid g^{-1} dry weight) were found in samples from the Murray and Boughton rivers collected during December and January. The highest concentrations of domoic acid were found in samples which contained the highest abundance of N. pungens cells. The decrease in domoic acid g^{-1} dry weight with time after mid-January was coincident with a decrease in both the absolute and relative abundance of N. pungens. This is illustrated by a positive correlation between the concentration of domoic acid and the number of N. pungens cells in the net tow samples (Fig. 9).

It should be noted that the Murray River sample, collected on December 31, 1987, had a low concentration of domoic acid (90 μg domoic acid g^{-1} dry weight), in spite of having a relatively high relative abundance (70%) of N. pungens. However, the number of N. pungens cells was extremely low in

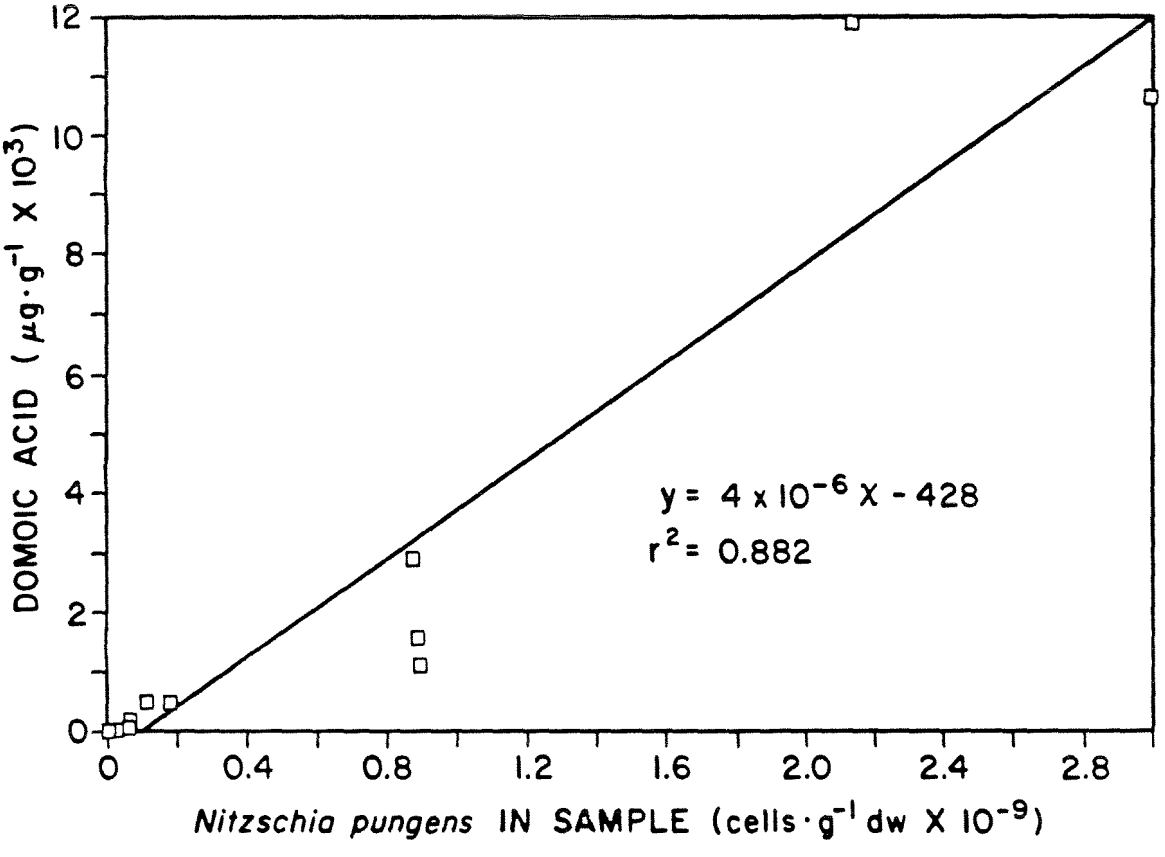


Fig. 9. Relationship between the concentration of domoic acid and the number of Nitzschia pungens cells from net plankton tow samples from the Cardigan River system.

