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5-8 April 2011

Gothenburg, Sweden



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Executive summary

Highlights

The 2011 meeting of the IOC-ICES Working group on harmful algal bloom dynamics was held in Gothenburg Sweden from 5 to 8 April at the Sweden's Meteorological and Hydrological Institute or SMHI. The meeting was successful with 22 scientists from 12 countries attending. A full schedule of terms of reference were worked through and this report is a summary of the deliberations of the group.

National Reports

The National Reports for 2010 were presented to the group on the status of HABs from all 12 countries that participated in this year's meeting, reports of presentations are given in this document. In general 2010 was a typical year across the ICES region, with the usual variability in the occurrence of HABs from country to country reported. Some of the highlights included:

France: Some unknown positive lipophilic bioassay results were detected in the Arcachon and the south Atlantic coast, but also some Medeterranean Lagoons were impacted with these unusual results. Low levels of AZA were detected in western Brittany. Low levels of Yessotoxins were also observed in some areas. PSP was lower than previous years again however *Alexandrium* is still present, but not at bloom levels. ASP was present in spring and present at considerable levels in several species of shellfish.

The Netherlands: 8th year in a row without any toxic occurrences.

Sweden: No major harmful algal blooms occurred in the area in 2010 other than the usual blooms of cyanobacteria.

Finland: Cyanobacterial blooms are the problem most notable in Finnish waters. In 2010 localized surface aggregations of predominantly *Aphanizomenon* spp, occurred locally. Cyanobacterial abundances started to increase in Finnish waters later than normal due to low water temperatures at the beginning of the summer. Cold weather was also the reason for the unusually low proportion of warm water adapted *Nodularia spumigena* in the early summer community.

Germany: In 2010 bivalve shellfish samples (78) were analyzed from the North Sea and the western Baltic Sea (16) for the presence of phycotoxins (primarily ASP-, DSP-, and PSP-type). None of the samples showed appreciable amounts of toxins apart from one collected in Sylt which had Azaspiracid, the origin of these was thought to have been from Ireland. The typical Cyanobacterial blooms, especially of the potentially toxic species *Nodularia spumigena* were present in the Baltic waters of Germany in 2010 causing some unsightly and nuisance fouling on touristic beaches.

USA: PSP was recorded along the West coast states of Alaska , Oregon and Washington which had very significant PSP year with many sites in North Puget Sound and Strait of Juan de Fuca hitting new records for toxicity levels. On the East coast in contrast no PSP toxicity was recorded in New Hampshire. Likewise no toxicity was recorded in Massachusetts. Maine experienced PSP toxicity but in much lower levels than previous years. **ASP** resulted in approximately 100 Sea Lion mortality / illnesses in California, with this toxin posing less of a problem further north in Oregon and Washington. **NSP** from K. brevis was present along the Florida coast but did not cause problems in Texas in 2010. Texas did however experience some Dinophysis ovum resulting in Okadaic Acid shellfish closures.

Canada experienced some salmon mortalities following blooms of *Heterosigma akashiwo*, *Chaetoceros concavicorne* and *Pseudochatonella* cf *verruculosa* along the west coast. No Mortalities were reported on the East coast. PSP in shellfish resulted in major shellfish shellfish harvesting areas being closed on the East Coast and alson in the St Lawrence Estuary and Bay of Fundy.

Ireland had problems from DSP whichdominated the south west for the summer months and this persisted through the autumn and into the winter in some areas.

UK: (Northern Ireland) Alexandrium, Dinophysis and Pseudonitzschia were present across the 31 sites sampled fortnightly, however only 2 samples of shellfish had DSP and ASP was confined to Scallop samples. (England and Wales) were also lightly affected by PSP following elevated counts of Alexandrium which was found up to concentrations of 292 000 cells/litre. DSP was detected in 20 samples following Dinophysis counts above 100 cells/l. There were no closures of shellfish production areas in England and Wales in 2010 due to the presence of ASP toxins even though Pseudonitzschia were present at considerable numbers on 14 occasions. (Scotland) The distribution of Alexandrium around the Scottish coast was similar to previous years and while some PSP was detected in some shellfish samples there were no closures for PSP toxins above the closure limit, DSP toxic events were widespread around Scotland in August.

