The Intergovernmental Oceanographic Commission of UNESCO 1993

HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms No. 7

Highlights of Sixth International Conference on Toxic Marine Phytoplankton

Nantes, France, 18-22 October 1993

The first New Zealand outbreak of human shellfish poisoning, an 'Ambush Predator' of fish and the apparent global increase in red tides dominated the Sixth International Conference on Toxic Marine Phytoplankton, held in Nantes, France, 18-22 October 1993.

In the austral summer of 1992/93, New Zealand reported 180 cases of shellfish poisoning. This was the first outbreak of Neurotoxic Shellfish Poisoning (NSP) outside the Atlantic and it involved a complex of poisons plus a new *Gymnodinium breve*-like species. Shifting winds and altered sea surface temperatures associated with 'El Niño' were implicated.

Lurking in the murky depths of the Pamlico Estuary (USA east coast) is a strange beast indeed: amoebas, sensing warmer waters and passing fish shoals, sprout flagella, then swarm up for a gruesome kill. Aerosolization of the unidentified toxins from this mainly bottom-dwelling dinoflagellate, called *Pfiesteria piscimorte*, caused eight days of amnesia in one investigator, and researchers now wear protective shields. Large fish-kills (>1000 fish) occurred in 1991, and recently from points north (Delaware) and south (Gulf of Mexico) of Pamlico.

A total of 122 papers were reviewed describing fish-kills from harmful algal blooms (HABs). Mechanisms include anoxia, intoxication (of fish and larvae), and clogging of gills (with spiked *Chaetoceros concavicornis* and *C. convolutus* in Alaska, British Columbia and Washington State). Nations dependent on fish for food or income are most immediately threatened.

The role of ship ballast water in 'hierarchical' dispersion of alien species (see Lockwood, 16 Oct. 1993, *The Lancet*) received considerable attention. Australian authorities have begun regular monitoring for toxic algae and for

(Cont'd on p. 3)

Gymnodinium catenatum in German coastal waters

The unarmoured, chain-forming dinoflagellate *Gymnodinium catenatum* Graham is the subject of intensive research in several countries because of its obvious association with outbreaks of paralytic shellfish poisoning (PSP). As part of its sexual life cycle, *G. catenatum* produces a brown, spherical, micro-reticulate resting cyst with a prominent red accumulation body. The occurrence and distribution of dinoflagellate resting cysts is of interest because cysts represent stable populations assuring geographical maintenance in contrast to the transient bloom from which they may be derived. Because of their small size, cysts are chiefly transported with residual currents and can infect areas where they are not endemic. The fact that resting cysts can be collected and enumerated during non-bloom periods, offers a potential tool for the prediction of future toxic blooms.

As pointed out by T. Wyatt in *Harmful Algae News* no. 2, the present-day distribution of cysts and vegetative cells of *G. catenatum* in European waters extends only to the Atlantic coast of the Iberian peninsula, where it was first recorded in 1976. This species has never been recorded in any of the numerous investigations of living phytoplankton from northern European waters (Elbrächter, pers. comm.). However, large numbers of subfossil *G. catenatum* cysts where found in sediments of the Kattegat area from a period about 2000 until 300 years ago and it is suggested that in former times the species was widespread throughout

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Who produces PSP?

It is well known that paralytic shellfish poisoning (PSP) toxins are produced by *Alexandrium catenella, A. tamarense, A. cohorticula, A. fundyense, A. fraterculus, A. minutum, Gymnodinium catenatum, Pyrodinium bahamense var compressum* and *Prorocentrum minimum*, but not by other species of *Alexandrium, Gymnodinium* and *Pyrodinium.*

Silva (Proc. IV IUPAC Symp. on Mycotoxins and Phycotoxins, Lausanne. *Proc. Pathotox Publ.* 1979) suggested that dinoflagellate toxin production may be due to bacteria living in association with the dinoflagellate. Recently Kodama & Ogata (*Mar. Poll. Bull.* 19, 559-564, 1988) also pointed out the possible association of bacteria in cell interiors with toxin production by *A. tamarense*, and suggested that toxin production is not a hereditary characteristic of *A. tamarense*. We thought that this hypothesis has not as yet been supported with sufficient evidence, given that (1) the algal cultures were not axenic, and (2) there were only a few bacteria in the nuclei of 10-15% of the many algal cells present. However, we cannot deny the possible association of factors other than bacteria, such as

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Update of
International Directory of Experts in Toxic and Harmful Algal Blooms

See back page for questionnaire

Toxic shellfish event in New Zealand attributed to *Gymnodinium* cf. *breve* and *Alexandrium minutum*

By January 1993 a full-scale dinoflagellate bloom was under way in Northland, New Zealand, and reports emerged of intoxicated animals, associated with eating shellfish scraps.

At this point events escalated rapidly. There were indications of illness in human shellfish consumers. Mouse bioassays carried out by the Communicable Diseases Centre in Wellington confirmed the presence of algal toxins in aqueous extracts of shellfish gathered from right around the country. The government ordered the closure of shell fisheries around the entire New Zealand coast.

Extensive surveys of the phytoplankton were undertaken, and aerial surveys carried out to help pinpoint bloom fronts. It soon became apparent that the South Island aquaculture regions, the Malborough Sounds and Big Glory Bay, had productive waters for the time of year, but with 'harmless' species predominating. Coccolithophores and diatoms were particularly abundant. With this information, and following an adjustment to the method for preparing shellfish for the mouse bioassay (a return to the use of 0.1N HCl instead of 1.0N HCl in the aqueous extracts), false positives were minimized and South Island was reopened to shellfish harvesting.

In the north-east of North Island, several dinoflagellate species came under suspicion. *Alexandrium minutum*, a species new to New Zealand waters, was found in Tauranga Harbour, and this linked well with both the epidemiological data and the mouse bioassay results. Paralytic shellfish toxins were later confirmed in shellfish from this area by HPLC analysis. However, this species was not widespread, and did not explain the majority of the illnesses.

During the second week of February, four things occurred almost simultaneously, implicating a *Gymnodinium* species which had persisted as a sub-dominant throughout the bloom.

First an outbreak of coughing amongst the residents of Orewa Beach, on the north-east coast, followed exposure to sea-spray aerosols, generated by heavy surf at the tail of a cyclone. (This well known phenomenon in the Gulf of Mexico/Florida area in the USA is related to blooms of *Gymnodinium breve*).

Reports followed of mass mortalities of marine fauna on reefs within the more northern Bream Bay, again a classic phenomenon associated with blooms of *G. Breve*. Thirdly, a sophisticated analysis of the contaminated shellfish by Professor Yasumoto, Tohoku University, Sendai, Japan, revealed the presence of toxins closely resembling the 'brevetoxins'. Fourthly, samples taken from Orewa Beach by Dr. Hoe Chang (New Zealand Oceanographic Institute), and samples received at that time by the Cawthron Institute from Coromandel (further to the south), contained abundant *Gymnodinium* cells that on scanning electron microscope examination closely resembled *G. breve*. Although *G. breve* has tentatively been identified outside USA waters, there have never been records of an association with shellfish toxicity elsewhere, until now.

