Oceanography Committee **ICES CM 2003/C:06**
Ref. ACME, F

Report of the

ICES-IOC Working Group on Harmful Algal Bloom Dynamics

Aberdeen, UK
17–20 March 2003

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

Palægade 2–4 DK–1261 Copenhagen K Denmark

TABLE OF CONTENTS

1 WELCOME AND OPENING OF THE MEETING

The ICES-IOC Working Group of Harmful Algal Bloom Dynamics (WGHABD) meeting was hosted by Fisheries Research Services Marine Laboratory (FRS), Aberdeen, Scotland from 17–20 March 2003. 29 scientists from 17 countries participated. The list of participants is presented in Annex 1. The meeting agenda is presented in Annex 2.

The meeting was opened by Dr. Colin Moffat from FRS on 17 March 2003 and the participants were introduced with respect to their names, institute, national affiliation and fields of expertise. The agenda was agreed and Eileen Bresnan and Henrik Enevoldsen elected as rapporteurs.

Being a joint ICES-IOC working group, the IOC every year announces the possibility for its Member States outside the ICES area to attend the WGHABD. Most years these participants are supported financially. In 2003 the IOC received applications to participate from Iran, Nigeria, New Zealand, Philippines, and Uruguay. The applicants' CV's were reviewed by the IOC Secretariat, the WGHABD Chair and the Chair of the IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB). Participation and financial support was offered to the applicants from the Philippines and New Zealand. Due to problems with obtaining a visa and flight bookings, Prof. Rhodora Azanza (from the Philippines) was unable to attend the meeting. Patrick Holland attended representing New Zealand.

The chair outlined the comments and review from the ICES Oceanography Committee relating to the WGHABD report from the 2002 meeting. The committee felt the report was well organized and encouraged the WG to publish their comprehensive reviews on HABs for the broader scientific community and to maintain the HAB database. The proposed Workshop on Real-Time Observations (WKHABWATCH, Villefranche, France in June 2003) designed to provide observational and modelling experience was encouraged. They made reference to the lower attendance than normal (13) and the extensive (ten) Terms of Reference. It was suggested the WG address their plans for the large datasets that they have collated.

The Committee noted that this Group was originally established to consider HAB dynamics. While concerned with HABs, the WG was not addressing the dynamics as extensively as previously. Nevertheless, some members of the Committee felt that this Working Group should further address the issue of HAB dynamics. The Terms of Reference for 2003 were reviewed and adopted.

2 TERMS OF REFERENCE

At the 90th Statutory Meeting (2002), Copenhagen, Denmark the council approved the WGHABD (2002) terms of reference (C. Res. 2C07).

The ICES-IOC Working Group on Harmful Algal Bloom Dynamics [WGHABD] (Chair: J. L. Martin, Canada) will meet in Aberdeen, UK from 17-20 March 2003 to:

a) compare and assess historical and retrospective data sets on phycotoxins in shellfish, related to phytoplankton abundance and phytoplankton community structure with reference to HAB population dynamics;
b) review the reports and products of recent workshops on molecular probe technology, and the development of technologies of direct use in studies of field populations of HAB species, with special attention to novel approaches that were not considered in the 2002 meeting;
c) evaluate the outcome of the Workshop: "Contrasting approaches to understanding eutrophication effects on phytoplankton" (CM2002/C:05) from a HAB perspective;
d) review effects of HABs on survival and fecundity of wild fish, and the relationship (if any) to recruitment;
e) prepare a resolution for a workshop on "New and classic techniques for the determination of numerical abundance and biovolume of HABspecies – evaluation of the cost, time-efficiency and intercalibration methods";
f) evaluate the usefulness and feasibility of creating HAEDAT maps directly from the HAEDAT-database;
g) review the application of methods for the detection and quantification of phycotoxins in eukaryotic microalgae and cyanobacteria, and related components of pelagic food webs, in coastal marine and brackish waters of the ICES area;
h) review the previous submissions to HAEDAT with a view to improving the accuracy of the information and increasing the utility of the database;
i) report on the ECOHAB-EUROHAB Workshop on joint research on HABs;
j) consider the potential of ITIS as a common taxonomic system within ICES.

3 SUMMARY AND CONCLUSIONS

The group discussed the name of the group – WGHABD and the form, objectives and functioning of the group. It was noted that this group is special in that it brings biologists, chemists and physicists together, although there is never a very large contingent of physicists and/or modellers.

Techniques for analysis and prediction of the population dynamics of HABs are not well developed and measures of species-specific growth rates and mortality rates are very difficult. Monitoring is an important aspect of HAB research and the WG needs to interact with monitoring programme designs and data interpretation. For example, more environmental data is often needed and sampling should be rationalised with local hydrography such as mixed layer depth, circulation patterns, frontal dynamics, etc. Historical data and time series data from sediment and climate studies are important in looking for historical occurrences of HABs. Increase and decrease in population size is important to bloom dynamics.

The importance of the WG approach and focus on population dynamics of specific HAB species and not on phytoplankton ecology in general was emphasized. The economic, resource and environmental effects of HABs are included within the WGHABD. In addition, often phytoplankton ecology models are usually biomass, nutrient, and carbon cycling and in many cases, cannot define, explain or predict HAB dynamics. In the past we have had joint meetings with SSO and modellers to try and incorporate physics and HAB dynamics into the models.

The WG felt that the existing ToR were related and important to dynamics.

Term of reference a: Compare and assess historical and retrospective datasets on phycotoxins in shellfish, related to phytoplankton abundance, and phytoplankton community structure with reference to HAB population dynamics

Three presentations were made on this topic.

Jennifer Martin (Canada) made a presentation on the analysis of a phytoplankton community data set (with particular reference to *Alexandrium* populations) from the Bay of Fundy using PRIMER-E. A preliminary descriptive summary of the long-term shellfish toxicity dataset since 1944 was presented.

Bengt Karlson (Sweden) presented the results from preliminary analysis of a dataset containing diarrhetic shellfish toxin data in the blue mussel (*Mytilus edulis*) in Sweden from 1988–2002. This analysis has highlighted large interannual and geographic variations in DST values that have been recorded.

Einar Dahl (Norway) detailed the dataset collected at the Institute of Marine Research, Norway. It contains, as well as extensive physical and chemical measurements, extensive studies of a *Gyrodinium aureolum* (*Karenia mikimotoi*) bloom that occurred in 1981, a *Chrysochromulina polyepsis* bloom that occurred in 1988 and *Chatonella* spp. blooms that occurred in 1998 and 2001. Analysis of historical deep water oxygen data showed a shift in levels during the 1970s possibly due to increased sedimentation which may in turn reflect a change in the phytoplankton species composition.

The WG determined that there were a number of datasets and they were becoming more long term and in the future there should be comparisons of analyses and between datasets.

Term of reference b: Review the reports and products of recent workshops on molecular probe technology, and the development of technologies of direct use in studies of field populations of HAB species, with special attention to novel approaches that were not considered in the 2002 meeting.

Caroline Cusack (Ireland) reported on the workshops 'Molecular Probe Technology for the detection of Harmful Algae', Galway, Ireland 20–24 May 2002 and 'Analysis of Single Cells in the Marine Phytoplankton' Alfred Wegner Institute (AWI) Germany, 15–21 April 2002.

The Workshop 'Molecular Probe Technology for the detection of Harmful Algae' in Galway, Ireland 20 – 24 May 2002 was endorsed by GEOHAB and attended by 50 scientists from 18 countries. A number of topics were covered including: real-time PCR, fluorescent whole cell oligonucleotide probes using fluorescent microscopy, cells homogenate assays, sandwich hybridization techniques and hand held microchip readers. Discussions carried out during the workshop highlighted the demand for inter-laboratory calibration trials using this probe technology. A CD of the presentations given at this workshop has been produced and distributed to the participants.

The workshop 'Analysis of single cells in the marine phytoplankton' in Germany, 15–21 April contained many presentations from two European projects; AIMS (Automated Identification of Microbial Subpopulations) and PICODIV (Monitoring the diversity of photosynthetic picoplankton in marine waters. This workshop focused on practical techniques using flow cytometery, artificial neural networks and molecular probes. The meeting report from this workshop has been published in the journal Protist (2002, Vol. 153, pp. 193 – 195).

Patrick Holland (New Zealand) reported on the use of DNA Probe Technology in the monitoring programme in New Zealand.

Whole cell DNA probes have been introduced to the monitoring programme in New Zealand following extensive trials and validation studies. Probes for seven species of *Pseudo-nitzschia* species and four toxic *Karenia* species (*K. mikimotoi, K. papilionacae, K. brevisculata* and *K. selliformis*) are routinely used. The protocol for the use of these probes has been optimised and all assays are conducted under strict quality controlled conditions. The need for on-going quality control and validation of such techniques was highlighted.

Term of reference c: Evaluate the outcome of the Workshop: "Contrasting approaches to understanding eutrophication effects on phytoplankton" (CM2002/C:05) from a HAB perspective.

Einar Dahl (Norway) reported on the Workshop: "Contrasting approaches to understanding eutrophication effects on phytoplankton" (CM2002/C:05) from a HAB perspective. This workshop was found to be well focussed and the final report produced is a useful document summarising the current state of knowledge about this subject. The workshop was comprised of four thematic sessions i) case histories, ii) driving forces, iii) physiology and nutrients iv) mass balance and modelling. The final plenary session focused on the current knowledge relating to eutrophication and phytoplankton responses. Four topics were addressed in this session, including the effects of anthropogenic nutrient supply on coastal waters, the response of phytoplankton to eutrophication, the potential to model this response and what knowledge is needed to quantify the response of nutrient enrichment.

This workshop was found to be well focussed and the final report produced is a useful document summarizing the current state of knowledge about this subject. The group noted that the outcome of the workshop documents a change in the way we discuss euthrophication and its effects on phytoplankton, although some older and too simplistic views such as regarding flagellates as indicators of eutrophication, still occur. In this context the WGHABD recommended that WGPE consider adding to its future terms of reference whether results from mesocosms can be extrapolated to in-situ. The WGHABD regretted that only one member of WGHABD was invited.

Term of reference d: Review effects of HABs on survival and fecundity of wild fish, and the relationship (if any) to recruitment.

Markku Viitasalo presented results from the Baltic where the effects of cyanobacteria and their toxins on biota were examined. It was determined that: certain copepod species can produce eggs during bloom decline; cyanobacterial filaments interfere with predation efficiency in crustaceans; nodularin is transferred through the food chain; and fish larvae tend to have slower growth rates when exposed to cyanobacteria.

Maija Balode highlighted research with *Microcystis aeruginosa, Nodularia spumigena* and zooplankton in the Baltic where zooplankton egg production and survival of egg-carrying copepods decreased with increased toxicity in the food source. In herring, the early stages of development appeared to be impacted.

Term of reference e: Prepare a resolution for a workshop on "New and classic techniques for the determination of numerical abundance and biovolume of HAB-species – evaluation of the cost, time-efficiency and intercalibration methods".

The WGHABD discussed the concepts behind this workshop at length. Currently most HAB monitoring progammes use classical microscope methodology to enumerate the harmful species present. These comprise of a variety of different techniques depending on how many cells are present and these methods require standardization. Current developments in whole cell and sandwich hybridisation molecular probe technology show great potential in being applied in HAB monitoring programmes. However a number of problems have been encountered using these probes on field material and the variability of their effectiveness with different cell nutritional states was highlighted. The WG agreed that an intercalibration workshop was needed to examine these methods further. The preferred forum for this workshop would be to combine it with a planned 'Classical techniques' workshop, described by Bengt Karlson (Sweden). The main objective of this combined workshop would be to compare traditional microscope methods for measuring phytoplankton abundance and to also compare them with molecular probe technologies. A reference

counting method would be identified and inaccuracies or deficiencies exposed would be targeted for future research. Potential members for a Steering committee were identified and a provisional planning time scale discussed.

Term of reference f: Evaluate the usefulness and feasibility of creating HAEDAT maps directly from the HAEDAT-database was combined with **Term of reference h:** Review the previous submissions to HAEDAT with a view to improving the accuracy of the information and increasing the utility of the database.

These ToRs were combined as the feasibility of creating HAEDAT maps directly from the HAEDAT database was directly related to the accuracy of the information entered.

Catherine Belin (France) and Monica Lion (Spain) both reported on this combined topic. A major point that was highlighted was that the regions used on the HAEDAT maps need to be defined and these same regions should also be used on the HAEDAT forms. Then, software could be used to automatically create the maps from the database. After some discussion by the WGHABD, Monica Lion agreed to print out existing forms that have been entered in the database and send them to the delegates. The delegates would divide their countries into regions and amend the forms so that the position of the event would observe these regional divisions. The amended maps along with a definition of the regions would then be returned to Monica Lion so the HAEDAT database could be updated.

Term of reference g: Review the application of methods for the detection and quantification of phycotoxins in eukaryotic microalgae and cyanobacteria, and related components of pelagic food webs, in coastal marine and brackish waters of the ICES area.

Presentations were made by Patrick Holland (New Zealand) on the use of LC-MS in the New Zealand Monitoring Programme and Bernd Lukas (Germany) on an LC-ESI-MS method for the simultaneous determination of algal and cyanobacterial toxins in phytoplankton.

LC-MS methodology has been adopted in New Zealand as an alternative method to the bioassay and an ASP/DSP multi-toxin method has been developed that determines ASP and 14 other lipophillic toxins. The New Zealand Food Safety Authority (NZFSA) has approved this method and it has been in routine use for 15 months. The introduction of new LC-MS methods to regulatory testing highlights a number of issues. i) harmonisation of these techniques between laboratories is required. ii) interlaboratory studies for the development of new methods is recommended and iii) the need for approved standard material is again highlighted if these methods are to be used in regulatory programmes.

A Liquid-Chromatography-Electro-Spray – Ionisation-MassSpectrometry (LC-ESI-MS) technique has been developed for the simultaneous determination of various algal and cyanobacterial toxins. This method allows the quantification of a number of toxins such as saxitoxin, anatoxin – A, domoic acid, nodularin, microcystins, okadaic acid and DTX-1 with a single chromatographic run. This method has been tested during a number of research cruises in the Baltic Sea.

Term of reference i: Report on the ECOHAB-EUROHAB Workshop on joint research on HABs.

Don Anderson (USA.) presented a report on this workshop. For the first time joint research from Europe and the USA is being proposed to address the problems of HABs. This has great potential as skills from both Europe and the U.S.A. can be combined and also different comparable systems in both areas can be studied. Financial support from Europe will come from the EU while in the USA it will come from the National Science Foundation (NSF). This initiative was launched at the workshop in Trieste and has been endorsed by GEOHAB. The funding for the EU-US programme is not finalized at this time although further developments are expected in 2003.

Term of reference j: Consider the potential of ITIS as a common taxonomic system within ICES.