Spain: The usual shellfish poisoning events affecting the shellfish industry were registered during 2010. Some exceptional events were: i) Report of the first ASP event in Catalonia; ii) Huge densities of *Ostreopsis* both attached to macroalgae and in floating scums in Llavaneres beach also in Catalonia; iii) Record densities of *D acuminata* in hose samples from Ria de Pontevedra (Galician Southern Rias).

<u>New findings</u> that were reported to the working group included: The impact of algal toxins on key copepods in the North East of Scotland; Trends and patterns in phytoplankton populations from monitoring in the Bay of Fundy (eastern Canada); The failed forecast of a major *Alexandrium fundyense* bloom in the Gulf of Maine in 2010 and feeding experiments and uptake of the toxins of *Azadinium spinosum* by Blue Mussels (*Mytilus edulis*) in Ireland.

<u>New Chair</u> of the group Dr Bengt Karlson from the SMHI in Sweden was selected for the period 2012 to 2014.

1 Welcome and opening of the Meeting

The **ICES-IOC Working Group on Harmful Algal Bloom Dynamics** meeting for 2011 was held in Gothenburg, Sweden from 5 to 8 April. Our local hosts for this meeting was the Sveriges Meteorologiska och Hydrologiska Institut (Sweden's Meteorological and Hydrological Institute or SMHI), Oceanographic Unit, and the venue was at the Nya Varvet Studios which is a small conference centre located near the SMHI Oceanographic Unit. On behalf of the host and the venue local organisers Bengt Karlson (SMHI) and the working group Chair, Joe Silke (Marine Institute, Ireland), opened the meeting and welcomed the participants to the working group meeting.

In the opening of the meeting it was recognised that this expert group meeting provides an excellent opportunity to meet and collectively address the terms of reference that we have set ourselves and have been passed to us through ICES and IOC. Both ICES and IOC have recognised the importance of the work carried out by our working group. ICES have stated on their website that the activities of this group was fundamental to the work of the Oceanography Committee, and this has been the view of the new Steering Group on Human Interactions on Ecosystems to whom we now report. The work of this ICES-IOC WG is deemed high priority.

The agenda was agreed and Eileen Bresnan (UK) and Jennifer Martin (Canada) were elected as joint rapporteurs.

22 Scientists representing 12 countries participated in the meeting. For the first time we included 2 participants from PICES to attend our meeting, and we also had 2 representatives from ICES Secretariat to attend. The list of participants is presented in Annex 1. The meeting agenda is presented in Annex 2. The meeting was very successful and with a full agenda of challenging and diverse terms of reference. An ICES SharePoint site was made available before and during the meeting. This proved to be a valuable tool to speed up the work and make exchange of information more efficient. Over the course of the 5-day meeting the group made presentations addressing the terms of reference this report presents a summary of these and subsequent discussions. Along with ICES, the IOC is a joint organiser of WGHABD, and provides valuable interaction regarding data collection and management of HAB data through the development of the HAEDAT database. As co-ordinators of the Intergovernmental Panel on HABs, the participation of IOC in WGHABD forms an important linkage between the working group and this panel. Last year a request from IPHAB to examine the importance of HAB species transferral through ballast water was passed on to WGHABD and along with the working group on ballast and other ship vectors, WGBOSV a workshop was held and the report of this was presented to WGHABD for discussion. The IOC also takes responsibility to promote the working group among IOC Member Countries outside the ICES area to attend WGHABD and some years is in a position to offer travel support. In 2011 there were no attendees from outside the ICES area however linkages to other ICES and IOC groups in developing countries are maintained by the interactions between IOC and WGHABD.

WGHABD is an important forum for ICES and IOC to review and discuss HAB events and to provide advice and updates on the state of HABs in the region on an annual basis. It also facilitates interaction between scientists working in diverse areas of HAB science and monitoring and provides a useful forum for interchange of useful terms of reference on various approaches to HAB research. The present working group was established in 1994 following a study group on the Dynamics of Algal Blooms, established two years earlier; however its origins go back further into the 1980s and evolved from other study groups within ICES. The group

In the opening session the Chair, Joe Silke (Ireland), gave a summary of the presentation of the WGHABD 2010 report to the parent Steering Group on Human Interactions in the Environment (SSGHIE) at the ASC meeting in Nantes, France. The report was very well received and feedback indicated the report was well organised, informative and the meeting was well attended. The participants then introduced themselves and gave a short review of their scientific activities.

