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Proceedings of the Second Canadian Workshop on Harmful Marine Algae Bedford Institute of Oceanography, Dartmouth, N.S. October 2-4, 1990

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Les numéros 1 à 456 de cette série ont été publiés à titre de rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

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PROCEEDINGS OF THE SECOND CANADIAN WORKSHOP ON HARMFUL MARINE ALGAE

Bedford Institute of Oceanography
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October 2-4, 1990

Edited by

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ABSTRACT

The Second Canadian Workshop on Harmful Marine Algae was hosted by the Department of Fisheries and Oceans, Scotia-Fundy Region, at the Bedford Institute of Oceanography on October 2-4, 1990. Over 100 people attended with representation from government research and management agencies (both federal and provincial), universities and the aquaculture industry. Thirty five poster and oral presentations summarized recent Canadian research on amnesic shellfish poisoning (ASP), paralytic shellfish poisoning (PSP) and diarrhetic shellfish poisoning (DSP). Discussion groups were held on laboratory and field studies. Federal research programs were reviewed and research priorities were considered in a panel discussion. Highlights of the Workshop are recorded in these proceedings.

RÉSUMÉ

La région de Scotia-Fundy du ministère des Pêches et des Océans a accueilli le deuxième atelier canadien sur les algues marines nuisibles, à l'Institut océanographique de Bedford, du 2 au 4 octobre 1990. Plus de 100 personnes y assistaient, dont des représentants des organismes de recherche et de gestion des gouvernements fédéral et provinciaux, des universités et de l'industrie de l'aquiculture. Trente-cinq affiches et exposés oraux résumaient les travaux effectués au Canada en ce qui a trait à la recherche sur l'IAM, l'IPM et l'IDM. Au cours de ces deux jours, des groupes de discussion ont traité des études effectuées en laboratoire et sur le terrain. Un panel discussion a également examiné les programmes de recherche fédéraux et les priorités en matière de recherche. Les actes présentés ici exposent les faits saillants de cet atelier.

INTRODUCTION

In recent years there has been growing scientific and public interest in marine phycotoxins, both in Canada and worldwide. The reasons for this interest are many and include: a) increasing incidence of paralytic shellfish poisoning (PSP), b) the discovery of domoic acid in eastern PEI, c) confirmation of diarrhetic shellfish poisoning (DSP) along the Atlantic coast of Nova Scotia, d) increasing frequency of algal blooms in general, perhaps due in part to nutrient enrichment in the coastal zone and e) evidence that toxic species can be carried great distances in ships' ballast water.

The scientific and resource management community has responded by setting up new research and monitoring programs. Effective communication among participants is essential. There clearly is a need to: a) promote the exchange of new scientific information on harmful marine algae and their effects in a timely fashion, b) foster the development of cooperative and collaborative scientific programs and c) encourage new research and monitoring initiatives. These objectives can be attained by holding periodic scientific workshops. In 1989, the Department of Fisheries and Oceans decided to sponsor such a series of workshops to be organized under the general guidance of the Phycotoxin Working Group, a national body in charge of coordinating marine phycotoxin research Canada-wide.

The first Canadian Workshop on Harmful Marine Algae was held at the Gulf Fisheries Centre in Moncton, NB on September 27-28, 1989. It was very well attended (130 registrants) and brought together representatives from government, universities and industry in both Canada and the USA. The proceedings have been published (Bates and Worms 1989).

This second Canadian Workshop on Harmful Marine Algae, held at the Bedford Institute of Oceanography, built upon the groundwork that was laid at Moncton but experimented with some different approaches. For example, more effort was made to solicit poster papers. To increase the time for discussion, no limits were placed on questions following the oral presentations and one half day was devoted to discussion groups. In addition, poster papers were set up immediately outside the auditorium where they could be easily viewed during coffee and lunch breaks.

This report contains the abstracts from all oral and poster presentations, discussion group summaries, overviews of federal agency phycotoxin programs, recommendations for future research and a list of participants.

References

Bates, S.S. and J. Worms (eds.). 1989. Proceedings of the First Canadian Workshop on Harmful Marine Algae, Gulf Fisheries Centre, Moncton, NB, September 27-28, 1989. Can. Tech. Rep. Fish. Aquat. Sci. No. 1712.

PROGRAM SCHEDULE

Tuesday, October 2

0900	Welcome	S.B. MacPhee
0905	Opening remarks	D.C. Gordon
0915	Overview of recent meetings concerned with harmful marine algae	J. Worms
0930	Contributed talks	W. Watson-Wright, Chair
1000	Coffee break	
1030	Contributed talks	W. Watson-Wright, Chair
1200	Lunch	
1300	Contributed talks	C. Enright, Chair
1500	Coffee break	
1530	Contributed talks	C. Enright, Chair
1700	Reception	

Wednesday, October 3

0900	Contributed talks	C. Enright, Chair
1000	Coffee break	
1030	Poster viewing and discussion session	
1130	Introduction to discussion groups	D.C. Gordon
1200	Lunch	
1300	Discussion groups	A. Cembella and J. Wright, Chairs
1500	Coffee break	
1530	Discussion groups	A. Cembella and J. Wright, Chairs
1700	Reception	

Thursday, October 5

0900	Discussion group reports	W. Watson-Wright and K. Haya
1000	Coffee break	
1030	Reviews of federal phycotoxin programs	D.C. Gordon, Chair
1100	Panel discussion on research recommendations	D.C. Gordon, Chair
1230	Workshop closing	D.C. Gordon
1330	Meeting of DFO Phycotoxin Working Group	

MAIN HIGHLIGHTS FROM RECENT MEETINGS ON MARINE TOXINS

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Since the first Canadian Workshop on Harmful Marine Algae, there have been numerous conferences either totally dedicated to, or featuring special sessions on, various aspects of harmful algal blooms. This may be an indication of the interest and concern raised by phycotoxins within a wide range of scientific disciplines. The following list, although not exhaustive, gives an idea of the diversity of meetings held in the last few months:

ICES WORKING GROUP ON PHYTOPLANKTON AND THE MANAGEMENT OF THEIR EFFECTS
April 2-5, 1990, Oban (Scotland)

WORLD AQUACULTURE 90
Special Session: Living With Plankton Blooms
June 14, 1990, Halifax (Canada)

CANADIAN FEDERATION OF BIOLOGICAL SOCIETIES
Natural Toxins in the Marine Environment
Sponsored by the Society of Toxicology of Canada
June 16, 1990, Halifax (Canada)

CANADIAN INSTITUTE OF CHEMISTS (CIC) ANNUAL MEETING
Marine Chemistry: Toxins to Therapeutics
July 16, 1990, Halifax (Canada)

OCEAN 90
Seattle, Wash. (USA)

200th AMERICAN CHEMICAL SOCIETY MEETING
Symposium on Natural Toxins
August 1990, Washington, D.C. (U.S.A.)

Based mainly on notes and preliminary proceedings, the following are the main highlights from the first three meetings on the above list:

1. More blooms are reported involving more species from more classes (*Cyanophycae, Haptophycae, Dinophycae, Bacillariophycae*).

2. Reasons for this apparent increase are still elusive/debated (increased awareness, better detection methods, coastal eutrophication, global warming, etc.).

3. Monitoring of phytoplankton and/or bioaccumulators provides an efficient way to protect consumers and the aquaculture industry.

4. Trend analysis, whenever possible, will better our predictive capabilities on a site-specific basis.

5. Better knowledge of basic processes and mechanisms is required especially for new toxin-producing species. This involves working with all ecological compartments from producers to end consumers.

6. Availability of analytical standards is critical to the development and implementation of new or improved detection/quantification methods.

The following phycotoxin-related meetings will or have taken place during the next twelve months:

IXth INTERNATIONAL SYMPOSIUM ON MEDICAL OCEANOGRAPHY
October 22-24, 1990, Nice (France)

INTERNATIONAL SYMPOSIUM ON MARINE BIOTOXINS
January 30-31, 1991, Paris (France)

ICES WORKING GROUP ON PHYTOPLANKTON AND THE MANAGEMENT ON THEIR EFFECTS
March 18-21, 1991, Vigo (Spain)

GORDON CONFERENCE ON MYCOTOXINS AND PHYCOTOXINS
June 24-28, 1991, Plymouth, NH (U.S.A.)

FIFTH INTERNATIONAL CONFERENCE ON TOXIC MARINE ALGAE
October 28 - November 1, 1990, Rhode Island (U.S.A.)

THIRD CANADIAN WORKSHOP ON HARMFUL MARINE ALGAE
Winter 1992, Mont-Joli, Quebec (Canada)

ABSTRACTS

AMMONIUM OR NITRATE: IS THERE AN INFLUENCE ON THE GROWTH AND DOMOIC ACID PRODUCTION BY THE DIATOM *NITZSCHIA PUNGENS*?

Stephen S. Bates, Luc Bourque, Paryse Cormier, Claude Léger, John C. Smith, and Jean Worms

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The pennate diatoms *Nitzschia pungens* f. *multiseries* (clone NPARL from eastern Prince Edward Island, and clone MD#1 from Glaveston Channel, Texas), and *N. pungens* f. *pungens* (clone BRUD-A, from eastern P.E.I.), were grown in five concentrations (55, 110, 220, 440, and 880 µM) of nitrogen in the form of nitrate or ammonium. The centric diatom *Skeletonema costatum* (clone SCOST from eastern P.E.I.) was grown for comparative purposes. The responses of all three *N. pungens* clones to the form and concentration of nitrogen were similar, except that there was no confirmed domoic acid production by f. *pungens*. *Nitzschia pungens* clones did not survive at 880 µM ammonium-N, whereas the division rate of *S. costatum* was only slightly reduced at this concentration. Division rates and cell yields of *N. pungens* clones were reduced at 220 to 440 µM ammonium-N relative to growth with the same nitrate-N concentrations. Domoic acid production by *N. pungens* f. *multiseries* (NPARL and MD#1), however, was enhanced at these ammonium-N concentrations. Growth at 55 to 110 µM nitrogen, whether in the form of nitrate or ammonium, resulted in decreased production of domoic acid, possibly due to nitrogen limitation of final cell yields. In contrast to growth on nitrate, *N. pungens* f. *multiseries* grown on 440 µM ammonium-N produced a significant quantity (up to 2 pg $cell^{-1}$) of domoic acid during the exponential growth phase. The difference in domoic acid production between ammonium-and nitrate-grown cells was greatest (17-fold) during early stationary phase, and decreased (to 2-fold) as culture age increased. The enhancement of domoic acid production by ammonium-N could be an important factor for determining the rates at which shellfish become toxified, given the likelihood of high concentrations of ammonium-N associated with bivalve excretion. However, it must yet be determined if domoic acid production by *N. pungens* f. *multiseries* cells growing in the natural environment with ambient concentrations of ammonium and nitrate, present simultaneously, is influenced in a similar way as was shown in these laboratory experiments.

PHYTOPLANKTON MONITORING AS A TOOL IN EARLY WARNING OF PHYCOTOXIN OCCURRENCES IN MOLLUSCS, GULF REGION

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The Inspection Branch of the Gulf Region has evaluated the use of a phytoplankton monitoring program as an early warning of phycotoxic occurrences in molluscs. The program has shown to have good potential in terms of predicting Amnesic Shellfish Poisoning (domoic acid) occurrences and identifying

new phytoplankton species of concern, such as those related to Diarrhetic Shellfish Poisoning. The program was not able to evaluate the use of phytoplankton monitoring in the case of Paralytic Shellfish Poisoning since no such toxic events have occurred in the region since 1988.

La Direction des services de l'inspection de la région du Golfe a évalué l'utilisation d'un programme de surveillance du phytoplancton pour la prédiction d'événements phycotoxiques chez les mollusques. Ce programme a démontré un bon potentiel pour la prédiction des cas intoxication amnésique par les mollusques, ainsi que pour l'identification de nouvelles espèces notamment celles reliées à l'intoxication diarrhéique par les mollusques. Le programe n'a pu évaluer l'utilisation de la surveillance du phytoplancton dans les cas de l'intoxication paralysante par les mollusques puisque les espèces de phytoplancton concernées n'ont pas été signalées depuis 1988.

THE PHYSICAL AND CHEMICAL OCEANOGRAPHIC ENVIRONMENT OF PHYTOPLANKTON MONITORING SITES

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The general purpose of the phytoplankton profiling program is to establish the phytoplankton composition of selected inlets in Atlantic Canada; i.e., to determine the abundance, types and time of occurrence of various species throughout the year for a period of three years. With this information, it may be possible 1) to determine what areas and times are favorable or unfavorable for shellfish or finfish aquaculture in terms of both the presence of toxin producing species and the carrying capacity of the location; 2) to indicate times when screening for toxins should be more or less frequent if a consistent species succession can be established; and 3) to provide background information for gauging whether observed phytoplankton events are normal or whether changes in biomass and species diversity may be related to exceptional meteorological events, changes in oceanic forcing or anthropogenic activity. There are five sites in the Scotia-Fundy Region where sampling is handled by Biological Sciences Branch at the Bedford Institute of Oceanography. These are: Tor Bay, Ship Harbour, St. Margaret's Bay, Wood's Harbour and Annapolis Basin.

To achieve these goals, it is crucial that a clear understanding of the physical factors controlling phytoplankton dynamics such as nutrient supply, radiation, stability of the water column, temperature and salinity at each site be obtained as an integral part of the program.

The sites have been chosen for their differing physical characteristics as well as their proximity to aquaculture operations. Ship Harbour and Annapolis Basin (Digby) have widely different tidal forcing, yet similar ratios of freshwater discharge to non-tidal volume. The differences in the physical-chemical environment resulting from the different forcing are strongly reflected in the phytoplankton populations of each region.

Sampling frequency at the five sites varies from once per month in winter to weekly in summer. Meteorological events, for example, may flush an entire bay on time scales shorter than this sampling frequency could resolve. To monitor these events thermograph moorings have been established at each of the sampling sites.

KINETICS OF PARALYTIC SHELLFISH TOXIN UPTAKE AND DETOXIFICATION IN BIVALVE MOLLUSCS

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The kinetics of Paralytic Shellfish Poisoning (PSP) toxin uptake, assimilation and detoxification, including compositional changes, were determined by high-performance liquid chromatographic (HPLC) analysis of tissue extracts of two species of bivalve molluscs. The blue mussel *Mytilus edulis* and the hard clam *Mercenaria mercenaria* were maintained in controlled ecosystems and fed independently upon two species of the toxic dinoflagellate *Alexandrium*, which differed in specific toxicity per cell and toxin composition. In mussels, saturating toxin concentrations of approximately 4.5×10^4 µg saxitoxin equivalents (STXeq) 100 g^{-1} shellfish tissue were achieved within two weeks of exposure to *A. fundyese* cells at environmentally realistic concentrations (~250 cells L^{-1}). Release of toxin from contaminated mussels after the cessation of feeding on *Alexandrium* was essentially biphasic; a rapid drop in toxin content within 24 hours, particularly from the viscera, was followed by a gradual decline in toxicity over several weeks.

Previous reports based on field studies have suggested that *Mercenaria* does not accumulate PSP toxins above regulatory limits (80 µg STXeq 100 g^{-1}), even when exposed to blooms of *Alexandrium*. However, when fed upon toxic *A. fundyense* cultures supplemented with the diatom *Thalassiosira weissflogii*, the hard clam readily ingested *Alexandrium* (3.4×10^5 cells g^{-1} d^{-1}) and retained substantial quantities of PSP toxins. As with *Mytilus*, saturating toxin levels were reached within two weeks of exposure to the toxic dinoflagellate, although peak toxicity (1.1×10^4 µg STXeq 100 g^{-1} tissue) was several times less than for mussels. The results indicated that the risk of PSP toxin accumulation by *Mercenaria* is enhanced in mixed phytoplankton assemblages in which *Alexandrium* is dominant, rather than monospecific.

In general, the toxin profile of accumulated PSP toxins in various tissues of both mollusc species reflected that of the dinoflagellate strain used as the food source, particularly during the early stages of the uptake experiments. Nevertheless, there was also evidence of *in vivo* toxin conversion and differential partitioning of toxin components among tissues, effects which became more pronounced during the later phases of detoxification.

