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HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms *No. 16*

◆ USA, Washington

Heterosigma bloom and associated fish kill

A bloom of the harmful marine phytoplankton, *Heterosigma carterae* occurred in upper Case Inlet, south Puget Sound, Washington in late September, 1994, correlating with the presence of at least 35 dead salmon. This marks the first time that this alga has been closely correlated with a wild fish kill; in the past it was thought to be associated with kills of penned fish at fish farms only. We were informed of the presence of a possible harmful algal bloom and dead salmon near the town of Allyn on 27 September and a team was formed to investigate. We arrived at the Allyn waterfront at 17:30 hours the same day. Prior to our arrival, state agency personnel walked approximately two miles of shoreline from the powerlines north of the dock, to the mouth of Sherwood Creek and conducted the only official count of dead fish present along the shore consisting of 12 coho salmon *(Oncorhynchus kisutch),* 11 chum salmon *(Oncorhynchus keta),* 12 chinook salmon *(Oncorhynchus tschawytscha),* one flat fish, and one sculpin on the morning of 9/27. Since previous harmful blooms of *Heterosigma* have resulted in the majority of net penreared salmon sinking to the bottom of pens, and only approximately two miles of shoreline were sampled, it is suspected that many more exposed fish may have succumbed than were counted. Witnesses who explored the east side of the bay reported seeing many dead salmon there as well, but no counts were made. State agency personnel who observed the fish kill reported seeing "dying fish coming to the beach, gulping at the surface, trying to get out of the water." Scavengers were seen consuming the salmon carcasses; these included two harbor seals, a house cat, and Hymenopteran insects. None suffered any noticeable acute ill effects. Although precise cause of death has not been ascertained, visual inspection of the reproductive organs from a deceased male chum salmon found on the shore at Allyn confirmed that the fish was not yet reproductively mature and therefore did not die from completion of the spawning process.

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◆ Brazil

PSP Outbreak in Brazil

This communication reports the first appearance of PSP toxins in cultured mussels from Santa Catarina Coast, in southern Brazil, observed during a pilot HAB monitoring program. The program was set first in a culturing area in order to start HAB studies to support mussel culturing, which has been growing fast along Santa Catarina's coast in the last decade (HAN No. 12/13, 1995). Due to algal distribution and former cases of massive intoxication characterized by gastrointestinal disorders, our first attempt was to verify the occurrence of DSP toxins. In august 1995 we first detected mussel toxicity by the mice bioassay in acetone extract. The extract was analyzed by HPLC and a small amount of okadaic acid was detected in the sample (1) and later analysis showed the presence of traces of PSP in the same sample. After this finding, we started to carry out regular analysis of mussel toxicity by the mice bioassay in a pilot HAB monitoring program at Armação do Itapocoroy (27° S). On the afternoon of Friday 26 of

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◆ USA

ECOHAB Program underway

The U.S. has just initiated its first national research program on the ecology and oceanography of harmful algal blooms (ECOHAB). ECOHAB, a partnership of four Federal agencies, the National Oceanic and Atmospheric Administration, National Science Foundation, Environmental Protection Agency, and Office of Naval Research, is coordinated by Dr. Kevin Sellner in NOAA's Coastal Ocean Program and is designed to support long-term research on the physiology, behavior, ecology and toxicity of HAB species and the environmental factors responsible for bloom expression. There are two types of research considered in ECOHAB, large, multidisciplinary regional studies that provide a regional understanding of physics, basin characteristics and water quality that support the physiology and ecology of the HAB species, in the end generating a predictive model for forecasting regional blooms. Targeted studies comprise the second research element. These projects include one to several researchers and focus on identifying specific characteristics of a bloom species that will assist in the general understanding of the organism's cellular characteristics, ecology or behavior.

Following competitive peer review, two regional and seven targeted projects were selected for support. The regional studies focused on *Alexandrium tamarense* in the Gulf of Maine (D. Anderson

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et al., WHOI) and on *Gymnodinium breve* in the Gulf of Mexico (K. Steidinger et al., Florida Department of Environmental Protection). Targeted studies included projects on brown tide population genetics (J. Stabile and I. Wirgin, New York University), trophic effects of *Prorocentrum minimum* and *Gyrodinium aureolum* (G. Wikfors et al., National Marine Fisheries Service) and *A. tamarense* (E. Durbin et al., U. Rhode Island), including sea otters and shorebirds (R. Kvitek, California State University Monterey Bay), ecophysiology of *Pseudo-nitzschia* (D. Garrison et al., U. of California, Santa Cruz), algicidal bacteria and *G. breve* (G. Doucette, U. of South Carolina) and macroalgal overgrowth in tropical reefs (V. Paul, U. Guam).

A second ECOHAB announcement has just been published providing support for regional and targeted studies as described above and a new area addressing *Pfiesteria* and related species. These taxa have been identified in the Mid-Atlantic and South Atlantic states of the eastern U.S., associated with fish lesions, fish kills and in some cases, human health problems. In 1997, *Pfiesteria* and related species severely impacted fish in several small tributaries on the eastern shore of Chesapeake Bay and more dramatically, caused acute short term memory losses and skin rashes in people exposed to *Pfiesteria*-containing water or aerosols. This threat to human safety resulted in a Federal response, providing immediate funding for state monitoring and assessment teams as well as public health studies. Using the U.S. national HAB plan as a model ("*Marine Biotoxin and Harmful Algae: A National Plan*"), a comprehensive strategy was developed for *Pfiesteria* and related taxa and HABs in general providing priorities for immediate, short term and long range efforts in monitoring, assessment, research and public health and education ("*National Harmful Algal Bloom Research and Monitoring Strategy: An Initial Focus on Pfiesteria, Fish Lesions, Fish Kills and Public Health*"). Proposals are due in late February, 1998 and following peer review, the second round of ECOHAB awards will be issued in mid- to late summer.

The U.S. ECOHAB Program provides a foundation for a long-term national program to support research on the increasingly frequent HABs in U.S. coastal waters. Through ECOHAB, a national predictive capability for HABs along the U.S. coast is anticipated, providing coastal communities with forecasting abilities to mitigate bloom impacts on coastal resources and local economies and ensuring public safety. ECOHAB research should also enhance development of cell and toxin assays, extremely important in pro-active monitoring programs in coastal waters. Finally, ECOHAB might be applicable for designing investigations of HABs common in other parts of the world, as exemplified by recent IOC support of ECOHAB as a model for its participating members (Paris, 1997) and IOC-SCOR discussions of an international workshop for development of an international HAB program along the lines of the ECOHAB Program. With increasingly frequent HABs all over the world, international multi-disciplinary investigations of blooms and their causes would greatly enhance our global predictive capabilities, potentially providing bases for predicting benefits from bloom control strategies throughout our coastal ecosystem once effective control strategies have been identified.

Additional information on the U.S. ECOHAB Program, including Announcements of Opportunity, as well as other relevant information on U.S. HAB activities can be obtained from our web site (under "Projects" on the HAB page at http://hpcc.noaa.gov/cop/projects/HAB.htm), the National Office on Marine Biotoxins and Harmful Algal Blooms at http://www.redtide.whoi.edu/hab/ or Kevin Sellner, ECOHAB Coordinator, at ksellner@cop.noaa.gov.

◆ Global

27 countries meet to discuss HAB priorities

On 30 June - 2 July 1997 the IOC Intergovernmental Panel on Harmful Algal Blooms held its fourth session (IPHAB-IV) in Vigo, Spain. The Panel was hosted by Instituto Español de Oceanografía, and more than 40 participants from 27 countries met to review activities completed since the Third Session of the Panel in 1995, discuss priorities, and to decide upon new initiatives to be taken.

The Panel noted that the awareness of problems caused by harmful algae is constantly increasing on a global basis. It was therefore proposed to develop an international science agenda on the ecology and oceanography of harmful algae to help IOC Member States in setting national priorities and in particular to promote the establishment of national and international research programmes. The Panel made a number of recommendations to promote science and capacity building in relation to harmful algae; (i) the Panel encouraged a stronger participation of scientists from IOC Member States in the ICES-IOC Working Group on the Dynamics of Harmful Algal Blooms; (ii) it was recommended to support on-going long-term studies and inclusion of phytoplankton species composition analysis as a routine part of monitoring programmes (including GOOS and LOICZ); (iii) the regional aspect was emphasised, and a strengthened coordination between harmful algae activities of organizations in the Pacific (IOC/WESTPAC, ASEAN, APEC, PICES) was stressed; (iv) The formation of an IOCARIBE working group on harmful algae in the Caribbean and adjacent regions was recommended; (v) The necessity of enhancing an integrated and coordinated research effort in Europe was stressed; (vi) Improved management and mitigation of the effects of harmful algae was referred to as a high priority; (vii). It was recommended that the IOC, in cooperation with APEC, organizes an international workshop, directed towards dialogue and improved design of management and monitoring of harmful algae and algal toxins; (viii) To ensure continuity in the implementation of the HAB programme, and to follow up on the recommendations of the IPHAB, it was reiterated that the HAB Programme Office requires one additional staff member to be seconded by Member States. Dr. Adriana Zingone, Italy, was re-elected Chair and Dr. Rhodora Azanza, the Philippines, was re-elected Vice-Chair of the Panel.

A full report of the IPHAB-IV is being published and can be obtained from the IOC Secretariat and from the IOC WWW-site.

◆ Argentina

Ceratium hirundinella blooms in Argentine reservoirs

Ceratium hirundinella (O.F. Müller) Schrank is a widespread freshwater dinoflagellate ocurring in temperate and subtropical lakes and reservoirs of the Northern Hemisphere. Records for Southern Hemisphere are, in contrast, relatively scarce, with a few mentions for Africa, southeastern Australia and Patagonia (1). In fact, this species has previously been recorded in South America as extremely rare in several Andean lakes of Southwestern Argentina (2, 3) and Chile (A. Boltovskoy, pers. comm.).

In recent years, however, dense blooms of *C. hirundinella* were detected in western Patagonia, mainly in reservoirs located along the rivers Limay and Neuquen. The highest population densities (275 cells.ml^{-1}) were recorded in E. R. Mexia and Arroyito Reservoirs (November 1996 and December 1996 respectively). Unfortunately, there are no quantitative data for Neuquen River reservoirs. All these man-made lakes are used for hydroelectric power generation; nevertheless, no harmful effects have been so far reported.

During early summer 1996-1997 *C. hirundinella* was first recorded in Paso de las Piedras Reservoir (located in southeastern Pampean Plains), the drinking water supply for the towns of Bahia Blanca (pop. 270,000) and Punta Alta (pop. 56,000). *Ceratium* population increased very rapidly and attained a maximum density of 2,000 cells.ml^{-1} by mid-summer (February 1997), raising chlorophyll a concentration from 38 to 130 mg.m^{-3}, the highest since the reservoir was built. The bloom of this large-sized plankter resulted in the clogging of the sand filters in the treatment plant and the consequent shortening of filter runs. This event was accompanied by a quite noticeable fishy to septic odour.

Massive developments of *C. hirundinella* and its rapid expansion in Argentine water-bodies are recent phenomena whose determining causes are still not clear. During their life cycle these dinoflagellates usually form resting cysts under unfavorable conditions and then sink down to the sediments. When favorable conditions return, the nutrients stored in the cysts allow the germinating vegetative cells to iniciate a bloom, independently of the nutrient concentration in the water (4). This competitive strategy suggests that *C. hirundinella* will persist as a troublesome algae for reservoir management and water quality in an early future.

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(Cont'd from p. 1. "PSP Outbreak in Brazil")

July of 1997 (austral winter), we detected by mice bioassay the first PSP positive in cultured mussels (*Perna perna*). Although positive, sample toxicity was under 400 MU.100g $^{-1}$. The presence of PSP was latter confirmed by post column oxidation FLD-HPLC analysis and toxicity was attributed to GTX_1, GTX_2, GTX_3, GTX_4 and STX. Concentrated net samples showed a similar toxin profile. Several dinoflagelates were present in the water, including species of *Alexandrium*, *Gonyaulax*, and *Gymnodinium*. Action was taken in order to notify local public health authorities which stayed on alert, and to sample and analyze samples from other culturing areas. All samples produced visual symptoms in the mice but toxins were only detected in samples from Armação do Itapocoroy. The toxicity increased a little and after two weeks it decreased to well under 400 MU.100g^{-1} following a the passage of a cold front system. These results have confirmed the necessity of a more comprehensive monitoring program in the region. From an ecological point of view, we still have to find the "guilty" alga or algae and attribute its toxicity. One possibility is that the causative organism was *Alexandrium tamarensis* which causes PSP in Argentina and Uruguay, and was observed on the high abundance in 1996 at Rio Grande Coast (2), about 700 km south of Armação do Itapocoroy. Other PSP producers in the southern Atlantic include *Gymnodinium catenatum* and *A. catenella*. These findings brings new elements to the discussion: is PSP spreading from the southern east Atlantic to northern waters or was it always there but not detected?

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(Cont'd from p. 1.
"Heterosigma bloom and associated fish kill"

Noticeable red algal streaks were present in the water during the sampling period on 9/27, which coincided with vertical salinity and temperature stratification of the water column and Secchi disk depths of 0.5 m. The water appeared clear the following morning, 9/28 with Secchi disk depths of 3 m until the tide began to flood, at which time vertical temperature and salinity stratification became established, characteristic red algal streaks reappeared, and Secchi disk depths decreased to 1 m. Unusually low nitrate concentrations of 0.22 µM were recorded throughout the water column during low tide on 9/29, however ammonium and orthophosphate concentrations were relatively normal. Numerous dead salmon carcasses, presumably not included in the fish count, were noted floating past the dock during the sampling period from 9/27-9/28.

Investigation of the area encompassed by the bloom on 9/28 suggested that it extended north to the end of the bay and south as far as Sherwood Creek and Gills Cove. Red discoloration in the water column and dead chum salmon, presumably not included in the fish count were seen on the bottom of Gills Cove and around the mouth of Sherwood Creek. Reports from residents around Allyn suggest that the bloom began on 9/24, and 9/28 was the last day we saw evidence of the bloom.

A review of climatological data provided by the National Oceanic and Atmospheric Administration for Seattle Tacoma Airport provides insight regarding conditions that may have promoted bloom development and cessation. In general, September, 1994 was 1.5 to 2.3°C warmer than normal at westem Washington recording stations. The warmest day of the month was September 22, just two days prior to the bloom's first appearance near Allyn. September 23 was the second warmest day of the month and the entire period of the bloom was marked by relatively warm air temperatures. Cessation of the bloom coincided with daily high temperatures dropping to below 29°C. The air temperature regimes correlated directly with the percent of possible sunshine index from the Weather Bureau. This index indicates that sunshine percentage was at least 88% of the maximum from 9/21-9/28 and dropped to 25% on 9/29, the day after the bloom. Rainfall was 1.69 inches in September at the nearby airport reporting station. This was only 0.19 inches less than normal, but most of the rainfall occurred in the first half of the month. Wind velocity did not change markedly in the period before to after the bloom, but wind direction changed from northerly to southerly on September 29. This is significant because the inlet is oriented almost north and south and hence is more susceptible to wind-driven mixing when winds are from the south. Additionally, a construction site was located approximately one mile north of the town of Allyn which was scarred with deep runoff trenches that had occurred as a result of a heavy thunderstorm approximately 2 weeks prior to the appearance of the bloom. Unconfirmed reports of grass seed being planted and fertilized the afternoon prior to the storm were stated by a local resident. If this were the case, such a storm may have introduced a large pulse of nutrients into nearby Case Inlet and provided the appropriate nutrient seed for an algal bloom. It should be noted that the unusually low nitrate levels reported here were sampled the day after the bloom and algal cells may have assimilated the available nutrients prior to and during the bloom.