2 Adoption of the agenda

The group reviewed the agenda (Annex 2) and this was adopted without any change.

3 Terms of Reference

At the 97th Statutory Meeting (2010), Nantes, France, the ICES Science Committee approved the WGHABD 2010 Terms of References as follows:

2010/2/SSGHIE08: The **ICES–IOC Working Group on Harmful Algal Bloom Dynamics** (WGHABD), chaired by Joe Silke, Ireland, will meet in Gothenburg, Sweden, 5–8 April 2011 to:

- a) Review progress in entering data onto the database;
- b) Review draft chapters of the proposed CRR;
- c) Review developments in automated HAB sampling devices in FerryBox systems and on other platforms;
- d) Discuss the need for and logistics of a demonstration workshop on automated in situ techniques for quantitative harmful algal bloom species analysis;
- e) Review output from the intersessional meeting of members from WG HABD and WG BOSV to generate a list of HAB species with the potential to become invasive through transportation in ship's ballast and other vectors;
- f) Review the pre-column oxidation technique for routine analysis of PSP toxins in shellfish (Lawrence method);
- g) Review the ICES special session on HABs that will be held during the Annual Science Meeting from 20–24 September 2010 in Nantes, France (La Cité des Congrès);
- h) Draft aims and propose a structure for a joint meeting with the ICES Working Group on Physical–Biological Interactions (WGPBI) during 2012;
- i) Report on new findings in the area of harmful algal bloom dynamics;
- j) Deliver National Reports on harmful algal events and bloom dynamics for the year 2010;
- k) Evaluate potential for collaboration with other EGs in relation to the ICES Science Plan and report on how such cooperation has been achieved in practical terms (e.g. joint meetings, back-to-back meetings, communication between EG chairs, having representatives from own EG attend other EG meetings).

4 Term of Reference A

4.1 Review progress in entering data onto the database

Monica Lion (IOC Spain) gave a presentation on the HAEDAT system

This indicated that while a number of countries had entered data into the system, there were still considerable a considerable number of member countries that were not up to date or had submitted no data.

While the site has obtained ~5000 visits, the system is still password protected preventing access to the data. A discussion of the map facility raised issues with linking the data to this format. No easy solution to this was identified, barring time consuming manual linking of individual records. A discussion of when to make the data set public ensued.

It was agreed that the HAEDAT be opened before end of April. PICES will be asked to agree to this. It was agreed that all data provider in ICES be contacted by IOC and ICES with a strong urge to submit data before 1 October 2011 and that this letter be accompanied with examples of the new maps (Ifremer) with a signature clearly showing the countries where data is missing or where no data at all have been received.

For the 2012 mtg the ICES and IOC data managers will be invited to attend to continue discussions on data input and overall development of HAEDAT.

Catherin Belin (IFREMER France) gave a subsequent presentation on new decadal maps of toxin distributions. The issue of missing data was discussed , and that non events are not recorded. A disclaimer clarifying the limits of the data set was agreed to be added to the maps. Subsequently an action to open the HAEDAT data base to the public one month from now (6 May 2011) was agreed, conditional on approval of the PICES contributors.

Neil Holdsworth of the ICES data centre in Copenhagen gave a presentation outlining the activities of the data services unit and its relevance for WGHABD.

The presentation covered the following areas:

- Desirable data set traits
- The various types of ICES data sets
- OBIS and its links to ICES data sets
- The ICES portal Ecosystem Data
- The GIS spatial visualisation facility and ICES geo-network
- European infrastructures such as MyOcean

Subsequent discussion topics included:

- The links with IOC
- The lack of lower trophic levels in ecosystem data sets, with the issue being identified as a lack of supplied data, perhaps due to a lack of a common format for data collection a need for simpler protocols was identified
- The geographical extent of the ICES domain it was clarified that the data centre would accept data from anywhere as long as it was collected by and ICES country
- The open data policy

Finally there was a short demonstration of how to access data.