TABLE 3. Domoic acid content of plankton tow samples from eastern Prince Edward Island as related to the presence of Nitzschia pungens.

		N. pungens IN PLANKTON		DOMOIC ACID IN PLANKTON		DOMOIC ACID IN *N. pungens*	TOTAL CARBON IN SAMPLE	*N. pungens* CARBON IN SAMPLE	CARBON ACCOUNTED FOR BY *N. pungens*
		A	B	C	D	E=C/B	F	G=B x f[1]	H=G/F
LOCATION	DATE	ABUNDANCE (%)	(CELLS/g d.w.) (X 10^6)	(µg/g d.w.)	(µg/g CARBON)	(pg/cell)	(%)	(%)	(%)
Cardigan River	16-Dec-87	>99	870	2890	19300	3.3	15	6.1	41
Cardigan River[2]	20-Dec-87	100	2990	10620	48300	3.6	22	21.0	95
Murray River	31-Dec-87	69	32	90	1170	2.9	8	0.2	3
Cardigan River	1-Jan-88	>99	2130	11900	56700	5.6	21	15.0	71
Boughton River	2-Jan-88	93	885	1560	7100	1.8	22	6.2	18
Cardigan Bay	7-Jan-88	98	890	1110	7380	1.2	15	6.2	42
Seal River	18-Jan-88	77	64	170	1140	2.7	15	0.5	3
Seal River	24-Jan-88	43	177	460	2100	2.6	22	1.2	6
Seal River	27-Jan-88	26	110	500	3330	4.5	15	0.8	5
Seal R. bridge	14-Feb-88	12	60	40	210	0.6	18	0.4	2
Cardigan dump	14-Feb-88	0	0	1[3]	N/A[4]	N/A	18	N/A	N/A
Cardigan bridge	14-Mar-88	<1	25	5	20	0.2	22	0.2	1

[1] f has the value of 70 pg carbon per cell, determined by converting cell volume measurements of Nitzschia pungens to carbon values (Strathman, 1967).

[2] A second plankton tow sample collected ca. 10 Km offshore from the Cardigan River, had a N. pungens abundance of >99% and contained 1600 µg domoic acid ml^{-1} of plankton concentrate.

[3] At the detection limit of the technique.

[4] N/A = not applicable.

this sample, being less than 0.01% the concentration of N. pungens in the sample collected only one day later from the Cardigan River (Table 3). The Murray River sample consisted mostly of inorganic sediment particles with a relatively low carbon content of 8%, of which only about 3% was accounted for by N. pungens biomass (Table 3). Other diatom genera such as Thalassiosira Cleve, Navicula Bory and Rhizosolenia were present (Appendix A), and could have accounted for most of the carbon in the sample. The carbon content of all the other samples was much higher and showed relatively little variation, ranging from 15% to 22% (Column F, Table 3). But, as expected, the proportion of carbon accounted for by N. pungens decreased during December, 1987 to March, 1988, from a high value of 95% to low values of 3% or less (Column H, Table 3), dependent on the number of N. pungens cells in the sample.

Estimates of domoic acid per cell, obtained by assigning the domoic acid content of the sample to the number of cells in the sample, resulted in remarkably similar values, ranging from 1 to 6 pg domoic acid per cell for samples collected over the first six week period from mid-December, 1987, to the end of January, 1988 (Column E, Table 3). Samples collected in February and March had much lower values. However, the error associated with these latter values is probably quite large owing to the very low incidence of N. pungens, and the correspondingly low levels of domoic acid in

these samples. We note that a domoic acid content of 3 pg per cell represents a very large intracellular pool (2 to 3% of the total cellular carbon) of a simple metabolite, particularly in the case of a diatom the size of N. pungens. In the same context, mussels feeding mainly on N. pungens containing this much domoic acid would readily accumulate sufficient toxin in their digestive glands to account for the levels reported in Table 1.

