Proceedings of the First Canadian Workshop on Harmful Marine Algae
Gulf Fisheries Centre, Moncton, N.B.
September 27 - 28, 1989

S.S. Bates and J. Worms (Editors)

Department of Fisheries and Oceans
Gulf Fisheries Centre
P.O. Box 5030
Moncton, New Brunswick
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CANADIAN WORKSHOP ON HARMFUL MARINE ALGAE

First Workshop

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ABSTRACT

The first Canadian Workshop on Harmful Marine Algae was hosted by the Department of Fisheries and Oceans, Gulf Region, at the Gulf Fisheries Centre in Moncton, New Brunswick, on September 27 - 28, 1989. The theme of this first workshop was "Marine Phycotoxins in Canadian Waters: their Production and Fate". The workshop was divided into three sessions:

1. Production of marine phycotoxins,
2. Fate of marine phycotoxins in the food web and the environment, and
3. Non-biological aspects of marine phycotoxins.

The proceedings consist of abstracts of 23 oral and 8 poster presentations, summaries of the keynote address, the three sessions and the panel discussion, outlines of research programs operated by federal and provincial agencies, research priorities derived from the workshop, and a list of participants.

RÉSUMÉ

Le premier Atelier de travail canadien sur les algues marines nuisibles s'est tenu les 27 et 28 septembre 1989 au Centre des Pêches du Golfe, à Moncton, Nouveau-Brunswick, à l'invitation de la Région du Golfe du Ministère des Pêches et des Océans. Le thème de ce premier atelier était: "Les phycotoxines marines dans les eaux canadiennes: production et devenir". L'Atelier était organisé en trois sessions:

1. Production des phycotoxines marines;
2. Devenir des phycotoxines marines dans la réseau alimentaire et dans l'environnement; et
3. Aspects non-biologiques des phycotoxines marines.

Les présents comptes-rendus contiennent les résumés des 23 présentations orales et des huit affiches, les rapports sur la conférence d'ouverture, les trois sessions et le débat-discussion, un aperçu des programmes mis en place par diverses agences fédérales et provinciales, les priorités de recherche qui se sont dégagées de l'atelier et une liste des participants.

INTRODUCTION

The first Canadian Workshop on Harmful Marine Algae was held at the Gulf Fisheries Centre in Moncton, New Brunswick, on September 27 - 28, 1989. Over 130 people from 8 provinces and 6 states took part in the workshop, which was organized by the Gulf Region of the Department of Fisheries and Oceans (DFO). The workshop will rotate annually among DFO regions involved in phycotoxin research, each workshop having a unique theme. The theme for this first workshop was "Marine Phycotoxins in Canadian Waters: their Production and Fate". The rationale for holding such workshops is described in the Terms of Reference drawn up by the Phycotoxin Working Group, an ad-hoc committee composed of representatives from six DFO regions:

> "Following the 1987 mussel toxicity event in eastern P.E.I. and considering the growing interest shown by the scientific community in the field of marine toxins and other harmful effects of marine algae, there is a need to establish a mechanism that will (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 initiatives. As this research is carried out by individuals and teams from a variety of disciplines, from government, academic and private institutions, the interface must be informal and flexible to allow for the ready entry of whoever has a contribution to make or an idea to share."

The organizing committee of this first workshop has strived to achieve these goals. We were fortunate in having the participation of such a large number of interested researchers and industry representatives. We are also grateful for the high caliber of the 23 oral and 8 poster presentations. We thank the Office of the Regional Director, Science, and the Communications Branch, Gulf Fisheries Centre, for their assistance.

What follows are abstracts of the presentations, summaries of the keynote address, the three sessions and the panel discussion, outlines of research programs operated by federal and provincial agencies, research priorities derived from the workshop, and a list of participants.

Stephen S. Bates, Convener
First Canadian Workshop on Harmful Marine Algae

PROGRAM SCHEDULE

SEPTEMBER 27, 1989

9:00 - 9:15 **Welcoming Remarks**
E.R. (Ted) Gaudet, Regional Director General, Gulf Region
DFO, Gulf Fisheries Centre, Moncton, New Brunswick

9:15 - 9:30 **Official Opening**
Barry S. Muir, Director General, Biological Sciences
DFO, Ottawa, Ontario

9:30 - 10:15 **Keynote Address**
The global epidemic of harmful phytoplankton blooms in the sea: Apparent causes and consequences.
Theodore J. Smayda, University of Rhode Island, Kingston, Rhode Island (USA)

10:15 - 10:35 ***Coffee Break***

SESSION I - Production of Marine Phycotoxins

Chaired by Greta Fryxell, Texas A&M University (USA)

10:35 - 10:55 Distribution of *Nitzschia pungens* in British Columbia coastal waters.
Roderick Forbes, Institute of Ocean Sciences
DFO, Sidney, British Columbia (Canada)

10:55 - 11:10 Domoic acid production in Passamaquoddy Bay, New Brunswick.
Jennifer Martin, St. Andrews Biological Station
DFO, St. Andrews, New Brunswick (Canada)

11:10 - 11:25 Domoic acid in mussels *Mytilus edulis* from Passamaquoddy Bay, New Brunswick.
Les Burridge, St Andrews Biological Station
DFO, St. Andrews, New Brunswick (Canada)

11:25 - 11:45 Toxic blooms of the domoic acid-containing diatom *Nitzschia pungens* in eastern Prince Edward Island in the fall of 1988 and late summer of 1989.
John C. Smith, Gulf Fisheries Centre
DFO, Moncton, New Brunswick (Canada)

11:45 - 12:05 Overview of Atlantic Zone Phytoplankton Monitoring Program.
Donald Gordon, Jr., Bedford Institute of Oceanography
DFO, Dartmouth, Nova Scotia (Canada)

12:05 - 13:30 ***Lunch Break***

13:30 - 14:30 **SESSION I (continued)**

Chaired by Irwin Judson, P.E.I. Department of Fisheries and Aquaculture Charlottetown, P.E.I. (Canada)

13:30 - 13:50 Domoic acid: Some biological and ecological considerations on the production of this neurotoxic secondary metabolite by the diatom *Nitzschia pungens*.
Anthony de Freitas, Atlantic Research Laboratory
NRC, Halifax, Nova Scotia (Canada)

13:50 - 14:10 Factors influencing the production and release of domoic acid by the diatom *Nitzschia pungens*: Nutrient and light limitation.
Stephen S. Bates, Gulf Fisheries Centre
DFO, Moncton, New Brunswick (Canada)

14:10 - 14:30 Production of domoic acid by the diatom *Nitzschia pungens* in mass culture.
Randall B. Angus, Miminegash Research Station
DFO, Gulf Region, Miminegash, Prince Edward Island (Canada)

SESSION II - Fate of Marine Phycotoxins in the Food Web and the Environment

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Chaired by David Scarratt, DFO, Halifax, Nova Scotia (Canada)

14:30 - 14:50 A recirculating flume with automatic addition of phytoplankton for bivalve mollusc feeding studies.
David Wildish, St. Andrews Biological Station
DFO, St. Andrews, New Brunswick, (Canada)

14:50 - 15:10 ***Coffee Break***

15:10 - 15:30 Anatomical distribution of PSP toxins in soft-shell clams.
Jennifer Martin, St. Andrews Biological Station
DFO, St. Andrews, New Brunswick (Canada)

15:30 - 15:50 Distribution of PSP toxins in mussels cultured in Deadman's Harbour, New Brunswick.
Katsuju Haya, St. Andrews Biological Station
DFO, St. Andrews, New Brunswick (Canada)

15:50 - 16:10 Paralytic shellfish toxins in mackerel, *Scomber scombrus*, from southwest Bay of Fundy, Canada.
Brenda Waiwood, St. Andrews Biological Station
DFO, St. Andrews, New Brunswick (Canada)

16:10 - 16:30 Neurotoxins and physiological mechanisms of ion channels.
Carl Boyd, Department of Oceanography
Dalhousie University, Halifax, Nova Scotia (Canada)

SEPTEMBER 28, 1989

8:30 - 10:00 **SESSION II (continued)**

Chaired by John C. Smith, Gulf Fisheries Centre, DFO, Moncton, New Brunswick (Canada)

8:30 - 8:50 Strategies for stimulating uptake and depuration of domoic acid in blue mussels (*Mytilus edulis*).
Irené Novaczeck, Atlantic Veterinary College
University of P.E.I., Charlottetown, Prince Edward Island (Canada)

8:50 - 9:10 Recent progress in understanding uptake and depuration of domoic acid by mussels.
David J. Scarratt, DFO, Halifax, Nova Scotia (Canada)

9:10 - 9:30 Selected topics from the 4th International Conference on Toxic Marine Phytoplankton.
Ewen Todd, Bureau of Microbial Hazards, Health Protection Branch
Health and Welfare Canada, Ottawa (Canada)

9:30 - 10:00 A review of the effects of algal blooms on shellfish and aquaculture.
Sandra Shumway, Maine Department of Marine Resources
West Boothbay Harbor, Maine (USA)

10:00 - 10:20 ***Coffee Break***

10:20 - 11:10 **POSTER PRESENTATIONS**

(authors will be available for questions)

SESSION III - Non-Biological Aspects of Marine Phycotoxins (Invited speakers)

Chaired by Roger Foxall, Atlantic Research Laboratory
National Research Council of Canada, Halifax, Nova Scotia (Canada)

11:10 - 11:40 Human health aspects.
Trish Perl, College of Medicine
The University of Iowa, Iowa City, Iowa (USA)

11:40 - 13:00 ***Lunch Break***

13:00 - 13:30 The DFO Inspection Program: Phycotoxin monitoring.
Régis Bourque, Gulf Fisheries Centre
DFO, Moncton, New Brunswick (Canada)

13:30 - 13:50 Analytical methods for the detection of marine phycotoxins.
Allan B. Cembella, Institut Maurice Lamontagne
DFO, Mont-Joli, Québec (Canada)

13:50 - 14:15 Instrumental analytical methods for marine toxins.
Michael A. Quilliam, Atlantic Research Laboratory
NRC, Halifax, Nova Scotia (Canada)

14:15 - 14:45 Toxic phytoplankton: How it is affecting the mussel culture industry.
Wayne Somers, President, P.E.I. Mussel Growers' Association
Murray Harbour, Prince Edward Island (Canada)

14:45 - 15:15 **Short Presentations of Research Programs Operated by Federal and Provincial Agencies.**

National Research Council of Canada
Jeffrey L.C. Wright, Atlantic Research Laboratory
Halifax, Nova Scotia

Health and Welfare Canada
Ewen Todd, Health Protection Branch, Bureau of Microbial Hazards
Ottawa, Ontario

Department of Fisheries and Oceans
Jean Worms, Gulf Fisheries Centre
Moncton, New Brunswick

Atlantic Veterinary College and University of Prince Edward Island
M.S. Nijjar, Atlantic Veterinary College
Charlottetown, Prince Edward Island

P.E.I. Department of Fisheries and Aquaculture
Irwin Judson, Charlottetown, Prince Edward Island

15:15 - 15:30 ***Coffee Break***

PANEL DISCUSSION - Future Research Directions

Chaired by Aivars Stasko, Department of Fisheries and Oceans
Ottawa, Ontario (Canada)

15:30 - 16:30 Open discussion with the participation of Session chairpersons and invited speakers.

16:30 **Closing of the Workshop**

Note: Co-authors and their affiliations are found in the collection of abstracts.

LIST OF PRESENTATIONS
(Listed in order of their presentation)

Note: Underlined authors presented the talks.

ABSTRACTS

KEYNOTE ADDRESS

THE GLOBAL EPIDEMIC OF HARMFUL PHYTOPLANKTON BLOOMS IN THE SEA: APPARENT CAUSES AND CONSEQUENCES

Theodore J. Smayda

Graduate School of Oceanography, University of Rhode Island, Kingston, RI 02881, U.S.A.

Evidence is presented that there is a global outbreak, spreading, increased frequency, and greater species' participation in nuisance algal blooms in the sea. The exact causes of the species-specific outbreaks vary with taxon, region and season. There appears to be a linkage between increased annual primary production rate and the increased occurrence of unusual, often inimical, phytoplankton blooms. Shifts in species and/or functional group predominance followed by major bloom events in subsequent years may also occur. Observations from the North Sea, Wadden Sea, Kattegat, Baltic Sea and Black Sea will be presented to illustrate this apparent linkage between parallel increases in primary production and bloom events. The apparent association between increasing primary production and increased phytoplankton bloom frequency appears to reflect a response to two basic attributes associated with increased primary production. A progressive change in the chemical environment, particularly with regard to the ratios of N:P:Si, is evident. Such progressive increases in primary production may also be a destabilizing event which alters community organization, phytoplankton niche structure, and favors blooms of ephemeral or successful immigrant species and indigenous components of the community. The resultant community reorganization and bloom events have characteristics suggestive of a trophic imbalance.

ORAL PRESENTATIONS

DISTRIBUTION OF *Nitzschia pungens* IN BRITISH COLUMBIA COASTAL WATERS

J. Roderick Forbes and Kenneth L. Denman

Department of Fisheries and Oceans, Institute of Ocean Sciences, P.O. Box 6000, Sidney, B.C. V8L 4B2

We have reviewed our records of the distribution of *Nitzschia pungens* in British Columbia coastal waters from 1980 to 1988. Sampling has been limited to the months of April to October. *N. pungens* occurs throughout the continental shelf, most frequently appearing as a minor component of the large diatom populations that develop off the southwest coast of Vancouver Island in late July and August. Occasionally, it becomes the dominant species at the tail end of these blooms. It is less common in the Strait of Georgia and is more sporadic north of Vancouver Island. Maximum concentrations recorded are 1 x 10^6 cells.L^{-1} in Hecate

Strait in early July 1983 and 5 x 10^5 cells.L^{-1} off southwest Vancouver Island in late August 1986. Discriminant function analysis showed that samples containing *N. pungens* could be distinguished from those without on the basis of higher chlorophyll and lower nitrate concentration, and narrower range of salinity. The two sets of samples were not dissociated by depth or temperature. Scanning electron microscopy has shown that both *N. pungens* forma *pungens* and *N. pungens* forma *multiseries* occur in the area. Current work involves attempting to obtain sufficient material to analyze for domoic acid.

