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Proceedings of the Fourth Canadian Workshop on Harmful Marine Algae

J.R. Forbes [ed.]

Institute of Ocean Sciences
Sidney, B.C., V8L 4B2

1994

Canadian Technical Report of Fisheries and Aquatic Sciences 2016

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Abstract

Forbes, J.R. [ed.]. 1994. Proceedings of the Fourth Canadian Workshop on Harmful Marine Algae. Can. Tech. Rep. Fish. Aquat. Sci. No. 2016: 92 p.

The Fourth Canadian Workshop on Harmful Marine Algae was hosted by the Department of Fisheries and Oceans, Pacific Region, at the Institute of Ocean Sciences, Sidney, B.C., on 3 - 5 May 1994. The workshop was attended by 72 Canadian and foreign participants. The workshop provided a forum for discussion of all issues related to research and management of the effects of harmful algal blooms. There was an increased focus on industry, management, public health and medical issues over past workshops and, for the first time, a review of freshwater Cyanobacteria toxins and microcystins in the marine environment. The report contains abstracts and extended abstracts of oral and poster presentations at the workshop, and reports of four discussion groups dealing with toxin sources, pathways and sinks, aquaculture issues, and human health issues.

Key words: harmful marine algae, red tide, toxins, PSP, DSP, domoic acid, microcystins, aquaculture

Résumé

Forbes, J.R. [ed.]. 1994. Proceedings of the Fourth Canadian Workshop on Harmful Marine Algae. Can. Tech. Rep. Fish. Aquat. Sci. No. 2016: 92 p.

Le quatrième Atelier de travail canadien sur les algues nuisables s'est déroulé du 3 au 5 mai 1994, à Sidney ne C.-B., sous les auspices de la Région du Pacifique du ministère de Pêches et des Océans et de l'Institut des sciences de la mer. Souixante-douze participants du Canada et de l'étranger étaient présents. On y a discuté de toutes les questions liées à la gestion des effets des proliférations d'algues nuisables. On a examiné plus attentivement que lors des derniers ateliers les questions liées à l'industrie, à la gestion, à la santé publique et à la médecine et l'on a, pour le première fois, fait le point sur les microcystines et autres toxines produit par les Cyanobactéries d'eau douce en milieu marin. Le rapport contient des résumés détaillés de présentations orales et graphiques de l'atelier, ainsi que les rapports de quatre groupes de discussion sur les sources, les voies et les puits des toxines, les questions d'aquaculture et les questions de santé humaine.

Mots clés: algues marines nuisables, eaux rouges, toxines, toxine paralysante des coquillages, toxine diarrhéique des coquillages, acide domoïque, microcystines, aquaculture

Acknowledgements

The Fourth Canadian Workshop on Harmful Marine Algae was sponsored by the Department of Fisheries and Oceans through its Phycotoxins Working Group. I thank fellow members of the PWG, in particular those who were involved in the organization of previous workshops, for their advice and assistance.

The advice of the Scientific Advisory Committee consisting of Richard Addison, Edward Black, Rudy Chiang, Max Taylor and Ian Whyte, is appreciated. They provided much assistance in developing the program and in proposing themes and discussion group topics.

Most of the logistical tasks (including correspondence, registration, organization of meeting rooms, arrangements for the workshop dinner, and coordination with the IOS cafeteria) were very efficiently handled by Brenda LaCroix. Thank you Brenda.

I also thank Beth Bornhold, Harold Lloyd, Steve Romaine and Linda White for their help during the meeting.

A financial contribution from the B.C. Ministry of Agriculture, Fisheries and Food assisted in some of the expenses associated with the workshop.

Introduction

Harmful marine algae have a serious impact on the Canadian shellfish and finfish aquaculture industries. For example, mortality of farmed salmon due to *Heterosigma carterae* and *Chaetoceros* spp. have exceeded $4,000,000 per year in British Columbia in a number of years. The Department of Fisheries and Oceans maintains a significant infrastructure for monitoring levels of shellfish toxins, to provide protection to consumers of wild harvest and aquaculture product.

Paralytic shellfish poisoning (PSP) is endemic to most or all of the Pacific coast and to much of Atlantic Canada, including the Bay of Fundy and the Gulf of St. Lawrence. A number of occurrences have been recorded in inlets of Newfoundland since 1982. Recently, expanded monitoring programs have revealed PSP toxins in a variety of molluscs not previously known to be toxic, as well as in lobster hepatopancreas. Amnesic shellfish poisoning (ASP), produced by the toxin domoic acid, was first recognized in farmed mussels from Prince Edward Island in 1987. Since then, both the number of phytoplankton species known to produce domoic acid and the areas affected have increased. These include molluscs in the Bay of Fundy, Scotian shelf, Gulf of Maine and Georges Bank on the east coast, and a number of areas on the west coast, in particular, the west coast of Vancouver Island, the North and Central coasts, and the Queen Charlotte Islands. The first North American record of diarrhetic shellfish poisoning (DSP) was from the south shore of Nova Scotia in 1990. *Dinophysis* spp. and DSP toxins have since also been found in the Bay of Fundy, the Gulf of St. Lawrence, and eastern Newfoundland. *Dinophysis* spp. that have produced DSP problems elsewhere also occur in British Columbia coastal waters.

Toxins of unknown origin have been found in freshwater mussels in the Northwest Territories. While there are no known toxic marine cyanobacteria in Canadian waters, the hepatotoxin, microcystin-LR, has been found in mussels on the Atlantic coast and is considered responsible for netpen liver disease in farmed Pacific salmon.

Canada continues to face new challenges in the management of harmful algal blooms and research activities required to support this. After the first occurrence of domoic acid in 1987 the Department of Fisheries and Oceans established a national advisory body, the Phycotoxins Working Group. Its role is to develop advice on coordinating, planning and setting priorities for research on phycotoxins and other harmful aspects of marine algal blooms, and the organisms producing them. One activity of this Working Group has been to sponsor national workshops on harmful algal blooms, to exchange information on new research results about topics related to harmful algae. This exchange provides significant assistance to the Phycotoxins Working Group in developing its recommendations to the Department on research priorities.

The first workshop was held in Moncton, N.B., in 1989 (Bates and Worms 1989), the second in Dartmouth, N.S., in 1990 (Gordon 1991), and the third in Mont-Joli, P.Q., in 1992 (Therriault and Levasseur 1992). In addition, a regional workshop on Pacific coast research on harmful algal blooms was held in 1991 (Forbes 1991). This report comprises the proceedings of the fourth national workshop, which was held at the Institute of Ocean Sciences, Sidney, B.C., from 3 - 5 May 1994.

People who presented oral or poster papers at the workshop were requested to submit an abstract or extended abstract. These have been collated as submitted, with only minor editing to ensure consistent style and, in a few cases, consistent and current taxonomy.

Workshop Proceedings:

Bates, S.S., and J. Worms [ed.]. 1989. Proceedings of the First Canadian Workshop on Harmful Algal Blooms, Gulf Fisheries Centre, Moncton, N.B., September 27 - 28, 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1712: 58p.

Gordon, D.C. Jr. [ed.]. 1991. Proceedings of the Second Canadian Workshop on Harmful Marine Algae, Bedford Institute of Oceanography, Dartmouth, N.S., October 2 - 4, 1990. Can. Tech. Rep. Fish. Aquat. Sci. 1799: 66p.

Therriault, J.-C., and M. Levasseur [ed.]. 1992. Proceedings of the Third Canadian Workshop on Harmful Marine Algae. Can. Tech. Rep. Fish. Aquat. Sci. 1893: 154p.

Forbes, J.R. [ed.]. 1991. Pacific coast research on toxic marine algae. Can. Tech. Rep. Hydrogr. Ocean. Sci. 135: 76p.

Controlling fish farm losses to algal events

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Frequently, algal events cause multi-million dollar losses in the salmon farming industry in British Columbia. The same is true of marine fish culture in many other jurisdictions, including Japan, Chile, Norway, New Zealand and Scotland. In spite of the widespread occurrence of the problem no systematized approach to preventing these losses has been developed for marine fish culture.

An integrated approach for managing the effects of algal blooms on marine fish farms was discussed. It extends from siting and design of the farm through setting up a plankton watch, to mitigation of bloom effects during the production cycle and disposal of dead fishes. It is the basis for a new publication in the B.C. Salmon Farming Hand Book.

Temporal and spatial distribution of *Gyrodinium aureolum* Hulburt (*Gymnodinium* cf. *nagasakiense*) in the St. Lawrence (Quebec)

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A dense bloom of *Gyrodinium aureolum* (6×10^5 cells.L^{-1}) was observed at Mont-Louis, in the Gaspé Current, on 6 September 1993. Analysis of phytoplankton samples of other areas indicated that *G. aureolum* was often present upstream in the lower estuary, all along the coast of Gaspé and in the Gulf of St. Lawrence during a major part of the summer season.

Taxonomical identification using an immunochemical tagging method indicated that the species found in the St. Lawrence is phenotypically identical to the species found in northern Europe. This is the first time that the presence of *G. aureolum* (*Gymnodinium* cf. *nagasakiense*) has been reported in the St. Lawrence. In European Waters, *G. aureolum* is responsible for major red tides frequently associated with mortality of fish and other marine organisms, and represents a potential threat for young oysters, mussels and sea-scallops.

Toxins of Cyanobacteria

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'Algal bloom' is a term used to describe the proliferation of planktonic algae in aquatic habitats up to millions of cells per liter. This phenomenon is often beneficial for aquaculture, fisheries and in the natural cycles of aquatic organisms. Increasingly, however, algal blooms produce a negative economic and human health effect. It is in this context that the issue of 'harmful algal blooms' (Hallegraeff 1993, Anderson et al. 1993) is receiving increased international attention. Most of the problems with harmful algal blooms occur from marine algae especially the red tide organisms. These algae produce toxins responsible for paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP) and ciguatera fishfood poisoning.

Harmful Cyanobacteria blooms cause the same (Carmichael and Falconer 1993, Carmichael 1992, Pearson 1990) types of problems as do other harmful algae. Their toxins (cyanotoxins) are just as potent as other phycotoxins and some are chemically the same as the paralytic shellfish toxins. There are etiological differences which contribute to our reduced understanding and attention regarding cyanotoxins. They are known almost exclusively from fresh and brackish water Cyanobacteria and only recently have been found in the marine environment. They are, therefore, a threat to animals and humans through drinking water and through the food chain. Cyanotoxins known to cause or be implicated in animal and human poisonings include the neurotoxic alkaloids anatoxin-a(s), saxitoxin and neosaxitoxin (and other PSP toxins) plus the hepatotoxic liver tumor promoting peptide microcystins, nodularins and the hepatotoxic alkaloid cylindrospermopsin (Table 1).

The biotoxins of Cyanobacteria constitute a major source of natural produce toxins that are found in surface supplies of freshwater. Species and strains in all of the common planktonic cyanobacterial genera, including *Anabaena, Aphanizomenon, Microcystis, Nodularia, Nostoc* and *Oscillatoria*, produce biotoxins. Other important genera, including *Coelosphaerium, Cylindrospermopsis, Fischerella, Gloeotrichia, Gomphosphaeria, Hapalosiphon, Schizothrix, Scytonema, Tolypothrix*, and *Trichodesmium*, have been found to be toxic but as yet no toxin has been isolated and characterized from these genera. These cyanotoxins produce intermittent but repeated cases of animal poisoning in many areas of the world. Poisoning cases, known since the late 19th century, involve sickness and death of livestock, pets and wildlife following ingestion of water containing toxic algae cells or the toxin(s) released by the aging cells. No acute lethal poisoning of humans by freshwater Cyanobacteria, such as occurs with paralytic shellfish poisoning, has been confirmed. Humans are probably just as susceptible as other mammals, but people are repelled by the idea of using water containing an algae bloom. Furthermore, there are no known food vectors, such as shellfish, which concentrate toxins of freshwater Cyanobacteria in the human food chain. However, the decreasing water quality and increasing eutrophication of our freshwater supplies mean that large growths or water blooms of Cyanobacteria are becoming more

Table 1

Species of cyanophyceans with recognized toxin-producing strains. The list presents species of cyanophyceans that have been confirmed to have toxin-producing strains (Skulberg et al., 1993).

Species

Unicellular

Coelosphaerium kützingianum Näg.
Gomphosphaeria lacustris Chod.
Gomphosphaeria nägeliana (Unger) Lemm.
Microcystis aeruginosa Kütz *
Microcystis cf. *botrys* Teil.
Microcystis viridis (A. Br.) Lemm. *
Microcystis wesenbergii Kom. *
Synechococcus nägeli sp. (strain Miami BCII 6S)
Synechocystis sauvageau sp.

Multicellular

Anabaena circinalis Rabenh. *+
Anabaena flos-aquae (Lyngb.) Bréb. *+
Anabaena hassallii (Kütz.) Wittr.
Anabaena lemmermannii P. Richt
Anabaena spiroides var. *contracta* Kleb. *+
Anabaena variabilis Kütz *+
Anabaenopsis milleri Woron.
Aphanizomenon flos-aquae (L.) Ralfs *+
Cylindrospermum Kützing sp.
Cylindrospermopsis raciborskii ++ (Wolos.) Seenaya & Subba Raju
Fischerella epiphytica Ghose
Gleoetrichia echinulata (J.E. Smith) P. Richter
Hapalosiphon fontinalis (Ag.) Born.
Hormothamnion enteromorphoides Grun.
Lyngbya majuscula Harvey
Lyngbya wollei Farlow ex Gomont +
Nodularia spumigena Mertens *
Nostoc linckia (Roth) Born. et Flah.
Nostoc paludosum Kütz.
Nostoc rivulare Kütz. *
Nostoc zetterstedtii Areschoug
Oscillatoria acutissima Kuff. *
Oscillatoria agardhii Gom. *+
Oscillatoria agardhii / rubescens group *
Oscillatoria nigro-viridis Thwaites *
Oscillatoria Vaucher sp.
Oscillatoria formosa Bory
(*Phormidium formosum* (Bory) Anagn. & Kom.)

Table 1 continued

Pseudanabaena catenata Lauterb.
Schizothrix calcicola (Ag.) Gom.
Scytonema pseudohofmanni Bharadw.
Tolypothrix byssoidea (Hass.) Kirchn.
Trichodesmium erthraeum Ehrb.

*	known producers of microcystins or nodularins
+	known producers of anatoxins or paralytic shellfish poisons (PSP)
++	produces the hepatotoxic alkaloid cylindrospermopsin

common. Tests on these water blooms are now more frequently positive for the two common groups of cyanotoxins - the alkaloid neurotoxins and the cyclic peptide hepatotoxins. Survey reports over the last few years indicate that a significant number of blooms are toxic in any given area. In the U.S. many states have reported blooms of toxic Cyanobacteria.

Acute hepatotoxicosis involving the hepatotoxins (liver toxins) is the most commonly encountered toxicosis involving Cyanobacteria, These toxins are produced by strains of species within the genera *Microcystis, Anabaena, Nodularia, Oscillatoria*, and *Nostoc*. These hepatotoxins are now known to be a class of cyclic heptopeptide (microcystins) and pentapeptides (nodularin). There are about 50 known microcystins all of which have a similar structure and activity (Table 2). All are potent liver toxins that act by inhibiting certain protein phosphatases.

Table 2

A listing of most known microcystins plus their molecular weight and formula (Carmichael and Falconer 1993; Carmichael unpublished data).

Microcystin	Molecular weight and Formula	
microcystin-LA	909	$C_{46}H_{67}N_7O_{12}$
microcystin-LAba	923	$C_{47}H_{69}N_7O_{12}$
microcystin-AR	952	$C_{49}H_{68}N_{10}O_{12}$
microcystin-YA	959	$C_{49}H_{65}N_7O_{13}$
[D-Asp^3,Dha^7]microcystin-LR	966	$C_{47}H_{70}N_{10}O_{12}$
[D-Asp^3]microcystin-LR	980	$C_{48}H_{72}N_{10}O_{12}$
[Dha^7]microcystin-LR	980	$C_{48}H_{72}N_{10}O_{12}$
[DMAdda^5]microcystin-LR	980	$C_{48}H_{72}N_{10}O_{12}$
microcystin-LR	985	$C_{52}H_{71}N_7O_{12}$
microcystin-LR	994	$C_{49}H_{74}N_{10}O_{12}$
[D-Asp^3,D-Glu(OCH$_3$)^6]microcystin-LR	994	$C_{49}H_{74}N_{10}O_{12}$
[(6Z)-Adda^5]microcystin-LR	994	$C_{49}H_{74}N_{10}O_{12}$
[L-Ser^7]microcystin-LR	998	$C_{48}H_{74}N_{10}O_{13}$
microcystin-LY	1001	$C_{52}H_{71}N_7O_{13}$
microcystin-HilR	1008	$C_{50}H_{76}N_{10}O_{12}$
[D-Asp^3,ADMAdda^5]microcystin-LR	1008	$C_{49}H_{72}N_{10}O_{13}$

Table 2 continued

[D-Glu-OCH_3^6]microcystin-LR	1008	$C_{50}H_{76}N_{10}O_{12}$
[D-Asp^3,Dha^7]microcystin-RR	1009	$C_{47}H_{71}N_{13}O_{12}$
[L-MeSer^7]microcystin-LR	1012	$C_{49}H_{76}N_{10}O_{13}$
[Dha^7]microcystin-LR	1014	$C_{51}H_{70}N_{10}O_{12}$
[ADMAdda^5]microcystin-LR	1022	$C_{50}H_{74}N_{10}O_{13}$
[D-Asp^3,ADMAdda^5]microcystin-LHar	1022	$C_{50}H_{74}N_{10}O_{13}$
[D-Asp^3]microcystin-RR	1023	$C_{48}H_{73}N_{13}O_{12}$
[Dha^7]microcystin-RR	1023	$C_{48}H_{73}N_{13}O_{12}$
microcystin-FR	1028	$C_{52}H_{72}N_{10}O_{12}$
microcystin-M(O)R	1028	$C_{48}H_{72}N_{10}O_{13}S$
[Dha^7]microcystin-HphR	1028	$C_{52}H_{72}N_{10}O_{12}$
[D-Asp^3,Dha^7]microcystin-HtyR	1030	$C_{51}H_{70}N_{10}O_{13}$
[Dha^7]microcystin-YR	1030	$C_{51}H_{70}N_{10}O_{13}$
[D-Asp^3]microcystin-YR	1030	$C_{51}H_{70}N_{10}O_{13}$
microcystin-YM(O)	1035	$C_{51}H_{69}N_7O_{14}S$
[ADMAdda^5]microcystin-LHar	1036	$C_{51}H_{76}N_{10}O_{13}$
microcystin-RR	1037	$C_{49}H_{75}N_{13}O_{12}$
[(6Z)-Adda^5]microcystin-RR	1037	$C_{49}H_{75}N_{13}O_{12}$
[D-Ser^1,ADMAdda^5]microcystin-LR	1038	$C_{50}H_{74}N_{10}O_{14}$
[ADMAdda^5,MeSer^7]microcystin-LR	1040	$C_{50}H_{76}N_{10}O_{14}$
[L-Ser^7]microcystin-RR	1041	$C_{48}H_{75}N_{13}O_{13}$
[D-Asp^3,MeSer^7]microcystin-RR	1041	$C_{48}H_{75}N_{13}O_{13}$
microcystin-YR	1044	$C_{52}H_{72}N_{10}O_{13}$
[D-Asp^3]microcystin-HtyR	1044	$C_{52}H_{72}N_{10}O_{13}$
[Dha^7]microcystin-HtyR	1044	$C_{52}H_{72}N_{10}O_{13}$
microcystin-(H_4)YR	1048	$C_{52}H_{76}N_{10}O_{13}$
[D-Glu-$OC_2H_3(CH_3)OH^6$]microcystin-LR	1052	$C_{52}H_{80}N_{10}O_{13}$
microcystin-HtyR	1058	$C_{53}H_{74}N_{10}O_{13}$
[L-Ser^7]microcystin-HtYR	1062	$C_{52}H_{74}N_{10}O_{14}$
microcystin-WR	1067	$C_{54}H_{73}N_{11}O_{12}$
[L-MeLan^7]m.icrocystin-LR	1115	$C_{52}H_{81}N_{11}O_{14}S$

Aba:	Aminoisobutyric acid
ADMAdda:	*O*-Acetyl-*O*-demethylAdda
Dha:	Dehydroalanine
DMAdda:	*O*-DemethylAdda
(H_4)Y:	1,2,3,4-tetrahydro-tyrosine
Har:	Homoarginine
Hil:	Homoisoleucine
Hph:	Homophenylalanine
Hty:	Homotyrosine
MeLan:	*N*-Methyllanthionine
M(O):	Methionine *S*-oxide
MeSer:	*N*-Methylserine
(6Z)-Adda:	Stereoisomer of Adda at the Δ^6 double bond.

The finding that microcystin activated phosphorylase and new work being done with the marine dinoflagellate diarrhetic shellfish poison (DSP) okadaic acid which showed it to be a potent inhibitor of protein phosphatases type 1 (PP1) and type 2A (PP2A) led Fujiki and his group at the National Cancer Research Institute in Tokyo to investigate the effect of MCYST-LR on protein phosphorylation. This work has shown that several of the microcystins and nodularin inhibit PP1 and 2A in a manner similar to okadaic acid. The inhibition of protein phosphatases by microcystins goes a long way towards explaining a possible mechanism of cytoskeletal alteration by microcystins since it is reasonable that the morphological changes observed in microcystin-treated hepatocytes are a result of increased phosphorylation of cytoskeletal components.

These results also show that microcystin and nodularin, like okadaic acid, are potent tumor promoters since PP1 and PP2A are probably the key enzymes that reverse the action of protein kinase C. It has been shown that the microcystins promote liver tumors *in vivo*.

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A sensitive, microtiter plate-based receptor assay for paralytic shellfish poisoning (PSP) toxins

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Paralytic shellfish poisoning (PSP) represents such a ubiquitous and potentially serious threat to human health that more than 20 countries have either established or proposed regulatory limits for one or more of the causative toxins. The toxins responsible for PSP include a total of 21 derivatives of the parent compound saxitoxin (STX), each exhibiting a different molar specific potency and highly variable relative abundance in natural samples. PSP toxin levels in seafood products are generally estimated using the standard AOAC mouse bioassay, which is a non-specific assay with limited sample throughput, poor precision, and low sensitivity. While this method is still considered to yield the best approximation of total toxicity for regulatory purposes, an acceptable alternative to the mouse bioassay is clearly needed.

We have developed a sensitive, high capacity assay which exploits the highly specific interaction of all PSP toxins with their biological receptor (i.e., voltage dependent sodium channel) that is based on the toxins' function/activity rather than an antigenic determinant. The assay competes PSP toxins present in a reference standard or sample against [^{3}H]STX for receptor sites in a preparation of rat synaptosomes, and is performed in a microtiter plate with wells occluded by membrane filters. Following a 1 h incubation, radioactivity retained on the filters is determined using standard liquid scintillation counting, and results are available within 24 hours.

The half maximal response (IC_{50}) for this assay is 4.96 ± 0.63 nM STX (equivalent to ca. 0.38 ng STX per microtiter plate well), and it has proven effective in testing against crude extracts of several sample matrices, including mussels, clams, oysters, and PSP producing dinoflagellates.

We are currently attempting to adapt the assay to microplate scintillation technology, which will reduce the total assay time to 3-6 h, and plan to implement this assay in monitoring and dockside inspection programs.

Influence of bacteria and bacterial extracts on domoic acid production by *Pseudonitzschia pungens* f. *multiseries*

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Strains of *Pseudonitzschia* (formerly *Nitzschia*) *pungens* f. *multiseries* that were rendered axenic using antibiotic treatment continued to produce the neurotoxin domoic acid (DA) but they invariably produced less toxin than non-axenic parent cultures. Both mixed bacterial populations and individual strains of bacteria were isolated from non-axenic *P. pungens* f. *multiseries* cultures and re-introduced to axenic cultures of the diatom. The results of these treatments ranged from no effect to increases in DA production beyond those observed in the original 'wild' strains. These results demonstrate that there was no irreversible damage to toxin production during preparation of axenic cultures, and that bacteria can have an important role in influencing DA production by *P. pungens* f. *multiseries*.

We have examined the possibility that biochemical toxin elicitors associated with the bacteria might be responsible for enhancing DA production. These experiments were conducted using a bacterial strain that enhanced DA production when introduced into axenic cultures of *P. pungens* f. *multiseries*. Several litres of this bacteria were grown in pure culture, the bacteria were harvested by centrifugation, and extracts were prepared using chemical extraction or mechanical cell disruption. In several cases, molecular weight fractionation was used to separate size classes of the cell-free material associated with the bacterial culture. Introduction of the bacterial extracts at a variety of concentrations into axenic cultures of *P. pungens* f. *multiseries* did not enhance DA production for any of the treatments. Our results suggest a bacterial mechanism of enhancement of toxin production that requires dynamic interaction between the diatoms and live bacteria.

