Shellfish biotoxins and algal blooms recur in New Zealand during 1994

Since the first closures due to biotoxin contamination in the north of New Zealand in early 1993, there has never been a period when the entire coast of the country has been open for shellfish harvesting. The summer and autumn of 1994 saw a repeat of the widespread closures of 1993, however this time most of these were in the South Island and, unlike the 1993 incident, no human illness occurred.

The monitoring programme carried out by the New Zealand Marine Biotoxin Management Board has proved to be effective in detecting biotoxin contamination incidents. Currently the programme involves the analysis of shellfish for water (PSP and domoic acid) and lipid (NSP and DSP) soluble biotoxins from an average of 120 sites every week. It has prevented suspect products appearing on the market and has proved valuable in directing research efforts.

**Paralytic shellfish poisoning (PSP)**

1994 began with a positive mouse bioassay test for PSP being returned by a sample collected from a mussel farm in the outer Marlborough Sounds region.

This was not the first time that PSP had been detected in cultivated mussels in the Sounds (there was an earlier minor incident in November 1993), but it was the first significant incident close to the centre of the major mussel production region. For a period the mussel industry held its breath as an area of the outer Sounds was closed and it was feared a more extensive closure might follow. Fortunately this was not necessary.

The initial examination of plankton samples revealed that moderate numbers (5.5 x 10^6 cells / litre) of *Alexandrium minutum* were present at the shellfish sampling site. More extensive sampling showed cell numbers up to 1.3 x 10^7 cells / litre existed in nearby waters, though samples from a number of sites soon established that this organism was only present in outer Pelorus Sound. Overall the cell numbers were low and *A. minutum* was not at any time the dominant phytoplankton species.

When the first positive bioassay results became known to the industry there was a voluntary closure of the bay and surrounding areas, although the levels of toxin in the mussels were still, at that stage, below the regulatory closure level (>80 μg / 100g). As further shellfish testing was carried out in adjacent areas, it was found that there was a good correlation between the results of the plankton analyses and the toxicity bioassays.

Because of this scrutiny it was obvious at all stages how extensive the problem was and how the situation was changing over time. This allowed the industry to continue business as normal throughout most of the Marlborough Sounds.

In retrospect it was obvious that had the plankton samples from this area been examined as a matter of routine, it would have been possible to predict the appearance of the toxicity

(Cont’d on p. 10)

**IOC opens science centres on harmful algae (see page 14)**

*The IOC Science and Communication Centre on Harmful Algae in Copenhagen is opened by the IOC Executive Secretary, Dr. G. Kullberg, and Professor Ø. Moestrup, University of Copenhagen.*
Potentially toxic algae along the Dutch coast in 1994

In 1994 high concentrations of algae were found in water samples off the Dutch Coast, on the Noordwijk transect. The seasonal sequence started with a bloom of *Phaeocystis* sp. in April. In June we observed *Pseudo-nitzschia pungens forma pungens* (Grunow in Cleve & Müller) Hasle with 380.10^6 cells/l. In July a *Dinophysis acuminata* bloom of long duration with maximum concentrations of 8.10^6 cell/l started. In the coloured water masses during August 1994, the Raphidophycean species *Heterosigma akashiwo* was found, a new species for Dutch coastal waters. The water column was slightly stratified (see table below).

In an extra sample, taken from Station 1 (from the pink water), large numbers of small species, like *Kato-dinium rotundatum*, solitary Chaetoceros cells and flagellates were observed. In this last group we identified the Raphidophycean *Heterosigma akashiwo* with the following details: two flagellae, width = 20 μm, length = 15 μm (with some size variation), numerous yellow/brown chromatophores, more or less flat in side view. The characteristic rotating movement was recorded on video. The total numbers per litre were 2.4 x 10^4. This species is known to be toxic to fish^{1,2}, but no dead fish were observed.

The video tapes with *Heterosigma akashiwo* from the Dutch Coast were shown at a phytoplankton workshop held in Büsum, Germany, 2-3 March 1995. At that meeting, Dr. Malte Elbrächter from the List Biological Station showed cultures of *Heterosigma akashiwo*. Good agreement was found between the cultured material and the video taped field material. The material from Dr. Elbrächter as well as the material from the Dutch Coast could not be preserved with Lugol or Formalin: after preservation no identification could be made; the cells changed into a cluster of small unrecognizable particles.

*Heterosigma akashiwo* was reported by Dr. Elbrächter on 26 August and 15 December 1994, at sampling station Weststrand off List and Wadden Sea 1, respectively, in the German Waddensee.

Environmental data on 4 August 1994

<table>
<thead>
<tr>
<th>Sample</th>
<th>Temperature</th>
<th>Nutrients</th>
<th>Salinity</th>
<th>Chlorophyll a</th>
<th>Phaeocystis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>10.49</td>
<td>0.04</td>
<td>3.02</td>
<td>21.91</td>
<td></td>
</tr>
<tr>
<td>5 m</td>
<td>6.83</td>
<td>29.62</td>
<td>1.64</td>
<td>20.53</td>
<td></td>
</tr>
<tr>
<td>10 m</td>
<td>6.91</td>
<td>31.05</td>
<td>1.28</td>
<td>19.96</td>
<td></td>
</tr>
<tr>
<td>Lower</td>
<td>17.53</td>
<td>27.05</td>
<td>4.09</td>
<td>24.5</td>
<td></td>
</tr>
<tr>
<td>5 m</td>
<td>7.08</td>
<td>29.85</td>
<td>1.46</td>
<td>20.5</td>
<td></td>
</tr>
<tr>
<td>10 m</td>
<td>6.73</td>
<td>30.73</td>
<td>1.3</td>
<td>20.07</td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>8.27</td>
<td>29.35</td>
<td>2.93</td>
<td>21.75</td>
<td></td>
</tr>
<tr>
<td>5 m</td>
<td>7.07</td>
<td>29.98</td>
<td>1.32</td>
<td>20.41</td>
<td></td>
</tr>
<tr>
<td>10 m</td>
<td>7.24</td>
<td>30.37</td>
<td>1.34</td>
<td>20.21</td>
<td></td>
</tr>
</tbody>
</table>

Marion Rademaker, Louise Janmaat, TRIPOS BV, Applied Marine Biological Research, Postbus 95125, 1090 HC Amsterdam, The Netherlands;


Satellite imagery

When multispectral data provided by satellite sensors first became generally available in the late 1970's, various attempts were made to detect and follow algal blooms, or at least chlorophyll, in coastal waters. Not all were successful. A serious brake on some of these studies was due to the fact that the optical characteristics of coastal and especially estuarine waters are usually very distinct from those of oceanic waters, and it was for the latter that the computational algorithms with which the sensor data was interpreted had been developed.

But there have been some successes. Dundas et al^{30} used the long-wave bands from a NOAA satellite to follow the advection of the 1988 *Chrysochromulina* bloom in Scandinavian waters, and Ligi et al^{22} of Telespazio, Rome, are using SPOT-HRV and LANDSAT-TM (Thematic Mapper) channels to establish the distributions of gelatinous aggregations in the northern Adriatic. By combining these images with the long wavebands of the NOAA-AVHRR (Advanced Very High Resolution Radiometer), from which surface flow fields can be derived with a resolution of about 0.1°C, they have shown that the aggregations and filaments of gel are clearly related to local gyres and thermohaline fronts. Some of these features are of the order of 100 km in extent.

This group now provides periodic bulletins to authorities in the region as to where along the coast problems associated with the stranding of these gels can be anticipated. The spatial resolution of this system is about 10 km. These are just two examples which indicate how satellite data may afford short-term forecasts of use to coastal zone managers.

*Trichodesmium* blooms have also been monitored by means of AVHRR signals, for example by Hakansson and Moberg^{26} in the Baltic. Subramanian and Carpenter^{47} took advantage of the high reflectivity of *Trichodesmium* which results from its contained gas bubbles and the strong absorption at 550 nm due to its phycocerythrin, and showed that the species can be successfully detected even when mixed with other phytoplankton. They used NIMBUS-CZCS data.