DOMOIC ACID PRODUCTION IN PASSAMAQUODDY BAY, NEW BRUNSWICK

Jennifer L. Martin and David J. Wildish

Department of Fisheries and Oceans, Aquaculture and Invertebrate Fisheries, Biological Station, St. Andrews, N.B. E0G 2X0

Domoic acid was detected in soft-shell clams (*Mya arenaria)* and blue mussels *(Mytilus edulis)* in the Passamaquoddy Bay region from late July through October, 1988. Toxin levels were above the acceptable level for harvesting at Chamcook Harbour, Pottery Cove, Northern Harbour, McCann's Cove and the Magaguadavic River. The diatom *Nitzschia pseudo-delicatissima* was the most abundant organism observed between August and October in weekly water samples collected at the surface, 10 m and 1 m above bottom from 17 locations in the Fundy Isles region. Highest concentrations were observed the last week of September when 1.8 x 10^7 cells.L^{-1} were observed in samples collected at an offshore station near the Wolves and 9.6 x 10^6 cells.L^{-1} were found inshore at Chamcook Harbour. Analysis and subsequent culture of phytoplankton from affected areas suggested *N. pseudodelicatissima* to be a major source of domoic acid.

DOMOIC ACID IN MUSSELS, *Mytilus edulis*, FROM PASSAMAQUODDY BAY, NEW BRUNSWICK, CANADA

K. Haya, L.E. Burridge, J.L. Martin, and B.A. Waiwood

Department of Fisheries and Oceans, Biological Station, St. Andrews, N.B. E0G 2X0

Mussels (*Mytilus edulis*) were suspended from long-lines (cultured) in Brandy Cove and Deadman's Harbour from May 1988 to November 1988. These mussels and a wild population from the intertidal zone in Chamcook Harbour were sampled periodically and analyzed for domoic acid. Domoic acid was detected in mussels from Brandy Cove and Chamcook Harbour during August 15 to October 10. No domoic acid was detected in mussels from Deadman's Harbour. The concentration of domoic acid was 90 and 160 µg.g^{-1} wet weight in digestive glands of mussels from Chamcook Harbour (September 27) and Brandy Cove (September 19), respectively. The distribution of domoic acid in mussel was: 96.5% in digestive gland; 2.4% in gills; and not detected in mantle, gonads and foot. A plankton tow obtained from Chamcook Harbour on September 30 had a domoic acid concentration of 3.5 µg.g^{-1} wet weight of plankton. Clams (*Mya arenaria*) sampled September 26 from Chamcook Harbour had a domoic acid concentration of 80 µg.g^{-1} wet weight.

TOXIC BLOOMS OF THE DOMOIC ACID-CONTAINING DIATOM *Nitzschia pungens* IN EASTERN PRINCE EDWARD ISLAND IN THE FALL OF 1988 AND LATE SUMMER OF 1989

John C. Smith[1], Randall Angus[2], Kevin Pauley[1], Paryse Cormier[1], Stephen Bates[1], Louis Hanic[3], Jean Worms[1], and Thomas Sephton[1]

[1]Department of Fisheries and Oceans, Gulf Fisheries Centre, P.O. Box 5030, Moncton, N.B. E1C 9B6; [2]DFO, Miminegash Research Station, Miminegash, P.E.I. C0B 1SO; [3]Department of Biology, University of Prince Edward Island, Charlottetown, P.E.I. C1A 4P3

As in 1987, blooms of *Nitzschia pungens* again appeared in eastern Prince Edward Island in the fall of 1988. These blooms contained the toxin domoic acid and were associated with domoic acid toxicity in cultivated mussels. The time course of the development of the *N. pungens* blooms and their relationship to the uptake and depuration of domoic acid in mussels are described. Monitoring the phytoplankton population provided an early warning of the impending domoic acid build up in mussels. The concurrent changes in physical, chemical and meteorological variables suggest that the blooms were initially limited by the supply of nitrogen and declined because of low winter temperatures and irradiance. The initial supply of nitrogen was correlated with rainfall patterns and wind events, but the precise source and mechanism of nitrogen supply are yet to be determined.

Beginning in early August 1989, blooms of *N. pungens* occurred in eastern Prince Edward Island and New London Bay (northern P.E.I.) and have continued to the present. Thus far there have been two distinct series of blooms in most locations. The first series produced many cells (up to 1,000,000 cells.L^{-1} in New London Bay on 9 August), but domoic acid was not detected, and the *N. pungens* cells consisted entirely of forma *pungens*. A second series of blooms began following a strong wind event on 25 August and, although fewer cells were observed at the peak of these blooms (maximum 150,000 cells.L^{-1} in the Brudenell River on 29 August), measurable quantities of domoic acid were produced in most locations. At the beginning of the second bloom in the Brudenell River, about 30% of the *N. pungens* cells were forma *multiseries* and this increased to about 90% by the peak of the bloom; it is clear that domoic acid is closely associated with the presence of forma *multiseries*. Although these latter blooms have declined considerably, significant numbers of *N. pungens* persist in the water column (9,000 to 60,000 cells.L^{-1}, depending on location), and, given the proper conditions, these could give rapid rise to a major toxic event.

OVERVIEW OF ATLANTIC ZONE PHYTOPLANKTON MONITORING PROGRAM

Donald C. Gordon, Jr.

Habitat Ecology Division, Department of Fisheries and Oceans, Scotia-Fundy Region, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2

Improved prediction of toxic algal blooms in the coastal waters of Atlantic Canada requires a better understanding of seasonal changes in phytoplankton species composition and controlling physical/chemical processes. A phytoplankton monitoring program was initiated in the Quoddy region by the St. Andrews Biological Station in 1987. This program was expanded in 1988 as

other Department of Fisheries and Oceans (DFO) laboratories added stations along the Atlantic coast and the Gulf of St. Lawrence. Participating scientists from DFO's Gulf, Quebec, and Scotia-Fundy Regions have met on several occasions to standardize procedures to ensure data comparability. Sampling frequency varies from weekly in summer to monthly in winter. In addition to phytoplankton species, variables routinely measured include temperature, salinity, chlorophyll, and nutrients. The program is expected to run for at least three years.

DOMOIC ACID: SOME BIOLOGICAL AND ECOLOGICAL CONSIDERATIONS ON THE PRODUCTION OF THIS NEUROTOXIC SECONDARY METABOLITE BY THE DIATOM *Nitzschia pungens*

Anthony de Freitas[1], Stephen S. Bates[2], Roger Pocklington[3], and Jeffrey L.C. Wright[1]

[1]Atlantic Research Laboratory, National Research Council of Canada, 1411 Oxford St., Halifax, N.S. B3H 3Z1; [2]Department of Fisheries and Oceans, Gulf Fisheries Centre, P.O. Box 5030, Moncton, N.B. E1C 9B6; [3]Department of Fisheries and Oceans, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2

The biosynthesis, utilization, accumulation, and release of many structurally dissimilar low molecular weight metabolites is a common feature in the normal cell cycle of microalgae. Intracellular free fatty acids and amino acids, for example, are considered to be "primary" metabolites with defined functions in maintaining cell homeostasis. Domoic acid, okadaic acid and other phycotoxins are referred to as "secondary" metabolites in that they are not always present in the cell and do not appear to have any known direct role in maintaining homeostasis. Domoic acid, as a nitrogen-containing product of amino acid metabolism, represents a particularly interesting type of secondary metabolite. Factors controlling its production by the diatom *Nitzschia pungens* forma *multiseries* are just beginning to become understood. Results with laboratory cultures of this organism conducted over a fairly wide range of growth conditions demonstrate that domoic acid production occurs only in non-actively dividing cells such as in cultures forced into stationary phase by silicate limitation, all other nutrients including nitrate remaining in excess. Cells in stationary phase remain viable for many weeks during which they continue to produce and excrete domoic acid into the medium. We raise the suggestion that domoic acid production by *N. pungens* is an example of "luxury consumption" of nitrogen, in a way analogous to the well-established phenomenon of phosphorus luxury consumption. This and other potentially important and ecologically relevant aspects of domoic acid production will be discussed.

FACTORS INFLUENCING THE PRODUCTION AND RELEASE OF DOMOIC ACID BY THE DIATOM *Nitzschia pungens*: NUTRIENT AND LIGHT LIMITATION

Stephen S. Bates[1], Anthony de Freitas[2], Roger Pocklington[3], Michael Quilliam[2], and John C. Smith[1]

[1]Department of Fisheries and Oceans, Gulf Fisheries Centre, P.O. Box 5030, Moncton, N.B. E1C 9B6; [2]Atlantic Research Laboratory, National Research Council of Canada, 1411 Oxford St., Halifax, N.S. B3H 3Z1; [3]Department of Fisheries and Oceans, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2

The pennate diatom *Nitzschia pungens* forma *multiseries* (isolate NPARL) was grown in batch culture under nutrient or light limitation. Domoic acid was never detected during the exponential growth phase. In medium f/2, cellular domoic acid reached a maximum (7 pg.cell^{-1}) about 7 days after the beginning of the stationary phase and thereafter slowly declined; domoic acid released by the cells into the medium continued to increase. The total amount of domoic acid produced by the culture was proportional to the cell density during the stationary phase in silicate-limited f/2 medium. Domoic acid production ceased when the growth medium containing an excess of nitrate was replaced during the stationary phase with nitrate-deficient medium. Replacement with media deficient in silicate or phosphate did not alter the rate of domoic acid production, as long as nitrate was present. Domoic acid was not detectable in cultures grown in the absence of added nitrate. The cellular domoic acid level was negligible (0.1 - 0.4 pg.cell^{-1}) when *N. pungens* was grown to stationary phase induced by nitrate limitation (0.5 mM nitrate). When additional nitrate (1.0 mM) was supplied to the nitrogen-deficient medium during the stationary phase, cell division resumed and cell number reached a new plateau limited by silicate concentration. Domoic acid production then also resumed at a rate comparable to that of the nitrogen-replete control.

Light limitation slowed the growth rate during the exponential phase and delayed the attainment of the stationary phase by about 10 days relative to the control. However, the rate of domoic acid production was comparable to that of the control once stationary phase was reached. On the other hand, cells placed into complete darkness during the stationary phase ceased their production of domoic acid, as did cells during the dark period of a light:dark cycle. We conclude that at least three conditions are required for the production of domoic acid by isolate NPARL: cessation of cell division, availability of nitrate during the stationary phase, and the presence of light. Growth in culture medium f/2, which contains limiting silicate and excess nitrate, fulfils these requirements.

PRODUCTION OF DOMOIC ACID BY THE DIATOM *Nitzschia pungens* IN MASS CULTURE

R.B. Angus[1], J.C. Smith[2], M.F. Stephenson[1], S. Myers[1], P. Cormier[2], and S. Bates[2]

Department of Fisheries and Oceans, Gulf Region. [1]Miminegash Research Station, Miminegash, P.E.I. C0B 1S0; [2]Gulf Fisheries Centre, P.O. Box 5030, Moncton, N.B. E1C 9B6

Nitzschia pungens forma *multiseries* (isolate NPMIM from November, 1988) was grown in batch culture for large-scale feeding experiments. Cultures of up to 220 L with cell concentrations of up to 260,000 cells.mL^{-1} were incorporated into the weekly production schedule of a shellfish research laboratory. Methods of culture, various morphological changes, division rates, numbers of dead cells, and domoic acid production and release are discussed. Domoic acid was produced during the stationary phase (days 6 - 18) at a temperature of 21°C, allowing harvesting with a predictable level of domoic acid.

A RECIRCULATING FLUME WITH AUTOMATIC ADDITION OF PHYTOPLANKTON FOR BIVALVE MOLLUSC FEEDING STUDIES

D.J. Wildish[1] and D.D. Kristmanson[2]

[1]Department of Fisheries and Oceans, Biological Station, St. Andrews, N.B. E0G 2X0; [2]Chemical Engineering Department, University of New Brunswick, Fredericton, N.B. E3B 5A3

Previous work on the environmental and physiological factors which control the filtration rate of bivalve molluscs has suffered from various methodological problems. They include an inability to provide, near the experimental subjects, a controlled ambient flow and a constant seston supply. Our purpose was to devise an experimental system which overcame these problems and to present some preliminary results obtained with it.

The experimental apparatus consisted of a recirculating flume with a volume capacity of 80-90 L and an open-test section which was 0.8 x 0.16 x 0.2 m in dimensions. Flows near the experimental molluscs could be varied from 0.5-35 cm.s^{-1} before standing waves or impeller-induced aeration became a problem. Flow profiling and localized flow measurements near the mollusc inhalant siphon were made with up to 5 Nixon Stream Flo probes. Seston concentration was maintained by an infrared light switch which controlled a pump supplying algal culture to the flume. A multi-channel data logger linked to a PC computer recorded temperature, flows and the time that the algal pump was activated, hence providing a temporal record of bivalve feeding.