The WGHABD discussed this website at length and noted that ITIS does not reflect recent taxonomic changes in classification. Concerns were raised about the maintenance of this taxonomic system. Another concern is that ITIS is a North American System and its' compatibility with the European register of Marine Organisms ICES reference list of phytoplankton (by WGPE) etc is not known. The WGHABD is aware that ITIS is a partner in the global biodiversity information facility (GBIF) but it was not clear is this implies endorsement of GBIF of this ITIS code.

In conclusion, the WGHABD felt the ITIS system to be not well developed and a number of clarifications must be made by ICES if they are to adopt it as their reference system.

4 HISTORICAL DATA SETS

Term of reference a: to compare and assess historical and retrospective data sets on phycotoxins in shellfish, related to phytoplankton abundance, and phytoplankton community structure with reference to HAB population dynamics;

Alexandrium fundyense and temporal and spatial variations in phytoplankton populations, eastern Canada

Jennifer Martin (Canada) presented results (from collaborative work with Fred Page) on analyses of the Bay of Fundy phytoplankton dataset. The spatial and temporal variation in abundance of *Alexandrium fundyense* in relation to the spatial and temporal variation in phytoplankton community structure at monitoring stations within the Bay of Fundy was examined through a multi-dimensional scaling ordination analysis conducted on data collected in 1991. The results indicated a strong seasonality in both the abundance of *A. fundyense* and in the degree of spatial similarity in the community structure. *A. fundyense* was abundant at only a few of the sampling stations and was most abundant during July. The temporal distribution of *A. fundyense* may therefore be related to the seasonality in the phytoplankton community or its correlates, such as the environmental conditions. Within a season, the spatial distribution of *A. fundyense* appeared to be independent of the phytoplankton community structure.

Dinophysis and DST in Sweden

Bengt Karlson (Sweden) reported on the preliminary analysis of a dataset containing information on the concentration of diarrhetic shellfish toxins (DST) in blue mussels (*Mytilus edulis*) from the Swedish West Coast (1988-2002). This large data set shows a strong seasonal variation in DST concentrations. A typical year has low DST-values from March to mid July when the concentrations start to rise. During autumn high concentrations are common and may remain until spring. However, in some areas DST-concentrations are low also when high values are found in other areas. The interannual variation may be quite large. In 1995 almost 5000 µg/kg was detected while in 1998 the maximum was ca 200 µg/kg. Also geographical variation may be substantial. In the Koljö fjord a large increase in DST-concentrations starting in 1998 was observed. Some of the results presented have been published in the 'Swedish National Report on Eutrophication Status in the Kattegat and the Skagerrak', OSPAR Assessment 2002.

Historical and long-term data of relevance to harmful algal blooms at the Institute of Marine Research, Flødevigen Marine Research Station

Einar Dahl (Norway) reported on long term monitoring at the IMR, Norway. For more than 70 years oxygen has been measured annually in September and October at fixed stations along the Skagerrak coast. The stations are located in fjords, skerries and the open coast and many are sub-sea basins. Analysis of this dataset has shown that a change in oxygen levels occurred in the mid 1970s. The change is thought to be due to increased sedimentation because of a shift in phytoplankton species composition.

Nutrients have been measured monthly together with chlorophyll *a* along the Arendal-Hirtshals transect in the Skagerrak since 1980, and roughly fortnightly in the coastal current outside Arendal since 1990. Chlorophyll *a* and phytoplankton in the 0-3 m depth, with emphasis on particular HABs, has been monitored consistently and frequently in the Flødevigen Bay outside Arendal since 1989, and less regularly from 1982–1989.

In 1981 a large bloom of *Gyrodinium aureolum* (*Karenia mikimotoi*) was extensively studied during its maxima. Later, other blooms of this particular species as well as other harmful species were extensively documented, i.e. *Chrysochromulina polylepis* (1988), *Chattonella* sp. (1998 and 2001).

Group discussion that followed indicated the importance in analyses of the long term datasets in determining patterns and trends in HAB populations and will ultimately aid in the future prediction of blooms. A comparison between analyses and different regions should be undertaken in the future.

5 MOLECULAR PROBES

Term of reference b: Review the reports and products of recent workshops on molecular probe technology, and the development of technologies of direct use in studies of field populations of HAB species, with special attention to novel approaches that were not considered in the 2002 meeting

Caroline Cusack (Ireland) reviewed recent Workshops held in Ireland and Germany and Patrick Holland reported on progress in DNA probes in New Zealand.

At the "Microalgal Gene Probe Workshop", convened by Chris Scholin at MBARI (March 2001) it was recognised that there was a need for periodical meetings to review molecular probe technologies in use by researchers worldwide. It was proposed that a workshop should be organised the following year to discuss new findings and consider how studies might develop. Lesley Rhodes (Cawthron Institute, New Zealand) put forward a suggestion that a website would a useful means for probe developers and users to deposit and retrieve information on the subject of molecular probes technologies for the detection of potentially toxic and harmful phytoplankton species. The website (www.geneprobes.org) was set-up the following year.

The next workshop, "Molecular Probe Technology for the detection of Harmful Algae" was endorsed by GEOHAB and jointly convened by the Irish Marine Institute (MI) and the National University of Ireland, Galway (NUIG). Fifty scientists from 18 countries attended the meeting at the Martin Ryan Institute, NUIG, (20–24 May 2002), forty of which took part in the practical demonstrations. Six keynote addresses, 14 oral presentations, 7 practical demonstrations, 7 roundtable discussions (participation of all attending) and 8 poster presentations were made. The main objective of the workshop was to demonstrate a variety of techniques that used molecular probes to detect and enumerate target HAB species.

These included:

1) Real Time-PCR amplification assay (using quantification in real-time) demonstrated by Holly Bowers, University of Maryland at Baltimore, USA;
2) A fluorescently labelled oligonucleotide probe-based whole cell hybridisation assay using fluorescent microscopy developed by Chris Scholin and co-workers from Monterey Bay Aquarium Research Institute (MBARI);
3) Cell homogenate assays
    - sandwich hybridisation assay employing colourimetric detection (Saigene Corporation, USA and their colleagues from MBARI)
    - hand-held DNA microchip reader coupling electrochemical detection, and sandwich hybridisation (Linda Medlin, Alfred Wegener Institute, Germany).

Other demonstrations and tutorials included useful information on culturing of microalgae (Santiago Fraga, Instituto Español de Oceanografía, Spain), Denaturant Gel Electrophoresis (Siobhán Kavanagh, NUI, Galway, Ireland), Bioinformatics (Donal Eardly, NUI, Galway, Ireland) while other participants presented their latest findings in the field. Further information on the workshop including presentations and the workshop booklet can be requested through the www.geneprobes.org website.

Discussions carried out during the workshop highlighted the demand for inter-laboratory calibration trials of the assays developed with a view to the provision of validation and certification of assays for monitoring. Sampling protocols need to be standardized. Further research is needed to understand how the physiological state of the cells affects the results obtained in cell homogenate assays. Once the question of reproducibility of the existing methods is resolved it is very important that the commercial assays are available to the users.

The provisional date, 22–26 March 2004 for the next Workshop on Molecular Probes ("GENE-HAB") has been set and will be convened by Arturo Sierra-Beltrán, México.

The "Analysis of Single Cells in the Marine Phytoplankton" (ASCMAP) workshop organised by Linda Medlin, Klaus Valentin and René Groben took place at the Alfred Wegener Institute (AWI), Germany from 15–21 April. An international participation of 90 scientists (more then 12 countries) attended with up to 40 talks and 40 posters presentations. Many of the presentations reported results from two European projects; AIMS (Automated Identification of Microbial Subpopulations) and PICODIV (Monitoring the diversity of photosynthetic picoplankton in marine waters). The workshop focused on Flow Cytometry (analyses of single cells using optical properties, characterisation of phytoplankton cells by measuring optical properties), Artificial Neural Networks (computer programes modelled on the operation of the brain and attempt to solve problems difficult or impossible by conventional methods e.g. computer generated recognition systems) and Molecular Probes (whole cell and sandwich hybridisation based methods). Experts in these fields encouraged the participants to take part in hands-on testing of the three techniques covered at the workshop. The meeting report of this workshop has been published in the journal, Protist (Vol 153, pp. 193–195, September 2002 http://www.urbanfishcher.de/journals/protist)

DNA probes in New Zealand

DNA-probes have been introduced to the New Zealand Marine Biotoxin Management Programme (NZMBMP) and are administered by the NZ Food Safety Authority to improve the robustness of HAB identifications. Phytoplankton monitoring for toxic algae constitutes the first tier of monitoring and functions to give an early warning of potential blooms. The Cawthron Institute Phytoplankton Laboratory analyses water samples from up to 250 representational sites around the coast of N.Z. Risks associated with toxic species are defined and conservative trigger levels are used to initiate flesh testing, with regulatory decisions being based on the flesh test results. Routine analysis of samples employs the Utermöhl method of marine phytoplankton counting. A 10 ml sample of preserved, integrated seawater is analysed using an inverted light microscope. All toxic species are recorded, along with all dinoflagellates, raphidophytes and the dominant diatom species. Calcofluor staining is used to aid identification of *Alexandrium* species. A suite of DNA probes has been developed for *Pseudo-nitzschia* spp. and *Karenia* spp. to confirm the species composition for blooms above the trigger levels. The collaboration and advice of Dr. Chris Scholin (MBARI, California) is gratefully acknowledged.

The introduction of whole cell DNA probes into regulatory monitoring has followed an extensive programme of research, development and validation. For *Pseudo-nitzschia* spp., specific probes are used for *P.australis* and 6 other potentially toxic species. The probes were selected for low cross-reactivity and validated against SEM on frustules from extensive algal selections. The fixation and probe incubation conditions were also optimized. The fluorescent cell counts are expressed as percentages of a Universal probe. For *Karenia*, specific probes have been selected for the four toxic species of most significance for NZ: *K. mikimotoi, K. papilionacae, K. brevisculata* and *K. selliformis*. Lugol treated samples are used with decolourisation to avoid low probe counts due to disintegration of the fragile *Karenia* cells. Cross-reactivities were established as low under optimal assay conditions. Routine use of the probe assays is conducted under conditions of close quality control using fully documented protocols. Accreditation (ISO17025) for the probe protocols and laboratory staff has followed internal and external audits. The necessity is highlighted for a rigorous approach to validation, approval and on-going quality control if new technologies such as DNA-probes are to provide reliable and credible data for phytoplankton within regulatory programmes.

The WG noted the rapid advances in technology in the past year and advances anticipated in the near future with plans for an upcoming Workshop in Mexico. Concern was expressed with the use of the new technologies and the need for intercalibration studies with existing methods such as microscopic techniques (Section 8) and the recommendation by IPHAB.

6 REVIEW OF THE WORKSHOP 'CONTRASTING APPROACHES TO UNDERSTANDING EUTROPHICATION EFFECTS ON PHYTOPLANKTON'

Term of reference c: Evaluate the outcome of the Workshop: "Contrasting approaches to understanding eutrophication effects on phytoplankton" (CM2002/C:05) from a HAB perspective

Reported by Einar Dahl (Norway).

The workshop in Den Hague attracted a wide expertise with a total of 45 scientists from 17 different countries. Most participants came from European countries, and participants from China, Korea, Japan and United States also attended. The Workshop was held just after the ICES-IOC Working Group on Harmful Algal Bloom Dynamics in Bermuda 7-10 March 2002, which made it difficult to participate fully in both meetings.

The aim of the workshop was to provide new perspectives into the effects of altered nutrification on coastal phytoplankton communities and into investigative procedures. A total of 33 oral presentations were made in four thematic sessions;

i) Case Histories; where experiences from field studies and monitoring of phytoplankton in many different areas around the world were presented. The possible role of nutrients for primary productivity, occurrence of biomass and species composition was emphasized;
ii) Driving forces; which focused on coupling between climate variability and coastal marine eutrophication, and the role of freshwater inputs and variations in nutrient ratios;
iii) Physiology and Nutrients; more specific aspects of phytoplankton ecology and physiology, possibly related to the nutrients conditions were presented;
iv) Mass Balance and Modelling; different available models and their potential as tools in studies of phytoplankton ecology were examined.

A plenary session at the end of the workshop aimed to seek a consensus about the current knowledge on the relationship between nutrification of coastal waters and phytoplankton responses and the research needed to quantify this relationship. To structure this session the participants were invited to prioritize 3 out of 10 topics presented for detailed discussion. All topics listed were very relevant to the possible effects linked to eutrophication, and could also be potential terms of reference for future ICES-Working Groups dealing with nutrient and phytoplankton/HAB dynamics. After much discussion the number of topics were increased to four. The first topic asked if there was evidence that anthropogenic nutrient supply of coastal waters can alter phytoplankton dynamics and/or communities. The consensus was that this was generally the case, and it was clearly demonstrated that eutrophication leads to increases in biomass and primary productivity. There were, however, fewer and more mixed responses in the discussion on to determining if nutrification might influence species composition in a way that can be highlighted by "indicator-species" of eutrophication. The second topic discussed was the generic level of the response e.g., extrapolatable as first principles, or primarily site-specific consequences of narrower interest? The common answer was that results of nutrification on phytoplankton species level is often site-specific, while effects on biomass and functional groups may be more universal. The third topic discussed was the potential to create reliable models to predict the effects of nutrification on phytoplankton mass balance, species-specific and functional group behaviour. It was concluded that presently the models of mass balance type are available first and foremost and useful to managers. Finally the participants were asked the question; what knowledge and technical gaps are impeding progress towards quantification of nutrient enrichment impacts on phytoplankton? Nine needs were recognized which form a starting point for further progress in this complicated field of nutrification effects on phytoplankton within the ICES-community. The practical and theoretical experiences gained from this trans-disciplinary workshop on eutrophication, were positive and can also be applied to the organization of similar workshops in the future.

The workshop report (ICES CM 2002/C:05) is available at the ICES website. Most of the contributions presented during the workshop are expected to be published in a forthcoming special issue of the Journal of Sea Research.

The WG discussed the document and summary as submitted to ICES. All participants felt that the Workshop was worthwhile and provided a good summary of research to date and where future research should concentrate. The WG agreed with the conclusions from the Workshop and felt that the WGPE should continue to focus on issues as such relating to the whole phytoplankton community. It was emphasized that eutrophication and over-use of mesocosms cannot be used as a global explanation for the occurrence or increase in HABs. The final report produced is a useful document summarizing the current state of knowledge about this subject. The group noted that the outcome of the workshop documents a change in the way we discuss euthrophication and its effects on phytoplankton, although some older and too simplistic views such as regarding flagellates as indicators of eutrophication, still occur. In this context the WGHABD recommended that WGPE consider adding to its future terms of reference whether results from mesocosms can be extrapolated to in-situ. The WGHABD regretted that only one member of WGHABD was invited.