(Cont'd on p. 3)
Domoic acid (DA) first occurred in intertidal razor clams on the Oregon/Washington coast and in the viscera of Dungeness crabs in late October 1991. Other bivalves, including commercially grown oysters and mussels were tested, and did not contain the toxin. The source of the DA in this incident was never identified, but *Pseudo-nitzschia australis*, *P. pungens f. multiseriata*, and *P. pseudodelicatissima*, all known DA producers, have been found in local waters\(^{1,2}\).

In October 1994, the Office of Shellfish Programs of the Washington Department of Health (WDH), in conjunction with the US Food and Drug Administration, started a weekly phytoplankton monitoring programme at 20 sites in coastal and inland waters of the state. Net tows are collected by WDH personnel at sites where mussel cages (used as sentinel organisms for PSP and DA determinations) are located, and by local shellfish growers, county health departments, and native tribes. The samples are analyzed for species presence and relative abundance.

The first samples collected from Hoodsport, southern Hood Canal on 8 November 1994, contained a bloom of *Pseudo-nitzschia pungens*. This species remained dominant in samples collected weekly until 15 December. On 29 November, mussels from a cage at Belfair contained about 10 µg/g DA. No phytoplankton samples were collected there before that date, but phytoplankton collected on the 29th contained about 14 µg/g wet weight toxin. Both *P. pungens f. pungens* and *P. pungens f. multiseriata* were present in acid-cleaned samples examined by SEM.

Washington Department of Ecology (WDE) personnel sampled at a number of sites in Hood Canal on 1 December 1994 as part of their Puget Sound Ambient Monitoring Program. Data from some of the stations, including phytoplankton counts, are given in the adjacent table. The last few days of November were rainy with about 3.5 cm of rain falling on 30 November. Runoff was high and the water appeared brown from sediment.

Other species, including *Skeletonema costatum* and *Asterionellopsis glacialis*, increased in early December, and by early January 1995, *S. costatum* and *Thalassiosira* spp. (some forming auxospores) became dominant. The *S. costatum* bloom continued at least into mid-March (when this was written). Few *Pseudo-nitzschia* cells were present in January and February.

L. Hanson (WDH) collected weekly phytoplankton samples; L. Eisner and S. Albertson (WDE) collected the 1 December samples; C. Hattig (National Marine Fisheries Service, Seattle) analyzed the phytoplankton samples for DA; the WDH laboratory, Seattle, analyzed the mussel samples; and R. Horner did the phytoplankton enumeration and SEM identification.


\(^{*}\) *Pseudo-nitzschia pungens* (incl. both f. pungens and f. multiseriata)
\(^{**}\) *Skeletonema costatum*
\(^{***}\) *Asterionellopsis glacialis*

(Cont’d from p. 2, “Satellite imagery”)

PC-based satellite receiving systems now provide access to AVHRR data in real time at reasonable cost. Gower\(^{5}\) has now shown how the red (580-700 nm) and near infrared (720-1000 nm) wavebands of this sensor can be used to monitor at least some of the intense algal blooms of concern to the aquaculture industry in British Columbia waters. Blooms potentially detectable by this approach are those which cause increased backscatter, and the algorithm used is designed to reduce the effects of atmospheric haze and sunglint which interfere with their recognition. A good discussion of some of the technical problems associated with the interpretation of satellite images can be found in Brown and Yoder\(^{6}\). In coastal waters, increased backscatter is also due to suspended sediments delivered to near-surface waters by runoff or resuspension of bottom sediments by winds and tides. So the application of Gower’s procedures to regions where these processes are very active may lead to more ambiguous results, and considerable intuition will be required to tune the algorithms for other locations. But in the relatively clear waters of British Columbia this approach is now a routine component of the red tide monitoring programme. The spatial resolution is 1.1 km at nadir and 3 to 5 km at the edges of the swath scanned by the satellite.

Tim Wyatt
In the Philippines ...

Benthic dinoflagellates of the brown seaweed Sargassum spp

Sixteen dinoflagellate species were identified associated with various Sargassum species collected around the island of Cebu in the Central Philippines from August 1994 to January 1995. These dinoflagellates comprise nine genera in five families and three orders (all identifications are still tentative and are being sent for confirmation). The ciguatera causing Procentrum occurred most frequently, and other DSP-causing species were also found, namely: Procentrum concavum, P. lima, P. minimum, P. rhathymum and Ostreopsis lenticularis. Environmental factors (water temperature, salinity) and morphological characteristics of Sargassum (age of thalli, fertility) did not seem to affect the population density of dinoflagellates.

Such baseline studies are important now to establish the occurrence of potentially toxic species, especially since no toxin poisoning cases have so far been reported in these islands. Further health research should also be conducted to establish if these dinoflagellates are really not causing problems now. There is some anecdotal evidence of certain species (pufferfish, small tuna, sea hare, shellfish) having seasonal toxicity. Clearly, many studies still need to be done, here and in similar tropical areas. Research is slow due to lack of funds, good microscopes and literature. Collaboration is much needed in these areas.

Training on plankton and water quality methods for aquaculturists

The Marine Biology Section of the University of San Carlos held a training course on "Plankton Identification and Water Quality Methods for Aquaculture Ponds", 21-25 February 1995. The purpose was to help the aquaculture industry face up to mass mortalities of prawns, which occur in their ponds, and which are so far unexplained.

This training aims to provide them with the basic technical expertise to identify the microscopic plants and animals in their culture ponds, and to be able to assess the environmental conditions. Harmful algal species are presently the prime suspects responsible for prawn deaths, and there is thus an obvious need to obtain baseline data now before the problem affects our dollar-earning aquaculture industry. From this training, we hope to come up with appropriate management options and a comprehensive manual.

Problems expressed by the participants, who come from Cebu and Negros, are that they lack the technical and biological expertise for such work. Also, they do not have all the equipment needed. Moreover, the government agencies and consulting companies are not always up to date, and are expensive. Hence, most of the aquaculturists tend to have their own individual laboratories, and work on common problems facing the industry is poorly coordinated. This training provided a step forward towards the implementation of more standardized methods.

Perpetua Oloroso, Filipina Sotto, Jason Young, Marine Biology Section, University of San Carlos, Cebu City 6000, The Philippines.

New PSP occurrence

(Compiled from news reports from the Manila Bulletin and Daily Inquirer)

The first sign of trouble came late April 1995 when a red tide alert was raised by the Bureau of Fisheries and Aquatic Resources (BFAR), extending from the north of the archipelago (Manila, Bataan, Pangasinan, ...) to the South (Samar, Tacloban, Camiguin, Zamboanga, ...). No description of the bloom nor the causative organism, however, was included. On 18 May, the government's Red Tide Task Force ordered a ban on the harvesting, selling and eating of shellfish from the entire Manila Bay area, when a 4-year-old girl died and 31 others fell ill after eating the "tahong" (mussel). In a report to the Health Secretary, the Task Force recorded patients, mostly fishermen, complaining of numbness in the mouth, lips and extremities five hours after eating mussels. The Task Force had banned earlier the harvesting and selling of shellfish. Authorities immediately released results that the "red tide poison had exceeded the allowable level of 40 micrograms per 100 gram of shellfish".

This news only merited space on page 12 of the newspaper. Radio announcers carried the warning in their daily news, and this was the best form of communication to reach the poor shellfish gatherers and consumers.

May 19 - Mussels and other shellfish were impounded by the police in markets around Manila when vendors continued to sell their harvest. Three other children, ages 2 to 12 years, are reported to die from shellfish poisoning. Ninety-three people are also admitted to hospitals due to food poisoning.

May 20 - Health authorities ask local government officials to ensure compliance of the ban. Police said those found selling shellfish will be arrested. Senator criticizes the Department of Agriculture and the Department of Health in the delay of the issuance of public warnings. No report of the causative organism and extent of the bloom is still reported.

In the Visayas region, central Philippines, the Department of Health and the Department of Agriculture quickly issued warnings to consumers to refrain from eating mussels harvested in the eastern provinces of Catbalogan and Camiguin where "red tide cells have been found in shellfish". Mussels from other areas in the region are safe.

May 21 - President Fidel Ramos declares a state of calamity in 13 coastal areas in the Manila area and neighbouring provinces. In this case, the National Disaster Coordinating Council headed by the Secretary of Defense is enabled to "implement measures to cushion the effects of the red tide phenomena on the livelihood.