Preliminary results with giant scallops, *Placopecten magellanicus* Gmelin, suggest that flow-induced inhibition of filtration is a positive function of seston concentration. This confirms results obtained earlier in a flume in which the initial seston concentration could not be maintained during the experimental period.

ANATOMICAL DISTRIBUTION OF PARALYTIC SHELLFISH TOXINS IN SOFT-SHELL CLAMS

Jennifer L. Martin[1], Alan W. White[2], and John J. Sullivan[3]

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Soft-shell clams *(Mya arenaria)* were collected from two sites in the southwestern Bay of Fundy in January, April, May, July, September and November, 1986. The sites were Crow Harbour, where clams exceed the quarantine toxin level year-round, and Lepreau Harbour, where clams exceed the quarantine level only during the annual, summer *Gonyaulax* bloom. Clams were dissected into digestive gland, gonad, gills, muscles (siphon, foot, pallial muscle, and adductor muscle), and "remainder" which consisted of a small amount of material near the digestive gland and included the kidney and heart. Toxic determinations were made by mouse bioassay and HPLC. The "remainder" contained the highest total toxin concentration (unit weight basis) throughout the year in all samples except one. Toxin levels in the "remainder" were up to 10 times greater than those for the next highest samples. In July, during the *Gonyaulax* bloom, the digestive gland also contained high toxin concentrations; much lower levels occurred in the gills and gonads. In the other months, the gills contained about the same toxin concentrations as the digestive gland. The patterns of abundance of the individual toxins in the various anatomical parts were similar within and between collection sites. In July, the sequence of abundance of the individual toxins in the clams was GTX I, GTX IV and GTX III, with STX usually the lowest of the six or seven detectable toxins. The sequence in July plankton dominated by *Gonyaulax* was GTX IV, GTX III, NEO and GTX I. Clam samples from the other months showed a striking difference from the July pattern; STX was nearly always present at the highest level, often followed by NEO, and GTX IV was always the lowest. Results indicate that soft-shell clams contain high toxin concentrations in parts other than the digestive gland. Further, results suggest that interconversion or selective retention of the toxins occurs within these animals.

DISTRIBUTION OF PSP TOXINS IN MUSSELS CULTURED IN DEADMAN'S HARBOUR, NEW BRUNSWICK

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Mussels, *Mytilus edulis*, were suspended from long lines at a maximum depth of 1.0 m below the surface and 1.0 m above the bottom (5 m below surface at low tide) in Deadman's Harbour, New Brunswick, from June 1988 to April 1989. During July 1988, a small bloom of *Alexandrium fundyense* occurred in Deadman's Harbour. A water sample taken on July 22 had the highest concentration of *A. fundyense* (4,000 cells.L^{-1}). The maximum concentration of cells observed in Brandy Cove (control site) during the experimental period was 100 cells.L^{-1}. The mussels were sampled periodically and analyzed by the official AOAC mouse bioassay

procedure to determine the concentration of PSP toxins. Mussels that were held near the surface were most toxic (432 µg SXT_{eq}.100 g^{-1} wet wt) on July 18. By October 17, the toxicity had decreased to 52 µg SXT_{eq}.100 g^{-1} wet wt. The mussels held at least 5 m below the surface did not accumulate PSP toxins. The distribution of PSP toxins in mussel were: 87.7% in digestive gland; 3.9% in gonads; 1.5% in mantle; 1.5% in gill; 0.4% in foot; and 5% in remaining tissues.

PARALYTIC SHELLFISH TOXINS IN MACKEREL, *Scomber scombrus*, FROM SOUTHWEST BAY OF FUNDY, CANADA

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During July 1988, a small bloom of *Alexandrium fundyense* occurred in Southwest Bay of Fundy, New Brunswick, Canada. The highest concentration of cells observed in a surface water sample was 7.5 x 10^3 cells.L^{-1} on July 12. From July to the end of September, Atlantic mackerel (*Scomber scombrus)* were sampled from 9 locations. Concentrations of PSP toxins in liver extracts were measured by mouse bioassay and ranged from 40 to 209 µg saxitoxin (STX) equivalents per 100 g wet wt. PSP toxins (2-26 µg STX_{eq}.100 g^{-1} wet wt.) were detected by HPLC analysis in 5 of 6 gastrointestinal (GI) tract extracts. In all but one sample, saxitoxin was greater than 97% of the total PSP toxin content in liver. One liver and one GI tract extract contained only neosaxitoxin. B2, GTX II and GTX III were also detected in some of the extracts from both tissues. The data suggest that mackerel do not accumulate PSP toxins exclusively from *A. fundyense.*

NEUROTOXINS AND PHYSIOLOGICAL MECHANISMS OF ION CHANNELS

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Recent progress in electrophysiology and the determination of the molecular structure of ion channel proteins in cell membranes has considerably increased our understanding of the configuration and functioning of ion channels. Many neurotoxins, including saxitoxin and domoic acid, act by blocking specific ion channels in other organisms. As a result, cells lose control of the ionic composition which insures their vital function; muscle cells become unable to contract, nerve cells to transmit messages. "Patch-clamping" provides an outstanding technique for studying the effect of neurotoxins on individual ion channels, and the polymerase chain reaction (PCR) offers an equally powerful means of probing specific categories of ion channels. The physiology of ionic regulation is both different and similar in plant and animal cells. The way plant cells, such as *Gonyaulax tamarensis* and *Nitzschia pungens*, capitalize on this difference and at the same time remain immune from their own toxin is central to the ecology and neurophysiology of marine organisms.

STRATEGIES FOR STIMULATING UPTAKE AND DEPURATION OF DOMOIC ACID IN BLUE MUSSELS (*Mytilus edulis*)

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Multilamellar liposomes with diameters ranging up to 20 µm have been tested as vehicles for the delivery of food to mussels. Using fluorescent dye and monastral blue particles in the liposomes, it was demonstrated that the liposomes are ingested and broken up in the stomach of the mussel. Liposomes containing domoic acid were fed to mussel at 7°C over a 5-day period. Uptake of toxin as measured in mussel extracts by HPLC was very limited. Absorption of domoic acid from solution was also found to occur only to a very small extent. Further experiments will involve liposomes bearing more concentrated solutions of domoic acid, the use of salinity change to try to enhance uptake and depuration, and the use of radiolabelled domoic acid so that small amounts can be monitored using scintillation counting and autoradiography.

RECENT PROGRESS IN UNDERSTANDING UPTAKE AND DEPURATION OF DOMOIC ACID BY MUSSELS

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In the absence to date of a guaranteed source of domoic acid-producing cultures of *Nitzschia pungens*, we have been obliged to use naturally-contaminated shellfish as experimental animals. Blooms sufficiently high to merit closure of clam flats occurred in Passamaquoddy Bay, N.B., in the late summer of 1988. Domoic acid content of clams and mussels held in quarantine at 13°C dropped from 43 to 15 ppm in 24 hours, but traces still remained as much as 6 days after harvesting. Similar runs on mussels harvested in the Cardigan estuary, P.E.I., in Fall 1988 showed depuration from 130 to 20 ppm in 4 - 6 days at 15°C, and 7 - 9 days at 7°C. Traces remained after 14 days depuration at the cooler temperature. In all cases the mussels were starved, and the question arises whether the presence of food other than *Nitzschia* would accelerate the depuration process. Clearly, the earlier 1987-88 observations that depuration occurred more rapidly in the lab than in the wild might be attributed to mussels continuing to feed on lingering traces of the *Nitzschia* bloom.

Progress in culturing *Nitzschia* at the Halifax Laboratory has been slow due to recurring problems with contamination by bacteria and flagellates, however, there has been some success in producing bulk cultures (230 L) at cell densities upwards of 200,000 cells.mL^{-1}, but not to the point where detectable quantities of domoic acid were produced. Results of the routine domoic acid monitoring program are being followed carefully so that any new bloom of *Nitzschia* in the Cardigan or elsewhere can be exploited to provide both a natural uptake experiment and a source of contaminated mussels, and other transplanted commercial species, for further depuration experiments.

SELECTED TOPICS FROM THE 4TH INTERNATIONAL CONFERENCE ON TOXIC MARINE PHYTOPLANKTON

Ewen Todd

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Ciguatera poisoning (A.R. Freudenthal)

Reports of ciguatera poisoning in northern residents returning from Caribbean and Bahamian island holidays indicate that this intoxication continues to be a public health problem. Tourists poisoned after consuming local fish products (mostly groupers and red snappers) become ill, and return home to seek medical assistance. Knowledge of case histories can provide a better understanding of trends and patterns in this highly-variable illness, thus adding to the information base needed for management purposes. There is an obvious need for increased clinical recognition, reporting and consumer education, especially in northern areas.

Diarrhetic shellfish poisoning (DSP)

- Mouse bioassay

The Japanese have introduced a standardized mouse bioassay procedure. Shellfish digestive glands are extracted (acetone and diethyl ether), and the combined solution is evaporated. The residue is resuspended in 1% Tween 60 solution, and serially-diluted suspensions are injected intraperitoneally into mice weighing 17-20 g each. The minimum amount of toxin required to kill a mouse at 24 h is defined as one (1) mouse unit. The maximum allowance level of toxins in shellfish is 5 mouse units per 100 g of shellfish meat.

- Origin of dinoflagellate toxins (T. Yasumoto)

Okadaic acid (OA), dinophysistoxins (DTX) 1 and 3, yessotoxin (DTX 2), 45-hydroxy yessotoxin, and pectenotoxins (PTX) 1, 2, 3, 6, 7 (4 and 5 were found in too small a concentration to characterize) have been isolated from the hepatopancreas of scallops feeding on *Dinophysis* in Japan. All the following *Dinophysis* species produce OA and its derivatives: *fortii, acuminata, mitra, tripos, rotundata, norvegica* and *acuta.* Shellfish metabolic activity converts DTX 1 to DTX 3; PTX 2 is converted to PTX 1, then to PTX 3 and PTX 6.

- Histopathological changes in rat intestinal mucosa due to okadaic acid (S. Lange *et al.*)

Rat ligated loop assay is another bioassay for OA. Fluid accumulation is measured after injection of 2 mL of toxic extract. The histopathology of the gut lining shows that the surface epithelial cells become filled with fluid and are edemateous with eventual sloughing off (in 30 min after 1 µg challenge of OA). The tips of the villae contain only goblet cells (no mucus release). This same effect can be shown in the colon lining. If OA is injected intravenously, it has the same effect as in the loop, suggesting that enterocytes are specific target cells for OA. Aplysiatoxin (from sea hare) and teleocidin (from *Streptomyces mediocidicus*) similarly induced fluid accumulation without mucus release. All three compounds are tumor-promoting agents. Intravenously-injected antisecretory factor (ASF) from porcine pituitary glands was inhibited by OA but not with the characteristic morphological changes. The ASF inhibition was 54% for *Dinophysis*, 58% for *Campylobacter* and 56% for *E. coli* ST (Heat-Stable Toxin).

- Effects of toxins in mice (K. Terao)

Sequential ultrastructural changes were studied in mouse digestive tracts, liver and lymphoid tissues, after IP injection of toxins isolated from certain species of dinoflagellates. Dinophysistoxin-1 (DTX 1) caused degeneration and desquamation of mucous epithelium from the lamina propria within 1 h after over 100 $\mu g.kg^{-1}$ given IP. In contrast to DTX 1, goniodomin A (GDA), a metabolite of the dinoflagellate *Goniodoma pseudogoniaulax*, produced marked necrosis in the centrilobular region of hepatic lobules, perihepatitis and non-fatty vacuole-formation in the hepatocytes. GDA also induced massive necrosis of lymphocytes in the cortical layer of the thymus at a dose of 0.5 $mg.kg^{-1}$. Maitotoxin (MTX), a water-soluble phycotoxin, is produced by *Gambierdiscus toxicus*. Treatment with a single dose of 200 $ng.kg^{-1}$ caused multiple erosion in the stomach, myocardial injury and marked reduction of lymphocytes in the thymus. A single injection of 200 $ng.kg^{-1}$ of MTX also induced a marked increase in calcium content in the adrenal glands and in plasma cortisol concentration within 1 h. It is suggested that MTX first stimulates calcium influx in the adrenal glands, which then causes the release of cortisol into the blood. Repeated injections of MTX (45 $ng.kg^{-1}$, 13 times) resulted in marked reduction of lymphocytes in blood, thymus and spleen. Prominent autophagosomes were seen in cells of zona reticularis of the adrenal glands. The mode of action of the immunotoxic effect of MTX is thus based on the increased content of calcium in adrenal glands.

- *Dinophysis acuta* as cause of DSP in Sweden (L. Edler and M. Hageltorn)

Field experiments have demonstrated the origin of DSP in Swedish mussels. HPLC quantification of OA showed more toxicity in mussels at a depth of 1 m than at 10 m. Size fractionation of plankton samples showed maximum toxicity in the 50 µm sieve fraction. Regression analysis of OA concentrations and cell counts showed that *Dinophysis acuta* ($r = 0.93$) was the most likely plankton to produce the OA ingested by the mussels, and probably caused the fall 1987 outbreak on the Swedish west coast. Next was *D. acuminata* ($r = 0.59$). 150,000 cells of *D. acuta* can kill mice.

- *Dinophysis* off Portuguese coasts (M. Sampayo *et al.*)

Although no illnesses due to DSP has been reported from Portuguese mussels, these do accumulate OA in association with *D. acuta* and *D. sacculus*. Depuration seems to be faster in the presence of non-toxic phytoplankton species. One questioner argued that depuration can take place within a few days, not over two weeks as suggested by the authors.