IMMUNOLOCALIZATION STUDIES OF SAXITOXIN IN *ALEXANDRIUM*

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An immunocytochemical approach was employed to determine the subcellular distribution of saxitoxin (STX) in the marine dinoflagellate *Alexandrium fundyense* (strain GTCA29). The closely-related *Alexandrium tamarense* (strain PCT183) served as a non-toxic control. Specimens were prepared using both conventional chemical fixation and rapid freezing (i.e. cryo-fixation) techniques. Primary antisera tested included two rabbit polyclonal antibodies (Ab) and one mouse monoclonal Ab against STX, in

addition to normal serum controls. Results obtained with chemically-fixed material indicate that STX is associated with the permanently-condensed dinoflagellate chromosomes. Supporting evidence was provided by negative non-toxic and normal serum controls, and also the elimination of chromosome labelling in *A. fundyense* by incubating a STX Ab with STX prior to staining. In the case of cryo-fixed specimens, both toxic and non-toxic isolates exhibited chromosome-associated labelling with not only two of the STX antibodies, but also normal rabbit serum. Virtual elimination of chromosome staining by pre-incubating a STX Ab with non-dinoflagellate DNA suggests that this labelling pattern resulted from the "non-specific" binding of a DNA component(s) by the antiserum. Additional data showing a reduction in the electrophoretic mobility of non-dinoflagellate DNA fragments, following incubation with sera that labelled chromosomes in cryo-fixed material, are consistent with a DNA-immunoglobulin interaction. While we are confident of the STX-chromosome association as demonstrated in chemically-fixed *A. fundyense*, it appears that a "non-specific" DNA-immunoglobulin interaction, which overshadows any STX-specific labelling, currently prohibits the localization of STX in our cryo-fixed specimens. It is possible that the unique, permanently-condensed nature of dinoflagellate chromosomes may preclude the use of "gentle" fixation methods (e.g. certain cryo-techniques) in studies attempting to localize antigens occurring in close association with these structures.

SOLUBILITY OF DOMOIC ACID IN WATER AND IN NON-AQUEOUS SOLVENTS

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The solubility of domoic acid in water and in several non-aqueous solvents was measured by NMR and UV spectroscopy. The solubility in water increases markedly between pH 3 and pH 7, corresponding to the increasing level of ionization of the molecule. The solubility in alcohols decreases rapidly with increasing hydrocarbon chain, but is much higher than the solubility of the closely related glutamic acid. The octanol-water partition coefficient for domoic acid at pH 5.32, K_{ow} = 0.0037, was obtained independently by UV and NMR. The low value of K_{ow} leads to the prediction that aquatic organisms will not bioconcentrate domoic acid.

SEASONAL AND GEOGRAPHIC DISTRIBUTION OF *NITZSCHIA PUNGENS* f. *PUNGENS* AND *N. PUNGENS* f. *MULTISERIES*

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Nitzschia pungens f. *multiseries* has now been found along the coasts of the northwest Gulf of Mexico, southwestern Korea (Jinhae Bay), and western Canada, and in estuaries of Prince Edward Island and in the Gulf of St. Lawrence. These sites are in addition to the five originally described by Hasle: Drobak, Norway; Chesapeake Bay; Atlantida, Uruguay; Quenquen, Argentina; and the Oregon coast. More than a year of monthly collections from six diverse sites around Galveston Island, Texas, have resulted in isolations of over 50 clones of *N. pungens* and provided material for a pilot study on the population dynamics of the toxic form (*N. pungens* f. *multiseries*) as well as *N. pungens* f. *pungens*.

Both forms have been successfully maintained at TAMU at 20°C in salinities of 25 or 30 PPT with natural northern light, although 31 clones of *N. pungens* f. *pungens* were isolated from samples collected at temperatures of from 27.9-31.5 C and salinities from 10-33 PPT. Twenty-one clones of *Nitzschia pungens* f. *multiseries* have been successfully isolated from sites with water temperatures from 20.0-30.0 C and salinities of 20-33 PPT. After establishment in microtest plates in Guillard's f/2 medium, survival of clones has been capricious for routine transfer in test tubes, better in flasks if medium is added as growth demands, and best in Petri dishes. The occasional addition to 10^{-8} M selenous acid in the growth medium does not seem to affect the toxic form, but has proved helpful in maintaining the nominate form.

The six Galveston sites sampled are diverse in water quality and history. One is adjacent to the 13 m deep dredged Galveston Ship Channel, another is on the open Gulf of Mexico coast, two are on the tidal inflow and outflow of a lagoon, and two are on a highly polluted bayou off West Bay, an arm of Galveston Bay. The weather during collections has ranged from Texas "blue northers" to an incoming hurricane. Samples have been taken following oil spills from both the MEGA BORG explosion offshore and a barge sinking in the Houston Ship Channel. The common pattern from these collections has been small populations of *N. pungens*, averaging less than 5% of the diatoms in net hauls; occasional samples can have much more, and one was close to 50%, all *N. pungens f. multiseries*. Analysis of which form is present has been labor intensive, but interesting that *N. pungens* f. *pungens* is most abundant during the heat of the summer and into the autumn, while *N. pungens* f. *multiseries* completely dominates once the periodic winter fronts start sweeping across the Bay.

To summarize, both forms of *N. pungens* are present in the same geographical region, but they dominate during different seasons and weather regimes.

ESCALATION OF PARALYTIC SHELLFISH POISON IN VISCERA OF GEORGES BANK SCALLOPS (*PLACOPECTEN MAGELLANICUS*) FROM 1988 TO 1990

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Intensive sampling of sea scallops (*Placopecten magellanicus*) from the Canadian portion of Georges Bank was undertaken in 1988 in conjunction with the commencement of the 'roe-on' scallop industry in Canadian waters. Scallop hepatopancreas and gonadal tissue (roe) were separated, extracted and analyzed for Paralytic Shellfish Poison (PSP) using the A.O.A.C. mouse bioassay (N=1650). PSP was nondetectable in all of the 80 bioassays performed on adductor muscle. Hepatopancreas PSP levels did not exceed 62 $\mu g\ 100\ g^{-1}$ during April and May but rose sharply during June to 800 $\mu g\ 100\ g^{-1}$ in Grid 16. These levels continued to rise in early July reaching peaks of 1300 $\mu g\ 100\ g^{-1}$ in Grid 16 and 1440 $\mu g\ 100\ g^{-1}$ in the adjacent Grid 9. Thereafter hepatopancreas PSP declined, the high in August being 55 $\mu g\ 100\ g^{-1}$. In roe, PSP was nondetectable until July when the peak level was 44 $\mu g\ 100\ g^{-1}$ in Grid 16, well below the acceptable level of <80 $\mu g\ 100\ g^{-1}$. Roe PSP was nondetectable in August and September of 1988. In contrast, PSP levels in roe had exceeded the tolerance level in a number of grids by mid-July of 1989, and by late May of 1990, necessitating the closure for roe-on scallop fishing of the majority of the Canadian portion of Georges Bank during those years. The closures remained in effect until the end of the roe-on seasons of both years. The intensive monitoring of toxin levels in these molluscs by industry

as well as government ensures that commercial distribution is restricted to product which is safe for human consumption. Nonetheless, the demonstrated temporal and geographical variability in PSP levels in scallop viscera and roe obtained from these waters suggests that longer term data collection would be of benefit to both science and industry.

UTILITY OF PHYTOPLANKTON NUTRIENT STATUS MEASUREMENTS AT AQUACULTURE SITES IN PHYCOTOXIN MONITORING

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Physiological measurements aimed at understanding the nutrient sufficiency of natural phytoplankton populations near an aquaculture site and offshore in St. Margaret's Bay, Nova Scotia, were made during August and September. The nutrient status measurements demonstrated that the offshore stations were primarily nitrogen deficient with phosphorus deficiency developing as stratification broke down. The inshore aquaculture sites were occasionally nitrogen deficient but after destratification, there was no evidence of control by nitrogen and one of the sites was phosphorus limited. Similar measurements were made using batch cultures of *Nitzschia pungens* in various growth phases and grown under different nutrient regimes to understand how a) the abundance of *Nitzschia pungens* and the production of domoic acid may be under specific nutrient control and b) how the nutrient status of phytoplankton at aquaculture sites can differ from typical coastal conditions and may favour the growth of toxic algae and toxin production.

STUDIES OF THE HISTOLOGY OF BIVALVE MOLLUSCS FED ON *NITZSCHIA PUNGENS*

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During the episode of domoic acid toxicity in late 1987, no clear evidence of histopathological changes in mussels (or other bivalves) was reported although digestive glands of mussels showed histological changes interpreted as representing abnormally high levels of feeding activity. Since domoic acid is an excitatory amino acid known to cause severe neuropathological changes in vertebrates and to affect neurons and neuromuscular junctions in invertebrates, a histological survey was initiated to determine whether feeding on toxin-producing *Nitzschia pungens* produces neuropathological changes in *Mytilus edulis* and *Argopecten irradians* which could be correlated with observed changes in digestive gland histology. To date, no evidence of acute or subacute neuropathological changes has been seen in experimental or field-exposed bivalves. Mapping of neurosecretory cells is currently under way, since this may provide a sensitive histological marker for non-specific excitatory effects at a level below the threshold for observable neuronal damage.

POTENTIAL THREAT OF TOXIC ALGAE INTRODUCTION BY BALLAST WATERS

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Ballast water transported by shipping is clearly the dominant vector for the global transfer of living aquatic organisms by human activity. Massive quantities of living organisms, representing a wide spectrum of taxonomic groups, are daily transported among biogeographic regions by this essentially unregulated activity. Prominent among the diverse assemblage of potential colonizing species is a wide variety of phytoplankton species, both marine and freshwater, that are known to be undesirable, harmful, or pathogenic to humans or to valued species of aquatic organisms. There is now abundant proof that ballast water, together with associated tank sediments, is routinely capable of transporting harmful organisms, such as toxic dinoflagellates. Less quantifiable, because proof is difficult to establish after the fact, and because few regulatory agencies have sought evidence, is the extent to which this vector is responsible for the observed global increase in occurrences of harmful species in regions where they were previously undetected. Competing hypotheses include the view that environmental changes, such as coastal eutrophication, stratospheric ozone depletion, long-range transport of atmospheric pollutants, and global warming have modified habitats to the extent that species changes are the inevitable consequence. I suggest these views are not exclusive, and the current traffic in ballast water affords a speedy, efficient means to ensure that inimical organisms, including many phytoplankton species, can rapidly become established on a global scale wherever changed conditions favour their survival.

A COMPARATIVE EVALUATION OF A RAPID IMMUNOASSAY TECHNIQUE FOR THE DETECTION OF PARALYTIC SHELLFISH TOXINS

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The accumulation of derivatives of the neurotoxin saxitoxin (STX) in filter-feeding shellfish results in an intoxication syndrome known as Paralytic Shellfish Poisoning (PSP) in human consumers of contaminated shellfish. In order to develop a rapid test for the detection of these compounds, a polyclonal anti-STX antibody was prepared by covalently coupling STX to a synthetic polypeptide, poly-alanine lysine (Pal), in the presence of glutaraldehyde. The specificity and cross-reactivity of the anti-STX antibody was evaluated against a number of purified STX derivatives, including neosaxitoxin, gonyautoxins 2 and 3, and a mixture of the low potency N-21-sulfocarbamoyl compounds. The STX-antibody exhibited a high affinity for STX, while cross-reacting to various degrees with the other derivatives tested. No reactivity was evident with the sulfocarbamoyl derivatives of STX, nor against the tetrapurine tetrodotoxin. Other non-tetrapurine microbial toxins, including domoic acid, okadaic acid and *Staphylococcus* enterotoxin B also failed to cross-react.

The anti-STX antibody has been incorporated into a rapid diagnostic kit ("Saxitoxin Test") based upon a competitive inhibition enzyme-linked immunosorbent assay (ELISA). The ELISA technique was

compared with two other standard methods for the detection of PSP toxins in shellfish samples, the AOAC mouse bioassay and the fluorescence HPLC method, in a double blind experiment among four independent laboratories. Quantitative and qualitative data on the efficacy, specificity, and sensitivity of these respective methods will be presented. The ELISA test has a theoretical sensitivity (1 pg saxitoxin equivalent [STXeq] per assay) several orders of magnitude greater than the alternative techniques, although it may be configured to yield optimum detection in the range 1 to 80 µg STXeq $100g^{-1}$ shellfish tissue - the range of greatest interest for regulatory purposes. The results of the PSP analyses by the ELISA kit and the HPLC and mouse bioassay methods were highly correlated; the ELISA test yielded most accurate values when the relative concentration of STX in the samples was highest. While the ELISA assay clearly requires further refinement, the relatively broad antigen specificity of the STX-antibody incorporated into the rapid test kit demonstrates its usefulness as a rapid diagnostic screening technique for PSP toxins in contaminated shellfish.

THE TOXIC PHYTOPLANKTON MONITORING PROGRAM IN THE QUEBEC REGION

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The results from the first toxic phytoplankton monitoring program in the Quebec Region are presented. The main objectives of this monitoring program are two-fold: 1) to provide DFO Inspection Branch and shellfish producers with rapid information on the occurrence of potentially toxic blooms; 2) to accumulate a database of toxin bloom events and associated ecological factors, for future aquaculture site selection, toxic bloom risk evaluation and trend analysis, and to establish the seasonal population dynamics and general spatio-temporal distribution of toxic phytoplankton in the St. Lawrence estuary and northwestern Gulf of St. Lawrence. In 1989, 10 stations were sampled for toxic phytoplankton using net tows and discrete water samplers. Salinity and temperature data were collected simultaneously. Most stations were sampled weekly between June and November. The data collected in 1989 showed that at most stations only one annual bloom of *Alexandrium* spp. occurred, typically during June and July. At certain stations where the sampling was initiated in early June, the link between the PSP toxicity in shellfish and the toxic *Alexandrium* blooms was clearly established.

One station in the Magdalen Islands drew particular attention, due to the historical lack of PSP toxicity in this important shellfish cultivation area. During the summer of 1989, relatively low but constant levels of *Alexandrium* were encountered, although PSP toxicity in shellfish was not confirmed. The toxic phytoplankton monitoring program thus highlighted the potential risk for PSP outbreaks, even in areas where shellfish toxicity has not previously been reported.

The toxic phytoplankton sampling program also demonstrated the wide geographical distribution of potential DSP-producing species, as *Dinophysis* spp. were found at all stations in the Quebec fisheries region. Particularly high concentrations were noted in the Baie de Gaspé and Baie des Chaleurs, in areas which are presently used for shellfish culture or where future exploitation is planned.
The omnipresence of these potentially toxic species underscores the importance of acquiring more information on their distribution and specific toxicity in eastern Canada. In light of the 1989 results in the Quebec region, some modifications to the program were proposed for the following years. These

changes are aimed at improving the geographical coverage of the sampling regime and the quality of the samples collected at each station.

DETERMINATION OF PARALYTIC SHELLFISH TOXINS USING PRECHROMATOGRAPHIC OXIDATION AND LIQUID CHROMATOGRAPHY

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A method for quantitating Paralytic Shellfish Poison (PSP) toxins in shellfish has been developed using pre-chromatographic oxidation of the toxins to fluorescent purines. Oxidation is accomplished under mildly basic conditions with hydrogen peroxide or periodate. Peroxide oxidation yields fluorescent derivatives with the non-hydroxylated PSP toxins such as saxitoxin, gonyautoxins 2 and 3, B-1, C-1 and C-2. Periodate oxidation yields fluorescent derivatives with all PSP toxins studied including, in addition to the above, neosaxitoxin, gonyautoxin 1, B-2 and C3. As low as 3-5 ng g^{-1} each of the non-hydroxylated toxins and 10-20 ng g^{-1} of the hydroxylated compounds could be detected in shellfish. Reversed-phase liquid chromatography was unable to separate all oxidation products. The three gonyautoxins studied eluted with the same retention time. Also, C-1 and C-2 eluted together as did neosaxitoxin and its sulfamate analogue, B-2. The repeatability coefficient of variation for the oxidation reactions ranged from 3-8% for the peroxide reaction and from 4-11% for the periodate reaction depending upon individual toxin analyzed and its concentration in the extract (0.04-0.55 $\mu g\ g^{-1}$). The method was compared to the mouse bioassay and the post-column oxidation procedure. In most cases results were comparable.