Geographic and temporal variability of domoic acid in samples collected for seafood inspection in British Columbia, 1992 - 1994

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Data from the Department of Fisheries and Oceans monitoring program for algal toxins in seafood has been used to examine the distribution of domoic acid in bivalve shellfish and crabs along the British Columbia coast. The data sources are the DFO mussel monitoring program for marine toxins, commercial batch samples submitted for analysis, and special samples collected in areas of known high toxin levels by the Fisheries Operations branch.

Although domoic acid is found in marine invertebrates along most of the coast of British Columbia (Fig. 1), the majority of positive samples were collected in the Barkley, Clayoquot and Quatsino Sound areas of the west coast of Vancouver Island. Both crabs and bivalves were contaminated in a limited number of events happening in relative synchrony. High levels of domoic acid appeared in crabs in the Quatsino Sound area in late August 1992 and early October 1993. Similarly, bivalves in Barkley Sound and other areas of the west coast of Vancouver Island became contaminated in the first week of September 1992 and in mid-October 1993. There were earlier events on the north coast in 1993. Crabs accumulated much higher concentrations of domoic acid than bivalves, and their depuration rates were much slower (months compared to a few weeks for the majority of bivalves). The proportion of crabs contaminated was also much higher (generally 50 to 80%) than bivalves (generally less than 10% except in Barkley Sound where rates were frequently much higher, including 100% in December 1992) (Table 1).

The information derived from this sampling program can be used to develop hypotheses regarding the sources for domoic acid, and differences in sources or routes of contamination of bivalve shellfish compared to crabs.

Table 1

Proportion of bivalve shellfish and crab samples contaminated with domoic acid in three management areas on the west coast of Vancouver Island (Number of samples positive for domoic acid, total samples analyzed, percentage positive).

	Bivalve shellfish			Crab		
	Positive	Total Samples	%	Positive	Total Samples	%
Area 23 (Barkley Sd.)	79	452	17		None	
Area 24 (Clayoquot Sd.)	25	466	5	11	25	44
Area 27	8	188	4	139	161	86

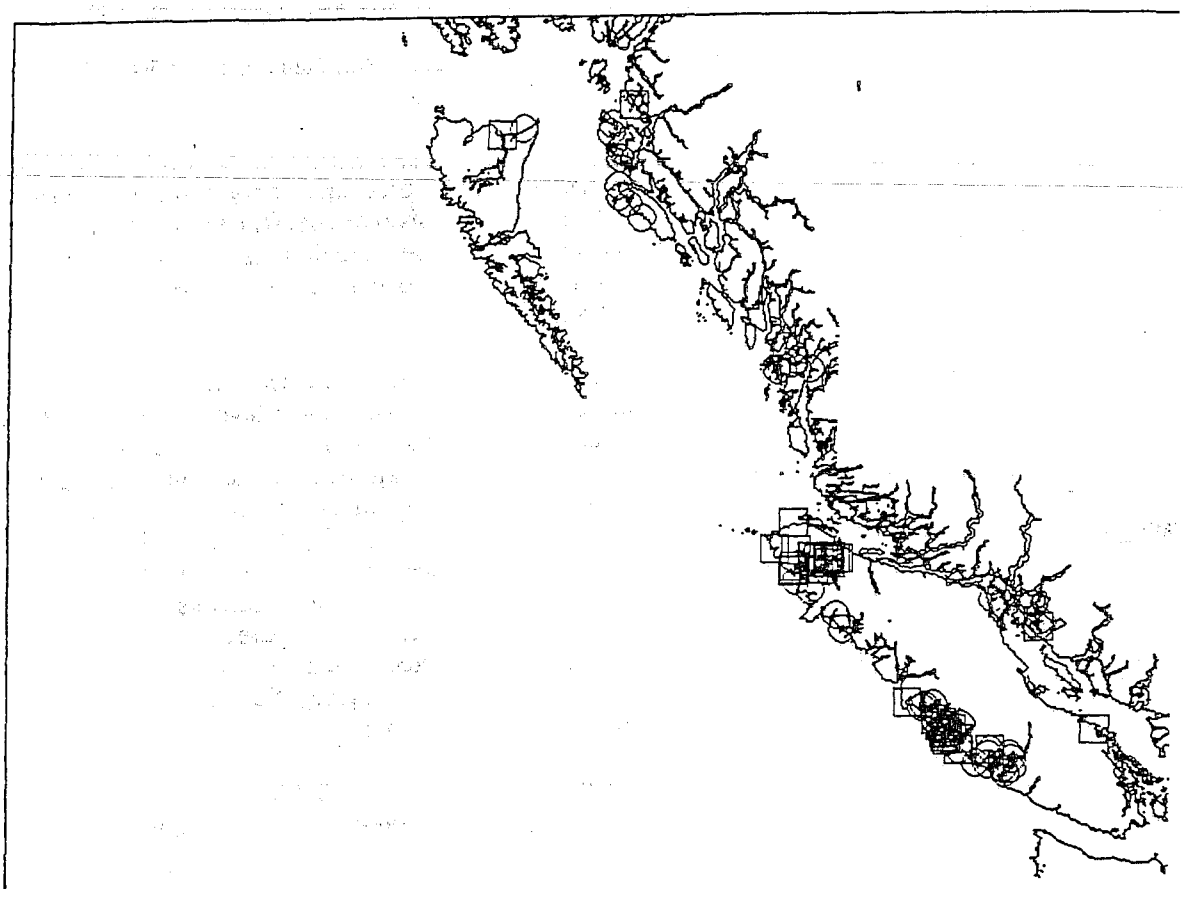

Figure 1

Locations of seafood inspection samples positive for domoic acid. Squares represent crab samples; circles represent bivalve shellfish samples.

The occurrence of a serious diarrhetic shellfish poisoning episode in mussels from Newfoundland during the late Autumn of 1993

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The occurrence of DSP in North American shellfish was first established in 1990 when DTX-1 was established as a contaminant of aquacultured mussels from Mahone Bay, Nova Scotia due to the joint efforts of the Department of Fisheries and Oceans, Inspection and Institute for Marine Biosciences (National Research Council of Canada). The presence and identity of the DTX-1 toxin was confirmed as the sole DSP toxin involved through the use of HPLC/MS.

A similar series of poisonings occurred in Newfoundland during October 1994 related to the consumption of mussels from two separate locations, Alexander Bay (Bloody Reach) and Magics Arm (Fig. 1). Analysis by HPLC-fluorescence showed that the suspect shellfish contained high levels of DTX-1 (up to 4 μg DTX-1.g^{-1} digestive glands), but no detectable okadaic acid. Examination of shellfish from areas in the vicinity of the two toxic episodes established that many of the embayments in an extensive area were contaminated to variable amounts by the toxin.

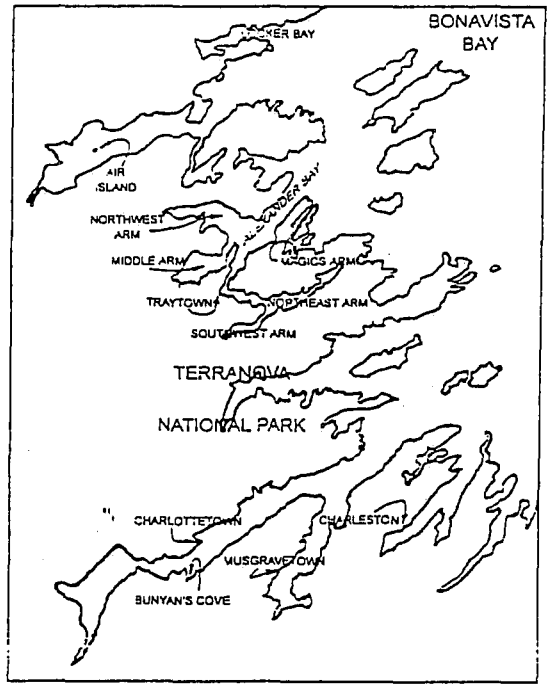

Figure 1

A map indicating most of the sample sites in the vicinity of Bonavista Bay, Newfoundland.

The presence of the DTX-1 was initially confirmed by a positive immunoassay and finally by HPLC/MS. With HPLC/MS the column effluent was monitored for the mass ions 805, 787 and 769 for okadaic acid, and 819, 801 and 783 for DTX-1. As by the HPLC-fluorescence, no okadaic acid was detected by HPLC/MS but DTX-1 was clearly present.

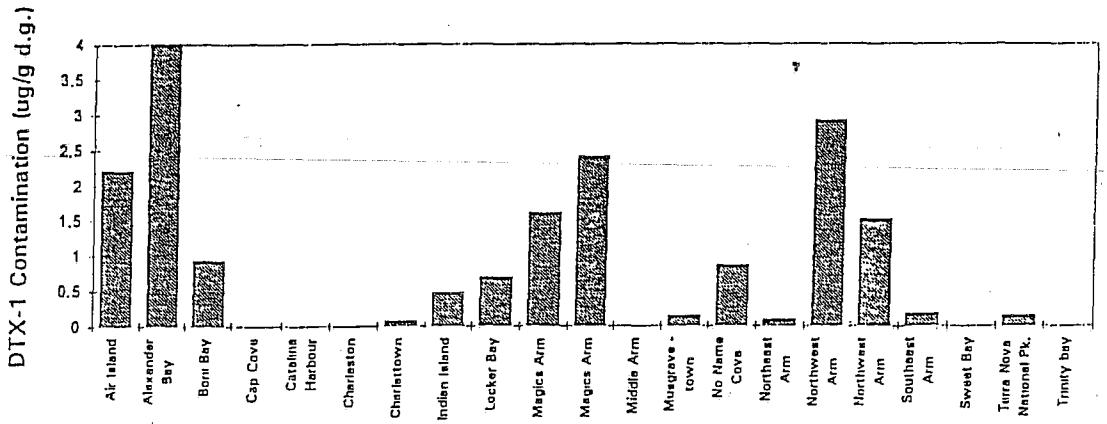

Figure 2

A summary of the occurrences of DTX-1 in blue mussel (*Mytilus edulis*) samples from several locations in the vicinity of Bonavista Bay, Newfoundland detected during the follow-up of the DSP poisoning episode in October-November 1993.

Since the water temperature in the vicinity of growing sites was approximately 6°C, clearly most of the Atlantic Canada will be at risk for DSP intoxication for most of the year if only water temperature were the determinant. Examination of water samples for the presence of phytoplankton showed that *Dinophysis norvegica*, a relatively common dinoflagellate in these waters, was present in significant numbers. Whatever the source organism, the fact that only DTX-1 was present in these shellfish, the same as has been the case in Nova Scotia, suggests that the source organism may be the same in both cases.

Since both Newfoundland and Nova Scotian shellfish have now been observed to become intoxicated with DSP, it seems likely that the whole of the Maritimes is at risk since the conditions at both locations where the toxic shellfish originated do not seem to be unique. It is therefore likely that many locations are periodically affected, perhaps to the extend that shellfish in the area could accumulate unacceptable levels of toxin.

Results of monitoring DSP toxins in aquacultured mussels from Mahone Bay, N.S. during 1993

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After the occurrence of consumer poisonings in 1990, the diarrhetic shellfish toxin, dinophysis toxin-1 (DTX-1), was detected in mussels from Mahone Bay, N.S. due to the joint efforts of Department of Fisheries and Oceans, Inspection, and the Institute for Marine Biosciences (National Research Council) in Halifax. Since then, this site has been watched for a recurrence of a serious intoxication. During 1993, mussel samples from the site were monitored at weekly intervals from spring to late fall using a variation of the HPLC-fluorescence chemical analysis. Multiple peaks in the level of toxin accumulation in the mussels were detected in the digestive glands of cultivated blue mussels (*Mytilus edulis*), but never at levels high enough to assist in determining the nature of the intoxicating phytoplankton, nor high enough to cause sickness in humans if the mussels were consumed.

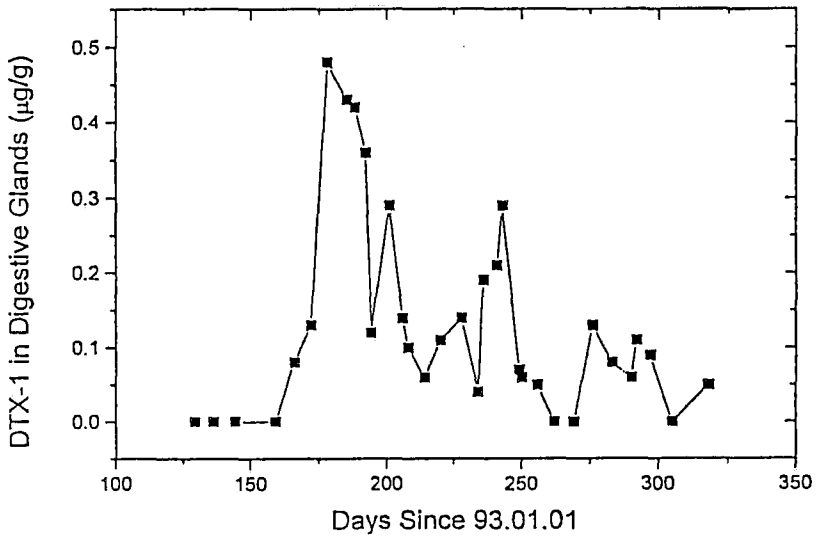

Figure 1

The abundance of DTX-1 in the digestive glands of cultivated mussels from Mahone Bay, N.S. during 1993.

While it has not been the intention to monitor all possible sites in Scotia-Fundy Region for DSP toxins, in the course of investigating consumer complaints, low levels of DTX-1 have been detected in shellfish from at several other sites in Nova Scotia and New Brunswick. Some occurrences, as determined by HPLC-fluorescence, were confirmed in many cases by either or both immunoassay or HPLC/MS. It is therefore presumed that the occurrence of the toxin is widespread but at a level too low to cause acute illness. If this is correct, it has human health significance which should be of concern to health agencies, since DTX-1 is considered to be a tumour promoter.

Satellite images and numerical modeling for plankton bloom monitoring

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The Institute of Ocean Sciences has developed numerical models and satellite image processing techniques that can be used for the monitoring and prediction of plankton blooms. In the last 10 years, the GF series of models have been developed to give increasing precision in predicting the temperature and salinity of water in the Strait of Georgia and Juan de Fuca Strait system.

The latest, GF-9, provides 2 km spatial resolution in 9 vertical layers, spaced to allow prediction of near surface properties in the presence of freshwater in-flow from the Fraser River. In the same time period collection of satellite data has been improved to include direct reception of NOAA AVHRR images with 1 km resolution of surface temperatures and water brightness. Starting in 1995, more detailed water colour information should come available from the Seawifs satellite-borne scanner.

By combining satellite and model data we plan to assess the possibility of predicting conditions, especially high surface temperatures, that lead to 'red tides', as well as to use the satellite data to detect and monitor some of the events as they happen. Examples will be shown of surface temperatures predicted by the model, and shown by satellite images, and of water brightened by plankton blooms, imaged by the satellite.

Program for cultured scallop toxicity testing

A joint venture between the Nova Scotia Department of Fisheries, the Federal Department of Fisheries and Oceans and the Aquaculture Association of Nova Scotia

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The idea of farming the giant sea scallop, *Placopectan magellanicus,* has become an important part of the aquaculture industry in Nova Scotia during the past few years. In the fall of 1992, at the urging of several aquaculturists a program was set up to test scallops for PSP, DSP and domoic acid. This program was funded through the Canada/Nova Scotia Cooperation Agreement on Fisheries Development.

Each grower who wishes to market whole scallops must submit a protocol which meets the requirements of Inspection. Since 1992 toxicity data have been collected from ten aquaculture sites throughout the province, enabling these growers to sell their product to markets in Canada and the United States.

Prior to this testing program, toxicity data on whole sea scallops were limited mainly to offshore populations. Because of the high PSP levels and slow depuration times typically observed in these populations, there was some scepticism as to whether a whole scallop market was a feasible venture. To date, only low or negligible levels of PSP have been observed in scallops from inshore sites, with estimates never exceeding 196 $\mu g.100g^{-1}$ in the hepatopancreas or 46 $\mu g.100g^{-1}$ in the whole animal. These peak levels are typically seen in June and decline significantly by mid- to-late August.

Profile of paralytic shellfish poisoning toxins in lobsters during uptake and depuration

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Lobsters, *Homarus americanus*, were held at 10°C, and fed every second day for eight weeks with a variety of formulated diet (scallop digestive glands that were contaminated with paralytic shellfish poisoning (PSP) toxins were included in the formulation), scallop mantle and digestive gland. Lobsters were sampled every two weeks and the PSP toxin concentration of the hepatopancreas was measured by the official AOAC mouse bioassay. The PSP concentration in the hepatopancreas was 2150 µg equivalents of saxitoxin (STS) / 100g of wet weight after four weeks and was 1554 µg equivalent STX / 100g wet weight after eight weeks. After eight weeks of PSP contaminated feed, the feed was switched to that of non-detectable concentrations of PSP toxins. The PSP toxin concentration in the hepatopancreas decreased exponentially with a half life of 26.3 days. PSP toxins were below regulatory levels by 70 days. PSP toxins were never detected in the muscle.

During the uptake phase of the experiment, the PSP toxin profile in the lobster hepatopancreas was similar to that found in the scallop digestive gland that was used for feed. However, C1 and C2 were clearly detected in the scallops, but were not detected in lobsters. The proportions of the PSP toxins having the N-hydroxy moiety (GTX1, GTX2 and neo-STX) in the lobster hepatopancreas were much less than those in the diet. This suggests that the N-hydroxy toxins are readily metabolized (degraded or dehydroxylated). During the later stages of depuration, proportions of saxitoxins (STX, neo-STX and dc-STX) increased and was correlated to the decrease in proportions of GTX-1 to GTX-4. This suggests that either the lobster hepatopancreas has a higher affinity for the more basic toxins or that the gonyautoxins are metabolized to saxitoxins.

Blooms of *Pseudonitzschia* spp. in western Washington waters

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Members of the diatom genus *Pseudonitzschia* H. Peragallo, *P. pungens* f. *multiseries* (Hasle) Hasle, *P. australis* Frenguelli, and *P. pseudodelicatissima* (Hasle) Hasle, that produce domoic acid, have been recognized in western Washington waters and elsewhere on the U.S. west coast. Their distribution is not well-known, probably because they are often misidentified. However, they appear to be relatively common and may be abundant.

Pseudonitzschia blooms associated with the first occurrence of domoic acid poisoning (DAP) on the west Coast in 1991 occurred off the sparsely populated open coast and were apparently part of a widespread bloom extending at least from Monterey Bay, CA, to Alaska. The bloom may have been caused by unusually warm weather conditions during an El Niño. In western Washington, *Pseudonitzschia* blooms in 1991, 1992, and 1993 all occurred after warm, dry periods followed by rain.

The appearance of domoic acid on the west coast signaled a new problem with regard to harmful algal blooms and the management of two important local fisheries, i.e. the sport harvest of razor clams (*Siliqua patula* Dixon) and the commercial harvest of Dungeness crabs (*Cancer magister* Linnaeus). We believe that *Pseudonitzschia* blooms and DAP will continue, spreading to new areas and shellfish species, and causing additional economic losses and health hazards.

Influence of associated bacteria on the algal production of paralytic shellfish poisons under nitrogen and phosphorus limitation

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Toxigenic *Alexandrium excavatum* was grown at 10°C under axenic and non-axenic conditions in large (8 L), aerated batch cultures of natural seawater medium. The seawater was supplemented with vitamins, trace elements, and replete (550 µM and 40 µM) or limiting (10% or 0% of replete) concentrations of nitrogen and phosphorus, respectively. The populations of dinoflagellates in each culture were separated into motile cells versus non-motile cells which included temporary cysts, planozygotes and other non-motile cell types; data was collected from each of the two subpopulations. Growth and autofluorescence of the dinoflagellates was monitored by flow cytometry for up to 30 d. Production of PSP toxins by the dinoflagellates was followed by testing AOAC extracts for toxicity in the mouse neuroblastoma cell bioassay.

Relative counts of the individual species of bacteria present in the non-axenic cultures were not found to be associated with toxin production or growth. Therefore, in later experiments, the density of all bacterial species was followed using a modification of the hydrophobic grid membrane filter (HGMF) technique. Sterility in the axenic cultures was determined by plating sonicated and untreated culture aliquots onto marine agar. Extracellular concentrations of nitrogen and phosphorus were determined during the course of the experiments.

As expected, toxin production was higher under phosphate limitation than under nitrogen limitation. Toxin production was also higher in axenic cultures than in non-axenic cultures; the data taken together suggest that mineralization by bacteria increases availability of nitrogen and phosphorus and reduces nutrient stress.

The effects of domoic acid on haemolymph pH, PCO_2 and PO_2 in the Pacific oyster, *Crassostrea gigas* and the California mussel, *Mytilus californianus*

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Changes in haemolymph pH, PCO_2, and PO_2 were measured in *Crassostrea gigas* and *Mytilus californianus* when exposed continuously to *Pseudonitzschia pungens* f *multiseries*, for a period of 48 h followed by 120 h of depuration.

A general stress response by the oyster was characterized by respiratory acidosis from shell closure 4 - 8 h after introduction of the algae. Haemolymph pH in the oyster returned to control levels after 36 h, although hypoxic conditions persisted during the 48 h exposure and returned to control levels only after 24 h of clearance. Significant hypercapnia and elevated haemolymph bicarbonate occurred in the oyster on exposure to the algae for 8 h which was rectified significantly by 36 - 48 h from exposure. Total body burden of domoic acid was greatest in the oyster after 4 h of exposure. At 120 h of continued clearance the gill, muscle and soft tissue still revealed detectable domoic acid concentrations.

M. californianus exposed continuously to toxic algae exhibited no significant change in haemolymph pH, but a slight transient alkalosis was noted that is associated with increased filtration and gas exchange. Haemolymph PCO_2 was within the range of established control values throughout the experiment, whereas haemolymph PO_2 declined significantly during 4 h of exposure. Total domoic acid body burden peaked after 4 h exposure and the highest level was observed in the gill tissue. No detectable concentration of domoic acid was observed in any tissue after 120 h of clearance.

Growth and domoic acid production by *Pseudonitzschia pungens* f. *multiseries* in chemostat culture

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The diatom *Pseudonitzschia pungens* f. *multiseries* produces domoic acid (DA) in chemostat cultures limited by silicate or phosphorus and grown at division rates of 0.3 to 0.8 per day. DA production and cell division rates showed a diurnal pattern when irradiance was provided as a 12 h light:12 h dark cycle. Growth decreased to zero at the beginning of both the light and dark phases, indicating that cell division in the chemostat was partially synchronized. These periods of low division rate were accompanied by periods of increased production of DA.

The conditions under which DA is produced in the chemostats, i.e. nutrient limitation and low to zero cell division, are consistent with those found in batch cultures when DA is produced, i.e. when cell division ceases during silicate- or phosphate-limited stationary phase.

A second observation was that DA production was not constant during periods of 6 to 18 d in spite of the fact that the culture was in 'steady state', as defined by the less than ± 20% variation in cell number. The increase or decrease in DA production may be due to a corresponding change in the concentration of bacteria in the non-axenic cultures. To test this hypothesis, we added glucose to the chemostats in order to stimulate bacterial growth. Glucose addition resulted in a 3-fold increase in bacterial numbers, during which time the DA per cell doubled. Removal of the glucose resulted in an immediate decrease in bacterial counts and in DA production. These results are consistent with batch culture studies, which show an important role of bacteria in DA production. We conclude that chemostats are a useful tool for studying the physiology of DA production.

In vitro interconversions of paralytic shellfish poisoning (PSP) toxins

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PSP toxins are a heterogeneous group of more than twenty analogs of saxitoxin. C toxins, which have both 11-O-sulfate and N-21 sulfamate groups in the molecule, are usually predominant in marine algae. In shellfish, the gonyautoxins (GTX's), with only the 11-O-sulfate, often dominate the PSP toxin profile. Extraction of shellfish meat with hot, dilute hydrochloric acid simplifies the toxin mixture by acid hydrolysis of the N-21 sulfamate so that the four C toxins are converted to their equivalent GTX's. B1 and B2 similarly yield saxitoxin (STX) and neosaxitoxin (NEO), respectively.

The kinetics of sulfate loss from C-11 and N-21 with time, temperature and HCl concentration were studied in pure solutions and in shellfish homogenates. GTX's were the main products of these reactions, however, 11-hydroxysaxitoxin was also produced with HCl concentrations greater than 0.3 M. 11-hydroxysaxitoxin has not been previously reported. It co-eluted with STX in the Sullivan HPLC method, but the two compounds can be separated by the Oshima HPLC method and by capillary electrophoresis.