7 EFFECTS OF HABS ON SURVIVAL AND FECUNDITY OF WILD FISH

Term of reference d: Review effects of HABs on survival and fecundity of wild fish, and the relationship (if any) to recruitment.

Reported by Markku Viitasalo (Finland) and Maija Balode, (Latvia).

Cyanobacteria ecosystem studies in the Baltic Sea

Markku Viitasalo

The zooplankton research group of Finnish Institute of Marine Research and University of Helsinki has recently investigated the effects of cyanobacteria and their toxins on the Baltic Sea biota.

Highlights of recent results include: (i) a large part of the cyanobacteria blooms do not sediment but decay in the water column, thus fuelling the microbial loop; (ii) certain copepod species produce eggs efficiently within the decaying bloom, despite the large amount of nodularin in water, (iii) cyanobacteria filaments interfere with the predation efficiency of crustacean predators, such as mysid shrimps on zooplankton; (iv) nodularin is transferred from cyanobacteria to fish through zooplankton, even when the fish were not exposed to cyanobacteria; and (v) fish larvae grow slower when feeding on zooplankton exposed to cyanobacteria toxins than when feeding on "toxin-free" zooplankton.

Future efforts will concentrate on verifying the laboratory results under field conditions. Emphasis will be put on determining the distribution and feeding rates of pelagic fish during cyanobacteria mass occurrences. For the first time

in the Baltic Sea, multiship surveys will be conducted to reveal the distribution of zooplankton and fish in relation to stratification, upwelling areas and cyanobacteria blooms.

Cyanobacteria and zoplankton

Maija Balode

Studies on the effect of cyanobacterial strains *Microcystis aeruginosa* (MAGR – 2 and PCC – 7820) and *Nodularia spumigena* (NSGR - 2) on dominant Baltic zooplankton species show a negative effect of cyanobacteria on egg production and survival of the egg-carrying copepod *Eurytemora affinis* and the free-spawning copepod *Acartia bifilosa*. The survival of copepods decreased with increasing toxicity of the food source, which suggested that the toxic effect is dependent on the toxin content in the tested algal strains. In the presence of cyanobacterial strains a difference in the survival of the dominant copepod species was observed. *A. bifilosa* showed higher mortality in comparison with *E. affinis*. *A. bifilosa* was more sensitive in the presence of *Microcystis* compared with *Nodularia*. Toxic resistance of copepods depended on the sex of the animals. The negative influence of cyanobacteria on the males was considerably higher than on the females. *M. aeruginosa* PCC-7820 had the most serious negative impact on the survival of *E. affinis.*

There was a difference in the egg production between egg-carrying (*E. affinis*) and free-spawning (*A. bifilosa*) copepod species. The presence of potentially toxic cyanobacteria caused a decrease in egg production in *E. affinis*. In the presence of *Nodularia* the development time of egg sacs was longer than in the control, but in the presence of the Baltic *Microcystis* strain MAGR – 2, no new egg sacs were produced (egg production of *E. affinis* was not detected in the presence of *M. aeruginosa* PCC - 7820 due to the high mortality of copepods). No significant effect of the toxic algae on egg production in *Acartia bifilosa* was observed. High production of eggs in the presence of *N. spumigena* and *M. aeroginosa* PCC-7820 (150% and 142% of the control, respectively) could be explained by stress conditions for *A. bifilosa.* The relatively high egg production of *Acartia bifilosa* that were fed *Nodularia* and the Brazilian *Microcystis* might be explained by the transfer of *Acartia* from optimal growth conditions to an inadequate, low-quality diet. Stress conditions also could cause considerable variability in egg production among the tested individuals.

Studies on the effects of cyanobacterial strains on embryonic and larval development of Baltic spring spawning herring (*Clupea harengus membras*) showed that the greatest negative impact was on the early stages of development. In the embryonic stages the influence of strains resulted in an increase in deviation from the normal pattern of development and a higher mortality. The impact of cyanobacterial strains upon the larval development of herring was moderate.

8 INTERCALIBRATION WORKSHOP

Term of reference e: Prepare a resolution for a workshop on "New and classic techniques for the determination of numerical abundance and biovolume of HAB-species – evaluation of the cost, time-efficiency and intercalibration methods".

The topics, potential participants, practical organization and possible publication of the workshop were discussed thoroughly by all the participants at the working group. The WGHABD took into consideration Recommendation IPHAB-VI.4 (Annex VI hereto) of the IOC Intergovernmental Panel on HAB which requests the WGHABD to consider the coordination of this workshop with the third international workshop on molecular probes.

Background

Currently almost all HAB-monitoring for aquaculture is performed using classical techniques for determining abundance and biomass of HAB-species. Also, most studies of HAB dynamics use these techniques. New probe-based techniques show great potential for studying HAB dynamics with much higher resolution in time and space than available previously, making it possible to understand biological processes leading to HAB events in detail. However, the validation of the new techniques in the field is limited.

The classic microscope based techniques are not standardised. Developments using filtering and centrifuging for fast sample throughput need to be intercalibrated with sedimentation chamber techniques. One example is the Calcofluor staining technique that is extensively used by some institutes for the identification of thecate harmful dinoflagellates. The problem of determining abundance of HAB-species that occur in low cell densities and still render shellfish toxic needs to be revisited. Also developments in computer aided microscopy for determining biovolume need to be intercalibrated with manual methods.

The HAB scientific community has been working towards the development of species- or strain-specific "probes" that can be used to label only the cells of interest so they can then be detected visually, electronically, or chemically. Progress has been rapid and probes of several different types are now available for many of the harmful algae, along with techniques for their application in the rapid and accurate identification, enumeration, and isolation of individual species. Reviews of these methods are available in Anderson (1995), Scholin and Anderson (1998), Anderson *et al.,* (2001) and in a new chapter (Scholin *et al.*, in press) in the second edition of the Manual for Harmful Marine Microalgae (Hallegraeff *et al.,* in press).

With respect to applications on HABs in natural waters, the sandwich hybridization assay (SHA), as well as "whole" or intact cell assays using rRNA probes, have been used in field trials in several areas of the world, including both the east and west coasts of the U.S. (C. Scholin and D. M. Anderson, unpub. data), off the coast of Scotland (John *et al.*, 2002), and in several countries where *Pseudo-nitzschia* species cause ASP toxicity (C. Scholin, unpub. data). The most extensive field applications of PCR-based molecular probe technologies to HAB species are probably in the monitoring for *Pfiesteria piscicida* and other *Pfiesteria*-like species in the southeastern US.

One problem area has arisen with the application of both whole cell and SHA technologies to field populations – namely the agreement between cell counts made with different methods. For example, *A. fundyense* counts using an rRNA probe in the whole-cell format agreed to a variable extent with SHA analyses of the same samples from the Gulf of Maine (D. Anderson, unpub. data). At some stations and at some depths, agreement was excellent between the two methods, but for others, the SHA counts were 2 to 20X higher than the manual counts. It is possible that this discrepancy is due to grazing, perhaps resulting in the incorporation of *A. fundyense* cells and/or rRNA in fecal pellets or other detritus that was detected by the SHA, but not by the whole-cell method. Laboratory experiments, however, have not supported this hypothesis, so the reason for the discrepancy remains unknown.

In a similar manner, Allan Cembella reported to the ICES-IOC WG meeting (2002) on studies of *Alexandrium* populations off the coast of Scotland in which bright-field microscope counts of Utermöhl samples were consistently higher than whole-cell counts using species-specific oligonucleotide probes (John *et al.,* 2002). Here again, the discrepancies are significant – an order of magnitude or more. In this case, the differences are between probe-based, whole-cell counts and standard microscope counts, whereas in the Gulf of Maine data cited above, the differences were between the whole-cell probe approach and the SHA.

It is important to recognize the fundamental differences between these the different assay types that have been developed. For example, a successful whole-cell assay requires detection of molecules inside intact, recognizable cells and those molecules must: a) be accessible to the probe, and b) be of sufficient quantity to visualize that cell above background. Furthermore, the target cell must survive treatment from sample collection through processing and be visible to be counted. Results of a whole-cell assay are thus operationally defined – even if a target cell is present it may not always be detected with this approach. Furthermore, anything that causes cells to clump or otherwise be hidden (e.g., large quantities of particulate organic matter, fecal pellets) will affect results of a whole-cell assay, as shown for *Pseudo-nitzschia* by (Scholin *et al.*, 1999) and *Heterosigma* by Tyrrell *et al*. (2001). Similarly, results of cell homogenate assays are operationally defined. The basic concept of the SHA is to detect molecules freed from particulate matter, analogous to detection of algal toxins or DNA sequences in phytoplankton samples. Target cells, or even remnants thereof, need only survive the initial collection step. Successful detection of the target molecule then depends on: a) extracting the target molecule, b) a sufficient quantity of the target molecule to elicit a positive reaction, and c) minimal interference (signal suppression/enhancement) from the sample matrix.

With these considerations in mind, several possible explanations for the observed discrepancies in cell count estimates can be offered. For the whole-cell approach, cellular uptake of the probe may vary (independent of rRNA concentration) due to permeability differences, such as those associated with life history transformations or nutritional condition. Temporary cysts, for example, are readily formed by *Alexandrium* species when subjected to sudden mechanical or environmental stress (Anderson and Wall, 1978). This could lead to weakly stained cells, and lower counts. Alternatively, cells may be more prone to lysis under certain physiological conditions, reducing the number of cells observed by the whole-cell assay. These differences might be enhanced by the different processing and preservation procedures followed for the different probe-based assay methods. In particular, the formalin/methanol fixation used in some whole-cell assay may lyse more cells relative to the liquid nitrogen typically used in SHA assays. The extent of lysis may vary depending on physiological condition of cells, so counting differences might be expected in this regard.

The nutritional, temperature and light history of the cells may also have a significant effect on the results of the different assay methods. This again might reflect cell permeability differences, or the accessibility or structural form of the target rRNA in whole cells versus a cell homogenate.

Clearly, more work is needed before probe-based cell counts can be accepted as an alternative to more traditional approaches. The differences between these probes and classical methods, and between different types of probe-based methods are significant and raise important questions about what actually should be counted, or is being counted, in research and monitoring programs focused on HAB species.

8.1 Need for an Intercalibration Workshop

The Working Group discussed the above issues, and generally agreed that an intercalibration workshop of some type was needed, given the need for accurate information on cell abundance is needed for research and modelling programs on HAB dynamics, as well as for general HAB monitoring activities. The general idea would not be to demonstrate technologies, or to rain others in their use, but to conduct rigorous intercalibration.

One possible approach would be to combine the WG activity with the planned 3rd International Workshop on Molecular Probe Technology, scheduled to be held in La Paz, Mexico in 2004 (referring to IPHAB Recommendation IPHAB-VI.4). That workshop, however, is intended to be a training effort, especially for HAB workers from Latin America. The possibility of adding a few days to that workshop in which the experts would conduct intercalibration exercises once the training participants had left was discussed, and this remains a viable possibility. A more favoured option, however, was to combine the Working Groups probe intercalibration exercises with another planned workshop described by B. Karlson, of Sweden. The main objective of that workshop is to compare extensively used traditional microscopic methods for estimating abundance and biomass of harmful algae in marine waters with novel techniques based on molecular probes, as well as to compare methods for concentrating and preserving phytoplankton. The goal is to produce scientifically based recommendations on choice of methodology for HAB-monitoring programmes in conjunction with e.g. aquaculture. Another deliverable will be step by step descriptions of methodology. “Classic” techniques to be compared include the Utermöhl method, Calcofluor stain-filter technique, freeze transfer technique, Palmer-Maloney chamber counts etc. The original idea was for a workshop of this type involving only Nordic countries, but the organizers agreed that by combining this workshop with one focused on probe-based counting methods, considerable synergism would result. In particular, common cultures could be used, as well as common plankton matrices. The WG concluded that flow cytometry and other flow-based systems would be evaluated at a later date to keep the workshop size small and manageable.

8.2 The workshop will have the following deliverables

Through laboratory exercises, presentations, and discussions:

1) A comparison of traditional methods for concentrating, preserving, and counting common HAB species using light microscope techniques;
2) A comparison of molecular probe-based methods for cell enumeration with the traditional techniques;
3) Recommendations for further research and development efforts targeted at identified inaccuracies or deficiencies in the methods being evaluated;
4) Identification, where possible, of a reference counting method against which other methods can be calibrated;
5) Assessment of the usefulness and cost efficiency of the available numerical methods in routine monitoring.

Fifteen months of preparation between approval and the workshop are required and it is recommended that the Steering Committee members meet within 4 months of the approval of the workshop to plan the workshop in full detail.

The scope and objectives of the workshop are consistent with the objectives of WGHABD and GEOHAB.

8.3 Publication of deliverables

Proceedings of the workshop will be published in one or more formats. An editorial committee will evaluate the feasibility of publishing a technical report with contributions on the principal topics. This option will be pursued if a strong commitment from a lead editor is obtained and adequate resources are secured.

The committee would also develop an alternate model of publication to be implemented in parallel or in place of the technical report, such as a web site, which would allow easy access.

8.4 Potential Convener:

Odd Lindahl, Kristineberg Biological Station, Sweden

8.5 Potential members of a steering committee:

Bengt Karlson, SMHI, Göteborg, Sweden

Don Anderson, WHOI, USA

Caroline Cusak, Marine Institute, Ireland

Odd Lindahl, Kristineberg Biological Station, Sweden

8.6 Potential members of editorial committee

Dr. xxxx, Chief Editor

8.7 The resolution

A workshop on new and classic techniques for the determination of numberical abundance and biovolume of HAB-species – evaluation of the cost, time-efficiency and intercalibrative methods will be held in xxxxxxxx, xxxxx, sponsored by ICES, IOC, GEOHAB, and EU with Dr. NN and Dr. NN as Conveners.

Priority:	ICES should take an active role in developing the implementation plan of the GEOHAB programme. The topic of intercalibration is relevant for GEOHAB and also fits well into ICES profile.
Scientific Justification:	Almost all HAB monitoring and dynamics studies are performed using classical techniques for determining abundance and biomass. New probe-based techniques show great potential for studying HAB dynamics and will make it possible to understand biological processes leading to HAB events. However, the validation of the new techniques is limited. Classic microscope techniques need to be compared with species and strain specific molecular probe methods as well as methods for preserving and concentrating phytoplankton. The goal is to produce scientifically based recommendations on choice of methodology for HAB-monitoring programmes.
Relation to Strategic Plan:	Implementation of the GEOHAB programme is relevant to the quantifying of human impacts on the on the marine ecosystem. The workshop will produce scientifically based recommendations on
Resource Requirements:	Conveners and lecturer's work time is required. Travelling and accommodation costs are needed for meeting participants. Laboratories, appropriate equipment and convenient access to coastal waters are required during the workshop. Technical support would be required for publication.
Participants:	Experts in relevant fields from around the world would be invited to participate.
Secretariat Facilities:	The Secretariat will be involved as normal in general professional and secretarial support, and the Secretariat should provide direct assistance during the workshop. The Secretariat might provide web space for the proceedings.