The main factor contributing to late diagnosis of the toxin, and consequently the microalgal culprit, was the failure to determine the toxicity of lipid extracts of shellfish.

Initially, because the nature of the predominant toxins involved was unknown, observation of mice following the injection of aqueous shellfish extract was continued for up to 24 hours. A positive toxicity score was regarded as causing the death of 2 out of 3 mice during that time. Subsequently it was shown that most of the toxicity was associated with a lipid soluble fraction. The delayed toxicity observed with aqueous extracts was shown to be irrelevant to human intoxications and the 1 to 24 hour observations were reduced to 1 hour, to monitor for PSP toxicity only. Once this was done the situation immediately became clearer, with lipid-soluble breve-like toxins being the predominant toxicity.

Research relating to the toxic event is still being carried out, and cyst surveys should help determine whether a seed bed exists from which further toxic blooms might arise in the future.

In retrospect, the event, involving both microalgal species and toxins new to New Zealand, was handled conservatively. This has resulted in confidence amongst consumers. New Zealand will certainly be well prepared for any future events, should they occur.

Lincoln MacKenzie and Lesley Rhodes, Cawthron Institute, Private Bag 2, Nelson, New Zealand.

IOC Manual on Harmful Marine Microplankton

A chapter on 'Algal Culture Collections' is being prepared by Robert A. Andersen, Susan Blackburn, F.J.R. Taylor and Carmelo Tomas for the forthcoming *IOC Manual on Harmful Marine Microplankton*. The goal of the chapter is to list all strains of toxic marine algae — **public and private holdings** — as well as the corresponding information: scientific name, collection site, isolator, toxins (when known), etc. If you maintain toxic strains of marine microplankton, please contact Robert Andersen as soon as possible at the following fax: (1-207) 633 9641. We are particularly interested in strains that are held privately in individual laboratories. Providing us with information does not obligate you to distribute your strains; it merely serves as a means for disseminating toxic strain information that is often difficult to locate.

(Cont'd from p. 1, 'Highlights')

Vibrio cholerae (Hallegraeff, Rigby, personal communication), and with the emergence of *V. cholerae* non-01 CT+ in Asia and new algal blooms occurring in Indonesia, Australia, New Zealand and the Philippines, the need to monitor ballast water and coastal algal blooms for vibrios is even more urgent. The International Maritime Organization is considering measures.

Marine bacterial-algal interactions were another subtheme. Included are: bacterial production of saxitoxins, bacterial enhancement of algal toxins (domoic acid), and algae as vectors of vibrios and other bacteria. In addition reports of toxic Cyanobacteria are increasing. In 1990 and 1992, in the Gulf of Finland, blue-greens produced centimetre-sized aggregates, which emphasizes the interaction of physical with biotic factors (e.g. with gels and fibres) that may enhance net growth and provide protection from grazing.

Explanations for the apparent spread of HABs could be: (1) increased awareness, (2) natural variability or (3) a real increase in the extent and the number of species involved, as a result of anthropogenic factors. The factors considered include eutrophication (nutrients), loss of wetlands, altered predation pressures (from over-fishing and fish diseases), and superimposed global change. The New Zealand outbreak and one off South Africa involving the Benguela Current involved changes in winds and sea surface temperatures associated with El Niño, whose increased frequency in the past decade (4 x) suggests increasing climate instability.

Everyone agreed that the costs of HABs to finfish harvesting, aquaculture and tourism have grown considerably. The apparent spreading of coastal algal blooms and their role as a reservoir and dispersal mechanism for cholera increases the urgency for an early warning system.

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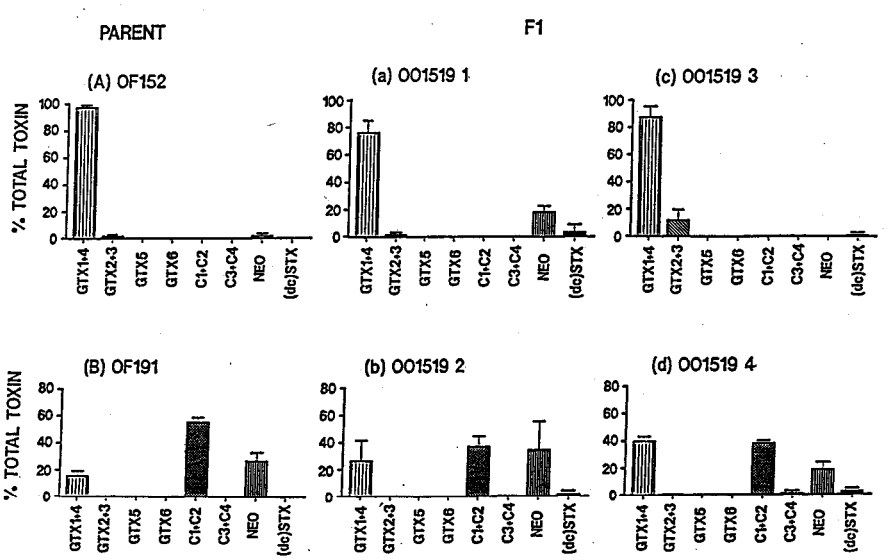
***Editors note**: If, as some forecasters feel, the El Niño system is 'locked in the on mode' (see Richerd Kerr,* Science, *262: 656-657, 29 Oct. 1993), New Zealand's troubles may be just beginning. A shortened version of this article appeared in:* The Lancet, *342, 1108, 30 Oct. 1993.*



Average toxin composition (as % total toxin, +1.0 S.D. n=8) of A. tamarense, parent strains OF152(A), OF191(B) and those 4 F1 strains (a-d).

(Cont'd from p. 1, 'PSP')

diphtheria toxin production by phage-infected cells.

We tried to determine whether the source of PSP toxins is encoded by nuclear DNA, chloroplast DNA, mitochondrial DNA, or bacterial DNA in *Alexandrium*, by studying the inheritance of toxin composition by F1 cells from parents with different toxin compositions. *A. catenella* and *A. tamarense* were examined in clonal-axenic cultures.

We noted that the mole percentage toxin composition in several strains did not change significantly until the late exponential phase. This indicates that PSP toxin composition in *A. catenella* and *A. tamarense* is a stable genetically determined trait.

Crosses of parents (mt+ and mt-) with different PSP toxin compositions were made and the toxin composition patterns of the F1 cells compared with those of the parents. Chromosome genes in crosses are inherited in a 2:2 Mendelian pattern, whereas chloroplast genes and mitochondrial genes are inherited uniparentally. Other symbiotic factors such as bacteria phages and plasmids are also inherited uniparentally or at random. Which gene codes for PSP toxin-synthesizing enzymes of *Alexandrium*?