In the preparation of standards for calibrating analytical methods we have concentrated on STX, NEO and the four GTX's because they are the most frequently encountered in shellfish extracts and they can be obtained directly or indirectly in substantial quantities from toxic dinoflagellates. *Alexandrium* can accumulate up to 2% of the dry weight as PSP toxins. However, because a high proportion occur as C toxins it is advantageous to be able to chemically convert them to less abundant forms. STX and NEO, for example, can be prepared from their respective 11-O-sulfates (GTX's and C toxins) using thiol reagents.

Dynamics of paralytic shellfish toxin accumulation in Atlantic mackerel

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Mortalities of Atlantic mackerel (*Scomber scombrus*) are often reported along the Scotian shelf during their spring migration toward their spawning grounds in the Gulf of St. Lawrence. The exact cause for the mortality is not known. The mortality has been attributed to either depletion of energetic reserves, PSP intoxication or discard at sea by fishermen.

In June 1993, we determined the PSP toxin content of the liver and muscles of 73 dead and live fish from Cape Breton (Nova Scotia). PSP toxins were present in the liver of the dead fish (36 ± 13 µg STX equ.$100g^{-1}$ of wet weight), but in concentrations well below values previously reported in live mackerel from the Bay of Fundy (288 µg STX equ.$100g^{-1}$ of wet weight). Consequently, the death of the mackerel cannot be formally attributed to the ingestion and accumulation of PSP toxins. Saxitoxin concentration per liver increased linearly with the age of the fish, indicating a gradual bioaccumulation of the toxin during its entire life.

Toxic and potentially toxic *Pseudonitzschia* in Danish coastal waters

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Following the serious incidents of ASP in Canada and USA, a study of the Danish *Pseudonitzschia* species was begun at the University of Copenhagen. Four species (*P. pseudodelicatissima, P. delicatissima, P. seriata, P. pungens* f. *pungens*) have been established in culture and have been tested for domoic acid using the FMOC-HPLC method of Pocklington et al. (1990). Unexpectedly, the only species which tested positive was *P. seriata* (three different isolates). All isolates were grown at 4°C and 15°C. The toxicity seemed to be both strain and temperature dependent.

As shown previously with *P. pungens* f. *multiseries*, domoic acid was produced at a detectable level only in late exponential phase (0.20 pg cell^{-1}) and increasing to a higher level in the stationary phase, reaching maximum when the culture was senescent (13.7 pg cell^{-1}) The concentration per cell (whole culture) is in agreement with the concentration found in *P. pungens* f. *multiseries*. The two species have approximately the same volume.

The Danish results are yet another example of the existence of both toxic and non-toxic strains of the same species. In Canada, *P. pseudodelicatissima* has previously been tested toxic and *P. seriata* non-toxic. The Danish findings also represent the first records of domoic acid in a marine plankton alga outside North America.

Temporal and spatial dynamics of *Alexandrium fundyense* cysts during 1981-83 and 1992 in the Bay of Fundy

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The Bay of Fundy has a long history of occurrences of *Alexandrium fundyense* which cause closures (generally summer, but sometimes annual) of shellfish harvesting areas due to unacceptable levels of paralytic shellfish toxins in their tissues. Studies of *A. fundyense* overwintering cysts were conducted in January 1981, November 1981, November 1982, October 1983, and February 1992. Results from January 1981 have been previously reported by White and Lewis (1982). The objectives of the study were to determine: where the highest concentrations of cysts tend to be located, annual variability, where the major summertime blooms of *A. fundyense* are initiated, and whether wintertime cyst numbers in sediments could be used as a predictor for the summertime bloom densities. Fig. 1 indicates locations where sediment samples were collected.

Highest densities during the five years sampled were found during January 1981 when 6000-8000 cysts.cm^{-3} were detected at stations located east and north of Grand Manan Island. During the following four years sampled, highest concentrations were found in the same general region.

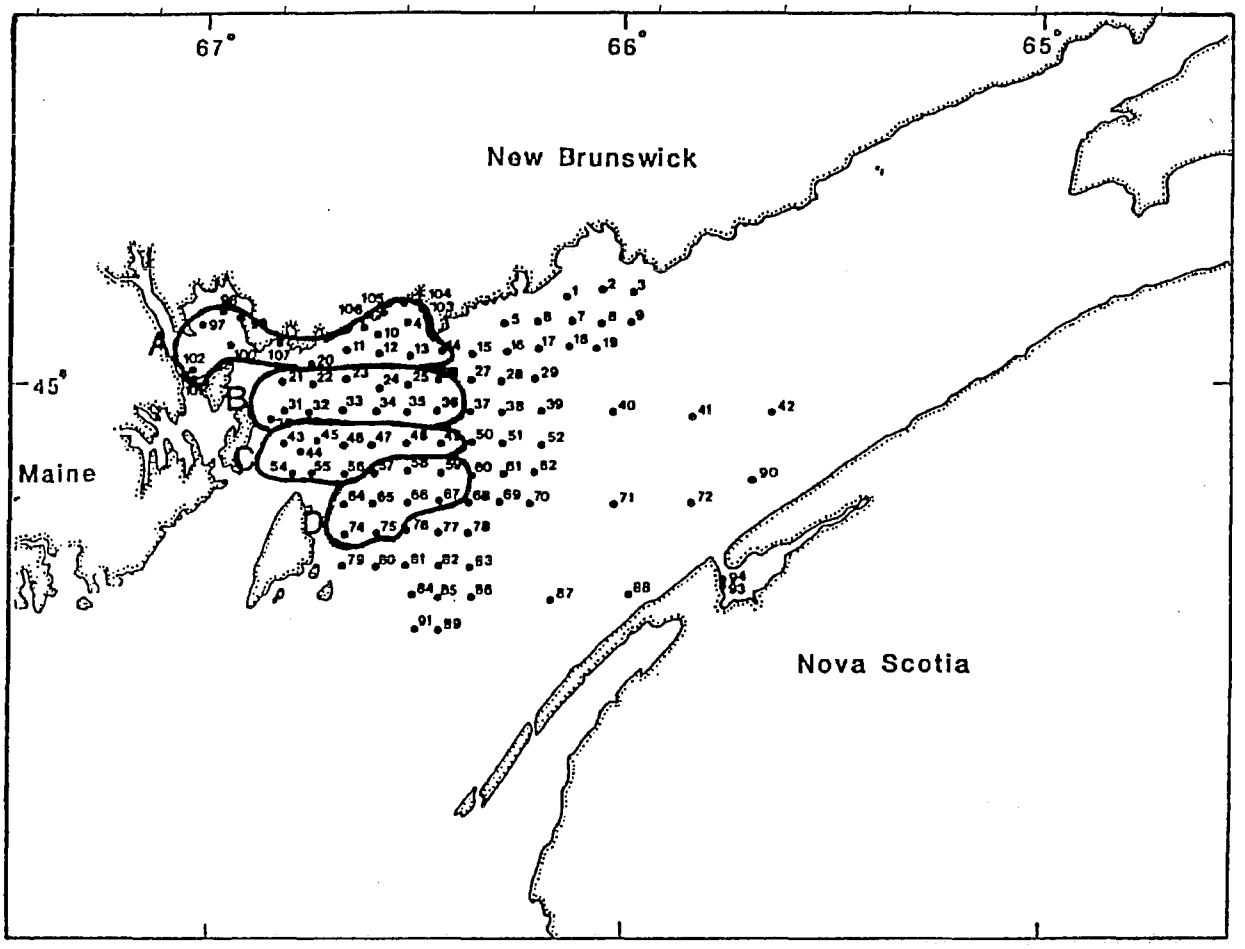

Figure 1

Locations of sampling stations in the Bay of Fundy during 1981 - 83 and 1992.

Table 1

Mean number cysts.cm^{-3} found at Areas A - D from Fig. 1. Ranges are indicated in brackets.

Month, Year	Area A	Area B	Area C	Area D
Jan. 1981	250 (20 - 60)	900 (150 - 2690)	1358 (30 - 5870)	2676 (600 - 7440)
Nov. 1981	106 (30 - 240)	409 (75 - 900)	545 (0 - 1335)	1542 (225 - 5025)
Nov. 1982	246 (0 - 1340)	654 (0 - 1470)	403 (105 - 1305)	726 (270 - 1305)
Oct. 1983	203 (0 - 1185)	431 (75 - 1200)	424 (45 - 890)	1478 (255 - 3330)
Feb. 1992	204 (0 - 1020)	480 (90 - 1740)	435 (450 - 690)	1507 (420 - 3015)

This region where the highest concentrations were found had a fine brown mud or clay sediment type and was 80-160m in depth. The Bay of Fundy, a very dynamic system with strong tidal currents, has a natural gyre centered in this region which may result in higher cyst deposition.

Lowest concentrations of cysts were found in inshore areas on the New Brunswick coast and areas that had rock or gravely bottom such as those on the Nova Scotia coast and portions of the head of the Bay. The sampling area was divided into four areas in order to compare regions of the Bay of Fundy as well as occurrences from year to year. Areas that were known to have rocky and sandy bottom and with low to nonexistent numbers of cysts were not used in the comparison. The four areas are indicated on Fig. 1 and identified as Areas A-D with Area A being the most inshore. Table 1 shows results from these areas for each year. The numbers of cysts increased in order from Area A through Area D. Cysts were observed at all stations in Area D whereas in all other areas there was at least one location or several (as in the case with Area A) where there were none found. January 1981 had the highest mean number of cysts.cm^{-3} for all areas.

A possible explanation for the highest numbers of cysts during January 1981, was that during the previous summer more than a million cells.L^{-1} of *A. fundyense* were observed in the Bay of Fundy at certain locations - enough cells to turn the water red. This is the only year that we have records documenting this phenomena. This abnormal bloom could have resulted in an abnormally high number of cysts in sediments.

It is unlikely that the numbers of cysts observed annually in the inshore Bay of Fundy can support the numbers of *A. fundyense* cells normally observed during the major bloom period. Therefore, the rich deposits of cysts located east and north of Grand Manan must play a role in these blooms. Indications are that cysts that hatch and rise in the water column from this region are dispersed throughout the Bay and result in the large numbers of cells observed in the inshore regions where many shellfish harvesting areas are located, resulting in increased toxicity.

Preliminary analysis indicates that winter cyst numbers alone may not be good indicator of upcoming summer bloom densities. For example, following 'red tide' proportions observed during the summer of 1980 and the subsequent high numbers of cysts found the following winter, one would have expected there to be high numbers the following year. Unfortunately, this was not the case. It is known that cysts may remain dormant for several years and conditions in the summer of 1981 may not have been ideal for the hatching of large numbers of cysts. Analyses of core samples from November 1981 indicated that cysts were present in sediment to a depth of 20 cm- which is considerably deeper than the upper layers where newly deposited cysts rest. It appears likely that various environmental, meteorological and hydrographic influences are more responsible for *A. fundyense* dynamics than cyst deposition.

References:

White, A.W., and C.M. Lewis. 1982. Resting cysts of the toxic red tide dinoflagellate *Gonyaulax excavata* in Bay of Fundy sediments. Can. J. Fish. Aquat. Sci. 39: 1185-1194.

Identification of protein phosphatase inhibitors of the microcystin class in the marine environment

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We have developed a quantitative environmental bioscreen which will detect, with unprecedented sensitivity, cyclic peptides of the microcystin class in marine and freshwater organisms. Our method employs capillary electrophoresis (CE) coupled with liquid chromatography (LC)-linked protein phosphatase (PPase) enzyme assay. Hepatotoxins from the microcystin class have been identified in northeastern Pacific Ocean, eastern Canadian and European mussels at levels up to three fold higher than accepted quarantine levels for the diarrhetic shellfish toxin okadaic acid. Recently, application of the LC/CE -linked bioassay protocol allowed for the effective detection of microcystin-LR in copepods collected from salmon netpen environments in British Columbia. The widespread presence of microcystins in the marine environment demonstrates the need for stringent and quantitative testing guidelines for diarrhetic shellfish toxins and microcystins.

A *Dinophysis norvegica* bloom and its implications in the occurrence of a diarrhetic shellfish poisoning episode in mussels from Newfoundland during the late Autumn in 1993

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In October of 1993 in Bonavista Bay, Newfoundland, several persons who had consumed mussels developed symptoms of what appeared to be diarrhetic shellfish poisoning. Mussel tissue was tested and was confirmed to contain DTX-1. Water samples collected from several locations within Bonavista Bay were examined to determine the phytoplankton organisms present in the water column.

The water samples were found to contain primarily *Dinophysis norvegica*, however, other dinoflagellates were also present. Some samples where the highest mussel toxicity had occurred contained 2,000 *D. norvegica* cells.L^{-1}. Examination of the digestive tissue of the contaminated mussels revealed *D. norvegica* concentrations of up to 40,000 cells per mussel. This is the first confirmed occurrence of diarrhetic shellfish poisoning in Newfoundland and only the second such occurrence in North America.

Alexandrium cyst distribution in sediments collected from shellfish aquaculture sites in Atlantic Canada (Newfoundland)

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Sediment cores from seven aquaculture sites were collected by SCUBA divers in November and December of 1992 to determine the distribution and abundance of cysts in the coastal waters of Newfoundland in Atlantic Canada. These cores were analyzed using a density gradient method to concentrate the cysts. They were then examined microscopically for identification and enumeration.

Sediments from six of the seven sites tested contained cysts (< 4 - 1130 cysts.cm^{-2}), mostly only in trace amounts. The site with high cyst concentrations has been permanently closed to shellfish aquaculture. Cyst distribution within the closed site was studied along three transects through the cove, and-a vertical profile for each core was compiled. The bottom sediments of this cove contained of fine silt and clay over a meter deep. Highest cyst concentration (1130 cysts.cm^{-2}) occurred in shallow (3m) sediment on the eastern edge of the cove.

Determination of domoic acid by two versions of a competitive enzyme-linked immunosorbent assay (ELISA)

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The enzyme-linked immunosorbent assay (ELISA) for domoic acid developed by Newsome et al. (1991) for use with urine samples and blood sera is highly specific, but lacks the sensitivity and range of concentrations measurable (lower limit 0.16 ng.mL^{-1}) we desired. Accordingly, we undertook to improve upon the original method which depended on a physical absorption of domoic acid to the microplate and also required a prior tube incubation as part of the operational procedure. Two satisfactory versions resulted.

The first version still relies upon physical absorption of domoic acid, but the method has been simplified and shortened and now incorporates the critical element of washing with a high ionic strength phosphate buffered saline solution. The second version relies on chemical coupling; the most effective coupling of domoic acid to the secondary amino group on CovaLink NH plates (Nunc, Denmark) was produced using 15 mM N-hydroxysuccinimide (NHS) and 12.5 mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC). Both assay procedures are conducted entirely in the microplate wells and utilize the high ionic strength phosphate buffered saline solution. A steeper inhibition curve is obtained with both new versions and the detection range for both is from 1 pg.mL^{-1} to 10 μg.mL^{-1}. The coefficients of variation for version 1 range between 0.4 and 7% and for version 2 (CovaLink NH plates) from 0.9 to 11%. No cross reactivity with kainic acid, glutamic acid and proline was observed when using either of the two ELISA systems. These two improved versions will provide for accurate determinations of domoic acid at substantially lower concentrations.

References:

Newsome, H., J. Truelove, L. Hierlihy and P. Collins. 1991. Determination of domoic acid in serum and urine by immunochemical analysis. Bull. Environ. Contam. Toxicol. 47: 329-334.

Parasitism of *Pseudonitzschia pungens* by a marine fungus

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Unusually wide cells of species of the pennate diatom genus *Pseudonitzschia* which have been described as 'auxospores' in the literature are shown here for *P. pungens* to actually be a result of parasitism by a marine fungus which appears to be an oomycete.

Using bisbenzimide (Hoechst 33342) as a fluorescent nuclear stain, it is shown that once the fungus has penetrated the algal cell, the nucleus and probably the protoplasm of the cell gradually disappear while the chloroplasts remain morphologically intact with apparently unaltered fluorescence properties. Following this, fungal spores, which stain strongly with bisbenzimide, appear in the region formerly occupied by the nucleus. These then proliferate producing at least 2^6 spores. This process is accompanied by a gradual widening (hypertrophy) of the algal cell brought about by the synthesis of several new pairs of girdle bands. An internal sporangium, which stains with the fluorescently labelled lectin FITC-WGA and calcofluor white, develops within the infected cells and contains the spores. When developed, biflagellate (visualized by vital crystal violet staining) zoospores become active and swim out of the parasitized cells via a pore in the silica frustule which is characteristic of this type of infection. The zoospores were first observed to become active near the start of the period of natural darkness.

These observations have been made on natural samples, but, in a wide variety of trials, it has so far not been possible to induce the infection of algal clones in the laboratory. The natural infection rate appears to be less than 0.5 per cent (but there are unconfirmed reports of much higher infection rates) and the possible effects of this fungus on the population dynamics of the domoic acid toxin producer *P. pungens* f. *multiseries* is not known. A similar, possibly identical, fungus attacks other members of the genus in local waters, including *P. pungens* f. *pungens*, *P. seriata* and *P. delicatissima*.

References:

Cupp, E.E. 1943. Marine diatoms of the west coast of North America. University of California Press. 237p.

The extent of tagging of *Pseudonitzschia pungens* cells by the fluorescently labelled lectin FITC-WGA in relation to growth rate and domoic acid production

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The extent of binding of the fluorescently labelled (by fluorescein isothiocyanate (FITC)) plant lectin wheat germ agglutinin (FITC-WGA) to the cell surface of the domoic acid toxin producer *Pseudonitzschia pungens* f. *multiseries* varies among clones and during different phases of the growth of this organism in batch culture. In 3 out of 4 clones of f. *multiseries*, immediately following inoculation of a batch culture, nearly 100% of the cells bound the FITC-WGA tag. When growth began, this proportion decreased to from less than 70% to less than 10%, depending on the clone, and the timing of this decrease was also clone dependent. When exponential growth ended and the growth rate became limited (due to silicate limitation), the percentage of cells binding WGA increased and again attained 100% at about the time stationary phase began. Domoic acid production took place in stationary phase following the attainment of 100% labelling by WGA. In one of these clones, the extent of binding of WGA was quantified in individual cells and found to vary during growth in a similar manner. In the fourth clone, the extent of labelling of cells with WGA varied similarly during growth but did not attain 50% during stationary phase. Two clones of the non-producer of domoic acid, *P. pungens* f. *pungens* did not significantly bind WGA during any phase of growth. Since the toxic and non-toxic forms of *P. pungens* cannot be distinguished using light microscopy, the presence of even the non-toxic f. *pungens* in significant numbers in phytoplankton monitoring samples necessitates expensive increases in sampling and toxin analyses. The use of FITC-WGA tagging could permit substantial economies to be realized by making such incremental efforts superfluous.

Neurological risk of phycotoxin exposure: distinguishing neuroexcitatory from neurotoxic effects of domoic acid in the intact animal

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We have investigated the action of the active component of amnesic shellfish poisoning, domoic acid, in the mouse brain following systemic exposure. Serum levels of domoic acid increased with intraparenteal doses up to 2.0 mg/kg as determined using the domoic acid radioreceptor assay. Domoic acid (4 mg/kg causing 1.2 μg/mL @ 60 min.) induced c-fos mRNA within 15 min., remained elevated for one hour and declined within two hours. Dose response studies indicated that domoic acid at doses as low as 1.0 mg/kg induced c-fos mRNA.

Within one hour, domoic acid caused the nuclear accumulation of c-Fos immunoreactivity in the hippocampal formation, lateral septal nucleus and olfactory bulb. The c-Fos immunoreactivity was most prominent in the granule cells of the dentate gyrus both in terms of earliest detectable response and intensity of the immunoreactive product. Domoic acid also caused a strong induction of c-Fos in two adjacent regions of the medulla; the area postrema and the medial region of the nucleus of the solitary tract. These results indicate that an early and prominent action of domoic acid administered to intact animals is on memory processing regions (i.e. hippocampus) and medullary regions controlling gastrointestinal function (area postrema and nucleus of the solitary tract).

We next examined the irreversible toxic effects of domoic acid. Mice treated with 4.0 mg/kg domoic acid for 72 h showed extensive degeneration in the hippocampus and its projections. Degeneration was evident in the granule cell layer and anterior olfactory nucleus, lateral septal nucleus, pyramidal cells of the CA1 and CA2 regions of the hippocampal formation; however, no degeneration was evident in the dentate gyrus or medullary regions. Taken together these studies demonstrate that domoic acid has neuroexcitatory effects on the hippocampus and its projections, area postrema and nucleus of the solitary tract whereas its permanent neurotoxic effects in mice are limited largely to the CA1 and CA2 regions of the hippocampus, lateral septal nucleus and olfactory bulb.

Harmful effects of the marine diatom *Chaetoceros concavicornis* on Atlantic salmon: the role of gill mucus.

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The effects of the spiny, chain-forming marine diatom *Chaetoceros concavicornis* on seawater-acclimated Atlantic salmon were studied in laboratory bioassays. Partial pressure of blood-oxygen (PO_2) in fish exposed to environmentally common and greater concentrations of the diatom was significantly less than control fish; partial pressure of blood-carbon dioxide (PCO_2) was elevated.

Histopathology of fish exposed for short periods (2 - 12 hours) showed massive discharge of gill mucus that obstructed interlamellar spaces. Longer exposure (24 - 48 hours) caused exhaustion of the supply of mucus cells and mucus and led to lamellar degeneration and separation. None of the several hundred sections of gill tissue examined showed compelling evidence of penetration by the diatoms' primary spines, as previously reported from observation of wet mounts. Scanning electron microscopy showed that cells and chains of *C. concavicornis* may lodge between the secondary lamellae of the gills, but many diatoms were also present in overlying mucus if special tissue preparation techniques were used.

Fish cough rate frequency was generally correlated with diatom concentration or morphology. Chains and spines of *C. concavicornis* grown in non-aerated cultures were longer than those grown in aerated cultures; fish exposed to longer chains and spines had lower PO_2 and increased cough rates.

Acid-cleaned cells of *C. concavicornis* had similar effects on blood-oxygen as normal cells. Cell filtrate and sonicated cells had no adverse effects on the fish. These data support a prior researcher's conclusion that the diatom has a physical action on the fish gills, not a chemical one.

Overall, fish gills appear to be protected from the effects of the diatom by mucus production which bundles the algal cells and allows them to be removed by coughing and normal flow of water over the gills. Conversely, if mucus production is too great, blood-hypoxia leads to immediate death of the fish.

Although oxygen supplementation could be an effective mitigation measure for short-term exposure of aquaculture fish to *C. concavicornis*, longer exposure could exhaust the supply of mucus cells and lead to major degradation of the gill epithelium and death. The economics of supplying supersaturated oxygen to net-pens appears to be unfavorable, compared to other preventive and management practices.

Diurnal variation of toxin content and composition in cage-cultures of *Alexandrium tamarense*

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Diurnal variations in toxin content of *Alexandrium tamarense* were investigated in turbidostat cage-cultures. Three 10 L cultures were grown at 18°C, 16:8 L:D, 150 micromol.m^{-2}.s^{-1} irradiance for five days. Samples were taken five times per day for toxins, cell doublet frequency, culture density and chlorophyll *a* (chl *a*).

A peak in the cell doublet frequency near midday indicated that mitosis was occurring in the late morning, rather than at night as is usually reported in this species. The cellular concentration of both chl *a* and toxins increased during the day and declined at night, suggesting that toxin synthesis is light-dependent and closely related to overall biomass changes.

Toxin composition also changed, with the proportion of GTX-3 and GTX-4 increasing in the day and declining at night. Concentrations of GTX-3 and 4 were in general much more variable than total toxin. Transfer of one culture to 24:0 L:D revealed that cell division continued, while diurnal toxin stopped, suggesting that the toxin synthesis cycle is controlled by light, not an endogenous clock. Transfer of one culture to a reduced irradiance of 85 micromol.m^{-2}.s^{-1} reduced the proportion of gonyautoxins in the cells, but not the total toxin content. In conclusion, toxin synthesis is light-mediated with the gonyautoxins being much more variable than the sulfocarbonyl derivatives.

Detection of feeding deterrents and phycotoxins from marine phytoplankton using a new bioassay technique - ecological and commercial applications

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It has been known for the last three decades that some marine phytoplankton can produce chemical compounds, referred to as feeding deterrents, which make them unpalatable to zooplankton, and thus protect them from grazing. There have been two main approaches to studies on feeding deterrents: (1) a number of species of phytoplankton have been demonstrated to reduce feeding of certain zooplankton; and (2) several well-known phycotoxins have been shown to be feeding deterrents. However, very little research has been done on the chemistry and physiology of feeding deterrent compounds, and no feeding deterrent compound has been isolated and structurally characterized from a marine phytoplankton using a bioassay specifically designed to detect feeding deterrents.