Financial:	Travelling support is needed for participants. Funds will be asked from IOC, EU, SCOR and other relevant organizations.
Linkages to Advisory Committees:	Harmful algal blooms are continuing issues in ACME.
Linkages to other Committees or Groups:	Support can be anticipated from the Baltic Committee, WGPE, SGGIB.
Linkages to Other Organizations:	GEOHAB is sponsored by IOC and SCOR.

9 HAEDAT MAPS AND FORMS

Term of reference f: Evaluate the usefulness and feasibility of creating HAEDAT maps directly from the HAEDAT-database combined with **Term of reference h:** Review the previous submissions to HAEDAT with a view to improving the accuracy of the information and increasing the utility of the database

These ToRs were combined as the feasibility of using the HAEDAT database to create the HAEDAT maps relies heavily on the accuracy of the information entered in the database.

The WGHABD evaluated the usefulness and feasibility of generating HAE-DAT maps from the ICES-IOC Harmful Algal Event Database HAEDAT.

It was agreed to clarify in the guidelines for submission of records to HAEDAT that cyanobacteria events in brackish-waters be included.

Monica Lion (IOC-IEO SCCHA, Spain) reported on the analysis of the differences between decadal maps and full HAEDAT records. While decadal maps represent on a separate map the information related to: PSP, DSP, ASP, NSP, CFP events, observation of animal or plant mortality, observation of other toxic effects such as cyanobacteria toxicity, the information collected by HAEDAT covers not only the associated syndrome but also the information on the nature of the event and the species affected, the location and dates of the events.

In HAEDAT the "Nature of the event information" includes descriptive information under the following headings: water discoloration, high phytoplankton concentration, seafood toxin, mass mortalities, foam/mucilage in the coast, and other. Information on the affected species is compiled in the following categories: humans, other terrestrial, birds, aquatic mammals, planktonic life, shellfish, benthic life, natural fish, aquaculture Fish and the syndrome such as PSP, DSP, ASP, NSP, AZP, CFP, cyanobacteria, or other. For each of these three categories it is possible to do independent searches.

The main problems in using the information available in HAEDAT to create maps directly through GIS software are the differences that exist in location information reported in the reports. In some reports just the name of the area and the number of the ICES region are indicated, whereas in other cases location information is given as latitude and longitude using the decimal system and in others this information is given in degrees and minutes.

It was decided to proceed with a system in which the HAEDAT input form is modified to include the location of an event in a predefined grid map. The grid for each area is to be defined as appropriate and practical as possible by each focal point. Any subareas may or may not correspond to existing ICES grids depending on which is best. These areas will be assigned a HAEDAT code and will serve to define the locations for the dots on the HAEDAT decadal maps.

This suggests that HAEDAT will provide the detailed information behind the decadal maps. In parallel HAEDAT will be modified so that maps can also be generated directly from the detailed data.

Monica Lion will provide copies of existing records from the HAEDAT database to all country representatives in order to assign these records a position in the new HAEDAT area numbering system as described above as well as improve the accuracy of the information. These modifications to the input data will improve data quality. All records are to be returned to Monica Lion prior to 1 May 2003.

10 ANALYTICAL METHODS

Term of reference g: Review the application of methods for the detection and quantification of phycotoxins in eukaryotic microalgae and cyanobacteria, and related components of pelagic food webs, in coastal marine and brackish waters of the ICES area

Reported by Patrick Holland (New Zealand) and Bernd Lukas (Germany)

Implementation of LC-MS methods in the New Zealand Marine Biotoxin Monitoring Programme

The New Zealand aquaculture industry and the Food Standards Authority (NZFSA) are committed to a new programme of marine biotoxin management in shellfish that will reduce the dependence on animal bioassays and increase the speed and quality of information flow without significantly increasing costs (Holland et al 2001). NZFSA has issued guidelines for the validation of new methods for use in shellfish monitoring and defined a process for gaining regulatory approval for their routine use. These procedures follow international best practice for performance-based methods. LC-MS has been chosen as the primary tool to meet this goal, although other analytical technologies are also under evaluation.

Cawthron Institute has developed and validated two new LC-MS methods. The ASP toxin method provides some advantages over the standard LC-UVD methods (Holland et al 2002) and an analysis of the uncertainty of measurement gave insights to factors influencing precision of LC-MS techniques (Holland et al 2003). Of greater significance is the ASP/DSP multi-toxin method that determines ASP toxins and 14 other lipophillic toxins and their metabolites in a single run. This scope is unprecedented and covers all toxins in the recent EC SANCO decisions establishing regulatory limits for shellfish. A thorough within-lab validation for four shellfish matrices establishing precision, accuracy and detection limits as well as some other issues such as extractability and robustness. This has been followed by an inter-laboratory sample exchange study involving 8 labs internationally that also demonstrated the adequate performance of the method when used with a variety of instruments. NZFSA has approved this method for regulatory testing of shellfish and its routine use over a period of 15 months has enabled further confidence in the reliability of the method.

Issues arising from introducing LC-MS methods to regulatory testing of toxins in seafood products, that also apply to other new analytical technologies, include:

i) International harmonisation is required on the criteria and procedures for acceptance of new methods. Although many bodies are formalizing procedures for food (Codex, EC, US-DA), the rigid application of strict fitness-for-purpose criteria for say veterinary drugs in meat, may not justified for natural toxins in seafood where the current 'reference' methods are widely acknowledged to be completely inadequate in relation to many key criteria.
ii) A related issue is the organization and funding of inter-laboratory studies for new methods. Such studies are acknowledged to the most credible means of establishing method suitability. However, studies designed to the AOAC/IUPAC criteria for full collaborative study require considerable resources and time. This is significant impediment to full adoption of new methods and some intermediate stages such as interim international approval are needed for the marine biotoxin field.
iii) Certified analytical standards and reference materials are essential for use of new performance-based methods in regulatory testing. Small quantities of isolated toxins have been circulated by research groups. These materials have been invaluable for establishing new methods and establishing some of their performance characteristics. However, they do not meet the needs for regulatory testing because of their limited availability and uncertain purity/concentrations. Cawthron Institute is providing crude toxin materials (gymnodimine, PTX2, yessotoxin) isolated from algal concentrates (field collections or cultures) to IMB, NRC, Halifax, Canada for production of certified standards. This and related initiatives will by mid-2003 overcome many of the current difficulties relating to availability of standards for key toxins.

Many of the above issues relating to introduction of new technologies will be covered at the HABTech03 workshop to be held in Nelson 26-30 November. 2003 with APEC as the main sponsor Plenary lectures and practical demonstration sessions are planned to cover recent developments in techniques for monitoring of marine biotoxins and hazardous algae. Planning is well advanced. See: http://www.cawthron.org.nz/habtech03

An LC-ESI-MS based method for the simultaneous determination of algal and cyanobacterial toxins in phytoplankton from marine waters and coastal zones

The threat to human consumers from algal and cyanobacterial toxins in the marine environment and the related economic problems for the fishery industry have been described extensively. Preventive measures for consumer's

protection, as well as basic scientific research on harmful algal blooms, require powerful analytical methods. Such methods have been developed for most of the toxin groups. Due to the structural differences among the toxins, analytical methods are typically based on ion-pair chromatography, ion-exchange chromatography and reversed phase chromatography coupled to UV or fluorescence detection. These toxin-specific methods have their benefits and are in use at numerous analytical laboratories. However, there is a need for simultaneous detection methods especially if large batches of samples have to be screened for HAB toxins. Recently, Liquid-Chromatography-Electro-Spray-Ionisation-Mass-Spectrometry (LC-ESI-MS) methods became available, which combine efficient separation power with generic detection for the different toxin groups. By application of the highly selective mass detector, reliable results are obtained and sample preparation protocols are usually simpler and less laborious.

A liquid chromatography based method with mass spectrometric detection was developed for simultaneous determination of various algal and cyanobacterial toxins extracted from phytoplankton occurring world-wide in marine waters and coastal zones. The method enables quantification of saxitoxin, anatoxin-A, domoic acid, nodularin, microcystins, okadaic acid and dinophysistoxin-1 with a single chromatographic run. In addition, the applied chromatographic conditions allow isolation and identification of substances suspected to be "new" microcystins (cyclic peptides) by fraction collection, hydrolysis, derivation of resulting free amino acids with the modified chiral Marfey´s reagent (L-FDVA) and enatioselective analysis of the amino acid derivatives by LC-ESI-MS.

Observations made during research cruises in the Baltic Sea confirmed that naturally occurring phytoplankton communities often consist of numerous algal and cyanobacterial species. A broad spectrum of HAB toxins, related to various toxic syndromes, was measured under the applied LC-ESI-MS conditions. In addition, the method was applied to different phytoplankton cultures of algae and cyanobacteria grown under laboratory conditions.

Comparison of the accuracy of quantification of phytoplankton toxins in values around 10%, similar to those values obtained with other methods. This is remarkable, since the multitoxin method is based on a universal extraction solvent (water / methanol (50:50; v/v).

LC-ESI-MS multitoxin methods allow rapid determination of the relevant toxins within one chromatographic run. Therefore, these methods are ideally suited for on board analyses of phytoplankton during harmful algal blooms.

11 ECOHAB-EUROHAB WORKSHOP

Term of reference i: Report on the ECOHAB-EUROHAB Workshop on joint research on HABs.

Don Anderson, (USA) reported on 'The EU-US Programme on Harmful Algal Blooms: A Joint Initiative by the European Commission – Environment and Sustainable Development Programme and the US National Science Foundation'.

For decades harmful algal blooms (HABs) have been studied on both sides of the Atlantic, but the underlying reasons for these blooms, the ability to predict their occurrence, and the means to mitigate them when they do occur, have not been well known. For the first time, joint research in Europe and the US is being proposed to address these problems of mutual concern, through financial support from the European Commission (EC) and the US National Science Foundation (NSF). This effort builds on a collaborative agreement signed between the European Commission and the NSF to foster scientific collaboration. This initiative was subsequently launched by a joint workshop in September 2002 in Trieste, Italy, during which scientists collectively assessed the state of the science, identified gaps in our knowledge, and developed a plan for cooperative, comparative studies. This workshop was endorsed by GEOHAB. A workshop and planning document will be available through the GEOHAB website.

There are several areas where such collaboration will lead to significant progress that would not be possible if similar studies were undertaken independently. First, there are comparative environments in the EU and in the US where comparisons of similar processes controlling bloom dynamics should lead to new understanding. Second, apparently similar species occur in the EU and in the US, but they differ in growth dynamics and expression of harmful attributes. Third, major anthropogenic and/or natural forcings, such as nutrient loading and climate variability, appear to have some differing impacts on HAB bloom dynamics in the EU and the US, and understanding this gradient of responses may lead to better insight and better management of HAB events. Advances will be mutual and both research communities will benefit.

The funding status of the EU-US programme is not finalized at this time, but looks promising. Once the report from the Trieste workshop is finalised and distributed, the NSF and the EU will make decisions as to whether HABs will be one

of the first areas to be explored under the agreement that was signed between the two entities. These decisions are expected in the summer, 2003.

12 STUDY GROUP ON IMPLIMENTATION OF GEOHAB IMPLIMENTATION IN THE BALTIC SEA (SGGIB)

The Study Group on GEOHAB Implementation in the Baltic Sea had originally met, under the chair of Kaisa Konen, two days prior to the WGHABD meeting in Dublin, Ireland (2001). There had been discussion on conducting a cooperative HAB Baltic study under the GEOHAB umbrella that would act as a model to addressing HAB dynamics in brackish water systems.

In the interim, the Chair of the group resigned and Markku Viitasalo (Finland) was recently asked to assume the duties. There was a danger in the group being disbanded as there were four original national members of the SG and three had indicated that they were no longer able to participate. In order to try and proceed with the action plan for the group, the Chair requested that the issue be brought up at the WGHABD meeting, as some of the countries that had initially indicated support would be in attendance. The Chair, M. Viitasalo, made a brief presentation on the SG and its dilemma.

Following discussion it was determined that development of Harmful Algal Studies in the Baltic Sea would provide a unique opportunity to study HABs at the scale of one ecosystem. Implementation in the Baltic Sea of scientific activities related to HABs in coherence with the GEOHAB Science Plan is essential. Members of the WGHAB bordering the Baltic Sea expressed interest in continuing the project. Therefore, WGHABD recommends that, despite its present difficulties, SGGIB be continued under the new chairmanship of M. Viitasalo and a meeting be convened. It was suggested that the SGGIBS meet jointly with the WGHABD in 2005.

13 ITIS TAXONOMIC REFERENCE LIST

Term of reference j: consider the potential of ITIS as a common taxonomic system within ICES

The WGHABD discussed the Integrated Taxonomic Information System (ITIS) based on the information available at the ITIS web site. ITIS includes plants, animals, fungi and microbes of 'North America and the world'. It is a partnership of US, Canadian, and Mexican agencies.

The WGHABD is aware that ITIS is a partner in the Global Biodiversity Information Facility (GBIF), but it was not clear to the WGHABD if that implies endorsement of GBIF of the ITIS code system, or if this is an issue GBIF will advice on. The WGHABD found that any adoption by ICES of a taxonomic reference system should take into account the recommendations or strategy of GBIF as to which taxonomic reference systems are to be used internationally for biodiversity databases.

The WGHABD noted that ITIS at the moment does not reflect most recent systematic or taxonomic changes (at all levels from kingdoms to species) and does not include all species of e.g. potentially harmful microalgae (based on a random check). Unless a given taxonomic reference system is maintained at a very high level in accordance with most current research, it will not be followed or used. ITIS does not either include in its expert roster key microalgae taxonomists (only US scientists appear to be included).

The WGHABD had potential concern that ITIS is a North American undertaking which at present is not maintained and developed by an international mechanism Also, it is of concern how a potential adoption by ICES of ITIS will be compatible with e.g. the European Register of Marine Organisms, the ICES Reference List of Phytoplankton (by WGPE) and the IOC Taxonomic Reference List of Potentially Harmful Microalgae, HELCOM lists, etc.

The discussion also led to a strong agreement that ideally such a taxonomic reference system should be linked to a database with information for each species with references, micrographs (type specimens where feasible), bio-volume formulas, etc.

If ICES adopted ITIS as its taxonomic reference system, the implications for national data management may be far reaching. The WGHABD questioned how ICES could implement ITIS and what would motivate national data centres to assign resources to adapt their systems to comply with ITIS codes.

In conclusion, the WGHABD found that ITIS in its present form is not mature in terms of taxonomic and geographical coverage or management structure for adoption by ICES as its taxonomic reference system, and that the questions

raised above have to be clarified before it can be fully assessed whether it is desirable for ICES to adopt the ITIS system.