5 Term of Reference B

5.1 Review draft chapters of the proposed CRR

Discussions were held during the working group meeting and further communications between the lead chapter authors are planned for later in 2010 to make progress on the drafting and compiling of data necessary for the Cooperative Research Report. The use of the HAEDAT data was recognised as being important for report and the compilation of national data into this database was urged.

Bill Anthony of the ICES publication group gave a presentation to inform WGHABD members of ICES protocols prior to the drafting of the proposed CRR.

Topics included:

- The format of documentation and the re-formatting carried out by ICES
- Figure quality
- Referencing
- Grammatical style
- Typography
- Copy editing

The subsequent discussion covered the following topics:

- Inclusion of photographs
- Timelines for manuscript submission and publication approximately eight weeks from submission to publication

A deadline of the end of July was set for the WG to complete the draft CRR. Subsequent to the working group meeting however, this was extended to November to allow for the chapter leads to assemble information and draft chapters to incorporate information from ICES member states.

6 Term of Reference C

6.1 Review developments in automated HAB sampling devices in FerryBox systems and on other platforms

Bengt Karlson presented different automated sampling systems from the perspective 'Why automated water sampling?' Water samples for microscopy is usually a necessity for species identification to improve temporal and spatial resolution and to lower cost compared to e.g. research vessels. The CPR – the Continuous Plankton Recorder is constructed for sampling of multicellular zooplankton, however, it is not fully quantitative and likely to underestimate small and fragile HAB species. SAHFOS is currently developing a water sampler in collaboration with CEFAS and funding from DEFRA for use on CPRs. The intention is that this instrument will collect and store 10 samples of 125ml of seawater with preservative if required. The samples will be taken at timed intervals along a CPR route. It is hoped that this instrument will reveal information on Harmful Algal Bloom species and other delicate phytoplankton taxa that may be under-estimated by CPRs.

The FerryBox water sampler, is also used in sewage treatment plants.

Denise Smythe-Wright (NERC, UK) presented by Skype automated sampling for phytoplankton community structure. Automated sampling for phytoplankton community structure is interesting because primary production by plants and algae forms the base of all marine ecosystem processes and are liable to change over next century, because Phytoplankton are fundamental to climate change studies e.g. uptake and export of carbon, and because a wide range of micro and macro-algae are known to produce halogenated trace gases through their metabolic processes. Working alongside a standard FerryBox system NERC have developed an automated robotic system with trace Gas equipment. The ferry operates two return journeys per week throughout the year and crosses a number of oceanic and biological provenances, thereby providing data over a variety of temporal and spatial conditions. The Pride of Bilbao ferry route starter April 2002 and operated till September 2010. In comprised Online "Web sensors" @ 1Hz, conductivity, temperature and chlorophyll-fluorescence and at @30secs O2 (start 2005) and pCO2 (mid 2005-7). Monthly water samples were taken from February 2003 for NO₃, Si, PO₄, Chl a, Salinity, O₂ (2004 -), and alkalinity, TCO₂ (2005-), pH (2007-). The robotic arm collects three types of sample. An injection system fills racks of amber glass bottles and cryovials (containing appropriate preservatives) for taxanomic identification by microscopy and flow cytometry. A filter head which filters seawater samples through a series of filter holders for plant pigment analysis. Where appropriate the robotic arm moves the samples to -20 °C and -80°C freezers. Trace gas equipment consists of an autonomous membrane-inlet purge and trap system, taking samples from the ship's seawater intake, coupled to a GC-MS which is installed within a specially designed laboratory area aboard the MV Pride of Bilbao. Co-axial stainless steel/silicone tubes act as a membrane for the gas transfer from sea water and the gas is trapped and pre-concentrated using a carboxen trap. All coupled to an Agilent 6890GC/5973 MSD, fitted with a 30 m CB Sil-5, 0.32 mm id column. The system, including data collection, is PC controlled and the carrier gas is helium throughout. Future work includes awaiting results of funding round for Arctic work; to develop a smaller more compact robot system, and to install on Tromso-Svalbard Ferry route. The aim is to quantify seasonal dynamics in phytoplankton and its control on carbon dioxide concentrations; to compare and validate satellite ocean colour data; and to develop biogeochemical models of arctic In collaboration with Norwegian colleagues.