(Cont'd on p. 5)
Baltic algaline in internet

In 1992 the Finnish Institute of Marine Research started unattended measurements of phytoplankton and related parameters on merchant ships in the Baltic Sea\(^1\). The data consist of high-frequency recordings of chlorophyll \(a\) fluorescence, temperature, and salinity in the surface water as well as phytoplankton species composition and nutrient concentration analysis in discrete water samples. In 1993 the system was developed to serve as an early warning method for exceptional algal blooms. Based on recordings on several ferries, it has been possible to deliver almost on-line information on the development of algal blooms. In 1994 the weekly Baltic Algafax was delivered to about 40 environmental authorities around the Baltic Sea.

In 1995 the results from unattended measurements of phytoplankton on ferries are available on the Internet World Wide Web (WWW) page with URL of http://www.fimr.fi. The e-mail address of the project is <algafax@fimr.fi>. In July-September, satellite images processed by the Finnish Meteorological Institute, showing the extent of the blue-green surface accumulations, will be included too. Local information will be added on the page according to the information delivered to the project by various research institutes around the Baltic Sea. The data can be sent by e-mail or using FTP. The algaline team (Seija Hälfors, Juha-Markku Leppänen, Eija Rantajärvi) is ready to help everyone in using the Internet connection and hopes to get information in order to improve the WWW page.

In 1993, twenty-two potentially toxic phytoplankton taxa were detected in the Baltic Sea, but only the bluegreen algae Nodularia spumigena formed extensive blooms\(^2\). In 1994 the situation was similar to the previous year.


Rantajärvi Eija
Finnish Institute of Marine Research
Tel.: (358-0) 61394570; fax: (358-0) 61394494; e-mail: eija@fimr.fi

---

Chlorophyll \(a\) concentration (mg/m\(^3\)) as measured on ferry Finnjet and research vessel Aranda on 5 May in the Baltic Sea.

(Cont'd from p. 4, "New PSP occurrence")

of shellfish gatherers and growers". About 20,000 fishermen are estimated to be directly affected. The NDCC Chief also added that the "spread of red tide toxins in Manila Bay was triggered by the recent rains".

May 22 - Las Pinas, Metro Manila, the Mayor "immediately responded to the urgency of the problem, most especially to the family of a red tide fatality and promised burial assistance and food aid. The Mayor also made inspection rounds in the market". In Bataan, south of the Luzon region, the chairman of the provincial Task Force points out that the "first step they will undertake is to look for alternative sources of income for the farmers dislocated by the red tide".

May 26 - The red tide continues to "wreak havoc in Metro Manila and other neighboring areas". In Bulakan, south of Manila, considered the largest producer of shellfish in Luzon, the ban is strictly observed. Health authorities order its personnel to "take shellfish samples to determine if they are contaminated with red tide toxin".

Jason G. Young, Marine Biology Section, University of San Carlos, Cebu City 6000, Philippines; fax. (63-32) 460351 / 217183; email: jgyoung@mangga.usc.edu.ph

---

Proceedings of the Sixth International Conference on Toxic Marine Phytoplankton

One hundred copies of the above mentioned proceedings, 1993, are available free of charge to libraries of marine science institutions in developing countries from the IOC Science and Communication Centre on Harmful Algae in Copenhagen.

Please send your request to:

The IOC Science and Communication Centre on Harmful Algae, University of Copenhagen, Botanical Institute, Dept. of Mycology and Phycology, Oster Farimagsgade 2D, DK-1353 Copenhagen K, Denmark; tel.: (45-33) 13 44 46; fax: (45-33) 13 44 47; e-mail: hab@bot.ku.dk
Workshop on the Physiological Ecology of Harmful Algal Blooms

Plans are underway to convene an Advanced Technical Workshop on the Physiological Ecology of Harmful Algal Blooms. This is being organized by a working group co-sponsored by the Scientific Committee for Ocean Research (SCOR) and the IOC. This working group was asked to assess the state of knowledge on the physiological ecology of harmful algae, and to identify areas for future research. At a working group meeting held in October 1993, the working group decided that the best way to accomplish these goals was to convene an advanced workshop.

A proposal is now being submitted to NATO to provide support for this meeting as a NATO Advanced Study Institute (ASI). If support is received from NATO (with supplementary funds from SCOR and the IOC), the meeting will follow the ASI format which requires a 10-day meeting with lectures, field and laboratory demonstrations, and group discussions.

The working group decided that the goal of the workshop would be to summarize major advances in two main areas: (1) The autecology of critical groups of toxic phytoplankton, such as Alexandrium, Gymnodinium, Heterosigma, Pyrodinium, Gambierdiscus, and Pseudo-nitzschia, and (2) the ecophysiological processes and mechanisms that affect toxic bloom formation and the production of phytoxins. The focus on "toxic" rather than "harmful" algae is deliberate. Also, given the complicated array of trophic processes involving toxic algae, it is important to narrow the focus even further by excluding such issues as toxicology, toxin biosynthesis, transport, shellfish toxin uptake and depuration, management, epidemiology, ballast water transport, and physical or hydrodynamic forcings. It was agreed that a variety of techniques should be presented (e.g., measurement of in situ growth rates, use of molecular probes), but that physiological or ecological questions, rather than techniques, should be the driving force behind all activities. It is hoped that one outcome of this approach will be the elucidation of common factors that underly blooms of related species as well as of toxic species in general.

The purpose of this notice is to alert the HAB community that this workshop is tentatively scheduled for 27 May-6 June 1996, at the Bermuda Biological Station for Research (BBSR).

The number of participants at the workshop will be restricted as well. If NATO funding is obtained, a maximum of 80-100 participants is allowed. The organizers are targeting 80 participants, based on the housing available at the BBSR. Some travel assistance will be available, but selection of participants will in part be determined by the degree to which each individual can provide his or her own travel funding. Since it is likely that there will be more than 80 individuals interested in participating, a steering committee will select participants on the basis of the expertise that applicants can bring to the workshop, country of origin, willingness to help with demonstrations or lectures, relevance to the physiological ecology theme, professional status and funding support.

Note that this meeting is still only "tentative", as funding is not yet secured. Nevertheless, it is important to place this date on your calendars and begin thinking not only of travel support, but also about topics you might be able to offer to the workshop participants. Demonstrations of new technologies or approaches are of interest. Further details of this meeting will be announced in this newsletter at a later date, as well as through advertisements in major scientific publications, notices on e-mail bulletin boards, etc. If you have ideas, comments, or questions, please address these to the chairman of the working group: Don Anderson, Biology Department, Redfield 3-32, Woods Hole Oceanographic Institution, Woods Hole, MA 02543-1049, USA; e-mail: danderson@whoi.edu.

Ireland: Extended "toxic season" in 1994

The Fisheries Research Centre of the Department of the Marine has in place an ongoing monitoring programme for the detection of DSP toxins in shellfish in Ireland. Since monitoring began in 1984 there have been regular closures of shellfish growing areas, particularly those situated in the Southwest of the country, due to the presence of DSP toxins in both mussels (Mytilus edulis) and oysters (Crassostrea gigas). In general, the period during which closures have been in force (the "toxic season") has been the summer months of June-September, and the growers and processors have adapted their marketing strategy to take account of these events. During 1994, however, the presence of toxins was detected for a period extending well beyond the traditional "toxic season". In the neighbouring Roaring Water, Dunmanus, Bantry and Kenmare Bays, on the southwest coast of Ireland, toxins were first detected by standard rat bioassay in mid-May/early June and positive bioassay results were obtained in weekly samples from these bays until February 1995. Chemical analysis of mussel extracts by LC-MS methods confirmed the presence of DSP toxins in the samples, and showed that both okadaic acid (OA) and DTX-2 were dominant, reaching a peak of 12 µg/g of hepatopancreas in mid-August. While OA concentrations decreased to <0.1 µg/g by late September, the concentration of DTX-2 was an order of magnitude higher, and remained at concentrations >2 µg/g throughout the winter. The high pulse of DTX-2 recorded in August was associated with a pulse of Dinophysis acuta of up to 20,000 cells/litre recorded in inner Bantry Bay at the same time. While both Dinophysis acuta and Dinophysis acuminata were recorded in the water column during the June-September period, no toxic species were recorded after this time, and the reason for the persistence of DTX-2 in the mussels is still unclear. It is clear, however, that a monitoring programme involving the surveillance of toxic or potentially toxic species alone is insufficient to prevent shellfish with high concentrations of toxins being placed on the market, and that bioassay and confirmation by chemical methods is essential.