- Modelling *Dinophysis* blooms (A. Menesguen *et al.*)

Predictive modelling of *Dinophysis* blooms was attempted, taking into account temperature, light, inorganic nitrogen (low until late September), phosphorus (builds up in late summer) and chlorophyll. Vertical migration and sea state are more important than zooplankton grazing and cyst formation. Wind-induced vertical turbulence, causing sediment resuspension and light attenuation, is important in preventing blooms.

- Modifications to the HPLC method of Yasumoto *et al.* (N. Ileby and A. Fiksdahl)

1. Stability of OA and DTX 1: OA, DTX 1 and their derivatives are sensitive to light, heat and oxygen and should therefore be handled in the dark under N_2-atmosphere and stored in the cold. However, decomposition may still occur. A DTX 1 derivative solution decreased in activity after 2 to 4 d storage at -30°C, and gave rise to new peaks as a result of an unknown chemical reaction. The same phenomenon was observed for OA, but to a lesser degree. Such instability leads to underestimation of toxin levels in shellfish and to poor reproducibility.

2. Reaction time: Analysis of methanol-extracted OA from hepatopancreas depends on adequate derivatization and proper rinsing. For pure toxin standards used for reference purposes, the reaction curve for derivatization with ADAM was the same as that reported for fatty acids. Derivatization was only about 30-50% complete after 1 h, and reached a maximum after 24 h. For shellfish samples, however, increased reaction time did not enhance the detection of toxins, indicating a different type of reaction. Insufficient reaction time with ADAM for toxin reference substances may result in a low calibration curve for OA and DTX 1, and an overestimation of the toxin content in shellfish.

3. Loss of toxin during HPLC: The toxin derivatives are purified on a SiO_2 cartridge column before injection into the HPLC. This resulted in a 30-50% loss of DTX 1 in the pre-fraction. The method was modified to avoid this loss by collecting and combining a part of the pre-fraction with the toxin fraction. Since the problem does not occur in all Scandinavian laboratories, this loss must be dependent on the quality of SiO_2 packing material, solvents, etc. OA is not lost in the pre-fraction in the same way.

4. Internal standard for the HPLC method: Genuine toxin standards are unstable and difficult to obtain pure, and are required for identification purposes only. Alternatively, an internal standard, deoxycholic acid, was used because it has chemical and physical properties similar to OA and DTX 1. The internal standard is added to the shellfish sample and is derivatized with ADAM, giving a chromatographic peak with a longer retention time than DTX 1. Continued work is required before the internal standard method works satisfactorily.

5. Interlaboratory calibration study: Consistent results using this HPLC method were found within each laboratory, but variations among laboratories were unacceptable. Laboratories using this method for quality control in shellfish products should report acceptable intercalibration results before being given approval to carry out testing. Toxin allowance levels must be defined with a large safety margin to take into account the great analytical variation among laboratories.

Role of bacteria in PSP-producing blooms

- Bacteria as a source of dinoflagellate toxins (M. Kodama)

Alexandrium tamarense exhibits large differences in toxicity within specific clones grown under the same conditions, as well as within subclones derived from single cells. Toxin production, therefore, is probably acquired, not genetically transferred. This variation could be caused by intracellular bacteria with the ability to produce toxins, as was reported by Silva. Bacteria (*Moraxella* spp.) found in the nucleus of *A. tamarense* were released by homogenization, and were cultured. Some GTX IV and lesser amounts of GTX I, GTX III, GTX II and neosaxitoxin were produced by the bacteria under starvation conditions. Antibiotics added to the bacterial cultures reduced the toxicity. Bacteria were isolated only from toxic dinoflagellate strains, and different *Moraxella* spp. were found in different dinoflagellate species. Bacteria may also play a role in water and on the surface of dinoflagellates. In scallops, toxicity increased at the end of a toxic dinoflagellate bloom. Water collected near the scallops was filtered through 20, 5 and 0.45 µm pore-size filters, and showed most PSP toxins (mainly GTX) in the 0.45 µm fraction. Bacteria from this fraction were cultured, clones were isolated, and antiserum was prepared against the above *Moraxella* spp. Non-toxic *A. tamarense* could not be made toxic by addition of the *Moraxella*. Treatment of toxic *A. tamarense* with the antiserum did not render it non-toxic, although toxicity was reduced. Bacteria were not observed on the surface of the dinoflagellates, but an SEM examination was not done. If these bacteria occur in the water near the scallops, they could be on the surface as well as intracellular. An immunofluorescence probe could be used to detect surface attachments. Chemical identification of the bacteria in the dinoflagellate cells could be done by gas chromatography.

- Bacteria associated with PSP blooms (T. Ogata *et al.*)

Bacteria from toxic and non-toxic *Alexandrium* and *Gymnodinium* strains were cultured in seawater medium. After 10 d, the bacterial extracts were tested on mouse neuroblast cells for sodium channel blocking, and were also checked with anti-*Moraxella* serum. Bacteria isolated from high-toxicity dinoflagellate strains blocked sodium channels and reacted with the serum. Bacteria other than *Moraxella* spp. were also isolated and produced toxins: *Bacillus* spp. from a *Gymnodinium* (GTX IV, GTX I, GTX III, GTX II, and saxitoxin), and *Pseudomonas* spp. from an *Alexandrium* (GTX IV, GTX I, GTX III, GTX II). These results indicated a close relationship, which seems to be strain as well as species specific, between toxic dinoflagellates and associated bacteria which produce toxins.

Toxicity of marine toxins for laboratory workers (D. Baden)

Toxins become effective through receptors in various body organs: PSP toxins activate or deactivate enzymes; okadaic acid destroys protein phosphatases, thus limiting protein synthesis or releasing hormones or transmitters. The chronic effects may be severe and could lead to premature death. Workers should know the symptomology, antidote (if any) and treatment for each toxin worked with, and this should be posted in the laboratory. For certain toxins, fume hoods, masks and gloves should be used, and proper decontamination procedures be followed. Inhalation of aerosols is a real possibility, especially if silicate gels are used for chromatography. DMSO should be used with care because it can mobilize toxins into the body. Long-term chronic effects (e.g., brain edema and lesions) have been noticed in animals. Federal Express will accept the shipment of toxins if they are labelled "ETIOLOGICAL AGENTS, NOT OTHERWISE SPECIFIED", and provided the amount is less than one human lethal dose.

A REVIEW OF THE EFFECTS OF ALGAL BLOOMS ON SHELLFISH AND AQUACULTURE

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Toxic algal blooms occur worldwide and in some areas they are a common and seasonal occurrence. Historically, attention has been focused on blooms of toxic dinoflagellates (e.g. *Protogonyaulax tamarensis*). More recently, attention has been turned to other species (e.g. *Dinophysis, Aureococcus, Gymnodinium*). These blooms often present problems with respect to optimal utilization of the shellfish resources and the magnitude of economic losses can be catastrophic. Nevertheless, successful culture facilities and commercial harvests persist in areas prone to toxic algal blooms. This paper reviews the literature available on occurrence of toxic algal blooms, the means by which harvesters, managers and industry cope with the problems associated with toxic algal blooms, and makes recommendations for the most efficient and successful utilization of resources in the face of environmental instability.

INVITED TALKS

HUMAN HEALTH ASPECTS

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Many intoxications are associated with ingestion of fish and shellfish. Excluding the infectious complications, paralytic shellfish poisoning (PSP) is the most common in Canada. More recently, Canada experienced an outbreak related to toxic mussel ingestion which caused an illness now called amnesic shellfish poisoning (ASP). These intoxications are caused by marine phytoplankton which secrete metabolites toxic to man when ingested in high concentrations. In the case of shellfish intoxications the plankton is concentrated in the digestive tract of the molluscs which are then consumed. Unfortunately, no serum test exists to confirm the diagnosis. It is usually based on clinical symptomology, and biological and chemical testing for various toxins in the suspected shellfish. The epidemiology and symptomology of these two common intoxications are listed below.

	AMNESIC SHELLFISH POISONING	PARALYTIC SHELLFISH POISONING
ORGANISM	*Nitzschia pungens* f. *multiseries*	*Alexandrium tamarense*
TOXIN	domoic acid	saxitoxin, neosaxitoxin, gonyautoxins
SEASON	November - January	May - November
VEHICLE	shellfish (mussels)	shellfish (clams, mussels, etc.)
LOCATION	Prince Edward Island (Canada)	NW Pacific, NE Atlantic
INCUBATION	15 min - 24 h (median 3 - 5 h)	within 30 min
DURATION	hours to months	6 h to 7 days
SYMPTOMS	nausea, vomiting, diarrhea, abdominal cramps, anorexia	nausea, vomiting, abdominal pain; diarrhea less common
Neurologic	decreased reaction to deep pain; long-term neuropathy	paresthesia, floating feeling
Motor	none	weakness, motor and repiratory paralysis
Cranial nerves	dysarthria, unusual ocular movements, paresis	dysarthria, dysphonia, dysphagia
Cerebellum	dizzyness, loss of balance, ataxia	vertigo, ataxia, nystagmus
Other	disorientation, confusion, memory loss, hallucinations, seizures	aphasia
Cardiovascular	blood pressure fluctuations, arrhytmias	tachycardia, ECG changes
Other	headache	headache, dysuria
LAB	increased CPK, WBC	increased CPK
THERAPY	??	supportive
FATALITY	3% - ?	2.6 - 23.2%

THE DFO INSPECTION PROGRAM: PHYCOTOXIN MONITORING

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The basis of the Canadian Fish Inspection Program is scientific. Science will even be more closely linked to the program in the future. In the scientific community the answer to one question leads to another. In an operational program such as the Inspection Program, "go" or "no go" decisions are the rule. On what basis can these two disciplines become compatible? Initial research and methods developed by the scientific community permitted DFO to set up an effective early monitoring program for phycotoxins. Data from the monitoring program are also proving useful to scientists. New knowledge, procedures and methods can improve the program efficiency.

ANALYTICAL METHODS FOR THE DETECTION OF MARINE PHYCOTOXINS

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Since the 1930's, when toxic phytoplankton were first recognized as a proximal cause of toxicity in marine bivalves, the detection of these toxins has generally been based upon conventional mammalian bioassays. In recent years, chromatographic techniques, including thin layer-, low pressure ion-exchange- and high performance liquid (HPLC)-chromatography, have been introduced, with the aim of supplementing or replacing to some extent these bioassay methods. Work has also advanced on immunological techniques, particularly radioimmunoassays (RIA) and enzyme-linked immunosorbent assays (ELISA), which may be incorporated into rapid high sensitivity test kits for specific toxins. Since the ideal analytical method for marine phycotoxins may not correspond with the requirements of a routine surveillance program, these various techniques will be compared with respect to sensitivity, specificity, ease of application, and cost. Comparative data for the AOAC mouse bioassay, the fluorescence-based HPLC technique, and the ELISA immunological method developed by the Institut Armand-Frappier for paralytic shellfish (PSP) toxins will be discussed. In this context, a brief introduction to similar approaches applied to the analysis of diarrhetic shellfish (DSP) toxins and brevetoxins (BTXs) will also be presented. This ongoing work will be placed in a historical perspective, with reference to potential future developments in routine analytical methods for marine phycotoxins.

INSTRUMENTAL ANALYTICAL METHODS FOR MARINE TOXINS

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Marine organisms produce many toxic metabolites, some of which are responsible for numerous seafood poisonings each year. As well as being hazardous to public health, such toxins can have a significant economic impact, as demonstrated by the 1987 toxic mussel incident in P.E.I. The most commonly used technique for detecting seafood toxins is the AOAC mouse bioassay. Although this method has the advantage of being non-selective and thus well suited for protection of the public, it is recognized that bioassay suffers from considerable variability and gives little information on toxin composition. Instrumental methods of analysis have the potential for more sensitive and selective detection of known toxins, and for the identification of new toxins. This paper will present an overview of present and future instrumental methods for three classes of toxins responsible for amnesic shellfish poisoning (ASP), diarrhetic shellfish poisoning (DSP) and paralytic shellfish poisoning (PSP).

TOXIC PHYTOPLANKTON: HOW IT IS AFFECTING THE MUSSEL CULTURE INDUSTRY

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The young Prince Edward Island mussel industry has had its share of problems since it started less than 10 years ago. Although most growers were aware of the potential risks associated with marine toxins, P.E.I. waters were reputed to be devoid of toxin-producing phytoplankton. The domoic acid episode therefore caught everybody by surprise. This outbreak has had both short-term and long-term effects on the industry, which has suffered from continued negative media publicity. More intensive monitoring and better records of bloom conditions may help to predict the occurrence of harmful blooms. At the same time, spreading mussel operations into different estuaries and stocking larger inventories at processing plants will minimize risks of production and market losses for individual growers.

The industry has developed faster than the scientists could keep up, resulting in a communications gap. More than one problem slows down the development of the P.E.I. mussel industry, and strong support is required from the scientific community. For example, summer die off of standing crop has occurred on a regular basis over the last few years, and has yet to be thoroughly investigated to help growers alleviate the consequences of such loss. The P.E.I. Mussel Growers' Association will strive this year to bridge the communication problem with government researchers by identifying ways of disseminating scientific information in a timely and effective manner. The P.E.I. mussel industry looks toward the future with reasonable optimism. Stabilization of production and prices, opening of new export markets, innovative processing techniques, and better promotion of the specific features of the Island Blue and its

home waters should give the industry the necessary strength to prosper. It can live with toxic phytoplankton provided that an adequate monitoring program is operated to safeguard it against unpredicted noxious blooms.