PSP toxins composition pattern of the parents OF152(mt+) and OF191 (mt–), and a representative pair of mt+ and mt– F1 progeny (OO1519-1 and 001519-3) are shown in figure. In the parental strain OF152, the major part corresponded to gonyautoxin GTX1+4. In the other parental strain OF191(mt–), the toxin composition corresponded to C1+C2, neosaxitoxin (neoSTX) and GTX1+4. The F1 progeny OO1519-1 and OO1519-3 showed the same toxin composition as the parental OF152, and the other F1 OO1519-2 and OO1519-4 showed the same toxin composition as the parental OF191. F1 progenies from *A. catenella* parents also show either one or the other parental toxin composition (Ishida et al., *Toxic Phytopl. Bloom in the Sea*, 881-887, 1993; Sako et al., *Biosci. Biotech. Biochem.*, 56, 692-694, 1992).

Thus toxin compositions of F1 progenies in *A. tamarense* and *A. catenella* are inherited on a 1:1 Mendelian pattern, referred to as biparental inheritance, and not in a uniparental pattern nor randomly. The genes for PSP toxin-synthesizing enzymes must be coded in the chromosomal DNA of *Alexandrium*.

However, more evidence is needed before a definitive statement can be made. Experiments continue to detect and isolate PSP toxin synthesizing enzymes, especially transforming enzymes (saxitoxin → gonyautoxin → C-toxins). The isolation of these enzymes and their DNA sequences should be decisive in settling this problem in the near future.

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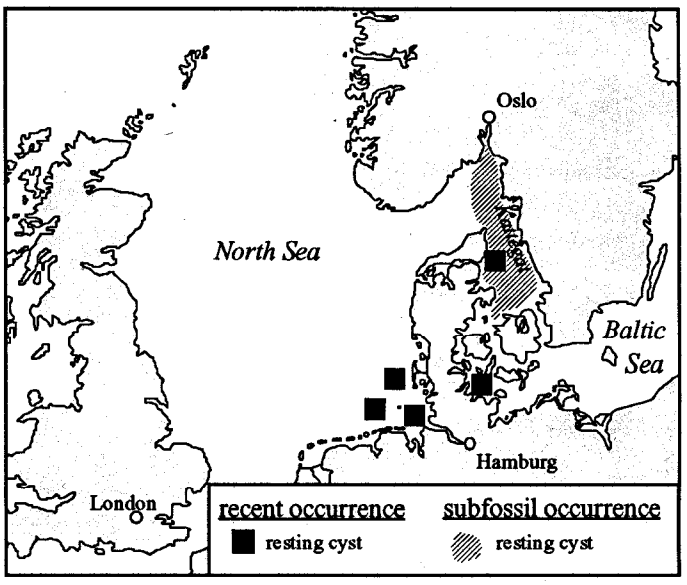
Further details appear in Sako, Y., Kim, C. H., and Ishida, Y., 1992. Mendelian inheritance of paralytic shellfish poisoning toxin in the marine dinoflagellate *Alexandrium catenella. Biosci. Biotech. Biochem.*, 56: 692-694 (eds.).

(Cont'd from p. 1, 'Gymnodinium')

the water column of the region (Dale et al., 1993. In: Smayda & Shimizu, *Toxic Phytoplankton Blooms in the Sea.* Elsevier, 47-52). Interactions between climatic change and local hydrography seem to be important factors regulating these fossil 'blooms', with the migration of relatively warmer waters contributing to the establishment of *G. catenatum.* It is suggested that the cooling of the Little Ice Age caused its disappearance in present times (Dale & Nordberg, 1993. In: Smayda & Shimizu, *Toxic Phytoplankton Blooms in the Sea,* Elsevier, 53-57).

This is in keeping with investigations from the adjacent North Sea area, especially in the German Bight in October 1991, which showed that *G. catenatum* cysts are apparently absent in recent sediments (Nehring, *Helgoländer Meeresunters.*, in press). This also holds true for other investigations of North Sea sediments (e.g. Dale, 1976, *Rev. Palaeobot. Palynol.* 22, 39-60; Reid, 1977, *Nova Hedwigia* 29, 429-463). However, in March 1992, living *G. catenatum* cysts were found at two stations in the German Bight (mentioned as 'cyst of Diplopsalid group' in Nehring, op. cit., in press) and were discovered in sediments of the Danish coast bordering the Kattegat (Ellegaard et al., *J. Phycol.*, in press). During an additional survey of the German Bight and Baltic Sea (Kiel Bight) in April 1993, living and empty cysts of *G. catenatum* were found in the topmost centimeter of sediments at numerous stations in both areas, showing maximal abundance of 17.0 living cysts/cm^3. The cysts of *G. catenatum* from Kiel Bight comply with the specimens isolated from German Bight sediments (30-35 µm in diameter) and with the description given by Ellegaard et al. (op. cit.) from the Kattegat area, but are slightly smaller than specimens from other parts of the world (for review see Ellegaard et al., op. cit.). The second important feature is that chains longer than two cells were never seen. This agrees with the comprehensive germination experiments and observations on motile cells of *G. catenatum* by Ellegaard et al. (op. cit.). Also they have found chains with only two cells under conditions where chain formation was induced in other studies (Blackburn et al., 1989, *J. Phycol.* 25, 577-590) and speculated that *G. catenatum* is in reality a species complex, with the Danish strain a different ecotype or a non-chainforming variant. The site nearest to northern Europe where motile cells of *G. catenatum* are known is the Atlantic coast of Spain (Estrada et al., 1984, *Inv. Pesq.* 48, 31-40) but Paulmier (1992, *Rapports internes de la Direction des Ressources Vivantes de l'IFREMER* – 92.007, 107pp.) recorded *G.* cf. *catenatum* from the Channel coast of France in 1983 and 1984 as single cells and chains of two cells.



Outline of Northwestern Europe showing distribution of recent and subfossil Gymnodinium catenatum *resting cysts.*

At present no motile cells or shellfish toxicity have been detected in waters of the North and Baltic Seas, but the positive germination experiments with filtered seawater from the sample locations show that the occurrence of *G. catenatum* in the water column is very likely, and has probably so far been overlooked.

These findings suggest that the toxic *G. catenatum* was introduced to German coastal waters by increased water influx through the English Channel into the North Sea and then transported with the residual currents to the Skagerrak/Kattegat area. This scenario is very likely because the current system of the North Sea should not allow infection of the German Bight with cysts from the Skagerrak/Kattegat area (see Lee, 1980, *North Sea: Physical Oceanography, II,* Elsevier Oceanogr. Ser. 24B). If so, cysts of this species must be present in recent sediments of the Atlantic and Channel coast of France as well as on the Danish North Sea coast and in the Kattegat area. In January 1993 a massive salt water influx from the Kattegat through the Great Belt into the Western Baltic took place, and may have infected this area with *G. catenatum* cysts.