UPTAKE, DISTRIBUTION AND CLEARANCE OF DOMOIC ACID BY MUSSELS (*MYTILUS EDULIS* L.)

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Radiolabelled (tritiated) domoic acid, kainic acid, and glutamic acid encapsulated in liposomes were fed to mussels (*Mytilus edulis*) to examine their uptake, tissue distribution and clearance. Only 1-2% of the domoic acid presented to mussels was incorporated; this was most concentrated in kidney and digestive gland tissues. Autoradiography showed uptake into amebocytes. Clearance of domoic acid from digestive gland over 72 h was minimal.

Kainic acid resembled domoic acid in its tissue distribution pattern. In contrast, glutamic acid did not accumulate in the kidney and was cleared more rapidly from the digestive gland. Domoic acid and kainic acid were predominantly found in the TCA-soluble fraction of digestive gland tissues. Glutamic acid was more highly associated with the TCA-insoluble fraction, indicating its ready incorporation into proteins.

(Funded by the National Research Council of Canada)

NITZSCHIA PSEUDODELICATISSIMA - A SOURCE OF DOMOIC ACID IN THE BAY OF FUNDY, EASTERN CANADA

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

PHYTOPLANKTON MONITORING IN THE FUNDY ISLES REGION

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The domoic acid incident in eastern Prince Edward Island in 1987, as well as the increased use of coastal waters in the Fundy Isles region for salmonid aquaculture, resulted in an environmental study being initiated to monitor phytoplankton populations and to establish a database. Four sites were selected: an offshore site to act as an early warning of populations in the Bay of Fundy and three inshore sites located either close to salmon leases, shellfish beds or upriver.

Since sampling began in 1987, more than 150 different species of diatoms, dinoflagellates, ciliates and smaller zooplankton have been observed. Organisms observed that have been known to produce toxins include *Alexandrium fundyense* (PSP), *Dinophysis* spp. (Diarrhetic Shellfish Poisoning) and *Nitzschia pseudodelicatissima* (domoic acid). The organism responsible for paralytic shellfish poisoning, *A. fundyense*, has occurred in the Bay of Fundy for a number of years and has been responsible for annual closures of shellfish beds to harvesting. Domoic acid was first detected in shellfish during 1988 in the Fundy Isles region.

Our study indicates that fewer cells of most species were observed inshore, where waters are well mixed, than offshore. Results indicate that at an inshore site (station number 3), highest concentrations of *A. fundyense* were observed in 1989 with 5.8×10^{4} cells L^{-1}, whereas highest concentrations of *N. pseudodelicatissima* and *Dinophysis* spp. were observed in 1990 with 1.12×10^{5} and 2.24×10^{3} cells L^{-1}. respectively. In the Fundy Isles area, highest densities of *A. fundyense* tend to occur between June and August; *Dinophysis* spp. between July and October; and *N. pseudodelicatissima* from August to October.

OCCURRENCE OF MICROPLANKTON SPECIES IN THE WATER COLUMN AND SHELLFISH TOXINS IN TISSUES OF THE GIANT SCALLOP, *PLACOPECTEN MAGELLANICUS*, AT TWO SITES IN NEWFOUNDLAND

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Water samples and scallop (*Placopecten magellanicus*) tissues were collected from two commercial aquaculture sites in Newfoundland during 1989. The water samples were examined microscopically (light and SEM), and microplankton identified. Scallop tissues were analysed to determine the occurrence of domoic acid and PSP toxins.

The domoic acid and PSP analyses of the tissue samples were negative. No causative phytoplankton species were detected.

Newfoundland has not, thus far, experienced shellfish toxin outbreaks of the frequency or intensity of those in other Atlantic areas. The data presented here indicate that scallops free of shellfish toxins can be produced in aquaculture facilities. The absence of the causative phytoplankton species supports the contention that frequent toxic events in these areas may be unlikely.

PHOTOSYNTHESIS AND GROWTH OF *NITZSCHIA PUNGENS* F. *MULTISERIES*, A NEUROTOXIN PRODUCING DIATOM

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The ubiquitous pennate diatom, *Nitzschia pungens* f. *multiseries*, implicated in the amnesic shellfish poisoning in eastern Prince Edward Island bays, is unique in forming monospecific blooms and producing domoic acid, a neurotoxin. To understand the photosynthetic function of this diatom, batch cultures grown at photon flux densities of 105 and 1100 $\mu E\ m^{-2}\ s^{-1}$ at 10°C were utilized. The relationships between photosynthesis and photon flux density were established on days 1, 4, 8, 15, 25 and 34 and were quantitatively described by the photoinhibition model of Platt et al. (1980). Cellular chlorophyll *a* of cultures grown at 105 $\mu E\ m^{-2}\ s^{-1}$ had higher assimilation numbers (P^B_m:$\mu g\ C\ \mu g\ Chla^{-1}\ h^{-1}$) but lower initial slopes (α: $\mu g\ C\ \mu g\ Chla^{-1}\ h^{-1}\ (\mu E\ m^{-2}\ s^{-1})^{-1}$) than those grown at 105 $\mu E\ m^{-2}\ s^{-1}$. Both P^B_m and α increased from day 1 to day 4, and then decreased as the cells aged. Maximum photosynthetic rates (P^B_m) were <2.2 $\mu g\ C\ \mu g\ Chla^{-1}\ h^{-1}$, which is low compared to data on other diatoms. The photoadaptation parameter, I_k ($\mu E\ m^{-2}\ s^{-1}$) was lower in the cultures grown at 105 $\mu E\ m^{-2}\ s^{-1}$ than those grown at 1100 $\mu E\ m^{-2}\ s^{-1}$. Temporal variations of growth rate were discussed using Gompertz growth model (1825). There were departures in their magnitude and time lag between chlorophyll *a*, cell density, carbon fixation and nitrogen accumulation.

EFFECTS OF GROWTH TEMPERATURE ON CARBON ASSIMILATION OF *NITZSCHIA PUNGENS* F. *MULTISERIES*

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The rate of photosynthesis (^{14}C uptake) as a function of photon flux density was established for *Nitzschia pungens* f. *multiseries* grown in batch culture for four days at 0, 5, 10, 15, 20, and 25°C. The specific growth rate (μ) increased from 0.2 d^{-1} at 0°C to 1.1 d^{-1} at 15°C and then maintained at 1.1 to 1.3 d^{-1} from 15 to 25°C. The initial slope of the phytosynthesis-photon flux densities curves (α) increased from 1.7 x 10^{-4} μg C μg $Chla^{-1}$ h^{-1} (μE m^{-2} $s^{-1})^{-1}$ observed at 0°C to 64.8 x 10^{-4} μg C μg $Chla^{-1}$ h^{-1} (μE m^{-2} $s^{-1})^{-1}$ at 20°C and then decreased to 49.1 x 10^{-4} μg C μg $Chla^{-1}$ h^{-1} (μE m^{-2} $s^{-1})^{-1}$ at 25°C. Assimilation numbers (P^B_m) increased from 0.05 μg C μg $Chla^{-1}$ h^{-1} at 0°C to 3.3 μg C μg $Chla^{-1}$ h^{-1} at 15°C and then decreased to 2.0 μg C μg $Chla^{-1}$ h^{-1} at 25°C. The assimilation numbers, even at optimum temperature, were low compared to values for other diatoms. Cellular chlorophyll *a* increased three-fold between 0 and 25°C, but carbon and nitrogen decreased to 44% and 55%, respectively. In the temperature range from 5 to 15°C, the Q_{10} for μ was 2.8, which is comparable to other algae. However, the Q_{10} for α and P^B_m was 10.3 and 16.9, which is about four to six times greater than that found in other algae. The information on the photosynthetic function of *N. pungens* under a wide variety of environmental variables (i.e. light and temperature) would be of considerable value in interpreting the physiological ecology of the monospecific blooms of this diatom which have been recurring in Cardigan Bay, P.E.I.

MONITORING DSP TOXIN PROFILES OF CULTURED DINOFLAGELLATES BY LC-MS USING A SCIEX API III MASS SPECTROMETER*

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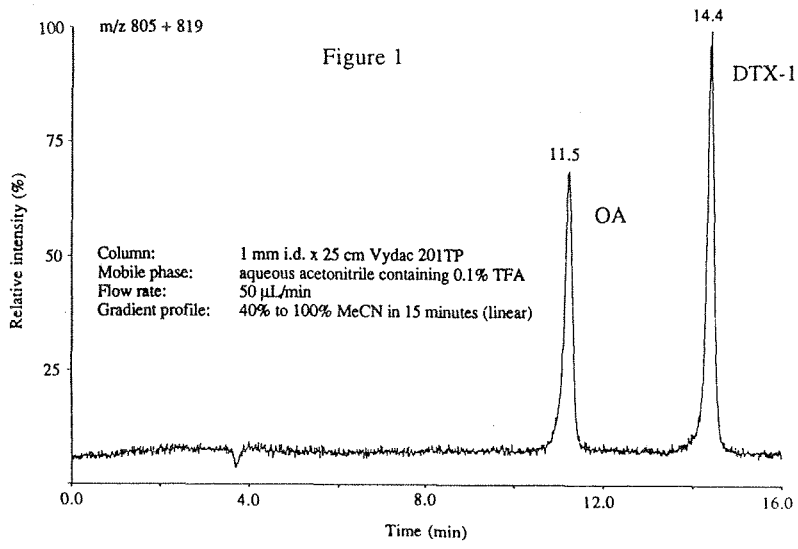
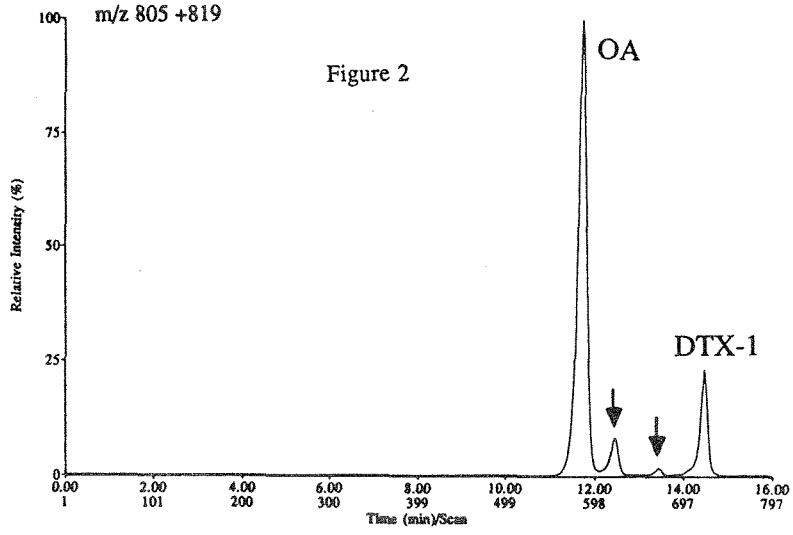
Ionspray mass spectrometry (ISP-MS) is a recently developed atmospheric pressure ionization (API) technique that is proving to be well suited to the analysis of polar and thermally labile molecules. We recently reported on an investigation of ISP-MS for the analysis of three marine toxins (domoic acid, saxitoxin and tetrodotoxin) and concluded that this technique has great potential for the analysis of trace levels of polar marine toxins by mass spectrometry. We have more recently described a combined liquid chromatography mass spectrometry (LC-MS) method using ISP ionization for the sensitive determination of okadaic acid (OA) and dinophysistoxin-1 (DTX-1), the principal toxins implicated in cases of DSP. This method was used to confirm the presence of OA in natural populations of *Dinophysis* spp. from eastern Canadian waters. In the present work, we report on the use of an improved gradient LC-MS method to profile DSP toxin production by different strains of *Prorocentrum* spp. grown in culture at our laboratory.

The analysis of standard OA and DTX-1 by LC-MS is shown in Figure 1. The DSP toxins have excellent retention characteristics on the reversed-phase column and are well resolved under the gradient elution conditions, allowing precise quantitation of both toxins. Using selected ion monitoring techniques we are

currently able to detect sub-nanogram levels of OA. For shellfish, this translates to a working detection limit of around 50 ng/g (whole tissue; 1 µL injection), although recent improvements in extraction and clean-up procedures have significantly reduced this limit.



Cultured dinoflagellates were harvested at five weeks and extracted into chloroform using sonication. The results of the LC-MS analysis of an extract from a strain of *P. lima* (NWCC #712) are presented in Figure 2. Comparisons with standard mass chromatograms confirm that this strain produces high levels of both OA and DTX-1. We have detected several previously unreported analogues of OA in a number of the strains analyzed, and this is evidenced by the additional peaks in the mass chromatogram presented. These analogues have been tentatively identified as the methyl ester of OA and isomers of both OA and DTX-1. These assignments are supported by full-scan analyses using the LC-MS system and by the observation of similar species in toxic mussels from Europe and the marine sponge *Halichondria melandociae*. In the case of the methyl ester we have also synthesized the derivative and obtained confirmatory LC-MS results. The isolation and structural elucidation of the isomers of OA and DTX-1 is currently being undertaken at IMB.



In conclusion, we have found that the LC-MS method is an extremely valuable tool in our DSP research program. The versatility of the technique is summarized below:

- Confirmation and determination of trace levels of underivatized DSP toxins both in plankton and shellfish
- Identification of novel toxins and related compounds
- Direction of isolation procedures
- Validation of biological and immunological assays
- Aid in the development and validation of extraction, clean-up, derivatization, and HPLC methods
- Monitoring of toxin production in cultured dinoflagellates

In the case of the last point, we have found that the DSP toxin profiles from different strains of *Prorocentrum concavum* and *P. lima* show significant variety in the quantity and type of toxin produced.

CONFIRMATION OF AN INCIDENT OF DIARRHETIC SHELLFISH POISONING IN EASTERN CANADA

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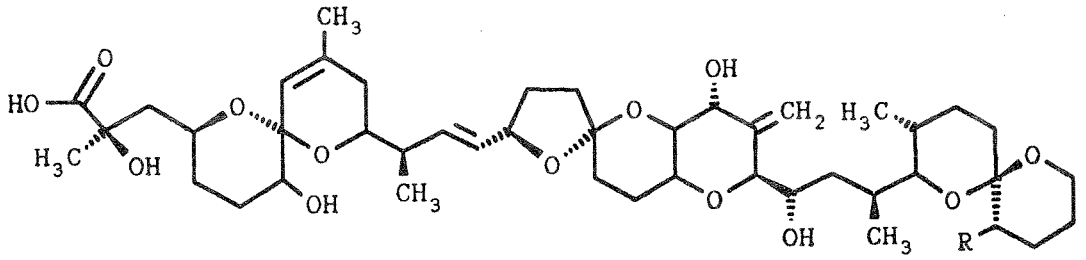
In early August 1990, at least 16 people developed symptoms of nausea, vomiting and diarrhoea shortly after eating cultured mussels from the Mahone Bay area in Nova Scotia. Diarrhetic shellfish poisoning (DSP) was suspected. Extracts of samples of raw mussels from a restaurant and cooked mussels from a residence were found to be toxic to mice (IP injection). Analyses by liquid chromatography-mass spectrometry (LC-MS) using the Canadian-built SCIEX API-III technology quickly established that there was a high level of the DSP toxin, dinophysistoxin-1 (DTX-1), present in the suspect mussels and



R = H Okadaic Acid

R = CH_3 DTX_1

not in control mussels (see Figure 1). Interestingly, okadaic acid was not detected. Conclusive proof of the toxin identity was provided by further experiments, including formation of chemical derivatives with the correct retention times in LC-MS and LC with fluorescence detection (see Figure 2) and isolation of the toxin followed by LSIMS mass spectrometry, accurate mass measurement, and proton NMR spectroscopy.

Extraction and handling procedures were improved during the investigation, allowing the quantitative analysis of survey samples. It was found that the incident was highly localized to one mussel growing lease and that by the end of August, the mussels had depurated all of their toxin load. The most toxic mussels, containing up to 1000 ng/g whole tissue (the legal level in Europe is 200 ng/g), appear to have been harvested on August 3. This suggests that a bloom of toxic plankton may have occurred near the end of July and led to contamination of the mussels.