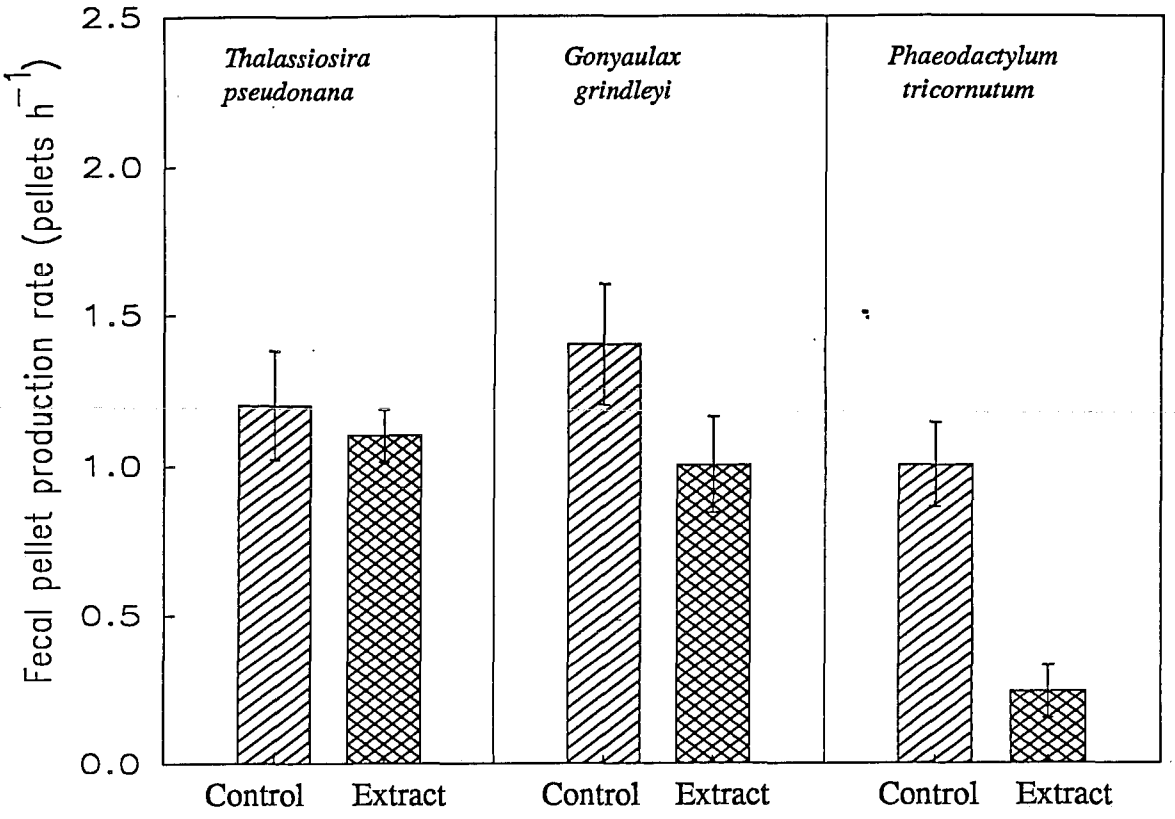
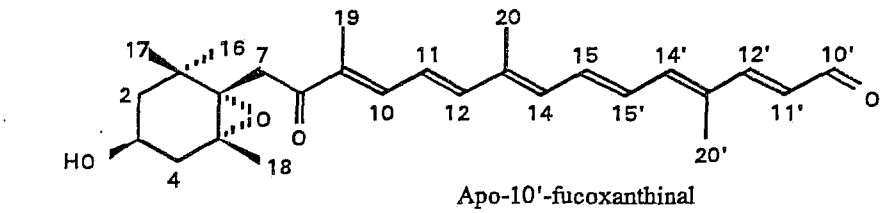


Figure 1

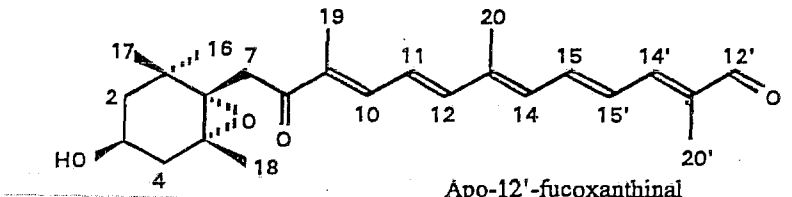
Feeding deterrent bioassay results for cellular extracts of three marine phytoplankton, *Thalassiosira pseudonana*, *Gonyaulax grindleyi*, and *Phaeodactylum tricornutum*.

In order to screen phytoplankton species for feeding deterrent production and to isolate and identify feeding deterrent compounds, a new, rapid, and reliable laboratory bioassay was developed. This bioassay used the harpacticoid copepod *Tigriopus californicus*, and measured inhibition of feeding by measuring the fecal pellet production rate. Using this bioassay, cellular extracts from the diatom *Phaeodactylum tricornutum* and the dinoflagellate *Gonyaulax grindleyi* gave feeding deterrent responses, while extracts from the diatom *Thalassiosira pseudonana* gave no feeding deterrent responses (Fig. 1). Live *P. tricornutum* cells also deterred feeding at densities of 6×10^5 cells mL^{-1}. Feeding deterrent compounds were isolated and characterized from *P. tricornutum* using bioassay-guided chemical fractionation. Spectroscopic techniques identified the isolated compounds as apo-10'-fucoxanthinal, apo-12'-fucoxanthinal, apo-12-fucoxanthinal, and apo-13'-fucoxanthinone (Fig. 2).

Preliminary studies on the physiology of production of these feeding deterrents were performed. In order to carry out these studies, an analytical HPLC method was developed to measure the apo-fucoxanthinoid concentrations in crude cell extracts. The apo-fucoxanthinoids were shown to be produced by *P. tricornutum* when the cells entered senescence due to phosphate limitation. As senescent cells are more susceptible to predation, the production of feeding deterrents during senescence may protect these cells from grazing until the limiting nutrient is replenished.



Apo-10'-fucoxanthinal



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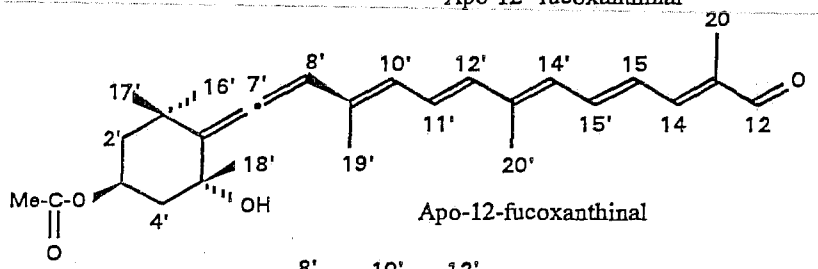
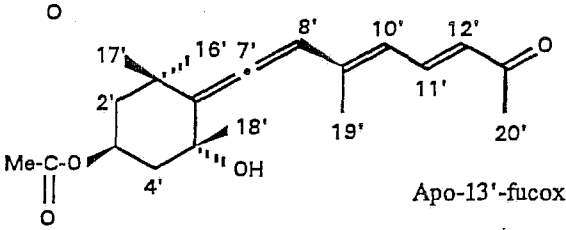


Apo-12-fucoxanthinal



Apo-13'-fucoxanthinone

Figure 2

Apo-fucoxanthinoid feeding deterrent compounds isolated from the marine diatom *Phaeodactylum tricornutum*

The toxic and feeding deterrent effects of the apo-fucoxanthinoids and several phycotoxins (okadaic acid, domoic acid, and microcystin-LR) on the copepod *Tigriopus californicus* were studied. IC50 (feeding inhibition) and LC50 (toxicity) curves were generated from these experiments using the equation:

$$y = y_0 e^{-kx^a}$$

For those compounds showing both feeding deterrent and toxic effects, the IC50 and LC50 curves were deconvoluted if they overlapped. Each compound was then classified as either toxin, feeding deterrent, both toxin and feeding deterrent, or inactive. The concentration of apo-fucoxanthinoids necessary to inhibit feeding of *T. californicus* by 50% ranged from 8.6 to 60 μM and was ≈ 1000 times lower than the concentration of total intracellular apo-fucoxanthinoids in *Phaeodactylum tricornutum*. These compounds are probably effective feeding deterrents at low concentrations such as would be expected in the natural environment. Three of the compounds tested (apo-12-fucoxanthinal, apo-13-fucoxanthinone, and microcystin-LR) showed only feeding deterrent effects in the range 0 to 50 μM, while domoic acid showed only toxic effects in this range, and the other compounds tested showed both feeding deterrent and toxic effects (Fig. 3). Compounds with only

feeding deterrent effects are probably detected by the copepod's chemoreceptors and not ingested, while compounds showing toxic effects are probably ingested, resulting in mortality. Thus, the bioassay developed for this research not only provides a valuable tool in screening phytoplankton for feeding deterrent compounds, and studying the chemistry and physiology of these compounds, but also to differentiate between toxic and non-toxic responses.

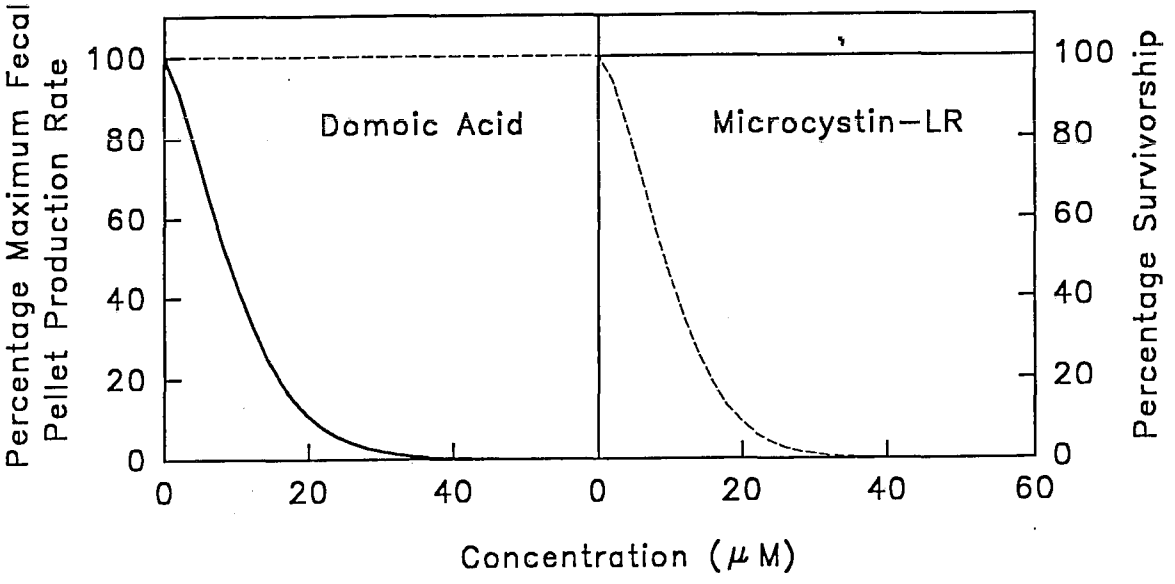


Figure 3

Comparison of LC50 and IC50 curves calculated from data for the effects of domoic acid and microcystin-LR on the copepod *Tigriopus californicus*. Solid line is the LC50 curve; dashed line is the IC50 curve.

Research on feeding deterrents and phycotoxins has major ecological significance. Screening various phytoplankton for feeding deterrent activity will determine which species of phytoplankton produce feeding deterrents. If phycotoxins have feeding deterrent activity, this may explain the purpose of the production of phycotoxins by phytoplankton in the natural environment. In addition to being potential human health problems, production of feeding deterrents by certain species of phytoplankton may control grazing and determine which species of phytoplankton will bloom and how long the bloom will persist. Thus, feeding deterrents may play an important role in phytoplankton species succession. Ultimately, these compounds may control the transfer of energy along some paths in the marine food web.

The results of this research may also have commercial implications. Phytoplankton are an important food source in mariculture. They are an essential component in the diet of marine bivalve molluscs (e.g. oysters, clams, scallops, and mussels), the larvae of some marine gastropods (e.g. abalone), larvae of salt-water shrimp (*Penaeus* and *Metapenaeus*), and zooplankters. Zooplankters, in turn, can be used as live food for rearing larvae of crustaceans (prawns, shrimp, crabs, and lobsters). Commonly used zooplankters are rotifers (*Brachionus*) and copepods

(*Tigriopus*). Today, more than 40 different species of phytoplankton are being used in mariculture. The food quality of these species varies greatly and experienced aquaculturalists frequently refer to 'good' and 'poor' food quality species. Production of feeding deterrents by 'poor' species may be the reason for decreased growth rates or increased mortality in mariculture species (e.g. oysters). Additionally, many mariculture facilities use natural, filtered seawater drawn from a local source. If this source is contaminated by blooms of phytoplankton which produce extracellular feeding deterrents, then the seawater itself may lead to decreased growth in the cultured organisms. Oyster beds and fish net pens exposed to blooms of feeding deterrent producing phytoplankton may also suffer decreased feeding resulting in decreased growth and health. A better understanding of which species produce feeding deterrents and what factors affect the production of these feeding deterrents would improve culturing practices in mariculture. Specifically, this research has shown that the diatom *Phaeodactylum tricornutum*, which is commonly used in mariculture, produces feeding deterrents and is not recommended for used as a food species, and that several common local species (*Gonyaulax grindleyi*, *Pseudonitzschia pungens*) produce compounds which may be harmful to exposed mariculture organisms.

An immunoassay for determining domoic acid concentrations in shellfish extracts

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Domoic acid (DA), a neuroexcitatory toxin produced by the marine diatom *Pseudonitzschia pungens*, can contaminate edible shellfish and is the causal agent of amnesic shellfish poisoning (ASP). Like paralytic shellfish poisoning (PSP), ASP poses serious public health concerns. The 1987 domoic acid outbreak in P.E.I. mussels resulted in 107 confirmed cases of ASP and four deaths. This crisis precipitated the near collapse of the east coast shellfishery. Although there have been no documented incidents of human ASP on the west coast, the shellfishery and the seafood industry in British Columbia view DA and the potential for ASP outbreaks as a serious economic threat. Furthermore, shellfish harvest closures in recent years have heightened public awareness of the problem, and as a consequence, the demand for DA testing is rapidly growing.

In Canada, the quality of shellfish for human consumption is assured in part by the Marine Toxin Monitoring Program, administered by the Inspection Branch of the Department of Fisheries and Oceans. Shellfish extracts are routinely analyzed for DA using standard high performance liquid chromatographic (HPLC) methods. While highly accurate and reproducible, HPLC methods can be expensive and time-consuming. Sensitive and cost-effective methods complementary to the HPLC procedure could be useful in processing the sheer volume of samples requiring DA analysis. Immunochemical methods, while generally meeting these criteria, can be adapted to formats which facilitate the rapid, simultaneous screening of many samples.

Table 1

Conjugation efficiency (eff.) of domoic acid (DA) to ovalbumin (OVA), keyhole limpet haemocyanin (KLH) and bovine serum albumin (BSA), and titre estimation of the anti-domoic acid sera against the various conjugates and carrier proteins in ELISA. Serum titre was estimated by the maximum serum dilution which allowed an absorbence of greater than 0.050 in the ELISA.

Antigen	Conjugation Eff. Molar Ratio carrier : DA	Test Antiserum Maximum antiserum dilution		
		anti-OVA-DA	anti-KLH-DA	anti-BSA-DA
OVA-DA	1:47	-[1]	1/35,000	<<1/500[2]
OVA[3]	1:0	-	<<1/1000	-
KLH-DA	1:44	1/50,000	-	<<1/500
KLH[3]	1:0	<<1/1000	-	-
BSA-DA	1:30	<<1/500	<<1/500	<<1/500[4]
BSA[3]	1:0	-	-	<<1/500[4]

1 not tested.

2 << - indicates no absorbence generated in the ELISA. The dilution indicated is the lowest dilution of antiserum tested.

3 indicates the unconjugated carrier protein.

4 indicates that the anti-BSA-DA was cross-absorbed with 100 $\mu g.mL^{-1}$ of BSA.

This paper describes a competitive enzyme-linked immunosorbent assay (ELISA) for determining DA concentrations in mussel extracts. The major objectives of this study were: (1) to produce an immunogen effective in raising a specific antiserum against DA; (2) to test the anti-DA serum in competitive ELISA; (3) to evaluate the accuracy of this ELISA by using it to determine the concentration of DA in mussel extracts prespiked with known levels; and (4) to compare the ELISA with the standard HPLC method in determining DA concentrations in extracts prepared from naturally contaminated mussels.

DA was coupled to ovalbumin (OVA), keyhole limpet haemocyanin (KLH) and bovine serum albumin (BSA) at molar ratios of 47:1, 44:1, and 30:1 respectively (Table 1) and injected into mice. Evaluation of the resulting antisera indicated that the titre of the anti-KLH-DA was

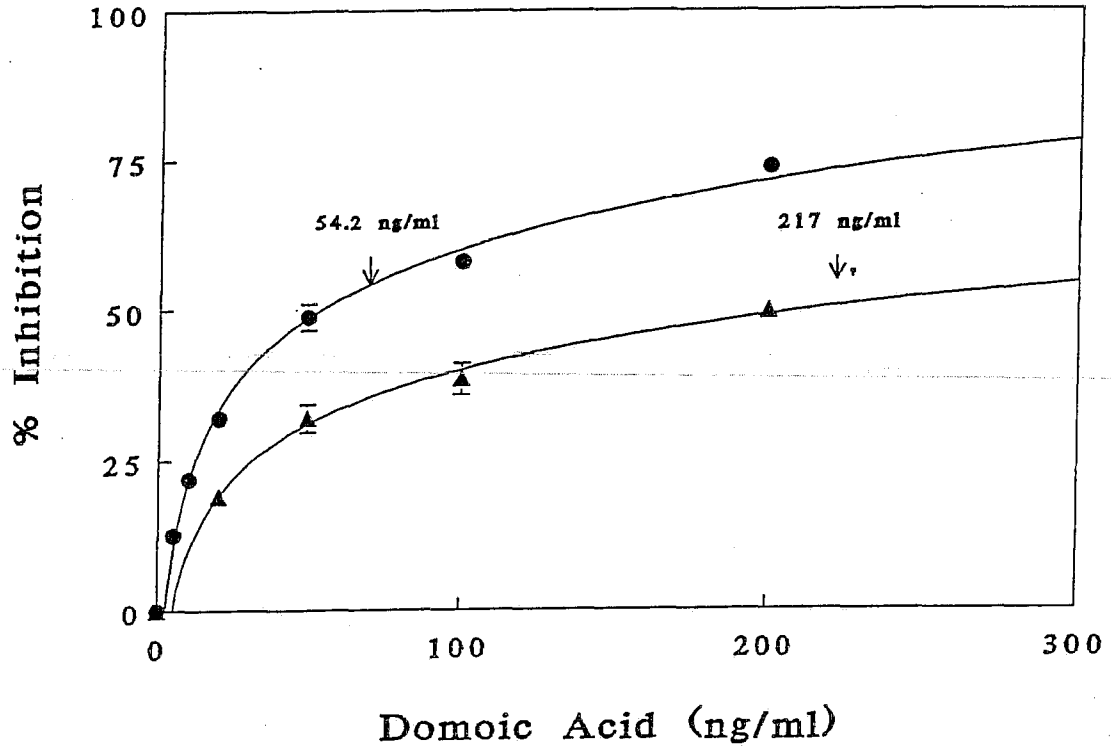

Figure 1

Performance of anti-OVA-DA (1/2000) (• r = 0.997), and anti-KLH-DA (1/2500) (♦ r = 0.996) sera in competitive ELISA for domoic acid. Arrows represent the indicated concentration of domoic acid required (anti-OVA-DA=54.2 ng.mL^{-1}; anti-KLH-DA=217 ng.mL^{-1}) to reduce the absorbence generated by the sera alone by 50%. Curves represent the least squares regression of y on log(x), and error bars represent the standard deviation around the mean of triplicate measurements.

1/35,000, while the titre of the anti-OVA-DA serum was approximately 1/50,000 (Table 1). The BSA-DA conjugate failed to produce measurable titres against either KLH-DA or OVA-DA (Table 1).

The anti-OVA-DA and the anti-KLH-DA sera were compared in the competitive ELISA. Free DA logarithmically inhibited absorbency generated with both the anti-OVA-DA (r = 0.997) and the anti-KLH-DA (r = 0.996) sera (Fig. 1). The concentration of free DA required for 50% inhibition with anti-OVA-DA was 54.2 ng.mL^{-1}, while 217 ng.mL^{-1} DA was required with the anti-KLH-DA (Fig. 1). Thus, the anti-OVA-DA serum had higher affinity for free DA than the anti-KLH-DA serum, and it was concluded that the anti-OVA-DA serum would therefore provide the more sensitive assay for DA. The anti-OVA-DA serum was also found to be highly specific, as neither kainic acid, glutamic acid, aspartic acid nor saxitoxin cross-reacted with the anti-OVA-DA serum (Table 2).

Table 2

Cross-reactivity of the anti-OVA-DA serum with various compounds in competitive ELISA as determined by the Student's t-test for paired observations.

Test Compound	Concentration µg/mL	Reduction in Absorbence (405 nm)[1] Mean ± S.D	*P*[2]
Domoic Acid	0.005	0.038 ± 0.004	<0.005[3]
Kainic Acid	50.0	0.000 ± 0.004	>0.5[4]
Aspartic Acid	50.0	0.001 ± 0.024	>0.5
Glutamic Acid	50.0	0.003 ± 0.011	>0.5
Saxitoxin	1.00	0.004 ± 0.009	>0.5

1 Reduction in absorbences (405 nm) generated in competitive ELISA by the test compound from the control (anti-OVA-DA alone). Data are given as the mean and standard deviation.) of triplicate measurements.

2 probability (*P*) that the observed reduction in absorbence (405 nm) is due to random variation.

3 indicates a significant reduction in absorbence from control.

4 indicates no significant reduction in absorbence from control.

The concentration of DA in MUS-1 (mussel standard reference material) was accurately determined to within 5% of the actual value in the aqueous extract (Table 3) with DACS-1 (DA calibration solution) used as a standard. The DA contents of DACS-1 and MUS-1 have been extensively analyzed and characterized (Quilliam et al. 1989a, Quilliam et al. 1989b, Pleasance et al. 1990, Wright et al. 1989, and Wright et al. 1990), with MUS-1 containing a higher and more varied concentration of DA isomers (Fig. 2). Because the total DA concentration in MUS-1 was accurately determined using the DACS-1, it would appear that this competitive ELISA provided a measure of total DA, which included the concentration of the various DA isomers present in MUS-1 (Fig. 2).

Total DA concentration was accurately determined to within 8% of the actual value in both aqueous and acid (Association of Official Analytical Chemists 1990) extracts and at 10, 5, 1.0, 0.5, and 0.25µg DA.mL^{-1} extract (Table 3). The lower limit of detection of DA in mussel extracts by this competitive ELISA has not been precisely determined. In sea mussel extracts, however,

Table 3

Spike-recovery experiment of domoic acid in aqueous and acid sea mussel extracts as determined by competitive ELISA.

Domoic Acid Spiked ($\mu g.mL^{-1}$)	Domoic Acid Determined ($\mu g.mL^{-1}$ (1); % recovery in parentheses) Extract	
	Aqueous	Acid
39.1 (2)	41.0 ± 1.60 (105%)	-
10	9.75 ± 0.82 (97.5%)	10.8 ± 0.84 (108%)
5	4.95 ± 0.24 (99.0%)	4.59 ± 0.39 (91.8%)
1.0	1.08 ± 0.30 (108%)	1.06 ± 0.22 (106%)
0.5	0.53 ± 0.06 (106%)	0.50 ± 0.04 (100%)
0.25	0.26 ± 0.08 (104%)	0.25 ± 0.00 (100%)

(1) mean and standard deviation of triplicate measurements over at least two dilutions.
(2) MUS-1 standard extract.

levels of DA not detected by HPLC were detected in competitive ELISA, (Table 4), suggesting that the lower limit of DA detection in ELISA is between 0.1 and 0.2 $\mu g.g^{-1}$.

Direct comparison of DA determinations with ELISA and HPLC analyses of sea mussel extracts indicated a good correlation ($r = 0.960$) between the two methods (Table 4). In most cases, the ELISA determination yielded higher DA concentrations than the HPLC analysis (Table 4). Again, the difference between the two assays may be attributed to the presence of DA isomers known to occur in toxic mussels (Quilliam et al. 1989a, Wright et al. 1990). Although DA and its isomers would have been included in the ELISA result, the fact that DA isomers do not co-elute with DA (Lawrence et al. 1989) strongly suggests that they were not determined in routine HPLC analyses.