It has been shown for Tasmanian waters that infection of the area with *Gymnodinium catenatum* cysts resulted in recurrent toxic blooms leading to human PSP and to temporary closure of shellfish farms (Bolch & Hallegraeff, 1990, *Bot. Mar.* 33, 173-192; Hallegraeff et al., 1988, *Aust. Fisheries* 47, 32-34). In the same manner *G. catenatum* cysts in North and Baltic Sea sediments may represent potential seedbeds for spontaneous bloom initiation. Phytoplankton monitoring programmes need to be aware of this. To get a comprehensive idea of the spreading of *G. catenatum* cysts in northern European waters, further surveys of the quantities and spatial and temporal distribution of these cysts in sediments are needed.

Cyst studies provide an important but relatively cheap additional tool for the evaluation of areas with potential toxicity problems. The potential impact of such species on commercial fisheries, aquaculture and the natural environment is of increasing concern. After three centuries of absence, a potential recolonization of *G. catenatum* in northern European waters may have taken place. With respect to global change, it is now a question of whether hydrodynamic, chemical and biological factors in the water column will favour germination of cysts and the proliferation of vegetative cells of *G. catenatum* in the future.

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HAB programme news

Advanced workshop on HAB ecophysiology

An advanced workshop on the ecophysiology of harmful algae was planned at the first meeting of the SCOR-IOC Working Group 97 on the Physiological Ecology of Harmful Algal Blooms. It was held at CREMA L'Houmeau, CNRS-IFREMER, La Rochelle, France, 23-24 October 1993. The WG is chaired by Dr. D.M. Anderson (USA).

Having outlined central questions in future research on the ecophysiology of harmful microalgae, the WG decided to organize an advanced scientific workshop to review the state of knowledge and to identify and discuss areas of future research.

The key questions identified included the roles of toxin production, bacteria, and mixo- and heterotrophy in the ecophysiology of harmful algae, as well as the importance of genetic variability in harmful algal species, and the role of UV on harmful algal succession and occurrence.

The WG decided to prepare a proposal for a NATO 'Advanced Study Institute'. A NATO ASI has a duration of 10 days and has 60 to 80 participants and includes both lectures and demonstrations. SCOR and IOC will co-sponsor the workshop, and the presentations and the results will be published.

The workshop is proposed for November 1994 or June 1996 in Bermuda. A detailed proposal was prepared and an organizing committee was identified.

A set of recommendations were adopted as follows:

(1) Convene within 3 years an advanced workshop to summarize the state of knowledge, exchange ideas, disperse new technologies, establish interaction, and identify themes of future research relating to the physiological ecology of harmful algae.

(2) Begin to contact individuals potentially interested in the workshop (at the direction of the Chairperson).

(3) Convene a second SCOR-IOC workshop in conjunction with the 7th International Conference on Toxic Marine Phytoplankton, Sendai, Japan, July 1995.

Joint ICES-IOC action planned for 1994

The joint Study Group of the International Council for the Exploration of the Sea (ICES) and IOC on the Dynamics of Algal Blooms has been re-established as an ICES-IOC Working Group on Harmful Algal Bloom Dynamics that works in close connection with the IOC Harmful Algal Bloom Programme. The Chairperson of the WG is Beatriz Reguera (Spain).

The setting up of this group was recommended at the last ICES Statutory Meeting by the Chairpersons of the Hydrography and the Biological Oceanography Committees. The terms of reference were: 'to plan and propose a programme to study the dynamics of HABs in coastal oceans'.

The activities to be carried out during 1994 are:

- ICES/IOC Workshop on 'Modelling the Population Dynamics of Harmful Algal Blooms', Vigo (Spain), 4-7 May 1994. Convenors: W. Fennel (Germany) and P. Tett (UK).
- Meeting of ICES/IOC Working Group on Harmful Algal Bloom Dynamics, Vigo (Spain), 9-12 May 1994. Chairperson: B. Reguera (Spain); including a joint session, 9-10 May, with the Working Group on Shelf Seas Oceanography, chaired by H. Dahlin.
- ICES/IOC Workshop on Intercomparison of *in situ* Growth Rate Measurements, Aveiro (Portugal), 25-29 July 1994. Chairperson: M.A. Sampayo (Portugal).

Participants from countries outside ICES, especially those interested in establishing HAB dynamic studies in other geographical regions, are invited to attend the WG meeting on Harmful Algal Bloom Dynamics and the Workshop on Modelling the Dynamics of HABs.

Intergovernmental panel sets priorities

The IOC-FAO Intergovernmental Panel on Harmful Algal Blooms held its Second Session in Paris 14-16 October 1993 to set priorities and identify resources for the Harmful Algal Bloom (HAB) Programme. Twenty-one Member States, WHO, ICES, IUPAC[1] and SCOR were represented.

The Panel decided to establish four Task Teams to address critical issues requiring specific priority and action:

- A Task Team on Algal Taxonomy will provide taxonomic recommendations, develop identification standards for preparation of manuals and training, and help coordinate activities;
- A Task Team on Aquatic Biotoxins will prepare a report on the current status of algal toxin chemistry and toxicology, and in particular facilitate the preparation and supply of algal toxins;
- A Task Team on Design and Implementation of Monitoring Programmes will assist in the preparation of a report where examples of monitoring systems on harmful algae worldwide are presented in detail, and assist in the organization of an international workshop directed toward improved design of HAB monitoring systems;
- A Task Team on HAB Project Development will work with the preparation of HAB project proposals.

The development of a comprehensive training and capacity building programme on harmful algae was agreed upon. The training programme will include courses and workshops on taxonomy, toxin chemistry, monitoring, and management of harmful algae. The first taxonomy course has already been held and both taxonomy and toxin chemistry courses are planned for 1994.

The Summary Report of the Second Session of the IOC-FAO Intergovernmental Panel on Harmful Algal blooms is available from the HAB Programme Office, IOC Secretariat.

[1] IUPAC = International Union for Pure and Applied Chemistry.

Ciguatera Management Workshop, Bribie Island, Australia

Overview

An international Workshop on Ciguatera Management was held on Bribie Island near Brisbane on 12-16 April 1993. The Workshop was sponsored by the Australian Fisheries Research and Development Corporation and the Queensland Department of Primary Industries (QDPI). Scientists, medical practitioners and fisheries managers attended and focussed on current research having implications for the management of ciguatera. *(Sources of comments are indicated in brackets.)*

A total of 56 people from Japan, mainland USA, Hawaii, France, French Polynesia, New Caledonia, Germany and Australia attended. The Workshop specifically addressed (i) the detection of ciguateric fishes and (ii) the management of ciguatera cases. P. Scheuer opened the scientific program with an historical perspective of modern ciguatera research initiated by the late A. H. (Hank) Banner and outlined some of the challenges for the future. The major themes of the Workshop were: chemical and immunological aspects of the detection of toxins involved in ciguatera; pharmacology and treatment of ciguatera; origin of the toxins involved in ciguatera; clinical aspects and epidemiology of ciguatera.