14 NATIONAL REPORTS

Representatives from Estonia and Spain did not attend the meeting, but submitted national reports to the Working Group.

Portugal sent HEADAT input forms to IOC-IEO SCCHA, Vigo, Spain.

Canada

PSP is an annual occurrence in many regions of Canada. STX equiv. above the regulatory levels were detected in the following regions: the West Coast (*Alexandrium catenella*), the St. Lawrence Estuary (*A. tamarense*) and the Bay of Fundy *(A. fundyense*).

Shellfish harvesting was closed as a result of unsafe levels of domoic acid in Newfoundland (probable cause – *Pseudo-nitzschia seriata*) and the St. Lawrence Estuary, with the largest closure on record observed. The majority of the southern Gulf of St. Lawrence was also affected with the following organisms detected: *P. multiseries, P. pseudodelicatissima, P. fraudulenta* and *P. pungens*.

The West Coast salmonid industry experienced moralities from the following species: *Heterosigma* (14,000,000 cells•L-1), *Chaetoceros convolutus* (180,000 cells•L-1), *Chatonella* (200,000 cells•L-1) and *Chaetoceros concavicornis*. In the Bay of Fundy, low levels of salmon moralities were observed during an extended *Eucampia zodiacus* bloom (962,000 cells•L-1).

Denmark

In Denmark PSP or ASP toxins were not recorded above regulatory levels in 2002. DSP was detected at concentrations above regulatory limits locally in the Isefjord (first record of DSP in this harvest area) as well as in a few harvest areas in the southwestern part of Kattegat during late summer and winter. In the earlier occurrence associated with toxicity (July-August), the most likely causes were *Dinophysis acuminata* and *D. acuta* which co-occurred. The late summer and winter toxicity was most likely caused by *D. acuta*, which occurred in low concentrations (100-200 cells•L-1). Toxicity continued into 2003 and did not disappear until March 2003 - at the time of the diatom spring bloom in the area. The timing and dynamics of the DSP incident is very similar to what is very often observed at the Swedish West Coast. No DSP was detected in the major harvest areas in the Limfjord.

High concentrations of *Pseudo-nitzschia* spp. were documented during the summer in the Limfjord as well as in the Kattegat area but as mentioned earlier, no domoic acid was detected.

No fish kills related to HABs were observed during 2003. *Chattonella* spp. was observed in low concentrations during the spring and early summer.

During the summer, high biomasses of phytoplankton (dominated by several *Ceratium* and diatoms from the genus *Pseudo-nitzschia*) were recorded which eventually was one of the triggering factors that lead to oxygen deficiency – which occurred in most of the Kattegat region. During this period fish and invertebrate mortalities were observed.

Estonia

Cyanobacterial blooms in the summer of 2002 in the Gulf of Finland were much like those during the record year 1997. High levels of phosphate phosphorus in the surface waters in the beginning of both summers contributed to increased cyanobacterial growth. In addition, the hot, sunny and calm weather was conducive to increased densities of the algae. In contrast to 1997, no large surface aggregations were observed after mid-August and the main biomass came from the nontoxic *Aphanizomenon*. In mid June the biomass of *Aphanizomenon* had increased to 2 mg l^{-1} at the 5 m depth. By the end of June the biomass had increased to 3 mg l^{-1}. The maximum level was observed during the first week of July when the biomass of *Aphanizomenon* was more than 4 mg l^{-1} in the Estonian coastal waters of the Gulf of Finland. At the same time the biomass of potentially toxic *Nodularia spumigena* was almost half that recorded in 1997 and 1999. In Estonian coastal waters it remained between 0.3-1.4 mg l^{-1}. High biomasses of *Nodularia* were detected in Narva Bay (2 mg l^{-1}) during the second week of June. Due to dense surface accumulations of cyanobacteria during the second half of

July several beaches near Tallinn were closed for a couple of days. At the same time no visible cyanobacterial blooms were detected in other Estonian coastal areas.

Finland

In the autumn/winter 2001, heavy storms mixed waters thoroughly in the Gulf of Finland. This resulted in large amounts of phosphate phosphorus being released from the anoxic bottom sediments to the deeper waters, and being transported to the surface. This created favourable growth conditions for cyanobacteria during the summer of 2002.

After the spring bloom in 2002, high levels of phosphate phosphorus in surface waters continued to be recorded, especially in the Gulf of Finland. Cyanobacteria were observed around Åland Island, in the Gulf of Finland, Bothnian Sea and the Bay of Bothnia during the second week in June. Concentrations were low and the dominant species was the nontoxic *Aphanizomenon flos-aquae*. Cyanobacterial growth accelerated towards the end of June but strong winds kept the surface waters mixed and cool. More nutrients were brought to the Gulf of Finland through upwelling along the south coast of Finland. During the second week of July, extensive cyanobacterial surface aggregations were built up as the weather calmed. From mid-July onwards, large surface aggregations were also observed in the Archipelago Sea.

Nodularia spumigena began to dominate the phytoplankton species composition in the water column and by the 3rd week of July completely dominated the community in the Gulf of Finland. The area in which strong blooms were observed encompassed almost the whole (central and western) Gulf of Finland and the Archipelago Sea. In mid-July, the blooms were as strong as those during the record year 1997. However, in contrast to 1997, the aggregations mostly did not reach the shoreline and no major blooms were observed after mid-August.

During the last week of July strong winds caused the major surface aggregations to disappear. In the beginning of August sporadic aggregations were observed over large areas in the Gulf of Finland and Archipelago Sea, with *A. flos-aquae* as the dominant species in most places. By mid-August all cyanobacterial surface aggregations had disappeared, except in the Archipelago Sea where some local blooms occurred. The continuing warm weather kept water temperatures above the long-term average and, in the end of August, some localized blooms were still observed in sheltered and nutrient-rich bays in the Gulf of Finland and the Archipelago Sea.

In the Gulf of Bothnia, cyanobacterial blooms were much less pronounced than in the Gulf of Finland (Also, compared to 1997 the blooms were less pronounced.) The first larger surface aggregations were observed on July 31 in the Bothnian Sea, with *N. spumigena* as the dominant species. The bloom persisted until the end of August. No mass occurrences occurred in the Bothnian Bay, except for local aggregations outside the city of Kemi.

As for other potential HAB species, local mass occurrences of the nontoxic dinoflagellates - *Heterocapsa rotundata* and *Prorocentrum minimum* - coloured the water red-brown in the Archipelago Sea in late August and September/October, respectively.

Despite intense blooms of cyanobacteria, no severe harmful effects (fish kills, health effects in humans, domestic animals or wildlife) due to cyanobacterial toxins were documented during 2002.

France

The French coast was mainly affected by DSP toxins in 2002. Fifteen sites were closed as a result of DSP toxins (compared to the 43 sites which are described on the whole French coast). Three sites were closed for ASP toxins, and none for PSP toxins.

As in previous years, DSP toxins were observed in shellfish from different areas: Seine bay, a great part of the Southern Brittany coast, two zones from the Atlantic coast (Pertuis Breton and Arcachon Bay), and a Western Mediterranean lagoon (Salses-Leucate). Toxicity above the regulatory level was observed in different types of shellfish such as mussels, cockles, clams, donax. All these observations were linked to the presence of *Dinophysis*. For the first time since monitoring was initiated, DSP toxicity was observed until December in several areas along the Atlantic coast.

In 2002, according to the European Community directives, the DSP mouse-test threshold was modified from 5 hours to 24 hours in the monitoring performed by IFREMER. The same methods and thresholds are now used for all toxins in IFREMER laboratories for shellfish from production areas and in Veterinary Services for shellfish from markets.

A systematic study was performed on 16 sampling stations, during one year (2002-2003), to evaluate the potential presence of AZPs both on mussels and oysters (oysters were not monitored until now for DSP toxins). To date, DSP

mouse-test positive results have only been observed during *Dinophysis* occurrences. Chemical analyses are in process, but at the present time, there is no evidence of AZPs presence in shellfish on the French coast.

Other chemical analyses were performed by LC-MS on several mussel and oyster samples, with two objectives to: i) compare mouse-test results and chemical results and to ii) evaluate the respective proportions of OA and DTX3. The two main results were: i) the absence of OA and DTX3 in mussels and oysters from the Salses-Leucate lagoon (Western Mediterranean) despite DSP positive mouse-test results, and ii) in the samples from other areas, the very large proportion of DTX3 (the ratio DTX3/OA+DTX3) was always between 50% and 100%).

There was no *Alexandrium* bloom last year along the French coast. Only one toxic PSP episode occurred in December in Arcachon Bay, with a maximum level of 86 µg STX equiv. per 100 g flesh in mussels. It is a recurrent event since low levels of PSP toxins have been observed every year in winter in this area, but a particular point is that it is not linked with the presence of a known PSP producing species. However it was noted that some *Goniodoma* and *Gonyaulax* species were always present at low levels during these episodes. A study is ongoing to determine the responsible phytoplankton species.

For the first time, ASP toxins were observed above the regulatory levels in Western Mediterranean shellfish (mussels and donax). The maximum level detected was 48 µg DA per g flesh. This episode occurred in April and the responsible species was identified as *P. pseudodelicatissima*.

Germany

Since the last report harmful algal events occurred in German coastal waters as they have each year, in the North Sea and in the Baltic Sea, and can be confirmed by the MURSIS-reports and the data provided to HAEDET.

North Sea

DSP – Blue mussels (*Mytilus edulis*) reached occasionally the critical value of 400 µg kg-1 several times, then mussel harvesting was prohibited. DSP-content of the mussels was correlated with the occurrence of dinoflagellates of the genus *Dinophysis,* mainly *D. acuminata*. Critical DSP-values in mussels are in most cases associated with *Dinophysis*-cell densities of about 1 000 cells l-1.

PSP –Blue mussels toxin levels exceeded the critical values of 800 µg kg-1, although *Alexandrium*-species (*A. tamarense*, *A. ostenfeldii*) [dinoflagellates] regularly occur in German coastal waters in low numbers. In the open North Sea *A. minutum* and *A. pseudogoniaulax* have also been recorded.

ASP was not recorded in blue mussels, although potentially toxic diatom *Pseudo-nitzschia* species regularly occur in German waters. The Alfred-Wegener-Institut at Helgoland has started a special study to document the species composition of *Pseudo-nitzschia* to detect ASP in natural samples and in isolated clones.

Azaspirazids have been detected in water samples from the northern North Sea but so far not from coastal waters or in seafood.

Cyanobacterial blooms have not occurred in the period of this report.

The dinoflagellate *Noctiluca scintillans* causes red water discoloration each year but no anoxia or other harmful effects have been reported.

The prymnesiophyte *Phaeocystis globosa* each year causes foam formation on the beaches causing adverse effects on tourism, although during 2002 blooms were short and not as intense as in other years. Prymnesiophytes of the genus *Chrysochromulina* are regular members of the phytoplankton community but no adverse effects have been detected.

Since 2000, a phototrophic euglenophyte has discoloured sandy intertidal beaches of German coastal waters. This organism is tentatively been identified (Prof. Melkonian, Univ. Köln) as cf. *Euglena viridis* var. *maritima*. The affected areas are increasing yearly. So far it is not known whether this will have adverse effects on other species. It is planned to investigate this phenomenon systematically, which will include bringing the species into culture (so far attempts have failed) and to perform biotests.

Baltic Sea

Cyanobacteria blooms associated with hepatotoxins have been documented each year in coastal waters of Germany but no incidents of animal deaths has been reported.

Species of the dinoflagellates Dinophysis and *Alexandrium* and the diatom *Pseudo-nitzschia* regularly occur but no intoxication was recorded.

Prorocentrum minimum regularly formed blooms but no adverse effect were reported.

Prymnesiophtyes of the genus *Chrysochromulina* are regular members of the phytoplankton community but no adverse effects have been detected.

Ireland

In general, 2002 did not exhibit the same severity of HAB events as recent years. However, four different groups of shellfish toxins necessitated management restrictions of either bay closure or restricted harvesting. In addition there were two blooms that resulted in water discolourations.

As is normal in Ireland the main HAB problem during 2002 was due to DSP toxins in shellfish. Early in the year a carryover from the previous year resulted in closures of *Mytilus edulis* from the south west (Castletownbere, Gerahies, & Adrigole). These levels were observed to decrease to below recommended threshold levels between January - April 2002. A further DSP event was determined from *M. edulis* samples in the South West (predominantly in the Bantry Bay area) from August to November 2002. Highest levels observed occurred in September with levels of okadaic acid equivalents >1.00 µg/g whole flesh in Castletownbere, South Chapel & Dunmanus Bay. High levels of *Dinophysis* sp. were observed in these areas during September with maximum cell counts of 12,160 cells•L-1.

PSP toxins were observed in Cork Harbour in both C. *gigas* and *M. edulis* for a 3 week period (mid – end July 2002) resulting in a closure. This area is prone to recurrent *Alexandrium* blooms from a cyst bed of these species in the area.

AZP exceeded 0.16 µg/g whole flesh on two separate short occasions. These were observed in *M. edulis* samples for 1 week in January (Castletownbere) & March (Adrigole). In contrast to 2001 when AZP was detected in phytoplankton net-hauls at several offshore locations, only trace levels were determined in 2002 in similar hauls. The levels of *Protoperidinium crassipes* were also much reduced as in the previous year.

ASP was predominantly observed in scallops (*Pecten maximus*), however, one *M. edulis* sample from the North West (Loughros Mor) had levels above 20 µg/g whole flesh. For P. *maximus*, the majority of samples with >20 µg/g domoic acid in the adductor muscle (highest recorded level 38.7 µg/g) were from Clew Bay on the west coast. The majority of samples >1000 µg/g domoic acid in the hepatopancreas were also from the West, predominantly Clew Bay.

At the end of July, there was a report of bright orange water in the East Coast of Ireland. This was sampled and was found to be due to a bloom of *Noctiluca scintillans*. The bloom was short lived and disappeared after one week. Highest cell counts were 3x106 cells•L-1.

A bloom of *Phaeocystis cf. pouchetii* off the South and south-west coast was intermittent between April and August. This was unusual as this species is usually only seen in early summer. Maximum cell counts of 28 x 106 cells•L-1 were observed in June.

Latvia

Eastern part of the Baltic Sea (Gulf of Riga)

The following HAB species were observed in Latvian territorial waters in 2002: *Dinophysis acuminata, Aphanizomenon flos-aquae, Anabaena spiroides, Nodularia spumigena, Microcystis aeruginosa, Chaetoceros danicus, Chrysochromulina* spp., and *Prorocentrum minimum.*

Although hydrological and hydrochemical conditions (high water temperature 18–21°C; low inorganic nitrogen concentrations, low DIN/DIP ratio) were favourable for the development of HAB species, increased HABs numbers

were not observed. This may be explained by windy conditions (1–9 m/s) in summer-autumn of 2002 disturbing the surface waters in the Gulf of Riga, as well as by low concentrations of phosphates (0.03 – 0.08 μM).