DISCUSSION

In any outbreak of food poisoning, establishing the initial source of the poisonous food component is a difficult task in itself. Identifying the toxin is more arduous, even though toxic-specific symptomology is well documented for many substances. The more difficult and often unsatisfactorily resolved part of the problem is establishing the initial source of the toxin.

The toxic shellfish incident documented in this report is unique in that it is the first known occurrence of human intoxication due to ingesting domoic acid. In this case, the toxic agent was identified rapidly (Wright et al., 1988). This information was crucial in deciding that the toxicant was not man-made or of terrestrial origin, but was a naturally-occurring compound most likely produced by one or more organisms in the marine food web.

Marine Food Web Sources

The major food items consumed by mussels are phytoplankton and detrital organic matter such as terrigenous particulate organic material and tissue fragments of aquatic macrophytes. The dense bloom of phytoplankton in the affected area suggested that phytoplankton may have acted as the contaminated food vector that caused the accumulation of toxic levels of domoic acid in the mussels. However, there was no evidence in the literature that domoic acid was produced by any

phytoplankton species. On the other hand, two species of benthic macroalgae (Alsidium corallinum and Chondria armata) were known to contain domoic acid (Takemoto and Daigo, 1958; 1960; Impellizzeri et al., 1975). The available evidence on their distribution rules out both species as potential sources of domoic acid in eastern P.E.I. However, a related species, Chondria baileyana, is found in Malpeque Bay on the northern coast of P.E.I. (Fig. 6) and similar embayments in the southern Gulf of St. Lawrence.

It should be kept in mind that bacteria or fungi could also be involved in domoic acid production. These organisms play a central role in habitat ecology, not only as decomposers but also as important sources of dissolved organic compounds. Such compounds could act as growth stimulants or as synergists in the de novo production of domoic acid by macroalgae as well as by phytoplankton. The following discussion deals with our results on macroalgae and phytoplankton as possible sources for the domoic acid in the contaminated mussels.

Possible Macroalgal Sources

Dried herbarium specimens (NRCC 6058) of C. baileyana, collected in 1973 from Malpeque Bay (Fig. 6), contained detectable amounts of domoic acid (Bird et al., 1988). This alga has not been recorded from eastern P.E.I., probably for lack of observation, as apparently suitable habitat occurs there. However, owing to its rarity in the Gulf of St.

Lawrence and its relatively small size (filiform, <15 cm tall), C. baileyana is unlikely to achieve sufficient biomass in the affected estuaries to form a major component of the mussel diet. In the Gulf, this species is thermally limited to one, occasionally two, generations per year (Novaczek et al., 1987) and, despite its ability to perennate via basal residua, an abnormally large biomass would require several years to develop. An early, low-level toxicity would probably have become apparent at least a year before the 1987 episode. Moreover, such a large well-established population would have been visible on the mussel culture systems, which provided a significant increase in the amount of solid substrata available for colonization by algae.

Although we could not sample the systems extensively, the absence of C. baileyana from toxic mussels and culture netting suggests that a large population of this alga had not developed in the affected area. Even had large quantities of C. baileyana been present, it is unlikely that the mussels could have acquired all their domoic acid content by direct consumption of algal detritus and spores. Autumn dieback of C. baileyana occurs by progressive cellular senescence and decay in situ, and fragments containing intact cells would not be liberated at any given time in sufficient quantity for capture by mussels to account for the high concentrations observed. Similarly, domoic acid released into the water by cellular disintegration of C. baileyana would be available at

only extremely low levels for direct uptake by shellfish or plankton. In principle, however, the water vector could be important, since mussels process huge volumes of water relative to their body size (Drinnan, 1964). This is probably not the case with domoic acid, particularly in view of its high solubility in water (Falk and Walter, in preparation), which indicates that its association with, and transport across, lipophylic biological membranes would not be an efficient process.