Automated filtering in situ includes so called Environmental Sample Processors (ESP). There is also automated sampling in plastic bags from Envirotech AquaMonitor used e.g. on mooring in Liverpool Bay. *In situ* imaging flow cytometers are manufactured by FlowCam. A new *in situ* version, FlowCytoBot is described in Olson *et al.* Deep-Sea Research I 50 (2003) 301–315, Olson and Sosik Limnol. Oceanogr.: Methods 5, 2007, 195–203, Campbell *et al.*, J. Phycol. 46, 66–75 (2010).

A discussion followed on the importance of sharing practical experience of using devices in the field.

D. Anderson informed about an automated sampler under development with funding from 'US Stimulus' which has allowed McClaine Inc to develop the device. It is equipped with ESP, two way communication, CTD, nutrient sensor; Challenges in data communication and power supply. Expected cost is several 100 000 USD.

MBARI also developing and now selling a sampler, but there was concern whether it is ready for use and reliable. The intention is to develop it as an AUV.

Other systems noted as in use were a Teledyne® ISCO [™] sampler (Ireland), which can be triggered remotely and takes a water sample. Triggering can be automated on

the basis of for instance florescence and salinity. In Helgoland there are stationary FerryBox system on land, In the UK, mid Irish Sea a system has been in operation since 1996. It measures plankton by collecting water samples, in situ measurement of nutrients. It was also noted that there are 6000 ARGO samplers in open ocean but not in coastal waters. ARGO includes CTD, O2 sensors, Chl sensors. The Coast Float (Optimare) is equipped with optical sensors. A major challenge in the development is movement in short water columns and this is not fully resolved.

The cost associated with using automated systems (it, power, communication, mooring, specialists, ship time, etc) is rarely considered in full and the total cost may be considerable. Less high tech may give more and better date at much lower cost. It was recognized that users need to clearly define what needs to be measured. We need a discussion of that to help guide technology development.

7 Term of reference D

7.1 Discuss the need for and logistics of a demonstration workshop on automated in situ techniques for quantitative harmful algal bloom species analysis

The group discussed the logistics and reasons for organizing such a workshop. It was felt that it is hard to convince manufacturers to bring equipment because shipping of instruments is very costly. At a minimum it was decided that it should be held in the US as most manufacturers in the US, Woods Hole area. The group asked how we ensure it will not just be glossy presentations by manufacturers. We need experience with full cost application and experience with performance. A goal of the workshop would be: to set out a demonstration of the current status of in-situ and other new technologies for monitoring and management technologies, to ask if this equipment delivers what they promise? Manufacturers would also have to provide real costs of operation (updates, support costs).

The development of this may continue at next year's meeting and a ToR for the following year be proposed.

One suggestion was that this could be the 3rd HABTech workshop to be convened in conjunction with an International Conference on Harmful Algae.

8 Term of Reference E

8.1 Review output from the intersessional meeting of members from WG HABD and WG BOSV to generate a list of HAB species with the potential to become invasive through transportation in ship's ballast and other vectors.

The ICES Workshop on harmful phytoplankton that could potentially be transported or introduced by ballast water (WKHABAL) met in Copenhagen, Denmark, on 14–15 October, 2010 and was attended by 11 participants from Denmark, Ireland, Sweden, the United Kingdom and the United States of America. The purpose of the meeting was to identify phytoplankton that could be transported via shipping vectors so that potential future invasive species could be identified and the risk managed. The meeting arose from a request from the IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB) to examine the importance of HAB species transferral through ballast water. The request was passed through ICES to WGHABD and the working group on ballast and other ship vectors, WGBOSV. Both groups convened a workshop was held and the report of this was presented ICES. The outcome and recommendations were discussed by WGHABD at this present meeting. The workshop produced two lists of phytoplankton, one marine and one freshwater based on the IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae and on the Great Lakes Invasive Species List respectively. The marine list focussed on known toxic species and also included some nuisance (but non toxic) species, the freshwater list was focussed on species known to have been transported by shipping but that were not necessarily problem species in terms of toxicity. The difference in the focus of the lists was owing to the availability of information. These lists were then expanded to include information regarding the characteristics of the species that may make them more likely to survive a long journey in a dark ballast tank. This included characteristics such as cyst forming ability and whether the species was phototrophic or heterotrophic. Where this information is known it could help identify which species were more likely to survive transport in ballast tanks. In addition to the lists the group also prepared background information that is contained in the body of the report to support the information in the table. This included a case study of a toxic marine dinoflagellate that may have been introduced by ballast water and more detailed background to the freshwater species list.