These conditions could only promote the development of nitrogen fixing or mixotrophic species. Higher concentrations of the potentially toxic cyanobacteria *Aphanizomenon flos-aquae* and the diarrhetic shellfish toxins (DST) producing dinoflagellate *Dinophysis acuminata* were observed, reaching 3 and 8 times higher biomass as in 2001 (500 mg/m3 and 1000 mg/m3). The highest concentrations of *Aphanizomenon flos-aquae* (183,000 filaments • L-1) were observed in the coastal zone of the Gulf (28.07.02). The highest density of *Dinophysis acuminata* cells (23,000 cells•L-1) was detected in the central part of the Gulf (8.07.02) in the presence of relatively high concentrations of organic nitrogen (27 - 29 μM) in the thermocline.

Although blooms of *N. spumigena* were observed in the open part of the Baltic Sea, no blooms of *Nodularia* were detected in the Gulf of Riga.

The Netherlands

Dinophysis acuminata

Okadaic acid was detected for the first time since 1995 in shellfish from the Dutch coastal waters in 2002. In August 2002, numbers of *Dinophysis acuminata* increased and persisted in coastal waters for several months. The numbers of *D. acuminata* were 200-500 cells•L-1from August to September. No DSP toxicity was detected in shellfish. In October, counts dropped to around 100 cells•L-1, and remained in the water constantly. *D. acuminata* persisted for another week at cell levels around 100 cells•L-1even after a severe storm at the end of October. After cell counts dropped to levels below 100 cells•L-1, DSP-toxins were detected by the traditional rat bioassay method in mussels in the Oosterom (Waddenzee) area and the area was closed to harvesting. After the report of toxicity in the Oosterom area, the cell counts of *D. acuminata* decreased further to below the limit of detection. However, in the following two weeks several other areas in the Waddenzee were affected with DSP toxins. After two weeks the entire mussel production area in the Waddenzee was contaminated with DSP toxins. Monitoring of the cockle (*Cerastroderma edule*) revealed positive rat bioassay results. Chemical analysis of the mussels indicated that the shellfish were contaminated with okadaic acid (source: RIVM/RIVO collaboration). The LC/MS detected the okadaic acid at levels lower then those detected using the rat bioassay. Toxicity persisted in the mussels for four to six weeks, and the affected areas were opened prior to Christmas.

In the same period that DSP-toxicity occurred in the Waddenzee, positive results were obtained by rat bioassay on shellfish from the Oosterschelde. No HAB species were detected in this area for this entire period. The toxicity in the Oosterschelde was very patchy and was undetectable (by rat bioassay) after four weeks.

Noctiluca

Several blooms of what probably was *Noctiluca* occurred in the North Sea from May to August. Most of these blooms were observed from ships and airplanes and could not be sampled. In samples that were taken by Rijkswaterstaat vessels, concentrations of *Noctiluca scintillans* ranged from 600 to 6000 cells•L-1.

Phaeocystis

Surprisingly, the spring bloom of *Phaeocystis globosa* did not occur in 2002. The probable causes for this absence are a very sunny early spring (March-April) and low Rhine discharge. A model for the *Phaeocystis* spring bloom that takes irradiance and discharge into account is presently being constructed in the EU-project HABES (Harmful Algal Bloom Expert System).

New Zealand

Major HABs with widespread and long-term effects are rare in New Zealand, with about a 5-year return period. *Karenia* spp. caused widespread NSP toxicity in shellfish from the Hauraki Gulf region (N. North Island) during December 1993 – March 1994. A large *Gymnodinium catenatum* bloom extended down the W. Coast of the North Island during May – December 2000 and moved through Cooks Strait onto the E. Coast before dying away. The scale and extended period of high PSP toxicity and harvest closures was unprecedented but the major Marlborough Sounds (N. South Island) was unaffected. There have been no subsequent major HAB events.

Localized blooms are common that result in ASP, PSP or DSP events of minor and often only short-term significance. There were few harvest closures in the period 2001-March 2003 but the recent advances in remote sensing, DNA-probes and LC-MS analyses have enabled many new insights from a number of these events. A *K. mikimotoi* bloom in the Hauraki Gulf (N North Island) during August-November 2002) was associated with an oceanic warm water intrusion and led to fish and abalone kills. Brevetoxins did not accumulate in shellfish, unlike the superficially similar 1994 event.

A large bloom on the NW coast of the South Island led to prolonged closures from DSP toxicity. The causative organisms were *Dinophysis acuta* and *Protoceratium reticulatum* with production of okadaic acid, pectenotoxins and yessotoxin. The later was persistent in mussels with different patterns of metabolism and depuration between greenshell and blue mussels. A new pectenotoxin (PTX11 – isomer of PTX1) has been discovered in association with PTX2 from *D. acuta*. Large-scale phytoplankton harvests from this event have enabled production of 10-50 mg quantities of PTX2 for preparation of standard reference material (cooperation with IMB-NRC, Halifax, Canada). This standards programme has also been assisted through provision of crude gymnodimine and yessotoxin from cultures of *G. selliforme* and *P. reticulatum* respectively.

A bloom of *D. acuminata* at Banks Peninsula (W. Coast, South Island) November-December 2001 led to DSP closures. LC-MS testing was used to monitor the event and clear harvest areas. Toxins in algae were dominated by PTX2 but mussels mainly contained okadaic acid and DTX1 (free and esterified forms) with PTX2 rapidly metabolized and depurated as the secoacid.

Recurrent but localized blooms of *D. acuminata* and *P. reticulatum* in an area of the Marlborough Sounds (N South Island) were studied in detail during 2003 with associations established between the dinoflagellate populations and toxin levels in algae, water and shellfish.

Pseudo-nitzschia australis is the diatom species causing ASP toxicity in NZ shellfish, although closure levels are rarely reached. LC-MS testing has revealed that a high proportion of events are associated with a P. *australis* strain that produces an isomer of domoic acid at levels 1-2 times that of domoic acid. Structural and toxicology studies are proceeding.

Norway

Detections of DSP-toxins in mussels in 2002 were above average for the last decade, and DSP-toxins above action levels were even detected in crabs (see more under "new findings"). The problems began in June, which is earlier than usual, and lasted throughout the year at some locations. Essentially most of the coastline in southern Norway, from the Swedish border to the Trøndelag-region was affected. The causative species, *Dinophysis acuta*, bloomed early and became widely distributed during the June-December period, although did not reach exceptionally high concentrations. Levels of PSP-toxins above action-levels were recorded at a few locations on the northwest coast of Norway, where these are recurrent problems. At a few locations along the south and west coasts of Norway the levels of yessotoxins (YTX) in mussels also exceeded regulatory action-levels for a short period in May. By the end of the year azaspiracid was detected in mussels at concentrations close to action-levels. There were no blooms of ichtyotoxic algae in Norway in 2002, but large amounts of gelatinous plankton caused severe losses in a few fish farms along the west coast.

Poland

In the Gulf of Gdansk (Southern Baltic) *Nodularia spumigena* blooms are annual occurrences. In 1994, an intensive bloom with cell numbers up to 16 million cells•L-1 (nodularin Nod concentration = 2590 µg/g dry wt) resulted in a beach closure along the coast of the Gulf of Gdansk. In 2001, during a short two-week *Nodularia spumigena* bloom at the beginning of July, Nod concentrations varied from 90 – 18130 µg/L (3000 –3520 µg/g dry wt). In 2002, the increased numbers of *Nodularia spumigena* cells were observed for the first time at the beginning of June, i.e. earlier than usual. In spite of favourable weather conditions (sunny and warm summer), the bloom did not reach the same intensity as in 2001. During 2001, the concentration of *nodularin* was three orders of magnitude lower. The maximum value of the Nod concentration was reached at the end of July and in August 12.6 µg/L (919 µg/g dry wt). In 2002, the *Nodularia spumigena* bloom lasted longer than usual, i.e. from the beginning of June until mid September. Apart from *nodularin*, anatoxin-a and microcystins were detected in coastal waters of the Gulf of Gdansk.

Scotland

Numbers of toxin producing dinoflagellates were much reduced this year. The maximum number of *Alexandrium* spp. recorded was 600 cells•L-1 at Scapa Bay in Orkney, compared with a more usual 2000 cells•L-1 recorded from the

same location in 2001. The incidence of PSP toxicity this year was one of the lowest on record. Similarly the maximum number of *Dinophysis* spp. recorded was 1,600 cells•L-1 at Stonehaven during August 2002 which contrasts to a maximum number of 33,000 recorded from Loch Inchard during 2001. DSP toxicities in mussels (*Mytilus edulis*) and queens scallops (*Chlamys opercularis*) were recorded during the summer. In contrast the maximum numbers of *Pseudo-nitzschia* spp recorded during 2002 (1,000,000 cells•L-1) from Loch Spleve was much greater then levels recorded in 2001. ASP toxins in offshore scallops (*Pecten maximus*) again remained a problem throughout the year.

No commercial fish kills as a result of a phytoplankton bloom were observed during 2002. Fatalities of farmed fish that occurred on the west coast during the summer were as a result of a jellyfish bloom.

Spain

Problems caused by harmful algae in the Galician and Andalusian communities of Spain are mainly due to toxic species that render shellfish unsuitable for human consumption at very moderate concentrations (< 10^4 cells•L^{-1}). In Cataluña (northern Mediterranean coast), visual contamination caused by red-tide-forming dinoflagellates represents the main impact as it affects tourist resources. During 2002, harmful events showed similar patterns to previous years. It is worth mentioning the very persistent DSP outbreaks in Galicia caused by *D. acuminata* and the first detection of homo-YTX, from an unknown origin in the Delta del Ebro (NE Mediterranean coast of Spain).

Andalucia

Western Andalucía - Atlantic coast

Intermittent diarrhetic shellfish toxins (DST) outbreaks were associated with increases of *Dinophysis acuminata* between May and August.

Eastern Andalucía- Mediterranean coast

Gymnodinium catenatum and PSP toxins above regulatory levels were reported in the summer (August-September) in the Alborán Sea coasta (Costa del Sol). Toxin accumulation, as usual, was especially high in the "corruco" or rugged clam (*Acanthocardia tuberculata*). Foam events were observed on the Eastern coast between September and December and associated with high levels of *Gonyaulax* cf *hyalina.*

Catalonia

PSP. Blooms of *Alexandrium catenella*, first recorded in the area in 1996, have become a recurrent event. Dense blooms were observed in Tarragona harbour and offshore waters between September and October in areas with no shellfish harvesting. In the Delta del Ebro region, PSP toxicity was just above regulatory levels.

DST above regulatory levels were detected in the Delta del Ebro when numbers of *D. sacculus* and *D. caudata* increased between November and December. Low levels of okadaic acid and homo-yessotoxin of unknown origin were detected in the same area between July and September.

Alexandrium minutum (February), *Pseudo-nitzschia* spp. (April-June), *Gymnodinium impudicum* (July-September), *A. taylorii* (June-August) and *Alexandrium catenella* (October) caused discolorations in localised harbours and beaches with no shellfish harvesting, but caused alarm to tourists.

Galicia

Northern Rías (North of Cape Finisterre)

Small scale localised high numbers of *Alexandrium minutum* and moderate (just above regulatory levels) concentrations of PSP toxins that lasted a few days were recorded. Very persistent DSP events (April-May; July-November) associated with increased cell numbers of *Dinophysis acuminata* and *D. sacculus* were observed. Low level ASP events associated with *Pseudo-nitzschia australis* were observed in April-May.

Rías Bajas (South of Cape Finisterre)

Extremely persistent DSP events caused by *Dinophysis acuminata*, that lasted up to 4 months (July-November) in different areas of the Rías Bajas (Muros, Arosa, Pontevedra and Vigo) and 6 months in some areas of Ría de Muros were recorded.

Mild ASP outbreaks caused by *Pseudo-nitzschia australis* were observed between April and September leading to intermittent short (few days to 2 weeks) closures of bivalve collection. Domoic acid levels in scallops remained above regulatory limits and remained unsafe for human consumption throughout the year.

Sweden

Skagerrak and Kattegatt: Harmful algae

No high biomass harmful algal blooms, such as *Chattonella*- or *Chrysochromulina*-blooms, occurred in this area in 2002. Dinoflagellates producing Diarrhetic Shellfish Toxins (DST) were found in the area throughout the year. *Dinophysis acuminata* was observed during 2002 with a maximum of over 2000 cells·L-1 recorded in July. *D. acuta* cells were observed from July to December with abundances up to 1000 cells·L-1. The Paralytic Shellfish Producing (PSP) dinoflagellate, *Alexandrium tamarense*, was observed in the Koljöfjord and the Havstensfjord in May with a maximum abundance of up to 2400 cells·L-1.

The potentially harmful algae *Karenia mikimotoi*, *Chrysochromulina* spp., *Pseudo-nitzschia* spp. and *Chattonella* sp. occurred in the area but in moderate abundance. Chattonella spp. now appears to be an established species in the area following the large blooms that occurred in 1998 and 2001.

Skagerrak and Kattegatt: Diarrhetic shellfish toxins in blue mussels (*Mytilus edulis*)

In some areas of the Swedish Skagerrak coast, concentrations of DSTs in blue mussels above the limits for marketing (160 µg/kg) were found during 2002. From the end of July to December, high concentrations were found in some areas while concentrations remained below the limit in others. The highest concentrations were observed in July and November-December. The maximum, ca 1400 µg/kg, was higher than in 2001 when the maximum level was 700 µg/kg.

Baltic proper

Substantial blooms of cyanobacteria were observed in the Baltic from the beginning of July to mid August. *Nodularia spumigena* occurred in very high amounts. Satellite images showed strong surface accumulations in large parts of the Baltic proper and in the Gulf of Finland. The maximum distribution was observed in mid July. Tourism in this area was badly affected since swimmers were advised not to go into the water. No toxin measurements or toxic effects were reported.

Bothnian Bay

No report of harmful algal blooms in the year 2002.

USA

2002 was basically a “normal” year for HABs in the U.S., with several noteworthy or exceptional events

PSP. As happens most years, PSP was recorded in the New England states (Maine) as well as in California, Oregon, Washington and Alaska on the west coast. In Maine, limited PSP toxicity was detected in western Maine, and in California there was limited distribution of toxins compared to previous years. The highest concentrations occurred approximately 50 miles north of San Francisco. Alert levels were also detected much further north in Del Norte county near the Oregon border. In Oregon, low levels of PSP toxins were detected, but below quarantine levels. In Alaska, only one PSP episode occurred, a reduction compared to past years. That event, however, involved the illness of seven people, one of whom was med-evacuated to a distant hospital. There were no fatalities. One unique and important event involving PSP toxins occurred in the Indian River Lagoon in Florida, where there were 14 cases of Puffer Fish Poisoning (PFP) confirmed to be from saxitoxin (not the usual puffer fish tetrodotoxin). Southern puffer fish (*Sphoeroides nephelus*) were confirmed to have saxitoxin in the Indian River Lagoon. The primary source for saxitoxin in the Indian River Lagoon was *Pyrodinium bahamense*. This is the first time this species has been reported to produce

saxitoxin in the U.S. Further information and links about the PFP event in 2002 and about puffer fish species in Florida are on a website at www.floridamarine.org. and abstracts about the puffer fish incident by Quilliam *et al.* and Landsberg *at al.* are posted at the Xth International HAB meeting web site http://www.xhab2002.com/ at p. 237 and p. 160 respectively.