DA content as determined by ELISA was underestimated in two samples, #19980 and #20053 (Table 4). This may have been due to the transformation of DA which can occur in storage (Lawrence et al. 1989). Additionally, potentially contaminating compounds, such as oxidized tryptophan, can co-elute with DA, and result in an overestimation of DA in the HPLC determination (Quilliam et al. 1989a).

Table 4

Comparison of domoic acid determinations using the HPLC and ELISA methods.

Sample	Domoic Acid ($\mu g.g^{-1} \pm$ S.D.[1])	
	HPLC	ELISA
20106	undetected	undetected
19761	undetected	0.16 ± 0.00
19885	undetected	0.16 ± 0.02
19785	undetected	0.24 ± 0.04
20093	undetected	0.30 ± 0.04
20062	undetected	0.36 ± 0.04
20090	undetected	0.36 ± 0.12
20092	undetected	0.36 ± 0.08
20105	undetected	0.36 ± 0.08
19845	undetected	0.36 ± 0.08
20164	undetected	0.51 ± 0.02
20163	undetected	0.58 ± 0.08
19884	undetected	2.80 ± 0.28
20107	0.4	1.00 ± 0.20
20096	0.7	2.80 ± 0.44
19940	0.9	1.40 ± 0.20
19844	1.3	2.00 ± 0.20
20050	1.4	3.00 ± 0.48
20051	1.4	2.00 ± 0.40
19981	1.9	2.60 ± 0.10
20064	2.1	1.76 ± 0.16
20065	2.1	1.56 ± 0.04
20095	2.2	2.64 ± 0.16
20068	2.2	4.76 ± 0.60
20048	3.5	3.80 ± 0.30
20067	4.2	5.00 ± 0.96
20054	5.2	8.20 ± 0.28
19939	7.9	8.70 ± 0.84
20030	9.9	12.2 ± 0.48
20053	18.2	13.7 ± 0.71
19980	47.4	35.4 ± 1.80
20029	54.3	70.4 ± 1.20

[1] standard deviation of duplicate or triplicate measurements.

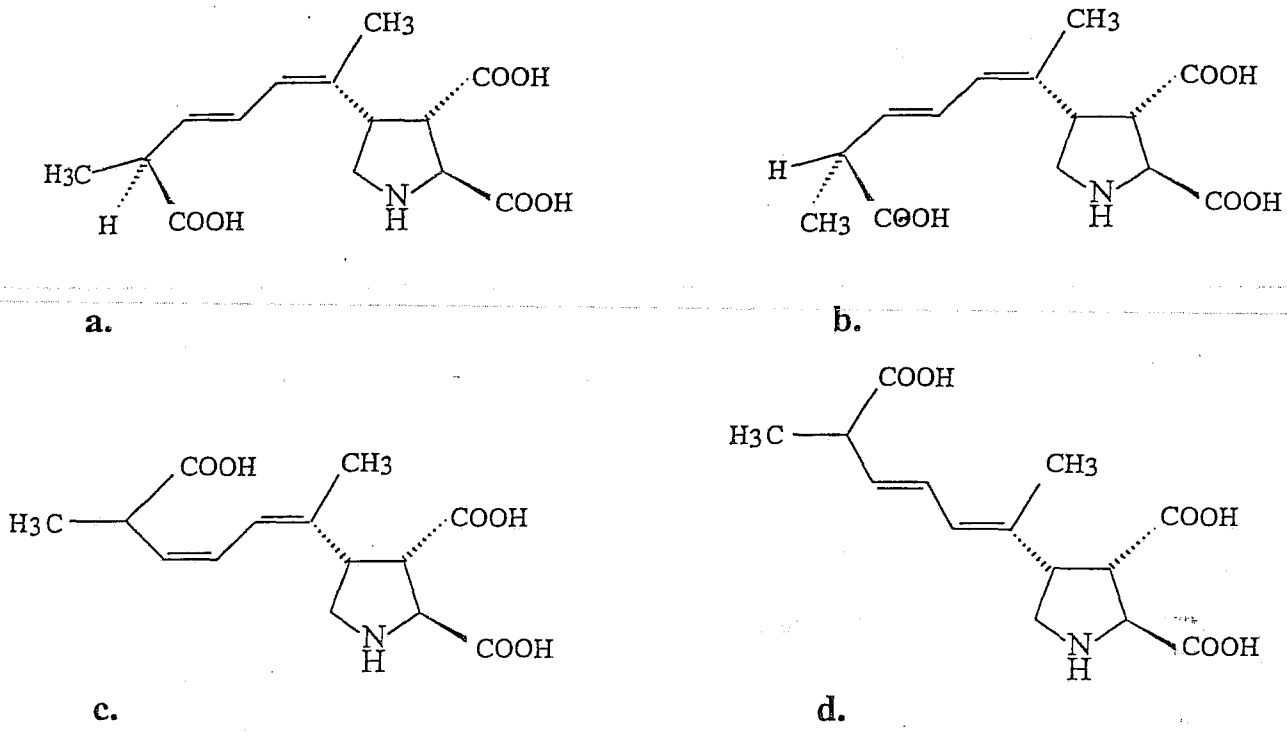

Figure 2

Chemical structures of domoic acid and domoic acid isomers; (a) domoic acid, (b) diastereoisomer of (a), (c) cis-trans isomer of (a), (d) cis-trans isomer of (a).

The vast majority of samples routinely tested for DA contain undetectable levels (Department of Fisheries and Oceans 1992). Immunochemical screening of mussel extracts would considerably reduce the volume of samples requiring DA analysis by HPLC. This would provide: (1) a higher turnover of positively testing samples for rapid confirmation by HPLC, (2) increased capacity to test suspect samples of other species, and (3) more rapid analyses of commercial shellfish and crab samples requiring official documentation of DA content which is demanded by some export markets.

Finally, the flexibility and portability of immunoassays makes them ideal for use as on-site testing tools. Given the sporadic nature of DA outbreaks, an on-site field test for this toxin would help to provide more timely analyses, especially in remote areas. Consequently, the monetary losses incurred by harvesters and processors when commercial lots are recalled due to DA contamination could be reduced.

In summary, an immunochemical method for determining DA concentration in shellfish extracts, such as the one described here, could provide a sensitive, rapid and economical procedure which effectively complements the standard HPLC procedure currently employed in the routine monitoring for DA in coastal waters.

Acknowledgments

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Relationship between paralytic shellfish poison toxicity and a 17 kilodalton protein in butterclam foot tissue

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Paralytic shellfish poisoning (PSP) affects many temperate coastal areas of the world and involves the accumulation of potent neurotoxins of dinoflagellate origin (*Alexandrium* sp.) in bivalve molluscs. Ingestion of toxic bivalves results in paralysis of the neuromuscular system and can be fatal in severe cases.

All along the North American coastline, PSP represents a serious problem not only with regard to public health, but also to the shellfish industry. In British Columbia, it is a damaging economic problem, affecting both the wild stocks and aquaculture of various species of clams, scallops and oysters. The imposition of blanket closures and the minimum period of six weeks for reinspection can result in job and market losses leading to deferred or lost income. Over harvesting of existing open areas is a consequence of this situation which is threatening the future viability of the shellfish industry. As a result there is growing need for sensitive and immediate screening methods for contaminated shellfish. It is hoped, for example, that such procedures could result in some site-specific exemptions from blanket closures (Vautier 1991, Spence 1991).

The original objective of this project was to develop serological screening assays for detection of PSP contamination in various shellfish species, augmenting the standard mouse bioassay by allowing more frequent monitoring of sites, as well as by expanding both geographical and species coverage. Former studies from this laboratory reported the presence of a high molecular weight protein found initially in *Hemigrapsus* crabs exposed to a red tide outbreak or acutely injected with saxitoxin (Barber et al. 1988). Subsequently a polyclonal antiserum developed against this protein, called saxitoxin induced protein (SIP) was found to cross-react with antigens unique to toxic shellfish (Smith et al. 1989; Kitts et al. 1992). A prototype ELISA for screening toxic butterclams (*Saxidoma giganteus*) was then developed (Kitts and Smith 1992).

In this study, all PSP toxicities were determined using the mouse bioassay (Association of Official Analytical Chemists 1990). Murine monoclonal antibodies were raised against proteins from foot tissue taken from butterclams contaminated with PSP. Most of the monoclonals isolated were specific for a 23 kilodalton (kd) protein, which did not appear to be associated with PSP toxicity. Western blot analysis demonstrated that one monoclonal line, 2B10, was highly specific for a 17 kd foot protein (FP; Fig. 1). Also, the density of the 17 kd FP band from butterclam foot extracts corresponded roughly to PSP toxicity over a broad range (Fig. 1).

Using purified 17 kd butterclam FP and monoclonal 2B10, a standardized ELISA was developed to quantify the concentration of the 17 kd FP relative to total soluble protein (TSP) concentration in foot tissue from 57 butterclam samples. Analysis of foot tissue from butterclams representing a range of PSP toxicities between 18 and 200 μg PSP / 100g shellfish indicated that there was a significant linear correlation between the relative level of 17 kd FP and total PSP toxicity ($r = 0.747$, $P < 0.01$) (Fig. 2). At toxicities greater than 200 μg PSP / 100g shellfish, the relative

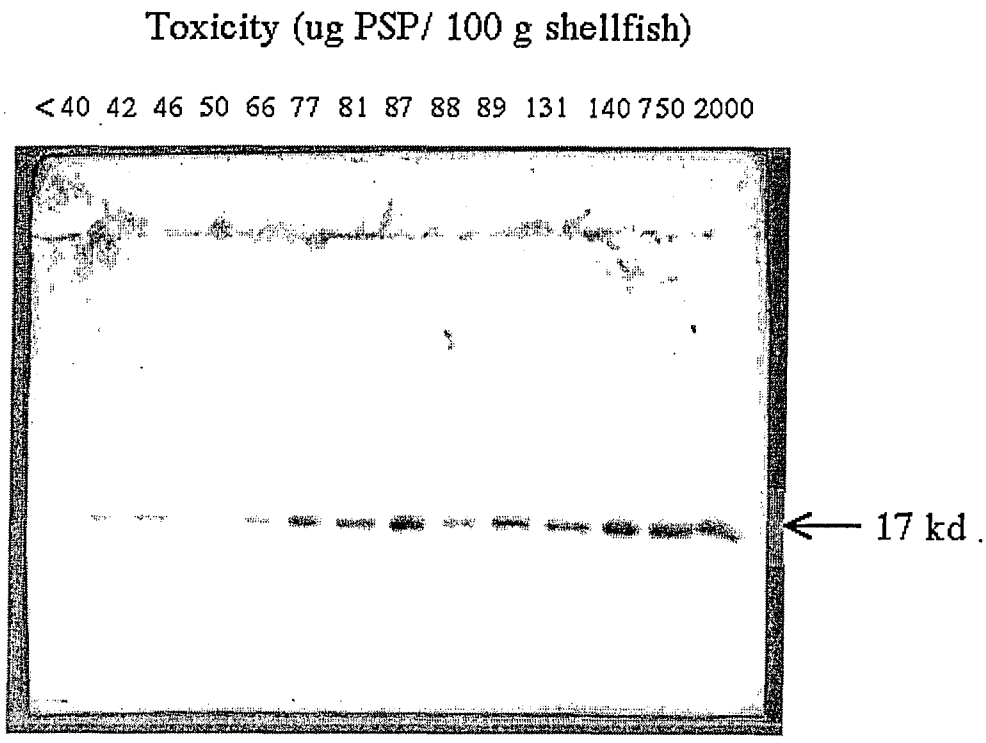

Figure 1

Western blot developed with monoclonal 2B10 indicating the relative amount of 17 kd protein in crude extracts of foot tissue from butterclams representing a broad range of paralytic shellfish poison (PSP) toxicities.

concentration of 17 kd FP did not continue to increase along this trend, but became much more variable (Fig. 2).

Generally, butterclam samples with toxicities greater than 79 μg PSP / 100g shellfish had greater than 100 μg 17 kd fp/mg TSP, a trend consistent over the entire range of samples tested (Fig. 3). This arbitrary criterion was used to successfully identify 51 of 57 samples as having either an acceptable level of toxicity (less than 79 μg PSP / 100g shellfish) or an unacceptable level of toxicity (greater than 79 μg PSP / 100g shellfish). Of the 57 samples tested, there were 6 false positives and no false negatives.

Although this trend was similar to that seen in the analysis of SIP-cross-reacting proteins in butterclam foot tissue, and the 17 kd FP does cross-react with the anti-SIP serum (Kitts and Smith 1992), a direct sample-by-sample comparison of the 17 kd FP and SIP equivalents (Fig. 4) suggested that the 17 kd FP did not represent the major cross-reacting antigen in foot tissue from toxic butterclams previously described (Kitts and Smith 1992). Although regression analysis of these data indicated a significant linear correlation between the two ($r = 0.555$), this probably reflected the fact that both the 17 kd FP and SIP equivalent antigens were associated with PSP toxicity, rather than a relationship of identity.

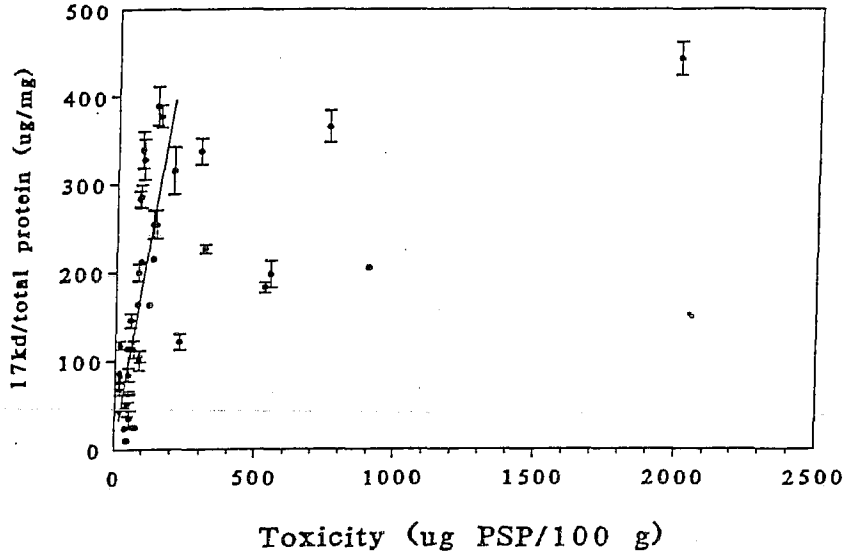

Figure 2

The relationship between paralytic shellfish poison (PSP) toxicity and the level of the 17 kdbutterclam foot protein relative to total soluble protein concentration. The linear portion of the curve represents the least squares linear regression (r = 0.747). Error bars represent standard deviation around the mean.

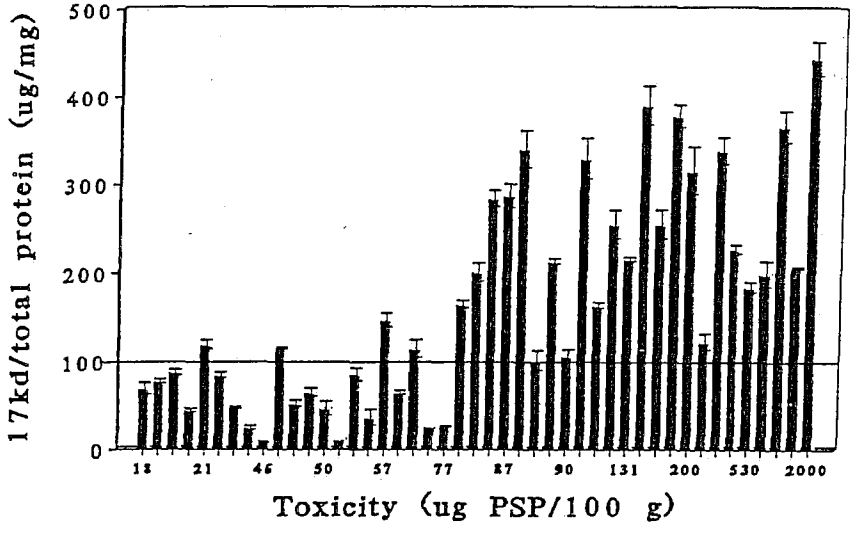

Figure 3

Screening of butterclams for paralytic shellfish poison (PSP) using the relative level of the 17 kd butterclam foot protein (BFP) as an indicator. The horizontal line at 100 µg 17 kd BFP.mg^{-1} total soluble protein represents a threshold for identifying toxic butterclam samples (with PSP concentrations exceeding 79 µg / 100g shellfish). Of 45 samples represented here, 41 are correctly identified as acceptably non-toxic or unacceptably toxic, based on the threshold criteria given above. Error bars represent standard deviation around the mean.

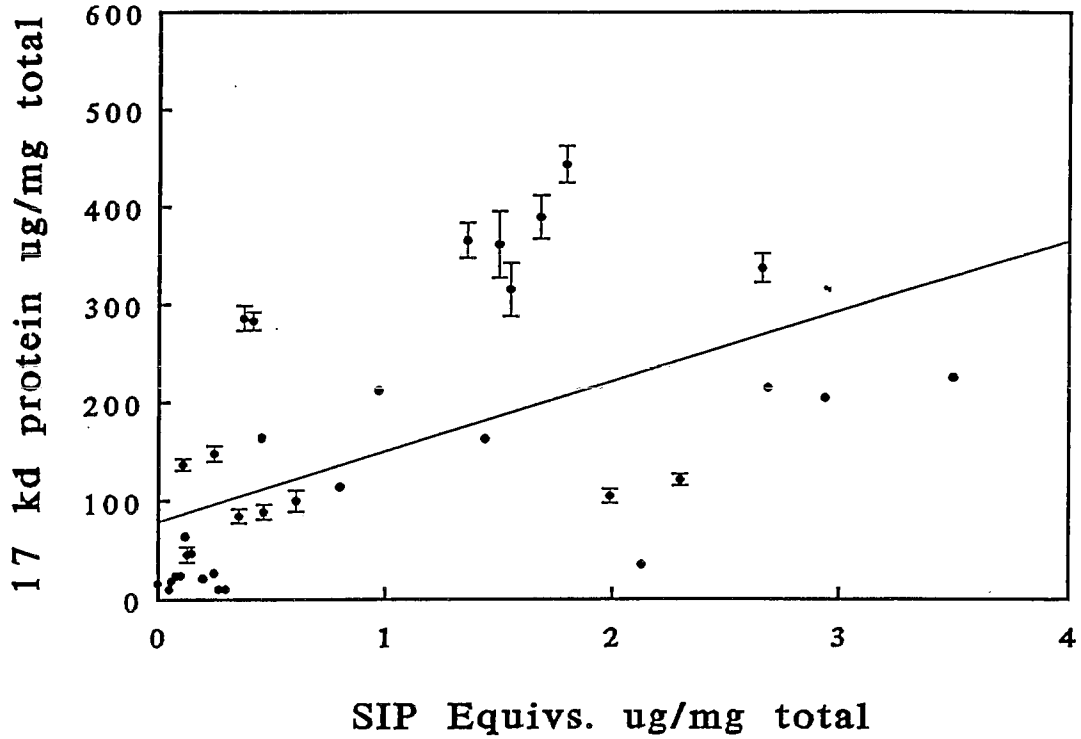

Figure 4

The relationship between saxitoxin-induced protein (SIP) equivalents and the level of 17 kd protein measured in foot tissue from butterclams representing a wide range of toxicities (undetectable to 2000 μg PSP / 100g shellfish). The curve represents the best fit of the least squares linear regression (r = 0.555). Error bars represent the standard deviation around the mean.

Of the 57 samples tested, there were 12 samples which contained undetectable levels of PSP toxicity. Of these, two had relatively high levels (greater than 100 $\mu g.mg^{-1}$ TSP) of 17 kd FP (Table 1). The remaining 10 samples displayed considerable variation in the level of 17 kd FP present, ranging from 16 to 85 μg 17 kd FP.mg^{-1} TSP. Relative levels of the 17 kd FP were more consistent, however, when compared within management areas and sampling dates (Table 1). For example, in area 17-04, the relative levels of 17 kd FP were high ranging between 65 and 85 $\mu g.mg^{-1}$ TSP, while in area 24, levels of the 17 kd FP were generally much lower, ranging between 16 and 27 $\mu g.mg^{-1}$ TSP (Table 1). This suggested that, unlike the saxitoxin-induced protein in *H. oregonensis*, the 17 kd FP in butterclams is probably not directly induced by PSP toxins, but its induction may represent a response to other factors associated with PSP.

Levels of 17 kd FP and observed temporal trends in PSP toxicity in butterclams appear to supported this hypothesis. In 1989 and 1990, butterclams from particular sites in different

Table 1

Proportion of 17 kd butterclam foot protein (BFP) per total soluble protein (TSP) in foot extracts from butterclams with very low or undetectable toxicity.

Sample	Date d/m/y	Area	Toxicity [1] µg PSP/100 g	17 kd BFP/TSP µg.mg^{-1}
RRI	27/12/90	17-04	none detected	85.1 ± 6.0
RR5	27/12/90	17-04	none detected	63.9 ± 4.9
RR6	27/12/90	17-04	none detected	76.3 ± 6.8
KIC	13/08/92	12-43	none detected	64.6 ± 3.8
KIB	28/10/92	12-39	none detected	420 ± 23[1]
KIE	28/10/92	12-38	<20	83.3 ± 7.8
797	10/07/90	24-10	none detected	18.3 ± 1.2
798	10/07/90	24-03	none detected	26.7 ± 2.5
803	10/07/90	24-13	<44	16.1 ± 1.5
796	10/07/90	24-11	<44	24.1 ± 1.2
1131	01/08/90	04-09	<44	137 ± 61

[1] Indicates false positive samples with greater than 100 µg 17 kd BFP.mg^{-1} TSP.

month. PSP toxicity records for these samples show that sites displaying both low PSP toxicity (less than 79 µg PSP / 100g shellfish) and low (less than 100 µg) 17 kd FP/mg TSP in butterclams during one month did not display an increase in PSP toxicity the following month (Fig. 5). However, at 4 sites in which butterclams contained low levels of PSP toxicity (less than 79 µg PSP / 100g shellfish), but high levels (greater than 100 µg) of 17 kd FP.mg^{-1} TSP, PSP toxicity had increased significantly in butterclams by the following month at these sites or closely neighbouring sites, exceeding 80 µg / 100 g shellfish (Fig. 5).

In conclusion it appears that the 17 kd FP is associated with PSP toxicity in butterclams, but the precise nature of this association remains unknown. There may be some practical potential in using the 17 kd FP as an indicator of PSP toxicity if a rate of approximately 11% for samples falsely testing positive can be tolerated. To date, observed trends indicate that quantifying relative levels of 17 kd FP may be useful in predicting impending PSP outbreaks in butterclams, however, these observations are preliminary and more research is required to investigate this further.

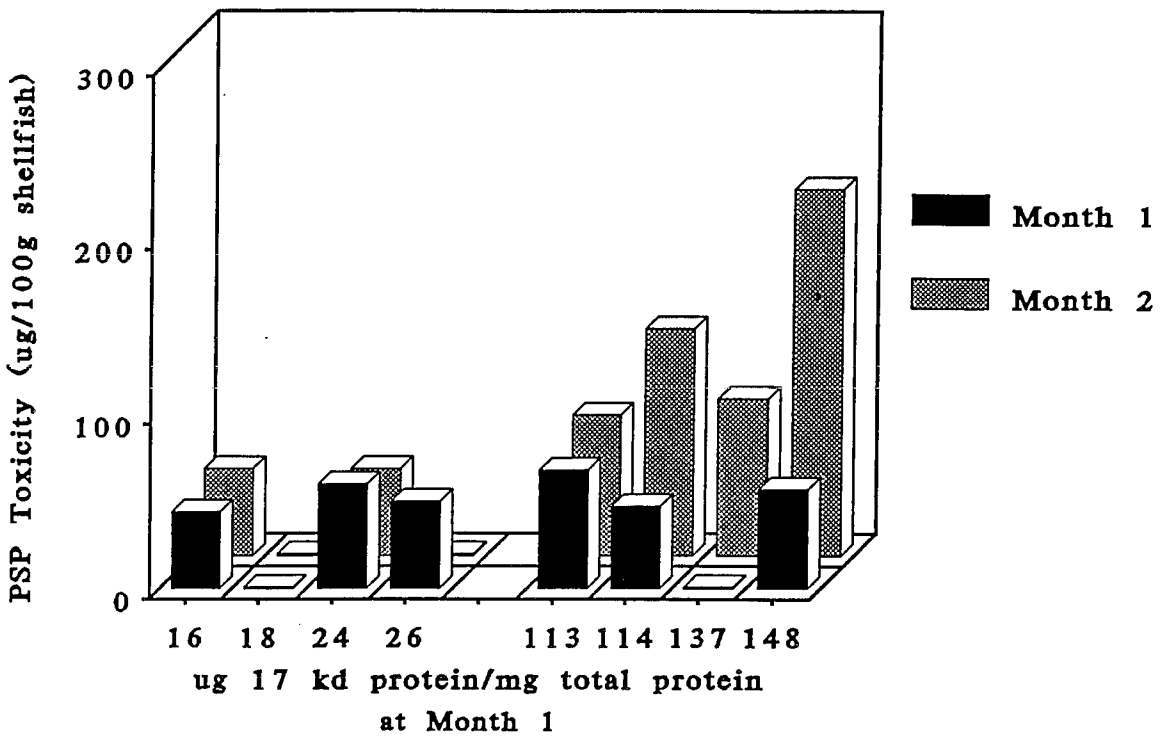

Figure 5

Temporal dimension in the relationship between the 17 kd butterclam foot protein (FP) and paralytic shellfish poison (PSP) toxicity indicating that concentrations of the 17 kd FP exceeding 100 µg/total soluble foot protein in butterclams of low toxicity (less than 79 µg PSP / 100g shellfish) may signal an impending increase in PSP toxicity in butterclams.