In the past we have observed two general time periods for the occurrence of noticeable *Heterosigma* blooms: late spring/early summer and the fall. Rensel (in press, Proceedings of Research '95, Puget Sound Water Quality Authority) discusses past blooms in Puget Sound pointing out that these blooms require quiescent weather conditions, often coupled with vertical stratification of the upper column. A major fall bloom in northem Puget Sound during 1989 occurred during a calm, warm period, but freshwater runoff at that time of the year from the nearby Fraser River was well past the annual maximum. Similarly in Case Inlet, riverine discharge during the late summer is typically very low, although a minor reduction of salinity was noted at the surface when the bloom was present. The very warm air temperatures and influence on surface water temperatures, high percentage of sunlight occurrence, lack of south winds, and potentially elevated nutrients from the construction site possibly combined to encourage formation of this bloom. The cessation of these conditions coincided with the termination of the bloom.

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◆ Spain

The unique microreticulate cyst?

Since 1976, *Gymnodinium catenatum* blooms have been frequent in the Rias Baixas of northwest Spain, and the principal cause of PSP toxicity in this region. The appearance of this species in Iberian waters is not confined to the Rias Baixas, and it also occurs along the Portuguese coast and the south coast of the Iberian Peninsula. With the aim of studying the cyst distribution of *G. catenatum*, sediment samples were collected from 27 stations on the Galician-Portuguese continental shelf between 40° 30' N and 42° 20' N in 1993.

The cyst of *G. catenatum* was first described by Anderson et al (1), and at that time (1988) was the only cyst of a naked dinoflagellate known to have a microreticulate external surface reflecting features of the vegetative cell. Since then, examples of this cyst have been found in sediments from various parts of the world, and in most cases have been identified as *G. catenatum* cysts due to the characteristic wall reticulation.

Two types of cyst can be differentiated in sediments from the Galician-Portuguese shelf, both similar to the cyst of *G. catenatum* (brown wall with microreticulation), but of different sizes. Fig. 1a shows the frequency distribution of cyst diameters, and the two clear modes correspond to the two types mentioned. *G. catenatum* cysts produced in laboratory cultures have a size range (fig. 1b) which corresponds with that of the larger size peak in fig. 1a. In addition, the morphology and arrangement of the paravesicles coincide with those of the original description of *G. catenatum* cysts. In contrast, in the smaller cysts found in the sediments, the paravesicles are larger, and there are fewer rows of them on the paracingulum. The vertical distribution and maximum concentrations of the two types in the sediments are also distinct.

The concentration of *G. catenatum* cysts (38-59 µm) diminished with depth in the sediments, with maximum values (20-500 cysts ml^{-1}) in the upper 8 cm; while the *G. catenatum*-like cysts (20-38 µm) had the opposite profile, and reached their maximum concentration (5000 cysts ml^{-1}) at 12 cm depth.

The two kinds of microreticulate cysts referred to here are known from the literature. Some descriptions correspond exact-

ly with the original description of *G. catenatum* cysts, eg. those of Blackburn et al (2) and Hallegraeff et al (3) from Tasmanian sediments. But from the same region, Bolch and Hallegraeff (4) have also mentioned a similar but smaller cyst (17-22 µm) which they called *Gymnodinium* sp 1 which germinated into "small (17 µm) diameter two-cell chains resembling *G. catenatum*". Their description suggests they may have been cells of *Gyrodinium impudicum*, recently described by Fraga et al (5), with morphological features very similar to those of *G. catenatum*. Finally, the cysts described by Nehring (6) and Ellegaard et al (7) from German and Danish coastal sediments respectively, and the fossil cyst described by Dale (8) all have diameters less than 40 µm, as well as microreticulation and paracingular patterns like those of the *G. catenatum*-like cysts (20-30µm) from the Galician-Portuguese shelf.

Attempts to germinate the smaller *G. catenatum* like cysts from the Galician-Portuguese sediments have so far failed, so that we do not yet know whether they are a second form of *G. catenatum* cyst, or whether they belong to another species of dinoflagellate. Ellegaard et al. (7) describe vegetative cell produced from a cyst very similar to those I have called *G. catenatum*- like, and they call them *G. catenatum* despite the smaller cell size and the absence of chains. They suggest that there is a *G. catenatum* "species complex". Whatever the case, it is clearly important that we learn more about this "complex" before we define the cyst distribution of *G. catenatum*.

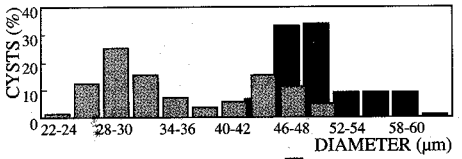

Figure 2. Size distribution of cysts, microreticulate cysts from sediment. *G. catenatum* cysts produced in cultures.

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◆ Brazil

14 South Americans trained on HAB

The IOC-FURG-DANIDA Regional Training Course: Taxonomy and Biology of Harmful Marine Microalgae was held at the Fundaçao Universidade do Rio Grande (FURG), Brazil between 4-14 March, 1997. It was the result of a joint effort between the IOC-DANIDA Science and Communication Centre on Harmful Algae in Copenhagen (SCCHA) and FURG.

Rio Grande, in south Brazil, was founded by the Portuguese in 1737 and presently has about 200,000 inhabitants. It is the major port of the province Rio Grande do Sul. The city lies on the shore of a channel connecting the Lagoa dos Patos (250 km long) to the Atlantic Ocean. Its economy is tightly bound to both marine and estuarine environments. The University of Rio Grande was founded in 1969 and several graduate studies in the humanities and scientific departments are dedicated to coastal-related subjects. The department of oceanography is oriented towards "the understanding of biotic structural components and functional aspects of marsh, estuarine and marine ecosystems".

The group of participants was composed of 14 advanced students and professionals from Latin American countries: Argentina, Brazil, Chile, Colombia, Cuba, Ecuador, Mexico, Peru and Uruguay. Each participant presented a description of research and management issues related to harmful algae in their countries.

The classes of Drs. Ojvind Moestrup and Jacob Larsen from the SCCHA, Botanical Institute, University of Copenhagen, on the taxonomy of Dinoflagellates, Prymnesiophytes and Raphydophytes were complemented by various contributions on other groups: Diatoms by Dr. Marta Ferrario (Universidad Nacional de La Plata, Argentina), *Prorocentrum* sp. and *Alexandrium* sp. by Lic. Rutt Askelman (INIDEP, Argentina; long-term collaborator of Dr. E. Balech), Cyanobacteria by Dr. Joao Sarkis Yunes (FURG) and the identification of dinoflagellate cysts by Dr. Kazumi Matsuoka (Nagasaki University, Japan).

Description and study of several species was carried with microscopic observation (transmission and fluorescence) of living or fixed material from culture collections or field samples, and supported with graphic material, such as TEM micrographs, which for instance, were necessary for descriptions of the genus *Pseudonitzschia*.

A day was dedicated to sampling of sediments for cyst determination, water sampling for quantitative and systematic studies on toxic microalgae, and for the establishment of algal cultures. Moreover, fixed field samples brought by the different participants were studied in order to facilitate the identification of species and favour descriptions.

In addition to theoretical lectures on taxonomy, technical sessions were performed on the identification of dinoflagellates (Dr. Jacob Larsen), quantitative methods of microalgae (Dr. Clarisse Oderbrecht, FURG), cyst preparation (Dr. Kazumi Matsuoka) and culture techniques (Dr. Virginia García, FURG).

Taxonomy training was complemented with presentations on the ecology of algal blooms (Dr. Clarisse Oderbrecht), on harmful algal blooms in South America (Lic. Silvia Méndez, INP, Uruguay), ciguatera and a general overview on microalgal toxins (Dr. Jorge Diogène, IOC-IEO Science and Communication Centre on Harmful Algae, Vigo, Spain).

Training courses in taxonomy respond to the demand clearly defined in the HAB Programme of the IOC in order to form scientists and improve their identification skills. Taxonomy constitutes a key-stone in the study of every HAB event. An immediate goal of such training courses should be that the participants become reference scientists in their own countries to assure the identification of harmful microalgal species.

Technical support was brought by FURG, the Brazilian Navy that collaborated during the field-trip, Zeiss-Germany that lent 15 microscopes and the IOC-DANIDA Science and Communication Centre on Harmful Algae, Copenhagen, that provided scientific publications to the participants.

Clarisse Oderbrecht, FURG

◆ Book review:

Patterns in the Ocean: Ocean Processes and Marine Population Dynamics

"The Sea Maid's Music: An Aid to Pattern Recognition", the title to the first section of the Introduction, boded well to Andrew Bakun's *Patterns in the Ocean – Ocean Processes and Marine Population Dynamics*, a book, like music, with several aims and recurring themes.

A preliminary glance at the eclectic-looking Table of Contents suggested that what was coming might complement Jumars (1993), Kaufman (1993) and Cushing (1995) in different ways.

The author has a double specialisation, physical oceanographer and fisheries scientist. This, coupled with a penchant for music and sitting on Pacific cliff tops watching the sea patterns change and decay, inspired him to write a book combining Fisheries, Oceanography, Climatology and Time series, with an eye to timely issues like carbon sequestering, global climate change and the "crisis" in fisheries science.

Jumars' book explained many topical issues concerning biological oceanographic processes in ways understandable to physical oceanographers: Bakun explains many basic physical oceanographic processes for biological oceanographers (like me). One muse he uses has been to banish all the 10 equations in the book, to a four-page appendix, which I hope to cherish evermore as a valuable reference.

So what has this to do with harmful algae? Although only 1% of the book is devoted directly to harmful algae (chain-forming dinoflagellates off the Galician rías), the indirect interest for HAN readers is that many phytoplankton species must be constantly selected to use fine-scale migrations to exploit ambient patterns of water movement to maintain suitable positions for their life stages over time, just as many fish (larvae and adults) and zooplankton do. The swimming speeds of organisms doing this will naturally limit the different scales that can be exploited, as Bakun discusses graphically for the life stages of different pelagic fish in Chapter 10.

In Chapter 6, "Thickening the Soup", Bakun introduces some of the myriad interfaces present in the ocean, at which organisms tend to concentrate, with the possibility of pushing concentrations over grazing thresholds for, say, fish larvae. He is clearly at ease here, exploiting his mastery of oceanographic processes combined with that of current fisheries issues, while keeping the "patterns" and the physical-biological interactions clearly in mind over 10^{-3} m to 10^{6} m scales.

Chapter 7, "Transport and retention: getting home", develops a recurring theme, that what happens to most pelagic larvae doesn't matter to recruitment: what does matter is what happens to the tiny percentage of survivors. He emphasises that the survivors frequently have been retained in physical oceanographic structures. Included amongst such stationary and moving retentive structures, he explains Taylor columns over shallow banks, and shelf-sea fronts, which tend to produce offshore-moving water at mid depths but shoreward moving water near both the surface and the bottom. Vertical shears, associated with practically all retentive structures are no doubt sometimes exploited for evolutionarily controlled position keeping and migration. They are also found, notably, in geostrophic currents associated with upwelling on eastern ocean boundaries in mid latitudes, where equatorward surface currents are countered by poleward flows at shallow depths. Onshore Stokes drift, produced in shallow and bottom layers by onshore waves "feeling" the bottom, may also provide shear structures, particularly off reefs subject to Trade Winds or swell, potentially exploitable for position keeping by plankton. A section, "Surface wind drift", includes much speculation on the complexities of Ekman transport. Apart for the *mean* transport, 90° to the right (N. hemisphere) of the wind, but more downwind (10° to 30°) at the surface, Ekman transport-induced circulation and the resultant structures are still too poorly understood quantitatively, even to allow speculation on the behaviour favoured by evolutionary pressures to exploit them, to which particular stocks organisms might be subject. One still has the right to guess, however, that such evolutionary pressure is likely to have honed certain populations to exploit some of the recurring patterns.

Bakun also cites authors reporting that slicks in surface convergences associated with underlying internal waves, move shorewards at 25 cm s^{-1}. He claims that neuston could thus "surf shorewards" at about this velocity. I wonder whether the phase velocity of a surface-film compression wave, with a slick at the moving zone of maximum compression, may not have been erroneously interpreted as surfing-type movement of the surface film itself. Shoreward movement of the slick might be produced, however, if there were also onshore wind stress, or shoreward movement of a surface layer thicker than the film, or both combined.

Showing how the intensity of oceanographic structures, such as upwelling zones, as well as phytoplankton productivity and feeding of fish larvae might vary in relation to turbulence, prepares the reader to associate recently perceived (Cushing 1995 and refs) similarity between peaks and troughs in landings of related fish species in different parts of the world with long-distance connections in climatic variability.

The dramatic nature of the cross-Pacific connections associated with the El Niño Southern Oscillation (ENSO) are now well known, but Bakun explains how Kelvin "waves" slowly propagating eastwards along the equator or polewards (equatorwards) on eastern (western) ocean boundaries provides a plausible mechanism for tele-connections delayed by a year or two, as do "slugs" of water in oceans, not mentioned by Bakun, but invoked by Cushing. One problem of perceiving this variety of possible tele-connection mechanisms, with variable time delays, is how to demonstrate from meteorological and other records, which mechanisms are acting, and to what extent. Much of the evidence in the literature unfortunately comes to resemble that in Bakun's Figure 13.9 (showing relationships between annual mean wind stress or atmospheric pressure at 16 localities from 1945 to 1990 around the Pacific and the Atlantic.

It is hard to "unravel the determinant mechanisms from happenstance" when functional relationships are, alas, frequently inferred from similar comparisons of selected and rather poorly correlated time series.

Although the importance of zooplankton food for fish stocks has been known at least since the early years of the century (see Hardy 1959 for the importance of *Calanus* to North Sea herring), Bakun's book has not shown much prowess in considering how plankton species composition determines the nature of the function *f*(physics, fish). This lacuna has unfortunately infected much of the fisheries and modelling milieux in recent decades. Fortunately, the pendulum is swinging back, as illustrated by the large European programmes within GLOBEC, ICOS (Investigation of *Calanus finmarchicus* migrations between Oceanic and Shelf seas off north-west Europe) and TASC (Trans-Atlantic Study of *Calanus finmarchicus*).

Since data on plankton variability are usually insufficient to estimate its role in any particular fishery at a given time, fishery workers have to fall back on trying to determine the linear part of *f*(physics, fish). This is even though they know that such relationships are, overall, likely to be:

1. non linear;
2. offset by an unknown and perhaps variable time;
3. subject to natural (e.g. volcanic and astronomic) and anthropic (e.g. pollution and fishing) forcing factors;
4. subject to the changes in gene frequencies in the fish species concerned or in their food, predators, parasites, pathogens or competing species (essentially unmonitorable for the foreseeable future).

Apart from short-term predictions, such as those of stocks and productivity modelled year by year from abundances of O-group larvae, perhaps we may have to face up to a scenario that the best available management policy is to prepare fishing fleets to cope with the unpredictable, to establish the limits of variability percentile probabilities, and to foster underfishing in general. Then, there should then nearly always be enough.

The kind of correlative research being undertaken in fisheries, climatology and many other branches of science with a view to improving forecasting, can rarely be justified as leading to much basic understanding of how things work. Fundamental knowledge, including of course that of how firstly processes and secondly correlative relationships work, can come only from understanding quantitatively the nitty-gritty mechanisms at work, including the passive properties and the reactive properties of the materials (here meant to include the organisms) that serve as their medium. Some understanding of form and function, of attractors, and of chaos, will also no doubt help.

One thing to emerge from this book, as from others on fisheries and other sets of processes taking place in turbulent continua is that even though there may be much understanding of the systems concerned at both scientific and intuitive levels, just as in modern weather forecasting, it becomes impossible to predict further ahead than a few times the timescale of the dominant structures or processes. In fisheries, a major time scale is that from egg to catchable size.