It is quite probable that other macroalgae produce domoic acid, particularly species of the genus Chondria. However, we can conclude with certainty that macroalgae were not the major source of domoic acid in the Cardigan River region.

Possible Phytoplankton Sources

It is clear from our results that appreciable amounts of domoic acid must have been present in the affected area as early as middle to late October, probably reaching maximum values during November and December, 1987. The total amount of domoic acid in all cultivated mussels at this time, estimated at about 6 kg (Wright et al., 1988), must have represented only a very small fraction (probably 1% or less) of the total domoic acid production in the affected area. We can expect, therefore, that the total production of domoic acid could have been as much as 1000 kg or more. The only obvious potential source of this much domoic acid was the intense bloom of phytoplankton dominated by the pennate

diatom Nitzschia pungens Grunow f. multiseries Hasle. This species has a broad temperature tolerance and is distributed world-wide (Hasle, 1965), but there are no reports of it forming dense unialgal blooms.

Our evidence on the spatial and temporal relationships between the abundance of N. pungens and the toxic levels in mussels provides strong circumstantial evidence that this diatom was the source of the domoic acid. When injected into mice, extracts of the phytoplankton tow samples produced symptoms identical to those produced by extracts of toxic mussels. In addition, the domoic acid levels in these plankton samples were often in excess of 1% dry weight, sufficient to account for the levels observed in toxic mussels.

In building a case for N. pungens as the source, it should also be noted that all toxic mussels harvested from the Cardigan in December, 1987, showed engorged digestive glands containing identifiable shell fragments of N. pungens. This was also true of toxic mussels harvested in October and November, but no information is available on the plankton composition in the Cardigan at that time. However, phytoplankton samples collected in the Boughton River estuary from early September to mid-November showed a relatively high abundance of N. pungens (Johnson, 1988). We can speculate that if a similar abundance of this diatom was present in the Cardigan River estuary in September, it could have developed

into a dense bloom by late October. Assuming that this was the case, it would still have to be explained how an exceptionally dense unialgal bloom of N. pungens could have been initiated and then maintained over the three-month period from October to December, 1987. No anomalous oceanographic conditions in eastern P.E.I. preceded the bloom (Drinkwater and Petrie, 1988). The summer weather was somewhat atypical, however, with very low rainfall in July and August. These dry conditions could have caused higher than normal levels of dissolved nutrients in the freshwater runoff during the sporadic heavy rain storms of autumn. Also, very few herbivores were found in the plankton samples, which might indicate that the insecticidal properties of domoic acid (Maeda et al., 1984) may have contributed to the continuance of the N. pungens bloom by inhibiting grazing by zooplankton.

In spite of these unknowns, the above discussion establishes a strong case in favour of N. pungens being the major source of domoic acid. This conclusion is strengthened even further by (a) the detailed analysis of the plankton samples (Table 3), which demonstrates clearly that the domoic acid content of plankton samples from the affected area must have been associated with the N. pungens component of the plankton, and (b) the demonstration of de novo production of domoic acid by laboratory cultures of N. pungens isolated

from the affected area (Subba Rao *et al.*, 1988; Bates *et al.*, in preparation).

A number of other diatom species present in the Cardigan River, including *Nitzschia seriata*, were also isolated, but failed to produce domoic acid in culture (Bates *et al.*, in preparation). Results from the St. Andrews Biological Station (J. Martin, personal communication) and from the University of Rhode Island (Y. Shimizu, personal communication) indicate that other microalgae, including an isolate of the pennate diatom tentatively identified as *Amphora coffaeformis* (Ag.) Kütz. from the Cardigan River, also produce domoic acid. However, we have been unable to observe domoic acid production in two other strains of *Amphora coffaeformis* (211M and 47M), or in 13 species of *Nitzschia* from the Provasoli-Guillard Center for Culture of Marine Phytoplankton (Bates *et al.*, in preparation).