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Distribution of the genus *Dinophysis* in the southeastern Gulf of St. Lawrence

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Species of the genus *Dinophysis* commonly occur in significant numbers (more than 5,000 and sometimes more than 10,000 cells.L^{-1}) in the southeastern Gulf of St. Lawrence from May until September. The greatest concentrations of these organisms are found in 3 general locations: 1) northern New Brunswick, along the south shore of the Baie des Chaleures and south as far as the estuary of the Miramichi River and Cap St. Louis, 2) off eastern Prince Edward Island and 3) in St. Georges Bay, Nova Scotia. The most prominent species present (in order of abundance) are *D. norvegica*, *D. acuta* and *D. acuminata*.

All these species are known to be producers of Diarrhetic Shellfish Poisoning (DSP) toxins in other parts of the world, but evidence for this in the southeastern Gulf of St. Lawrence is limited to the low levels of DTX-1 (a DSP toxin frequently found in eastern Canada) which were detected by the highly sensitive, radiometric phosphatase inhibition assay during a bloom of *D. acuminata* off eastern P.E.I. in August 1989 (Luu et al. 1993). Other analytical methods (HPLC/ADAM, HPLC coupled with ion mass spray spectrometry and immunological techniques) have so far proved negative or inconclusive. Whether these organisms are capable of producing DSP toxins, as

elsewhere, and the possible factors governing such toxin production remain major unknowns at this time.

At any given location, population levels of *Dinophysis* are characterized by a large interannual variance. Great variation is also found between inshore phytoplankton monitoring sites within a given area and within a particular site on an intraseasonal (week to week) basis. This short term variation in the concentration of *Dinophysis* at inshore monitoring sites may imply that these cells do not grow in situ, but are advected to the site from deeper, stratified water in a series of pulses possibly driven by meteorological forcing.

In general, where large *Dinophysis* populations persist and probably grow, the water column is stratified, with the cells concentrated at a depth usually characterized by very low nutrient levels, particularly for nitrogen. In the outer Miramichi Bay area, where stratification is probably due both to solar heating and freshwater discharge from the river, *Dinophysis* populations increase at stations further from the shore. The location of these populations outside sheltered waters and the concentration at depth in the water column make it difficult to monitor this potential hazard with the type of inshore monitoring program presently in place. Should DSP toxicity occur in such circumstances, we are vulnerable to a phycotoxin event with possible important human health and economic consequences. Determining effective ways of monitoring this risk is a major objective of the phycotoxins program in the Gulf Region.

Dinophysis species have not been successfully brought into culture for laboratory studies of the factors governing the production of DSP toxins by these species. However, we have developed methods of using nutrient enrichments in microcosms to induce these organisms to grow under controlled conditions. We are presently using these techniques to try to determine the factors which may lead to the production of DSP toxins by *Dinophysis* in the southeastern Gulf of St. Lawrence.

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Domoic Acid Utilizing Bacteria From Molluscs and Their Possible Involvement in its Elimination From Shellfish

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Domoic acid has been shown to be produced widely in nature and in quantity; as it does not appear to accumulate in nature beyond a certain point, mechanisms must exist for its degradation and disposal. As bacteria contained within the marine environment are prime choices to mediate this activity, a search for bacteria competent to utilize domoic acid was instituted.

Sources examined in detail for such bacteria included sea water and sediments from mussel culture areas of Cardigan Bay, Prince Edward Island (the origin of the domoic acid containing mussels giving rise to the original intoxication episode in 1987); Bedford Basin, Nova Scotia, seawater; bacteria from collections made by A. Boraie over the years from Bedford Basin and the Bay of Fundy, as well as our own collection of bacteria from various marine sources, were examined for growth on domoic acid and the capacity of resting cells to oxidize it using manometric procedures. Despite extensive and intensive trials, the results were uniformly negative; in some instances, the domoic acid appeared to be inhibitory. Clearly, the capacity to grow on and utilize domoic acid is not a common microbial attribute. As the original premise was still valid, a change in direction was required to find bacteria responsible for domoic acid biodegradation or to discover alternative explanations for its disappearance.

Published studies have shown that blue mussels (*Mytilus edulis*) routinely are capable of reducing the concentrations of accumulated domoic acid relatively rapidly; in contrast, the results from trials as well as anecdotal evidence indicate that the sea scallop (*Placopecten magellanicus*) eliminates domoic acid very slowly. Thus, it was decided to broaden the search to include an examination of the microflora of molluscan species in which domoic acid had been detected. Through application of enrichment techniques, using gill and digestive gland tissue, we showed that 45 of 46 individual mussels possessed bacteria, the growth of which was enhanced to a limited, but significant extent by domoic acid; in addition, five blue mussel soft tissue homogenates (representing, in all, another 50 or so mussels from different locations) also yielded similar bacteria. Nine of 20 softshell clams (*Mya arenaria*) (two groups of 10) had bacteria stimulated by domoic acid while only four of 60 scallops taken from six locations were positive for bacteria, the growth of which was stimulated by domoic acid. One sample (10) of red mussels (*Modiolus modiolus*) yielded two positive specimens.

Although a number of bacterial genera were represented in the isolates (*Alteromonas, Vibrio, Pseudomonas, Acinetobacter, Aeromonas, Flavobacter,* and *Alcaligenes*), the dominant genus appeared to be *Alteromonas* followed by *Pseudomonas* sp. Substrate utilization trials were carried out with five of the bacterial isolates showing the greatest growth in the presence of domoic acid. A significant portion of the substrate (depending upon the isolate) disappeared from the medium after incubation at 20°C.

It was concluded that the blue mussel virtually always possessed microflora which could utilize domoic acid, while the softshell clam was more variable. The sea scallop only occasionally had such organisms and the one sample of red mussels seemed to parallel the results obtained with the scallops. Domoic acid clearance from molluscan shellfish species, as judged from limited trials and anecdotal evidence appear to parallel these microbial findings. To account for the different microbial capacities evident in the different molluscan species, it is necessary to postulate a selection mechanism; this might involve molluscan lysozymes such as those described in publications on *M. edulis*. As these bacteria have the potential to play a significant and possibly a dominant role in toxin elimination in certain molluscan species, it could be profitable to explore these leads in considerable depth. If confirmed as a significant or primary toxin clearance mechanism in molluscs, practical applications of bacterial clearance could include detoxification procedures based upon favouring autochthonous domoic acid utilizing bacteria and possibly implanting relevant bacteria (or transferring their capacities to autochthonous bacteria) in those molluscan species which appear to select against the toxin utilizing bacteria.

Spatial and temporal distributions of harmful phytoplankton during the summers of 1992 - 93 in Barkley Sound.

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Barkley Sound is a large embayment (≈ 550 km^2) on the west coast of Vancouver Island (Fig. 1). It is divided into three channels: Loudon Channel on the western side is relatively shallow (40 m) and is the most productive (MacIsaac et al. 1991); Imperial Eagle Channel (approx. 100 m deep) lies in the centre and is most exposed to the offshore environment; Trevor Channel is narrow and deep (150 m) and is heavily influenced by the freshwater outflow from Alberni Inlet. Three stations were established in each of the three main channels (Fig. 1).

The general plankton composition differed during the two summers: the El Niño year 1992 experienced higher concentrations of flagellates than in 1993, whereas the trend was reversed for diatoms. There was also a greater occurrence of the potentially harmful species in 1992. Fifteen potentially harmful phytoplankton species have been found in Barkley Sound (diatoms: *Chaetoceros concavicornis*, *C. convolutus*, *Pseudonitzschia australis*, *P. pungens*; dinoflagellates: *Alexandrium catenella*, *Dinophysis acuminata*, *D. fortii*, *D. infundibulus*, *D. norvegica*, *D. ovum*, *Goniodoma pseudogonyaulax*, *Prorocentrum minimum*; others: *Heterosigma carterae*, Simoom raphidophyte, unidentified raphidophyte). Populations of harmful species can be divided into those which form within the inlet (autochthonous), those which are advected from offshore (allochthonous), and those which show mixed patterns.

Alexandrium catenella typically forms subsurface (5-10 m) populations below the pycnocline in Barkley Sound, except later in the season (lower light) when it congregates near the surface. There appears to be a resident population in Toquart Bay (NW corner of the Sound); however, the highest numbers we observed occurred in the upper 5 m of the central region during September 92.

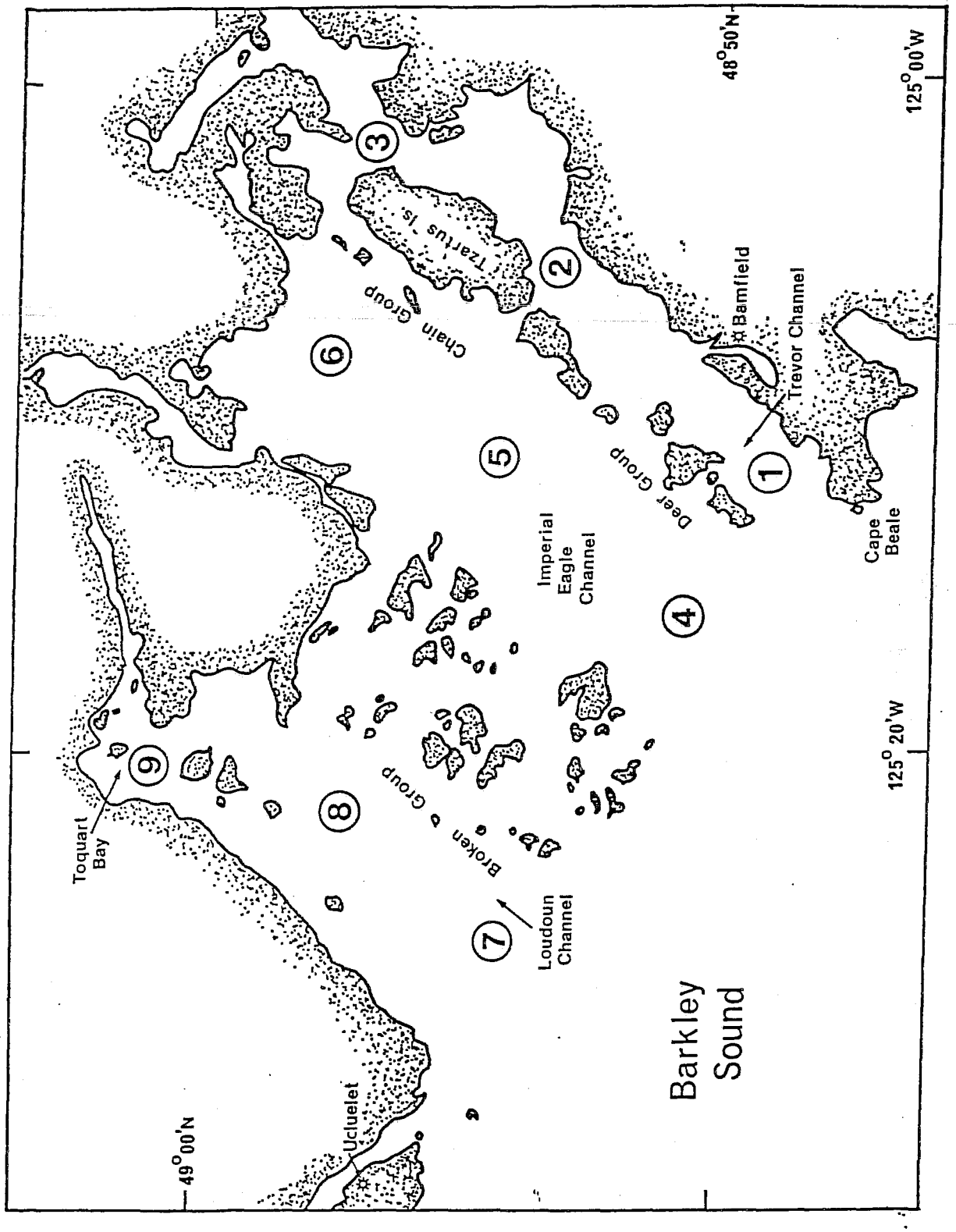

Figure 1

Barkley Sound, showing sampling locations and places mentioned in text

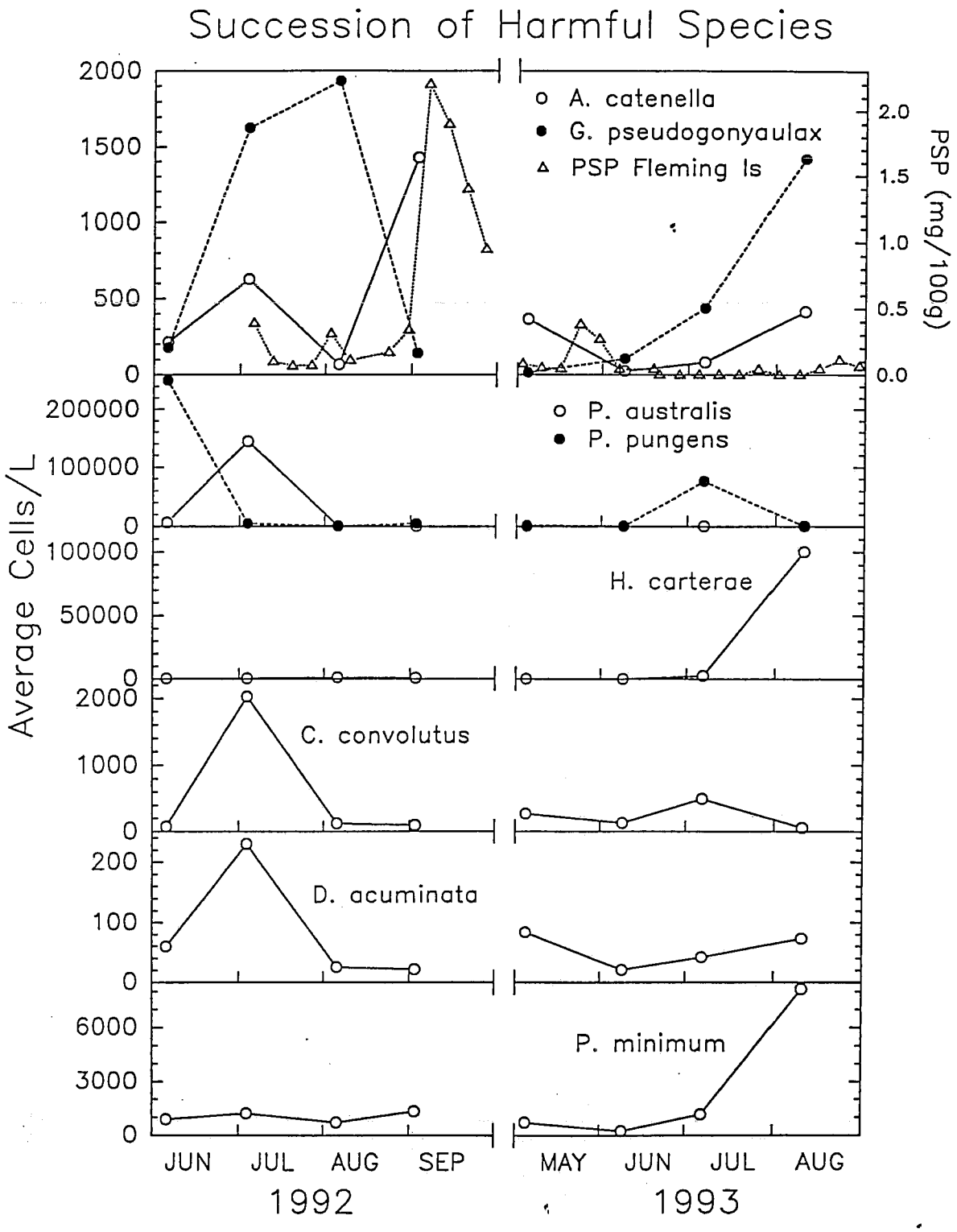

Figure 2

Succession of harmful species (June - Sept. 1992 and May - Aug. 1993), and PSP concentrations in shellfish at Fleming Island

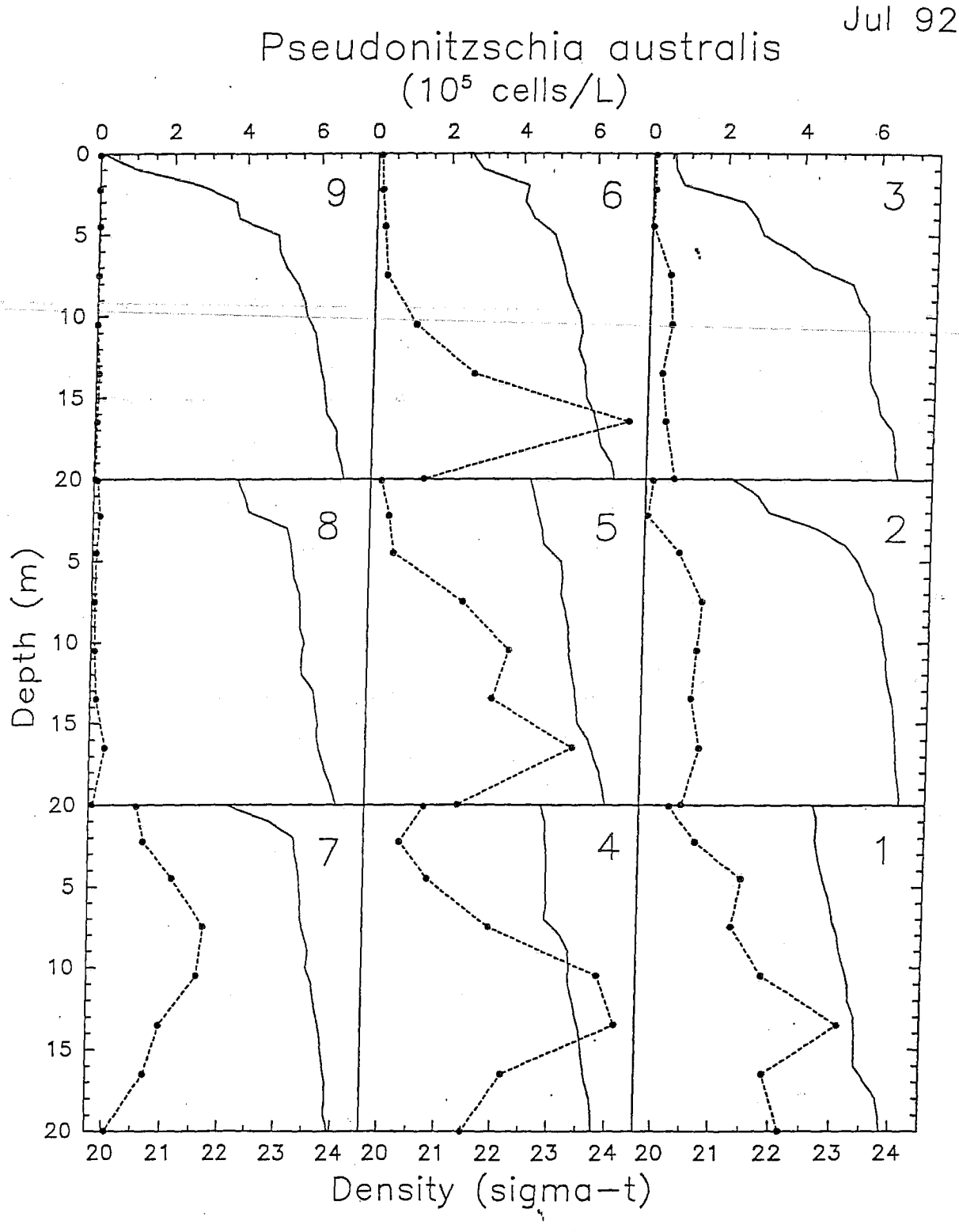

Figure 3

Concentration of *Pseudonitzschia australis* cells (- - - - -) and density (———) in July 1992 at the nine sampling locations

The flanking outer stations, however, did not have comparable cell numbers, suggesting that the population arose within the Broken Island Group. PSP data (DFO Inspection Branch) show a concurrent rise in the toxicity of shellfish at nearby Fleming Island (Fig. 2). Interestingly, the temporal distribution of the closely related species, *Goniodoma pseudogonyaulax*, suggests no link between this dinoflagellate and saxitoxin production.

The domoic acid-producing *Pseudonitzschia australis* was only abundant during the El Niño year 1992 (Fig. 2). During the July cruise of this year we observed this diatom being advected from offshore, primarily in the direction of the prevailing winds (SW). The bulk of the population occurred at 10-15 m and intruded down the central channel (Fig. 3). Away from the outer stations this diatom appeared to sink out. There was no correlation with the domoic acid data (DFO Inspection Branch).

Pseudonitzschia pungens was also more abundant during 1992 (Fig. 2) and was primarily advected from offshore in early summer (May/June). There appeared to be no connection with the occurrence of domoic acid, suggesting that these early offshore populations were *P. pungens* f. *pungens*. During late summer, offshore populations disappeared and deep resident ones formed in Toquart Bay. In 1992 the latter co-coincided with domoic acid concentrations of 100 ppm in this bay (Fig. 4), suggesting that this population was chiefly *P. pungens* f. *multiseries*. During 1993 no such co-occurrence was observed; however, domoic acid in Toquart Bay appeared during Oct/Nov when we did not sample.

Heterosigma carterae was not particularly noteworthy during our sampling periods. Highest abundance occurred during our August 1993 cruise when cell concentrations exceeded 1 million per litre in the surface waters of the most exposed station west of the Broken Island Group. There also appears to be periodic intrusions of *H. carterae* from Alberni Inlet during the late summer months (Jul-Sep).

The diatom *Chaetoceros concavicornis* was rarely present at our sample sites, making an appearance of note only during the September 1992 cruise (intrusion from offshore). This diatom would probably impact the Sound later in fall (Sep-Nov). The closely related species *C. convolutus* was always present during our sample times and was usually advected from offshore. Morphologically, this diatom was often smaller than usual with very reduced spines. There was evidence that *C. convolutus* can be advected from Alberni Inlet along a pronounced pycnocline. The distribution of harmful species in Barkley Sound suggests that Toquart Bay is potentially the most active site for toxin production. Its protected waters provide ideal conditions (temperature & salinity stratification, low wind turbulence, shallow bottom) for flagellated species. The dinoflagellates *A. catenella*, *G. pseudogonyaulax*, *D. acuminata*, and *P. minimum* form subsurface maxima at or below the pycnocline in this bay during summer and presumably have seed beds here. It also appears that Toquart Bay acts as a chemostat for introduced species such as *P. pungens*. Curiously, this bay does not provide a favourable environment for *H. carterae*, though a closely-related, fish-killing raphidophyte (which has yet to be described) formed a subsurface maximum at 15 m in July 1993.