Terry McMahon, Eugene Nixon and Joe Silke, Fisheries Research Centre Abbotstown, Dublin 15, Ireland.
Cyanobacterial overgrowth in inland Australia waters has recently received attention after the development of massive blooms of toxic organisms in the Darling River basin\(^{12}\). These overgrowths have been attributed to increased nutrients coming from town effluents, runoff from fertilized pastures and cattle feedlot waste, together with low river flow rates and water diversion. However, this is not a recent phenomenon, for, as described by Johnstone, toxicity at the mouth of the same river system was reported as long ago as 1878\(^{13}\).

There is evidence in Australia that cyanobacterial overgrowth and toxicity occurred in inland waterways well before the advent of closer settlement. Australia is dominated by old erodible soils, with prolonged dry periods broken by torrential rainfall resulting in significant transport of nutrients into aquatic systems even without the intervention of man. Where there is warmth, high evaporation with solute concentration, shelter from wind and relative stagnation, natural waters may support cyanobacterial blooms even without the further addition of waste materials.

A disease known as “Barcoo Fever” or “Barcoo Sprue” was once widespread in outback Australia and has been described by many early authors. Characteristic symptoms were nausea and vomiting, exacerbated by eating and often even by the sight or smell of food, fever, headache, initial constipation often followed by diarrhea, and variable myalgia. The disease may range in severity, all ages may be affected, and recurrent episodes were common. It does not have the features of an infectious disease and it is not due to either sun exposure or vitamin deficiency, as has been variously proposed. This disease, once very prevalent, has now disappeared from medical literature and is no longer known as such in outback Australia. However it does still exist and many habitual travellers in outback Australia, on questioning, can recall an episode of illness very similar to the old descriptions. There is good evidence that the disease is due to cyanobacterial toxicity; such an explanation would explain its original incidence and now virtual evanescence, recurrence in the one individual and marked variation in severity\(^{14}\).

The early explorers found cyanobacterial and algal blooms, suffered from their toxicity, and some died from them. Capt. Charles Sturt in 1844 was the first European to discover and name Coopers’s Creek, the stream in central Australia which receives the waters of the Barcoo River. He found “splendid sheets of water, some 200 yards from side to side with banks which rose up some 18 feet.” The water in the stream however was vivid green. Returning to his base camp at Fort Grey, Sturt found the camp deserted, with a message from his lieutenant Harris-Browne telling him that he had been obliged to retreat to another waterhole 100 km away. The water at Fort Grey had become putrid and was causing his men dysentery. The problem was all too evident; when they examined the little pool, they found it covered with slime that was green on top and red below.

In the disastrous Burke and Wills expedition of 1960-61, death occurred not only of the leaders of the expedition but also among members of the rear party during their futile attempt to reach the base camp on Cooper Creek. Herman Beckel, the German doctor with this party described their difficulties\(^{15}\): “Mr. Wright had miscalculated this time; the waterhole which lay about a mile from here was supposed to provide us with water for four to five days, but the horses drank it all at once so that nothing remained but a layer of green slime about two inches deep. Nonetheless, I was very glad to wet my lips with it when I came to it the following day with the camels. ... The ground near this water was covered with stalactite-like stones of a strangely rounded form, coated as with varnish — possibly sintered lime or silica.” (These appearances are seen when drying has occurred of water containing dense concentrations of algae or cyanobacteria.)

... “Late in the evening of 8 March, Hodgkinson returned. He had found Becker and the cook very wretched. For two days they had no drinkable water, which was not, however, our fault as the water we had taken to the camp could easily have lasted five days. ... Unfortunately Becker and the cook had become visibly more ill since I had last seen them, although the former was still in good spirits. Wright now wished to travel to Bulla with the whole party as soon as possible”. It was at Bulla that the two members of the party died.

Their symptoms were those of lethargy and inanition, symptoms which may well have been due to the forced ingestion of water containing toxic microorganisms. These two were the first to become ill and it was these two who were exposed to contaminated water. Although vitamin deficiency and simple starvation may have contributed to the deaths of other members of the party, there is no reason why the members of the rear party should have been so affected as they had adequate food supplies, both for themselves and for the forward party. It would seem very likely that toxic algae caused the death of these two persons and at least contributed to the deaths of others.

Aboriginal Australians, whose forebears have lived on the Australian continent for at least 40,000 years were well aware of the dangers of contaminated water. In many areas they would dig soakas in sand beside streams and draw water from these rather than from the main stream. Experienced settlers followed their example and survived where others before them had perished.

In summary, although regarded as a recent development, blue-green algae or cyanobacterial contamination of inland Australian waters has occurred for many years if not millennia. Organisms which have evolved thousands of millions of years before man, do not require man for their propagation.

John Hayman, Department of Pathology, Box Hill Hospital, Box Hill, Melbourne, Victoria 3128, Australia.
Did the majority of dinoflagellates perish in the great Permian extinction?

The dinoflagellates (*Phylum pyrrophyta*) constitute a very diverse group of protists, ranging from potent toxin producers to those responsible for the formation of red tides. The most remarkable feature of dinoflagellates is the unusual nature of their nucleus, exceptional amongst the Eukaryotes and which indicates their primitive origin (Loeblich, 1976). The dinoflagellate nucleus combines various ancestral characters representative of the Prokaryotes, and several typically eukaryotic features with other properties unique to dinoflagellate nuclei alone (reviewed by Herzog et al., 1984). Consequently, dinoflagellates were widely studied at the cell biology level, and new and controversial phylogenetic interpretations were proposed, suggesting, among others, the positioning of this group among the lower Eukaryotes (Taylor, 1980), or alternatively its consideration as a sister group of the actual Eukaryotes (Herzog et al., 1984).

Dinoflagellates were previously perceived to be Mesokaryotes, phylogenetic intermediaries between Prokaryotes and Eukaryotes (Dodge, 1965). However, with the onset of molecular phylogenetic criteria it was demonstrated that the dinoflagellates were far from primitive, being neither eukaryotic nor afore-stated phylogenetic intermediates. What was actually being considered were organisms distantly linked to ciliates and yeasts (Lenaers et al., 1989). Without doubt, from a molecular point of view, the Giardias, the Microsporidios, the Kinetoplasts, the slime-moulds, the Entamoebas, the Plasmodias, and also the Euglenas had separated from the main trunk of the Eukaryotic family tree before the dinoflagellates (Knoll, 1992). The fossil record (limited to cyst-forming species) suggests an evolutionary course beginning as a primitive Gymnodinioid-like dinoflagellate. Further species evolved by plate reduction, at first originating into Peridinioid- and Gonyaulacoid-like and then later into Prorocentroid-like dinoflagellates (Eaton, 1980; Loeblich, 1984). On the other hand biological considerations support a plate increase model, where Prorocentroids represent the most primitive condition and Peridinioids, Gonyaulacoids and later Gymnodinioids evolved by a progressive increase in the number of thecal plates (Loeblich, 1976; Taylor, 1980). A further model implicates thecal plate fragmentation as the evolutive process from Prorocentroid to Gymnodinioid and plate reduction for the further development to Peridinioid-Gonyaulacoid (Buijak and Williams, 1981). Recently, the primary molecular phylogenies have shown what was suspected, that it is not possible to establish a clear phylogeny for the principle groups. In fact, Prorocentroid, Gymnodinioid and Peridinioid species all appeared intermixed (Lenaers et al., 1991). A previous study indicates the Syndinophyceae (parasitic which one finds to be well established for LsUrRNA genes. The clock can be calibrated by means of well defined reference points in the fossil record. The figure below summarizes the results found using the average values cited by Knoll (1992) as source material to time scale the molecular change, and distance matrix methods to analyse the sequences of the LsUrRNA gene regions D1 and D2. Apparently the dinoflagellates separated from the ciliates as little as 600 million years ago. In a conventional interpretation, the separation between thecal and naked species would have occurred approximately 300 million years ago, while the separation between desmokonts and dinokonts would have happened over 236 million years ago. The Gymnodinioids would have radiated some 220 million years ago, and the Prorocentroids 215 million years ago, compared to the relatively

![Diagram: Major events in the evolutionary history of dinoflagellates: 1) separation between dinoflagellates and ciliates; 2) separation between thecal and naked dinoflagellates; 3) separation between desmokonts and dinokonts; 4) radiation of the gymnodinioids; 5) radiation of the prorocentroids; 6) radiation of the Alexandrium; ?? possible early radiations of the extinct dinoflagellates before or during the Permian period.]