POSTER PRESENTATIONS

EFFECTS OF *Aureococcus anophagefferens* ("BROWN TIDE") ON THE LATERAL CILIA OF 5 SPECIES OF BIVALVE MOLLUSCS

Christy Draper[1], Louis Gainey[1], Sandra Shumway[2,3], and Lynda Shapiro[3]

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Aureococcus anophagefferens had no effect on the lateral ciliary activity of *Mya arenaria*, *Geukensia demissa*, and *Argopecten irradians*. *Aureococcus* caused a significant decrease in the lateral ciliary activity in isolated gills of *Mercenaria mercenaria*, and *Mytilus edulis*. The lateral cilia of the species that were unaffected by *Aureococcus* were also unaffected by the neurotransmitter dopamine, while the lateral cilia of the species inhibited by *Aureococcus* were also inhibited by dopamine.

OYSTERS AND TOXIC ALGAL BLOOMS: ARE THEY IMMUNE?

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A literature survey has indicated that various species of oysters exhibit very low levels of toxicity when exposed to toxic algal blooms. A study was begun in our laboratory in the spring of 1988 when oysters (*Crassostrea virginica* and *Ostrea edulis*) were suspended in cages with mussels, *Mytilus edulis* in Boothbay Harbor, Maine. Samples were taken of all species on a bi-weekly basis to assess the toxicity due to *Alexandrium (Protogonyaulax) tamarensis*. Preliminary results indicate that oysters rarely become toxic, even when mussels show high levels of toxicity in the same area. Field data are presented in conjunction with a literature review.

DOMOIC ACID CONTAMINATION OF SHELLFISH IN ATLANTIC CANADA DURING 1988

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During the late fall of 1987, shellfish contaminated with domoic acid, probably from a toxic diatom, *Nitzschia pungens*, were responsible for severe illness in many Canadians. The occurrence of domoic acid contamination in shellfish from Atlantic Canada in 1988, was evaluated by HPLC analysis of extracts prepared by the AOAC procedure for PSP toxins. Several outbreaks of severely contaminated shellfish occurred. These were characterized by initial low-level preliminary contamination followed by an abrupt rise to severe levels and an equally abrupt decline in the domoic acid content. The toxin was first detected in mussels (*Mytilus edulis*) and soft-shell clams (*Mya arenaria*) collected from the shore in late July-early August in the Bay of Fundy and vicinity. Peak toxin accumulation (73 and 33 $\mu g.g^{-1}$, respectively) occurred in these species in September-October, then declined rapidly to not detectable. In the area which produced the toxic shellfish of the previous winter, Cardigan River, P.E.I. and environs, the toxin accumulation did not become significant until late November and peaked in December (max. 343 $\mu g.g^{-1}$).

Nitzschia pungens AND MUSSEL-DOMOIC ACID CONCENTRATIONS IN EASTERN PRINCE EDWARD ISLAND, NOVEMBER 1988 - FEBRUARY 1989

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Nitzschia pungens and mussel-domoic acid concentrations are given for Brudenell R., Cardigan R., Montague R., St. Marys Bay, Murray R. and Boughton R. for November 1, 1988 to February 19, 1989. In all systems *Nitzschia* concentrations rose rapidly, reaching a peak around November 10-14 (highest about 2×10^6 cells.L^{-1}), declining rapidly thereafter, reaching zero concentrations by December 15-31. Domoic acid levels followed closely with a lag of 9-14 days, peaking on November 25 (highest, 343 ppm in mussels). A minimum concentration of about 2-4 $\times 10^5$ cells.L^{-1} over a minimum of 3 to 4 weeks appears required to produce 20 ppm domoic acid (government-set limit) in mussels.

THE ULTRASTRUCTURE OF *Nitzschia pungens* forma *multiseries* (Grun ex Hasle)

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A natural population of *Nitzschia pungens* forma *multiseries* producing domoic acid was obtained from the Cardigan River system, P.E.I., in December 1988. The sample was examined by scanning and transmission electron microscopy for evidence of an extra-genomic vector for domoic acid production. This study described external and internal features as being normal. No evidence was found for viruses, ectosymbionts or endosymbionts. Domoic acid production is therefore likely to be due to an intrinsic physiological-genetic mechanism.

DIATOMS THAT PRODUCE DOMOIC ACID

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The toxic mussel episode of 1987/88 in Prince Edward Island marked the discovery of a new shellfish toxin, domoic acid, and for the first time implicated diatoms in shellfish poisoning. A bloom of the planktonic, chain-forming pennate diatom *Nitzschia pungens* f. *multiseries* was found to be the primary source of domoic acid in this instance. However, a much less abundant, benthic pennate species, *Amphora coffeaeformis*, was isolated from toxic mussels and was also observed to produce domoic acid. Subsequently, a second chain-forming species of *Nitzschia*, *N. pseudodelicatissima*, was determined to be a source of domoic acid in the Bay of Fundy. As an aid to plankton-monitoring services, we present optical and electron micrographs of these taxa, to illustrate key features for their identification. The two species of *Nitzschia* are also compared with closely related *N. seriata*, which was present in the affected area of Prince Edward Island during the 1987/88 episode but has not produced domoic acid in culture and appears to be innocuous.

DISTRIBUTION OF TOXIC PHYTOPLANKTON SPECIES IN SOUTHERN GULF OF ST. LAWRENCE COASTAL WATERS

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Several species of toxin-producing phytoplankton have been detected in the southern Gulf of St. Lawrence, mainly the diatom *Nitzschia pungens* (domoic acid) and the dinoflagellates *Alexandrium tamarense* (PSP), *Dinophysis norvegica, D. acuminata, D. acuta,* and *Prorocentrum lima* (DSP). Among water samples collected in more than 70 sites using bottles, nets or pumping, and analyzed under the microscope for the presence of any of the known or putative toxin-producing phytoplankton species, results from 18 sites were compiled. A qualitative visual summary is given of the occurrence of these species along the Gulf coasts of New Brunswick, Nova Scotia and P.E.I. between July 1988 and August 1989. In some instances semi-quantitative information is also given. So far, outbreaks of both domoic acid and PSP have been documented in the last three years in the area of interest. The presence of numerous phytoplankton species, known to be DSP-producing species in other parts of the world, suggests that it may just be a matter of time for this family of toxins to be found in molluscan shellfish from the southern Gulf of St. Lawrence. *N. pungens* is most abundant in late fall and early winter, when it may provoke toxification of filter feeders like the blue mussel, *Mytilus edulis*, as experienced in 1987, 1988 and 1989. DSP-producing species seem to prefer warmer waters as found in this region in summer and early fall. In October 1988, they had practically disappeared from this area. Although more fragmentary, our information on *A. tamarense* suggests that spring (as early as late March) to early fall (September) is the period to watch.

THE EFFECT OF DOMOIC ACID ON ZOOPLANKTON

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Zooplankton play an important role in the development and maintenance of phytoplankton blooms. As domoic acid has been demonstrated to have strong insecticidal properties and possesses functional similarity to the crustacean neurotransmitter L-glutamate, a series of bioassays was carried out to determine the toxicity of domoic acid towards zooplankton. The copepod *Pseudocalanus acuspes* was used as a test species. Domoic acid was found to be toxic to *P. acuspes* eliciting a 24-h LC_{50} at 84 $\mu g.mL^{-1}$ and a 48-h LC_{50} at 52 $\mu g.mL^{-1}$. Filtered seawater and L-glutamate controls did not produce significant mortality. These results and an outline of current research will be presented.

REPORTS FROM SESSION CHAIRPERSONS

Keynote Address

Theodore Smayda presented evidence from several seas that there is currently a global spreading and increase in frequency of phytoplankton blooms, both benign and harmful. Examples of toxic dinoflagellate species that have spread include: *Gyrodinium aureolum* from the north-east coast of the U.S. to the western Norwegian coast; *Gymnodinium catenatum* from southern Californian waters to shellfish rearing areas in Spain, Japan and Tasmania; *Ptychodiscus brevis* from the Gulf of Mexico to the North Carolina coast; the Indo-Pacific spreading of *Pyrodinium bahamense* var. *compressa*; and the spreading within Japanese waters of *Alexandrium tamarense*, *Gymnodinium catenatum*, *Dinophysis fortii*, and *D. acuminata*. The mechanisms for such spreading remain enigmatic, but transport of cyst stages in ballast water of cargo ships and in altered current patterns remain possibilities. It may also be that some of these species were indigenous, and previously went unreported because they remained a "hidden" component of the flora.

Examples of a long-term increase in the annual frequency of red tide-blooms at a given locality were presented for the Bay of Fundy (Canada), Tolo Harbour (Hong Kong), and the South African coast. There has also recently been an increase in the incidence of novel harmful phytoplankters: *Chrysochromulina polylepis* in Scandinavian waters, *Chaetoceros* spp. off the coast of British Columbia (Canada), and *Nitzschia pungens* forma *multiseries* in eastern Prince Edward Island (Canada). During the last decade there has been a shift from dinoflagellates to flagellates, particularly the Rhaphidiophyceae, implicated in red-tide blooms, and, as the above list shows, an emergence of harmful diatoms.

To address the possible causes of the global epidemic of harmful phytoplankton blooms, Dr. Smayda drew on long-term data sets collected in Nordic waters. He presented evidence from the Baltic, Kattegat, Skagerrak, Dutch Wadden, and North seas that there has been a progressive nutrient enrichment, in progress for about two decades, accompanied by increased primary production and the occurrences of exceptional, unusual or novel bloom episodes. For example, total nitrogen and phosphorus levels increased 5.3- and 1.7-fold, respectively, in surface waters of the Skagerrak during a 12-year (1971 - 1982) period, with a concurrent increase in primary production. Over a 25-year period (1964 - 1988) the waters of the Skagerrak, Kattegat and Baltic Sea generally have exhibited unusual blooms involving at least 11 different taxa, characterized by apparently newly-introduced, aggressive, toxic species. Within the Wadden Sea, coincident long-term increases were reported in phosphate concentrations and in primary production. In Dutch coastal waters, riverine inputs of nitrogen and phosphorus have increased 5- and 7-fold, respectively, since 1930. *Phaeocystis pouchetii* has been a major problem in these waters since 1976, with a significant increase in its annual abundance, its frequency of occurrence within the yearly cycle, and duration of its spring bloom. Similar examples were given for the German Bight, English Channel, and Black Sea. In the Seto Inland Sea (Japan), an increase in nutrient buildup and concomitant bloom frequency was followed by a decrease in the number of annual red-tide outbreaks after effluent controls were initiated in the mid-1970's.

Dr. Smayda then presented an hypothesis, based on the ionic ratios of essential nutrients, to explain the novel floristic changes that accompany the increased nutrient loading. He gave examples of how changes in the ionic ratios are associated with nutrient enrichment. In the Rhine River, the N:P and Si:P ratios decreased by about 6-fold over a 20-year (1955 - 1975) period. A long-term decrease in these ratios was also shown for the German Bight, the Baltic Sea, the Kattegat, and for Romanian coastal waters. At the same time, he showed that significant blooms of non-silicon requiring phytoplankton have emerged with increased frequency and persistence in these waters. For example, there has been an increased number, intensity and duration of *Exuviaella cordata* red-tide blooms in the Black Sea, extensive blooms of the blue-green alga *Nodularia spumigena* in the Baltic Sea, and the emergence and increased predominance of the dinoflagellate *Phaeocystis pouchetii* in Dutch coastal waters. Furthermore, he showed how changes in the Si:P ratio can gate the competition between diatoms and non-diatoms, and that changes in the N:P ratio can regulate competition between blue-green algae and chlorophytes, for example in the Baltic Sea. He emphasized that other factors are involved in regulating bloom events, but that he considers such nutrient ratio effects to be of major importance.

Papers by Dr. Smayda on this topic are published in: E.M. Cosper, E.J. Carpenter and V.M. Bricelj (Eds.), Novel phytoplankton blooms: Causes and impacts of recurrent brown tides and other unusual blooms, Lecture Notes on Coastal and Estuarine Studies, Springer-Verlag, Berlin (1989); and E. Granéli and L. Edler (Eds.), Proceedings of the Fourth International Conference on Toxic Marine Phytoplankton, Elsevier Sci. Publ. Co., N.Y. (1990).

Session I: Production of Marine Phycotoxins

10:35 - 12:05 (Greta Fryxell, Texas A&M University, U.S.A.)

Several main ideas were presented, and some common themes and promising future directions of investigation emerged. It is accepted that *Nitzschia pungens* forma *multiseries* produces domoic acid during stationary phase, apparently brought on as a response to nutrient pulses. In the field, the lag between the onset of a bloom and domoic acid production gives an advance warning of possible need for precautionary measures, and thus the monitoring of phytoplankton and domoic acid production is essential. A three-year monitoring program from 43 inshore sites in the Atlantic Zone has been instituted by the Department of Fisheries and Oceans. It has been found that possible closings of areas for harvest can probably be anticipated a week or even more in advance, as the onset of the third year of *N. pungens* forma *multiseries* blooms appears possible.

Domoic acid has been concentrated in the digestive glands of mussels suspended in the plankton; other parts of the animals have carried less toxin. Less domoic acid has been found in the soft-shell clams in the intertidal zone.

Samples from an early August bloom in Boughton River (Prince Edward Island) have lacked domoic acid at the time of testing, and *Nitzschia pungens* forma *pungens* dominated in one sample. One culture of *N. pungens* (forma *pungens* ??) from Brandy Cove (Passamaquoddy

Bay, New Brunswick) has not produced domoic acid. Previously, *Nitzschia seriata*, in the same section of the genus, has been found not to produce the neurotoxin when tested in culture. Thus, the capability of producing amnesic shellfish poisoning (ASP) toxin may not be in the taxa with the closest genetic relationships.