Microscopic examination of toxic mussel digestive gland contents revealed remnants of *Dinophysis* spp. (probably *D. norvegica*) (see Figure 3), an organism known to produce both okadaic acid and DTX-1. Control, non-toxic mussels showed no recognizable remnants of *Dinophysis*. Plankton tows from the waters adjacent to the affected area in mid-August did show several *Dinophysis* spp. (including *D. norvegica* as the dominant species), but at low levels in the water column (see Figure 4). LC-MS analysis of these plankton samples provided only a weak, unreliable signal for the toxin. Presumably the bloom of plankton had dissipated by the time of sampling.

To our knowledge, this is the first proven case of diarrhetic shellfish poisoning in North America.

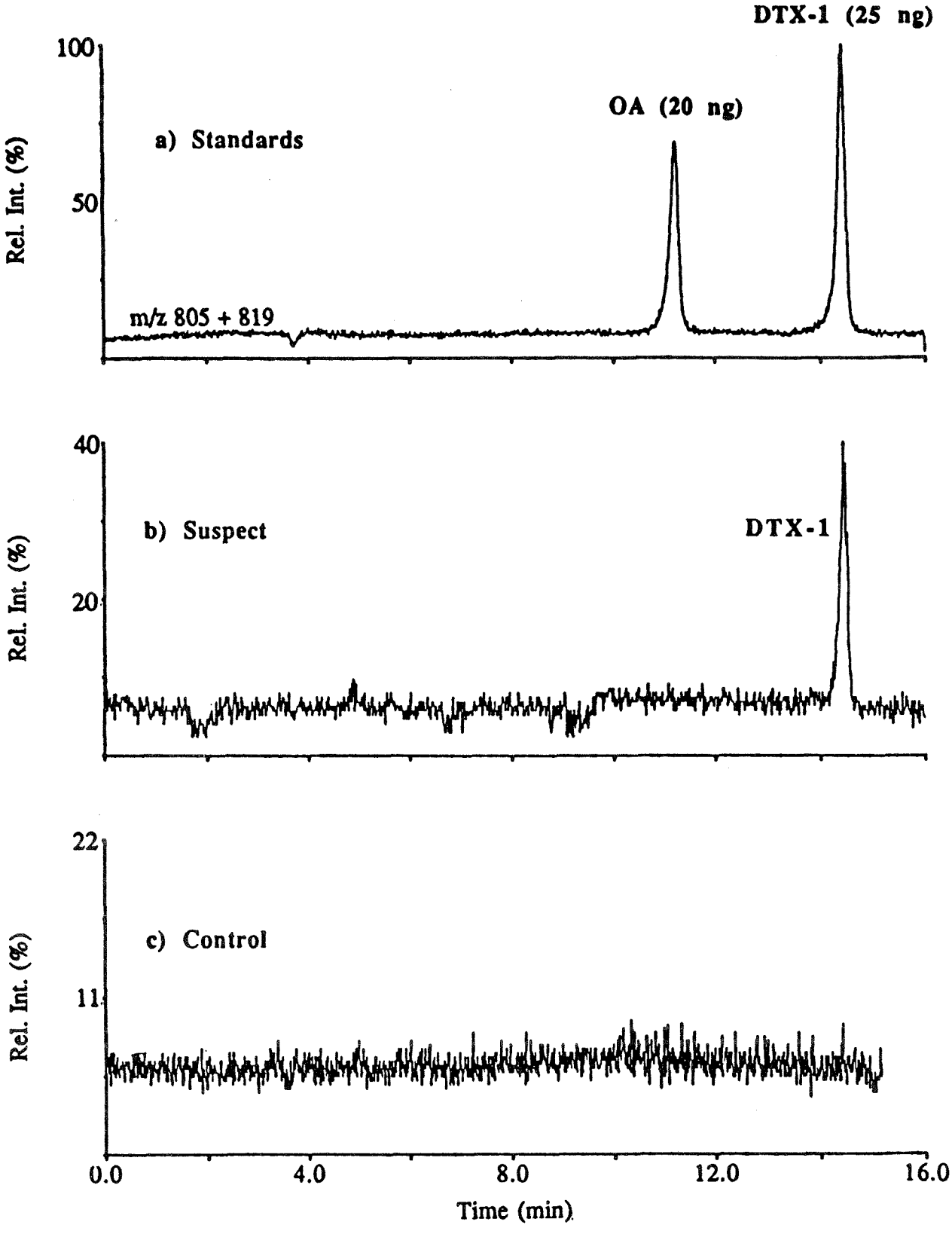


Figure 1: LC-MS analysis of DSP toxin standards (a) and the extracts of suspect (b) and control (c) mussel digestive glands.

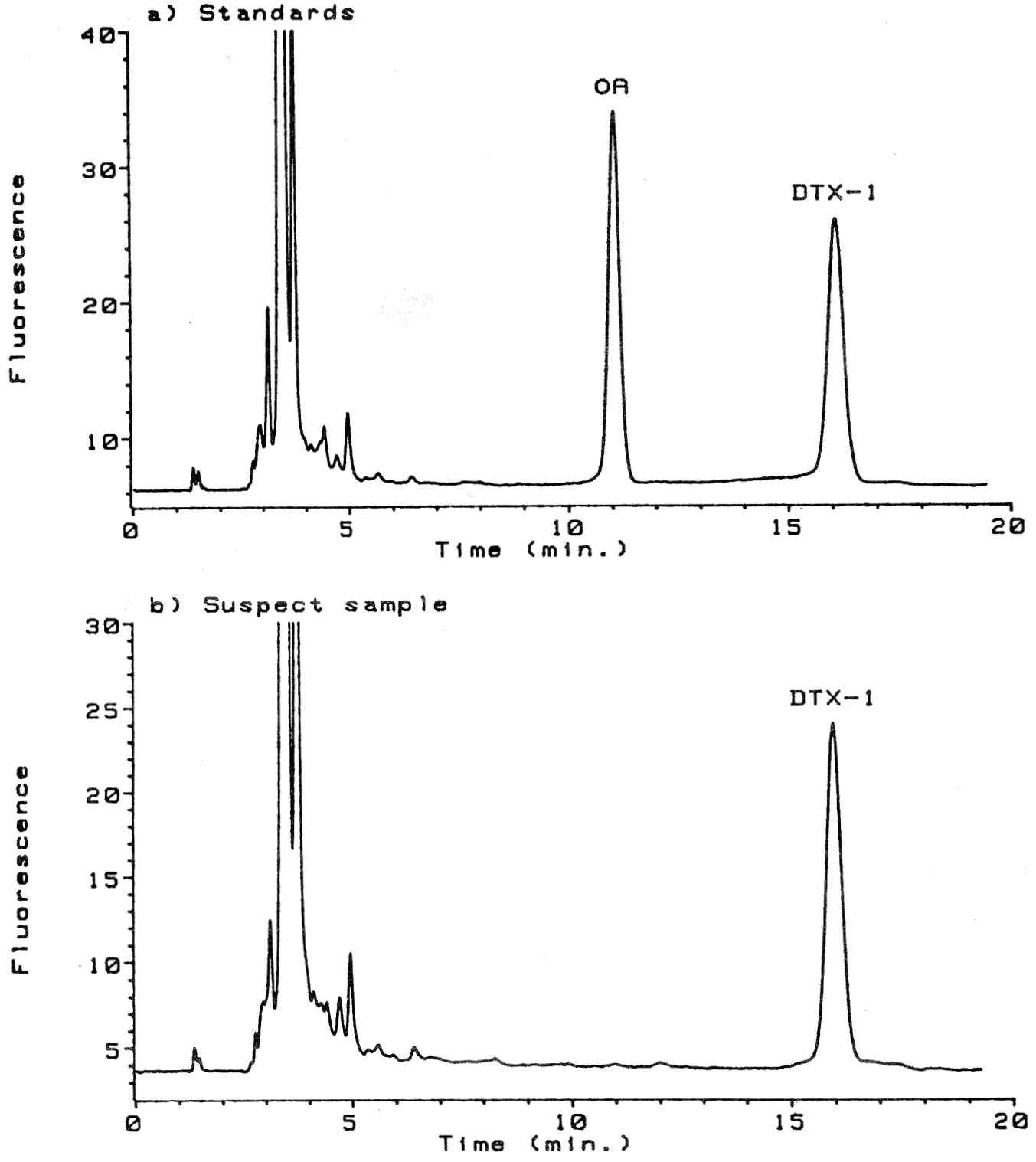


Figure 2: HPLC analysis of standards (a) and the toxin isolated from a suspect sample (b) using pre-column derivatization with anthryldiazomethane (ADAM) and fluorescence detection (reference 4).

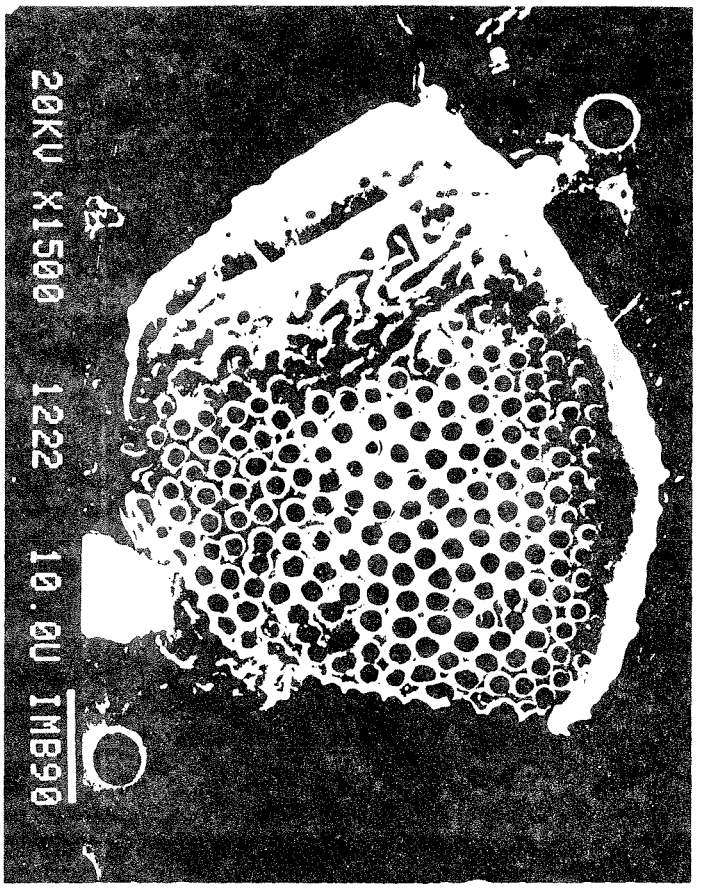
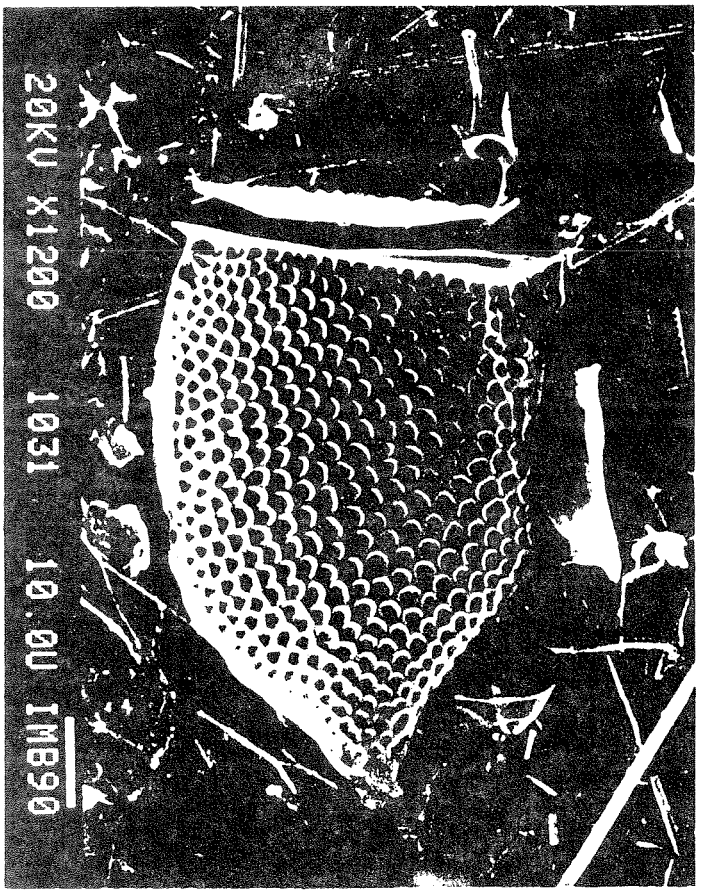
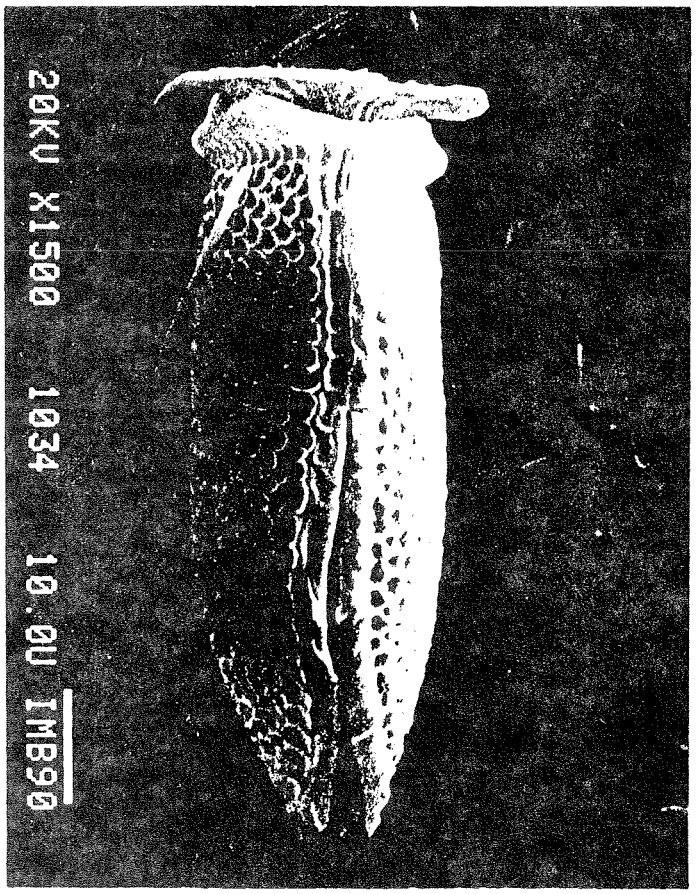


Figure 3 (right): Scanning electron micrograph of partially digested *Dinophysis* cell walls taken from a suspect mussel digestive gland. Scale bar equals 10 μm.

Figure 4 (lower left, lower right): Scanning electron micrographs (different view angles) of intact *Dinophysis* cells from a plankton tow of the Mahone Bay water column. Scale bar equals 10 μm.





MANAGEMENT OF MARINE BIOTOXINS BY THE INSPECTION SERVICES DIRECTORATE IN SOUTHWESTERN NEW BRUNSWICK

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The management of marine biotoxins has undergone dramatic changes in the southwest New Brunswick area. The establishment of a mouse bioassay unit in 1984 has permitted the rapid detection of PSP increases. A strong effort at communicating information to the public and industry has benefitted the safety and health of consumers. The introduction of the Enhanced Shellfish Monitoring Program since the detection of domoic acid in Prince Edward Island has created an increased workload and changed the focus of inspection priorities. Data are now electronically stored which allows managers easier access to information. The creation of a toll-free "clam line" means the public and industry can now verify the status of all shellfish areas in southwest New Brunswick. Continued research into the development of analytical methods will no doubt provide more accurate analysis of marine toxins and may allow the earlier detection of rising toxicity levels.