In an attempt to clarify the perplexing phylogeny of the major dinoflagellate groups, we sequenced the regions 1, 2, 9 and 10 of the LsUrRNA gene, in a sense a pseudogene of the same subunit. These sequences are to be found deposited in the GenBank with the access numbers L38623 to L38640 and will soon be published (Zardoya et al., in press). Using these sequences we can convert the genetic distance into a temporal distance assuming molecular change as a clock at constant velocity, late radiation of *Alexandrium* species just 80 million years ago. However, an event of even higher importance should be mentioned, an event that occurred over 286 million years ago, culminating in the greatest mass extinction in the history of the planet. This catastrophe caused the extinction of over 90% of all marine species, being especially severe amongst planktonic organisms where diverse groups, prosperous of old, disappeared forever. Viewing the phylogenetic reconstruction for the dinoflagellates, it could be considered that just two groups (thecal and naked) survived the great Permian extinction. Practically straight-
afterwards however, the dinoflagellates suffered a significant species radiation which coincides with that observed in the fossil record. Can it then be said that after separating from the ciliates, dinoflagellates existed for the greater part of their evolution without radiating, and then underwent a sudden diversification immediately after the great Permic extinction? We think not.

Thus according to our hypothesis, it seems reasonable to assume that dinoflagellates saw numerous radiations during their life history after separating from the ciliates. However, with the arrival of the Permic period they suffered an extinction of such grand proportions that it left a scarce few representatives of both naked and thecal dinoflagellates as the sole remnants of this evolutionary branch. The rest disappeared, falling foul of the Murphys law of the Permic period: extinction. In taking this secret – that many have tried to uncover – to their hidden graves, they have so deprived us of the possibility of verifying the evolution of the principle dinoflagellate groups forever.

How the radiation of the major dinoflagellate groups occurred seems ever more indecipherable with each passing moment. What is undeniable is that it is impossible to unravel the enigma of their evolution with only the dinoflagellates that have survived to this day. In this sense, it seems we are resigned to never knowing how the principle dinoflagellate groups radiated out from one another, although perhaps this polemic will survive some time yet. All is not woe however; one important fact can be indirectly ascertained from the re- construction of the dinoflagellate phylogeny: the origin of PSP toxicity. The capacity to produce toxins appears to be unrelated to any particular phylogenetic line. Toxic species can be seen for both Gymnodinioioids and Gonyaulacoïds, and toxic and non-toxic species can be found between species that are closely related phylogenetically. Paleontological data further complicates this problem by suggesting dinoflagellates caused high animal mortality various thousands of years ago, apparently by means of toxins similar in fashion to those responsible for PSP. Therefore it leads one to think that either the genetic base for toxicity was acquired from an external source (from bacteria?) or it is rooted in genes that manifest evolutionary similarities to pseudogenes. Hence, an interesting issue can be proposed: the list of toxic species is not nor will it ever be limited. It is highly probable that we will be witness to the appearance of toxicity in species that are, for now, free from our suspicions.

References

Eduardo Costas and Victoria Lopez-Rodas, Facultad de Veterinaria, Universidad Complutense de Madrid, 28040 Madrid, Spain.

Economic consequences of toxic algae in shellfish markets

A wide variety of economic losses result from toxic algae blooms. Typically the calculated losses are a function of lost revenues by fishermen, lost revenues by other businesses, such as the retail, restaurant and hotel industries, and affected tourism revenues. There are also the medical costs of illness and consequent loss of wages, of monitoring the water and seafood for toxins, and the costs associated with public announcements and advice are not trivial either.

Another impact of harmful algae blooms arises when public announcements warn of the dangers of consuming shellfish harvested from affected areas. Such public announcements, typically reported by the news media, generally influence the demand for associated consumer products. Public announcements that shellfish from particular areas are toxic, and hence should not be consumed, generally result in consumer fear and avoidance of that product, and usually spread to related products. Often the public agency's announcements specify exactly which areas are problematic and are not meant to alarm the public. However, news media reports may not be that specific, and may even be inaccurate, creating the potential for false perceptions of elevated risk. Consumers may not be able to differentiate between safe and unsafe products, and hence assume that all supplies are unsafe. A possible result is a decrease in demand for both affected and unaffected shellfish and seafood products. This decrease in demand may continue for only a short time, a few days or weeks, or may persist for an extended period after the effects of the algal bloom are over.

In an on-going research project funded by the Rhode Island Sea Grant Program, the fraction of the economic losses attributable to the decrease in consumer demand for mussels in Montreal, following the 1987 domoic acid contamination of Prince Edward Island (Canada) mussels, was estimated. In particular, losses for one Maine firm, whose mussels were not contaminated by domoic acid, were estimated at 15% of the firm's annual average sales to the Montreal market (actual sales numbers are confidential). These losses apply only to the one firm and only the Montreal market. Thus, these losses do not provide information on the full extent of economic losses to the Canadian and U. S. mussel industries due to a decrease in demand.

However, it does indicate that these economic losses may be comparable in magnitude to other more commonly quantified economic costs of toxic algae blooms. Quantifying these losses is necessary to compare the costs of toxic events with the benefits of reducing the frequency or severity of such blooms.

Cathy R. Wessells, Department of Resource Economics, University of Rhode Island, Kingston, RI 02881; tel.: (1-401) 729 4569; fax: (1-401) 782 4766.
in the shellfish in advance of the results of the toxicity tests becoming available. In August 1994 another minor contamination incident occurred in a nearby area. In this case the plankton samples were examined immediately after collection and a warning of the presence of low numbers of *Amphidinium* prevented the harvesting of mussels that, some days later, revealed traces of PSP toxicity.

The examination of a very large number of plankton samples from throughout the country over the last few years has revealed that *Amphidinium* is widespread but rarely plentiful, and then only for brief periods. It seems likely that closures due to this species will be necessary, from time to time, but these will probably be rather small scale local events, representing little real hazard to human consumers and an irritation rather than a threat to the shellfish industry.

**The Southland “Lipo-tox” Incident**

The major biotoxin event of 1994 began in mid-January due to the detection (by mouse bioassay) of a lipophilic toxin in dredge oysters (*Tiosstrea lataria*) from Foveaux Strait. As a result, a large area of the Southland coast was closed and over the following months the closed area was extended up the east coast of the South Island, until eventually in late April a part of the northern Marlborough Sounds was affected. As well as contaminating filter feeding shellfish there was evidence of associated mortalities of other fauna such as seabirds and crayfish. There was also some evidence to suggest that it made cultivated mussels more susceptible to an infectious viral disease that also appeared at this time.

The mouse bioassay of acutely toxic extracts of oysters first showed a toxic response of >39 MU / 100g on 14 January and these levels had risen to >850 MU / 100g by late February.

From the beginning it was apparent that the bioassay results were different from anything previously encountered. Very rapid death times of the mice (with an unusual symptomatology) resulted in high toxicity scores; however, when extracts were diluted to obtain quantitative estimates (i.e. MU values), the toxicity disappeared. Hence there appeared to be a dose-related threshold effect in the response of the bioassay mice.

The first plankton samples collected from Foveaux Strait on 21 February revealed high numbers of a variety of *Gymnodinium* cells. The most abundant cell type observed in these samples was one resembling *Gymnodinium mikimotoi*. This cell is distinguished by a centrally located nucleus in the hypotheca, extreme dorso-ventral flattening, chloroplasts distributed around the periphery of the cell and an apical groove. Other morphotypes were represented by cells with a different chloroplast morphology and a few cells closely resembling *Gymnodinium breve* were observed. Cell numbers of *G. cf. mikimotoi* in excess of 100 thousand cells per litre were uniformly distributed through the water column down to the bottom at 30 metres. Recently a detailed examination of the morphology of this dinoflagellate and comparison with a Japanese strain of *G. mikimotoi* has shown that this is most probably an undescribed species.