Detection of ciguateric fish

A cost-effective screen for ciguateric fish was recognized as an important management tool able to directly reduce the adverse effects of ciguatera on public health, fisheries, trade and tourism (R. Lewis, D. Park). Several different approaches to the detection of ciguateric fish were presented. Two approaches measured the interaction between ciguatoxin and the voltage-dependent sodium channel through either (i) the inhibition of brevetoxin binding to sodium channels in a rat brain synaptosome preparation (A. M. Legrand) or (ii) the cytotoxic effects of ciguatoxin on sodium channel-containing cell lines pre-exposed to ouabain and veratridine (R. Manger). Both assays were more sensitive than the mouse bioassay, and may replace *in vivo* assay in laboratories possessing the specialized equipment required. However, it is unlikely that these approaches, as they stand, can be used for the routine screening of suspect fish.

Antibody-based screens still appear to hold most promise for the cost-effective detection of ciguateric fish (R. Lewis). This approach is the basis of a potential commercial test to detect ciguateric fish being developed by Hawaii Chemtect International (Pasadena California, USA). D. Park presented a summary of the performance of this solid-phase immunobead assay (Ciguatect™) which was claimed to be able to detect most, if not all ciguateric fish. However, the test was reported to be unsuitable for detecting ciguatera toxins if the fish flesh being screened was slightly acidic (pH ≅ 6.5), a factor that may considerably limit the usefulness of the test. Y. Hokama commented that the test may not work satisfactorily because the solid-phase used in the Ciguatect™ test may not be as efficient at extracting ciguatoxins from fish as the correction fluid used for the solid-phase in the original stick test he developed (apparently the same antibody was used for both tests). When compared with the results of a well conducted mouse bioassay, predictive indices from 5% to 75% were obtained with the Ciguatect™ test in an independent study of ciguateric fish from the Caribbean (R. Dickey). These results suggest that the test may not be responding to the major toxins (as yet unidentified) present in these Caribbean fish. Lack of ready access to samples of pure ciguatoxin or its analogues, and an inability to independently validate the levels of ciguatoxins present in fish samples being screened, hamper attempts to validate (or otherwise) the Ciguatect™ test.

Pharmacology and treatment of ciguatera

Major advances are being made into how ciguatoxins cause human poisoning (J. Molgo, E. McLachlan, J. Brock, P. Hamblin, E. Beniot, K. Terao, C. Purcell, M. Capra). However, the precise mechanism by which mannitol, the present treatment for acute ciguatera, acts to relieve the symptoms of ciguatera remains unclear. A double-blind clinical study of mannitol treatment is being conducted (N. Palafox) but the results of this study were not available at the time of the meeting. Clinical experiences with mannitol therapy (D. Blythe, N. Palafox) continue to be positive, and mannitol remains the treatment of choice for acute ciguatera. Confirmation of the clinical findings would be assisted by the development of an animal model for ciguatera that responds to mannitol.

Clinical aspects and epidemiology of ciguatera

While most of the clinical features of ciguatera are well documented, the long-term effects of ciguatera and how frequently these occur are poorly understood (T. Ruff). Follow-up research on victims is required to establish the true extent of long-term effects, especially the allergy-like reactions that can persist after a single exposure to toxic fish. J. Pearn reported that ciguatera remains typically a poorly recognized and managed disease, despite the introduction of mannitol therapy. QDPI maintains a database that covers 27 years of ciguatera cases reported in Queensland. An analysis of this database using recently developed statistical modelling approaches revealed major shifts in the species and the nature of poisoning in Queensland (M. Chaloupka).

Origin and identification of toxins involved in ciguatera

Gambierdiscus toxicus is now widely accepted as the organism that produces ciguatera toxins. Indeed this organism may be the only source of these toxins. T. Yasumoto reported the structure for GTX-4A (52 epi-GTX-4B), the major gambiertoxin produced by a Rangiroa Atoll strain of *G. toxicus* grown in culture. This study confirms that *G. toxicus* is indeed the origin of the ciguatoxins isolated from fish. Long and short range inverse detected NMR of CTX-1 confirmed the structure originally proposed for ciguatoxin (R. Lewis). The structure of a maitotoxin produced by culture *G. toxicus* was also presented by T. Yasumoto. Maitotoxin is the largest non-repeating unit compound for which a structure is known and elucidation of its structure represents a significant milestone in natural product chemistry. Maitotoxin's structure is only distantly related to the ciguatoxins, thereby ending speculation that the maitotoxins may be a precursor of the ciguatoxins.

The environmental factors that cause

the upsurges of ciguatera remain poorly understood (M. Holmes, R. Lewis, Y. Hokama, D. Ichinotsubo, R. Bagnis, U. Kaly). The origin of ciguatera was questioned by J-P. Vernoux based on studies conducted in French Polynesia. A rapid extraction method for isolation of ciguatoxins from *G. toxicus* may assist studies on toxin production in culture (J. Babinchak). Studies of the vectors which transfer gambiertoxins to carnivorous fish revealed that shrimps may be involved in some communities, and herbivorous fish such as *Ctenochaetus striatus* in others (R. Lewis).

A record of the meeting will be published in a special issue of the '*Memoirs of the Queensland Museum*' by mid-1994. For further information on the published proceedings please contact: Richard J. Lewis, QDPI, Southern Fisheries Centre, PO Box 76, Deception Bay, Queensland 4508, Australia; tel.: (61-7) 203 1444; fax: (61-7) 203 3517.

Toxin action

It was shown using confocal laser microscopy that ciguatoxin causes oedema of adaxonal Schwann cells and nodes of Ranvier in myelinated nerve fibers, and that mannitol (a product successfully used in ciguatera therapy) acts via its osmotic effects (J. Molgo).

The association of ciguatoxin with specific proteins may explain its lack of toxicity to Na+ channels of fish, even though fish nerves respond to ciguatoxin in a similar manner to those of mammals (F. Capra).

Origin

For the first time ciguatera was reported (M. J. Holmes) in an area (Platypus Bay, Australia) not typically associated with ciguateric fish, without corals but with a sandy bottom covered by unattached green macroalgae (*Cladophora* sp.). Nevertheless, *G. toxicus* was attached on this algae and contained ciguatoxins, suggesting that this *G. toxicus* population is the origin of toxins found in ciguateric fishes caught in this bay. Thus the presence of corals is not essential for ciguatera development. In this area invertebrates such as shrimps were shown to contain detectable levels of ciguatoxin and to transfer these toxins in the ciguatera food chain (R. J. Lewis). It is the first time that shrimps were implicated in the ciguatera phenomenon.

Hindsight on Arctic toxic bloom

Toxic blooms of marine algae are often attributed to pollution, as in the case with *Chrysochromulina polylepis* in the North Sea in 1988. Therefore, a toxic bloom of *Chrysochromulina leadbeateri* in Norwegian coastal waters, to the north of the Arctic circle in June 1991, came as a real surprise and needed other models to explain it. The affected area was the Vestfjord, situated between the Lofoton Islands and the Norwegian mainland. The area is very sparsely populated and has little agriculture and no heavy industry. So, why here?