As would be expected the offshore environment substantially influences Barkley Sound. The harmful species which are consistently advected into the Sound during summer are chiefly diatoms (*C. concavicornis, C. convolutus, P. australis, P. pungens*). Intrusions appear to be primarily at depth along the axis of the prevailing onshore winds (SW). Mackas & Sefton (1982) found that mono-specific diatom populations were common on the outer shelf off Barkley Sound (including

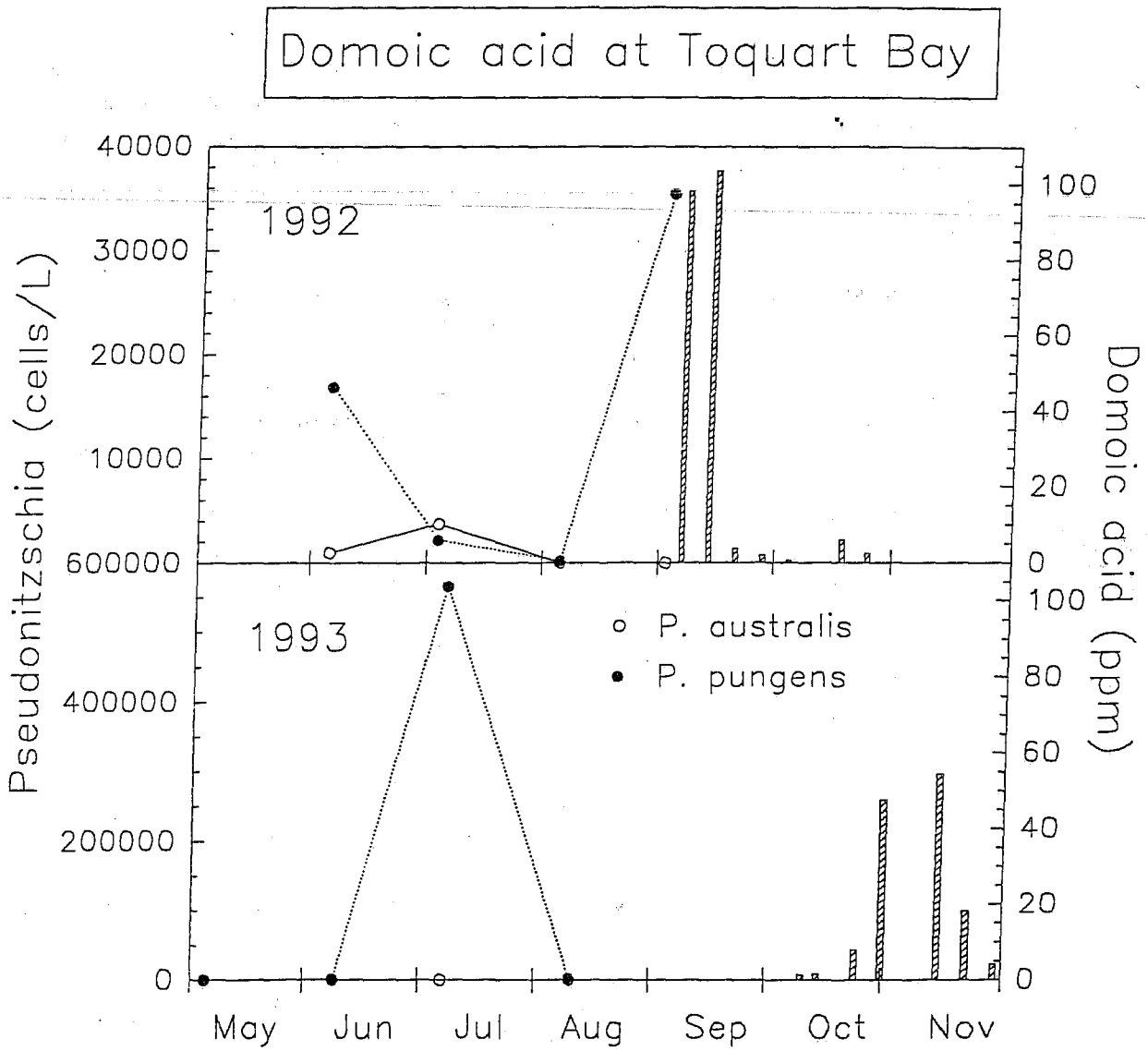

Figure 4

Concentrations of *Pseudonitzschia australis* and *P. pungens* at Toquart Bay in 1992 and 1993, and domoic acid concentrations in shellfish

Pseudonitzschia). Also, Forbes & Denman (1991) present multi-year distributions of *P. pungens*, indicating large offshore populations to the southwest. Given the strong prevailing onshore winds during summer, it is not surprising that intrusion takes place.

In summary, the potential for problems associated with harmful phytoplankton appears to be greatest in the Loudon Channel - Toquart Bay region and least in Trevor Channel. This corresponds more to water depth in each area than to relative wind exposure.

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Emerging diseases associated with harmful algal blooms and other waterborne agents

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Introduction

Paralytic shellfish, scombroid and ciguatera poisoning have been described for over a century, but within the last 20 years other diseases have been associated with seafood toxins, and appearances of toxic blooms in new locations are continually occurring.

For instance, paralytic shellfish poisoning (PSP) occurred for the first time in Newfoundland in 1982. Other recent Canadian concerns are PSP toxins in clams, mussels, oysters, and moonshells in the southern Gulf of St. Lawrence in 1988/89; *Alexandrium excavatum* in high numbers under the ice in the same region; and PSP toxins in lobster hepatopancreases in eastern Atlantic waters from 1990 onwards. Unique episodes of amnesic shellfish poisoning (ASP) and diarrhetic shellfish poisoning (DSP) were documented in 1987 (PEI) and in 1990 (Nova Scotia), respectively.

In the United States, PSP toxins were found in Long Island Sound for the first time in 1985, and domoic acid in shellfish on the west coast since 1991. In South America, exceptional *Alexandrium* blooms have been noticed off Argentina, Brazil and Chile. In France, DSP has been causing illnesses since 1983 and PSP since 1988 with spreading of the toxic dinoflagellates around the coasts. In the United Kingdom the largest toxic event occurred in 1990 with PSP toxins present in bivalves, crabs, lobsters and prawns in several locations. Scallop adductor muscles in Scotland also were found to contain PSP toxins. In Scandinavia, toxicity from *Alexandrium* has been spreading and large harmful algal blooms of *Chrysochromulina* and *Prymnesium* were recorded for the first time from 1988-91. Also, PSP toxins have been reported from more and more tropical countries causing illnesses and economic loss.

In addition, bacteria, such as *Vibrio cholerae*, and parasites are increasingly becoming recognized as fish-borne and shellfish-borne disease agents. There are several key factors that allow the emergence of such diseases and their recognition.

1) Natural environmental changes

Although increased awareness of illnesses from seafood toxins, and global expansion of aquaculture, has contributed to these reports, natural environmental factors are also important. These include global warming increasing the water temperature to allow warm water dinoflagellates to spread, e.g. *Gymnodinium catenatum* in the North Sea, the Kattegat and off Spain. Warming also increases upwelling of nutrient-rich cold water and a change in the phytoplankton distribution. PSP blooms are usually preceded by warm, calm periods of weather, and the increase in UV radiation, because of the weakening of the ozone layer in the Antarctic, may inhibit the zooplankton browsers in southern Chilean and Argentinian waters. Neurologic shellfish poisoning (NSP) has occurred in North Carolina and New Zealand because of unusual climatic

Table 1

Human influence on the aquatic environment leading to illness

Action	Consequence	Diseases that have occurred
Aquaculture	- New harvest areas in possible polluted water or water with risk of harmful algal blooms	- Norwalk-type viral diseases hepatitis, cholera, typhoid, ASP, DSP, PSP
	- Products distributed more widely with increased sales volume	- Large outbreaks of above
	- Use of human and animal feces as nutrient	- Norwalk-type viral diseases? hepatitis? cholera? typhoid?
	- Use of antibiotics to prevent fish and shellfish diseases	- Salmonellosis from antibiotic resistant strains?
Relaying of shellfish	- Introduction of new toxic species and pathogens	- Salmonellosis?, cholera?, PSP?
Eutrophication of water	- Increased algal blooms	- Cyanobacterial poisoning
Construction of harbours, ship wrecks, destruction of coral	- Exposure of inanimate surfaces to allow growth of benthic dinoflagellates	- Ciguatera poisoning
Cargo ships	- Dumping of contaminated ballast water in new locations	- Cholera, PSP
	- Transport of shellfish in fecally-contaminated barges	- Hepatitis A
	- International transport of contaminated foodstuffs (egg powder, cocoa, beans, coconut, spices, etc.)	- Salmonellosis from new serovars
Cruise ships	- Passenger consumption of contaminated food and water	- Shigellosis, *E coli* infections, salmonellosis, ciguatera poisoning

ASP: amnesic shellfish poisoning
DSP: diarrhetic shellfish poisoning
PSP: paralytic shellfish poisoning
? likely but not confirmed

conditions affecting currents and changing water temperature. Cholera is transmitted by *Vibrio cholerae* in water or food. The organism can exist in a non-culturable state in cooler water attached to freshwater aquatic vegetation and green algae, and can also be spread by ocean currents attached to drifting zooplankton. The non-culturable form has been detected in Chesapeake Bay, the Black Sea and the Antarctic.

2) Human influence on the environment

Harmful algal blooms can occur through excessive eutrophication which changes the algal population of a body of water, such as in the Black Sea and Seto Inland Sea; the colonization of new areas by dinoflagellates and pathogenic bacteria through dumping of aquarium water and ballast water; and relaying of imported shellfish stock containing undesirable organisms. Eutrophication favours growth of non-siliceous Cyanobacteria (bluegreen algae), such as *Anabaena, Microcystis, Nodularia* and *Oscillatoria*, over dinoflagellates in brackish or fresh water. These algae produce neuro-, hepato-, dermato- and cytotoxins in recreational and drinking water. Animal deaths resulting from ingestion of contaminated water have been documented worldwide, and there have been a few accounts of human gastroenteritis, respiratory distress and skin rashes. *Alexandrium* has been found in the ballast water of 12 of 340 cargo vessels in Australian ports, and has been linked to colonization of aquaculture areas. The seaweed *Caulerpa taxifolia*, released into the Mediterranean Sea in 1984, is now invading much of the coastline and reducing fish productivity. The consequences of human interventions in the aquatic environment are shown in Table 1.

3) Development of new culturing and fishing procedures

The expansion of aquaculture has led to an increase in organic matter from artificial feed and deposited feces which may encourage local phytoplankton blooms. There are also more opportunities for transfer of bacterial and parasitic pathogens from one fish or crustacean to another. New food items may increase the hazards, e.g. marlin exported to Canada and the United States causing scombroid poisoning, and moonshells as a potential marketable gastropod in the southern Gulf of St. Lawrence containing PSP toxins.

4) Social change towards eating habits

New eating habits arising from different traditions of immigrants and Canadians eating food abroad have created new risks of illness, such as ciguatera in tropical fish, PSP toxins in ungutted mackerel, tetrodotoxin in pufferfish, and parasites in sushi.

5) More awareness of fish and shellfish associated diseases

Once a new disease is recognized, it becomes easier to identify it again through awareness by public health officials and available detection methods for the etiological agent. Since domoic acid was established as the cause of ASP in 1987, this toxin has been found in diatoms and shellfish in several countries. Recently, an ASP-like illness occurred in South Korea after smoked mussels were consumed, but the batch of oysters eaten was not available for testing. Also, once the ambush predator *Pfiesteria* was identified, another species with a similar life cycle was found, indicating this type of dinoflagellate may be quite common. Enhanced monitoring of potentially toxic areas also increases the chances of detection of a toxigenic agent.

Conclusion

It appears that environmental change and non-traditional eating habits are increasing the risk to consumers of fish and shellfish related diseases from toxic algal blooms and microbial pathogens. The social and economic impact of such illnesses and harvest losses are sufficient to warrant investing in procedures to predict blooms, detect the responsible agents, and reduce the impact of environmental change.

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High capacity receptor assay for domoic acid: use of a recombinant glutamate receptor produced in a baculovirus expression system

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Amnesic shellfish poisoning (ASP) is a major human heath issue affecting several fisheries on the northeast and northwest coasts of North America. The most widely utilized method of analysis for ASP is the quantification of domoic acid and its isomers by HPLC with UV detection. However, a rapid, high throughput screening method for domoic acid is currently lacking.

To address this need we have developed a receptor assay for domoic acid and its isomers based on their competition with [^{3}H] kainic acid for binding to the kainate glutamate receptor. Binding competition is carried out in 96-well microplates by incubating [^{3}H] kainic acid (5nM) in the presence of the receptor and various concentrations of domoic acid or unknown sample for 1 h at 4 °C. Incubation is terminated by filtration onto a 96-place glass fiber filter mat, with rinsing to remove unbound label. The dried filter mat is then saturated with solid scintillant and the mat counted directly in a microplate scintillation counter. Using multiple microplates, simultaneous analysis of several dozen samples is possible and complete assay time is approximately 3 h.

The assay was first developed using frog brain (*Rana pipiens*) synaptosomes as the source of glutamate receptor. The limit of detection using frog brain synaptosomes is 1 nM domoic acid (0.3

ng.mL^{-1}), with an IC_{50} of 2.8 ± 0.33 nM (0.84 ng.mL^{-1}). The quantitative reliability of the frog brain assay has been assessed in several sample matrices including seawater extracts of *Pseudonitzschia pungens* f. *multiseries*, methanol extracts of mussel and crab hepatopancreas, and in mouse serum, with excellent correlation to HPLC analyses.

We are currently assessing the utility of a cloned glutamate receptor (GluR6) expressed in a baculovirus system in this assay. We have found that the cloned receptor yields similar sensitivity as the frog brain receptor, with an IC_{50} of 3.4 ± 0.42 nM (1.02 ng.mL^{-1}). We anticipate that the microplate receptor assay will provide a rapid assay method for monitoring and inspection programs. Application of the cloned glutamate receptor will minimize interlaboratory variability and eliminate the use of animals in the assay.

Is the mussel, *Mytilus californianus*, a suitable sentinel species for the detection of domoic acid on the coast of British Columbia?

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The mussel, *Mytilus californianus*, is used as a sentinel species for monitoring PSP and domoic acid in areas of B.C. harvested for shellfish. Barkley Sound on the west coast of Vancouver Island was closed to bivalve fishing in late 1992 and 1993 when domoic acid in sentinel mussels reached levels from 54 to 98 μg.g^{-1}. Rarely, however, is domoic acid detected in the mussels used in the monitoring program, despite the low detection level of 01.μg.g^{-1} flesh.

To understand whether this resulted from the lack of toxic blooms, or the kinetics of domoic acid uptake and depuration, the mussel was exposed to cultured *P. pungens* f. *multiseries* at levels considered to be within lower limits of occurrence in the natural environment. To simulate variable cell concentrations that mussels experience in blooms subjected to currents and tides in the nearshore, a pulsed feeding regime every 6 h was used rather than a constant metered concentration. The highest level of domoic acid accumulated by the mussel was 12.7 μg.g^{-1} wet weight of tissue, and the rate varied from 0.71 to 0.03 μg.g^{-1}.h^{-1} during the period of the 114 h of exposure. On depuration for 411 h the rate of toxin loss varied from 0.41 μg.g^{-1}.h^{-1} at the start of depuration, to 0.01 μg.g^{-1}.h^{-1} after 338 h, when domoic acid in the mussel tissue fell below the level of detection. After 250 h of depuration, domoic acid was still detectable at 0.1 μg.g^{-1} in mussel tissue.

Based on these data for mussels which had been contaminated under conditions simulating a minor toxic bloom, the time-frame for detection suggests that weekly monitoring of sentinel *M. californianus* would detect any minor bloom that produces domoic acid.

Seasonal variation in content and distribution of domoic acid in the razor clam, *Siliqua patula*, from different geographic locations in British Columbia.

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The occurrence of domoic acid in bivalves on the coastline of the northeast Pacific Ocean was first observed in Washington State on November 1991. Recreational harvesters of razor clams became ill on consuming the clams with tissue levels in excess of 20 ppm, the domoic acid upper level generally considered to be safe.

Razor clams, unlike other bivalves examined to date, appear to retain the toxin in the tissue in an irreversible manner which defies complete depuration. This affords a means of diagnosing the seasonality and historic records of former toxic bloom events in specific areas of B.C. A 2-year assessment was initiated on the domoic acid levels in razor clams collected monthly from Tow Hill beach on the north coast of Graham Island, Queen Charlotte Islands, and from Cox Bay, Long Beach on the west coast of Vancouver Island. Results to date have shown the consistent presence of domoic acid in clams collected monthly from Cox Bay, with peak levels of 25 ppm in September 1993 and 16 ppm in January 1994. Mean monthly levels ranged from 6.4 to 16.7 ppm but with the typical biological variance for toxin contamination.

Levels of domoic acid in the foot, siphon, gut (stomach and digestive gland) and remaining tissues of the September 1993 specimens were 10.3, 7.4, 6.4 and 8.4 $\mu g.g^{-1}$, and in the October 1993 specimens 5.3, 1.8, 3.5 and 3.9 $\mu g.g^{-1}$, respectively. Although the greatest accumulation was evident in the foot of the clam, substantially high levels were also evident in other tissues. By contrast, none to only trace (0.2 $\mu g.g^{-1}$) levels of domoic acid have been observed in the razor clams analyzed to date from the Queen Charlotte Islands., suggesting the absence of domoic acid producing algal blooms on that portion of the coast.

Effects of toxic and non-toxic *Pseudonitzschia pungens* on fecundity, in the amictic cycle, of the rotifer *Brachionus plicatilis*

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Marine toxins such as PSP are known to adversely affect larval finfish through transfer of the toxin by herbivorous zooplankton. Whether zooplankton are major biological vectors through which domoic acid can reach higher marine species or are themselves adversely affected by the toxin is unknown and formed the objective of this initial study.

Domoic acid in cells of cultured toxic *Pseudonitzschia pungens* f. *multiseries* increased significantly and in order of being cultured in 4 L flasks, 20 L carboys, 350 L columns and 500 L semi-batch bag cultures. Bag cultured *P. pungens* f. *multiseries* (DA 3.71 ± 1.40 pg.cell^{-1}) , non-toxic *P. pungens* f. *pungens* (DA <0.01 pg.cell^{-1}) and the diatom *Thalassiosira pseudonana* were fed at satiation levels to the rotifers during 15 days to cover at least two generations of amictic females. The definite cyclical peaks in fecundity of the females fed *T. pseudonana* were not as evident in those fed non-toxic *P. pungens*, but no fecundity peaks were exhibited by the female rotifers fed toxic *P. pungens*.

The mean fecundities of the rotifers fed the non-toxic algae were not significantly different from each other but were different from the rotifers fed toxic *P. pungens* at the 0.05 significance level.

Diarrhetic shellfish poisons: how they are made by *Prorocentrum lima* and why

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Despite their chemical complexity and the energy required to produce secondary metabolites, the role or function of these metabolites in organisms is often difficult to identify. The DSP toxins provide us with such a conundrum - highly intricate chemical structures requiring many biosynthetic steps, yet with no obvious function within the cell. A further consideration is that as potent phosphatase inhibitors, DSP toxins induce powerful biological effects on other eukaryotic cells.

Research at IMB has uncovered a possible role for these DSP toxins as allelopaths, though this raises some important questions regarding protection of the producer organism. In addition, using stable isotopes and NMR spectroscopy, we have established a novel biosynthetic pathway for DSP toxins from acetate units, presumably utilizing a novel polyketide synthase enzyme. This pathway contains several unique biosynthetic steps, and its relevance to other polyether toxins of dinoflagellate origin were also discussed.

New highly polar toxins from *Prorocentrum* spp.

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In addition to the diarrhetic shellfish poisoning (DSP) toxins, there are scattered reports in the literature of other toxic compounds produced by various *Prorocentrum* species. An extensive examination of the chemistry of *Prorocentrum maculosum* has resulted in the discovery of polar fractions that are highly toxic in the mouse bioassay.

Further purification of these fractions has yielded a series of compounds generically described as 'fast acting toxins'. The structures of these new compounds and their distribution in other *Prorocentrum* species was described. The mechanism of biological action, and their significance with respect to known marine toxins, was also discussed.

An effective method for reducing mortalities of salmonids when exposed to lethal concentrations of the harmful phytoplankter, *Chaetoceros concavicornis*

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Our previous work has shown that when the cells of the harmful diatom, *Chaetoceros concavicornis*, become trapped between the primary and secondary lamellae of salmonids, they cause the production and accumulation of excessive amounts of mucus, which greatly inhibits oxygen uptake by the fish. If the finfish become sufficiently hypoxic, mortalities occur.

Mucolytic agents, such as L-cysteine ethyl ester, can suppress mucus production of the secondary lamellae of salmonids. Our data show that oral treatment of coho salmon (*Oncorhynchus kisutch*) with L-cysteine ethyl ester, greatly reduces mucus production by the secondary lamellae upon exposure to lethal concentrations of *C. concavicornis*; these coho live in what would otherwise be a lethal concentration of *C. concavicornis* cells.

Oxygen-radical-mediated toxic effects of *Heterosigma akashiwo* on juvenile salmonids

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The phytoplankter, *Heterosigma akashiwo* (syn. *H. carterae*) has caused fish kills in many coastal seawaters in Europe, North America, Asia, Japan and New Zealand. We have found that when this species is grown under laboratory conditions, in a semi-defined medium, the culture become toxic to juvenile salmonids within two weeks.

A toxic culture produces superoxide radical and hydrogen peroxide. When the same toxic culture is treated with catalase (catalyzes the destruction of hydrogen peroxide) and/or superoxide dismutase -SOD (catalyzes the destruction of superoxide radical) immediately prior to a standard toxicity fish-bioassay, the culture becomes non-toxic. We suggest that the toxic mode of action of *H. akashiwo* is due to formation of toxic concentrations of oxygen radicals and hydrogen peroxide.

Efficacy of clays in mitigation of red tides

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Red tides and harmful blooms are a potential menace to wild and cultured species of shellfish and finfish. Relocation of mariculture structures (i.e. rafts, cages etc.) outside the harmful algal bloom areas is one of the practical measures employed to mitigate adverse effects. Alternatively, coagulation of the red tide organisms with clays and subsequent deposition is a potentially safe and cost-effective method.

This paper discusses the efficacy of several clays in mitigation of red tides and reviews studies on the interaction between clay particles and algal cells. Addition of the clay montmorillonite to cultures with cell densities that ranged between 0.02×10^6 L^{-1} and 250×10^6 L^{-1} was effective in coagulating algal cells and the proportion of algal cells removed increased with the quantity of clay added. Results on four of the most common species of red tide organisms in Chinese coastal waters showed addition of 500 mg montmorillonite L^{-1} of culture removed up to 90% cells of *Pseudonitzschia pungens*, 78% of *Skeletonema costatum*, 40% of *Prorocentrum minimum* and 25% in natural assemblages of *Noctiluca scintillans*. Results with kaolin with halloysite were most promising and showed that surface characteristics of clay particles affect the coagulation that plays an important role in the removal of red tide organisms.

Based on a theoretical model, we propose an improved system of clays with a removal efficiency of about 10 times more than the original clays. Mechanisms for coagulation of algal bloom populations at various phases of growth, and adsorption of nutrients by clays and its role in mitigating red tides are also discussed.

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DISCUSSION GROUP REPORTS

1. Sources of toxins
2. Pathways and sinks of toxins
3. Management and mitigation for human health protection
4. Management and mitigation for aquaculture industry protection

Report of Discussion Group #1

Sources of Toxins

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1. Introduction

The objective of the working group was to review the different sources of aquatic and marine algal toxins and, if possible, to try to find unifying concepts linking what often appear to be unrelated observations of toxin production. It was felt that this approach might increase our understanding of the production of marine toxins and would be useful in determining research priorities.

2. Sources of marine biotoxins

Members of the group agreed that toxins are considerably more widespread in aquatic and marine environments than was previously believed. Several of the major classes of microalgal toxins and the known biological source organisms are presented in Table 1. Based on this cursory overview, it is evident that microorganisms from widely different taxonomic groups may produce identical toxins. The production of PSP toxins by marine dinoflagellates, marine eubacteria and freshwater cyanobacteria is certainly one of the most striking examples of a variety of sources producing a single family of biotoxins. Production of DSP toxins by species of *Dinophysis* and *Prorocentrum* was also cited as indicative of the production of complex biomolecules by taxonomically and ecologically distinct algae. Conversely, there is a notable variety in the ability to produce toxin within closely related, and in some cases the same, algal species. The variation in toxigenic versus non-toxigenic strains within the *Pseudonitzschia* and *Alexandrium* spp. are examples of the later observation. It was also pointed out that many toxins may have previously gone unnoticed. This may be due either to their lack of effect on mammals or being produced at low concentrations. The recent rapid expansion of aquaculture effort, together with the toxin monitoring programs associated with this industry, have also increased availability of information on potentially harmful algal species.

3. Toward a unifying concept

It was suggested that biosynthetic pathways responsible for toxin production may be present but not functional due to a structural alteration or a lack of appropriate substrate. If this is the case, it follows that the genes coding for the biosynthetic pathways involved in toxin production are probably more widely dispersed than the toxins themselves. Toxins for which the biochemical machinery is present may fail to be produced because the biosynthetic pathway has not been "triggered" by the appropriate combination of external stimuli and/or vital nutrients. An example

Table 1 Some of the major classes of toxins and their sources

Toxin Class	Sources
Paralytic Shellfish Disease Saxitoxins, neosaxitoxin, and gonyautoxins	Dinoflagellates (e.g. *Alexandrium*, *Gymnodinium*, and *Gonyaulax* spp.); Cyanobacteria (e.g. *Aphanizomenon flosaquae*); and Eubacteria
Diarrhetic Shellfish Disease Okadaic acid and DTX's	Dinoflagellates (e.g. *Prorocentrum* and *Dinophysis* spp.)
Amnesic Shellfish Disease Domoic acid	*Pseudonitzschia* spp. (e.g. *P. pungens*, *P. australis*, *P. pseudodelicatissima*, and *P. seriata*)
Hepatotoxins Microcystins	Cyanobacteria (*Microcystis, Nostoc, Oscillatoria, Anabaena, Nodularia*)

is the unexplained pattern of biosynthesis domoic acid production, i.e. almost only during stationary growth phase, in toxic strains of *Pseudonitzschia*. The inconsistent correlation between the presence of DSP toxins and the abundance of *Dinophysis* spp. in many aquaculture settings may also reflect a requirement for appropriate triggering conditions. These examples underscore the need for an understanding of the mechanisms controlling toxin production.