Although the science of harmful algal blooms (HABs)is still an infant compared to fisheries science, HABs and fishes live in the same environments, so similar restrictions on predictability are likely to exist for both.

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◆ Finland

Fish kill by *Prymnesium*

An extensive fish kill occurred in the oligohaline and meromictic coastal lake Vargsundet (110 ha, two major basins) in Cland, SW Finland in late June and early July 1997, in connection with a mass development of *Prymnesium* sp. The cell concentrations exceeded 20.000 cells per.ml in the uppermost 4-5 m layer but few *Prymnesium* cells occurred in deeper strata. Several tonnes of fish were killed. Hundreds of gulls and terns removed much of the smaller fishes. Several white-tailed eagles ate of the dying and dead fishes. The fish mortality was almost total in the more shallow, southern basin of Lake Vargsundet, whereas some fish escaped to deeper layers in the stratified, northern basin (max depth about 32 m, but anoxic conditions below 15 m). The birds appeared healthy.

The bloom of *Prymnesium (*presumably *P. parvum)* developed during warm and very sunny conditions. The surface water temperature occasionally exceeded 24°C. The bloom persisted from late June to late July (at least). The bloom was present and still ichtyotoxic on July 26, 1997. All fish species known to occur in the lake died. Also freshwater crayfishes (*Astacus astacus*) died. The conditions of Lake Vargsundet are rather unusual, with a salinity of about 2 per mille but with both freshwater crayfish and a brackish (marine) algal bloom. *Prymnesium* is now and then observed in the plankton of Finnish coastal waters but there is only one similar earlier fish kill episode from Finland, in June 1990, in a smaller basin with about 6 per mille salinity.

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◆ Greece

Blooms in Thermaikos Bay

In December 1996, a red tide predominated by the photosynthetic ciliate *Mesodinium rubrum* was recorded in the inner Thermaikos Bay (North Aegean Sea, Greece. In this virtually monospecific red tide, *Mesodinium rubrum* reached abundance levels of $14.5x10^6$ cells.l^{-1} and a chl a concentration value of 487 mg.m^3 was mcasured. This has been the last incident in a series of intense phytoplankton blooms that occurred in the inner bay the past few years. These blooms were dominated mostly by dinoflagellates: such examples are the red tide incidents in May 1996 (by *P. minimum,* 10×10^6 cells.l^{-1}), September 1994 (by an unidentified Gymnodinioid species, P. *cf. dentatum* and *P. rostratum,* 17.7×10^6 , 3.1×10^6 and 1.3×10^6 cells.l^{-1} respectively), April 1994 (by *P. micans,* $37. \times 10^6$ cells.l^{-1}) and October 1993 by *P. cf. dentatum,* 210×10^6 cells.l^{-1}). Intense blooms of diatoms or coccolithophorids have also been observed in the bay: for example, in August 1996 a bloom dominated by the coccolithophorid *Pleurochrysis carterae* was recorded and an abundance of up to 72×10^6 cells.l^{-1} was measured. A bloom of *Mesodinium rubrum* was also recorded in December 1985. These red tides occurred during periods of calm weather, lasted a few days and were attributed to the eutrophication of the inner part of the bay due to the high nutrient inputs of the domestic and industrial wastewater of the city of Thessaloniki. However, none of the red tide incidents recorded so far was studied in detail throughout its development and no firm conclusions can thus be drawn concerning their origin and the factors that triggered them, affected their development and led to their decline.

The occurrence of several potentially toxic/harmful phytoplanktonic species has already been reported in Thermaikos Bay (1, 2). Examples of such species are the dinoflagellates *Alexandrium catenella, Ceratium fusus, Dinophysis acuminata, D. acuta, D. caudata, D. fortii, D. tripos, Gymnodinium breve, G. catenatum, Gyrodínium aureolum, Noctiluca scintillans* and *Prorocentrum minimum,* the diatoms *Pseudo-nitzschia delicatissima* and *P. seriata* and the haptophyte *Prymnesium parvum.* However, these species have not dominated any major bloom recorded so far nor have they been reported to have reached abundance levels high enough to cause fish mortality or shellfish toxification.

Mussel cultures spread along the northern and westem coasts of the bay with a production of 10,000 and 4,000 tonnes in 1995 respectively. According to the EEC Directive 91/492/EEC, bivalves from designated areas of culture should be examined for PSP and DSP toxins and also the waters should be monitored for toxic phytoplankton. In compliance with this directive, all samples examined so far by the Institute of Food Hygiene in Thessaloniki using mouse bioassay had PSP toxins below the tolerance levels i.e. <80 μg/100g meat (3). No human poisoning by algal toxins after consumption of shellfish harvested in the bay is known so far. The consequences of the blooms seem to have been restricted to small scale fish deaths due to oxygen depletion and occasionally they prevented fishing activities in the bay. However, the areas of mussel culture are not currently being monitored for toxic phytoplankton.

The phytoplankton of Thermaikos Bay has been monitored by the Department of Botany, School of Biology, Aristotle University of Thessaloniki for the past 7 years. The monitoring work was partially supported by the Greek Ministry of Environment, Physical Planning

◆ Scotland

Early reports of PSP

The first recorded instance of paralytic poisoning in Scotland arose from the consumption of mussels taken from the Firth of Forth at Leith in 1827 (Combe, 1828).

It took a further one hundred and thirty years before another episode was brought to the attention of the medical authorities. Gemmill & Manderson (1960) reported the first outbreak of neurotoxic or "paralytic" mussel poisoning ever recorded in Glasgow.

On June 20th 1958 three men employed by a Glasgow engineering works ate mussels left in a lorry which had previously carried a load of timber. The mussels had been attached to logs of Oregon pine taken from storage in the Junction Dock, Grangemouth in the Firth of Forth. The three men exhibited typical symptoms of PSP to a varying extent, however all eventually recovered.

Water samples from Junction Dock were examined for plankton content at the Marine Station, Millport and found to contain numerous flagellates dominated by *Euglenoids, Peridinians* and *Gonyaulax* including a small species of *gonyaulax* possibly *Gonyaulax tamarensis (Alexandrium tamarense).* The latter is one of the organisms most commonly associated with PSP in the northern hemisphere.

Gemmill & Manderson observed that in all nine previous European outbreaks and the latest reported, the incriminated mussels were invariably taken from waterways which were essentially enclosed, stagnant or polluted.

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and Public Works and the Ministry of Agriculture. A new plankton bloom monitoring program has been initiated in 1996 in Thermaikos Bay within the framework of the Long-term Program for Pollution Monitoring and Research in the Mediterranean (MED POL)-Phase 11. This monitoring program has been undertaken by the Department of Botany, School of Biology, Aristotle University of Thessaloniki under the co-ordination of FAO (GFCM) and MED UNIT. Special attention is to be given to potentially toxic phytoplankton species.

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Corrigendum to IOC-HAB Manual

Cover:

The *Alexandrium* cell illustration is optically reversed; the correct illustration appears on p. 293, Fig.15.

Cover page:

The address of G.M.Hallegraeff should be Department of Plant Science, University of Tasmania

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The following address is missing: Jorge C. Valderrama, Swedish Meteorological and Hydrological Institute, Oceanographical Laboratory, Byggnad 31, Nya Varvet, S-426 71, Vastra Frolunda, Sweden

Chapter 3. Culture Methods.

Figure legend 3.2. (p. 48) ; item D, Latex tubing should be 1/4 inch (6.35mm) Table 3.2 . (p.57); K_2CRO_4 concentration (first column, last line) should be 0.0194 [This error is also in the original Guillard and Hargraves 1993 paper].

Chapter 5. Post-column derivatization HPLC methods for Paralytic Shellfish Poisons.

The specific toxicity values for dcGTX2 and dcGTX3 are erroneous.

p.83, line 16 from below should read: For the calculation of toxicity from HPLC chromatograms, the following values of specific toxicity (MU/micromole) were determined: GTX1 (2468), GTX2 (892), GTX3 (1584), GTX4 (1803), GTX5 (160), dcGTX2 (382), dcGTX3 (935), C1 (15), C2 (239), C3 (33), C4 (143), STX (2483), neoSTX (2295) and dcSTX (1274).

Chapter 10 A. *In vitro* biochemical and cellular assays.

p.188, last line: competitive binding assay
p.191, line 3 from below: 0.89 ± 0.07 nM
p.192, figure legend 10.5: competitive binding curves
p.200, line 16 from below: 0.65 micrometer Duropore membrane (not mm).
p.200, line 8 from below: volume-200 microlitre (not mL), amount of radioactivity-50 microCi (not mCi)
p.200, line 7 from below: 50 microCi / (39 microCi / nmol); STX concentration= 6.40 microM (not mM)
p.200, line 4 from below: 3 microL [^{3}H] STX (not mL)
p.201. The Table on the top of the page should read:

		conc.of standard	conc.in assay
15 microL	100 microM STX + 235 microL HEPES/NaCl buffer	6.00×10^{-6}	1.0×10^{-6}
50 microL	6.00×10^{-6} solution + 450 microL buffer	6.00×10^{-7}	1.0×10^{-7}
50 microL	6.00×10^{-7} solution + 450 microL buffer	6.00×10^{-8}	1.0×10^{-8}
25 microL	6.00×10^{-7} solution + 475 microL buffer	3.00×10^{-8}	5.0×10^{-9}
50 microL	3.00×10^{-8} solution + 200 microL buffer	6.00×10^{-9}	1.0×10^{-9}
50 microL	6.00×10^{-9} solution + 450 microL buffer	6.00×10^{-10}	1.0×10^{-10}
Reference		Buffer only	0

p.201, line 2 in text below Table: 100 microL aliquots (not mL)
p.201, line 6 from below: 35 microL [^{3}H] STX (not mL)
p.201, line 5 from below: 35 microL STX standard (not mL)
p.201, line 4 from below: 135 microL synaptosome preparation (not mL)
p.202, line 2: 200 microL of ice-cold HEPES buffer (not mL)
Note that ALL OTHER "milli" (m)-designations in Chapter 10 are correct.

Chapter 15. Taxonomy of Harmful Dinoflagellates

p.289. The illustrations in Figures 15.10 i-j depict *Gyrodinium impudicum* (not *Gymnodinium catenatum*) . A correct illustration for *Gymnodinium catenatum* appears in Fig. 15.5 (p. 287).

◆ Modelling

Modelling the kinetics of PSP toxin uptake and release in mussels

The Galician rías (N.W.Spain) are the second world producer of mussels but mussel farming is being adversely affected by blooms of toxic algae, since the mussels accumulate phycotoxins leading to closure of the market so as to avoid human intoxications. This is especially critical in the case of the PSP producer *Gymnodinium catenatum* (Graham) because blooms of this species are recurrent along the Galician coast. The possibility of predicting the duration of closure periods can contribute to optimize the resource management and minimize the economic losses due to market bans. By means of modelling the PSP accumulation kinetics in mussels, it is possible to obtain an estimate how much toxin the mussels would accumulate and how long it would take to reach the market closure threshold. Modelling the PSP release kinetics it would be possible to predict how long mussel toxicity remains above that closure threshold. Up to now, few studies have attempted such modelling (Silvert and Subba Rao (1), with domoic acid ; Blanco et al. (2), with DSP; Silvert and Cembella (3), with PSP), only the first of them included in the model an environmental factor (temperature). As a first approximation to build such a model we have considered that the PSP toxins are organic compounds accumulated by mussels when they ingest the toxic algae and that their posterior processing in mussels are similar to that of the remaining organic matter ingested. Therefore, our simplest model includes: filtration rate, assimilation efficiency and depuration rate and some measure of the toxin concentration in the plankton that in its most useful expression would be pg STXeq/cell. As the seston concentrations in the Galician Rías are usually below the threshold of pseudofaeces production of 5 mg l^{-1} (4) the filtration and ingestion rate can be considered equivalent.

We applied four models sequentially (Fig 1) to the data of toxic cells and environmental variables obtained weekly, from 1993 to 1995, by the Centro para o Control da Calidade do Medio Mariño. We started with a very simple one-compartment model and modified or made more complex the aspects that would lead to better fitting to the toxicity data obtained by the Consellería de Sanidade and the Centro para o Control da Calidade do Medio Mariño using the mouse bioassay.

Filtration rate and assimilation efficiency were considered constant and the values were taken from a study by Navarro and his colleagues (5) carried out in the ría de Arousa. The concentration of toxins in the plankton was estimated from the number of *Gymnodinium catenatum* cells assuming a constant toxin content per cell of 40 pg STXeq (6). Depuration rate applied was 0.15d^{-1} (7).

When the one-compartment model was considered, a delay in the toxin maxima and underestimates of toxicity could be observed in most of the events (Fig 2). The degree of underestimation varies depending on the sampling point and the toxic episode. These inadequate predictions may derive from incorrect values of parameters as the filtration rate and/or assimilation rate and/or the depuration rate but also may originate from incorrect estimates of the concentration of toxins. The values of the first set of parameters needed to match the observed toxicity levels would be unrealistic. Therefore, the variable most likely affecting the model output is toxin concentration in the plankton. This has two components: the cell concentration and the toxin content per cell of *Gymnodinium catenatum*. The first component was measured in this study but it is subjected to counting and sampling errors. The toxin content per cell will be discussed more completely below. The percentage of variance of the data explained by this simple model was 45%.

A delay can be observed in fall of 1993 in all sampling points except Vigo. Also, in the first bloom studied (spring of 1993), in both Muros and Pontevedra, the delay was observed. In both Vigo and Arousa, in this same bloom, the general discrepancies were too high to discern any process from the error. In the fall of 1994 the delay was not observed. The optimization of the model fitting, by allowing the delay to vary, gave an esti-

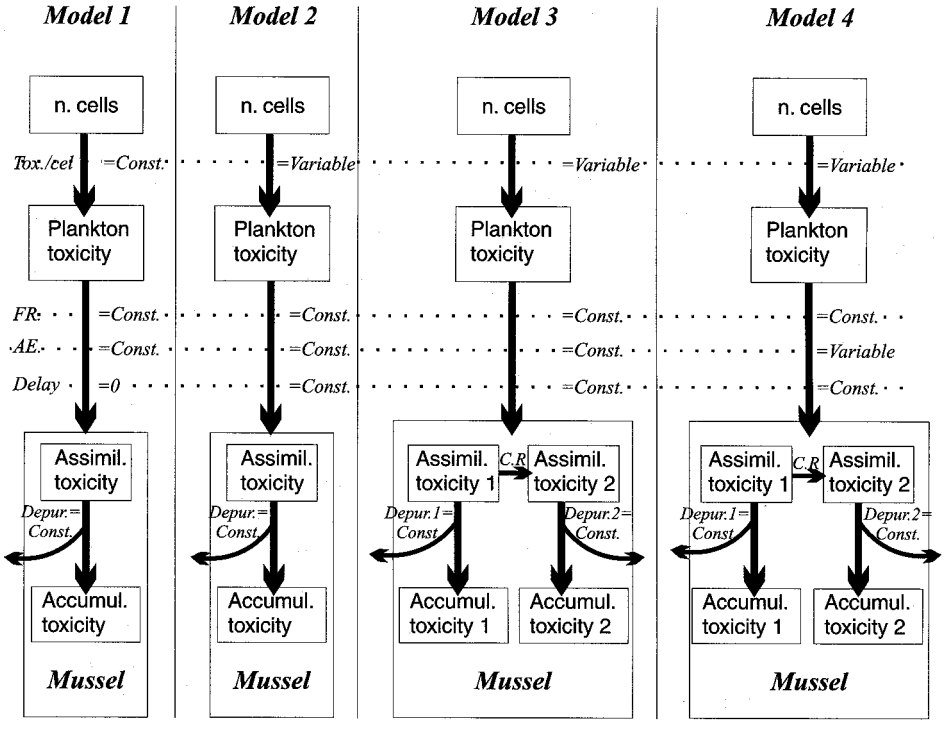

Fig. 1: Four models sequentially applied to the data of toxic cells.