The link between the toxicity in the shellfish and the dinoflagellate was suggested by the relationship between cell numbers and the results of the bioassays and was eventually proven by the establishment of the *Gymnodinium* species in laboratory culture and the bioassay of crude extracts of culture concentrates. These extracts gave a bioassay result identical in terms of symptoms, rapid death times and threshold effects to that observed in the contaminated shellfish.

Considerable effort has gone into investigating the nature of the toxin involved in this incident by several research groups and much progress has been made. Professor Yasumoto and co-workers at Tohoku University have succeeded in identifying a compound from the oysters that has been given the name "gymnodimine". These workers have also succeeded in isolating gymnodimine from the *Gymnodinium sp.* culture. As a result of rat feeding trials (Dr N. Towers, Ag-Research, pers. comm.), it has been established that the oral toxicity of this compound is very low if indeed it has any toxicity at all.

On the basis of these results, the Marine Biotoxin Management Board decided in September 1994 to change the bioassay procedure. This basically involves an additional confirmatory ether extraction procedure in the event of positive bioassays of acetone extracts of shellfish. The *Gymnodinium* sp. toxin is not apparent using this method and, by this criteria, gymnodimine is no longer regarded as a significant hazard to human health.

By early March, *Gymnodinium* cell numbers in Foveaux Strait had fallen substantially and by mid March they had all but disappeared from the plankton.

One of the most unusual features of this incident has been the very long retention of acetone soluble toxicity by the Foveaux Strait oysters. This appears to be a property of the shellfish themselves since other filter feeding molluscs have lost it rapidly in the absence of the causative dinoflagellate. One year after the initial contamination, acetone extracts of Foveaux Strait oysters are still returning toxicity scores over 100 MU /100g. Ether extracts of the same shellfish show no toxicity.

**Diarrhetic shellfish poisoning (DSP)**

It has been known for many years that blooms of a variety of *Dinophysis* species are common in New Zealand waters though (with one possible exception) no actual cases of human poisoning have ever been attributed to this cause. In addition to the conventional mouse bioassay for this toxin, in 1994 the screening of shellfish using an ELISA test ("DSP-Check") for DSP toxins was begun. As a result, on several occasions indications of the trace occurrence of DSP toxins have been observed in some South Island mussels. Recently the opportunity has arisen to examine this in more detail (using bioassay, HPLC and ELISA techniques) due to blooms of *D. acuminata/saccusculus* and *Dinophysis acuta*. The provisional results of these studies indicate that *D. acuminata/saccusculus* appears to be a long term resident of specific locations. It does produce some DSP toxins; however, the specific toxicity per cell is extremely low. Even under worse case conditions where mussels are exposed for long periods to high *D. acuminata/saccusculus* cell numbers (> 2.0 x10⁴ cells / litre) the levels of toxin accumulated by the
shellfish are below the action level. A prolonged bloom of *D. acuta* and the associated contamination of mussels with okadaic acid has been followed over the last summer in some detail. There has been a good correspondence observed between the levels of toxicity in inter-tidal mussels and cell numbers in surface waters. Work is continuing on the characterisation of DSP-toxin profiles in New Zealand, *Dinophysis* species and contaminated shellfish.

**Domoic acid**

During 1994, the routine screening of shellfish samples for domoic acid (DA) from a large number of sites throughout the country was carried out (by Environmental Science Research Ltd.). During this period, 1,957 samples were analysed of which 7% showed traces or larger amounts of DA. DA contamination seems to be quite widespread though most results were below the action level of 20ppm. Scallops appear to be particularly vulnerable to DA contamination. They have returned the highest results and contamination has persisted longest. Other species (except on one occasion in mussels) have only revealed very low-trace amounts of DA. DA is sequestered in greatest concentrations in the digestive glands of scallops, though the edible tissues (muscle and roe) have become contaminated to unacceptable levels at some times.

The relationship between DA acid contamination and *Pseudo-nitzschia* spp. blooms is not as clear cut as with some other biotoxins (eg. PSP). The reasons include: the lack of plankton data accompanying DA contamination incidents (and the brevity of some of these), rapid changes in specific DA production rates by the micro-algae during the growth cycle and the difficulty in the definitive identification of potentially toxic species using the light microscope.

Despite these problems there are data that indicate a relationship between *Pseudo-nitzschia* abundance and domoic acid contamination. This seems to occur during the decline phase of *Pseudo-nitzschia* blooms and these are therefore usually rather brief events.

There is recent evidence that some species or strains of *Pseudo-nitzschia* in New Zealand do produce DA. A New Zealand isolate of *P. australis* (from Bream Bay) has been shown to produce DA in culture. This species has been found in association with DA contamination incidents in the Marlborough Sounds and the far north (Whangara Hbr.). Other producers of DA (eg. benthic diatoms) also remain a possibility.

Further research is required to more fully elucidate the relationship between the phytoplankton and DA contamination; however a "rule of thumb" suggests there should be extra vigilance after the peak of *Pseudo-nitzschia* blooms is reached.

**"Red Herrings"**

In addition to the biotoxin incidents discussed so far, there have also been several occasions where the results of bioassays for lipid soluble toxins appear to have been misleading. From an industry perspective, the most damaging incident this year was the closure of a large part of the Marlborough Sounds mussel growing region in June 1994.

This incident resulted in a large product recall, and involved considerable expense in carrying out clearance tests on detained material. The reason for the closure was a single positive bioassay on a lipid (acetone) extract of cultivated mussels. A routine plankton monitoring programme was in operation at the time which revealed no correlation between the plankton observations and the results of the bioassay. It could be said with confidence that there had been no change in the composition of the plankton for a considerable period and no potentially toxic phytoplankton were in evidence.

None of the subsequent shellfish sampling at this location, or in the general vicinity, returned positive tests and there was no means of confirming the original test. This incident had all the hall marks of a false positive. It illustrates the problem associated with reliance on mouse bioassays for lipid soluble toxins and highlights the need for some means of independently validating these results. The cause of the original positive test in this case remains unknown.

Lincoln Mackenzie, Allison Haywood, Lesley Rhodes and David White, Cawthron Institute, Private Bag 2, Nelson, New Zealand;

Penny Truman, Communicable Diseases Centre, Kenepuru Drive, Porirua, New Zealand;

Don Hannah and Paul Jones, Environmental Science Research Ltd., PO Box 30543, Lower Hutt, New Zealand,

Bill Trusewich and Jim Sim, Marine Biotoxin Surveillance Unit, MAF Regulatory Authority, PO Box 2526, Wellington, New Zealand.
Bivalve mortality on southwest Atlantic shores

A mass mortality of yellow clams, *Mesodesma mactroides*, was recorded on the coast of Uruguay (Barra del Chuy) near the Brazilian frontier, beginning on 11 December 1994. It has been calculated that around 750,000 clams died, with a mean size of 44 mm and a mean weight of 12 g, equivalent to about 9 tons in total. A smaller quantity of cockles, *Donax hanleyanus*, with a mean length of 16 mm, also died. The water was clear with a temperature of 22°C, normal for this time of the year, and a salinity of 26.8 PSU, lower than that recorded 15 days earlier. Variations in salinity of this order are normal here, and do not generally affect the benthos. Mouse bioassays indicated that diarrhetic but not paralytic shellfish toxins were present.

Plankton samples contained a variety of diatoms and dinoflagellates dominated by *Asterionellopsis glacialis* and lower numbers of centric diatoms; *Dinophysis caudata* (4600 cells/l) and *D. acuminata* (3560 cells/l) and various ceratia, especially *Ceratium furca* (4000 cells/l) were the most abundant dinoflagellates. The abundance of silicoflagellates was also notable. Dense plankton blooms are common here at all times of the year, and wave energy considerable, and deoxygenation of the water can be discounted as a cause of the mortality, but it is possible that the siphons were blocked physically. The local prefecture recalls a similar event 15 years ago, but there are no records. INAPE (Instituto Nacional de Pesca) has regulated the extraction of clams from this beach since 1981 due to its commercial importance.

News of the occurrence was passed quickly to staff at FURG (Fundação Universidade do Rio Grande) in Brazil and sampling in the two countries was coordinated, following guidelines proposed during the IOC Regional Workshop on Harmful Algal Blooms, held in Montevideo, 15-17 June 1994 (IOC-UNESCO Report, Nº 101). A mortality of yellow clams was noted simultaneously in Brazil, extending about 12 km to Hermenegildio. Plankton samples taken there yielded similar results to those from Barra del Chuy. A massive mortality of yellow clams in southern Brazil took place in 1993, and extended for approximately 350 km. The present event was smaller since the population has not yet recovered.