The case developed from late May, when farmed salmon started to die and a diver reported a layer of yellow water below the cages. Eventually, a total of nearly 550 metric tons of salmon were reported lost, from different farms isolated from each other and with unharmed farms in between. In one case, dead and dying salmon were taken out of a farm while samples were taken close to the cages, showing that cell concentrations were lower than 2 x $10^6 l^{-1}$. So, if salmon mortality was not related to the abundance of toxic algae, to what was it related?

Several research institutes in Norway became involved in the operation, mapping the distribution of the bloom and doing experiments to explain its causes. Compared to the *C. polylepis* bloom, the *C. leadbeateri* bloom had lower maximal concentrations, occurred at lower temperatures and higher salinities. Its distribution was more restricted and it did not affect the natural flora and fauna.

C. leadbeateri was present in areas to the south of the Vestfjord as well, but was then part of a flora dominated by diatoms, causing Secchi depths to be 2-7 m. Secchi depths in the Vestfjord were 8-21 m and the flora consisted almost exclusively of *C. leadbeateri*. In the Vestfjord proper, as well as in branching fjords, the mixed layer was rather deep, often causing the highest concentration of the algae to be found in depth. The mixed layer contained little nutrients and the algae seemed to optimize its growth by tactical positioning in a gradient of light and nutrients, which occurred in a deeper layer. Growth experiments with added nutri-

(Cont'd on p. 9)

Detection

Ciguatera results predominantly from the effects of the most potent ciguatoxin (CTX-1) which is present at > 0.1 ppb (10-10 M/Kg) in the flesh of carnivorous fish. Significant levels of the less potent CTX-2 and CTX-3 also accumulate in fish (R. J. Lewis).

By using the specific binding property of ciguatoxins to voltage-dependent sodium channels two detection methods were developed: the first one (A. M. Legrand) measures the ability of ciguatoxins to displace by competition ratio-labelled brevetoxins from sodium channels. The second (R. Manger) measures the enhancement of toxicity caused by ciguatoxins when added with ouabain and veratridine to neuroblastoma cells; it is simple, uses readily available reagents (MTT), is well within the scope of even modest tissue culture facilities and has the potential to serve as an alternative and complementary method to the standard mouse bioassay. A new mouse bioassay protocol adapted to 50, 100 or 200 g of fish was presented by J. P. Vernoux.

Ciguatect™, the solid-phase immunobead assay for the detection of ciguatera-related toxins is available from Hawaii Chemtect International (D. L. Park). It has high potential for screening marketplace fish for ciguatera toxicity. Nevertheless, improvement of this kit is needed since a significant number of false negative and false positive values were obtained in an FDA trial (R. W. Dickey).

Derivatization experiments with the chromophore-poor ciguatoxin-1 yielded a fluorescent coumarin-carbamic acid ester of the biotoxin. HPLC with fluorimetric detection produces a moderately intense detector response to 2 nanograms of the CTX-1 derivate (R. W. Dickey). The derivate shows potential for the detection of ciguatoxins. To separate toxic from non-toxic fish, the detection limit by HPLC should be 1 picogram, a threshold of sensitivity which has never been obtained for ciguatoxins by any described procedure. This limits the use of HPLC with fluorimetric detection of ciguatoxins to the research laboratory only.

Jean Paul Vernoux, Université de Caen, Laboratoire de Physiologie Cellulaire et Moléculaire, Esplanade de la Paix, 14032 Caen Cedex, FRANCE.

South Korean oysters gave ASP symptoms

An illness similar to Amnesic Shellfish Poisoning (ASP) beset two persons after eating smoked oysters in South Korea. The event occurred as follows: On 16 February 1992, approximately 5 hours after eating six canned oysters each from a smoked Korean oyster product, the symptoms of ASP began to show. One person, a male, suffered seizures, disorientation, confusion, vomiting, and general weakness. Tests at a hospital for stroke, brain tumour, and heart attack were negative. The other person, a female, complained of a headache, was unable to make decisions and had swollen hands and feet. Approximately 24 hours after she had ingested the oysters, she was stricken with a vomiting spell, followed by seizures, and went into a coma that lasted for 48 hours. She had extremely low blood pressure, low sodium, malfunctioning kidneys and liver, and was left with muscle damage. At present, she suffers memory loss of these events. She was tested for the same disorders as the male victim as well as for tetanus, botulism, Rocky Mountain spotted fever, Lyme's disease and bacterial infection. All tests were negative. The house where the two victims live was tested for carbon monoxide and radon gas poisoning, and the water supply was tested for numerous toxins; all tests proved negative.

On 26 March 1992, both persons ate seafood gumbo. The following day, the male had another attack with a seizure and symptoms similar to the 16 February attack. Again he was rushed to the hospital and underwent the same tests as before. All proved negative.

Both persons were in good physical condition before the illness appeared. They did not drink or take drugs, and had not shown any previous reactions when eating shellfish. Neither of the victims suffered from stomach problems, diarrhea, paralysis, or experienced tingling sensations in the mouth during the attack.

Therefore it does not seem likely that the victims suffered from Paralytic Shellfish Poisoning. The symptoms shown by the two persons, however, indicate that they suffered from ASP. Common symptoms of ASP include choking, vomiting, diarrhea, abdominal cramps, incapacitating headaches, seizures, and permanent short-term memory loss (Perl et al., 1990).

We analyzed Korean shellfish products for domoic acid (DA) contamination, the causative agent of ASP. Twenty-five cans of smoked Korean oysters and two cans of smoked Korean mussels were tested with high performance liquid chromatography methods. The cans represented different brands and lot numbers including the same brand and lot number the two persons consumed on 16 February. Domoic acid was not detected in any of the smoked Korean products.

From two of the cans, oyster gut contents were analyzed for diatom frustules from the *Pseudonitzschia* complex. To date, only phytoplankton from this group of diatoms produce DA. No forms known to be toxic were found, but the non-toxic diatom *P. pungens* f. *pungens* was present.

The HPLC analyses do not provide supporting evidence that the Korean products were contaminated with DA. However, we believe that the products we analyzed may not have been representative of what the two people ate on 16 February, despite testing cans of oysters from the same brand and lot number that were consumed. Within a can of oysters variation in oyster size was small. However, variation in the oyster size between cans was large, even when the cans were from the same brand and lot number. This implies that the oysters were harvested from different locations or times, despite identical lot numbers on the cans.

Although smoked Korean oysters tested negative, it may have been that the oysters analyzed were harvested from a non-toxic area, and that the oysters consumed by the two people were harvested from a toxic area, or even a 'pocket' of toxicity. The oyster *Crassostrea virginica* accumulates DA at low levels over short feeding periods (Roelke, 1993). It is likely that oyster species from Korea are similar to *Crassostrea virginica* in their feeding behaviour and may accumulate DA if toxic diatoms are ingested over time.