CONCLUSIONS

From this investigation we conclude that the pennate diatom, *Nitzschia pungens*, was the source of the domoic acid contained in the digestive glands of toxic mussels from the Cardigan River region of P.E.I. In view of the global distribution of *N. pungens*, and the possibility that there may be other sources of domoic acid, contamination of

shellfish by this toxin could be wide-spread. We consider that it may be advisable to establish a world-wide monitoring program in conjunction with a comprehensive research program on its production and modes of action.

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REFERENCES

Anderson, D.A., and M.M. Morel. 1979. Toxic dinoflagellate blooms in the Cape Cod Region of Massachusetts, p. 145-150. In: D.L. Taylor and H.H. Seliger (eds.), Toxic dinoflagellate blooms. Elsevier/North-Holland, New York.

Bird, C.J., R.K. Boyd, D. Brewer, C.A. Craft, A.S.W. de Freitas, E.W. Dyer, D.J. Embree, M. Falk, M.G. Flack, R.A. Foxall, C. Gillis, M. Greenwell, W.R. Hardstaff, W.D. Jamieson, M.V. Laycock, P. Leblanc, N.I. Lewis, A.W. McCulloch, G.K. McCully, M. McInerney-Northcott, A.G. McInnes, J.L. McLachlan, P. Odense, D. O'Neil, V.P. Pathak, M.A. Quilliam, M.A. Ragan, P.F. Seto, P.G. Sim, D. Tappen, P. Thibault, J.A. Walter, J.L.C. Wright, A.M. Backman, A.R. Taylor, D. Dewar, M. Gilgan, and D.J.A. Richard. 1988. Identification of domoic acid as the toxic agent responsible for the P.E.I. contaminated mussel incident. Atlantic Research Laboratory Technical Report #56, NRCC #29083.

Boyle, J.A., and J.D. Pickett-Heaps. 1984. Valve morphogenesis in the pennate diatom Achnanthes coarctata. J. Phycol. 20: 563-573.

Couturier, C. 1988. Shellfish toxins aplenty. Bull. Aquaculture Assn. Can. 88-2: 11-27.

Debonnel, G., L. Beauchesne, and C. de Montigny. 1988. The "mussel toxin", domoic acid, produces its neurotoxic effect through kainate receptor activation: An electrophysiological study in the rat dorsal hippocampus. Can. J. Physiol. Pharmacol. In press.

Drinkwater, K., and B. Petrie. 1988. On the physical oceanography of the Cardigan Bay region of PEI: A report related to the toxic mussel problem of 1987. Canadian Technical Report of Hydrography and Ocean Sciences, Department of Fisheries and Oceans. In press.

Drinnan, R.E. 1964. An apparatus for recording the water-pumping behaviour of lamellibranchs. Netherlands J. Sea Res. 2: 223-232.

Edelstein, T., C.J. Bird, and J. McLachlan. 1974. Tetrasporangia and gametangia on the same thallus in the red alga Cystoclonium purpureum (Huds.) Batt. and Chondria baileyana (Mont.) Harv. Br. Phycol. J. 9: 247-250.

Falk, M. 1988. The infrared spectrum of domoic acid. Can. J. Spec. In press.

Hasle, G.R. 1965. Nitzschia and Fragilariopsis species studied in the light and electron microscopes. II. The group Pseudonitzschia. Videnskaps-Akademi Skr. I. Mat.-Naturv. Klasse. Ny Serie, No. 18.

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

Johnson, K.J. 1988. The phytoplankton community structure of two estuaries in eastern Prince Edward Island. Honours Certificate in Biology Thesis. Mount Allison Univ., New Brunswick.

McLachlan, J. 1977. Effects of nutrients on growth and development of embryos of Fucus edentatus Phy. (Plaeophyceae, Fucales). Phycologia 16: 329-338.