COMPARED RESPONSES OF FIVE LARVAL FISH SPECIES TO THE TOXIC *ALEXANDRIUM EXCAVATUM*

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The responses of fish larvae to the toxic dinoflagellate *Alexandrium excavatum* are reviewed using published and new data from comparable experiments. Larvae were exposed to variable cell concentrations of toxic (treatment) and non-toxic (control) strains. Response time (2-72 h) and symptoms varied with the toxicity of the treatment strain (0.03-105 pg STX eq. $cell^{-1}$) and species sensitivity. Larvae that died during the experiments had ingested significantly more toxic cells than surviving larvae. Mortality attributable to the toxins reached 95% d^{-1} in direct exposure (first-feeding larvae fed toxic cells) and 36% d^{-1} in vectorial exposure (carnivorous postlarvae fed tainted zooplankton). Instantaneous daily mortality increased with strain toxicity and with cell concentration in high toxicity strains. The size of toxic zooplankton prey determined mortality in carnivorous fish larvae (no mortality with zooplankton <250 μm). We conclude that the response of fish larvae to toxic dinoflagellates blooms in nature will be affected by several factors including cell concentration, strain toxicity, size of tainted zooplankton, species sensitivity and feeding behaviour.

PROGRESS IN UNDERSTANDING THE UPTAKE AND ELIMINATION OF DOMOIC ACID BY MUSSELS (*MYTILUS EDULIS*) AND BAY SCALLOPS (*ARGOPECTEN IRRADIANS*)

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In a series of experiments conducted jointly by staff from the Gulf and Scotia-Fundy Regions of the Department of Fisheries and Oceans, mussels and bay scallops were maintained in a 2000 L tank at 10°C. Cultured *Nitzschia pungens* was added regularly to maintain a more or less constant density of 1 x 10^6 cells L^{-1}, yielding a mean domoic acid content of 0.897 µg L^{-1}. Shellfish samples were taken at 12 hour intervals. After 84 hours, remaining shellfish were removed to tanks of filtered flowing water at 10°C. Concentration of domoic acid in digestive glands of mussels approached 60 ppm after 84 hours exposure and was near 5 ppm after 48 hours depuration. Clearly, experimental exposures will require to be considerably longer to simulate natural conditions. When held in the same experimental conditions, bay scallops accumulated domoic acid at approximately half the rate of mussels.

Progress in culturing *Nitzschia* at the Halifax Laboratory continues to be slow; however, there has been some improvement in producing bulk cultures (230 L) at cell densities upwards of 200,000/mL. Survival of cultures into the stationary phase has been disappointing, and domoic acid production has not exceeded 0.3 pg/cell. These cultures have been used for the histological program. Problems with contamination by bacteria and flagellates have been reduced but not eliminated. Results of the routine domoic acid monitoring program are being followed carefully. Any bloom of *Nitzschia* in the Cardigan or elsewhere will be exploited to provide both a natural uptake experiment and a source of contaminated mussels and other transplanted commercial species, for further depuration experiments.

DYNAMIC MODEL OF THE FLUX OF DOMOIC ACID THROUGH THE CARDIGAN BAY *MYTILUS* POPULATION

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Domoic acid is an episodically serious toxin found in populations of *Mytilus edulis* and other shellfish since 1987. The domoic acid found in *Mytilus* populations from Cardigan Bay, PEI, is believed to originate in blooms of the pennate diatom *Nitzschia pungens*. A dynamic model of the flux of domoic acid from the *Nitzschia* cells through *Mytilus* was developed to estimate whether this alga can supply enough domoic acid to account for the very high concentrations sometimes observed in *Mytilus*, and to see whether the processes involved are sufficiently well understood to make it feasible to predict the occurrence of high domoic acid levels in mussels. Preliminary results give reasonable agreement with toxin concentrations reported during most of the season, but the peak values of about 1000 ppm are difficult to account for. It appears that reduced depuration rates during the peak of the *Nitzschia* bloom may lead to a build-up of the toxin in the mussels; otherwise the ingestion of even heavily laden algal

cells (over 10^6 cells L^{-1} with 7 pg of domoic acid per cell) cannot account for the very high levels of domoic acid observed.

POPULATION DYNAMICS AND TOXICITY OF VARIOUS SPECIES OF *DINOPHYSIS* AND *NITZSCHIA* FROM THE SOUTHERN GULF OF ST. LAWRENCE

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Analysis of the spatial and temporal distribution and toxicity of known and putative phycotoxin-producing phytoplankton species reveals that phytoplankton monitoring is a valuable means of predicting toxic events, but one which can sometimes yield either false positive or false negative results. Thus we observed a large bloom of *Nitzschia pungens* in New London Bay (PEI) in mid-August 1989 which contained no domoic acid and did not toxify mussels. However, a subsequent, much smaller bloom led to significant contamination of the shellfish. The earlier, non-toxic bloom consisted entirely of *N. pungens* forma *pungens*, which is not known to contain significant amounts of domoic acid, whereas the later bloom was largely composed of f. *multiseries*, a strong producer of the toxin. As these two forms of the species cannot be distinguished using a light microscope, phytoplankton monitoring without associated instrumental analytical methods can result in a false positive indication of domoic acid toxicity. Similarly, large populations of *Dinophysis norvegica* and *D. acuminata* were observed in the Cardigan River in June and July 1990. These species are known producers of the diarrhetic shellfish poisoning (DSP) toxins okadaic acid and DTX-1, but LC-MS analysis failed to detect either toxin. In September 1989, however, a smaller population of *D. acuminata* was shown, by a phosphatase inhibition assay, to contain significant amounts of what was probably DTX-1. Thus, seasonal variation in cellular toxin production may also lead to false positive results for DSP. Reliable, rapid methods of instrumental analysis now exist or are under development for both domoic acid and okadaic acid, and these, coupled with appropriate phytoplankton monitoring, can give a valuable forewarning of toxin accumulation by shellfish while avoiding false positives.

However, there also exists the more serious possibility of obtaining false negative results from a phytoplankton monitoring program. Another *Nitzschia* species, *N. actydrophila*, appears to produce domoic acid in our laboratory, and the evidence for domoic acid production and shellfish contamination in the field by *N. pseudodelicatissima* is very strong. Although *N. actydrophila* contains only about 5 fg domoic acid per cell, the cells are very small and, on a cell volume basis, this amounts to more than that produced by certain clones of *N. pungens* f. *multiseries*. Further, we have observed domoic acid in phytoplankton samples when all known domoic acid producing species were absent and the population was dominated by a species of *Navicula*. Although the levels of domoic acid involved in these cases are rather small, such observations indicate the need for continued research, by instrumental and other means, into the subject of toxin production by phytoplankton.

A REVIEW OF MARINE AND FRESHWATER BIOTOXINS OF IMPORTANCE TO HUMAN HEALTH

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Introduction
Chapter 1. Ecology and Physiology of Toxic Dinoflagellates, Diatoms and Cyanobacteria
Chapter 2. Paralytic Shellfish Poisoning (PSP)
Chapter 3. Diarrhetic Shellfish Poisoning (DSP)
Chapter 4. Neurotoxic Shellfish Poisoning (NSP)
Chapter 5. Amnesic Shellfish Poisoning (ASP/Domoic Acid Intoxication)
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Chapter 7. Pufferfish Poisoning (PFP/Tetrodotoxin Intoxication)
Chapter 8. Cyanophyte Toxins
Chapter 9. Pharmacology of Marine Biotoxin Action
Chapter 10. Management of Human Intoxications
Concluding Remarks

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Status: In preparation. Most chapters are now being critically reviewed by experts; comments and suggestions received to date are being used to complete the final draft.

Anticipated completion and submission for publication: Spring 1991

GAMETOGENSIS IN *NITZSCHIA PUNGENS*, A TOXIC, BLOOM FORMING, MARINE DIATOM

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Senescent cells of *Nitzschia pungens* showed gametogenesis when subcultured into FE medium at increased light, cells in exponential growth phase did not. Anisogamous gametes, probably haploid, were isolated by combining conventional microscopy with flow cytometry. Zygotes from syngamy yielded cigar-shaped naviculoid cells, morphologically different from parent cells (heteromorphic). These cells could serve as a seed population and explain the origin and rapid progresion of the toxic blooms of red-water proportions that have been a public health problem in eastern Canada. Age of cells and increase in light were more important than salinity and nutrients is inducing sexuality in *N. pungens* f. *multiseries*.

PHYTOPLANKTON MONITORING PROGRAM AROUND NOVA SCOTIA

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To understand the qualitative and quantitative variations of toxic and non-toxic phytoplankton, a monitoring program has been carried out since October 1988. Tor Bay, Ship Harbour, St. Margaret's Bay, Woods Harbour, and Digby have been sampled at three depths, once every two weeks during autumn and spring, once a week during summer, and once a month during winter. Physical, chemical, and biological (including species identification) data are collected. More than 300 taxa have been recorded of which 120 species are common and less than 20 are dominant. The phytoplankton cycle shows a bimodal (spring and fall bloom) cycle, typical for temperate seas. However, regional differences between stations with respect to the magnitude and duration of the spring bloom exist.

Two diatoms, *Nitzschia pungens* f. *multiseries* and *N. pseudodelicatissima*, implicated in the production and accumulation of the neurotoxin domoic acid in shellfish, are present at all five sites at concentrations less than 10^6 cells m^{-3}, not sufficient to produce a toxicity problem similar to that in Cardigan, PEI. *Prorocentrum micans*, *Dinophysis norvegica*, and *D. accuminata*, dinoflagellates known to produce DSP toxins, are also observed at the monitoring sites in low concentrations.

PROTONATION OF DOMOIC ACID

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UV spectra, ^1H NMR and ^{13}C NMR spectra of the amnesic shellfish poison, domoic acid, have been studied in aqueous solution as a function of pH. The results provide spectroscopic parameters for the five protonation states of domoic acid, yield accurate pK values in both H_2O and D_2O solution, and enable each pK to be associated with a particular protonation site. Changes of NMR spectra with pH indicate unambiguously the following order of protonation with decreasing pH: 1) the imino group, 2) carboxyl-7, 3) carboxyl-5', 4) carboxyl-2.

MONITORING OF PSP IN HEPATOPANCREAS OF LOBSTER FROM ATLANTIC CANADA

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As an addition to its Enhanced Shellfish Monitoring Program, Inspection Services Branch of Fisheries and Oceans Canada (DFO) undertook the monitoring of lobsters (*Homarus americanus*) in the Scotia-Fundy

Region for the possible presence of paralytic shellfish poison (PSP) during the spring and summer of 1990. Initially, live animals which had been harvested from 32 sites around Nova Scotia and New Brunswick were purchased from industry. The lobsters were chilled and transported to the DFO extraction laboratory, where they were boiled, tissues (meat, hepatopancreas and stomach) extracted and analysed for the presence of PSP using the A.O.A.C. extraction and bioassay procedure. PSP could not be detected in any of the meat samples analysed but was detectable in stomach, albeit at lower levels than in the hepatopancreas of the same animal. PSP was detected in hepatopancreas of lobsters from 18 of the 32 sites initially sampled and quantifiable in 11 of those sites. Only four sites were found where PSP levels in hepatopancreas were higher than 80 $\mu g\ 100\ g^{-1}$ tissue, all of those being in the Bay of Fundy. Following the initial survey, twelve sites were chosen for continued monitoring throughout the lobster seasons. Samples containing quantifiable PSP were also purchased for the determination of effects of cooking and depuration. PSP in the hepatopancreas of lobsters boiled for 20 minutes ($64\pm40\ \mu g\ 100\ g^{-1}$, mean$\pm$SD) was significantly lower ($p<0.01$) than in raw lobsters ($124\pm89\ \mu g\ 100\ g^{-1}$) (N=20). Toxins could not be detected in juices trapped in the body cavity of cooked animals. Paired analysis of PSP levels in hepatopancreas cooked *in vitro* for 5, 10 and 15 minutes revealed no differences between cooked and raw ($p>0.05$), thus suggesting that PSP is lost to the cooking bouillon during boiling of whole lobsters. PSP in hepatopancreas of lobsters maintained in an aquarium showed no appreciable change after two weeks but was significantly lower than initially ($p<0.05$) after one month. The depuration study is ongoing. PSP in hepatopancreas of lobster from the Nova Scotia side of the Bay of Fundy peaked during the third week of June (mean = $100\ \mu g\ 100\ g^{-1}$ hepatopancreas) but declined to $<40\ \mu g\ 100\ g^{-1}$ within two weeks and remained there until the close of the lobster season at the end of July. Results from this limited number of samples indicate that consumption of lobsters from the Scotia-Fundy Region does not present a PSP risk to consumers but that more intensive monitoring should commence at the beginning of the 1991 lobster seasons.

THE EFFECT OF MARINE, MICROALGAL EXTRACTS ON THE SALMON SMOLT ELECTROCARDIOGRAM

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Smolts of *Salmo salar* L. recently acclimated to seawater were prepared for open heart recording of the electrocardiogram (ECG). Typical ECG responses were obtained with 1 mL aliquots of reference solutions placed directly on the heart producing bradycardia with acetylcholine chloride and tachycardia with atropine sulfate. ECG responses of 3-5 smolts were used to test the effect of marine microalgal extracts obtained by sonicating cells from unialgal cultures or from plankton net hauls. Cultured microalgae tested included: *Nitzschia pungens* forma *multiseries* from Prince Edward Island and *N. pseudodelicatissima* from the Bay of Fundy. Both caused tachycardia in salmon smolts with the threshold level occurring at different microalgal cell densities but at a similar domoic acid concentration of 1.75 ng mL^{-1} estimated from published data. A culture of *Alexandrium fundyense* caused marked bradycardia at all concentrations tested down to a 15-fold dilution. Plankton net haul extracts tested consisted of predominantly one microalgae but also other species. Two net haul extracts from the Bay of Fundy composed chiefly of *Scrippsiella trochoidea* and *Thalassiosira gravida* produced no ECG effects. An extract consisting chiefly of *Chaetoceros debilis* caused bradycardia after an initial delay in this response.

These preliminary results suggest the utility of the salmon smolt ECG bioassay in rapidly screening microalgae for effects on farmed salmon. Drawbacks of this bioassay are that it does not assess the availability of microalgal toxins as exocrines from living cells or assess the rate of uptake across the gills in salmon. Microalgae tested, with the exception of *N. pungens*, were selected because of their seasonal dominance in the Bay of Fundy salmonid mariculture area. Two species known to contain neurotoxins: *N. pseudodelicatissima* and *A. fundyenese* were identified. In addition, a microalga not previously recognized as harmful, *Chaetoceros debilis*, caused bradycardia and other responses similar to *A. fundyenese*, suggesting that further chemical and biological investigations of this microalga should be undertaken.

THE EFFECTS OF DOMOIC ACID AND *NITZSCHIA PUNGENS* ON ZOOPLANKTON: TOXICITY, FEEDING AND UPTAKE

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Little is known about the effects of domoic acid on marine organisms. The zooplankton have been chosen for study because of their key position in the marine food chain. Domoic acid was found to be moderately toxic to the copepods *Pseudocalanus acuspes* and *Temora longicornis* eliciting 72-h LC50 values of 37.5 and 135.3 $\mu g\ mL^{-1}$ respectively. These concentrations did not induce any mortality in the larger pelagic copepod *Calanus glacialis*. In feeding experiments both *N. pungens* f. *multiseries* and *N. pungens* f. *pungens* were accepted as food by *C. glacialis* and *T. longicornis*. No mortalities were noted during the course of the feeding experiments. The hypothesis that domoic acid may function as an antifeedant is discussed.

T. longicorinis was found to quickly accumulate domoic acid when fed *N. pungens* f. *multiseries*. Body burdens reached 6.63 ng $copepod^{-1}$ following five hours exposure to a cell concentration of 7,000 cells mL^{-1}. Interestingly, this burden did not significantly decline after holding previously exposed animals for five hours in filtered seawater. The copepods are therefore exposed to domoic acid through their diet and can function as a vector. An outline of current research is provided.

DISCUSSION GROUP I - LABORATORY STUDIES

J.L.C. Wright, Chair **K. Haya, Rapporteur**

Introduction

The main objective of this discussion group was to discuss "what has been done?" and "where are we going?" with laboratory-oriented research on marine toxins, particularly in Atlantic Canada.

The discussion was stimulating and vigorous with active participation by all those who attended this session. The topics were diverse, with several recurring concerns. In this report, they have been rearranged and summarized under: a) chemistry; b) toxicity assays; and c) culture of toxin-producing algae. Therefore, the following is not necessarily in the order or context of the workshop.