As stated earlier, a non-toxic diatom, *P. pungens* f. *pungens* was found in the gut contents of the oysters analyzed. Fryxell et al., (1992) and Dickey et al., (1992) reported that two forms of *P. pungens* are present together year-round in Galveston Bay, Texas, with the non-toxic form, *P. pungens* f. *pungens* more common in warmer months and the toxic form, *P. pungens* f. *multiseries* in colder months. *P. pungens* f. *multiseries* was found in small numbers from net hauls with many *P. pungens* f. *pungens* cells from Jinhae Vay, Korea, April 1983 (Fryxell et al., 1990). The same trend of seasonal relative dominance observed in Galveston Bay is a possibility in Korean waters as well.

During the preparation process of smoked Korean oysters, they are first shucked, then smoked, put into cans with oil, sealed in a vacuum, and finally sterilized by heating (Crown Prince, pers. comm.). Domoic acid is a heat-stable molecule. It does not appear likely that the preparation process for smoked Korean oysters would remove the toxin from the product.

The distribution of DA-producing diatoms is world-wide in temperate climates (Villac et al., in press). If appropriate environmental conditions arise that favour a bloom of these toxic species, ASP events could also occur world-wide in temperate climates. Phytoplankton monitoring for DA-producing species in Korean and Chinese waters has only just begun (Fryxell, pers. comm.). If a toxic bloom occurred in 1992 it is likely that it would have gone unnoticed.

In summary, we know that two people suffered from an illness similar to ASP after eating smoked Korean oysters. We know that a related oyster species along North America accumulates DA, and that at least one toxic diatom species occurs in Korean waters. We also know that the canning process of smoked Korean oysters is not likely to destroy DA. Finally, we know that a toxic bloom of diatoms in Eastern waters would have most likely gone unnoticed. There is, no direct evidence that an ASP event occurred due to consumption of a Korean oyster product, but these two cases serve as a warning.

As the scientific community increases its understanding of DA occurrences around North America and Europe, we also need to consider Eastern waters. It is essential that monitoring of the phytoplankton community be carried out in temperate climates worldwide where shellfisheries exist. The understanding of population dynamics and life strategy of the species we know to produce the toxin and the possible dis-

covery of other DA sources are crucial in our effort to better protect shellfisheries and consumers.

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Biology, Epidemiology and Management of* Pyrodinium *Red Tides

Limited copies of the above title, 1989 (Hallegraeff and Maclean, eds.) are available (US $25 inc. surface mail) through Dr. Rhodora A. Corrales, Marine Science Institute, University of the Philippines, Diliman, Quezon Sity 1101, The Philippines; fax: (63-2) 921 5967.

Raphidophyte algal bloom preceded New Zealand shellfish poisoning

Rhaphidophytes dominated an extensive bloom which occurred along the north-east coastline of New Zealand from August to December 1992. A dramatic increase in chlorophyll content was noted at that time in coastal waters off Leigh Marine Reserve, in Northland, New Zealand. The event was associated with the 'El Niño' phase of the Southern Oscillation Index, which resulted in unusually cold sea temperatures.

The microalgal species involved included the potentially ichthyotoxic species *Fibrocapsa japonica*, *Heterosigma akasiwo* (Raphidophytes), *Dictyocha speculum* (Silicoflagellate) and a dinoflagellate initially described as *Gyrodinium* cf. *aureolum*. On closer examination, by SEM (scanning electron microscopy), the dinoflagellate resembled the Florida red tide microalga *Gymnodinium breve*. The coccolithophore *Gephyrocapsa oceanica* was in constant and significant presence throughout the bloom. *Fibrocapsa japonica* had only been noted once before in New Zealand – it was observed in north-eastern waters the previous October. Early in the bloom, *Dictyocha speculum* occurred in its naked form, but by December, when the bloom had temporarily diminished, the unarmoured form was predominant.

Maximum cell numbers were reached in October, and although not particularly high for individual species (e.g. *Fibrocapsa japonica* reached no more than 107,000 cells per litre), the combined numbers result in a patchy discoloration of the coastal waters for over 200 km of the coastline. Bioassays for both shellfish toxins and for ichthyotoxicity were negative during this period, but there were many anecdotal accounts of unusual events. These included dieback of kelp beds, mass mortalities of penguins, skin irritations among fishermen and peppery tasting mussels in some areas.

The end of the raphidophyte-dominated bloom proved to be a brief respite. Dinoflagellates proceeded to dominate the water column along the same coastline, and in late December animal deaths and human illnesses linked to the eating of shellfish were reported. It is tempting to speculate that the conditioning of the water column by the earlier bloom, which was triggered by the unusual weather patterns, was a critical factor in the toxic event that followed.

Lesley Rhodes and Allison Haywood, Cawthron Institute, Private Bag 2, Nelson, New Zealand.

(Cont'd from p. 7, 'Arctic toxic bloom')

ents revealed that both nitrogen and phosphorus had to be added to gain growth in cultures.

A national report which sums up most of the observations, suggests that high freshwater outflow in winter led to stratification that was beneficial to *C. leadbeateri*. After the normal spring bloom in April, when diatoms and *Phaeocystis poucheti* deplete the nutrient reserves in the mixed layer, *C. leadbeateri* may have been the only species able to maintain a population under the prevailing conditions. The species seemed to become toxic in salmon farms localized in bays which exchange tidally mixed water through shallow channels. Thus, there is a possibility that admixture of new nutrients from deep water made the algae toxic. However, local eutrophication from the farms themselves should be considered, and also the saprobial faeces and feed-spill. The heterotrophic and bacteriophagous behaviour of *Chrysochromulina* genus could make fish farms feasible habitats matching its niche.

Another speculation suggests that wintering herring may have contributed to the conditions for algal growth. Most of the spring-spawning Norwegian herring spent the winter in the innermost part of the Vestfjord, leaving a water body characterized by low levels of oxygen and high levels of plant nutrients. In fact, ammonia, which was not recorded regularly during the bloom, turned up in high concentrations when measured, lending support to this view. The bloom terminated in late June, possibly due to competing algae or grazers. Thus, the research does indicate a complex matrix of causes and may have raised more questions than answers, not least because so many features deviated from previous experience.

Stig Skreslet, Kristin Heidal and Age Mohus, School of Fisheries, Nordland College, N-8002 Bodo, Norway.

Information on algal blooms and other hazardous events in Swedish Baltic

The Information Office for the Baltic Proper was established in June 1992 as a direct result of a Swedish government proposal. According to the proposal, three information offices should be established for those County Administrative Boards in whose counties Centres for Marine Research had already been established in 1989. The information offices are to report especially on the following topics:

- Algal blooms, predominantly on toxic blooms but also on the occurrence of non-toxic blooms of major extent. The reports should include information about the toxicity of the algae and how to avoid negative effects.
- Oxygen deficiency situations. It is valuable if early warnings about expected deficiencies can be given.
- Fish kills, partly in connection with oxygen deficiency and partly with other causes, like diseases. It is valuable if early warnings can be given.
- Extensive diseases and/or kills of organisms other than fish, e.g. seals and birds.
- Major spills of oil and chemicals which may cause harm to the marine environment.