The group acknowledged that there is a lack of information for many species and that this limits the amount of detail that can be provided for some species. However, these lists are a good starting point and can be updated and adapted as more information and feedback from users is incorporated.

9 Term of Reference F

9.1 Review the pre-column oxidation technique for routine analysis of PSP toxins in shellfish (Lawrence method)

Rapporteur: Allan Cembella (AWI, Bremerhaven, Germany)

9.1.1 Contributions by: Gemma Giménez & Jorge Diogéne (IRTA, Tarragona)

The AOAC mouse bioassay was the only certified reference method for detection of PSP toxins in shellfish for many decades. In spite of the fact that this bioassay has been well calibrated and is still widely employed for seafood quality control for the presence of these neurotoxins, concerns over animal rights, and technical factors such as low precision, specificity and the relatively high detection limit (approximately 400 μ g kg⁻¹ shellfish tissue) has led to the search for alternative methods. Most chemical analytical methods for PSP toxins are based upon the separation of toxin analogues by liquid chromatography coupled with fluorescence detection of their derivatives (LC-FD), although liquid chromatography coupled with mass spectrometry (LC-MS) is also frequently used, particularly for confirmatory analysis. The LC-FD methods for PSP toxin analysis can be divided into two major groups: pre-column oxidation procedures involve oxidation of the toxins to fluorescent derivatives prior to separation on the analytical column, whereas post-column oxidation methods first resolve the toxin components and then fluorescence derivatisation is carried out in a postcolumn reaction module before fluorescence detection. Both techniques require the oxidation of toxins to fluorescent analogues because the toxins do not possess a native fluorochrome.

In spite of the widespread application of LC-FD methods for analysis of these toxins in shellfish, plankton and other marine fauna in a research mode, until recently such methods have not been generally implemented into seafood safety regimes as a replacement for the mouse bioassay. The LC-FD methods have been considered as supporting technology, as an adjunct or complement to the AOAC mouse bioassay, within most regulatory frameworks. The technical reasons for this delayed implementation are manifold and complex, but include the limited availability of certified toxin standards and reference materials and incomplete knowledge of specific toxicity of toxin analogues (both problems now largely alleviated), as well as the cost and complexity of the instrumentation and technical training required for LC-FD. A further constraint has been the reluctance of regulatory authorities to accept methods based on toxin concentrations rather than on "toxicity", as the analytical methods do not detect unknown toxins or measure toxicity.

For many years, the implementation of LC-FD methods into regulatory regimes for shellfish toxins was also delayed by the lack of inter-laboratory calibration trials towards development of standardized and certified methods. Earlier attempts to conduct inter-laboratory calibration studies, particularly with the post-column approach, did not yield convincing and coherent results, due to differences in equipment, choice of analytical column and reagents, and the tendency of analysts to develop their own individual optimization strategies as variations on the basic theme. Nevertheless, concerted focus on the pre-column oxidation approach in inter-calibration exercises did lead to the certification of the Lawrence method for analysis of PSP toxins for regulatory purposes (AOAC Method 2005.06; AOAC, 2005), although it has only recently begun to be widely applied in regulatory protocols. One of the many variants of the post-column oxidation method has undergone a detailed single laboratory validation (van de Riet et al., 2009), and through a multi-laboratory collaborative validation under the auspices of the AOAC is also now (since 2011) recognized as an official method. Many countries are switching rapidly to the implementation of either the pre- or post-column method for PSP toxin regulation in shellfish. For example, in the US, the post-column approach has been accepted as a Type IV National Shellfish Sanitation Program (ISSC, 2007) method for the determination for these toxins in shellfish for regulatory decisions (ISSC, 2009).