It was generally agreed that the discussion would emphasize, but not be limited to, Amnesic Shellfish Poisoning (ASP), Paralytic Shellfish Poisoning (PSP) and Diarrhetic Shellfish Poisoning (DSP). A knowledge of all toxin-producing algae would help in the identification of new phytotoxins and aid in the understanding of algae of concern. It was also noted that the intensification of aquaculture may lead to changes in the environment, such as increased nutrients and other factors initiating blooms of harmful microorganisms or algae which previously had been observed in significantly lower concentrations.

Chemistry

Certified mussel tissue reference material (MUS-1) and instrumental calibration solution (DACS-1) for domoic acid are available from the Institute for Marine Biosciences (IMB), National Research Council, Halifax. Four PSP toxins (saxitoxin, neosaxitoxin and a GTX 2 and 3 mixture) have been isolated by IMB. The preparation of certified instrumental standards and of a PSP scallop tissue reference material is currently in progress. Also at IMB, large scale cultures of *Prorocentrum concavum* have been used as a source of okadaic acid. This is being used to develop a reference material. This material is also commercially available from Diagnostic Chemicals Ltd., Charlottetown, PEI. IMB has received mussels contaminated with DSP toxins and a feasibility study on the use of these mussels as reference material is in progress.

Some concern was expressed over the lack of correlation between the PSP standards obtained from Food and Drug Directorate (FDA), USA, and those obtained from Japan. It was stressed that only certified standards be used when accuracy and precision of the results are important. Requirements may be less stringent for comparative profiles within an intra-laboratory study. It was also noted that the FDA standard is not certified.

Most participants acknowledged that certified instrumental calibration standards and reference material for PSP and DSP toxins are in critical need. However, it was stressed that the separation, purification, and characterization of new toxins and their derivatives requires a large amount of biomass, time, and financial commitment. At least 15 mg of a chemical are required for unequivocal identification, and much more material is required for the preparation of certified standards. Application of monoclonal antibody-affinity chromatography technology in obtaining large quantities of a chemical may be useful in the preparation

of standards. The development of more sensitive toxicity tests would be helpful in the identification of new toxins.

It was recommended that monitoring for all known toxins should eventually use chemical techniques and that future developments in analytical methodology should strive to increase selectivity rather than sensitivity. IMB is currently examining the feasibility of a single extract, single instrument method for the simultaneous quantitative determination of PSP, DSP, and ASP toxins. This method is based on the use of the liquid chromatograph - ion spray mass spectrometer (SCIEX). It was suggested that DFO initiate a cost-effectiveness study on the use of one instrument to meet the regional monitoring requirements. One advantage would be the unequivocal confirmation of the toxin by mass spectrometry. It is possible that the enforcement of closures due to phycotoxins will require structural confirmation by mass spectrometry in the future.

Toxicity Assays

The general consensus was that, for the moment, the mouse bioassay cannot be replaced by any of the currently available tests which do not use live animals. However, it was appreciated that the mouse bioassay has its limitation as well. For example, ASP toxins are not detected by the currently employed mouse bioassay test in shellfish monitoring programs.

It was suggested that a protocol for responding to emergency situations or perceived problems be developed. Such an initiative, although essential, was nevertheless considered to be a reactive approach, and it was acknowledged that more effort is needed to develop proactive approaches. For example, all prevalent algal species in an area could be cultured and the cell extracts screened with a series of bioassays (specific and non-specific). The limitations to this approach are that toxin-producing organisms may not produce toxins in culture; it would require considerable effort and resources, and some algae are not easily cultured (for example, *Dinophysis*). Furthermore, extrapolation of bioassays to human toxicity may be difficult and new toxins with unknown mechanisms of action may not be detected. Although after the fact, epidemiological studies are very important because for some toxins the only real indicator may be toxic responses by humans. Despite these constraints, it was acknowledged that a carefully planned and selective approach could result in the discovery of hitherto unknown toxic algal species.

Other tests with potential for use in monitoring phycotoxins are: a) the *in vitro* cytotoxicity test with hepatocytes currently in use in Scandinavian countries for DSP; b) cell cultures of mouse neuroblasts for the assay of saxitoxin; c) synthetic membranes to detect sodium channel blockers (PSP toxins); d) immunological methods, for example, both enzyme linked immunosorbant assay (ELISA) and radioimmunoassay for the detection of domoic acid, and Armand-Frappier Institute's kit for saxitoxin. In the latter case, an inter-laboratory evaluation of the saxitoxin kit by DFO, Inspection Branch was not favourable. However, it was felt that a better presentation of the kit and more thorough training of the personnel would have resulted in a more positive review. IMB has purified sufficient neosaxitoxin to proceed with development of an immunological based test kit.

Studies by IMB indicate that the UBE Industries, Japan, test kit for DSP works well for the most common DSP toxin, okadaic acid, between 10-100 ng mL^{-1} solution (equivalent to 100 ng g^{-1} of shellfish homogenate). However, the kit is less sensitive to dinophysistoxin 1 (DTX-1). Thus, the levels of DSP toxins would be under-estimated in those contaminated with mainly DTX-1 (such as the recent DSP incident at Mahone Bay, NS). However, the kit can be used as long as one is aware of this limitation.

There was a consensus that field test kits, which can indicate phycotoxin concentrations below regulatory levels, are needed. These test kits should be simple, specific, sensitive and quick. A model example is the test kit which is available for ciguatoxin.

Culture of toxin-producing algae

Some of the reasons for culturing algae are:

- Identification of toxin-producing organisms,
- Provide sufficient amounts of biomass for the structural identification of new and known toxins, large scale production for the preparation of toxin standards and development of analytical methods, and sufficient amounts for toxicological testing (including uptake and depuration),
- Study physiological mechanisms of toxin production,
- Determine requirements for optimum growth and toxin production,
- Learn the basic biology of the algae under laboratory conditions to aid in the understanding of the biology of the organism in the field.

It is critical that unialgal, axenic cultures be produced for:

- The determination of the biosynthetic routes of toxins and the factors controlling biosynthesis.
- Unequivocal proof of the organism producing the toxin. It is especially important to answer the questions surrounding the involvement of bacteria in the production of PSP toxins and domoic acid.

Some other information items of relevance that were presented were:

- While PSP toxins have been produced by unibacterial cultures, studies at the Atlantic Veterinary College, PEI, have found no evidence of endosymbiotic bacteria in *Nitzschia pungens* f. *multiseries*.

- Studies with what appears to be axenic cultures of *Nitzschia pungens* f. *multiseries* are in progress at DFO, Moncton.

- Dr. Greta Fryxell, Texas A&M University, has had better success in culturing algae from the field when they were obtained during non-bloom rather than bloom conditions.

- Dr. Bob Anderson, Bigelow Laboratory for Ocean Sciences, Boothbay Harbor, Maine, apparently has a *Dinophysis* spp. in culture.

- One of the problems encountered by those wanting to make large batch or continuous cultures of algae is that the specialized equipment required is often not available. Workers are frequently using make-shift apparatus. It was suggested that some of the methods and technology currently employed in the pharmaceutical industry may be adaptable to phycotoxin production (e.g. fermentors).

Miscellaneous

A question was raised whether toxin-producing organisms could be identified using gene mapping techniques. For example, could common genomes be identified for enzymes responsible for the biosynthesis of toxins. While this appears to be a novel approach, this was not an area of expertise for most of the participants and the question could not be answered.

Another concern raised was the possibility that viruses may be able to induce toxin production in algae and that this may be contributing to the spread of phytotoxin incidences globally.

While it was recognized that research on transport of toxins through the marine food web to higher trophic levels and on the mechanisms of toxin production (biosynthesis) are important areas of study, there was insufficient time to discuss these topics during this workshop.

DISCUSSION GROUP 2 - FIELD STUDIES

A. Cembella, Chair **W. Watson-Wright, Rapporteur**

Oceanography of Atlantic estuaries and coastal inlets

Clive Mason and Gary Bugden of the Physical/Chemical Sciences Branch of DFO, Scotia-Fundy Region, gave an overview of coastal physical oceanographic programs that interact with the phytoplankton monitoring program. An inlet classification study is presently putting together a database concerning the dynamics of 71 inlets in the Region with a view to generalizing to others which may possess similar physical characteristics. The database for the existing program will be finished next year but the classification system could be expanded to include other Regions in the Atlantic Zone. An ultimate goal of the physical oceanographic studies is to correlate inshore/offshore events with meteorological events and to build interactions between physcial/chemical oceanographers and biologists. Knowledge of the physics is important in designing the details of a biological sampling program (e.g. depth and temporal scales). Plans are also underway to study particle dynamics and how flocculation processes affect blooms.

Dave Scarratt then raised two questions: a) can we respond to events that are not captured by the oceanographic program (e.g. *Dinophysis* bloom in Mahone Bay) and b) are there any opportunities for an ongoing, collaborative role for the shellfish industry? Peter Darnell (Indian Point Marine Farms) commented that government-generated information and programs of this type are of benefit to aquaculturists but that aquaculturists can also be of benefit to scientists (e.g. providing boats and sampling platforms, making daily observations, etc.). There was much discussion on the need for increased continuing collaboration but that in many cases 'ad hoc' arrangements appear to work better than formal ones.

Phytoplankton monitoring program

Primary discussion centred on the different approaches and objectives of DFO Science and Inspection. Two primary viewpoints emerged. In general, the Science Branch has the primary objective of collecting extensive data on a few stations in order to ultimately use that to establish basic mechanisms and enable prediction. Turnaround time is not critical. The Inspection Services Branch, on the other hand, wants limited data on a large number of sites. Its primary goal is to enable early warning of impending 'blooms' and potential toxicity in order to cut losses to industry. Rapidity of results and reporting of same are of extreme importance.

The comment was made that there appears to be a lack of communication among branches in DFO concerning these differences. The need for increased consultation between Science and Inspection was recognized. However, recognition must be given to the collaboration which has existed for years in southern New Brunswick, the Gulf Region and the Quebec Region between Science and Inspection, to the extensive monitoring which has been carried out, and to the marriage of the shellfish toxin data to the 'bloom' data which has taken place. It was felt that since the original phytoplankton monitoring program is approaching the end of its first three years, there is a great need to reassess how it should continue and perhaps be modified. When the program is examined, there will be a need to consult Inspection, other Regions, and other branches.

Other concerns expressed included:

- If growers participate in phytoplankton monitoring, there will need to be very defined steps as to what actions should be taken if potentially toxic algae are found.

- There is an immediate need for Health and Welfare to develop a tolerance level for DSP.

- Regardless of how programs proceed, there is a need for high quality data. We must consider whether high coverage means low quality and whether low coverage necessarily means high quality.

- Since much data have been gathered thus far from the program, it is important at this stage to put it together to assist in planning further field work. Science may wish to continue some part of the program or may wish to transfer it to Inspection.

Field program planning

It was stressed that there is a need to be more flexible in work plans and to be able to respond to 'events' such as toxic blooms. A rapid deployment plan for taking advantage of blooms was suggested (i.e. ability to acquire a vessel). It was felt that upper management may need to be convinced of the importance of these blooms before such a suggestion could be taken seriously.

A number of field-oriented research programs currently underway were briefly reviewed. Of particular note are the multi-disciplinary studies underway in the L'Etang Inlet of southern New Brunswick which are examining the environmental effects of salmon aquaculture. The comment was made that although observation is important, there is a need to continue from there and to perform experiments in the field as well as in the laboratory.

Data management

The consensus was that each research group should proceed in the most efficient way and would be responsible for its own database, analysis and publication. Those present felt it was not essential that all groups use the same database structure. However, it was recognized that all groups must be prepared to share their data and that in time it might be necessary to develop a zonal database for the overall phytoplankton monitoring program.

It was recommended that someone be charged with creating an index of available data related to toxic algae, including its nature, format, location and contact person.

Causes, prediction and effects of toxic blooms

Allan Cembella offered some ideas on what has advanced the state of our knowledge about toxic algal blooms thus far which served as a basis for discussion.

- "Seed-bed" hypothesis

This hypothesis, proposed by Prakash and modified by Steidinger, states that benthic cysts can act as reservoirs of toxicity. Therefore, a baseline survey of sediments may be useful. There was discussion as to whether this would apply only to cyst-forming species. It appeared to be generally felt that resting stages seem important for blooms, not necessarily toxic ones, and therefore that gathering this type of data could indeed be useful.

The possibility was raised that cysts may move with shellfish when the shellstock is moved from one site to another and that their subsequent release could be an important factor in extending the range of potentially harmful phytoplankton species.

Information was offered to suggest that the concept of a "global spread" of algal blooms may in fact be overstated. According to some, we are presently in a phase of recession regarding *Alexandrium* blooms in the Gaspé. Evidence apparently exists that the peak was 11,000 years ago following the last glacier age. Similarly, *Gymnodinium* was apparently found much further north in Europe 14,000 years ago.

- Water column stability/stratification

Do hydrodynamics control bloom dynamics? It appeared to be generally agreed that information on the water column is important for monitoring programs since it should assist in deciding when to sample and how often. Clive Mason commented that it is feasible to consider expanding the Scotia-Fundy physical and chemical measurements to other Regions but that it must be done in a proactive fashion. The system should be set up early and not be expected to be instituted in response to a crisis. In addition he emphasized the need for a process-oriented approach in one location where there would be a good signal. Extrapolation of physical and chemical data from one inlet to another should be possible.

- Nutrient Dynamics

Eutrophication can also be responsible for blooms. Stephanie Guildford commented that because of the concern over the impacts of toxic blooms on aquaculture there is a need to concentrate research programs on present and potential aquaculture sites. However, the comment was also made that we are equally concerned about the impacts on wild stocks (e.g. soft-shelled clams, scallops, lobster, etc.).

- Allelopathy

The deleterious effect of one species upon another was mentioned but lack of time precluded discussion of this topic.

The discussion group ended on a somewhat positive note with the feeling that somehow, someday, we will be able to model and predict toxic blooms. Aivars Stasko commented that he hoped we were being optimistic with cause. The final item raised was the possibility of holding a practical workshop in which industry, Inspection, Science, and the province(s) would participate. The purpose of such a workshop would be to acquaint industry with the importance of phytoplankton monitoring and to transfer the knowledge necessary in order for them to actively participate in such a program. Peter Darnell suggested that he go back to his colleagues in industry and that industry put forth a formal request for such a workshop outlining the particulars of their needs.

Summary of Recommendations

- Upon receipt of a request from industry, sponsor a workshop with the industry with a view to transferring knowledge and techniques about toxic phytoplankton and how to monitor them.

- Support the expansion of the classification study of bays and estuaries by the Physical/Chemical Sciences Branch.

- Continue phytoplankton monitoring but recognize that Science and Inspection have different needs and objectives and that the two branches and industry will benefit from ongoing collaboration and sharing of knowledge and techniques.

- Create an index of available data on toxic algae along with the locations of such data.

- Suggest investigation of the possibility of transfer of toxic algal cysts and/or motile populations with shellfish transfers.

REVIEWS OF FEDERAL AGENCY RESEARCH PROGRAMS

A. Health and Welfare (Bureau of Chemical Safety) Research Program on Marine Toxins

James F. Lawrence
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Health Protection Branch
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Health and Welfare Canada is currently carrying out research programs in several areas of marine toxins. These include both analytical and toxicological studies.

Paralytic Shellfish Toxins

A simplified liquid chromatographic (LC) method has been developed which involves pre-chromatographic oxidation of individual PSP's to fluorescent products which are then separated by LC. The method is about 10 times more sensitive than the post-column oxidation method. However, all gonyautoxins elute as a single peak, making their individual quantitation difficult. The method has been applied to a number of shellfish types without difficulty. Work is continuing to compare the technique to the post-column method and the mouse bioassay.

Domoic Acid

A confirmation technique for domoic acid in mussels is presently being developed. The method is simple and uses the same LC equipment as the direct analysis. Additional sample cleanup is not required to confirm positive findings above 5 $\mu g\ g^{-1}$ domoic acid in a variety of shellfish tissue. The confirmation involves reaction of domoic acid with butyl isothiocyanate to form a thiourea derivative which does not increase greatly the UV absorption of the molecule but does cause a retention time shift which is useful for confirming the presence of domoic acid in shellfish extracts. Recoveries, reproducibility, detection limit, etc., are being evaluated.