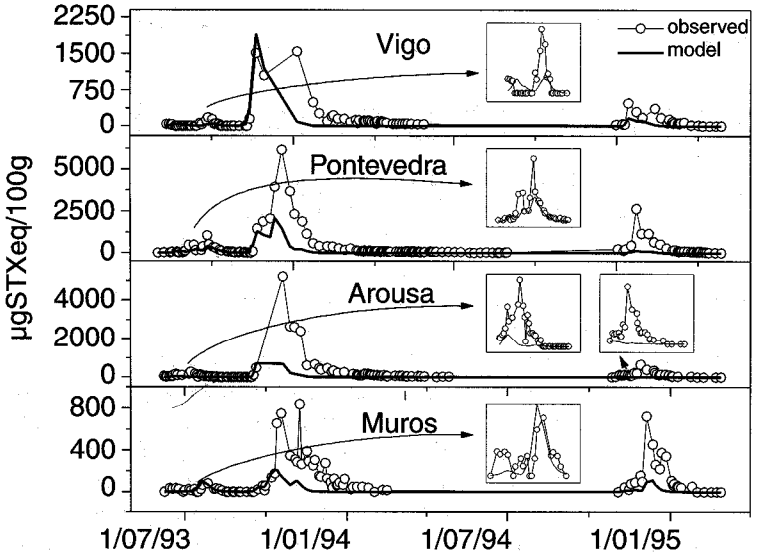

Fig. 2: Fitting of a one compartment model with constant toxin content per cell in the four Rías.

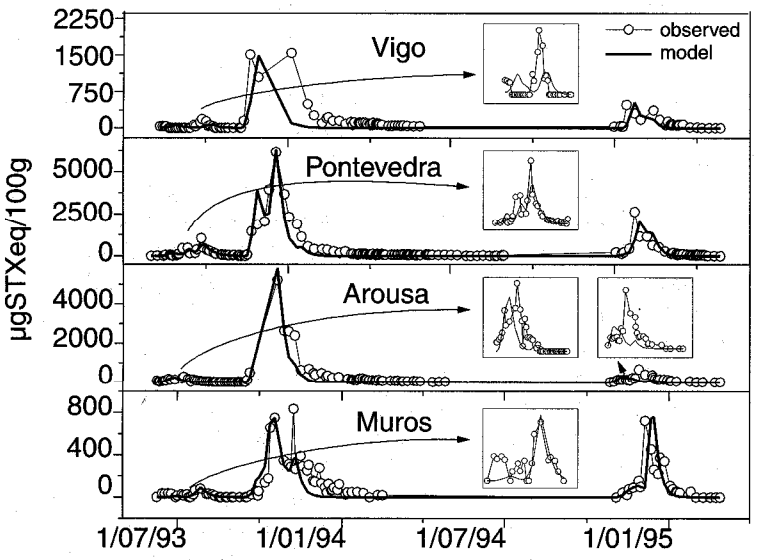

Fig. 3: One compartment model with variable toxin content per cell fitting in the four Rías.

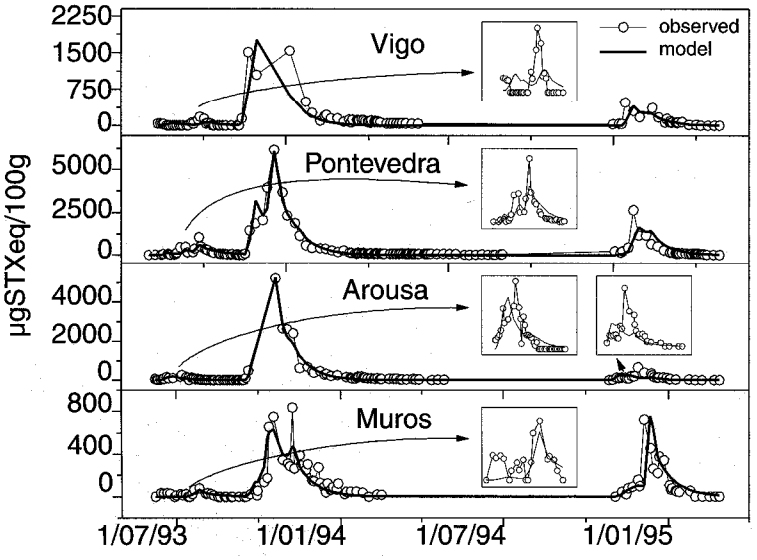

Fig. 4: Two compartment model fitting in the four Rías.

mation of 4 days for that delay. We can not discard that a real delay exists but we think that it might be an artifact due to sampling or counting errors – mainly because of the heterogeneity in the *Gymnodinium catenatum* distribution – or to errors derived from the interpolation of the plankton data. We included the delay in the model and optimized again the fitting but this time varying the toxin content per cell of *Gymnodinium catenatum* in each sampling point and each toxic episode.

Once optimized, the model fitted the data fairly well. The variance explained 80% of the deviation (Fig 3) but two obvious deviations were still present: a) some toxic values did correspond to samples in which a very small number of *Gymnodinium catenatum* cells were detected, and b) the initial portion of each detoxication process coincides in the model and in the actual data, but in the final part of the detoxication period the observed toxicity values are higher than the expected ones. The first discrepancy can be attributed to heterogeneity in the *Gymnodinium catenatum* distribution and thus sampling which is not representative. In order to avoid the second discrepancy we introduced a two-compartment model in which compartment 1 has a depuration process faster than compartment 2 and a toxin flow from compartment 1 to compartment 2. We optimized again the model fitting with a fixed depuration rate for compartment 1 ($0.21d^{-1}$ (7)) and a varying depuration rate of compartment 2 (the change rate between compartments and toxin content per cell of *Gymnodinium catenatum,* to each sampling point and each toxic episode). This change did not produce obvious differences in the expected toxin level during the early detoxication of each episode, mainly because most of the toxins were in the first compartment, but it produced a significant alteration of the detoxication in the middle-final portion, increasing substantially its fitting. So, this optimization gave a better fitting of the model to most of the toxic events and accounted for the 89% of variation in PSP data (fig 4).

The inclusion in the model of a new variable, the toxic quality, has been found to be relevant in laboratory studies (8). This coefficient was defined by analogy with seston quality for nutrition (5) as toxic content per unit volume of particulate matter. The volume of particulate matter was obtained from light transmission data by linear regression. The toxic Quality (Q_{tox}) was included in

the two-compartment model and a new optimization in the same conditions as the previous one led to a similar fitting and as expected to a positive effect of the toxic quality on assimilation efficiency.

We checked by multiple regression the effect of salinity, estimated growth rate of *Gymnodinium catenatum* (day^{-1}), and nitrate concentration on the estimated toxin content per cell of *Gymnodinium catenatum* (to each toxic episode and each sampling point). The three variables had a significant effect and in the case of nitrate the relation was found to be parabolic. By means of this regression a 80.7% of the variance was explained. Taking into account all this data, and the fact that no other variable in the model could affect so substantially the output, we think that the toxin estimations are correct and make further experiments on this subject indispensable.

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8) Moroño et al. in preparation

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◆ South Africa

New regional emergence of cyanobacterial toxicosis

Although blooms of cyanobacteria are common in South Africa, animal deaths due to hepatotoxins were, prior to 1994, confined to the central and northern provinces (1). This pattern changed during the last weeks of 1993 when the first stock losses, attributable to cyanobacterial toxicosis, occurred in the south-western Cape (2). Subsequently, and until December 1996, 7 stock and domestic animal deaths were recorded in this region and along the southern Cape coast (Fig 1). During the same period a single incident of stock loss was reported from the Northern province. Together, these losses represented a financial loss of 1.1 million SA Rands (1 USD = R4.50).

The first south-western Cape poisonings occurred between December 1993 and March 1994 in the Malmesbury-Darling area (Fig 1). In both cases the stock losses, 3 cattle plus 5 exhibiting photosensitivity, and 29 sheep, respectively, occurred in camps where animal deaths with identical clinical signs had previously been observed (2). These earlier, deaths, however, had not been linked to toxins produced by the cyanobacteria. *Nodularia spumigena* was found in the drinking water supplies in both incidents. No analyses for the presence of nodularin were performed as appropriate methodology was not available locally at the time. These two incidents have been described in detail elsewhere (2).

During March 1994, a bloom of *Nodularia spumigena* in Zeekoevlei, a shallow lake near Cape Town (Fig 1) resulted in the death of a bull terrier bitch. HPLC analysis (3) of algal material collected from where the dog drank contained a nodularin concentration of 3479 g g^{-1} freeze-dried (f-d) material. This incident, described fully elsewhere (1), was interesting in that this was a short-lived appearance of *Nodularia* in a lake which has been dominated by *Microcystis aeruginosa* forma *flos-aquae* for the past five decades.

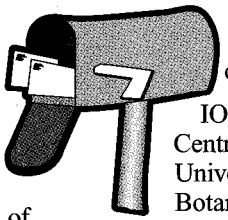
In May of the same year, a bloom of *Microcystis aeruginosa* forma *aeruginosa* near the town of Paarl (Fig 1) resulted in the death of 11 sheep and induced-photosensitivity in a further 20.

The cyanobacterial hepatotoxin, microcystin-LR, was detected in the algal bloom at a concentration of 1340 g g^{-1} f-d weight, and a second, unidentified, microcystin variant at 585 g g^{-1} f-d weight. The case history of this incident is detailed in Van Halderen *et al* (2).

After a lull of more than two years, a *Microcystis aeruginosa* forma *aeruginosa* bloom in the George district (Fig 1) resulted in the deaths of three calves and photosensitivity in a further 30. Analysis of lyophilized material from this bloom revealed the presence of the microcystins-LR and -YA at a combined concentration of 1720 g g^{-1} f-d weight.

The George case was followed by a massive stock loss of 290 in-milk dairy cows on a farm in the Kareedouw district (Fig 1). A further 70 animals presented with acute photosensitivity and

Identification Service



The IOC Science and Communication Centre on Harmful Algae, Copenhagen, and the Asian Natural Environmental Science Center offers an identification service. If you have problems with identification of harmful algal species, the Centres may assist you. You should send samples to one of the two addresses given below. It should be noted that this assistance is not given for routine identification/ monitoring of samples, but applies to particulary difficult species, or to species that require special techniques e.g. electron microscopy, for identification.

IOC Science and Communication Centre on Harmful Algae,
Univeristy of Copenhagen,
Botanical Institute,
Øster Farimagsgade 2D,
DK-1353 Copenhagen K., Denmark

Asian Environmental Science Center,
The University of Tokyo,
Yayoi 1-1-1,
Bunkyo-ku, Tokyo 113,
Japan.

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http://www.unesco.org/ioc/isisdb/html/habd.ht

had to be slaughtered. Although the clinical signs and symptoms were consistent with acute hepatotoxicosis, the water supply was flushed out soon after the incident and no toxins could be detected at the scene of the incident. Microscopic examination of the rumen contents of two of the dead animals showed the presence of filaments of *Anabaena*, and a mat of *Oscillatoria* filaments was found growing on the walls of the cement-lined reservoir from which the animals had drunk. HPLC analysis of rumen and bile revealed a hydrophobic, microcystin-like component possessing a UV_{238} spectrum closely-typical of the microcystins. The results of further tests on the rumen contents, performed by Professor Geoff Codd (University of Dundee, Scotland), and using ELISA immunoassay and protein phosphatase inhibition, were consistent with the presence of microcystins.

During December 1996 another incident was reported, again from the Malmesbury district. Here three 8-month old lambs died after drinking water containing *Microcystis aeruginosa* forma *aeruginosa*, and containing the microcystins -LR, -YR and LY at a total concentration of 1890 **g g^{-1} f-d** weight. No clinical signs or symptoms were evident prior to the deaths. Post-mortem of the affected animals showed severe lung congestion and oedema, widespread endo- and epicardial haemhorrages, severe liver damage with yellow colouration, and moderate swelling of the kidneys. There were also widespread haemhorrages in the skeletal muscles. The histopathological examination showed pannecrosis of the liver, and acute nephrosis of the kidneys.

During the period encompassed by these incidents, a case of a death of goats was reported from the Northern Province (Fig. 1). Eighteen four-month old goats died after drinking from a cement-lined trough near the town of Alldays. As was the case at Kareedouw, the causative organism was found to be *Oscillatoria*. Post-mortem examination revealed swollen and congested livers, congestion of the kidneys with some nephrosis, and oedema of the lungs. The results of the histopathological examination were consistent with the effects of acute cyanobacterial poisoning. HPLC analysis of a water sample from this incident contained 71g^{-1} of an unidentified, hydrophobic microcystin.

Although cyanobacterial blooms have long been common in South Africa, the incidents reported on here represent the emergence of related poisonings in the southern and south-western regions of the country. They also indicate the appearance of *Oscillatoria*-related incidents in a country where *Microcystis* and *Anabaena* are the commonly-expected dominants of cyanobacterial blooms. The incidents involving *Oscillatoria* differ from those typical of *Microcystis* or *Anabaena* in that a lesser amount of cyanobacterial biomass appears to be involved, and that the hydrophobic toxins produced appear to have a toxicity greater than equivalent amounts of the more hydrophilic, commonly-encountered microcystins.

The cases reported on here are the first from this region, although others may previously have been incorrectly-diagnosed or the cyanobacterial-link undetected. A heightened awareness of the problem is now emerging amongst the veterinary and stock farming communities, this leading to improved sample collection and incident investigations. The growth of algal mats on the walls of drinking troughs and dams, as opposed to the readily-observed, buoyant scums of algae, indicates a requirement for increased vigilance regarding water supplies if stock losses are to be avoided.

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3) Lawton, L.A., *et al*, 1994. *The Analyst*, 119: 1525-1530

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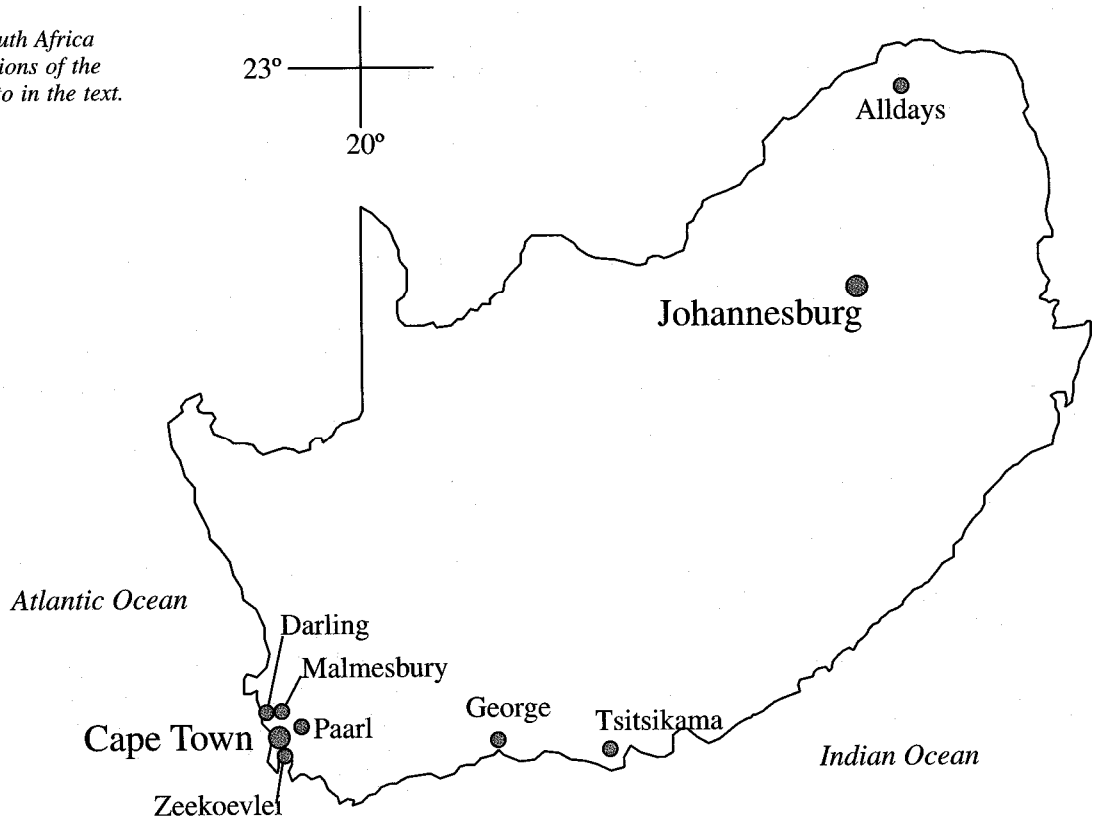

Figure 1: Map of South Africa illustrating the locations of the poisonings referred to in the text.