Maeda, M. T.,T. Kodama, T. Tanaka, Y. Ohfune, K. Nomoto, K. Nishimura, and T. Fujita. 1984. Insecticidal and neuromuscular activities of domoic acid and its related compounds. J. Pesticide Sci. 9: 27-32.

Moore, M.N. 1985. Cellular responses to pollutants. Mar. Pollution Bull. 16: 134-139.

Novaczek, I. C.J. Bird, and J. McLachlan. 1987. Phenology and temperature tolerance of the red algae Chondria baileyana, Lomentaria baileyana, Griffithsia globifera, and Dasya baillouviana in Nova Scotia. Can. J. Bot. 65: 57-62.

Parker, M., and P. Tett (eds.). 1987. Exceptional plankton blooms. Rapp. P.-v. Réun. Cons. int. Explor Mer. Vol. 187. Conseil International pour l'éxploration de la Mer, 114 p. Copenhagen.

Perl, T.M., L. Bedard, T. Kosatsly, J. Hockin, and R.S. Remis. 1988. Gastrointestinal and neurological illness related to mussels from Prince Edward Island: A new clinical syndrome associated with domoic acid. Epidemic Intelligence Service Conference, Center for Disease Control, Atlanta, Georgia, 19 April, 1988 (unpublished).

Quilliam, M.A., P.G. Sim, A.W. McCulloch, and A.G. McInnes. 1988a. High performance liquid chromatography of domoic acid, a marine neurotoxin. Can. J. Chem. (submitted).

Quilliam, M.A., P.G. Sim, A.W. McCulloch, and A.G. McInnes. 1988b. Determination of domoic acid in shellfish tissue by HPLC. Atlantic Research Laboratory Technical Report #55, NRCC #29015.

Strathman, R.R. 1967. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol. Oceanogr. 12: 411-418.

Subba Rao, D.V., M.A. Quilliam, and R. Pocklington. 1988. Domoic acid - a neurotoxic amino acid produced by the marine diatom N. pungens in culture. Can J. Fish. Aquat. Sci. In press.

Takemoto, T., and K. Daigo. 1958. Constituents of Chondria armata. Chem. Pharm. Bull. 6: 578-580.

Takemoto, T., and K. Daigo. 1960. Uber die Inhaltsstoffe von Chondria armata und ihre pharmakologische Wirkung. Archiv der Pharmazie. 293: 627-633.

Taylor, D.L., and H. H. Seliger (eds). 1979. Toxic dinoflagellate blooms, 505 p. Elsevier/North-Holland. New York.

Thibault, P., M.A. Quilliam, W.D. Jamieson, and R.K. Boyd. 1988. Mass spectrometry of domoic acid. Biomedical Environ. Mass. Spec. In press.

White, A.W. 1987. Relationships of environmental factors to toxic blooms in the Bay of Fundy. Rapp. P.-v. Réun. Cons. int. Explor. Mer 157: 5-8.

Wright, J.L.C., R.K. Boyd, A.S.W. de Freitas, M. Falk, R.A. Foxall, W.D. Jamieson, M.V. Laycock, A.W. McCulloch, A.G. McInnes, P. Odense, V.P. Pathak, M.A. Quilliam, M.A. Ragan, P.G. Sim, P. Thibault, J.A. Walter, M. Gilgan, D.J.A. Richard, and D. Dewar. 1988. Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from Eastern Prince Edward Island. Can. J. Chem. (submitted).

APPENDIX A: General Description of Plankton Samples

I. Cardigan River system

Cardigan R., 16 Dec. 1987. Heavily dominated by Nitzschia pungens. Only a few other diatom taxa present, and these represented by relatively few individuals: Pleurosigma, Gyrosigma, Navicula, Melosira and a non-colonial Nitzschia. Moderate quantity of sediment and detritus including vascular plant debris, a few tintinnid loricae, Calothrix crustacea (Cyanophyta) and dead diatom frustules.