Two immunoassay techniques (ELISA and RIA) have been developed for the determination of domoic acid at levels of less than 1 $\mu g\ g^{-1}$ in biological samples for toxicological studies. Current ongoing research will evaluate these methods for routine monitoring of shellfish at levels greater than 1 $\mu g\ g^{-1}$ domoic acid with a comparison to the LC method.

Several toxicological studies are underway or planned to generate more information on the toxicity and behavioral effects of domoic acid on various animal species. These will hopefully lead to a more accurate regulatory guideline for domoic acid in shellfish.

Diarrhetic Shellfish Poisons

Method development for okadaic acid will start in the near future. Standards of okadaic acid and reagents have been ordered. The Bureau of Chemical Safety is currently evaluating existing toxicological data on DSP in order to establish a regulatory guideline for DSP levels in shellfish.

B. National Research Council of Canada Shellfish Toxin Research

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Much of the research on shellfish toxins has been reviewed in detail quite recently (Canadian Chemical News, May issue, pp. 18-27, 1990). The general thrusts and more recent developments are outlined here, and are subdivided according to toxin group.

Domoic Acid (ASP):

Some of the physiological parameters governing domoic acid production by the diatom *Nitzschia pungens* forma *multiseries* have been investigated. The optimum source of nitrogen is being studied in collaboration with Dr. Bates at DFO in Moncton, while the luxury consumption of phosphorus is being studied at the Institute for Marine Biosciences (IMB). In collaboration with Dr. Fryxell in Texas, a number of other strains of *N. pungens* are being investigated for their ability to produce domoic acid. As yet, no axenic strain of *N. pungens* has been obtained, and some attempt is being made to produce one at IMB.

Some of the physical-chemical properties of domoic acid, including the pKa's and order of protonation of domoic acid, have been established, as has the solubility of domoic acid in water and non-aqueous solvents.

Methods for the extraction, cleanup and instrumental analysis of domoic acid and related compounds in plankton and shellfish have been further refined in collaboration with Dr. Pocklington at the Bedford Institute of Oceanography in Dartmouth.

A certified instrumental calibration standard and mussel tissue reference material have been developed and are available from the NRC Marine Analytical Chemistry Standards Program.

The chemical structures of eight domoic acid isomers and related compounds, present in mussel tissue and phytoplankton, have been established. In some cases, these compounds have been synthesized in the laboratory. Detailed NMR studies have shown that these isomers have different spatial requirements than domoic acid. In collaboration with Dr. Hampson, at the University of Toronto, these compounds are being used in a structure-activity study with the kainate receptor protein.

Little is known about the effects of domoic acid upon marine organisms. In a study at IMB, zooplankton have been chosen because of their unique position in the food chain. Preliminary results show that domoic acid has acute toxicity to smaller copepods, and that domoic acid is retained by these herbivores, even hours after consuming the toxin-producing diatom.

Paralytic Shellfish Poisons (PSPs):

Large-scale purification of several PSPs has been achieved. Four toxins are currently on hand: saxitoxin, neosaxitoxin, and a mixture of GTX 2 and 3. These materials are of very high purity. Considerable NMR, IR, and MS spectral data for these compounds have been accumulated. Sources for the remaining PSPs of importance are being investigated. The HPLC-fluorescence system for monitoring PSPs has been developed to operate routinely and has been automated to maintain a high throughput. Using the high-grade neosaxitoxin produced at IMB, a new HPLC response factor has been developed for this system.

Developments continue in the analysis of PSP toxins by combined liquid chromatography and ionspray mass spectrometry (LC-MS). Some progress has been made in finding a suitable mobile phase that reduces suppression effects and does not result in interferring peaks in the mass spectrum. All the PSPs examined to date display interpretable spectral data, and tandem mass-spectrometry (MS-MS) of these toxins appears to be useful in distinguishing stereoisomers.

Another promising technique for the separation and analysis of PSPs is capillary electrophoresis (CE). This approach, using UV detection, permits the quantification of underivatized toxins. Using a CE-MS interface, mass spectrometric detection of the toxins following electrophoretic separation is also being investigated.

A feasibility study for the preparation of a PSP scallop tissue reference material is underway, and another for the preparation of selected PSP standards has just begun.

Diarrhetic Shellfish Poisons (DSPs):

Large-scale laboratory cultures of the tropical dinoflagellate *Prorocentrum concavum* have been used as a source of okadaic acid (OKA), the parent DSP toxin. Substantial amounts of very pure material have been prepared. This material is commercially available through Diagnostic Chemicals Ltd., Charlottetown, PEI. Two other new diol esters of OKA have also been isolated and characterized. Dinoflagellate sources of other DSP toxins are being investigated.

In collaboration with scientists at Fenwick Laboratories in Halifax, methods for the chemical analysis of DSPs are under active investigation. The reported fluorescent tagging method using the ADAM reagent has been made to work reliably, and can be used to monitor for OKA and the related DTX-1 in phytoplankton and shellfish. Unfortunately, the method results in many interfering peaks in the final chromatogram, but this has been improved by a simple cleanup step. Other tagging reagents are also being screened, and several commercially-available reagents hold some promise.

An instrumental analytical method, using combined LC-MS, has been found to be very effective in detecting the known DSPs in a variety of matrices. For example, it has been useful in alerting IMB researchers to the presence of other new DSPs present in laboratory cultures of dinoflagellates. At present, this is still the front-line analytical method at IMB for detecting DSP toxins.

In addition, the LC-MS method has been used to evaluate the antibody-based ELISA assay kit for DSP toxins produced by UBE Industries, Japan. Preliminary results indicate good correlation with the LC-MS data when only OKA is present, but the kit does not respond equally to DTX-1. However, using DTX-1 standards, it is possible to obtain a good correlation with the LC-MS data. Thus, it appears imperative to know if DTX-1 is present in the sample being investigated.

Many or all of these approaches were used recently to assist DFO Inspection Services Branch in Halifax during an outbreak of DSP poisoning in the Mahone Bay area. The culprit toxin was shown to be DTX-1, and the producing organism is believed to be *Dinophysis norvegica*. This is the first fully-documented incident of DSP poisoning in North America.

Currently, studies are underway to develop an OKA instrumental calibration standard, as well as a mussel tissue reference material.

C. Projects Operated by the Department of Fisheries and Oceans on Harmful Marine Algae

Jean Worms
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Science Branch, Gulf Region
Department of Fisheries and Oceans
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Moncton, N.B. E1C 9B6

Under the umbrella of the DFO Marine Toxins Program, a variety of research projects are operated mainly in the Quebec, Gulf, and Scotia-Fundy Regions of DFO. The following provides a quick review of the main research themes with indication of the target phycotoxin(s). In most cases, each theme will be the object of several projects. The Phycotoxins Working Group of DFO is in charge of coordinating regional planning to ensure that no undue duplication or obvious lacuna may subsist.

Chemical Methodology

- Development/improvement of detection and quantification methods (PSP, DSP, ASP)

Phytoplankton Dynamics

- Monitoring of phytoplankton populations in space and time (PSP, DSP, ASP)
- Causal relationships between toxic blooms and environmental factors (PSP, DSP, ASP)
- Design and testing of predictive models (ASP)

Molluscan Physiology

- Uptake and depuration mechanisms (PSP, DSP, ASP)
- Selective grazing by the blue mussel (PSP)

Biology and Biochemistry of Toxin Production

- Factors influencing growth and production of toxins (DSP, ASP)
- Bioactive metabolites and exudates (PSP)
- Genetic variability of *Alexandrium* (PSP)
- Development of axenic cultures (ASP)

Aquatic Toxicology

- Toxicity of phycotoxins to zooplankton, fish, and fish larvae (PSP, ASP)

Fate of Phycotoxins in the Environment

- Biodegradation of domoic acid (ASP)

RECOMMENDATIONS FOR FUTURE RESEARCH

A. Invited Response

Peter Darnell
Indian Point Marine Farms Ltd.
Mahone Bay, Nova Scotia

All shellfish aquaculturists should be involved in phytoplankton monitoring at their own sites, whether this is carried out by the growers themselves, DFO Inspection, or by a contractor. A phytotoxin workshop should be held for shellfish growers with a view towards better understanding of the problems and the importance and methodology of phytoplankton monitoring. A clear course of action to be followed by growers when possibly toxic algae are identified should be delineated. In addition, when phytotoxins are detected, an "emergency response team" should proceed to the problem area and do more indepth sampling in order to get rapid feedback to the grower.

The technology to identify DSP must be transferred into DFO's Inspection laboratories. Since DSP is now documented in Atlantic Canada, DFO must routinely test for it. Research must be carried out to understand why *Dinophysis* is sometimes toxic and sometimes not. National Health and Welfare must deal with the DSP issue and set regulatory limits, and DFO Inspection must then assume its responsibilities in enforcing these limits. Further research is required into the implications of shellstock movement and spreading of phycotoxin blooms by cyst movement or other mechanisms.

James F. Lawrence
National Health and Welfare
Ottawa, Ontario

It is recommended that a high priority be placed on the preparation of pure analytical standards of marine toxins in sufficient quantity to be available for use in routine monitoring programs. Without pure standards analytical methods research, chemical monitoring, and toxicology studies will be hampered.

It is also recommended that the preparation of certified reference materials, representative of different types of shellfish from different geographical locations containing various levels of marine toxins (PSP, DSP, or domoic acid, for example) be carried out. These might serve as secondary analytical standards in the absence of pure reference standards.

National Health and Welfare strongly supports the current research efforts of the Institute for Marine Biosciences, NRC, in preparing pure analytical standards and reference materials. This work should continue to receive high priority and support from the various government departments involved in marine toxin work.

Jeffrey L.C. Wright
National Research Council
Halifax, Nova Scotia

These recommendations are arranged according to the three toxin groups that are currently of importance to the eastern Canadian fishery, and there are some general recommendations at the end. Some of these recommendations are of particular relevance to NRC, but have a direct impact upon other agencies including DFO and NHW.

Domoic Acid (ASP Toxin):

Most of the work concerning the chemical analysis of this toxin has been completed. A calibration standard and reference material are available through the NRC Marine Analytical Chemistry Standards Program (MACSP).

There is still an important requirement for domoic acid in order to complete all the pharmacological testing that needs to be done. This is vital to verify the current tolerance level. Partially-purified material exists at DFO that, if processed, could yield several grams of the toxin. It is important to establish how much domoic acid is required by NHW and also to assess how much is required by DFO scientists for their experiments. Agreement should then be reached on how and who should complete the purification.

In the same vein, NHW has expressed an urgent requirement for radio-labelled domoic acid. This material is essential for studies of mammalian toxicity and metabolism of domoic acid. The currently available material is labelled non-specifically and is of poor quality. Appropriately labelled material would be useful to a number of research groups involved in studies of domoic acid action and metabolism. IMB is currently considering the feasibility of an approach to produce this material.

It appears that an axenic strain of *Nitzschia pungens* f. *multiseries* may have been obtained although this still requires some further confirmation. This is a key point in our understanding of domoic acid production, and it is recommended that current efforts by DFO and IMB to obtain and examine axenic cultures be maintained or augmented. Complementary work to examine the role (if any) of associated microorganisms should also be supported.

Three years after the event, we are still not certain if ASP is an eastern Canadian and northeastern United States problem only. Despite anecdotal information that blooms of *Nitzschia* have occurred in other parts of the world, we have no confirmed information as to the species involved and whether any toxin is produced. It is recommended that an individual in DFO be given the task of assimilating and coordinating this global information and that this individual be empowered to offer analytical services to these areas or countries that at present cannot analyze for domoic acid.

Okadaic Acid and DTX-1 (DSP Toxins):

In recent months, IMB and Fenwick Laboratories have made significant progress in the chemical and instrumental detection and analysis of the two principal toxins of the DSP group. Using *Prorocentrum* spp. as a source of the toxin, many milligrams of okadaic acid have been isolated and purified. Using this material, existing and new chemical analytical methods are being investigated. One new instrumental method (LC-ionspray MS) has been developed. In view of the recent crisis in Nova Scotia, it is important to achieve a number of important short-term goals. IMB and Fenwick Laboratories are developing reliable

chemical analytical methods for the detection and analysis of DSP toxins, and these will be introduced into DFO Inspection laboratories as soon as possible.

Following on this, there will be an immediate need for calibration standards of okadaic acid and DTX-1. IMB is proceeding with this on an urgent basis and a certified calibration standard for okadaic acid may be available within 6-9 months.

Although several species of dinoflagellates associated with DSP toxins have been found in the Maritimes, they do not (thankfully) always appear to produce toxins. The reasons for this are unknown. It is recommended that IMB and DFO collaborate in a significant biological and chemical effort to begin to understand this phenomenon and the possible factors controlling toxin production. This would involve both field and laboratory studies. It is also recommended that DFO determine the distribution of these DSP-producing dinoflagellates throughout the Maritimes.

Saxitoxin and Derivatives (PSP Toxins):

These toxins and the microalgae that produce them are the best understood of the three groups of toxins considered here. Nevertheless, they are the most difficult to accurately quantify and analyze by chemical means. To date, they are detected using the standard mouse bioassay, though this gives no information on the profile of the toxins, and is not as sensitive as certain chemical assays. Unfortunately, these chemical assays are particularly difficult to perform and have an absolute requirement for standards.

In order for existing analytical methods to be refined and new ones to be developed, it is essential that a number of PSP standards be made available. IMB is proceeding as quickly as possible and one or two certified standards may be available within the next 12 months.

IMB is also investigating other instrumental analytical procedures utilizing ionspray MS and CZE.

General Recommendations:

There are a number of recommendations that can be made which are generic to any toxin group or problem.

It may be useful to hold an annual workshop on analytical methods (chemical and biological) currently in use, under development, planned, etc., for shellfish toxins. This workshop would be primarily for all analytical and regulatory labs and would provide workers with an opportunity to discuss their day-to-day problems, solutions and long-term goals.

There is a unanimous opinion that a rapid and economical test should be developed for each one of the toxin groups. Such a test would be an initial front-line procedure with very general specificity and would be used to highlight only those samples that require further investigation. It is recommended that work commence to develop such an economical test using pure toxins already isolated.

The mechanism of uptake, storage, and elimination or depuration of toxins in shellfish is of special importance. As yet, little is known about the dynamics of this phenomenon and the factors controlling it. It is recommended that DFO support well-defined, achievable goals to investigate this process.

It is also recommended that DFO and IMB give careful consideration to a long-term plan to discover new toxins present in microalgae that are found in the Maritime region. The selection of algae for this research effort would be based upon a number of factors, and would be guided by the presence of biological activity (toxicity). Such a chemical effort would complement the results of the phytoplankton monitoring program.

It is also strongly recommended that DFO establish a "hot-line" for reporting the occurrence of new, potentially toxic blooms. The discovery of such a bloom would be reported to each agency or department. A person from each agency or department would be designated to receive this information and pass it on to colleagues.

Irené Novaczek
Atlantic Veterinary College
Charlottetown, Prince Edward Island

Firstly, I think it bears repeating that although the phytoplankton monitoring program may be tedious and time consuming, it is an essential component of the ongoing research on phytotoxins. Baseline floristic data on areas not yet affected by toxins as well as the systematic tracking of toxic blooms are most valuable and every effort should be made to collate and distribute the data from this program. The monitoring net should not be allowed to shrivel from lack of funds or interest while our energies are diverted by more exciting, short-term projects.

Secondly, I am happy to see that a small number of molecular geneticists have joined our ranks. I hope that in future, geneticists apply their newly-developed technology on a broad range of topics relevant to the phytotoxin problem - from the minutiae of biosynthetic pathways to global biogeography of toxic algae.

Thirdly, I must emphasize that a great deal remains to be learned about the animals in the food webs arising from toxic microorganisms. We need to know more about the fate of marine toxins - how they are taken up, to what degree they are retained and passed on to predators, and the mechanisms and time scales of depuration. We cannot rely on research conducted in other parts of the world but must investigate the responses of our local hydrography of the Gulf of St. Lawrence. We are dealing with a unique blend of Arctic, temperate and warm temperate species which, because of their varying degrees of historical genetic isolation, may act differently from similar organisms in other regions. I hope that our scientific efforts will help us understand the fate not only of natural marine toxins, but also of man-made toxins in the marine environment.