Now that AOAC certification has been obtained for both pre- and post-column oxidation methods, the decision to adopt either approach is subject to a number of technical and logistical considerations. In general, pre-column oxidation methods have the advantage of employing simpler and less expensive hardware, because a postcolumn reaction system is not required. However, without a series of different oxidation procedures, thereby requiring multiple injections into the chromatographic system, it is not possible to determine the native toxin profile of the sample. This in turn complicates the calculation of toxicity. A recent review addresses the comparison of the pre- and post-column approaches in the latest configurations for toxin analysis in shellfish, based upon multiple laboratory trials (DeGrasse et al. 2011). The conclusions can be summarised as follows: the pre-column method offers an easier chromatographic set-up and slightly better correlation with the mouse bioassay, but also requires more complicated and time-consuming sample preparation to yield the complete toxin profile and demands higher skill in the data processing and interpretation. An automated preparation system for solid phase extraction of samples highly desirable for the pre-column oxidation procedure, for efficiency and high throughput.

Detailed comparisons are also available from national and international projects to evaluate these respective methods. For example, the national JACUMAR (Sea Harvest Advisory Board) in Spain has coordinated such a project, with participation of institutions from the three main aquaculture regions (Andalucia, Catalonia and Galicia). The main participants include Jorge Diogéne, Coordinator (IRTA, Tarragona, Catalonia), Luz Mamán (LCRRPP, Huelva, western Andalucía), José M. Franco (IIM, CSIC, Vigo, Galicia) and Juan Blanco (CIMA and INTECMAR, Vilagarcia de Arousa, Galicia). The project focuses on the comparison of available methodologies (mouse bioassay, HPLC pre-column, HPLC post-column, LC-MS/MS, cellbased assays, commercial kits) for the evaluation of PSP toxins and the implementation of these methodologies for the optimal management of the shellfish industry in Spain. Analyses are focused on autochthonous shellfish species (mainly the mussel, *Mytilus galloprovincialis*) and on toxins produced by phytoplankton species responsible for PSP outbreaks in Spain: *Alexandrium minutum* and *Gymnodinium catenatum*.

The Spanish project has not finished, but differing views have arisen during its execution (communicated here by G. Giménez and J. Diogéne, IRTA, Tarragona). Regarding the HPLC pre-column oxidation method, participants have various opinions depending on the toxin profile that they usually find. The toxin profiles produced by *Alexandrium minutum* are less varied and complicated than those produced by *Gymnodinium catenatum*, thus affecting the performance of the analysis (faster, clearer, etc.). Laboratories in charge of monitoring programmes have serious doubts about the correct implementation of this method for routine analyses, because they must work fast (with short time between sampling and results) and under quality rules (ISO 17025, etc.). This adds to the cost due to the required controls and the number of points to validate. Nevertheless, the approach seems appropriate for responding to the EU requirement of reducing the use of animals for control purposes.

Specific comments are provided here by J.M. Franco, IIM, CSIC, Vigo (unpublished commun.). The pre-column oxidation method is very time consuming because a solid phase extraction with two cartridges plus two derivatizations - one with oxygen per-oxide (H₂O₂) and the other with periodate - are required to ensure resolution of the qualitative issues. The protocol may be shortened in the case of samples with a known toxin profile, and depending on the profile one of the cartridge clean-up steps and one oxidation procedure may be eliminated.

Oxidations generate other fluorescent peaks in addition to those specifically characteristic for each toxin peak, i.e. depending on the toxin standard as many as three peaks for each may be produced. Furthermore, different oxidation products generated by some isomers are not well separated. Thus, GTX4 and GTX1 elute together, as do GTX3 and GTX 2, dcGTX3 and dcGTX2, and the C-toxins (CXs). Therefore, the toxicity values in μ g STXeq \cdot kg⁻¹ mollusc flesh are only approximate. Extraction is carried out with acetic acid, rather than with 0.1 M hydrochloric acid, so results are difficult to compare directly with those obtained from mouse bioassays.