Cardigan R., 20 Dec. 1987. Virtually all N. pungens, with trace quantities of other diatoms: Navicula, Licmophora, Pleurosigma, Achnanthes, sigmoid Nitzschia, Rhizosolenia and Chaetoceros. Dinoflagellates rare: Ceratium, Dinophysis, Prorocentrum, and others. Some floc and amorphous organic debris, also a few tintinnid loricae.

Cardigan R., 1 Jan. 1988. Almost entirely N. pungens, with rare to occasional individuals of Navicula, Licmophora, Fragilaria, Pleurosigma, Thalassiosira, Rhizosolenia and Coscinodiscus, Dinophysis and other dinoflagellates.

Cardigan Bay, 7 Jan. 1988. Nitzschia pungens constitutes nearly all the diatom flora. Small numbers of Pleurosigma, Gyrosigma, Fragilaria, Achnanthes, Licmophora, and other Nitzschia, Rhizosolenia, Melosira and small diatoms. A few dinoflagellates and skeletons of the silicoflagellate Distephanus speculum.

Seal R., 18 Jan. 1988. Nitzschia pungens dominant, followed by Rhizosolenia. Other diatoms, in markedly lesser quantity: Pleurosigma, Navicula, Licmophora, Fragilaria, non-colonial Nitzschia, Thalassionema, Melosira, Chaetoceros, other centric species. Moderate amount of detritus.

Seal R., 23 Jan. 1988. Dominated in aspect by Rhizosolenia and a small centric diatom, but N. pungens numerically equivalent. Other, occasional diatom genera: Navicula, Licmophora, Pleurosigma, Thalassionema, Fragilaria, Thalassiosira, Melosira, Coscinodiscus. Fragments of filamentous algae rare: Microcoleus lyngbyaceus (Cyanophyta), an ulotrichalean chlorophyte, and acrochaetioid rhodophytes. Occasional Dinophysis and other dinoflagellates. Moderate amount of detritus including protoplasm of broken Rhizosolenia and fragments of small crustacea.

Seal R., 24 Jan. 1988. Ostensibly dominated by Rhizosolenia, but considerable N. pungens still present. Other diatoms: Fragilaria, Melosira, Thalassiosira, Coscinodiscus, other centric species. Dinophysis rare; small crustacea occasional.

Seal R., 27 Jan. 1988. Rhizosolenia dominant, N. pungens noticeably less abundant than previously. Other diatoms: Fragilaria, Navicula, Pleurosigma, Licmophora, Melosira, Thalassiosira, Coscinodiscus, other centric species.

Dinoflagellates and small crustacea occasional. Detritus includes fragments of filamentous Phaeophyta.

Upper Seal R., 13 Feb. 1988. Dominated by Licmophora (a different species from that of the estuarine sites). Other diatoms present: Nitzschia, Fragilaria, Gomphonema, Striatella (?), Pleurosigma, Synedra, Melosira. Other organisms: Pandorina, Pediastrum, Closterium (Chlorophyta); Synura and Tribonema (Chrysophyta).

Seal R., 14 Feb. 1988. Rhizosolenia dominant, and N. pungens relatively uncommon. Other diatoms: Fragilaria, Pleurosigma, other Nitzschia spp., Licmophora, Navicula, Chaetoceros, Thalassiosira, Biddulphia, Melosira, Coscinodiscus, small centric species. A few crustacea, dinoflagellates and fragments of filamentous algae.

Dump site, 14 Feb. 1988. Dominated by a broad Fragilaria. Other diatoms: Melosira, Licmophora, non-colonial Nitzschia, Synedra, Epithemia, other pennate species. Among other organisms: fragments of Rhizoclonium (Chlorophyta), fungal hyphae, ostracods. Much floc, mineral chips and organic debris, including protozoan loricae.

Seal River, 26 Feb. 1988. Dominants: Rhizosolenia and a small centric diatom. Fragilaria occasional. Also present: N. pungens (rare), Pleurosigma, Navicula, other pennate diatoms, Melosira, dinoflagellates. Detritus moderate, including contents of broken diatoms and fragments of small crustacea.