Mike Gilgan
Department of Fisheries and Oceans
Halifax, Nova Scotia

The topics listed below are of interest to Inspection Branch since they would assist in the ready identification of phytoplankton and in the detection and measurement of toxins. The interest should not be taken to mean that financial support would necessarily be available.

Bioassays

a) Immunologic

- For the identification of phytoplankton
- Immunoassays for toxins (such as mixed monoclonal antibodies, mixed in inverse proportion to the toxicity of analytes, e.g. PSP toxins).

b) Other bioassay test animals or procedures

- To avoid over-dependence on the mouse bioassay
- To improve sensitivity and specificity

c) Evaluation of toxicity of known species of phytoplankton from local waters

- Evaluations should be done on pure cultures of organisms
- The final evaluation of the toxins should be done with a mammalian bioassay to avoid artifactual toxins detected by special assays (such as phosphatase) but which are not toxic under ordinary circumstances
- Perhaps the evaluations should include organisms other than the usual, such as picoplankton and bacteria

Chemical Assays

a) Standards and reference materials for DSP and PSP toxins

- The standards are crucial since no serious chemical analyses can be done without them
- The reference materials are highly desirable

b) Improved method for DSPs in shellfish

- These are needed for the whole group (DTXs, okadaic acid, PTXs and yessotoxins)

c) Confirmation procedures for all toxins which can be done by an ordinary analytical laboratory without heroic efforts in time and technique

d) A "library" or repository of standards for most of the known marine toxins should be established at one of the institutions, such as the IMB of NRC, which is accessible by scientists and analysts concerned with shellfish toxins

- To reduce the reaction time when a "new" toxin is encountered
- Particularly for toxins which are a perceived threat to aquaculture in temperate waters (e.g., ciguatera toxin(s), brevetoxins)

e) Rapid PSP chemical analysis procedures and confirmation

- Procedures which might be substituted for animal bioassay and which could be automated

f) Rapid, simple, and portable detection methods for all toxins

- Such as is being developed for the detection of domoic acid by pyrolysis

3. Establishment of a Shellfish Toxin Occurrence Notification Centre

- An individual or group which would be notified in the event of a serious alert and which would be responsible for alerting other people concerned with such events
- The people directly involved with handling the problem are frequently too busy to spend a lot of time contacting indirectly-concerned people or agencies

Jean Worms
Department of Fisheries and Oceans
Moncton, New Brunswick

First, it is worth mentioning that, since the 1987 "mussel crisis" the momentum has been maintained at its original level and research on issues of concern to all of us is progressing in a satisfactory manner while the monitoring program operated by DFO Inspection effectively protects both consumers and the shellfish industry. However, from the presentations and discussions heard yesterday and today, it appears that some areas deserve more attention.

Most participants stressed the necessity of being proactive. This obviously necessitates a tight regulatory-oriented monitoring program supported by active research. As new results come from research projects, monitoring programs need to be fine-tuned. It also requires more emphasis to be placed on process-oriented studies including trend analysis and ecological modelling in order to better our predictive capabilities.

The efficiency of research programs depends in part on the quality and quantity of information flowing between the various disciplines involved.

From the detection/quantification viewpoint, the most critical aspect remains the availability of purified standards and reference material.

The development of immunological methods has shown promise but is far from having reached an adequate level of effectiveness. Also, likely problems with the use of mammalian bioassays renders the need for an alternative general screening assay to be more critical than ever.

Aside from these general comments, I would like to mention some more specific points which deserve additional attention:

- Culture of *Dinophysis* and research on DSPs.
- Kinetics of domoic acid production by axenic cultures of *Nitzschia pungens* forma *multiseries* and other domoic acid producers and the possible role of bacteria.
- Biosynthetic pathways of domoic acid and other phycotoxins.
- Adaptability of NSP (neurotoxic shellfish poisoning) producing organisms to different environmental conditions.

Catherine T. Enright
Nova Scotia Department of Fisheries
Halifax, Nova Scotia

I specifically asked the Workshop Chair to allow me to be the final panel member speaker because, in many ways, the Nova Scotia Department of Fisheries is one of the major end users, charged with the responsibility of applying the results derived from the many research, inspection and monitoring programs discussed during this workshop.

It is the mandate of the Nova Scotia Department of Fisheries to promote and facilitate sound aquaculture practises; operations that make ecological, social, and financial good sence, in both a short- and long-term perspective. The Minister of the Nova Scotia Department of Fisheries, in accordance with our Provincial Aquaculture Act and Regulations (R.S.N.S. 1989, Chapter 18) has sole legislative responsibility to grant or cancel aquaculture licenses and restrict the size and production levels of aquaculture sites in Nova Scotia.

Presently, there are 737 aquaculture sites either leased, or under review for leasing in Nova Scotia. Eighty-four percent of the applications are for shellfish farming. The aquaculture leasing and licensing decisions made by the Nova Scotia Department of Fisheries relies greatly upon the research and expertise of the aquaculture network, in addition to inhouse professional and responses from public hearings. The aquaculture network consists of ten provincial and federal departments which include all the Departments represented here today, as well as others. The Nova Scotia Department of Fisheries has limited scientific resources and, by agreement, relies on the federal research projects for the critical data upon which aquaculture decisions are based. Thus, the Nova Scotia Department of Fisheries strongly recommends continuation and expansion of present research inspection and monitoring programs to keep pace with the needs of our quickly growing aquaculture industry.

Nova Scotia Department of Fisheries funding is targeted towards development projects, for example, in the past the development of culture techniques and equipment design has been supported. Some aspects of the harmful marine algae problem can be addressed within development programs.

The Nova Scotia Department of Fisheries, Aquaculture Division, loans data recording instruments for the purposes of collecting information for site selection and expansion decisions. Furthermore, the Division attempts to work closely with each aquaculturalist in establishing development plans for their site and encourages the aquaculturalist to initiate a site monitoring protocol and be aware of any self-polluting practises. Growth and production data in conjunction with available temperature and phytoplankton information are analyzed to ensure that aquaculture farms operate below the carrying capacity level for their site.

Cooperation in regional development projects is being fostered among aquaculturalists. The Water Quality Monitoring Programme for Mussel Growers, coordinated by Michael Brylinsky, Acadia Centre for Estuarine Research, Wolfville, N.S., was the first monitoring project of its size. Unfortunately, this project was met with limited sustained industry participation, in part due to its novelty and the unfortunate interruption in aquaculturalist's cash flow, resulting from the domoic acid episode which caused many mussel growers to panic and pursue alternate income generating activities. Nevertheless, the Nova Scotia Department of Fisheries continues to increase its extension services for aquaculturalists and fosters an appreciation of the potential value of coordinated monitoring of aquaculture sites. Presently, there are two joint development projects in progress. One evaluates the use of salt marshes as nursery sites for various

shellfish species and the second is a reciprocal transplant-growth experiment, with selected native oyster populations. Both projects serve to coordinate and train as many as five aquaculture companies in monitoring protocol, data collection and the importance of shared information and expertise among researchers, government and neighbouring aquaculturalists.

The Nova Scotia Department of Fisheries recommends that coordinated development projects, which contain a monitoring component, be continued. Such projects should emphasize industry's direct participation and provide the necessary education, training, and understanding of the projects significance, through workshops and field extension services.

The Nova Scotia Aquaculture Coordinating Committee (representatives from both the Scotia-Fundy and Gulf Regions of the Department of Fisheries and Oceans, the Nova Scotia Department of Fisheries, the Aquaculture Association of Nova Scotia and independent aquaculturalists) meet approximately monthly to proceed with the revisions to the Nova Scotia Aquaculture Development Strategy text and make recommendations on generic proposals suitable for funding under the Economic Regional Development Agreement (ERDA) Fisheries Subagreement. The Province recommends that development projects designed to produce generic solutions to problems resulting from harmful marine algae be submitted to this Committee for review.

In response to the newly-discovered outbreak of DSP in Mahone Bay, a fact-sheet should be established to inform shellfish growers of this new finding and solicit their assistance in the early detection and reporting of any recurrence of a similar toxic bloom. Shellfish growers should be increasingly wary of any reports of illness resulting from the consumption of their product and should be made aware of all the symptoms which can result from the consumption of contaminated product. Further, the press should be encouraged to deal with outbreaks, such as the recent DSP bloom, in a manner which is responsible to the consuming public, the aquaculture industry and the regulatory agencies.

A review of quality control procedures for aquaculture products is suggested. While the Aquaculture Association of Nova Scotia has made great headway with its Scotia Pride trademark program, further work must be done to maintain and enhance consumer's confidence in product quality. In 1992, depuration procedures will be mandatory for shellfish products in Europe. In order to compete internationally, an evaluation of depuration facilities and programs to ensure sustained product quality should be instituted.

B. Response From the Floor

Roger Foxall
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We need to take steps to improve coordination and cooperation among all agencies involved in phycotoxin research. The Institute for Marine Biosciences could provide more support if if was loaned manpower to work on specific projects. We must not limit our research programs on phycotoxins to aquaculture applications. Wild stock are at risk as well, as shown by the occurrence of PSP on Georges Bank. Offshore sites are needed in the phytoplankton monitoring programs.

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It would be highly desirable to ensure that the knowledge presented at this workshop be made available to clinical researchers whose job it is to study and develop antidotes to, and treatments for, marine toxins. Chemical and physiological knowledge would be particularly precious in that regard.

It may be somewhat anticlimatic to raise this topic after spending much time and effort to elaborate, implement and describe algal bloom prediction and product inspection mechanisms. It may, however, be wise to have the humility to accept the possibility that toxins may strike again despite all of our efforts. It is the job of research to anticipate such events and to give the medical profession means to treat victims.

Clinical research should be encouraged because treatment methods are necessary. The most sophisticated monitoring and prevention mechanisms cannot prevent all accidents; there is no insurance against ignorance, imprudence and greed. A short review of the history of Paralytic Shellfish Poisoning (PSP), and of the mortalities it caused, should be enough to convince doubters. One well-publicized death can set the industry back for years.

The emerging opposition to bioassays using mammals reinforces the need for treatments. The mouse bioassay has, for the most part, served us well. The humble mouse serves as an integrator of toxin effects; any one lethal individual toxin or any lethal combination of toxins and/or derivatives causes the animal to become sick or to die. If animal right movements were to force an early abandonment of the mouse bioassay, we may have to rely more on chemical detection methods.

Modern analytical instruments can identify and quantify known toxins. It is less certain that they can pinpoint and predict ill-effects of yet unknown toxins, and of combinations of marine substances and of their derivatives. As there was a first time for domoic acid, there may be a first time for something else equally undesirable. In such eventuality, the existence of generic antidotes or easily available treatment methods could not only save lives, but also spare the industry severe, if not irrepairable, damage.

CONCLUSIONS

The second Canadian Workshop on Harmful Marine Algae gave over 100 participants an excellent opportunity to hear about and to discuss the latest developments in marine phycotoxin research in Canada. At the first Workshop, most of the presentations focused on the *Nitzschia pungens*/domoic acid issue which was responsible for stimulating increased interest in phycotoxin research after the PEI mussel crisis in late 1987. At this second Workshop, more attention was given to other kinds of phycotoxins, in particular PSP and DSP.

The following recommendations, unprioritized, came out of the workshop:

A. Recommendations on aspects within the DFO mandate:

1. In view of the recent Mahone Bay incident and results from general phytoplankton monitoring, increased research focus should be placed on DSP, including culture of DSP-producing organisms, detection methods and bioassays.

2. DFO's regulatory role requires a broad-spectrum screening test. There may be increasing pressures from animal rights groups to develop alternatives to the mouse bioassay. Effort should be made to develop a broad-spectrum, socially acceptable phycotoxin assay method as a substitute for the mouse bioassay.

3. To better meet the needs of Inspection Services and to facilitate integration of analytical methodology into routine regulatory testing procedures, priority should be given to selectivity rather than sensitivity when developing and fine-tuning analytical methods for toxin detection. Joint planning between NRC and DFO is appropriate for development of such methods.

4. For detection of phycotoxins in the field, efforts to develop accurate, reliable and easy-to-use immunological methods should continue. Priority should be given to improving the saxitoxin (STX) test kit, and developing a neoSTX kit using neoSTX isolated by NRC.

5. There is increasing suspicion that bacteria may play a role in the production of certain marine toxins. There is also a distinct possibility that viruses may serve as vectors for transferring genetic material controlling the production of a given toxin to other phytoplankters. Unialgal and axenic cultures must be developed to provide unequivocal proof that a particular organism produces toxins.

6. Although significant progress has been made, continued effort is needed to understand factors that influence the growth of toxin-producing phytoplankton and the production of phycotoxins with an emphasis on integrating laboratory and field studies.

7. Interpretation of bloom dynamics and the prediction of blooms of toxin-producing algae requires an understanding of the physical parameters of bays and estuaries. Consideration should be given to expanding the Scotia-Fundy Estuary, Inlet and Coastal Embayment Classification Program (Coastal Oceanography Division, BIO) to other DFO Regions.

8. Although the present phytoplankton monitoring programs operated in the various Atlantic Regions serve both DFO Inspection and Science Branches, the two have different objectives. For regulatory purposes, Inspection Services require limited data from numerous sites, with a short turn-around time in order to act rapidly in case a harmful bloom develops. Science requires extensive data on few sites to understand the seasonal and longer-term changes in phytoplankton communities, and the underlying causative mechanisms. The existing phytoplankton monitoring programs should be evaluated, monitoring technology transferred to Inspection, and approaches/plans developed jointly for work in 1991-92 and beyond.

9. Recent toxic episodes have demonstrated the need for rapid responses to transitory events. A rapid communications mechanism should be developed among DFO, NRC, NHW, universities and provincial agencies so that interested research groups are informed quickly of unusual blooms.

10. Industry has expressed an interest in becoming more involved in efforts to understand and minimize the impacts of toxic blooms, and to be informed of toxic bloom events. DFO should support industry initiatives (e.g., workshops for industry) to develop their own capability to monitor harmful phytoplankton at aquaculture sites.

11. In view of the impact of toxic phytoplankton blooms on the shellfish industry, research on the uptake and depuration of phycotoxins should be continued.

12. In view of past and recent toxic episodes on the Pacific coast and lack of west coast representation at the second Canadian Workshop on Harmful Marine Algae, PWG should review the harmful marine algae situation on the Pacific coast.

13. To facilitate access to existing data on marine toxins, an index of all available databases on marine toxins in Canada should be compiled.

B. Recommendations falling outside of the DFO mandate:

14. At present, there is no specified tolerance level for DSP in Canada. A DSP tolerance level in food should be established for regulatory purposes by National Health and Welfare.

15. Availability of analytical (calibration) standards and reference material is critical to many DFO research projects and Inspection activities on phycotoxins. NRC should clarify which standards and reference materials they will develop and in what time frame. For those standards and reference materials that NRC is not expecting to produce in a useful time frame, alternative sources should be investigated.

16. Basic research on understanding metabolic control factors of toxin biosynthesis could lead to culture methods for optimizing the biosynthesis of toxins in large-scale culture. Basic research is also required for an eventual elucidation of the gene loci that control toxin production, and for possible biotechnological manipulation of toxin-producing phytoplankton. DFO should encourage studies to elucidate the biosynthetic pathways of toxin production.

The informal nature of this Workshop encouraged active discussion, both in the formal sessions and in the halls during the breaks. The response was positive, indicating that this format should be pursued at

future workshops. The discussion groups were especially beneficial. One disadvantage, however, was that they were structured in a way which artificially separated laboratory from field studies. Numerous scientists stressed the point that laboratory and field work must be tightly linked and recommended a different organization of discussion groups at the next workshop.

The Workshop was stimulating and informative. It permitted the sharing of valuable new information and generated many personal contacts which will hopefully result in additional collaborative research projects in the coming year. Considering the success of the first two workshops, the Department of Fisheries and Oceans will host a third workshop in the winter of 1992 to be held at the Institut Maurice Lamontagne in Mont-Joli, Quebec.

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