Not only *Nitzschia pungens* f. *multiseries* from Prince Edward Island, but also *Nitzschia pseudodelicatissima* from Passamaquoddy Bay (New Brunswick) was reported to produce domoic acid in the field and in culture. The only samples which contained domoic acid were from the Passamaquoddy Bay region, and these samples were dominated (95 - 98%) by *N. pseudodelicatissima*. *Nitzschia pungens* was rarely seen, and when it was present, it represented less than 1% of the total cell numbers. It was therefore unlikely that the domoic acid from that region originated from *N. pungens*.

Different methods were used to ascertain the presence of domoic acid in the above studies, and if they prove to be comparable in the future, the capability to produce domoic acid under certain conditions may be widespread. Thus, it is essential that methods be compared before the source of the toxin associated with diatoms, possibly even a bacterium, is to be searched out, dealt with directly, and regulated effectively.

In contrast, off British Columbia, records for the last decade have been kept from the continental shelf, not inshore, and they indicate that during April to October, *Nitzschia pungens* only occasionally dominates off the southwest coast of Vancouver Island. Scanning electron micrographs, taken from a sample off Brooks Peninsula during August 1989, indicate that *N. pungens* f. *pungens* is most abundant, but that f. *multiseries* is present. Although *Nitzschia pungens* has not yet been shown to produce domoic acid on the west coast, its presence is of concern, and samples will continue to be collected for analysis.

13:30 - 14:30 (W. Irwin Judson, Prince Edward Island Department of Fisheries and Aquaculture, Charlottetown)

The second part of Session I consisted of presentations summarizing the culture of *Nitzschia pungens* under laboratory and mass culture conditions. The primary theme common to all three presentations was the attempt to understand the biological and biochemical mechanisms of domoic acid production, and the limiting factors (e.g., light, nutrients) that control the production of this toxin.

It has been well documented that domoic acid production does not occur during the exponential growth phase, but only when cell division ceases. It seems apparent from the work of Anthony de Freitas and Stephen Bates that both light and nutrients play a part in the production of domoic acid. They showed that there is no production of domoic acid during darkness by *N. pungens* in culture and that nitrate must be available to the cells during the stationary phase for domoic acid production to proceed.

In the culture of *Nitzschia pungens* for the production of domoic acid to be used in feeding experiments, it is important to be cognizant of the fact that, at the present level of expertise in such culture, the cells become progressively smaller through successive generations. Consequently, one must take into account the size-selectivity of the cells by mussels during feeding experiments, especially when non-toxic control cells of a different size are being used.

A rather interesting phenomenon noted by Randy Angus was a morphological abnormality in certain nutrient-limited cultures. In this instance a peculiar zig-zag shape was seen in the individual cells of certain *N. pungens* chains.

Another general observation common to these discussions concerned the possibility that fungi and bacteria could be playing a role in the production of domoic acid by *N. pungens.* This will be a moot question until such time as axenic cultures of *N. pungens* are available, and it is recommended this line of research be pursued.

The presentations show that, although considerable progress has been made in understanding the production of domoic acid, much research remains to be done to study the inter-relationships of the various nutrients, what the triggers are that switch on the cell to produce the toxin, why the toxin is produced, and to relate these findings to natural conditions.

Session II: Fate of Marine Phycotoxins in the Food Web and the Environment

14:30 - 16:30 (David J. Scarratt, Department of Fisheries and Oceans, Halifax)

It is clear that in addition to determining the abundance and distribution of toxic phytoplankters, a detailed knowledge of their fate is essential. This includes not only the environmental and commercial implications of toxic blooms, but also the mechanisms for the breakdown and dissipation of the toxins themselves. This session started with a description by David Wildish of a recirculating flume for conducting bivalve feeding studies. The flow rate of water over the bivalves can be maintained at a preselected level and seston automatically introduced by means of a pump controlled by an infrared light switch. A multichannel data logger provides a temporal record of bivalve feeding.

Jennifer Martin discussed the anatomical distribution of paralytic shellfish toxins in clams from two closely-adjacent sites in Charlotte County, N.B., which differed in that clams were toxic year-round at one site, and seasonally at the other. While high toxin concentrations were found in the digestive gland, highest values were in the kidney and heart and other soft tissues surrounding the digestive gland. The sequencing of the various toxins changes throughout the year, suggesting either interconversion or selective retention of different toxins. By contrast, Katz Haya showed that in mussels from a third Charlotte County site, nearly 90% of the toxin is located in the digestive gland. Mussels held at the surface were most toxic, whereas those at 5 m depth or more did not accumulate toxicity. Brenda Waiwood discussed the occurrence of PSP toxins in mackerel following a bloom of *Alexandrium fundyense.* The data strongly suggest that mackerel do not accumulate PSP toxins exclusively from *A. fundyense.*

Carl Boyd gave an illuminating overview of the mechanisms by which neurotoxins affect the ionic control mechanisms of cells. Their molecular structure is such that specific ion channels through the cell are blocked, and cells lose the capacity to function normally. This phenomenon may be used as a research tool in its own right.

08:30 - 10:00 (John C. Smith, Department of Fisheries and Oceans, Moncton)

The second part of Session II consisted of presentations concerning the uptake and depuration of domoic acid in molluscs, the effects of algal blooms on shellfish and aquaculture, and a summary of selected topics from the 4th International Conference on Toxic Marine Phytoplankton held in Lund, Sweden in June 1989.

In order to study the uptake and depuration of domoic acid in the laboratory, it is necessary to produce toxic shellfish under artificial conditions. This can be achieved by feeding with cultured toxic *Nitzschia pungens* or with an artificial food source containing domoic acid; preliminary results of work utilizing both approaches were described during this session. Irené Novaczek used synthetic liposomes as a carrier for domoic acid and discussed the advantages and disadvantages of this method. The advantages are that liposomes are easy to produce, and mussels do in fact clear such liposomes from the surrounding medium. It is also possible to encapsulate a radiolabelled molecule in these liposomes, thus enabling the study of the deposition of domoic acid in various shellfish tissues by autoradiography as well as clearance rates by liquid scintillation counting. It is also possible to produce synthetic liposomes with a variety of concentrations of domoic acid. Some reservations were expressed from the floor, however, that the 2 to 5-μm diameter synthetic liposome particles might not be large enough to be suitable as a mussel ration. Results of these studies so far show that, although mussels can clear a significant proportion of the synthetic liposomes from the medium, only a very low proportion of the domoic acid contained therein is actually retained by the shellfish, and that this retained fraction is very resistant to depuration. It appears possible that manipulation of the cytosolic free amino acid concentration by variations in salinity could facilitate the clearance of this residual domoic acid, although this is not liable to be important unless the action level for closing harvesting areas is lowered significantly.

David Scarratt described the culture of *Nitzschia pungens* in his laboratory. At 15°C, reasonable growth was obtained in carboys (in excess of 200,000 cells.mL^{-1}), but difficulty with contamination was experienced when attempting to scale these cultures up to 230 L cylinders. Based on results by Dr. Michael Gilgan that have shown a large variance in domoic acid concentrations among individual mussels, Dr. Scarratt has calculated that, for uptake and depuration studies, he will study a large sample of 300 market-size mussels and thus will require approximately 40 L of mature *N. pungens* per day, for 10 to 15 days, at a concentration of 200,000 cells.mL^{-1} with an average of 5 pg domoic acid per cell. Toxic *N. pungens* as a source of domoic acid has the advantage of being a natural food, but the provision of such quantities is a difficult and time-consuming task.

Studies with naturally-toxified mussels have shown that they depurate to the action level of 20 ppm quickly (120 ppm to below 20 ppm in 3 - 4 d) when placed in a clean environment, but that it takes a long time (up to one year in some cases) to completely clear the residual domoic acid. An increased temperature accelerates the depuration process. Why it takes so long to clear the residual domoic acid remains an interesting question for the future. Dr. Scarratt has also studied the behavior of mussels in laboratory suspensions of *N. pungens* and has found that the concentration of cells at which mussels will shift over to pseudofeces production is approximately 1000 cells.mL^{-1}, which is similar to the cell concentrations that can be encountered during *N. pungens* blooms. Under natural conditions, however, mussels accumulate very large quantities of *N. pungens*. This discrepancy, and the possibility of a neurological effect of domoic acid on mussel feeding, remain to be investigated.

Sandra Shumway reviewed the detrimental effects of toxic blooms on the shellfish industry and also stressed the fact that noxious (as opposed to toxic) blooms could have an equally deleterious effect. These outbreaks not only pose threats to public health, but have also been responsible for mass mortalities of shellfish. The problem of toxic phytoplankton outbreaks appears to be spreading, and both the frequency and severity of these events seems to be increasing. Additionally, the list of phytoplankton species implicated in toxic blooms is lengthening. Anthropogenic input to the coastal zone environment appears to be an important factor in the enhancement of toxic bloom formation and shifts in floristic composition, but it is also necessary to consider the possible effects of long-term climate changes on these phenomena. Toxic algal blooms have a drastic impact on the shellfish industry because of harvesting and marketing restrictions that are necessary to avoid public health dangers. These restrictions cause unemployment for fishermen, processors and those involved in marketing, and result in hesitancy of new growers to enter the industry, and in consumer wariness that gravely affects sales.

Several factors were mentioned that can influence the rates of toxification and detoxification of shellfish. These include the initial toxicity of the bloom, temperature and salinity effects, biological factors such as the age, size, and sexual stage of the shellfish, the toxin binding capabilities of various shellfish species, and the exercise of feeding preference or avoidance by shellfish with respect to various toxic phytoplankton species. Previous work was described which showed that the accumulation of toxins can have a negative effect on shellfish, as demonstrated by abnormal feeding, decreased byssus production, limp syphons, gaping, decreased pumping rates, increased rates of valve closure, abnormal clearance rates, increased rates of mucous production and increased mortality. It was asserted that a monitoring program is presently the best management tool to detect blooms and to determine whether these blooms produce toxins. The early detection of such blooms is important so the industry and the public can be forewarned, and orderly and timely closures of harvesting sites can be assured.

Ewen Todd reviewed selected topics covered at the 4th International Conference of Toxic Marine Phytoplankton, the proceedings of which are published in: E. Granéli and L. Edler (Eds.), Proceedings of the Fourth International Conference on Toxic Marine Phytoplankton, Elsevier Sci. Publ. Co., N.Y. (1990). It has been established that several species of *Dinophysis* and *Prorocentrum* produce okadaic acid and its derivatives. Modelling of *Dinophysis* blooms suggests that vertical turbulence is important in preventing blooms since light does not penetrate very far. The stability of okadaic acid and its derivatives were studied and they proved to be light, heat and oxygen sensitive. Dinophysistoxin-1 (DTX 1) seems to be more unstable than okadaic acid. Hence reproducible results for DTX 1 analysis are more difficult to obtain than for okadaic acid. In the ADAM derivatization for fluorescence detection of okadaic acid, it appears that the reaction time with ADAM for toxin reference substances is commonly too short, and this may give low calibration curves for okadaic acid and DTX 1 and therefore too high toxin content results when used as basis for shellfish analysis. Some laboratories have experienced losses of the okadaic acid derivative on silica cartridges while performing a clean up step. This loss could depend on the quality of silica and solvents used, and should be investigated by each user. Because of the instability and difficulty in obtaining okadaic acid toxins, the development of an internal standard was recommended. One likely choice is deoxycholic acid, because it has chemical and physical properties similar to okadaic acid and DTX 1. An interlaboratory study of the HPLC method for DSP toxins, carried out recently, showed that there were consistent results within each laboratory, but unacceptable variations among the laboratories. In conclusion, it was recommended that laboratories using this HPLC

method in quality control of shellfish products should report acceptable intercalibration results before being given approval to carry out testing. Also, that toxin allowance levels must be defined with a large safety margin to take into consideration the great variation among different laboratories. The need for Canadian laboratories to adopt an appropriate method for the determination of DSP toxins was stressed.

At the conference, Kodama and Ogata presented several cases indicating that there is a close relationship between dinoflagellates and associated bacteria which produce PSP toxins. This relationship seems to be strain specific as well as species specific. There were counter-arguments by Shimizu, indicating the uncertainty of the role played by bacteria in the production of PSP toxins because DNA patterns for toxic and non-toxic strains are similar and strains do not lose their toxicity under different transfer and culture conditions. Also, DNA restriction enzymes used on the dinoflagellates and their associated bacteria do not give common sequences. Other laboratories have not yet been able to isolate the bacteria and repeat the results of Kodama and Ogata.

Session III: Non-Biological Aspects of Marine Phycotoxins

11:10 - 14:45 (Chairperson: Roger Foxall, National Research Council, Atlantic Research Laboratory, Halifax) (Rapporteur: Stephen Bates, Department of Fisheries and Oceans, Moncton)

This session consisted of five invited speakers who gave overviews on human health aspects, the DFO inspection program, analytical aspects, and the shellfish industry perspective. Trish Perl provided us with a clinical viewpoint of ciguatera poisoning, paralytic shellfish poisoning (PSP), and amnesic shellfish poisoning (ASP). It is possible to distinguish these poisonings on the basis of their unique symptomologies. However, the problem at present is that most physicians do not know much about shellfish poisoning and therefore may have difficulty in diagnosing it, especially since no clinical test is available. Of the 145 probable cases of ASP poisoning due to domoic acid, 107 met the strict clinical definition, and four deaths were officially recorded. Risk factors for ASP are: 1) the concentration of domoic acid in the mussels, and 2) host-risk factors, including age and, likely, renal function, which cannot be separated at present. The low illness rate (ca. 2%) of ASP is consistent with the low specific toxicity of this toxin, as was determined during the initial identification of the compound after the 1987 toxicity episode. Physicians learned from the initial ASP episode that the links between the monitoring branch of DFO and the federal and provincial health units were not optimal. Working better in concert would help to recognize any future toxicity problems and to define their frequency.