Silvia Méndez, Instituto Nacional de Pesca, Constituyente 1497, 11200 Montevideo, Uruguay.

Bibliographic notes

Dr. Felipina Sotto and her colleagues in the central Philippines recently suggested that their nationally planned red tide management programme is not flexible enough to respond rapidly to regional events, due to “the lack of coordination among agencies and the inadequate response of local government units”, and also the fact that “active involvement of both regional and municipal governments ... was not implemented ...”[1]. This newsletter has already drawn attention to the difficult political and social challenges faced there[2]. The Philippines archipelago has a total area of around 300,000 km², roughly the same as Italy or the Ivory Coast. But its coastline is amongst the longest in the world – only high latitude regions with fjords like in Chile or Norway are in the same league. Coasts like these are obviously nightmares for the agencies responsible for comprehensive nationwide monitoring programmes, given the spatial and temporal scales of toxic phytoplankton events.

A red tide in November 1992 which affected green mussel farms in Samar provoked the establishment of a regional task force in Cebu, headed by the Department of Health there, and a rapid response to the threat of contaminated imports from the neighbouring region. According to this account, a local credibility problem between exporters and health authorities was quickly resolved, and some technical constraints on more effective regional monitoring were highlighted.

The court life of the Heian period of Japanese history is familiar to many through the widely translated 11th-century novel, *The Tale of Genji*. This period saw the establishment of Kyoto as the capital and the rise to power of the Fujiwara clan. Less familiar will be the red tides recorded then (8th and 9th centuries of the western calendar) and summarized by Akashio Takano[3]. A thorough search of ancient Japanese sources would surely reward us with much more of interest in this context, as has been done for the Pacific sardine fishery, documented in a semi-quantitative way as far back as the 14th century.

Modern Japan, as is well known, sometimes suffers severe economic losses due to red tides. The main species responsible are *Gymnodinium*.
nagasakiense, Chattonella antiqua, C. marina, and Heterosigma akashiwo. Tsuneso Honjo of the Nansei Fisheries Institute has recently provided a concise precis of the biology of these four organisms, together with an assessment of some empirical methods for anticipating when they might bloom. These methods are site-specific. In another paper, Honjo runs briefly through the kinds of evasive actions which are used to protect cultured stocks when algal threats are imminent.

It is a long way from the 9th century Kyoto court to 17th century England, but the longest available instrumental temperature record, the Central England time series or CET, began there in 1659 A.D. A recent analysis of this time series by David Thomson of the AT&T Bell Labs in New Jersey suggests that the dominant seasonal cycle is timed in accordance with the anomalistic year which runs from perihelion to perihelion (when the sun is closest to the earth), rather than with the tropical year (which runs from equinox to equinox and measures the normal calendar year). Since the difference between these two ways of measuring the year is only one part in 26,000, the distinction may at first sight appear rather esoteric. Not so. It is important to recall that the position of perihelion "precesses", that a complete circuit of the earth from one perihelion to the next is not exactly 360°. There is thus a small phase change, called the precession constant, which amounts to about 30 arc seconds per year. The startling feature of Thomson's analysis of the CET is that the phase of the temperature data follows that of precession for 300 years, and then alters dramatically, in about 1940 A.D. Some other records show similar features, while others do not, and there is some evidence of phase capture triggered by solar forcing. Interested readers should consult Thomson's paper for details. What has all this to do with harmful algal blooms, or mediaeval Kyoto for that matter?

For more than a decade, there has been a fascinating debate, initiated by Ted Smayda, which is based on his contention that there has been a worldwide increase in the frequency and intensity of algal blooms. The debate is about whether it is true, and if so, what is causing it. Changes induced by climate change or greenhouse forcing provide an attractive alternative to competing hypotheses. Thomson concludes his analysis with the comment that "the effects of increasing greenhouse gases may be worse than previously thought", since removal of the precessional signal from temperature time series increases the importance of the increase observed in recent decades. What we need then from our special perspective is reliable time series of HAB events covering at least the last 100 years, to compare with the trends being uncovered by meteorology and other disciplines. Archival studies like that of Takano are one route to the compilation of such time series, and if they become available, will help us to understand better what is going on. But for the time being political and technical innovations such as those being dealt with by Soto and her colleagues in the Philippines and by Honjo and others in Japan are of more immediate practical concern.

References:
2) HAN No. 3, p. 1; No. 4, p. 1; No. 8, p. 5.

*We are grateful to Dr. Honjo for supplying us with this paper.

Tim Wyatt
IOC opens science centres on harmful algae

To provide a better service to Member States facing serious problems caused by harmful algae, the IOC at its Seventeenth Assembly in 1993, decided to establish Science and Communication Centres on Harmful Algae. The Centres will in particular facilitate the implementation of the HAB Training and Capacity Building Programme. In 1993, Denmark and Spain offered the IOC to establish Centres at the University of Copenhagen and at the Instituto Español de Oceanografía, Centro Vigo, respectively. The Centre in Copenhagen was opened by the IOC Executive Secretary, Dr. G. Kullenberg, and Professor Ø. Moestrup, on 5 May 1995. The Centre in Spain is expected to be operational early 1996. The two Centres are established for a five year period, are planned to be complementary and will be coordinated by the IOC Secretariat.

The Centre in Copenhagen is jointly sponsored by IOC, the Danish International Development Assistance (DANIDA), the University of Copenhagen, the Danish Institute for Fisheries Research, and the National Environmental Research Institute, and the activities of the Centre will be directed specifically towards developing countries. The Centre will offer practical and direct assistance with identification of problematic species of harmful algae, establish a data base on harmful algae literature, and build up a reprint collection which will be available free of charge to research institutions in developing countries. A major activity will be recurrent training courses on the taxonomy and biology of harmful marine phytoplankton in particular to train trainers. A first course was held already in 1993 at the University of Copenhagen. The second course is scheduled for 31 July - 11 August this year. Up to 15 phytoplankton scientists will attend each course. Courses will also be given regionally. In 1995, a course for the Northern and Western Indian Ocean is planned for 4-14 September 1995 at the University of Mauritius.

The Centre will also offer supervision of PhD students from developing countries. PhD projects will be offered in collaboration with the sponsoring Danish institutions.

The research activities of the Centre will concentrate primarily on applied aspects of the biology and toxicology of harmful algae. Research institutions in developing countries will be invited to take part in both the planning and implementation of the projects. Activities are expected to include: (i) publication of material for identification of toxic algae; (ii) establishment of cultures and reference collections of harmful algae to be used for teaching purposes and for species intercalibration in difficult taxonomic fields; (iii) investigations on the effects of algal toxins on caged fish.

The Centre will initially have three staff members: PhD Jacob Larsen, Associate Professor, MSc Henrik Enevoldsen, IOC Project Coordinator, and a third post which has not yet been filled. The focal point at the Botanical Institute is Prof. Ø. Moestrup.

For more information, please contact: IOC Science and Communication Centre on Harmful Algae, University of Copenhagen, Botanical Institute, Dept. of Mycology and Phycology, Øster Farimagsgade 2D, DK-1353 Copenhagen K, Denmark, tel.: (45-33) 13 44 46; fax: (45-33) 13 44 47; e-mail: hab@bot.ku.dk.

In the next issue of HAN, we will bring more news on the Centre in Vigo, Spain.