This special section is sponsored by the Organizing Committee of the VIII International Conference on Harmful Algae

HIGHLIGHTS OF VIII INTERNATIONAL CONFERENCE ON HARMFUL ALGAE

The *VIII International Conference on Harmful Algae* was held in the Cultural Centre "Caixavigo" from 25 to 29 June 1997 in Vigo, Spain. 470 scientists and 41 accompanying persons from 58 countries attended this event. The conference was hosted by Instituto Español de Oceanografía, Ministerio de Sanidad y Consumo, and Xunta de Galicia. It has only been possible to bring you these reports thanks to the enthusiastic collaboration of those people who agreed to chair the scientific sessions and round tables and to act as rapporteurs.

A considerable number of people obtained grants to attend: 45 scientists from developing countries/economies in transition (supported by IOC, SCOR, UNEP, and the organizers); 16 young Europeans (Federation of European Microbiological Societies); 6 US doctorands (US-National Science Foundation) and 16 Spanish doctorands (Spanish Universities and the organizers).

There were 10 keynote and 62 oral communications, all presented in plenary sessions; 2 poster sessions where 332 posters were exhibited; six round tables on the most relevant or controversial topics identified by the organizing committee, and a special session about the "Standard, Measuring and Testing Programme" of the European Union.

Before the conference, the abstracts of all accepted communications (403) were edited and organized as a database. They were available to the International Scientific Community since the preceding May through the web site of IOC at internet: http://www.unesco.org:80/ioc/oslr/habcon7.htm.

Here we provide synopses of the scientific sessions and round tables held during the conference. The proceedings of this event will be co-published by the Galician Government and IOC. About 2000 copies will be printed by the end of 1997. Libraries from developing countries will have free copies distributed through the IOC Science and Communication Centres on Harmful Algae in Copenhagen and Vigo.

Beatriz Reguera, Instituto Español de Oceanografía.

SCIENTIFIC SESSIONS

Opening session

Chair: Marta Estrada, Spain

The opening session of the VIII Conference on Harmful Algae was devoted to the opening address by Ramón Margalef (Spain) and the contribution of Colin Reynolds (U.K.) and Ted Smayda (USA). Both lectures considered aspects of harmful algal events within a general ecological framework. Margalef remarked on the diversity of dinoflagellates and the complexity of their adaptive responses to hydrodynamic and chemical constraints. In this context, accumulation of toxic compounds might be interpreted as a result of continued chemical synthesis under conditions leading to limitation of cell division. The role of toxins could be compared with that of many secondary metabolites in flowering plants.

The second lecture, by Colin Reynolds and Ted Smayda reviewed the conceptual representation (also known as Margalef's mandala) of phytoplankton life-forms as "adaptation syndromes" responding to two main selective factors: nutrient supply, as related to growth rate, and water turbulence. In this representation, red tide sequences follow a path comparable to that of a typical phytoplankton succession, but displaced towards situations with higher stratification. Reynolds reaffirmed the conclusion that life-form and function are better predictors of fitness than phylogenetic affinities and pointed out that the ability to predict the exact composition of assemblages remains subject to a strongly stochastic element.

Population dynamics of harmful algae – physical forcing mechanisms – case studies

Chairs: Patrick Gentien, France
Kaisa Kononen, Finland
José I. Carreto, Argentina

Mainstream biological oceanography has been mainly concerned with biomass production in open waters, and formed the background to Victor Smetacek's (Germany) keynote talk, which looked at the potential for cross-fertilization of ideas between "normal" phytoplankton ecology and HAB ecology. Even though considerable accomplishments have been achieved in the understanding of speciation between diatoms and dinoflagellates due to conditions of new or of regenerated production, it is now recognized that so-called exceptional blooms, which tend to be harmful or a nuisance from the human point of view, do not fit in the above scheme. Knowledge of factors determining species or even group dominance in the open ocean is still vague. A major impediment is our lack of understanding of factors shaping succession.

The study of harmful algae blooms stemmed from the need to understand toxic events. Conceptual "species of interest" models are developing and take into account, not only the regulation of growth by inorganic subtrates, but all the processes the particular species is involved in during its whole life cycle. A large amount of the detailed physiological knowledge commonly used in biomass models, although necessary, has been shown to be insufficient to answer the question: "Why does this species bloom and why is it toxic?"

The life cycle of the species of interest has to be considered, starting from the inoculation and wintering processes, to the selection by substrate and competition, selective grazing pressure, behaviour at the cell and population levels in the physico-chemical field, and mortality factors. Cysts beds due to sexual reproduction are of primary importance, in that they determine the inoculation zone. *Alexandrium*, for instance, spends most of the year as an overwintering cyst, deposited on the sea-floor and transported as sediment particles. The processes affecting cyst resuspension will determine, when growth conditions become favorable, the success of the population. Hans Eilertsen (Norway) and Tim Wyatt contribution showed clearly the rôle of tidal kinetic energy phased on the lunar cycle in determining the success of a bloom event.

Results of a tentative more classical model based on competition between species for nutrients, presented by Keith Davidson (U.K.) were not shown to be as successful. It was clearly stated that simulation of the appearance of harmful algal blooms by applying models which are just sufficient to simulate monoculture data of the particular species of interest is unlikely. Mixotrophy, and grazing pressure have been presented as determining processes which have to be considered.

Compared to previous symposia, there has been an increasing number of contributions related to meso-scale oceanographic processes and controls of harmful algae blooms. Although the most detrimental effects of toxic blooms are often detected in seafood cultures in coastal or estuarine areas, the dynamics of bloom development and concentration is tied to off-shore or coastal oceanic processes rather than local estuarine conditions. Different presentations have shown that, in many cases, populations originate offshore where they are often confined in physical structures like fronts and are transported, episodically, inshore. Close relations have been shown between meso-scale circulation and bloom appearance and extension in Argentina (José Carreto et al.) South Africa (Grant Pitcher et al.), Bay of Biscay, France (Patrick Gentien et al), Celtic Sea, Ireland (Terry MacMahon et al.), Galicia, Spain (Francisco G. Figueiras), Portugal (Teresa Moita et al.), and Gulf of Mexico (Parsons et al., USA.). Mechanisms of onshore/offshore transport of HAB populations need to be included for an understanding of HAB events in coastal and estuarine regions. An interesting finding was the observation that the growth of the domoic acid producing diatom *Pseudo-nitzschia* was affected by neither N/P ratio nor salinity, but by small-scale turbulence. These findings give a lead towards the establishment of predictive models, provided that the pertinent ecophysiological processes already known are estimated in terms of their relative importance to population development and set into the real world, which is, in this case, the ocean at meso- and small scales.

Uptake and elimination of toxins by shellfish

Chair: Sandra Shumway, USA

In the plenary lecture (Bricelj and Shumway), Monica Bricelj (Canada) presented an in-depth overview of the kinetics of PSP toxin transfer in bivalve molluscs. Based on a suite of factors contributing to their detoxification kinetics, bivalves were broadly classified into two groups: **slow detoxifiers** (e.g., *Saxidomus giganteus, Spisula solidissima, Placopecten magellanicus* and *Mya arenaria*) and **fast detoxifiers** *(Mytilus edulis, M. galloprovincialis)*.

It was noted that a bi-phasic, two-compartment model can describe detoxification kinetics in some species. Biotransformation of toxins was shown to be most pronounced in species capable of enzymatic decarbamoylation. The use of bivalve toxicity to predict the timing and source of toxic blooms was also discussed. The modelling of aspect of toxin kinetics was further discussed by Bill Silvert et al (Canada) and Angeles Moroño et al (Spain). Bill Silvert presented a dynamic model of uptake, detoxification, and biotransformation that simulates the development of toxicity in different functional compartments. The model takes into account the effects of increasing body size, addresses the changes in toxicity caused by biotransformation and can be used to test hypotheses regarding pathways and detoxification processes. Moroño showed that DSP and PSP detoxification kinetics in mussels (*M. galloprovincialis)* can be described by two and one compartment models respectively, and that the effect of environmental conditions on those kinetics cannot be estimated independently from the model structure. While real progress has been made in realistically modelling toxin kinetics in bivalve molluscs, much work remains to be done if these models are to be available for general applications.

The presentation of Fletcher et al. (New Zealand) dealt with detoxification of Pacific oysters, and emphasized the interactions of intrinsic and extrinsic factors and the detoxification process in bivalve molluscs. In the final oral paper (Vasconcelos et al., Portugal) the focus was shifted to the toxic cyanobacteria and a proposal for a monitoring programme in the Tamega River (Portugal) was discussed.

The oral presentations concerning shellfish toxicity were supplemented by a large number of poster presentations. Topics covered included: accumulation and depuration of major toxins (PSP, DSP, ASP, brevetoxins) and others (microcystins), and seasonal changes in toxicity in some geographical regions not previously described (e.g. Cuba, Morocco).

Application of cellular and molecular probes in taxonomy and genetics of harmful algae

Chair: Donald M. Anderson, USA

This session began with a review by Chris Scholin and Don Anderson (USA) of recent progress in applying antibody and DNA probes to the identification and quantification of HAB species and then proceeded to direct demonstrations of developments and applications of these technologies. A number of poster contributions also related to the theme of this session. The review highlighted the **tremendous expansion in the number of HAB species for which there are now molecular probes of various types**. At least one probe-type, and often several, is available for nearly every one of the key HAB species or genera. **However**, the review pointed out that only a few actual field applications had thus far been attempted. Clearly, probe technology developed using unialgal laboratory cultures is not easily transferred to complex field assemblage, and significant work remains to make these tools useful in field settings.

A related application of molecular technologies was demonstrated in several studies of genetic diversity that relied on comparisons of rRNA sequences, RFLP or RAPD analyses of those sequences, and lectin or antibody binding to the cell surfaces of strains of individual HAB species. Populations of *Gymnodinium*

catenatum ((Chris Bolch et al., Australia), *Gambierdiscus toxicus* (Chinain et al., French Polynesia), *Microcystis aeruginosa* (Victoria López-Rodas et al., Spain) and other species exhibit considerable diversity over small spatial and temporal scales when examined in this manner. There is considerable genetic variability within blooms or regional populations of HAB species that is not evident using traditional methods for enumeration or identification. Progress was also reported on procedures to count or separate HAB species from co-occurring organisms (Louis Peperzak et al., The Netherlands). Flow cytometry applications were described by Sako et al. (Japan) and Nicole Poulton and Don Anderson (USA), and a novel method to separate *Alexandrium* cells from samples using antibodies and magnets was presented by Angeles Aguilera et al. (Spain). Here again, direct applications to field populations were on a trial basis at best, with no sustained usage in major studies for any of these techniques.

In the discussion that followed, Chris Bolch was asked whether genetic analysis of *G. catenatum* had shed any light on the long-standing hypothesis that ballast water discharges had introduced that species to Tasmania. The response was that no foreign strains had yet been identified that were genetically identical to Tasmanian isolates, so the putative "source" population remains to be found. Interestingly, the isolates that most closely resembled Tasmanian populations were from mainland Australia. Another general comment was that despite the increased resolution obtainable with the molecular techniques, the "big picture" of HAB species bloom dynamics or ecology is still lacking. Perhaps time and resources devoted to probe development or to resolution of the genetic diversity within regional populations should be re-directed to field studies of organism ecology and dynamics, which still remain largely unknown for most HAB species. One response to this observation is that many of those involved in probe design or genetic analysis are also actively involved in field programs, and thus have strong motivations to develop and use the tools needed to quantify HAB species abundance, distribution and physiological rates on appropriate temporal and spatial scales. Conventional methods have proven to be too coarse and time-consuming to provide the coverage that is needed. Another response is that the increased resolution may be needed to explain variability in population abundance or toxicity caused by different genotypes with different levels of inherent toxicity or growth requirements – i.e., that variability reflects the dominance of different genetic strains rather than control by external environmental factors.

Overall, progress continues to be rapid in molecular approaches to HAB problems, and the potential remains high that the tools will soon be heavily used in autecological studies of HAB species. Priority research topics for the future include:

- development of new probes for HAB species for which none are presently available;
- development and marketing of kits and instruments that allow molecular probes to be used by skilled and non-skilled users alike;
- application of probe technologies in field studies investigating bloom dynamics and ecology;
- development of methods to obtain species-specific physiological measurements using molecular techniques;
- expand regional biogeographic studies of key HAB species to include globally distant populations.

Toxin detection methods

Chair: Donald H. Hannah, New Zealand

This session included one keynote address and six submitted papers. The keynote address was given by Michael Quilliam (Canada) on the use of LC-MS. He posed the question "Is LC-MS a universal method for biotoxin detection?", and then proceeded to show its efficacy for the main situations in ASP, DSP, NSP and PSP and also for ciguatera (CFP) and many of the compounds and toxins associated with these seafood poisonings. The method was shown to be very versatile, capable of detecting virtually all the relevant compounds, though not in one single analysis. The major negative problem with the technique is the capital cost for purchasing the equipment (more that US$250,000). Quilliam explained that in situations where there are a large number of samples LC-MS is a cost effective and profitable technique.

Greg Boyer et al. (USA) described the development and application of an electrochemical detector for PSP toxins in the HPLC analysis. The method used the multiple isocratic separations of Y.Oshima but replaced the post-column fluorescence reaction method with a sensitive and specific electrochemical technique. The development and application of the neuroblastoma cell based detection of PSP toxins was outlined by Joanne Jellett et al. (Canada). This technique has been applied at 3 levels, a yes/no kit, a semiquantitative kit and a fully quantitative kit. J. Jellet outlined the collaborative testing with AOAC that is currently planned.

Kevin James et al. (Ireland), presented HPLC, radioactive protein phosphatase and electrospray mass spectrometric methods to determine cyanobacterial toxins in Irish freshwaters. Mercedes Vieitez et al.(Spain) outlined the use of 4-methylumbelliferyl phosphate and fluorescein diphosphate as substrates for the protein phosphatase inhibition assay for DSP toxins; this method is sensitive, fast and simple to use and this presentation represents a further example of the utility of the method. Two poster presentations by Roberto della Loggia et al (Italy) and Yuzuru Shimizu et al. (USA), also discussed the use of fluorescence substrates for this assay.

Armando García et al. (Cuba), presented an innovative method, based on the survival of neuronal cells, as a comprehensive assay for ASP, DSP and PSP toxins. The method has potential to be an in vitro based alternative assay to the mouse bioassay. The comparision of, and utility of, the neuroblastoma cell assays and the radiolabelled competitive binding assay with the mouse bioassay for the detection of NSP toxins was presented by Penelope Truman et al. (New Zealand). Again the two in vitro assays show great promise as alternatives to the mouse biassays.