As mentioned above, there are several instances of the existence of a complex toxic molecule in widely different organisms, suggesting either the existence of a common ancestor or (less likely) a parallel evolution of rather complex biosynthetic pathways. In the case of the production of PSP toxins by dinoflagellates, cyanobacteria and eubacteria species, it remains unclear what, if any, common background is involved. It is notable that bacteria are often present within PSP-producing dinoflagellates and that some of these may be capable of autonomous toxin production. Work is needed to determine the connection between the occurrence of the intracellular bacteria in dinoflagellates and PSP toxin production. In summary, there is good reason to speculate that a common ancestor in involved in the development of these complex biochemical pathways.

4. Role of marine biotoxins

It was proposed that we may find some common ground by investigating the ecological "roles" of toxins. Most of the known biotoxins are deemed to be secondary metabolites; in most cases non-toxigenic strains also exist in natural environments. It is widely suspected that, although the production of toxin is not essential, these toxic secondary metabolites play significant physiological or ecological roles. Given the energy cost involved in synthesis, it seems likely that secondary

metabolites are useful to the producing organisms. Natural selection will favour the transmission of genes coding for secondary metabolites increasing the fitness of the species. Secondary metabolites may play either an intra- or extracellular role. In support of an extracellular role, there is increasing evidence that some toxins have allelopathic function, i.e. reducing the ability of other species to grow. They may also function as feeding-deterrents. However, other than some suggestion that PSP toxins may act as nitrogen reservoir, demonstrations of intracellular roles of biotoxins are still rare.

It was noted that the production of toxic secondary metabolites must result from a long evolutionary process. The preservation and transmission of these sophisticated biosynthetic pathways in so many different procaryote and eucaryote organisms suggests that the toxins increase their overall fitness.

Mammals are not the primary target of many of the marine biotoxins. In most cases, it may be chance that the bioactive secondary metabolites are toxic for mammals. Certainly, it is difficult to postulate a sound hypothesis that leads a microalga to produces a mammalian neurotoxin such a domoic acid. It is notable that the modes of biological action of toxins are surprisingly limited. They appear to fall into three major categories: phosphatase inhibitors, ion channel blockers, and glutamate agonists.

5. Recommendations

It was concluded that one of the most important shortcomings in our understanding of marine toxins is that of the mechanisms that code for toxin biosynthesis at the molecular genetic level. Research aimed at elucidating the genetic information responsible for the pathways of toxin biosynthesis could lead to major breakthroughs. Further, once the molecular genetic sequences for biosynthetic pathways have been established, it will be possible to "probe" organisms freely. This would lead to a considerably more complete understanding of the extent to which the potential to produce toxins is distributed among aquatic and marine microorganisms.

Closely related questions are those the "triggering" and control of the level of toxin production. Elucidation of the apparently complex combination of physical, chemical, and biological factors which control production is critical to the understanding of toxins including several with strong commercial importance, e.g. domoic acid and the toxins responsible for PSP. The continuing mystery surrounding sources of DSP production is another critical area where determining how toxin production is controlled will prove particularly valuable.

Finally, results of recent work have demonstrated several examples of involvement of marine bacteria in production of toxins that were originally thought to be produced solely by microalgal mechanisms. Research directed at defining the roles of bacteria will lead to a better understanding of toxin production in both freshwater and marine environments.

Report of Working Group #2

Pathways and Sinks of Toxins

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1. Introduction

This discussion group considered a range of topics related to pathways of toxins through marine and freshwater food webs, and the resulting distribution of toxins. Major topics discussed included domoic acid distribution, particularly on the Pacific coast of Canada and the United States, PSP toxin dynamics, including impacts on juvenile fish, freshwater toxins in the Northwest Territories, microcystins in the marine environment, and anthropogenic introductions. The requirement for further development of reference standards for toxins that are presently causing substantial problems within Canada and those that we may require future action, such as brevetoxins and ciguatoxins, was stressed by a number of participants. The need for developing prescreening tools was also recognized, both as a tool for industry and as a tool to assist in response to a major toxin crisis or episode.

2. Domoic acid

A number of questions remain about the dynamics of domoic acid on the west coast of Canada and the United States. We do not understand how crabs accumulate the high concentrations of the toxin that are observed. Principal potential sources are consumption of phytoplankton directly following mass sink out of blooms, consumption of toxic bivalve shellfish, or consumption of other benthic producers. Observations are required on distribution of crabs, the range of movement by individuals, substrate and diet.

The number of organisms known to produce domoic acid is increasing, suggesting that other agonists need to be considered. These producers are pennate diatoms and red macroalgae of the genus *Chondria*. There is little or no information on the extent of testing of other benthic algae; the benthos should be examined for possible additional sources. Tests have been conducted on diatoms that comprise the surf zone community in Washington State; the results were negative. However, *Pseudonitzschia* spp. do occur in littoral waters along the exposed outer beaches.

Another question raised was how quickly does domoic acid 'leak' from cells. Sediment traps were recommended as a tool to investigate some of the problems.

3. PSP toxin dynamics

Discussion focused initially on sources of blooms. Knowledge remains limited in a number of locations about where and how blooms originate. In British Columbia they seem to be primarily by local seeding from cyst beds. In the Scotia-Fundy region on the east coast many blooms originate to the south and are advected to affected coastal areas.

It was pointed out that large seed beds do not necessarily translate to sources of blooms. Rather, physical environment and water properties dictate excystment, and small beds may be an important and overlooked source. In the Bay of Fundy, there is generally a small increase in PSP in June, while the large seed beds in the central area of the bay typically hatch in July. Here there is a two week lag seen from the appearance of *Alexandrium* cells in the water column to the rise in toxicity in shellfish. There is evidence also from several areas of seasonal control on excystment.

The impact of PSP toxins on fish populations and recruitment is still not well understood and may be substantial. Experiments have demonstrated that a single herring larva can be killed by one *Alexandrium* cell. In one experiment, results also showed that herring physiological condition was correlated with *Alexandrium* concentration. Results of experiments in feeding *Alexandrium* to salmon fry demonstrated an effect under some conditions associated with size and timing of saltwater entry. Copepods are viewed as a key vector for transfer of toxins from phytoplankton to fish, with experimental evidence showing little or no effect of PSP toxins on copepods.

In Atlantic Canada there have been a number of fish kills in which PSP toxins may have played a role. These include herring kills in fish weirs in the Bay of Fundy, and mackerel kills in the Gulf of St. Lawrence and Cabot Strait. One topic that needs to be examined is what concentrations of PSP are normal for fish at age.

It was observed that mackerel accumulate PSP in the liver. It is unusual for water-soluble compounds to be stored here, indicating that there must be some compartmentalization. It was suggested that the toxins might be bound to melanin in some way. This would explain retention in siphon tips of shellfish such as butter clams. Finally, it was recommended that the occurrence of PSP in the adductor muscles of scallops be reexamined, to verify that the observations are not artifacts of the analysis.

4. Freshwater toxins in the Northwest Territories and marine microcystins

PSP-like toxins have been found in freshwater mussels in the Northwest Territories. Water samples showed low biomass of phytoplankton, with few unicellular Cyanobacteria. Mouse bioassays have shown that acidic extracts produce positive bioassay results while methanolic extracts do not.

The modest variation of toxin concentrations in the mussels by season, and the fact that the toxins are not restricted to the viscera led to a suggestion that the toxins might be endogenous. There was a consensus that priority should be given be to identification of the toxin. It was suggested that microcystins should be investigated as the source, although these give a unique symptomology in mouse bioassays that does not appear to be consistent with observations. However, the symptomology observed is consistent with that produced by Cylindrospermopsin, presently known only from semi-tropical areas.

There was a brief discussion of marine microcystins. The source for these remains enigmatic. There are observations of microcystins from copepods (*Calanus marshallae*) and crab larvae from areas with severe net pen liver disease. Clearly there is a need to investigate this whole issue more extensively.

5. Anthropogenic introductions

There is experimental evidence to show that viable cells are contained in ships' ballast tanks following ocean voyages. There is also evidence of actual cases of introductions in some locations. However, the level of risk remains unquantified. In order to document introductions, accurate knowledge is required of both the current and the paleofloras. Further development of genetic biomarkers is required to provide the capability to demonstrate specific cases of introductions and the source locations.

One participant raised the issue of risk of introductions from exchange of cultures within the research community. For example, the only cyanobacterial neurotoxins found in Australia are PSP. It is readily apparent that there is a risk of introduction of other freshwater toxins there and, conversely, the introduction of the Australian cyanobacterial toxin producers elsewhere, through careless handling of culture material.

Report of Working Group #3

Management and Mitigation for Human Health Protection

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1. Agents (toxins)

In Canada the toxins of concern are Paralytic Shellfish Poisoning (PSP), Domoic Acid Poisoning (DAP, also referred to as Amnesic Shellfish Poisoning or ASP), Diarrhetic Shellfish Poisoning (DSP), ciguatera and possibly others (eg. scombroid poisoning - this is a product of decomposition).

2. Implicated Food Sources (Vectors)

Bivalve molluscan shellfish (PSP, DAP, DSP), predatory gastropods and some crustaceans (PSP, DAP) and other food fishes (ciguatera).

Some work is being carried out in tracing DAP toxin through the food chain. It has not been determined how DAP and PSP toxins accumulate in Dungeness crab hepatopancreas nor how PSP toxin develops in lobster hepatopancreas.

3. Distribution and consumption of toxin laden foods.

There are two types of activities (i.e. commercial and recreational) where phycotoxins may place consumers at risk. With respect to commercial harvesting and processing activities Canada's DFO's biotoxin monitoring program for PSP and DAP and patrolling of closed areas provides a high level of protection for the consuming public. There have been no PSP incidents from consumption of commercial shellfish for years and no DAP intoxications have occurred in Canada since DFO has implemented routine DAP toxin testing.

These efforts, however, cannot totally eliminate all the problems of intentional illegal harvesting by locals for personal consumption or sale* (eg. peddlers, road side stands etc.) and occasional accidental harvesting by tourists.

* Distribution and sale of shellfish within individual provinces is a provincial jurisdiction.

4. Preventative Methods

A number of preventative methods are currently in use. Most of these are proactive and directed toward harvest sites prior to and during harvest. These include harvest area monitoring (shell stock - presence of PSP and DAP, phytoplankton - presence of toxin-producing phytoplankton), enforcement of toxin closures (sign posting and patrol) and processing plant inspection (product analysis and record keeping).

Additional initiatives by DFO include production and distribution of:

1. Posters* and brochures* providing information on bacterial and shellfish toxin (PSP and DAP) concerns related to local or regional shellfish harvesting. Some examples are *Maritimes Coastal Mollusc Harvesting Guide* (March, 1994)**, *Clam Digging in Southwestern New Brunswick, Where to Harvest Shellfish?* (Quebec) and *British Columbia Guide to Safe Mollusc Harvesting***

* Available from regional DFO Communications Branches and local DFO offices.

** Published in conjunction with Environment Canada and provincial authorities

2. Information articles - DFO Underwater World© factsheet - *RED TIDES** and Backgrounder©♦ - *Paralytic Shellfish Poisoning* and *Domoic Acid.*

* Available from regional DFO Communications Branches and local DFO offices.

♦ Available from DFO Communications Branch, 200 Kent Street, Ottawa, Ontario, K1A 0E6

3. Video on PSP - Originally produced by the State of Maine and now available, on loan, to fisherman and the public from many local DFO Fisheries and Habitat offices.

4. Regional or area Toll-free or 1-800 information lines indicating which local harvest areas are currently open.

5. Media releases to local radio and newspapers on harvest area openings and closures.

6. DFO Advertisements in commercial tourist publications.

7. Educational programs in local schools.

The application of these initiatives varies considerably between DFO Regions and even between Areas within those Regions. To a large extent this variability reflects the potential for phycotoxins blooms and intoxication of local shellfish. Generally more of these items are utilized in areas which have a higher the frequency and intensity of toxin problems (eg. Bay of Fundy, Gaspe Peninsula and the B.C. coast).

5. Limits (guidelines or action levels) and Methods of Detection

These have been addressed for both PSP (80 μg/100g - mouse bioassay) and DAP (20 μg/g - HPLC).

Canada currently has an interim policy on DSP. This policy has been discussed and agreed to by Fisheries and Oceans and Health Canada. The interim policy has an action level of 1 μg/g of digestive tissue (chemical analysis for okadaic and/or DTX-1, singly or together). The policy is reactive in that DSP testing will only occur in suspect harvesting areas or as a result of consumer complaints where symptoms would indicate possible DSP intoxication. The U.S. has no official guideline nor method of detection.

Neither Canada nor the U.S. has an approved method for testing for ciguatera toxin although a mouse bioassay is available. No guideline or action level exists.

6. Risk assessment

Health Canada (formally Helath and Welfare Canada) is currently preparing risk assessments for all seafood products. This exercise should permit authorities to direct their efforts to the toxin(s) of greatest concern.

Recommendations:

1. Direct highest amount of effort to the appropriate people at the proper time of year.

Tourists, recreational and commercial harvesters should be reminded of the potential risks just prior to and during major harvesting seasons. It would be impractical to provide information on appropriate harvest areas to consumers, in locations like Calgary, Montreal and Toronto, who are obtaining their shellfish in restaurants or grocery stores.

2. Improve dissemination of existing information.

This can be aided by supplying posters, brochures and notices to tourist bureaus, national and provincial parks, campgrounds etc. This is occurring in some locations but dissemination could be implemented or increased in other locations.

3. Improve Education.

- DFO Inspectors and Fishery Officers

These individuals are often the first point of contact for commercial harvesters and tourists. If DFO officials have a clear, general understanding of the potential risks involved they can impart that information to the people they come in contact with.

- The Local Community

Local people, in areas prone to biotoxin problems, are often the ones that ignore posted and announced closures. Convincing them that these closures are necessary is often difficult. Education programs directed at Quebec school children has appeared to have a greater impact than trying to convince their parents directly.

- The Medical community

Many medical staff, especially those far removed from biotoxin prone areas, have never knowingly been exposed to patients suffering shellfish poisoning symptoms. Preferably introduction to concerns should be made through colleagues who have treated patients suffering from shellfish poisonings. Focus should be on the causes of shellfish poisoning, typical symptoms and appropriate medical action.

Efforts should be directed not only to medical personnel in toxin prone areas but also in large urban areas where the shellfish are consumed. Doctors should be encouraged to notify DFO Inspection, Health Canada or other health officials of suspected intoxications.

- The Judiciary

Often presiding judges and attorneys representing the Crown are not fully aware of the resulting risks, to the general consuming public, that could occur should biotoxin-contaminated shellfish be made available through illegal harvesting. The fines and penalties applied to individuals convicted of illegal harvesting, therefore, vary widely across the country.

General information about the risks from toxic shellfish could be directed to judges and Crown consul outside individual case situations (i.e. general education). An alternative approach would be to provide information to Crown consul prior to individual trials. This information could include previous trial transcripts successfully prosecuted, and the fines and sentences issued.

4. Presentation of Information

- Tone of the message

Although a health and safety message, an announcement of closures due to biotoxins, must impart a clear warning it must not be overly alarmist.

A positive response was made with respect to the toll-free information phone lines. These recordings indicate which areas are open and not which are closed.

- Duration, Frequency and Placement of Closure Signs

Frequently biotoxin closures in general, and PSP closures in particular, remain closed over long periods of time. Posting of these closures is done using DFO signs specifically designed for this task. During prolonged periods of closure these signs may become faded or torn. This could potentially make the information on them appear out of date*. Another risk is the willful destruction of signs by vandals and illegal harvesters.

All efforts must continue to be made to keep closure signs posted, up to date and replaced when necessary.

* Closure signs are required to be removed when any area is re-opened to harvesting.

5. Action Levels and Analytical Techniques

Work should continue to refine "official" analytical methods and action guidelines for phycotoxins especially for those without them.

Report of Working Group #4

Management and Mitigation for Aquaculture Industry Protection

Facilitator: Edward A. Black

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There are differing focuses of interest in algal effects on aquaculture on the east and west coast of Canada. The singular concern on the west coast is the effect algal blooms have on fish culture practices. Direct mortality of fishes on the west coast accounts for an average annual loss of stock valued at between $2.5-3.0 million dollars. Fish farming on the east coast has yet to experience any mortalities of cultured fish which can be attributed to algal events.

On both coasts the shellfish industries consider phtyotoxin contamination a serious quality control problem which affects marketability of their product. Unlike the experience of west coast finfish culture industry, mortality of shellfish stock due to an algal bloom is not a common occurrence.

As a result of these differences industries' need for support in dealing with algal effects differ by region and industry. For example in the west coast fish farming industry the major needs are for methodologies which can predict the development and transport of dangerous blooms and for information on effective methods of managing stock through a bloom with minimal fish mortalities. In contrast the shellfish industries desire technologies which can improve the extent and frequency of product testing for phytotoxin contamination of products.

In addition to these broader needs of the finfish and shellfish industries some more detailed problems within our existing data gathering and knowledge base need to be addressed. For example, at present it would be difficult to differentiate, from the information supplied by the human epidemiology professionals, whether an outbreak of illness associated with consumption of shellfish is due to contamination by DSP toxins or if it is due to fecal coliform contamination of the product. It would also be useful to be able to determine if the geographic distribution of toxic events such as contamination with PSP toxins is changing over time and to what degree practices such as the dumping of ships' ballast waters drive any detected changes. Another area of research which needs to be investigated is whether shellfish can be selectively bred to limit their ability to take up phcotoxins.

For the finfish industries there are a series of associated problems attached to the development of the industry and the development of techniques to husband fish stocks through algal blooms.

The rapid development of the industry has led to concerns in the regulatory agencies that nutrients from fish farms may play a significant role in the development of toxic blooms. While there does not appear to be evidence supporting this view there is also no conclusive proof that farms are unlikely to have a significant role in the development of blooms. This uncertainty is believed to put pressure on the industry to unnecessarily monitor macrontrient concentrations or to seriously limit industry's access to new sites.

In deciding how to mitigate the effect algal blooms have on stocks a number of issues need to be resolved. These include the identification of precisely which types of problems the industry is likely to face as it changes its geographic distribution on the coast of British Columbia, and what approaches to mitigating the affect of blooms will be available.

The fish farming industry in B.C. has moved out of the area of the Strait of Georgia into more open or more northerly locations. With this move there has been some suggestion that industry is loosing fish to previously undescribed algal species. For example in 1989 in Simoon sound fish mortalities were associated with abundance of a previously undescribed algal species. It was also suggested that an algae associated with fish mortalities in Japan, *Chattonella* sp., has been detected on this coast. Is this a threat which will be come more apparent with the change in the distribution of the industry?

Some clarification is also needed on what will constitute acceptable practices for mitigating the effects of a bloom. Two broad approaches are available. One involves the use of a cemotherapeutant incorporated into the fishes feed which might be administered by veterinarians on a as needs basis. There is evidence that such an approach may have potential to limit the loss of fish however obtaining registration of such a product is financially beyond the interest of the fish farmers or makers of therapeutants. Registration of such a product will require support from government.

The other approach to mitigating the effects of blooms involves temporarily towing the fish cages out of the path of the bloom or manipulating the water supplied inside the cages through their use of such things as upwelling systems or barrier screens around the cages. At present there is inadequate information on how effective these methods are at isolating the fish from the bloom or on how the fish respond to the presence of the bloom (for example if the fish have deep cages will the fish move to deeper water to avoid the bloom).

For the aquaculture industries these areas of concern are critical to the development of an industry which can cost-effectively compete in the international seafood market place. For healthy aquaculture industry in the future that is ultimately, where they my be able to compete.

Appendix 1: Workshop Agenda

Fourth Canadian Workshop on Harmful Marine Algae

Tuesday 3 May

0930–0945	Opening	

Algal Toxins – Their role and production

0945 – 1020	Wright	Diarrhetic shellfish poisons: how they are made by Prorocentrum lima and why
1020 – 1035	Coffee	
1035 – 1055	Laycock	In vitro interconversions of paralytic shellfish poisoning (PSP) toxins
1055 – 1115	Jellett & Stewart	Influence of associated bacteria on the algal production of paralytic shellfish poisons under nitrogen and phosphorus limitation
1115 – 1135	Douglas et al.	Influence of bacteria and bacterial extracts on domoic acid production by Pseudonitzschia pungens f. multiseries
1135 – 1155	Wright et al.	New highly polar toxins from Prorocentrum spp.

Algal toxins – emerging analytical techniques

1155 – 1215	Doucette et al.	A sensitive, microtiter plate–based receptor assay for paralytic shellfish poisoning (PSP) toxins
1215 – 1235	Van Dolah et al.	High capacity receptor assay for domoic acid: use of a recombinant glutamate receptor produced in a baculovirus expression system
1235 – 1345	Lunch	
1345 – 1405	Shaw et al.	Detection of feeding deterrents from marine phytoplankton using a new bioassay technique – ecological and commercial implications
1405 – 1425	Smith and Kitts	Relationship between paralytic shellfish poisoning toxicity and a 17 kilodalton protein in butterclam foot tissue

Oceanography and monitoring topics

1425 – 1445	Gower and Murty	Satellite images and numerical modelling for phytoplankton bloom monitoring
1445 – 1500	Coffee	

1500 – 1520	Taylor and Haigh	Spatial and temporal distributions of harmful phytoplankton during the summers of 1992–93 in Barkley Sound
1520 – 1540	Martin and Wildish	Alexandrium fundyense resting cysts in the Bay of Fundy during five years
1540 – 1600	Whyte et al.	Is the mussel, Mytilus californianus, a suitable sentinel species for the detection of domoic acid on the coast of British Columbia
1600 – 1620	Forbes and Chiang	Geographic and temporal variability of domoic acid in samples collected for seafood inspection in British Columbia, 1992 – 1994

Wednesday 4 May

Freshwater algal toxins and microcystins

0900 – 0935	Carmichael	Toxins of cyanobacteria
0935 – 0955	McCready	Production and distribution of microcystins in the marine environment
0955 – 1015	Kent	Microcystins and fish health

Harmful algal blooms and aquaculture

1015 – 1035	Black	Controlling fish farm losses to algal events
1035 – 1050	Coffee	
1050 – 1110	Yang and Albright	An effective method for reducing mortalities of salmonids when exposed to lethal concentrations of the harmful phytoplankter, C. concavicornis
1110 – 1130	Yang et al.	Oxygen–radical–mediated toxic effects of H. akashiwo on juvenile salmonids
1130 – 1150	Rensel	Harmful effects of the marine diatom Chaetoceros concavicornis on Atlantic salmon: the role of gill mucus
1150 – 1250	DISCUSSION GROUPS – SECTIONS A AND B: SESSION 1	
1250 – 1350	Lunch	

Health Issues

1350 – 1410	Todd	Emerging diseases associated with harmful algal blooms and other waterbourne agents
1410 – 1430	Avery	Emergency medical treatment for PSP
1430 – 1450	Ramsdell et al.	Neurological risk of phycotoxin exposure: distinguishing neuroexcitatory from neurotoxic effects of domoic acid in the intact animal

1450 – 1550	Coffee and POSTER SESSION	
1600 – 1700	DISCUSSION GROUPS – SECTIONS A AND B: SESSION 2	

Thursday 5 May

Toxin Dynamics

0845 – 0905	Whyte et al.	Effects of toxic and non–toxic Pseudonitzschia pungens on fecundity in the amictic cycle of the rotifer Brachionus plicatilis
0905 – 0925	Scarratt	Diurnal variation of toxin content and composition in cage–cultures of Alexandrium tamarense
0925 – 0945	Stewart et al.	Elimination of the neurotoxin, domoic acid, from molluscs
0945 – 1005	Jones et al.	The effects of domoic acid on haemolyph pH, PCO2 and PO2 in the Pacific oyster, Crassostrea gigas, and the California mussel, Mytilus californianus
1005 – 1020	Coffee	
1020 – 1150	DISCUSSION GROUPS – SECTIONS C AND D: SESSION 1	
1150 – 1315	Lunch	
1315 – 1345	DISCUSSION GROUPS – SECTIONS C AND D: SESSION 2	
1345 – 1430	Discussion Group Reports and Conclusions	

Appendix 2: Workshop Participants

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