The information office arranges telephone conferences with a limited number of scientists involved in, or in close contact with, field sampling programmes within the area of interest. These conferences are held at predetermined intervals (ranging from about one week up to about every second month) depending on the expected development of the situations monitored. If necessary, conferences can be held at very short notice. During the conferences the participants present available information and formulate a prognosis of the expected development. The information office staff puts the material presented, including the prognosis, into a short report or 'newsletter' (maximum about one A4 page). The report is then sent by telefax to interested receivers (all County Administrative Boards and local authorities along the coast within the area concerned, research institutes, the Coast Guard, the Navy, fishermen's organizations, mass media and others) immediately after the conference.

The Stockholm information office (Informationskontoret för egentliga Östersjön or Information Office for the Baltic Proper) is currently reporting only in Swedish. We hope to report also in English in the near future. Before that can be done we have to establish good contacts with suitable institutes/authorities in other Baltic countries in order to achieve a reliable system for the exchange of information. We are now trying to establish such contacts. Therefore, any suitable source of information in other Baltic countries is welcome to contact us. We are equally happy to be able to provide information to those interested in receiving it.

Our area of interest ranges from the northern Åland Sea to the southern part of the Sound (Øresund) between Sweden and Denmark, and includes primarily Swedish coastal waters and the open Baltic proper. The information office in Umeå (to be established) is to cover the Sea of Bothnia and Bothnia Bay. The office in Gothenburg covers the area from the southern part of the Sound up to the Norwegian border, including the Kattegat and the Skagerrak.

Contact persons are: Gunnar Anner and Kerstin Bohm, The Information Office for the Baltic Proper, Stockholm County Administrative Board, Department of Environment Protection, Box 22067, S-104 22 Stockholm, Sweden; tel: (46-8) 785 51 18 or 785 51 21; fax: (46-8) 651 57 50.

Future events

4th Canadian Workshop on Harmful Marine Algae; 3-5 May 1994; Sidney, B.C. Canada. *Contact*: Rod Forbes/Brenda Lacroix, Institute of Ocean Sciences, PO Box 6000, 9860 West Saanich Road, Sidney, B.C. V8L 4B2, Canada; tel: (1-604) 363 6443/6533; fax: (1-604) 363 6390.

5th International Phycological Conference; 26 June - 2 July 1994; Qingdao, P.R. China. *Contact*: The Secretary, 5th Int. Phyc. Conf., EMBL Institute of Oceanography, 7 Nanhai Road, Qingdao 266071, P.R. China.

Modelling & Control of Activated Sludge Processes; 21-23 August 1994; Copenhagen, Denmark. *Contact*: Mia Clausen, Conference Secretariat, c/o Dept. of Environmental Engineering, Technical University of Denmark, DK-2800 Lyngby, Denmark.

13th International Diatom Symposium; 1-7 September 1994; Acquafredda di Maratea, Italy. *Contact*: Jean Gilder Congressi snc., Via G. Quagliariello 35/E, I-80131 Napoli, Italy; tel: (39-81) 546 3779 or 545 4617; fax: (39-81) 546 3781.

Coastal Zone Canada '94; 20-23 September 1994; Halifax, Nova Scotia, Canada. *Contact*: Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N. S., B2Y 4A2, Canada; tel: (1-902) 429 9497; fax: (1-902) 429 9491.

24th International Conference on Coastal Engineering (ICCE '94); 23-28 October 1994; Kobe, Japan. *Contact*: Secretariat, c/o Inter Group, Shiroguchi Bldg., 2-15, Kakutacho, Kita-ku, Osaka 530, Japan; fax: (81-6) 372 6127.

Pollution of the Mediterranean Sea; 3-4 November 1994; Nicosia, Cyprus. *Contact*: M. Nicolaou, WTSAC, PO BOX 1735, Limassol, Cyprus.

IOC International Oceanographic Conference – 'Towards sustainable use of the oceans and coastal zones'; 7-12 November 1994; Lisbon, Portugal. *Contact:* Secretary, IOC, 1 rue Miollis, 75732 Paris cedex 15, France; fax (33-1) 4056 9316.

HARMFUL ALGAE NEWS

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Production Editor: Gary Wright, IOC/MRI.
Layout: Micheline Turner.

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ISSN 0020-7918

International Directory of Experts in Toxic and Harmful Algal Blooms

Questionnaire

An updated and expanded edition of this international directory is being prepared as a joint effort of the Intergovernmental Oceanographic Commission (of UNESCO) and the NOAA National Marine Fisheries Service of the United States. The purpose of the directory is to assist countries facing toxic and harmful algal bloom emergencies by facilitating rapid access to scientists, fisheries managers, public health officials, and physicians who are experienced in dealing with toxic and harmful algal blooms and their consequences to fisheries, aquaculture, and public health. The directory will also serve to expedite contact among the international community of experts in harmful algal blooms and related issues.

Please complete this questionnaire if you are involved in basic scientific, fisheries, public health, or medical aspects of harmful algal blooms, and wish to be included in the directory. Please copy this form for use by other appropriate colleagues in your country. A copy of the directory will be sent to all those who reply to the questionnaire. Send the completed questionnaire to the address on the reverse side.

Surname (*last name*): .....

Given names (*first names*): .....

Title (*Prof., Dr., Mr., Ms., etc.*): .....

Academic degrees: .....

Position (*Director, Chairman, Research Scientist etc.*): .....

Institution or affiliation (*include department if applicable*): .....

.....

Postal address: .....

.....

City: .....Country: .....

State or province: .....Postal code: .....

Telephone (*include country and city codes*): .....

Telex: .....

Fax: .....

E-mail (*include system, e.g. BITNET, INTERNET, OMNET, etc.*): .....

OVER PLEASE

General expertise (*you may indicate more than one*):

Biology	_____	Taxonomy	_____	Public Health	_____
Chemistry	_____	Physiology	_____	Medicine	_____
Physics	_____	Toxicology	_____	Vet. Medicine	_____
Ecology	_____	Fisheries	_____	Management	_____
Genetics	_____	Aquaculture	_____	Other (specify)	_____

Harmful algal bloom specialty/expertise (*summarize in a few sentences your background, experience, and specific subject areas in which you feel most able to provide expert advice regarding harmful algal blooms and their effects*):

.....

.....

.....

Species of harmful algae, shellfish, fish, etc., with which you are most familiar:

.....

Algal toxins with which you have worked most:

.....

Number of years experience with harmful algae/biotoxins:

.....

Geographic areas of the world where you have worked:

.....

Regional/international programmes related to harmful algal blooms, in which you participate:

.....

.....

Languages in which you have a working knowledge:

.....

Citizenship: .....

Please send completed questionnaire by 28 February 1994 to:

Dr. Alan W. White
Northeast Fisheries Science Center
National Marine Fisheries Service
166 Water Street
Woods Hole, MA 02543, USA