ASP. ASP was recorded in California, Oregon, and Washington. The outbreak in California was extensive, stretching from Los Angeles to northern California, and achieving toxin levels as high as 230 ppm of domoic acid. There were no human poisonings, but marine animal moralities were reported.

NSP. NSP associated with *Karenia brevis* was reported in Florida at several locations, including the panhandle, where such episodes are not frequent. It was also reported in Texas. There were no illnesses from these events.

DSP. Traditionally, DSP has not been a problem in the US. In spring, 2002, a large bloom of *Dinophysis acuminata* occurred in the Potomac River, reaching a maximum cell concentration of 236,000 cells/L. Portions of the estuary were closed for shellfish harvesting on a precautionary level while toxin testing was being conducted. That testing eventually detected okadaic acid, but at trace levels well below quarantine limits.

Brown tide. An area of New York that has had brown tides in the past experienced a relatively low density blooms this year (up to 734,000 cells/L).

Pfiesteria. This year there were no reports of fish kills definitively attributed to *Pfiesteria* in North Carolina or Chesapeake Bay.

15 NEW FINDINGS

Modelling (3-D) of *Karenia mikimotoi* in the Bay of Biscay, French Coast: Vertical, Spatial and Temporal 6 - Years Validation

Reported by Patrick Gentien

Karenia mikimotoi blooms occur regularly along the French Atlantic coast, with large interannual variations in intensity and geographic extent. A species-specific model has been developed in an attempt to define the most important processes regulating *K. mikimotoi* population dynamics. This novel modelling approach does not necessarily imply a growth closure by inorganic nutrition. Formulation of the species dynamics is based on a detailed knowledge of the species biology. Growth rate depends on temperature and light, taking into account the plasticity of the species regarding light regimes. An original formulation of the mortality rate related to shear has been included. Since this species has been shown in situ to rely solely on ammonium from remineralization, it has been necessary to include this species model into a standard phytoplankton biomass model. These biological models are embedded into a 3D-hydrodynamical model of the whole continental shelf of the Bay of Biscay (5 nm mesh, 10 s layers, 2.5 turbulence closure). Mortality rate parameterization (tested in a 1-D vertical model under realistic forcing) reproduces confinement of the population in the pycnocline without any formulation of migration. 3-D simulation results without reseeding in winter have been validated against observed time-series for the same period (1990-1995). Confinement in the pycnocline is adequately simulated as well as timing of the blooms. The model reproduces correctly 5 years out of 6 in terms of geographic extent and interannual variations in abundance. This species of interest model requires tuning of only 7 parameters and as a result is probably more robust than a model which would take into account all the physiological processes observed and measured on this species.

DSP in *Cancer pagurus* in Norway

Reported by Lars Naustvoll, Norway

Several people became ill after eating crabs (*Cancer pagurus*) caught in shallow waters in the Arendal area (southern Norway). Chemical analyses showed the crabs contained high levels of DSP toxins. Blue mussels are an important part of the diet of crabs in nature, especially during the summer and autumn period, and in the Arendal area the blue mussels had high DSP levels from mid June in 2002. Laboratory experiments conducted at IMR, Flødevigen, confirmed that a diet of toxic blue mussels resulted in DSP accumulation in crabs. Toxins were only found in the “brown meat” whereas the “white meat” did not contain toxins.

From mid July to the end of October 2002, toxic crabs were observed from Hvaler (Swedish border along the southern coast of Norway) down to Mandal/Lindesnes. This area is rarely used for commercial crab fishing and warnings were

issued by the Norwegian Food Control Authority against eating crabs from the area while the blue mussels contained DSP toxins.

***Chattonella* – A new bloom forming harmful algal species in Scandinavian waters**

Reported by Lars Naustvoll, Norway

A short presentation relating to the national project on *Chattonella* sp. was given. The main objective of the project is to increase the knowledge about this harmful raphidophyte, which formed large blooms in the Skagerrak in 1998, 2000 and 2001. The following objectives will be addressed by this project:

i) identification and taxonomy using morphological studies, molecular biological analyses, and development of probes;
ii) life cycle studies of *Chattonella* with emphasis on cyst formation and excystment both in laboratory and field;
iii) studies on the autecology, tolerance and optimum for temperature, salinity, and irradiance;
iv) interactions between *Chattonella* and metazoan and protistan grazers, investigating the effect of *Chattonella* on survival and reproduction and potential predators;
v) bloom dynamic, analyses of monitoring and environmental data for a better understanding of environmental factors affecting the bloom dynamic. The bloom dynamics work will be coordinated with the EU project HABIL.

HABES Swedish West Coast pilot project results from field work 2001/2002

Reported by Bengt Karlson, Sweden

Odd Lindahl	Project leader
Marie Johansen	PhD student
Bengt Lundve	assistant
Joakim Strickner	student

This study is part of the EU-project HABES (Harmful Algal Blooms Expert System). Samples were obtained from two locations on the Swedish Skagerrak coast: i) Gåsö, west of the mouth of Gullmar Fjord is strongly influenced by the Baltic current and ii) Koljöfjord situated north of the island of Orust. Shallow sills restrict the water exchange in the Koljöfjord (inner site) and the stratification is much more stable than at Gåsö (outer site). Results showed most *Dinophysis acuta* were found above the pycnocline at both sites with higher concentrations recorded at the inner site. *Dinophysis acuminata* cells were observed to occur in the outer site mostly above the pycnocline, while at the inner site they were found within or below the pycnocline. Higher concentrations of *D. acuminata* were observed at the inner site.

Toxins were also measured in the phytoplankton. DTX-1 was associated with the occurrence of *D. acuta*. Okadaic acid could clearly be related to the occurrence of *D. acuminata* and the toxin content per cell varied in both species.

16 OTHER REPORTS

Patrick Gentian gave a presentation on the GEOHAB programme and on developments in the organization of the HABWATCH workshop.

GEOHAB - Global **E**cology and **O**ceanography of **H**armful **A**lgal **B**looms:

A programme of SCOR and IOC.

In 1999, a Scientific Steering Committee was established by SCOR and IOC with the major task of defining precise scientific goals and the strategy to address them. The GEOHAB overall goal is to improve prediction of HABs by determining the ecological and oceanographic mechanisms underlying their population dynamics, integrating biological, chemical, and physical studies supported by enhanced observation and modelling techniques. The **GEOHAB mission** is to foster international co-operative research on HABs in ecosystem types sharing common features, comparing the key species involved and the oceanographic processes that influence their population dynamics. The **Science Plan** (GEOHAB Report #1, 2001; http://ioc.unesco.org/hab/GEOHAB.htm or http://www.jhu.edu/scor/GEOHAB_2001.pdf) defines five Programme Elements that serve as a guide to establish research priorities. Although HABs also pose critical problems in freshwater ecosystems, the focus of GEOHAB will be on the manifestation of these blooms in marine and brackish waters.

The elements of GEOHAB and their overarching questions are:

1) Biodiversity and Biogeography. What are the factors that determine the changing distribution of HAB species, their genetic variability, and the biodiversity of associated communities?
2) Nutrients and Eutrophication. To what extent does increased eutrophication influence the occurrence of HABs and their harmful effects?
3) Adaptive strategies. What are the unique adaptations of HAB species and how do they help to explain their proliferation or harmful effects?
4) Comparative Ecosystems. To what extent do HAB species, their population dynamics, and community interactions respond similarly under comparable ecosystem types?
5) Observation, Modelling, and Prediction. How can we improve the detection, and prediction of HABs by developing capabilities in observation and modelling?

The rationale for each of these objectives and the anticipated outcomes are fully described in the GEOHAB Science Plan. The list of objectives is long and diverse and represents a significant challenge for the implementation of GEOHAB.

The GEOHAB strategy is to address these issues through several categories of research that differ in their scales of international and disciplinary co-operation. Details of these Core, Targeted, and Regional/National studies are given below. Framework Activities, which are not specifically research, will also be developed to achieve the mission of GEOHAB. GEOHAB is not a funding programme but rather, it provides a framework for the integration of resources and expertise. The central feature of GEOHAB programme implementation will be the Core Research in comparable ecosystems, as these promote the synthesis and integration of research conducted in many different countries and regions and on many different HAB species and the identification of commonalties.

Core Research is co-ordinated by the SSC. Core research comprises at present four projects:

- HABs in Upwelling. Contact person: G. Pitcher
- HABs in Semi-confined Eutrophic Zones and Estuaries. Contact : P. Glibert
- HABs in Fjords and Coastal Embayments. Contact: A. Cembella
- HABs in Stratified Environments. Contact: P. Gentien

For each of the core research projects, an Open Science Meeting (OSM) is scheduled in order to establish priorities, set a project committee, select study sites and plan field operations. The scientific community is invited to participate. The Upwelling OSM will occur in November 2003. The Fjords and Coastal embayments OSM is planned to occur in the first 3 months of 2004. The two remaining OSM should occur before the end of 2004.

Targeted Research addresses specific objectives outlined in the GEOHAB Science Plan. Targeted Research may be solicited by the SSC as the need arises from Core Research. Targeted Research includes, but is not limited to:

- Modelling procedures and approaches,
- Some laboratory experiments,
- Influence of water quality on ciguatera development

Regional/National Research includes activities relevant to the objectives of the Science Plan, but may have other overall objectives. Regional/National research is co-ordinated at a regional or national level rather than by the SSC. At the moment, the Chinese programme CEOHAB has been endorsed by GEOHAB, the Canadian and the Atlantic European are being prepared.

Framework Activities are activities that are not research, but will facilitate the implementation of GEOHAB. They serve to enhance the value of research by ensuring consistency and communication among researchers. They include:

- Scientific Networking and the Co-ordination of Resources,
- Data Management,
- Specification of Protocols and Quality Control,
- Capacity Building,

- Co-ordination of Modelling Activities,
- Interaction with other Programmes and Project

Activities sponsored to date:

1) Workshop on the life cycles of HAB species (LIFEHAB), Calvia, ES, October 2001;
2) Workshop on Gene Probes, Galway, May 2002;
3) The EU-US Programme on Harmful Algal Blooms, Trieste, Sept. 2002;
4) Workshop on Real-time Coastal Observing Systems for Ecosystem Dynamics and Harmful Algal Blooms (HABWATCH) 11–21 June 2003.

The GEOHAB Programme provides the opportunity for scientists to participate in an important multidisciplinary programme of ecological and oceanographic research. GEOHAB recognises that different levels of participation are possible, as described in the Implementation Plan. Scientists are invited to participate in GEOHAB by designing research studies in keeping with the goals and objectives of GEOHAB, by applying for endorsement of such research, and by participating in framework activities including workshops. An application form may be downloaded on the following web sites:

http:\\ioc.unesco.org\hab\GEOHAB.htm

http:\\.www.jhu.edu\scor\GEOHAB_2001.pdf

HABWATCH

Workshop on Real-time Coastal Observing Systems for Ecosystem Dynamics and Harmful Algal Blooms (HABWATCH)

The development and implementation of methods, instrumentation and systems for real-time coastal observation of HABs was identified as a GEOHAB priority. Accordingly, as a Framework Activity, GEOHAB has endorsed and contributed to the planning of an advanced workshop on this topic (Villefranche-sur-Mer, France, 11-21 June 2003). Recent advances in instrumentation, communications and modelling capabilities have led to the design of prototype real-time observation and prediction systems for coastal ecosystems. Nevertheless, many of the new approaches in ocean observation are unfamiliar to potential users, particularly within the community of HAB researchers. Certain systems that have been developed for other purposes (e.g., estimation of primary production, diagnostics of nutrient limitation, taxonomic probes, etc.) may be critical to the achievement of GEOHAB goals. Through plenary lectures, contributed presentations, demonstrations and practical tutorials, real-time systems applicable for observation, modelling and prediction will be considered, with special emphasis on relevance to HAB dynamics. Underlying theory, a review of existing knowledge, and potential advances and constraints for future research will be presented and published in a proceedings volume (Monographs in Oceanographic Methods, UNESCO). Annotated presentations and other information will also be disseminated via CD.

17 DRAFT RESOLUTIONS

Proposed Terms of Reference for the WGHABD 2004 Meeting

The ICES-IOC Working Group on Harmful Algal Bloom Dynamics [WGHABD] (Chair J.L. Martin, Canada) will meet in Corsica, 5-8 April 2004 to:

a) collate and assess national reports and update the decadal mapping of harmful algal events for the IOC-ICES harmful algal database, HAE-DAT, on a regional, temporal and species basis;
b) Review the plans of the intercalibration workshop for comparison of new and classical techniques for determination of numerical abundance and biovolume of HAB species;
c) Review progress in computerised production of decadal maps from country reports, including the revision of reports already in the database covering the last 10 years;
d) Suggest the types of analysis that should be performed using the IOC_ICES HAEDAT dataset and identify problems and gaps in this dataset that must be rectified before the analyses can be conducted;
e) Review report on real-time observation workshop;

f) Review existing phytoplankton population dynamics models with particular emphasis on prediction of HAB events;

g) Review biological loss processes of selected HAB species;

h) Consider the environmental dynamics and impacts of individual phycotoxins and their metabolites enabled by new analytical technologies;

i) Report and discuss new findings.

The WGHABD will report within 4 weeks (May 1) to the Oceanography Committee and ACME.