General Comments

The techniques presented, both in the oral and in the poster sessions, clearly indicate that there are a number of chemical and *in vitro* biological assays that could be suitable alternatives to the mouse bioassays. There is an urgent requirement to proceed with supervised inter-laboratory collaboration trials, especially for the neuroblastoma assay for PSP toxins and the fluorescence based protein phosphates assay for DSP toxins. There is, however, still a gap on the development of a non-animal based alternative to the mouse bioassay that will detect "unknown" toxins that may present a hazard to public health.

New toxins

Chair: Kevin J. James, Ireland

A reeview of new developments in seafood toxins was presented by Takeshi Yasumoto (Japan). Considerable progress has been made in elucidating the causes of a number of unexplained shellfish toxic incidents in Europe in recents years. Thus, a new toxin, KT-3, has been isolated from shellfish in Ireland and it is a polyether carboxylic acid with a novel spiroamine moiety. New reports of yessotoxins in Chile, New Zealand and Italy indicate their widespread occurrence but a new fluorimetric HPLC analysis, using DMEQ-TAD, should facilitate their determination and this reagent may also find application in the determination of pectenotoxins. Appreciation of the incidence of cyanophyte toxins in seafood was also highlighted with the implication of aplysiatoxin in in *Gracilaria* poisoning in Hawaii and lyngbyatoxin A in turtle poisoning in Madagascar.

New additions to the group of fast acting marine toxins, represented by the spirolides, was discussed by Jeffrey Wright (Canada) and the cyclic imine in these compounds is apparently crucial to their pharmacological activity. An intriguing theory on the characteristic methylation sequence in dinoflagellate polyether compounds should prove useful in the investigation of the source of new toxins.

Shigeru Sato et al (Japan) made an important contribution to the debate on the possible role of bacteria in the production of PSP toxins. Thus, it was shown that naturally fluorescent compounds may be confused with some PSP toxins using HPLC determinations. The complexity of the PSP group of toxins was highlighted by H. Onodera et al. (Japan). Remarkably, six new PSP analogues have recently been isolated from a freshwater cyanobacterium.

Physiology and toxin production

Chair: Stephen Bates, Canada

Seven oral presentations and 42 posters described how cell growth and toxin production are affected by cell cycle and life history events; inorganic and organic nutrient status; turbulence; salinity; and light intensity, quality and photoperiod. In his keynote, Kevin Flynn (U.K.) discussed how modelling ("simulation") is a useful tool for focusing objectives and showing cell physiologists which experiments could next be carried out, provided that these models are constructed with a sound physiological basis. The model presented was flexible and could simulate the interactions of various light and nutrients conditions with respect to toxin production. Current models still suffer from a lack of information about cell cycle and life history events, as well as growth rate data; some of this knowledge was provided in this session. For example, the timing of toxin production in relation to the light:dark cycle is becoming better understood for *Amphidinium operculatum* (Leighfield and Van Dolah, USA) and *Alexandrium catenella* (Young and Chan, Hong Kong). The study of light:dark cycles is being exploited to identify genes involved in saxitoxin biosynthesis by *Alexandrium fundyense*, by use of "differential display" of mRNA patterns, a novel genetic approach (Taroncher-Oldenburg and Anderson, USA). Growth rates of *A. fundyense* and *Prorocentrum micans* can be determined by flow cytometric analysis of the diel DNA cycle (Peperzak et al., The Netherlans). In contrast to looking at the molecular level, some basic biology of cell division and life history was given for *Prorocentrum* spp. (Pan and Cembella, Canada), *Alexandrium lusitanicum* (Honsell et al.), and *A. minutum* (Probert et al.). The pattern of sexual reproduction was described for two *Pseudonitzschia* spp. (Davidovich and Bates, Ukraine). This type of basic biology may help to understand the timing of blooms and of toxin production, and is still missing from several other algal groups.

The effects of nutrients on toxin production by different groups of toxic algae was summarized by Edna Granèli et al (Sweden); no simple correlations or patterns were found. It is essential to distinguish between toxin per cell and the rate of toxin production, and to take into account differences in cell volume (Bustillos-Guzmán and Diogène) in such comparisons. Several posters added to the information available on nutrient effects, but basically supported past findings. For example, phosphorus limitation seems to enhance toxin production by *Chrysochromulina polylepis* (Johansson and Granèli, Sweden), *Nodularia spumigena* (Panosso et al., Brasil), and *Alexandrium catenella* (Young and Chan, Hong Kong). What implication could this have for shellfish poisoning by *A. tamarense* in Hiroshima Bay, Japan, for example, where a decreasing trend in phosphate concentration may have resulted in phosphorus becoming a limiting factor for phytoplankton growth (Yamamoto and Tarutani)? In contrast, phosphorus limitation was shown to decrease microcystin toxicity in *Microcystis aeruginosa* (Hesse and Kohl, Germany) and it had no effect on okadaic acid production by *Prorocentrum hoffmannianum* (Morton et al., USA). In the above two examples of cyanobacteria (*N. spumigena* and *M. aeruginosa*), it would be interesting to determine why phosphorus limitation gave contradictory responses. New tools were presented for detecting phosphorus-limited cells: alkaline phosphatase was quantified in individual cells by use of a novel insoluble fluorigenic substrate (González-Gil et al., Spain), and two proteins (one, an alkaline phosphatase) induced by phosphorus stress were identified on the cell surface of *Prorocentrum minimum* (Dyhrman and Palenik, USA).

The major emphasis in this session was on macronutrients; there was less information on the effects of organic matter and trace metals on algal growth and toxin production. The concentration of humic substances, by controlling the supply of selenium via chelation, was shown to be important for the growth of *Gymnodinium catenatum* (Doblin et al., Australia). Likewise, the complex physiological and ecological roles of humic and fulvic acids in relation to nuisance algal blooms was demonstrated (C.Heil et al., Australia), as was their contribution to the nitrogen (Legrand and Carlsson, Sweden) and phosphorus (Panosso et al.) requirements for growth. Gambieric acid-A was shown to be an endogenous growth enhancing factor for *Gambierdiscus toxicus* (Sakamoto et al., Japan). What appears to be lacking (except for the study of Panosso et al.) is an examination in the same study of the effects of organic matter on both growth and toxin production. Trace metal limitation may have an important effect, but this was addressed in only one poster, where it was shown that iron limitation increased the toxicity of *Microcystis aeruginosa* (Kiefer and Lyck, Norway).

Another area that requires more attention than was devoted to it at this session is the study of differences in physiology and toxin production when several clones of the same species are examined under the same conditions. Characteristic differences were found among three strains of *Microcystis aeruginosa* with respect to growth and physiology; only one of the three strains was toxic (Hesse and Kohl). A comprehensive study of 20 unitrichome strains of *Anabaena circinalis* under controlled culture conditions revealed that none of the strains from outside Australia produced PSP toxins, and 11 of the Australian strains were non-toxic, indicating genetic divergence among the strains (Negri et al, Australia). The question of toxic vs non-toxic strains is becoming in-

creasingly evident for *Pseudo-nitzschia* spp., as seen in this session as well as the session on "Taxonomy; application of advanced techniques in taxonomy" (Rhodes et al., New Zealand). Two isolates of *P. pungens* from Washington and California, USA, were shown to produce low quantities of domoic acid (Trainer et al.); this species has been reported to be non-toxic elsewhere, except for one case in New Zealand. A parallel, independent study also demonstrated low toxicity in the same California clone of *P. pungens* (separate poster presented by Doucette and Scholin, USA).

This session showed a trend, continuing from past conferences, for an increased emphasis on the physiology of toxin production by cyanobacteria (Coyle and Lawton; Rouhiainen et al.; Rapala et al.; Ljungblom; Kiefer and Lyck, Hesse and Kohl; Panosso et al.; Negri et al.). Other algal groups appeared to be represented in proportion to their involvement in toxic events. Research on the physiology of toxic algae has benefited from techniques and approaches borrowed from other scientific disciplines. The study of HABs is still in its infancy, but one would soon hope for the construction of paradigms to better explain toxin production and bloom dynamics in relation to cell physiology.

Monitoring and management

Chair: Einar Dahl, Norway

The keynote talk of this session, by Geoffrey Codd (U.K.) reviewed toxicity problems in freshwaters, including drinking water supplies. Codd made a plea that some barriers need to be dismantled, since research and policy development with respect to toxins and their impacts on human activities are currently compartmentalized between freshwater and marine systems. Yet some of the toxins share common modes of action, and PSP is found both sides of the barriers.

This session included a description and rationale of the Galician monitoring programme (Joaquín Mariño et al., Spain), and emphasized that with only a small additional cost of the basic sampling requirements, it is possible to provide valuable research data. Capital and running costs, and staff requirements were detailed.

Moves to legislate in favour of mandatory ballast water exchange may under some circunstances be counter-productive. This was the conclusion of Elspeth MacDonald (U.K.), who showed that exchange may increase the risks of introducing harmful algae, at least in the regional seas of northern Europe. Motile dinoflagellates were found in most of 120 vessels arriving in ballast to Scottish ports, and cysts in more than 80% of them. *Alexandrium, Dinophysis* and *Pseudo-nitzschia* were common components of the ballast flora, and the diversity and abundance of diatoms and dinoflagellates was increased following ballast water exchange.

The fish-killing *Cochlodinium polykrikoides* causes severe losses to aquaculture in Korea. Hak Gyoon Kim (Pusan, Korea) described the success of spraying blooms with clay as a mitigation strategy. Water with 2-4 g l^{-1} of clay in suspension can remove 70-90% of the *Cochlodinium* in the water column within 20 mn.

Domoic acid in offshore *Pseudo-nitzschia* populations along the U.S. Washington coast presents a potential threat to razor clam resources. John Wekell et al. (USA) showed data indicating that they could become toxic very rapidly, making monitoring more expensive due to the sampling frequency needed to protect consumers.

Pedro Burdaspal et al. (Spain) described procedures used to reduce PSP levels in canned *Acanthocardia tuberculata,* a bivalve showing almost chronic low levels of PSP toxicity on the Mediterranean coasts of Spain and Morocco (Taleb et al.). The test showed by Burdaspal, after one year's appplication of an aproved exception in European Directives, showed that it leads to acceptably low toxin levels. ASP toxins associated with *Pseudo-nitzschia* spp. are being detected in a growing number of countries. Although the monitoring of ASP toxins in the European Union is not mandatory, some countries (Portugal, Spain..) have already included its control in their national/regional programmes.

Interactions between harmful microalgae and other microorganisms

Chair: Yuzaburo Ishida, Japan

In this session twenty eight contributions on (1) inhibitory effects of algal toxins on protozoa ingestion, copepods grazing (also egg production and egg hatching success) and fish, (2) competitive effects of algal toxins between algal species, (3) bacterial effect on PSP toxin production of dinoflagellates, (4) regulatory effects of bacteria and viruses on algal growth, and (5) others, were presented by oral and poster.

The relationships between toxic microalgae (PSP, DSP and microcystins) and grazing activities of protozoa and copepods are variable, and grazing may be influenced by food quality irrespective of its toxin content (Jeff Turner et al, USA; Jayatissa et al, Sri Lanka). The ecological interest in growth competition between algal species was that the okadaic acid-producing algae played a rôle of growth inhibition in the benthic dinoflagellates community (Linda Sugg and Frances VanDolah, USA), and that the breakage of *Gymnodinium catenatum* cells by *Polykrikos kofoidii* led to the end of a bloom of the former and rapid decline in bivalve toxicity (M. Antonia Sampayo, Portugal). On so-called PSP-producing bacteria, further experiments for identification of toxin compounds will be necessary; the expression "bacteria associated with dinoflagellates" is ambiguous as to whether "the association" means in natural environments or culture tubes or cell attachment. Finally research on regulation of algal blooms by algicidal bacteria and viruses is of ecological and economical importance (Yoshinaga et al.; Nagai et al., Japan). It may be possible to apply these bacteria and viruses as bioremediators to supress red tides.

Toxicology, pharmacology and mechanisms of action of phycotoxins in mammals

Chair: Lars Edebo, Sweden

The keynote lecture was given by Eisaburo Sueoka from the well-known research group at Saitama Cancer Centre Research Institute in Japan. This group, to which Hirota Fujiki and Masami Suganuma also belong, has discovered the tumour-promoting activity of DST (diarrheic shellfish toxins) and other structurally analogous, aquatic microbial toxins, and related it to inhibition of protein phosphatases 1 and 2A. Accumulating data indicate that this inhibition is a general mechanism for tumor promotion. It was emphasized that tumor promotion is the enhanced development of tumors after tumor initiation has taken place e.g. after exposure to chemical carcinogens. Tumor promotion might be mediated by the cytokine tumor necrosis factor (TNF) which is produced endogenously in the human body by a number of inflam-

matory stimuli. Early response genes are involved in the activation mechanisms and cellular cytoskeleton proteins such as cytokeratins appear hyperphosphorylated. Thus, this kind of toxins may participate in complex interactions with inflammation in a multitude of reactions at the cell and organ level.

DST are also toxic to neurons leading to apoptotic cell death possibly by inhibition of protein phosphatase 2A but not by the involvement of the protein kinases A and C (M.Teresa Fernández et al., Spain). Ciguatoxins, which are also polyether toxins, show more apparent neurotoxicity. At the cellular level it is seen as an increased number of coated vesicles and swelling of nerve terminals due to water influx (Jordi Molgo et al., France). Brevetoxins are also polyether compounds. In 1996 they caused a major epizootic of West Indian manatees killing more than 150 animals each ca. 700 kg. In succumbed animals toxin had accumulated in phagocytic cells in brain, spleen, liver, and kidney apparently leading to apoptotic cell death (Daniel Baden et al., USA).

Accumulating data seem to indicate that although the different groups of polyether marine toxins show specific reaction patterns, they use some common pathways. Furthermore, more toxins are being discovered, particularly with DST-like mechanisms, which draws attention to the possible health hazards of chronic exposure to subacute toxic doses.

Algae events and their impacts

Chair: Leonardo Guzmán, Chile

This session included five oral papers which demonstrated the wide variety of ways in which harmful algae can interact with human affairs. Anne Marie Legrand (French Polynesia) gave a wide-ranging review of many aspects of ciguatera, including the large scale geographical distribution and local patterns of patchiness in reef areas, the chemical structure of the main toxins and their mode of action, and how they are transferred through the food chain to reach man. Clinical treatment has advanced, but it is still necessary to identify substances capable of inhibiting or reversing the actions of ciguatoxins. Methods available to detect low concentrations of ciguatoxins in fish muscle need to be validated, and monitoring systems better defined.

Attention was drawn to the potential threat posed directly to humans via drinking water supplies, and to the fisheries in Patos Lagoon, Brasil, by blooms of *Microcystis aeruginosa.* Joao Yunes described the ecology of this cyanophyte and the toxin concentrations of perhaps four different microcystins detected in the blooms and the scums.

Arturo Sierra-Beltrán summarized potential problems in the coastal waters of Baja California, Mexico. Mortalities of fish, shellfish, birds and mammals have been recorded there since 1991, and linked to PSP. Scallops from Bahía Concepción yielded DSP toxins, first noted in 1992. *Dinophysis* and *Prorocentrum* present year-round in the bay are suspected sources. A pelecan (*Pelecanus occidentalis*) mortality at the southern end of the Baja California peninsula was probably caused by *Pseudo-nitzschia.* Domoic acid and *Pseudonitzschia* frustules were found in the stomachs together with remains of the presumed vector, the mackerel *Scomber japonicum.*

The well known *fish killer, Chatonella marina,* was linked to a major mortality of caged southern bluefin tuna (*Thunnus maccoyii*) in South Australia. The event took place in April 1996, and 1700 t of tuna (75% of the stock) were lost, with an estimated first sale value over $ 50 million. Gustaf Hallegreff argued that the relatively low cell concentrations encountered could have beeen responsible for the pathological signs observed in the tuna gills, since this species has a uniquely large gill surface area relative to body weight.