Régis Bourque gave a brief history of the DFO monitoring program. Prior to the 1987 ASP episode, only 40 - 50 samples were examined per year in the Gulf Region. In 1988, 270 sites were inspected on a regular basis, generating a total of 5,564 samples. Resources were also received to set up bioassay laboratories and/or to purchase HPLC instrumentation in five DFO regions, so that samples can now be analyzed locally. Communications must be kept open between the inspectors and the scientific community. As an example, the Science Branch of

DFO is working closely with the Inspection Branch to investigate the use of phytoplankton as an early warning system for the occurrence of toxic blooms. Such phytoplankton monitoring may also direct inspection efforts towards areas that should be looked at more closely.

Allan Cembella gave an overview and comparison of the use of bioassays, chemical methods, and immunological assays to detect and quantify marine phycotoxins. One can identify three distinct but complementary approaches: 1) national facilities for high performance analytical chemistry to identify novel compounds; 2) research laboratories to analyze toxins in studies of uptake, depuration and metabolism; and 3) facilities for routine monitoring of toxins, requiring a lower resolution but higher throughput. For regulatory purposes, the mouse bioassay technique shows a good correlation of results between laboratories, provides the required sensitivity, and thus has remained the current method of choice for paralytic shellfish toxins. The most commonly used HPLC method employing fluorescence derivatization (Sullivan technique) for the analysis of PSP toxins correlated well ($r = 0.88$) with mouse bioassay values when a broad range of toxin levels (42 to 10,000 µg saxitoxin equivalents per 100 g) were compared. However, within the critical range of 42 to 80 µg $STX_{eq}.100\ g^{-1}$ (the limits of detection for the mouse bioassay and the acceptable regulatory limit for human consumption, respectively) there was no correlation between toxin values obtained by these alternative methods. A peak corresponding to the diarrhetic shellfish poison (DSP), okadaic acid, was detected by HPLC in a plankton tow sample dominated by *Dinophysis* spp. A positive response was also obtained with the same sample using a DSP test kit. Immunological assays involve the use of either polyclonal or monoclonal antibodies. The Institut Armand-Frappier has developed an enzyme-linked immunosorbent assay (ELISA) test kit based upon a polyclonal antibody for saxitoxin (PSP). The kit provides adequate sensitivity (1 to 80 µg $STX_{eq}.100\ g^{-1}$) for regulation purposes. A good relationship was found between levels of saxitoxin detected by the ELISA kit and by HPLC, but total PSP toxin levels are underestimated by the current immunological technique. Although there is a relatively high binding affinity (and thus detectability) for certain other PSP toxin derivatives, including gonyautoxins 2 and 3, neosaxitoxin is only weakly bound, and the N-21-sulfocarbamoyl toxins ($C_{1,2,3,4}$) are not bound at all. Plans are under way to produce specific monoclonal antibodies to at least several of the PSP derivatives. It may not be necessary to have monoclonals for each of the PSP toxins, but rather several antibodies with cross-reactivity towards other members of the same sub-group of toxins, i.e., the saxitoxin or neosaxitoxin "family". A DSP kit, based on an enzyme-conjugated monoclonal antibody reaction, is available from UBE Industries in Japan. Good reactivity is found between okadaic acid and dinophysistoxin-1, but there is poor cross-reaction with yessotoxin and other DSP toxic components. Advances in methodology are hampered by the poor availability of pure PSP and DSP toxin standards.

As pointed out by Michael Quilliam, the mouse bioassay technique for toxin detection is the only USFDA-approved method, but suffers from some major flaws: it cannot detect cryptic toxins, does not provide specific identification, offers low dynamic range, salts (e.g., Zn) may interfere, sensitivity is too low for domoic acid (ca. 150 ppm vs 20 ppm action level), and the route of administration is different from human cases (IP injection vs oral ingestion). In addition, it is expensive, labour intensive and might soon be under pressure from animal rights activists, as is the case in western Europe. Instrumental methods of analysis are desirable for the detection, identification and quantitation of marine toxins, but these compounds present significant challenges since they are usually polar, labile, are often without a chromophore (with the exception of domoic acid), and are present in complex matrices. Several methods exist to detect and quantify domoic acid. Aqueous extraction offers the best recovery, but the AOAC

water/acid method gives comparable results, mainly due to the fortuitous cancelation of two errors. An HPLC-UV method, simple to implement, has a detection limit of 0.7 $\mu g.g^{-1}$ wet tissue. However, identification based solely on retention time may be misleading, and positive UV spectroscopic identification with diode array detection is advisable. A more sensitive method has been developed based on pre-column FMOC derivatization followed by HPLC with fluorescence detection (i.e., 15 $pg.mL^{-1}$ detection limit for seawater samples). An extra clean-up procedure is required for mussel tissues and tight control of blanks is essential. Ion-spray mass spectrometry is an attractive and effective method for most marine toxins. It has so far given spectacular results for both domoic acid and saxitoxin, and has shown promise for DSP toxins. Tandem mass spectrometry allows accurate finger printing for unambiguous confirmation. Instrumental methods are highly sensitive and specific, offer a wide dynamic range, are amenable to total automation, including sample preparation, and give the spectroscopic confirmation often legally required. However, these methods require the availability of reference materials and standards for their development and validation, as well as for calibration of instruments and quality assurance. The NRC Marine Analytical Chemistry Standards Program strives to provide such reference materials and has made available an instrument calibration solution (DACS-1), and a mussel tissue reference material (MUS-1), with documented concentrations of domoic acid.

Wayne Somers discussed the impact of toxic blooms on the shellfish industry on Prince Edward Island. The rapid growth of the mussel industry over the last 10 years has stabilized in large part because of the negative media publicity subsequent to the 1987 domoic acid episode. Mussel growers have a limited defense against natural blooms of toxic phytoplankton. However, they can ensure that more intensive monitoring takes place and they can keep better records pertaining to bloom conditions, thus helping to anticipate future blooms. It is unlikely, however, that mussel growers could cooperate to any greater extent (e.g., by monitoring phytoplankton with microscopes) because of financial and time constraints. The mussel industry can live with toxic blooms, provided that there is adequate monitoring of the blooms, because the domoic acid-producing phytoplankton do not remain for any extended period of time. Currently, the major problem for the industry is the die off of a percentage of the standing crop following spawning in August. Scientific research should be directed in this area. Growers can protect themselves by taking out recently-available aquaculture insurance policies, and by obtaining seed stock from different areas, as there may be a genetic component to die off susceptibility. A mandate for industry is to help bridge the communications problem the industry has with governments and research scientists. Existing aquaculture publications could be used to transmit news about technology advances, scientific breakthroughs, and practical information.

PANEL DISCUSSION

15:30 - 16:30 (Aivars Stasko, Department of Fisheries and Oceans, Ottawa)

Panel Participants:

Greta Fryxell	Texas A&M University, U.S.A.
Irwin Judson	P.E.I. Department of Fisheries and Aquaculture, Charlottetown
David Scarratt	DFO, Halifax
John Smith	DFO, Moncton
Roger Foxall	NRC, Halifax
Régis Bourque	DFO, Moncton
Allan Cembella	DFO, Mont-Joli
Michael Quilliam	NRC, Halifax
Wayne Somers	P.E.I. Mussel Growers' Association, Murray Harbour

The session chairpersons and the invited speakers constituted the panel to discuss future research direction. To start the discussion, Dr. Stasko briefly summarized the topics that were covered during the workshop, and suggested areas that might be addressed during the panel discussion, namely:

- Phycotoxin Production
- Environmental Fate of Phycotoxins
- Inspection Approaches
- Analytical Techniques and Standardization
- Prediction of New Phycotoxin Events
- Communicating Scientific Information to Industry

The course of the discussions, although not rigidly tied to the above list, did address the suggested topics, except the environmental fate of phycotoxins. The following is a brief summary of the discussions.

A question was raised about the most useful units for presenting data on toxin concentrations. In some situations, bivalves do not concentrate, but specifically reject, the toxic organisms. Thus, would it be useful to specify the concentration of toxins in the water, or in the mussel; the whole mussel or in the digestive gland? Concentration of toxins per unit of chlorophyll *a* was raised as another possible measure. The latter unit was deemed to be of limited value, since often more than one toxin-producing species is involved. The overriding consideration for which units are to be used in presenting toxicity data must, in the end, be determined by the needs of the user, be it researcher, grower or consumer of mussels.

Standardization of analytical methods is a concern. The National Research Council will soon have an ion-spray mass spectrometer, against which the chromatography methods can ultimately be calibrated. However, as long as a reference toxin standard is available, any method can be checked against the standard. Domoic acid is now available as an instrument calibration standard and as reference material. For inspection purposes, the same methods should be used consistently by all inspection units.

Standardization is also needed in identification of phytoplankton species. The consistency in identification must be improved through cross-calibration among laboratories. A linking is needed between the chemotaxonomy and morphotaxonomy through a field method to identify whether the toxin is actually present in the organisms.

In response to industry concerns about causes for mussel die off, it was suggested that this is a site-specific problem. Local experience and visual clues can predict when the problem will start and which mussel strings will be affected. Another industry concern is the carrying capacity of a particular location with regards to growth rates and disease. Some modelling on carrying capacity has been attempted in Europe, but each site has its own local controlling factors. Trial and error has been the practical way in the European experience. Generic models should be possible for biological aspects. However, the physical forcing functions (volume, tidal exchange, freshwater run-off) are what varies the most between locations; eventually, it should be possible to adjust for the physical factors in models. One opinion was that not modelling, but multivariate analysis of local data is needed.

Returning to toxins, a question was raised whether toxins unknown at present could be identified on the basis of chemical structure. The difficulty with this approach is the large number of organisms and chemical compounds that would have to be screened. It is just not practical to screen for as yet unknown compounds. Some indication of a problem, a "flag", is needed on which to focus. Even for ciguatoxins, the chemical structure was only recently determined. In the meantime we can be prepared by keeping in touch with work around the globe, and by keeping up our analytical skills. In Japan, marine biotechnology is becoming a high national priority area for industry, attracting major investment.

Communication between scientists is excellent in the phycotoxins field. But communicating scientific knowledge to the aquaculture industry requires different channels and can be improved. For instance, how will some of the more-pertinent items from the present workshop be communicated to the industry? Newspapers and aquaculture trade journals are useful channels that could be used more frequently. A ready-made channel for communicating with the industry is the bulletin, operated by the P.E.I. Department of Fisheries and Aquaculture, that reports results of the routine sampling to the shellfish industry. This vehicle is available for communicating broader scientific knowledge. Similar channels in other provinces should be utilized where such exist. On the Pacific coast, the B.C. Salmon Farmers' Association operates a 1-800 telephone number that reports on results from a monitoring network. Samples are collected and provided to a contracted person for analysis and reporting daily via the 1-800 number. This telephone system also allows callers to add to the daily message by reporting up-to-date observations in their area. This appears to be a successful and very rapid means of communication. Computer bulletin boards could be used if enough growers have computers. From the inspection viewpoint, the end goal must be adequate communication to avoid a crisis such as in 1987. One goal of communication should be to decide what are the right questions to ask of research. Getting the right answers to the wrong questions is wasteful. Research must be focused on real problems and conducted in a way that leads to solutions.

RESEARCH PROGRAMS OPERATED BY FEDERAL AND PROVINCIAL AGENCIES

NATIONAL RESEARCH COUNCIL OF CANADA

Before the domoic acid crisis in Nov/Dec 1987, the Atlantic Research Laboratory (ARL) of the National Research Council was studying the chemistry and analysis of the paralytic shellfish poisons (PSP) in a collaborative effort with DFO and NHW through an unsolicited proposal (UP) with Dr. Peter Wangersky of Dalhousie University. Dr. Wangersky is being funded by NRC until December 31, 1989 to continue supplying the NRC with dinoflagellate cells from his turbidostat cultures. Since the discovery of domoic acid as a new shellfish toxin, ARL's interests in shellfish toxin research has expanded to include domoic acid and related compounds, as well as the diarrhetic shellfish poisons (DSP), through a collaborative research agreement with OceanChem Research Ltd., Dartmouth, N.S. Recently Dr. Roger Pocklington (DFO) has been seconded to ARL to assist in the development of analytical methods for the detection of shellfish toxins. In point form, research activities in shellfish toxins include:

Domoic Acid:

1. Successful development and implementation of a routine LC-UV method for the analysis of domoic acid in a variety of matrices.

2. Development of a trace analytical method (LC-fluorescence) for domoic acid in seawater and plankton samples.

3. Development of a GC-MS method for analysis of domoic acid.

4. Studying novel LC-MS methods for the separation and detection of domoic acid and related compounds.

5. Introduction of an instrument calibration solution and mussel tissue reference material for domoic acid analysis.

6. Identification and laboratory synthesis of the major isomers of domoic acid found in mussel tissue and phytoplankton.

7. Assessing the biological activity of these isomers and derivatives.

8. Studying the effects of domoic acid towards zooplankton.

9. Successful medium-scale culture of *Nitzschia pungens* forma *multiseries* to produce domoic acid.

10. Examining physiological factors affecting the growth of *N. pungens* and the production of domoic acid.

11. Determining the capability of some other *Nitzschia* spp. to produce domoic acid.

Paralytic Shellfish Poisons:

1. Successful construction and operation of an HPLC-fluorescence system for analysis of PSP toxins, following the Sullivan procedures.