Justifications for the proposed Terms of Reference

Priority	The activities of this group are fundamental to the work of the Oceanography Committee. The work is essential to the development and understanding of the effects of climate and man-induced variability and change in relation to the health of the ecosystem. The work of this ICES/IOC WG is deemed high priority.
Term of reference	Scientific Justification
ToR a: collate and assess national reports and update the decadal mapping of harmful algal events for the IOC-ICES harmful algal database, HAE-DAT, on a regional, temporal and species basis.	The work of collating the national HAE reports and building up HAEDAT and the associated maps is an activity which is unique to the WGHABD. HAE-DAT is not yet established enough to stand alone. A critical step forward is to make HAE-DAT operational with input from regions/countries outside the ICES areas as originally envisaged. In 2001-03 the aim is to include PICES and South America and Caribbean countries (via IOC/FANSA and IOC/ANCA) in HAE-DAT. It should be endeavoured to include HAE-DAT and the associated decadal maps as a contribution to GOOS, thereby embedding these activities in a permanent setting and securing continuity.
ToR b: Review the plans of the intercalibration workshop for comparison of new and classical techniques for determination of numerical abundance and biovolume of HAB species.	This workshop is a complex activity requiring algal cultures, field material and a variety of different methodologies and thus detailed planning is necessary for success. The WGHABD has prior experience in conducting intercalibration workshops (in situ growth rate measurements) and can help the SC with this process..
ToR c: Review progress in computerised production of decadal maps from country reports, including the revision of reports already in the database covering the last 10 years.	The WGHABD feels its important that the decadal maps be tied directly to the IOC-HAEDAT reports. Currently the decadal maps are produced manually with limited consistency and quality control. Procedures and techniques developed under this group may be applied to other regional activities (PICES, IOCARIB etc).
ToR d: Suggest the types of analysis that should be performed using the IOC_ICES HAEDAT dataset and identify problems and gaps in this dataset that must be rectified before the analyses can be conducted.	HAEDAT is an extremely valuable dataset that has not been extensively utilised. There are inconsistencies that must be corrected before database queries can be effective over the entire dataset.

ToR e: Review report on real-time observation workshop.	The WGHABD is particularly interested in many of the techniques that will be demonstrated in the workshop. Consideration by the WG will contribute to a realistic assessment of the observation systems in monitoring HAB dynamics and foster linkage to the relevant theme within the GEOHAB programme.
ToR f: Review existing phytoplankton population dynamics models with particular emphasis on prediction of HAB events.	Modelling exercises aimed at understanding HAB population dynamics have been developed with a number of different approaches. These range from conventional biomass and species specific analytical models to black box models such as those using fuzzy logic. There is a need to evaluate progress and the potential for use in observing the dynamics of different HAB events. To this effect the WGHABD will review the relevant topics in the 2003 SGMPBI report and provide a synopsis of invited presentations.
ToR g: Review biological loss processes of selected HAB species	Basic understanding as well as modelling and prediction of population dynamics/development of HAB species relies on detailed understanding of the biological loss processes in general as well as detailed knowledge of the species to species interactions between the HAB species and their "predators". The relevance of this new insight to better understanding of the bloom dynamics of selected HAB-species should be reviewed, with aims of better interpretation of monitoring data and improving of monitoring programmes and management strategies.
ToR h: Consider the environmental dynamics and impacts of individual phycotoxins and their metabolites enabled by new analytical technologies..	Basic understanding of the role, redistribution and ecosystem effects of many toxins produced by phytoplankton has been limited by inadequate analytical methods. Recent advances in ELISA and LC-MS are enabling many new insights, especially for the lipophilic toxins and complex classes such as microcystins and PSP toxins. The relationships can now be explored between toxin production in alga, redistribution to water and their uptake, metabolism and depuration by affected species such as shellfish. Such data will be useful to formulate models for toxin behaviour that can be linked to phytoplankton population models to produce risk management tools for HABs.
ToR i: Report and discuss new findings	The forum for presenting new findings has been an excellent tool for promoting the discussions about topics of general interest. There are obvious reasons to continue with this topic as a term of reference.
Relation to strategic plan:	This work is relevant to the quantifying of human impacts on the marine ecosystem,
Resource requirements:	None specific.
Participants:	The 2003 meeting attracted 29 participants, demonstrating the importance and interest of this Group within ICES and IOC.
Secretariat Facilities:	None

Financial:	None
Linkages to Advisory Committee:	The WG reports to ACME
Linkages to other Committees or Groups:	The WGHABD interacts with WGZE, WGPE, SGGIB, SGBOSV
Linkages to other Organizations:	The work of this group is undertaken in close collaboration with the IOC HAB Programme. IOC should be consulted regarding ToR or discontinuation of the WG prior to the ASC. There is a linkage to SCOR through the interactions of the IOC-SCOR GEOHAB Programme.

Background to TOR 7:

Biological loss processes are potentially important as factors in control of the population dynamic of HAB species. New HAB species are continuously being added to the list of HAB species. Recent progress has been made in understanding species specific biological control processes such as grazing, parasitism and virus/bacterial infections. Knowledge on biological mechanisms controlling or terminating algal blooms, such as bacteria- and virus infections, selective grazing and parasitism, has increased substantially in recent years.

18 WGHABD RECOMMENDATION

Development of Harmful Algal Studies in the Baltic Sea would provide a unique opportunity in studying HABs at the scale of one ecosystem. Difficulties in planning GEOHAB activities have risen in the two last years.

Implementation in the Baltic Sea of scientific activities related to HABs in coherence with the GEOHAB Science Plan is essential.

Therefore, WGHABD recommends that, despite its present difficulties, SGGIB be continued under the new chairmanship of M. Viitasalo.

19 CONCLUDING BUSINESS

The Working Group thanked Eileen Bresnan for hosting the 2003 meeting and thanked Eileen Bresnan and Henrik Enevoldsen for generously agreeing to act as Rappouteurs for the meeting. Balloting was held for the venue of the 2004 meeting of the WGHABD. The WG proposes to meet in Corsica 5–8 April 2004 – to be hosted by the Université de Liege, Belgium.

ANNEX 1: LIST OF PARTICIPANTS

Name	Institute (Address, Telephone, Fax)	E-mail
Andersen, Per	Bio/Consult as, Johs. Ewaldsvej 42-44, 8230 Aabyhoej, Denmark. Tel: 45-86258111 Fax: 45 – 86258173	pa@bioconsult.dk
Anderson, Don	Biology Dept., MS #32, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA Tel: 1 508 289 2351 Fax: 1 508 457 2027	danderson@whoi.edu
Belin, Catherine	IFREMER Rue de Lile d'Yeu, 44037 Nantes cedex, France Tel: +33 240 374 110 Fax: + 33 240 374 073	Catherine.Belin@ifremer.fr
Balode, Maija	Institute of Aquatic Ecology University of Latvia, 8 Daugavgrivas St., Riga, LV – 1007, Latvia Tel: +371 947 1203 Fax: +371 794 554	Maija@hydro.edu.lv
Bresnan, Eileen	FRS Marine Lab., Victoria Road, Aberdeen AB119DB, Scotland Tel: 44-1224 876544 Fax: 44-1224 295511	Bresnane@marlab.ac.uk
Collins, Catherine	FRS Marine Lab., Victoria Road, Aberdeen AB119DB, Scotland Tel: 44-1224 876544 Fax: 44-1224 295511	Collinsc@marlab.ac.uk
Cunningham, Carey	FRS Marine Lab., Victoria Road, Aberdeen AB119DB, Scotland Tel: 44-1224 876544 Fax: 44-1224 295511	Cunninghamc@marlab.ac.uk

Cusack, Caroline	Marine Institute, Galway Technology Park, Parkmore, Galway, Ireland Tel: +353 91 730400	caroline.cusack@marine.ie
Dahl, Einar	Institute of Marine Research, Flødevigen Marine Research Station, N-4817 HIS, Norway Tel: +47 370 59040 Fax: +47 370 59001	einar.dahl@imr.no
Enevoldsen, Henrik	IOC Science and Communication Centre On Harmful Algae, University of Copenhagen, Øster, Farimsgade 2D 1353 Copenhagen K, Denmark Tel: +45 33 13 44 46 Fax: + 45 33 13 44 47	Henrike@bot.bu.dk
Fraser, Sheila	FRS Marine Lab., Victoria Road, Aberdeen AB119DB, Scotland Tel: 44-1224 876544 Fax: 44-1224 295511	Frasers@marlab.ac.uk
Gallacher, Susan	Victoria Road, Aberdeen AB119DB, Scotland Tel: 44-1224 876544 Fax: 44-1224 295511	Gallachers@marlab.ac.uk
Gentian, Patrick	CREMA Place du Séminaire BP 5 17137 L'HOUMEAU FRANCE Tel: 05 46 50 06 30 Fax: 05 46 50 06 60	patrick.gentien@ifremer.fr
Goffart, A.	Université De Liege, Crescendeau, Ave Van Benedon 25, 4000 Liège, Belgium. Tel: +32 4 366 5076	a.goffart@ulg.ac.be
Hale, James	FRS Marine Lab., Victoria Road, Aberdeen AB119DB, Scotland. Tel: 44-1224 876544 Fax: 44-1224 295511	halej@marlab.ac.uk

Holland, Patrick	Cawthron Institute, 98 Halifax St. E. Nelson, New Zealand. Ph: +64 3 548 2319 Fax: +64 3 546 9464	Patrickh@cawthron.org.nz
Karlson, Bengt	Oceanographic services, Swedish Meteorological & Hydrological Institute (SMHI), Nya Varvet 31 SE-42671 Vastra Frolunda, Sweden Tel: +46-31-7518958 Fax: +46-31-7518980	bengt.karlson@smhi.se
Lacaze, Jean-Pierre	FRS Marine Lab., Victoria Road, Aberdeen AB119DB, Scotland Tel: 44-1224 876544 Fax: 44-1224 295511	Lacazejp@marlab.ac.uk
Lion, Monica	IOC-IEO Science & Communication Centre on Harmful Algae Bloom, Instituto Espanol de Oceanografia, Centro Oceanografico Ide Vigo, P.O Box 1552, 36200 Vigo, Spain Tel: +34 986 492111 Fax: +34 986 492003	vigohab@vi.ieo.es monica.lion@vi.ieo.es
Lukas, Bernd	University of Jena, Institute of Nutrition, Dept Food Chemistry, Dornburger Sw, 25, D-07743 Jena, Germany Tel: +49-3641/9-49651 Fax: +49-3641/9-49652	Bernd.lukas@uni-jena.de
Mackenzie, M	FRS Marine Lab., Victoria Road, Aberdeen AB119DB, Scotland. Tel: 44-1224 876544 Fax: 44-1224 295511	mackenziem@marlab.ac.uk
Martin, Jennifer	Fisheries & Oceans Canada, Biological Station, 531 Brandy Cove Rd., St. Andrews, NB, Canada E5B 2L9 Tel: +1 506-529-5921 Fax: +1 506-529-5862	martinjl@mar.dfo-mpo.gc.ca

Mazur, Hanne	Institute of Oceanography, University of Gdansk, Al. Marzalka Pilsudskiepo 46 81 – 378 Gdynia, Poland Tel: + 4858 – 660 – 16 – 21 Fax: + 4858 – 620 – 21 – 65	Biohm@univ.gda.pl
McCollin, Tracy	FRS Marine Lab., Victoria Road, Aberdeen AB119DB, Scotland. Tel: 44-1224 876544 Fax: 44-1224 295511	Mccollint@marlab.ac.uk
Naustvoll, Lars-Johan	Institute of Marine Research, Flødevigen Marine Station, N-4817 H/S, Norway Tel: +47 370 59017 Fax: +47 370 59001	larsjn@imr.no
Poelman, Marnix	RIVO, P.O. Box 77, 7700 AB Yerseke, Netherlands Tel: +311 36 72314 Fax: +3111 3573477	m.poelman@rivo.wag.urnl
Silke, Joe	Marine Institute, Galway Technology Park, Parkmore, Galway, Ireland Tel: +353-(0)91-730603 Fax: +353-(0)91-730470	joe.silke@marine.ie
Smith, Nikola	FRS Marine Lab., Victoria Road, Aberdeen AB119DB, Scotland Tel: 44-1224 876544 Fax: 44-1224 295511	bresnane@marlab.ac.uk
Vitasalo, Markku	Finnish Institute if Marine Research, P.O. Box 33, Fin – 00931, Helsinki, Finland Tel: +358 9 613945500 Fax: +358 9 61394494	Markku.vitasalo@fimr.fi

ANNEX 2: AGENDA OF THE MEETING

MONDAY, March 17

09:00	Welcome; housekeeping issues Introduction of participants Review by the Oceanographic Committee Adoption of agenda/ Terms of Reference
09:45	ToR b: review the reports and products of recent workshops on molecular probe technology (Caroline Cusack will give a review of Workshop; Pat Holland – Cawthron progress with DNA- probes)
10:30	***Health break***
11:00	ToR f: evaluate usefulness and feasibility of creating HAEDAT maps from HAEDAT database (Catherine Belin/Monica Lyon)
12:30	***LUNCH***
13:30	ToR h: review previous submissions to HAEDAT with view to improving accuracy of information (Monica Lyon and additional participation)
15:30	***Health Break***
16:00	Country reports (2002)
18:00	Adjourn for the day

TUESDAY, March 18

09:00	ToR g: review the application of methods for the detection and quantification of phycotoxins in eucaryotic microalgae and cyanobacteria, and related components of pelagic food webs, in coastal marine and brackish waters in the ICES area (Pat Holland – development of LC-MS methods for ASP, DSP and NSP toxins as applied to alga, water and shellfish) Additional, relevant information welcome from participants.
10:30	***Health break***
11:00	ToR c: Evaluate the outcome of the "Understanding eutrophication effects on phytoplankton Workshop". Proceeding have not been published but summary was submitted to ICES (C:05) – available at http://www.ices.dk/reports/occ/2002/ (Any relevant information from participants)
12:30	***LUNCH***
13:30	Tor d: effects of HABs on survival and fecundity of wild fish, effects on recruitment. (Markku Viitasalo to present cyano-fish problematics; Pat Holland with comments on *Pfeisteria*).
14:30	ToR a: compare and assess historical and retrospective datasets.... (Bengt Karlson to present on historical data, Dinophysis and DST; Pat Holland - phytoplankton and shellfish database and historical perspectives on *Karenia, Gymnodinium*, etc.; Jennifer Martin - shellfish toxicity, community structure and *Alexandrium*) Additional, relevant information would be welcome from participants
15:30	***Health Break***
16:00	Country reports, report writing
18:00	Adjourn for the day

WEDNESDAY, March 19

09:00	ToR i: report on the ECOHAB-EUROHAB Workshop on joint research on HABs (Patrick Gentien will give an update)
10:00	SGGIB (Study Group on GEOHAB Implementation in the Baltic Sea) (Markku Viitasalo – Chair)
10:30	***Health break***
11:00	ToR e: prepare a resolution for a workshop on "New and classic techniques for the determination of numerical abundance and bio-volume of HAB species" (Pat Holland to discuss Cawthron experience with phytoplankton monitoring with depth increment samples; Additional, relevant information would be welcome from participants)
11:45	ToR j: consider the potential of ITIS as a common taxonomic system within ICES
12:30	***LUNCH***
13:30	New Findings
14:30	Report writing
15:30	***Health Break***
16:00	Report Writing; subgroup and plenary sessions to be decided on '*ad hoc*' basis
18:00	Adjourn for the day

THURSDAY, March 20

09:00	Adoption of all sections of the report
10:30	***Health Break***
11:00	ToRs for 2004; meeting location for 2004
12:30	***LUNCH***
13:00	Other business; completion of the report
16:00	Meeting adjournment