Seal River, 9 Mar. 1988. Similar to preceding sample, but with less Fragilaria. Thalassionema, Pleurosigma, N. pungens (rare), sigmoid Nitzschia, and other pennate diatoms.

Cardigan River bridge, 14 Mar. 1988. Presence of Chaetoceros, Cylindrotheca, Amphiprora, Ulothrix, Navicula, a low frequency of Nitzschia pungens and Nitzschia seriata, and other large and small pennate diatoms.

II. Other sites

Murray River, 31 Dec. 1987. Nitzschia pungens dominant. Other diatom genera: Thalassionema, Licmophora, Pleurosigma, Navicula, Thalassiosira, Rhizosolenia. Few other biota present, but much sediment and detritus.

Boughton River, 2 Jan. 1988. Nitzschia pungens dominant. Few other diatoms present: Licmophora, Pleurosigma, Navicula, Thalassionema, Thalassiosira.

Hillsborough River, 6 Jan. 1988. Dominated by Rhizosolenia, with considerable Thalassiosira as well. Other diatoms: Biddulphia, Chaetoceros, Skeletonema, Coscinodiscus, Melosira, naviculoid species.

St. Peters Bay, 11 Jan. 1988. Mostly small crustacea and tintinnid loricea. Little else present: Fragilaria, Coscinodiscus, and a few skeletons of Distephanus speculum.

Tracadie Bay, 12 Jan. 1988. Dominated by Biddulphia, tintinnid loricae and Distephanus speculum skeletons. Other, occasional diatom genera: Pleurosigma, Thalassionema, and

Coscinodiscus. Dinophysis and other dinoflagellates rare to occasional.

New London Bay, 13 Jan. 1988. Mostly small crustacea, tintinnid loricae, Biddulphia, Melosira. Also, considerable Dinophysis. Other components: Licmophora, Thalassionema, Pleurosigma, non-colonial Nitzschia, Fragilaria, Achnanthes, naviculoid diatoms, Coscinodiscus, Rhizololenia (fragments), dinoflagellates, fragments of Erythrotrichia carnea (Rhodophyta), Distephanus skeletons.

Alberton, 15 Jan. 1988. Almost entirely of small crustacea. A few Melosira, Coscinodiscus, Thalassionema and other pennate diatoms.

Bentick Cove (Malpeque Bay), 15 Jan. 1988. Dominated by small crustacea and tintinnid loricae. Other components sparse, mostly the filamentous diatom Leptocylindrus, and Distephanus skeletons. Dinoflagellates rare, also Licmophora, Fragilaria and Thalassionema.

Foxley River, 16 Jan. 1988. Dominated by small crustacea, detrital plant fragments and tintinnid loricae. Diatom flora sparse but varied: Fragilaria, non-colonial Nitzschia, Thalassionema, Licmophora, Striatella (?), Navicula and other pennate species: Melosira, Lithodesmium (?), Biddulphia, Leptocylindrus. A few Dinophysis present and, rarely, fragments of Microcoleus lyngbyaceus (Cyanophyta).

Grand River (Malpeque Bay), 17 Jan. 1988. Small crustacea and tintinnid loricea the major components. Other biota

sparse: Biddulphia, Melosira, Leptocylindrus, Licmophora and naviculoid diatoms; dinoflagellates; fragments of Pilayella littoralis (Phaeophyta) and other filamentous algae; Distephanus skeletons.

Kildare River, 18 Jan. 1988. Mostly detritus and sediment. Traces of N. pungens. Other diatom flora sparse but varied: Licmophora, Cocconeis, non-colonial Nitzschia, Gyrosigma, Amphora, Amphiprora, Thalassionema, Synedra, naviculoid species, Skeletonema, Biddulphia, Melosira, Chaetoceros. Also, fragments of Spirulina subsalsa (Cyanophyta), ulotrichalean sporelings (Chlorophyta), and Distephanus skeletons.