HAB Panel meets in Paris

During the first week of June (6-9 June 1995), the IOC-FAO Intergovernmental Panel on Harmful Algal Blooms had its third meeting at UNESCO/IOC Headquarters to evaluate the results of the IOC-FAO HAB Programme, to decide on new activities, and to work on the possible funding of those activities. Some highlights from the Session:

(i) The Harmful Algal Bloom Programme Office at the IOC Secretariat presently has one staff member, seconded by Denmark. The Panel strongly encouraged Member States to second staff members to the HAB Programme Office. (ii) The Panel welcomed the establishment of IOC Science and Communication Centres on Harmful Algae in Copenhagen and Vigo. The Panel also welcomed the proposal to investigate the possibility of establishing centres in the USA and Japan. (iii) The Panel recommended that a working group on harmful algal blooms in South America (COI-FANSA) be established. (iv) The Panel recognized the need for a WESTPAC Project Coordinator for developing regional projects and recommended IOC support for the coordinator. (v) Sweden and Chile offered to assist in the production of an information brochure on the IOC HAB Programme. (vi) The Panel recommended that the IOC Task Team on Aquatic Biotoxins should continue until its tasks can be charged to a permanent working group. (vii) The Panel considered it to be of the highest priority to facilitate the provision of toxin standards and reference material to developing countries. (viii) The Panel decided that the IOC Task Team on Design and Implementation of HAB Monitoring Programmes should continue, finalize a report on monitoring systems on harmful algae and assist in organizing an international conference/training workshop on this subject. The Panel stressed that monitoring of the occurrence of harmful algae should be seen as an integral element of the Global Ocean Observing System (GOOS). (ix) The Panel elected Dr. A. Zingone, Italy, as the new Chair, and Dr. R. Corrales, the Philippines, Vice-Chair. The Panel expressed its gratitude to the outgoing Chair, Dr. Bernt I. Dybøen, Sweden, for his considerable efforts in promoting the Panel’s work, as well as their thanks and appreciation for the hard work and dedication displayed by the staff of the IOC Secretariat. (x) In March 1995, FAO announced that it would cease to co-sponsor the Panel because of financial constraints and re-prioritization of activities, but confirmed its general interest in the activities of the Programme.
Analytical procedure to avoid interference of PSP in mouse bioassay for DSP

The Galician rias in northwest Spain support the largest raft cultivation of blue mussels in the world. It is quite common towards the end of summer, that Gymnodinium catenatum and species of Dinophysis are present in the same plankton samples, and the mussels may therefore contain both paralytic and diarrheic toxins. The same problem has been raised by scientists in Chile and Uruguay, and was discussed at the IOC-UNESCO Regional Workshop on Harmful Algal Blooms held in Montevideo, 15-17 June 1994 (Report N° 101).

The mouse bioassay is the method usually used to detect marine bio-toxins in shellfish for public health purposes, and most countries follow the official method (AOAC, 1990) for the paralytic shellfish toxins (PSP). For the diarrhetic shellfish toxins (DSP), biological methods are also used, but there are wide differences between countries in the type of bioassay employed and in the toxicity criterion used. The commonest test for DSP is the mouse bioassay described by Yasumoto (1978). In this method, the digestive glands of the shellfish are extracted with acetone. After evaporation of the acetone, the residue is resuspended in Tween 60, and 1 ml samples injected into each of three mice. Different times of death are used as the toxicity criterion. The method is not very selective, and for this reason is very suitable for public health purposes. Its main disadvantage is the possibility of analytical interference which can confuse the interpretation of the results and lead to false positives.

PSP toxins can alter the results of this method even when they are present in concentrations too low to be detected by the standard mouse bioassay. These toxins are extracted together with the DSP toxins during the acetone treatment, and the final extract is highly toxic to mice. They can therefore die with neurotoxic symptoms, and it is not then possible to observe the possible presence of DSP toxins in the extracts. This problem can be avoided by using a modification of the method (Yasumoto, 1984) in which the aqueous residue obtained following acetone evaporation is made up to a fixed volume with water and extracted three times with diethyl ether. The PSP toxins are removed on the water phase. The combined ether solutions are backwashed with water and then evaporated. The residue from this procedure is then resuspended in Tween 60 and 1 ml samples injected into mice as before. With this procedure, the possible PSP interferences are removed in the water layer. This assay is also very suitable in the case of commercial presentation of shellfish without glands. Shellfish must be tested anyway, and a higher amount of shellfish meat is required. The high amount of salts present in the muscle might produce mice deaths, unless water washing to remove them is carried out during the procedure. A disadvantage of this assay is the poor solubility of Yessotoxins in the diethyl ether, that could lead to false DSP negative when they are present in shellfish samples. By using dicyclohexanthane instead of diethylether, all the DSP toxins, even the most polar as YTXs, are detected (Yasumoto, personal communication). Another quite different debate is whether Yessotoxins as well as Pectenotoxins, that do not produce diarrhetic symptoms, must be excluded from the DSP group.


Just published:

UNESCO secondary school field guide curriculum on tropical marine environments

Study No. 7: The Tropical Marine Environment: Field Exercises for Teacher Training and Class Work, in the Series Contending with Global Change, was just published by and can be obtained from: UNESCO Office, Jakarta, Jln. M.H. Thamrin No. 14, Jakarta 10002, Indonesia (send orders to the attention of: Ms Nuning Wirjoatmodjo). Cost: US$ 2.5 per copy. A limited number of complimentary copies are available to institutions in developing countries.

Included is a section on practical work in the study of phytoplankton, which at the same time produces results usable in the study of phytoplankton in the target location, e.g. on phytoplankton responses to nutrient loads in the region of Jakarta Bay (Nostocalan scintillans) into the Pulau Seribu chain since 1990 and also involving Oscillatoria sp.

Framework: The objective of this issue within the series of publications, produced by the UNESCO Jakarta office, is to present an inter-disciplinary field studies curriculum that can prepare today’s and future generations by giving them an understanding of marine, terrestrial and associated systems as they relate to ecologically sound and sustainable development in a continually changing world. The programme also encourages the inclusion of training in navigation, basic and advanced onboard sailing skills, snorkeling, basic Scuba practices, as well as direct environmental monitoring using techniques such as shoreline debris calculations, resource assessment strategies, plankton sampling, secci-disk and water quality readings and so forth.

Case study: The publication includes an exemplary or model field trip structure involving a launch trip from Jakarta to a coral atoll in the Java Sea, about 85 km northwest of the north coast of Java. Here, over a period of three days the group conducts land-based forays into the island and related reef system in order to: (i) collect physical information and measure associated biological responses over distinctive environmental trends and (ii) carry out observations of in situ ecosystem elements. In cruising from the mainland city of Jakarta to the island, an activity schedule is defined for the sampling and counting of phytoplankton and zooplankton populations growing on the sewage and nutrient discharges from the city. Class data obtained annually over a seven-year interval (during the period 1986-1992) showed an apparent significant increase in population densities and the extent of the standing algal bloom out to about 60 km from Jakarta.

Selected events

Future events

Second IOC Regional Science Planning Workshop on Harmful Algal Blooms, 30 October - 1 November 1995, Mar del Plata, Argentina. Contact: Dr. J.I. Carreto, INIDEF, Casilla de Correo 175, 7600 Mar del Plata, Argentina; tel.: (54-23) 517818; fax: (54-23) 517442.

IUPAC-UNESCO/IoC-FAO International Workshop on Ciguatera, 8-10 November 1995, Santo Domingo, Dominican Republic. Contact: Prof. D. Park, Food Science Department, Louisiana State University, Baton Rouge, LA 70893-4200, USA; fax: (1-504) 388 5500.


Fifth Canadian Workshop on Harmful Marine Algae, September 1996. Contact: M. A. Parranjape, NAFC, Science Branch, Division of Ocean Ecology, PO Box 5667, St. John’s, Nf. Canada A1C5X1; fax: (1-709) 772 3207; e-mail: mparanjape@nflcorf.nwafe.nf.ca.

HARMFUL ALGAE NEWS

Compiled and edited by Tim Wyatt, Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, Eduardo Cabello 6, 38208 Vigo, Spain; tel.: (34-86) 23 19 30/23 19 73; fax: (34-86) 28 27 62; and Yolanda Pazos, Centro Galego para o Control de Calidade do Medio Maríno, Vilaxoan, Pontevedra, Spain; tel.: (34-86) 51 23 20.

The opinions expressed herein are those of the authors indicated and do not necessarily reflect the views of UNESCO or its IOC. Texts may be freely reproduced and translated (except when reproduction or translation rights are indicated as reserved), provided that mention is made of the author and source and a copy sent to the Editor.

Project Coordinator: Helle Ravn, IOC, UNESCO, 1 rue Miolles, 75732 Paris Cedex 15. Tel: (33-1) 45 69 36 41; Fax: (33-1) 40 56 83 16; E-mail: h.ravn@unesco.org

Production Editor: Gary Wright, IOC/MRI.

Layout: Michelle Turner.