2. Examining the isolation, purification and identification of selected PSP toxins on a mg scale for assessment of new or improved chemical analytical methods.

3. Supplying Institute Armand-Frappier with selected PSP toxins for development of mono- or polyclonal antibodies.

4. Examining the feasibility of producing a scallop-tissue reference material.

5. Studying the development of novel LC-MS methods for the separation and detection of PSP toxins.

Diarrhetic Shellfish Poisons:

1. Examining the efficiency and applicability of the published ADAM-HPLC-fluorescence method for the analysis of okadaic acid.

2. Examining other analytical methods such as LC-MS for the detection of DSP toxins.

3. In collaboration with the NRC laboratory in Montreal (Dr. Charles Holmes), assess the applicability and reliability of an enzyme bioassay for DSP toxins.

4. Attempting to obtain okadaic acid and other DSP toxins from alternate sources (e.g., sponges, plankton and shellfish).

5. Examining the feasibility of culturing DSP-producing dinoflagellates with a view to refining analytical methods and obtaining mg amounts of DSP toxins.

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NATIONAL HEALTH AND WELFARE, HEALTH PROTECTION BRANCH

BUREAU OF CHEMICAL SAFETY ON SEAFOOD TOXINS

Completed Activities:

1. Completed successful collaborative study of HPLC method for domoic acid in mussels.
2. Participated in interlaboratory study on extraction methods for domoic acid in shellfish.
3. Presented data relating to domoic acid toxicity in mice, rats and monkeys at Domoic Acid Symposium in Ottawa, April, 1989. Proceedings should be available soon.

Ongoing Activities:

1. Developing antibodies to domoic acid in rabbits. Assessing their use in development of RIA and ELISA procedures to permit rapid screening of domoic acid in blood and urine. Results promising.
2. Participating in a preliminary collaborative study of the Sullivan method for PSP toxins by HPLC post-column derivatization (Hungerford, FDA).
3. Investigating alternative HPLC procedures (pre-column chromatographic derivatization) for PSP toxins.
4. Initiating studies to assess disposition of tritiated-domoic acid in mice, rats and monkeys. C^{14}-labelled domoic acid would be preferable.
5. Studies will be initiated on analytical procedures for DSP when standards become available.

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DEPARTMENT OF FISHERIES AND OCEANS

Long before the domoic acid outbreak of December 1987, the Department of Fisheries and Oceans (DFO) had programs in place to monitor and study the existing populations of harmful algae. Most of the research and control was targeted at the PSP-associated species, especially in the Bay of Fundy, in the St. Lawrence estuary and in coastal waters of British Columbia. The mussel scare prompted the initiation of new projects with a coordinated interregional strategy in order to ensure optimal efficiency of resources. The following summarizes the main research thrusts on marine phycotoxins within DFO, indicates the toxins in question, and identifies the DFO regions where the projects are operated during the current fiscal year:

1. **Chemical Methodology:**
   Development and implementation of analytical techniques and assays to detect and quantify marine phycotoxins (3 projects: Québec, Scotia/Fundy; PSP; domoic acid).

2. **Phytoplankton Dynamics:**
   Large-scale monitoring of harmful phytoplankton blooms (Atlantic Zone Phytoplankton Monitoring Program) and their dynamics; biochemical, physiological and genetic studies (8 projects: Gulf, Newfoundland, Québec, Scotia/Fundy; PSP, DSP, domoic acid).

3. **Shellfish Feeding Preferences (Uptake and Depuration):**
   Uptake and depuration of marine phycotoxins by filter feeders; feeding behavior (6 projects: Gulf, Québec, Scotia/Fundy; PSP, domoic acid).

4. **Biological and Biochemical Aspects of Phycotoxin Production:**
   Biological and biochemical mechanisms of toxin production, including control factors and biochemical pathways (4 projects: Gulf, Scotia/Fundy; PSP, DSP, domoic acid).

5. **Aquatic Toxicology:**
   Direct impacts of marine toxins on marine animals, including larval and adult finfish, and zooplankton (4 projects: Québec, Scotia-Fundy; PSP, domoic acid).

6. **Fate of Phycotoxins in the Environment:**
   Fate of domoic acid once released into the water through cell excretion and/or cell decay (1 project: Scotia/Fundy; domoic acid).

7. **Inspection Directorate Program:**
   Wide-scale regional surveys of molluscan shellfish in order to detect the occurrence of marine toxins in tissues and to provide industry with early warnings (all DFO regions; PSP, domoic acid).

In addition to these in-house projects, several researchers are actively involved in collaborative studies with other agencies, e.g., fine tuning of immunological assays, and production of analytical standards for PSP toxins and domoic acid.

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ATLANTIC VETERINARY COLLEGE

THE UNIVERSITY OF PRINCE EDWARD ISLAND

Gerry Johnson (AVC; 902-566-0853):

1. Depuration of domoic acid from contaminated mussels.
2. Haemocyte disease in *Mytilus edulis.*
3. Histopathology of domoic acid intoxication in *M. edulis* from the Cardigan River.

M.S. Nijjar (AVC; 902-566-0802):

1. Measurement of domoic acid by HPLC in mussels to assist industry with their management decisions.
2. Development of HPLC methods to measure PSP and DSP toxins in extracts of aquatic species.
3. Domoic acid uptake, tissue distribution and elimination by mussels under different climatic conditions.
4. Cellular basis of domoic acid-induced neurotoxicity:
   - calcium uptake by nervous tissue
   - glutamate release from synaptosomes
   - signal-transduction in neuronal membranes, i.e., cyclic nucleotides and related enzymes

David Sims (AVC; 902-566-0812):

1. Determination of optimal fixation methods for *Mytilus edulis* tissues and *Nitzschia pungens* samples.
2. Histological comparison of control and domoic-acid-loaded mussels and *N. pungens.*
3. Determination of the possible presence of ectoparasites or symbionts in the mucous slime of *N. pungens.*

R.A.R. (Andy) Tasker (AVC; 902-566-0662):

1. Absorption, distribution and elimination kinetics of domoic acid in rodents.
2. Behavioral pharmacology of systemically administered domoic acid in rodents: effects of other agonists and antagonists.
3. Neuroanatomical changes in response to EAA agonists, including domoic acid, in rodents.
4. Effects of domoic acid on conditioned behavior (retention and acquisition) in rats.

Louis A. Hanic (UPEI; 902-566-0551):

1. Field survey of *Nitzschia pungens*: presence/absence and concentration (December 1987 - May 1988).
2. Ultrastructure studies of natural populations of *N. pungens.*
3. Large-scale harvesting of *N. pungens.*

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P.E.I. DEPARTMENT OF FISHERIES AND AQUACULTURE

Mussel Monitoring Service

Purpose:

1. To provide mussel growers with information on mussel meat yield, spatfall prediction, and temperature and salinity data for all of the major P.E.I. mussel-growing areas.

2. To monitor the presence of *Nitzschia pungens* and other harmful marine organisms.

3. To serve as a source of information, field trials and samples for academic and government research agencies.

Modus Operandi:

1. Visits are made in rotation to each of the 17 major mussel-growing estuaries approximately every 3 weeks during May to December. A mussel sample is taken (minimum 30 mussels) and a 50 L water sample is passed through a 64 µm mesh sieve. Temperature and salinity are recorded.

2. The mean length of the mussels is obtained, and the mussels are steamed and weighed to give a steamed-meat yield.

3. The water sample is inspected for the concentration and size of mussel larvae, as well as for the presence and concentration of *N. pungens*, and occasionally for other organisms such as oyster larvae and causative organisms of PSP and DSP.

4. Results, with pertinent observations and comments, are mailed the following day to all growers in the particular estuary. When harmful marine algae concentrations are noted, the information is transmitted to the Inspection Branch of DFO.

5. A final report is prepared at the end of the year, summarizing and graphing the data for each estuary. The report is not "peer reviewed", as it is designed to be, above all, a technical advisory service in layman's terms for the grower.

Historical Data:

Data are on file for each year since 1982. This historical data base was used during the 1987/88 domoic acid crisis, as it was the only environmental data, directly related to mussels, available at that time. Effort is currently being made to computerize the storage of files and the generation of daily and annual reports.

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CONCLUSIONS

The first Canadian Workshop on Harmful Marine Algae gave the 133 participants an excellent opportunity to hear about the latest developments in marine phycotoxin research in Canada. A good part of this research was initiated following the "mussel crisis", which crippled the molluscan aquaculture industry in December of 1987. This explains why the *Nitzschia pungens*/domoic acid tandem has received most of the newly-focused attention during the last two years and has motivated so many different studies by a variety of research teams around the Maritime provinces of Canada and elsewhere.

One of the primary targets of this workshop was to help the Department of Fisheries and Oceans identify any gaps that may exist in ongoing research projects. Although the range of subjects covered during the workshop was impressive, and taking into account its specific theme, a number of areas requiring more insight and targeted research were identified:

1. **Chemical methodology:**
   - Purify PSP and DSP toxin standards.
     A number of biochemical and physiological studies cannot proceed due to the lack of primary standards for most of the PSP and DSP toxins.

   - Intercalibrate and standardize analytical methods.
     This aspect is well under way for both PSP toxins and domoic acid. Much research remains to be done for DSP toxins before reliable standardized methods are available for their detection and quantification.

   - Investigate new instrumental methods.
     The need is evident to develop analytical methods which are simpler and less expensive than the HPLC and LC/MS approaches currently used.

2. **Phytoplankton dynamics:**
   - Elucidate and standardize the taxonomic status of toxin-producing phytoplankton species, especially *Nitzschia* and *Dinophysis*.
     The taxonomy of these and other genera is far from being clear. Serious studies into the genetic basis of the existing classification are needed in order to gain better insight into the mechanisms of toxin production and its intra- and interspecific variability.

   - Study the potential impact of aquaculture wastes (i.e., nutrients) on the development of toxic algal blooms.
     The potential link between intensive shellfish and finfish aquaculture, and the development of toxin-producing phytoplankton blooms needs to be addressed.

   - Assess results of the ongoing large-scale phytoplankton monitoring program and incorporate results into the design of a smaller long-term program.
     Such a program should include inshore control stations removed from aquaculture sites and at least one offshore station, such as Georges Bank.

- Synthesize field and laboratory data on toxin dynamics (*sensu largo*) gathered in different projects by using numerical models with a view to improving the prediction of toxic algal blooms.

The complexity of ecological interactions warrants the use of mathematical models to better synthesize and visualize the mechanisms involved in the development and disappearance of toxin-producing phytoplankton blooms.

3. **Uptake and depuration of phycotoxins by molluscan shellfish:**
   - Intensify studies on uptake and depuration.

   Although some relevant projects are already in place regarding domoic acid, the importance of these studies has to be re-emphasized. As a prerequisite, it is essential to master batch-culture techniques for the phytoplankton species of interest. This has recently been achieved for N. pungens *forma* multiseries.

4. **Biological and biochemical aspects of the production of phycotoxins:**
   - Develop methods for isolating and culturing indigenous and non-indigenous *Nitzschia, Alexandrium, Prorocentrum, Dinophysis* and other toxin-producing species.

   This aspect is an essential prerequisite to starting a wide range of other research projects including production of toxins for analytical standards, development of analytical techniques, kinetics of toxin production, uptake studies, taxonomy, etc. The difficulties of culturing species of the genus Dinophysis *are acknowledged, but it is felt that more efforts should be dedicated toward solving the problem.*

   - Intensify studies on the kinetics of toxin production.

   This is already under way this year for domoic-acid-producing N. pungens. *Hardly any research on DSP-producing species has been carried out in Canada, and efforts should be directed to this end.*

   - Study the potential role of bacteria in toxin production.

   More insight is needed into the possible role of exo- or endocellular microorganisms in the biosynthesis of marine phycotoxins, using axenic and bacterized cultures. So far, generation of axenic cultures has not been achieved for N. pungens, *although considerable effort has already been directed to that end.*

5. **Fate of phycotoxins in the environment:**
   - Study the environmental fate (i.e., biodegradation) of phycotoxins, especially domoic acid.

   Little is known of the stability of phycotoxins in seawater, or of their potential for biodegradation by bacteria and uptake by animals and plants.

6. **Non-instrumental methods for phycotoxin detection and quantitation:**
   - Continue to develop immunological test kits.

   The importance of easier and quicker-to-use detection kits with more specificity is recognized. Several such kits are at various stages of completion in Canada and other countries for okadaic acid, saxitoxin and other PSP toxins, and brevetoxin. Research in this direction is presently hampered by the lack of analytical standards (see item 1.).

- Monitor the development of other bioassays.

  In view of a likely ban on mammalian bioassays by EEC countries, several western European countries are investigating alternative bioassays, e.g. cytotoxicity tests (hepatocytes, Chinese Hamster Ovary), protein binding assays, forward and reverse mutation assays, etc. Canadian and U.S. agencies will eventually benefit from these research efforts.

The workshop was stimulating and informative. It permitted the sharing of valuable new information, and generated many personal contacts. These will hopefully result in additional collaborative research projects in the future. The presentations and discussions have demonstrated the need to continue this multidisciplinary effort in the years to come. In this regard, and considering the successful outcome of the first Canadian Workshop on Harmful Marine Algae, the Department of Fisheries and Oceans will sponsor a similar event in 1990. This second workshop, organized by the Scotia/Fundy Region of DFO, will be hosted by the Bedford Institute of Oceanography in Dartmouth, Nova Scotia.

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