In March-April 1996, there was a major manatee, *Trichechus manatus latirostris* amongst the barrier islands in the southwest part of the state (Landsberg et al., USA). The event was attributed to the exposure to *Gymnodinium breve* brought about by penetration of the algae into the inshore waters and their persistence there, and dispersal of the manatees from upstream refuges as the bay waters warmed up. Similar events,but with fewer deaths, were recorded in1963 and 1982.

Cell cycle – life history – behaviour

Chair: Malte Elbrächter, Germany

Knowledge of cell cycles and life histories as well as of the behaviour of Harmful Algae is essential for understanding and – in a second step – predicting Harmful Algal Blooms (HAB). The session described the difficulties in elucidating life cycles. It also demonstrated that the understanding of the mechanisms of HAB-formation has made progress since the last conference. Therefore prediction of HAB-events is now more feasible than before. Nicolai Davidovich (Ukraine) and Stephen Bates presented the "true" life cycle of the AST-producing diatom, *Pseudo-nitzschia.* Some years before a "life-cycle" had been presented for this genus which apparently showed a parasitic infection by a fungus. Howard Glasgow et al. (USA) showed that the fish-killing potential of *Pfiesteria* zoospores (dinoflagellate) depends on the nutrient load of the water column and – more importantly – on the earlier history of the life-cycle stages forming the fish-killing zoospores. Besides parasitic food uptake by attacking fish, *Pfiesteria* may also feed phagotrophically on plankton-organisms including other algae. *Pfiesteria* is even able to live by mixotrophy, using *kleptochloroplasts* of their food items. This and related studies are important steps towards better understanding and predicting the pattern of fish-kills caused by *Pfiesteria.*

Mixotrophy is apparently a common and important but so far mostly neglected nutritional strategy of harmful algae. Maria Faust (USA) demonstrated this for benthic toxic algae during the session and several poster tackled the same topic.

M. Grazia Giacobbe et al (Italy) analysed the cell division cycle of dinoflagellates belonging to the genera *Dinophysis* (DST-producer) and *Alexandrium* (PST-producer). These organisms multiply by binary cell division, but this cell division is syncronized in the day-night cycle so that all cells undergoing cell division do it at the same time. This behaviour is important as recently divide cells can be recognized even a few hours later. Therefore the population growth rate can be determined in natural populations. This is the starting point to differentiate between population increase due to growth or by physical accumulation of cells from a larger water body. Frances Van Dolah et al. (USA) investigated the biochemical mechanisms of this endogenous cell cycle, using the benthic dinoflagellate *Gambierdiscus toxicus,* causing ciguatera. These investigations may be the starting point for growth rate determinations in field populations of species showing no morphological criteria for cell division or for those species which do not divide synchronously.

In conclusion, the session covered very well the progress which is being made with respect to a better understanding of population dynamics and predicting the occurrence of HABs. Both provide essential information for efforts to mitigate the effects of Harmful Algae Blooms.

ROUND TABLE DISCUSSIONS

Emerging technologies for biological and biochemical assays of phycotoxins

Chair: Allan Cembella, Canada

Rapporteurs: Marion Gillman-Coughlan, Alan Bishop and Ireland.

Alternatives to the various mouse bioassays for phycotoxin detection and quantification were explored. Development and implementation of alternative methods was acknowledged as a high priority for seafood regulatory agencies, the aquaculture and harvest fishery industry, and the protection of public health. A distinction was made between an assay, which yields a single toxin concentration or toxicity value, but no direct information on the specific toxin composition, and an analytical method (e.g., HPLC, coupled mass spectrometry, capillary electrophoresis, etc.), whereby the toxin spectrum is resolved, and total toxicity is derived from specific toxicity values of the individual components.

The participants recognised that currently available *in vitro* biological and biochemical methods may supplement or partially replace the whole animal bioassays conventionally used in phycotoxin monitoring programs. In principle, *in vitro* assays could be configured to yield at least semi-quantitative measures of toxin concentration or toxicity in a simple, rapid, and sensitive format. In a kit configuration, such assays could be employed routinely for high-volume screening of putatively toxic seafood extracts on board ship, in rudimentary dockside laboratories, and at aquaculture installations, as well as in well-equipped regulatory laboratories responsible for toxin monitoring. Nevertheless, regardless of the high level of technical sophistication, sensitivity, low cost and ease of use of several in vitro assays for phycotoxins, few such methods are in widespread distribution as commercial products, and acceptance as official standardised methods (by AOAC, U.S. Food and Drug Agency, EU committees, etc.) is even rarer. The panel discussion addressed the reasons for this apparent contradiction between technical facility and implementation:

1) technical performance limitations of in vitro assays?;
2) high cost of development versus low rate of economic return?;
3) conservatism by regulatory agencies?;
4) lack of properly controlled inter-calibration trials?;
5) poor availability of standards and reference materials?; and
6) need for advanced training in the proper use of these methods?

Since the Panel participants demonstrated a wide spectrum of expertise in alternative assay approaches for various groups of phycotoxins (PSP, DSP, ASP, NSP, ciguatera toxins, microcystins), and they have been instrumental in the technical development and/or application of these methods, their comments focused primarily on the technical performance aspects – rather than on regulatory acceptance.

In accordance with the scheme outlined in the Manual on Harmful Marine Microalgae (IOC-UNESCO, 1995), four basic categories of in vitro biological and biochemical methods were defined:

1) immunoassays;
2) enzyme-inhibition assays;
3) cellular-based ("cytotoxicity") assays and
4) receptor-based assays.

These were divided further into structural versus functional assays – in the former type (e.g., immunoassays), toxin detection is contingent upon the molecular configuration of the analyte (toxin), but this may not be directly correlated with the toxicity towards target cells. Some participants noted that initial enthusiasm for immunoassays (especially, enzyme-linked immunosorbent assays [ELISA]) has begun to decline in favour of functional assays (e.g., receptor-binding or cytotoxicity assays), whereby the toxic effect is exerted directly upon the target system, e.g. ion channel or membrane. This is a particular weakness of immunoassays applied to samples containing a complex mixtures of toxins with a wide range of specific toxicities, using antibodies with highly specific cross-reactivity. It was suggested that the problem of limited cross-reactivity could be partially overcome by employing an antibody "cocktail". In any case, sensitivity levels are more than adequate for regulatory purposes (usually <nM detection limits) but some *in vitro* methods are plagued with false positive responses, matrix effects, or the failure to detect certain key toxin analogues. There was also concern expressed regarding the mats, poor stability of reagents and other perishable supplies provided in kit foralthough efforts are being made to alleviate this critical limitation.

It was acknowledged that proper application of in vitro diagnostic methods for phycotoxins will require further knowledge regarding the acute and chronic toxicity of specific toxins towards mammalian systems (e.g., non-phosphatase inhibitory DSP toxins). An additional challenge would be to optimise clean-up techniques to reduce interfering matrix effects and/or to simplify the complex toxin mixtures in samples via chemical conversion, and thereby determine "maximum potential toxicity".

There was no unanimity regarding the ideal approach for *in vitro* assay development and implementation, but a consensus emerged that *in vitro* methods will play an ever increasing rôle in phycotoxin monitoring programs and that the inherent technical problems are not insurmountable.

New issues in taxonomy

Chair: Øjvind Moestrup, Denmark

Three topics were raised:
1. Documentation for the identity of potentially toxic species of phytoplankton
2. The *Heterosigma akashiwo/carterae* issue
3. The *Prorocentrum/Exuviaella* issue

1. Identification of potentially toxic algae is becoming increasingly more difficult to the use of sophisticated techniques which allow for a better definition of the species but at the same time for a narrower species concept. It also results in the erection of many new species. This development is not recognized by non-taxonomists. Thus *Microcystis aeruginosa* is still the most commonly used name in papers dealing with *Microcystis*, yet taxonomists know that this

species is actually very difficult to identify. Similarly the name *Pseudo-nitzschia seriata* keeps being reported worldwide (including in the tropics), although is a species restricted to cold water in the Northern Hemisphere. The many incorrect identifications creeping into the literature give rise to misunderstandings, wrong ideas about biogeography and toxicity, etc, and participants discussed how this situation can be ameliorated. The following suggestions were brought forward and generally adopted.

Scientific papers should always contain photographic evidence for the identity of the organisms used, or must refer to a previously published photographic illustration. Drawings should always be accompanied by photographic evidence.

The whole cell should be illustrated (important e.g. for *Pseudo-nitzchia* species). If possible, permanent slides should be prepared, which are available for future confirmation, and material should be deposited at a place where it is generally available.

2. Taylor (1992) argued that the correct name for *H. akashiwo* is *H. carterae*, based on Hulburt's description of the organism, the first description of the species (as *Olisthodiscus carterae*). Unfortunately Hulburt's description is invalid (no type), as pointed out by Throndsen (1996). The name change suggested by Taylor is therefore not required. However, the author citation given by Throndsen needs to be modified, since the genus *Heterosigma* was not validly erected by Hada (no type species). It was validated by Sournia and the correct name for the organism which has had such a confused taxonomic history is therefore *Heterosigma akashiwo* (Hada) Hada ex Sournia, or since the code allows deletion of the name preceeding "ex", *Heterosigma akashiwo* (Hada) Sournia. Let us all hope that this remains the last change.

3. The identity of *Prorocentrum lima,* a species described originally by Ehrenberg (as *Cryptomonas lima*), is uncertain. At the same time there are indications from ultrastructure, toxicity studies and ecology that the genus *Prorocentrum* as presently understood comprises more that one genus. McLachlan *et al.* (1996) suggested splitting the genus and use of the name *Exuviaella* for the species related to *lima*, i.e. most if not all the toxic non-planktonic species known at the moment. This would result in renaming of *P. lima* as *Exuviaella lima*. Malte Elbrächter (Germany) spoke strongly in favour of rejecting the name *Exuviaella* as the identity of the type species of *Exuviaella*, E. *marina* is uncertain. Elbrächter recommended that the name *Exuviaella* be rejected and that all species be retained within the genus *Prorocentrum* until more data is available, including studies on more species. This recommendation was accepted after a long discussion. Elbrächter will present his arguments for rejecting *Exuviaella* separately.

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Re-evaluating DSP

Organizer: Takeshi Yasumoto, Japan
Chair: Donald Hannah, New Zealand
Rapporteur: Jim Sim, New Zealand

This session continued discussion begun in other fora including a round table at the VIIth Harmful Algae Conference in Sendai, Japan.

Takeshi Yasumoto (Japan) gave an introduction to and a summary of the toxicology of yessotoxin (YTX). Perspectives of the DSP issues were given by Tore Aune (Norway), Z. Amzil (France), Aurelia Tubaro (Italy) and Donald Hannah (New Zealand). Eisaburo Sueoka (Japan) gave a brief summary of the tumour promoting potential of DSP toxins. Sources of YTX contaminated material were described by Patricia Ciminiello (Italy) and Lincoln MacKenzie (New Zealand).

During the discussion, issues raised included:

- the genotoxicity of okadaic acid
- restricted or no access to the mouse bioassay
- the use of risk assessment methods to determine acceptable levels of DSP and associated toxins in seafoods
- methods to extract DSP toxins
- alternative methods to the mouse bioassay to detect DSP toxins
- what, if any, levels are acceptable for those compounds not regarded as DSP toxins

1. Consensus was reached that YTX should not be regarded as a DSP toxin;
2. There was widespread agreement, with some dissenting views, that Pectenotoxins (PTX) should not be regulated at the same level as DSP toxins;
3. Agreement, with some dissension, was reached that the protein phosphatase methods would be acceptable alternatives to the mouse bioassays for DSP toxins;
4. Discussion also focused on how screening for DSP toxins fits in with screening for other shellfish poisoning toxins, showing methods are required to cover all types of shellfish poisoning in order to provide acceptable levels of food safety;
5. Establishment of appropriate levels for the non-DSP toxins (such as YTX, PTX and gymnodimine, etc.) requires robust oral toxicological studies that determine dose-response curves, the not observable adverse effect levels (NOAEL) and the lowest adverse effect level (LOAEL).

Bacterial production of toxins

Chair: Susan Gallacher, U.K.

Discussion focused on bacterial production of Paralytic Shellfish Poisons (PSP). The chair defined this area as consisting of three issues:

1. Are bacteria capable of autonomous production of PSP and, if so, how does this influence dinoflagellate toxicity?
2. Do bacteria influence dinoflagellate toxicity in some manner, not necessary related to autonomous toxin production?
3. Do bacteria influence shellfish toxicity?

The following issues were raised during discussion:

a) The location of bacteria in relation to the dinoflagellate cell. Some parties claimed that bacteria can be seen in *Alexandrium* cells, whereas others disagreed, stating that such observations may be explained by artifacts in the sampling process. A study by Jane Lewis (U.K.) and Susana Franca (Portugal) designed specifically to answer these questions, will commence soon this year, and will be the first comprehensive study of its kind.

b) PSP is produced by "axenic" cultures of dinoflagellates in Yuz-

aburo Ishida's laboratory (Japan). The panel pointed out that at least 95% of marine bacteria are non-culturable by standard laboratory procedures, hence demonstrations of the bacteria-free status of dinoflagellates by routine culture methods are not sufficient. It was noted that epifluorescence microscopy was also used, but the panel considered that the best proof of "axenic" dinoflagellate cultures would be based on molecular techniques.

c) Although evidence for the autonomous production of PSP by bacteria is available using techniques such as the receptor binding and mouse neuroblastoma assays, as well as HPLC and selective ion monitoring CE-MS, several participants agreed that definitive tandem MS data was still required. Greg Doucette (USA), Susan Gallacher (U.K.) and Elizabeth Maas (New Zealand) plan to colaborate on a study aimed at generating sufficient quantities of bacterial cell extracts for such analysis. Mike Quilliam (Canada) has agreed to perform the tandem MS experiments and provide a critical assessment of the results. The panel is hopeful that such data will be available before the next conference in Tasmania in the year 2000.

Georgina Hold (U.K.) said that shellfish can be made toxic to low levels after exposure to PSP- producing bacteria, as measured by the mouse neuroblastoma assay. Concern was raised about the use of this technique and the panel explained that very thorough controls were used which negated any possible false results due to the sample matrix. However, it was agreed that future work should concentrate on attempts to increase shellfish toxicity to levels allowing HPLC and mouse bioassay data to be obtained.

Allan Cembella (Canada) raised the issue that in his experience the toxin content of shellfish could be fully explained by the toxin content of the dinoflagellates to which they were exposed. The panel raised the question of shellfish toxicity in the absence of any known PSP-producing dinoflagellate cells. There was some disagreement over the validity of this point.

Some information was presented to demonstrate how molecular biology could advance our understanding of bacterial PSP production. Arthur Hosie summarized his efforts to produce mutants of PSP-producing bacteria to identify genes which could be involved in toxin synthesis. This approach complimentes the work by Gaspar Taroncher-Oldenburg (USA) on differential display in dinoflagellates. A. Hosie explained that once genes were identified, probes could be developed which would be used for various purposes, including examining dinoflagellates for similar genes, and the detection of toxin producing organisms in the environment.

The issue of proof of toxin production by bacteria was again raised. It was pointed out that a genetic approach provides an alternative definitive way to address this question. For example, if genes involved in toxin biosynthesis can be identified, one long term aim would be to express these in non-toxic bacterial species and determine if this is sufficient to mediate toxin production. Molecular techniques also have the potential to be used to investigate the regulation of toxin production in response to environmental stimuli.

It is accepted that cyanobacteria produce PSP, and Geoffrey Codd (U.K.), indicated that the question of autochtonous toxin production by cyanobacteria, or symbiotic bacteria, was also becoming an issue in this field.

The point was raised that attention should be focussed on demonstrating a bacterial contribution to PSP toxicity in the natural environment. Linda Medlin (Germany) described how the development of gene probes to toxic bacteria could be used to determine if they are present during periods of shellfish toxicity.

Future research areas identified were:

1. To elucidate the influence of bacteria on dinoflagellate toxicity with the help of molecular approaches;
2. To develop gene probes to bacteria of interest and enhance our understanding of bacteria-dinoflagellate interactions;
3. To identify genes involved in toxin synthesis and to develop probes.

Cyanobacteria

Chair: Ian Falconer, Australia
Rapporteurs: Karina Sivonen, Finland; Susan Blackburn, Australia

An introduction to current problems with toxic cyanobacteria in drinking and recreational waters was given by Ian Falconer (Australia) and focussed on the following topics:

1. Ecological issues
2. Toxicological issues
3. Remedial issues
4. Safety of drinking and recreational waters.

1-2. Ecology and Toxicity Issues

Cyanobacterial blooms are a long standing problem, and pre-date intensive agriculture and high populations. They are aggravated by nutrient inputs such as superphosphate fertilizers. But in the Baltic (K. Kononen), while nutrient input is important near the coasts, the heaviest blooms occur in the least polluted areas. They are a natural occurrence, and are hugely variable due to hydrodynamic effects. Many factors influence the growth of bloom forming species (C. Reynolds). Not all cyanobacteria are equal and the scum-forming species are the most problematic. Temperature and water stratification effects are very significant in temperate regions. In Australia (S. Blackburn), *Anabaena circinalis* is less toxic in the tropics and more toxic in the temperate region and forms winter blooms in Tasmania and summer blooms in Queensland. Are the same factors the trigger?

So far, there are no clear answers as to the reasons for toxin development, which occurs in quantity equal to chlorophyll, and thus cannot be an accident C.Reynolds). Toxins may be a means of communication between cells in the population-intrapopulation signalling. The high level of variation in toxin content may reflect signalling changes.

Cylindrospermopsis is a new large-scale toxic hazard in water reservoirs, and is less obvious because it forms dense layers at 3 m or more depth, with no surface scums. The toxin causes kidney and liver injuries.

Dense blooms of *Aphanizomenon* occurred in Lake Kinneret (Sea of Galilea) in 1994 which contained cylindrospermopsin (Sukenik). Due to depth of the lake below sea-level, the 30 atm pressure charge in the water supply pipeline broke the cells and freed toxin into the water. This was a new occurrence and the ecology of the bloom has not been studied.

Of 75 cyanobacterial isolates screened for toxicity, 25% were found to be toxic, and 5 were highly toxic; they are used for fertilization of agricultural crops in the Nile Delta (Yanni). Falconer commented that cyanobacteria are also harvested for human food (*Spirulina* + *Aphanizomenon)* which may be a health risk.

PSP has been reported in *Cylindrospermopsis* in Brazil, and

cylindrospermopsin in *Aphanizomenon.* According to G.Codd this may be an issue of species identity; molecular taxonomy is required as well as morphology.

Increased blooms in the Gulf of Riga (M.Balode) may be related to increased ambient temperatures from climate change. *Microcystis* blooms are associated with hot summers. Fish kills, livestock illness and human skin irritation have been associated with *Mycrocystis* blooms in Lake Victoria, Kenya.

In Portugal (V. Vasconcelos), 90% of the human population drink surface water, and for 3-5 months of the year (summer) all of the rivers are full of cyanobacteria. There are also small shallow reservoirs surrounded by agricultural land, including pig production. Some reservoirs have year round blooms, even with very low nutrient concentrations in the water.

A seawater channel was cut in Western Australia at $ 80 million cost to increase salinity and decrease nutrients in an estuary subject to repeated toxic *Nodularia* blooms. The blooms now occur in the river mouths to the estuary, and dinoflagellates are appearing in the estuary itself (Falconer, Blackburn)

3. Remedial Issues

Remediation of a lake which was repeatedly subject to toxic blooms from the 1980's, one of the most eutrophic in Sweden, was carried out by restoration by top-down and bottom up processes. Farming was moved away from the lake shore. 430 tons of grazing fish were removed, and predatory fish returned. This has resulted in an increase in macrophytes, *Daphnia*, clearing of turbidity and cessation of algal blooms for 3.5 years.

Aeration at depth to destratify lakes and reservoirs is commonly used, but not always successfully, depending on the depth and other characteristics of the reservoir. No information is available on surface sprays. Water supply authorities often use copper sulphate to clear algal blooms. This cause releasing of toxins into the water.

4. Safety of Drinking and Recreational Waters

Drinking water needs to be demonstrably safe for human consumption. Recent toxic blooms in England, Canada, and Australia have lead to concern over toxins in top water. WHO established a task force on setting a safety guideline value for microcystins, which is likely to be 1µg/l of microcystin-LR or equivalent. The main unresolved issue is tumour promotion. Epidemiological studies in China indicate a risk, but there are also uncertainties over exposure routes. Liver tumour studies in rats suggest that the WHO guideline of 1 µg/l is likely to be safe (E.Sueoka).

Ciguatera

Chair: Richard Lewis, Australia
Rapporteurs: Michael Holmes, Singapore;
Jorge Diogéne, Spain

Richard Lewis introduced ciguatera with the definition "ciguatera is a disease caused by sodium channel activator toxins that accumulate in the flesh and viscera of fish to levels that harm humans". Areas for discussion were:

Epidemiology: identifying human threats from fish
Toxinology: characterising the mode of action and chemical nature of toxins involved
Origin: identifying the organism(s) responsible
Detection: development of a cost-effective and reliable assay

The global distribution of the disease and its variation between Oceans were summarised. The South Pacific Health and Epidemiological Information Services (SPEHIS) data base was described and a comment made that comparable data was not available for other ciguatera-endemic regions.

At least four Caribbean ciguatoxins (C-CTXs) are now identified. The C-CTXs have similarities to the known Pacific Ocean-CTXs (P-CTXs) both structurally and pharmacologically (John-Paul Vernoux, France). However, C-CTXs are sensitive to alkaline media, unlike P-CTXs. While most C-CTXs are slow-acting toxins (like the P-CTXs), there are also a number of fast-acting CTXs that induce deaths of mice of in about 10.0 min.

Maitotoxin (MTX) is the major toxin produced by the benthic dinoflagellate *Gambierdiscus toxicus*. MTX is not found in the muscle (flesh) of fish, only in the gut contents and viscera of grazing fish and therefore is unlikely to be involved in ciguatera (Anne Marie Legrand, French Polynesia). Thus ciguatera appears to be caused only by CTXs, which have been isolated from fish as well as from wild and some cultured *G. toxicus*. Approximately 20 CTX analogs have been isolated from toxic fish of the Pacific, but many of these have not been structurally characterised; CTXs and brevetoxins (PbTxs) bind to site 5 of the Na^+ channel. CTX causes Na^+ channels to open at resting membrane potentials when they would normally be closed; this causes membrane depolarisation, spontaneous action potential and finally inhibition of action potentials (Evelyne Benoit, France). High ciguatoxin concentrations may block Na^+ and K^+ channels. Associated with the increased sodium influx and the spontaneous action potentials, CTXs cause an influx of water resulting in cell swelling, especially at nerve terminals.

Since ciguatera appears to be caused only by CTXs, its origin is probably restricted to *Gambierdiscus toxicus*. M. Holmes and R. Lewis showed that CTX production by *G. toxicus* is strain dependent. This was confirmed by T.Yasumoto's and A.M. Legrand's groups. Another factor may be environmental and Sperr and Doucette suggest that high N:P media ratios induce CTX production in most strains of *G. toxicus*.

A lot of benthic dinoflagellates produce toxins but the accumulation of these toxins in fish to cause human poisoning is not proved for any species except *G. toxicus*. Yasumoto's group have shown that *O. siamensis* produces palytoxin which may be implicated in fish poisonings such as clupeotoxism. However, palytoxin poisoning is distinct from ciguatera, and causes elevated serum enzyme levels not seen in ciguatera victims. Okadaic acid and analogs are produced by a number of benthic species of *Prorocentrum* (*Exuviaella*) and OA has been reported from the flesh of fish. However, its detection in fish at concentrations sufficient to poison people needs verification.

Recently, Maria Faust described a new sand-dwelling species of *Gambierdiscus*, *G. belizeanus* from the Caribbean and the Indian Ocean; however, its rôle in ciguatera is unknown. While the link between *G. toxicus* and ciguatera has been confirmed in the Pacific, the origin of the C-CTXs has yet to be identified.

Immunodetection of CTXs was discussed (Serge Pauillac, French Polynesia). CTXs are lipophilic, non-immunogenic low molecular weight compounds, so that a hapten-protein conjugate must be used for immunisation. The CTX are also difficult to acquire; only a few mg have ever been isolated to purity. By using the JKLM ring of CTX coupled to a protein carrier, a CTX-specific antibody had recently been obtained. Miniaturised assays indicate that it may be possible to detect about a 0.3 pM of CTX using the JKLM antibody.

Future events

IOC-NorFa Nordic-Baltic Course on the Taxonomy and Biology of Harmful Microalgae, Vortsjärv Limnological Station, Estonia. 15-23 August 1998. The objective of the course is to improve the taxonomic skills of the participants in order to enable them to make reliable identification of phytoplankton species causative of harmful algal events in the Baltic region. The Course will cover also ecology and toxin chemistry particularly of blue-green algae. Seats are available for participants from the Baltic countries, Northwest Russia and the Nordic countries. Course lecturers will include: Dr. G. Cronberg, University of Lund, Dr. K. Kononen, Finnish Institute of Marine Research, Dr. J. Larsen, IOC Science and Communication Centre on Harmful Algae, Professor Ø. Moestrup, University of Copenhagen, Denmark; Dr. K. Sivonen, Finnish Institute of Marine Research. Deadline for applications: 1 April 1998. Application forms can be obtained from the address below and from the WWW at http://www.unesco.org:80/ioc/oslr/oslr.htm. Contact: IOC Science and Communication Centre on Harmful Algae, Copenhagen. (See below).

6th Canadian Workshop on Harmful Algae, St. Andrews, New Brunswick, Canada, 27-29 May, 1998. Contact: Jennifer L. Martin, Fisheries and Oceans Canada, Biological Station, St. Andrews, N.B. E0G 2X0, Canada.
E-mail MartinJL@mar. dfo-mpo.gc.ca,
Fax (1) 506 529 5862, Tel (1) 506 529 5921.

IOC Danida Training Course on the Taxonomy and Biology of Harmful Marine Microalgae, July 1998. This two weeks course will focus on identification and preparation techniques supplemented by lectures on different aspects of the biology of harmful algae. Teaching staff will include: Dr Yasuwo Fukuyo (Univ. of Tokyo), Prof. Øjvind Moestrup (Univ. of Copenhagen), Dr Jacob Larsen (Univ. of Copenhagen/IOC Centre), Organized by the University of Copenhagen, and IOC Science and Communication Centre on Harmful Algae, Copenhagen. For application forms, contact: the IOC Science and Communication Centre on Harmful Algae, Botanical Institute, Ø. Farimagsgade 2D, DK-1353 Copenhagen K, Denmark; fax: +45 33 13 44 47. Deadline 1. April 1998.

2nd European Phycological Congress (EPC2). Montecatini Terme, Italy. September 20-26, 1999. Prof. Francesco Cinelli, Dipartimento di Scienze dell'Uomo e dell'Ambiente, Universita di Pisa, Via A. Volta, 6, I-56126, Pisa, Italy.
Tel: 39-50-23054. Fax: 39-50-49694.
E-mail:cinelli@discat.unipi.it
Web page: http://www.area.fi.cnr.it/icaa.htm

Sixth International Conference on Modern and Fossil Dinoflagellates "DINO 6". Trondheim, Norway, 7-12 June 1998. Contact: NTNU Museum of Natural History and Archaeology, Attn. Morten Smelror, N-7004 Trondheim, Norway. Tel. +47 73 5921 47, Fax. 47 73 59 22 23, E-mail morten.smelror@vm.ntnu.no. More info at http://www.ntnu.no/vmuseet/dino6

4th International Toxic Cyanobacteria Symposium, 27 September-1 October, 1998
Duke University Marine Laboratory, Beaufort, North Carolina, USA. Symposium Co-Chairs: Hans W. Paerl & Wayne W. Carmichael. Themes: Toxin physiology and regulation; Detection and characterization of toxic Cyanobacteria, toxins and other harmful substances; Novel toxins, mechanisms, organisms, and toxic episodes; The functional roles of toxins from the cyanobacteria's perspective; From cultures to blooms: Clarifying harmful bloom dynamics in the laboratory and field; Ecology and biotic interactions of of harmful bloom cyanobacteria; Environmental monitoring and risk assessments of cyanotoxins, taste and odor causing compounds; Human alteration and manipulation of toxic cyanobacteria, including watershed management for bloom control and removal methods for toxins in water supplies; Manifestations and consequences of toxic cyanobacteria on environmental quality and human health: WHO guidelines for cyanotoxins (e.g. microcystins) in drinking and bathing waters;
Contact: Dr. Hans W. Paerl, Co-Chair, 4th ITCS, UNC-CH Institute of Marine Sciences, 3431 Arendell Street, Morehead City, NC 28557 USA, Hans_Paerl@UNC.EDU

For a more comprehensive overview of up-coming activities and events you should visit the IOC International Marine Meeting List at the IOC homepage at http://www.unesco.org/ioc/infserv/meets.htm. The list of meetings related to the International Year of the Ocean might also be of interest http://www.unesco.org/ioc/iyo/conferences.htm

Literature for developing country libraries

Limited copies of the following titles are available from IOC's HAB Centre in Denmark, to libraries of marine science institutions *in developing countries only*:

- The Genus Alexandrium Halim (Dinoflagellata). **E. Balech, 1994.**
- Algae. An Introduction to Phycology. **C. van den Hoek et al., 1995.**
- Identifying Marine Phytoplankton. **C. Thomas et al. (eds.), 1997.**
- Proceedings of the International Symposium on Ciguatera and Marine Natural products, Hawaii, 8-10 August 1994. Hokama et al (eds.) 1995.
- Proceedings of the First International Congress on Toxic Cyanobacteria, Denmark, 20-24 August 1995, and, Proceedings of the First Maj and Tor Nessling Foundation Symposium on 'Recent developments in Cyanobacterial Research', Finland 16-18 August 1995. Moestrup et al (eds.) Phycologia, Vol.35 No.6, 1996

Applications must be submitted and signed by the responsible librarian, and should indicate why the requested title is of specific interest to your institution and its ongoing research or teaching. No requests from individuals will be honoured. Address applications to:

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IOC publications

- Summary Report of the Fourth Session of the IOC Intergovernmental Panel on Harmful Algal Blooms, Vigo 30 june - 2 July 1997. Available in English, French, Spanish.
- Amnesic Shellfish Poisoning (ASP), Volume 1, IOC *Manuals and Guides*, No. 31.

These publications are free of charge and available from the IOC Secretariat (Paris)

- Manual on Harmful Marine Microalgae, IOC Manual and Guides No. 33.
- Harmful and Toxic Algal Blooms. Proceedings of the Seventh International Conference on Toxic Phytoplankton, Sendai, Japan, 12-16 July 1995.

The Manual and the Sendai Proceedings are free of charge but a fee of US$ 30 will be charged for handling. A cheque issued for the IOC SCCHA, University of Copenhagen, should accompany all orders. Only prepaid orders will be accepted. Excepted from the handling fee are marine science libraries and scientists in developing countries which can apply for delivery free of charge.

HARMFUL ALGAE NEWS

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