In comparison, the post-column oxidation method allows better toxin separation and quantification (each has only one characteristic peak) and therefore in principle provides more accurate estimations of μ g STXeq · kg⁻¹ mollusc flesh. The same extract as that prepared for the AOAC mouse bioassay can be analysed. The method requires two additional pumps for derivatization and a post-column reactor, thereby adding to the equipment and operating costs. The main (still unsolved) inconvenience is that shellfish sample extracts are usually very impure dirty, a fact that considerably shortens the mean life of the analytical columns (each column costs between 450 and 600€). Each column will yield optimal separations for only 100–200 samples, therefore a major effort must be made to extend the mean life of the columns.

Diverse opinions regarding the appropriate replacement method for the AOAC mouse bioassay will undoubtedly continue to manifest within regulatory frameworks. Nevertheless, the selection of pre- versus post-column oxidation chromatographic methods are now likely to be based more upon practical and logistical considerations, including knowledge and expectations of regional and specific toxin profiles in shellfish, than on the analytical validity and scientific merit of the respective methods.

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9.1.2 Comments from participants in the project: Comparison of methods for the determination of Paralytic Shellfish Poisoning (PSP) toxins in shellfish. Application to Spanish Aquaculture

National JACUMAR (Sea Harvest Advisory Board) coordinated Project, with participation of institutions from the 3 main aquaculture regions (Andalucia, Catalonia and Galicia) in Spain:

Participants (PIs and institutions):

- 1) Jorge Diogéne, Coordinator (IRTA, Tarragona, Catalonia)
- 2) Luz Mamán (LCRRPP, Huelva, western Andalucía))
- 3) José M. Franco (IIM, CSIC, Vigo, Galicia))
- 4) Juan Blanco (CIMA and INTECMAR, Vilagarcia de Arousa, Galicia)

The aim of this project included the evaluation of the Lawrence pre-column method as analytical tool for the monitoring of PSP toxins in three areas with important shellfish harvesting activities (wild shellfish bancs and rafts)

9.1.2.1 Comments from Gemma Giménez & Jorge Diogéne (IRTA, Tarragona)

The project focuses on the comparison of available methodologies (mouse bioassay, HPLC pre-column, HPLC post-column, LC-MS/MS, cell-based assays, commercial kits) for the evaluation of Paralytic Shellfish Poisoning (PSP) toxins and the implementation of these methodologies for the optimal management of the shellfish industry in Spain. Analyses are focused on autochthonous shellfish species (mainly mussel, *Mytilus galloprovincialis*) and on toxins produced by phytoplankton species responsible for PSP outbreaks in Spain: *Alexandrium minutum* and *Gymnodinium catenatum*.

The project has not finished, but some opinions have arise during its execution. Regarding HPLC pre-column method, participants have different opinions depending on the toxin profile that they usually find. Thus, toxins produced by *Alexandrium minutum* are less varied and complicated to solve than those produced by *Gymnodinium catenatum*, thus affecting the performance of the analysis (faster, clearer, etc...). Laboratories in charge of monitoring programmes have serious doubts about the right implementation of this method for routine analyses, because they must work fast (with short time between sampling and results) and under quality rules (ISO 17025, etc.; that add cost due to the required controls and the variety of points to validate). Nevertheless, the approach used by CEFAS seems a good way for responding to the EU requirement of reducing the use of animals for control purposes.

9.1.2.2 Comments from José M. Franco (IIM, CSIC, Vigo)

Pre-column method: The protocol takes too long. You need to make a solid phase extraction with two cartridges plus two derivatizations - one with oxygen peroxide (H₂O₂) and the other one with periodate - to ensure the qualitative issues. The protocol may be shortened in the case of samples with a known toxin profile, and depending on the profile itself you may be able to eliminate one of the cleaning cartridges and one oxidation.

Oxidations generate additional peaks to the toxin peaks, i.e. depending on the standard you use you may get up to 3 peaks. Different oxidation products generated by some isomers do not get well separated. Thus, GTX4 and GTX1 come out together, as GTX3 and GTX 2; dcGTX3 and dcGTX2, and the CXs do. Therefore, you have to make approximations when you estimate the values of μ g STXeq \cdot kg⁻¹ mollusc. Extraction is carried out with acetic acid, so then results cannot be compared with those obtained from mouse bioassays.

Post-column method: allows a good separation and quantification and therefore, more accurate estimations of μ g STXeq \cdot kg⁻¹ mollusc. The same extract than that prepared for the mouse bioassay can be used. The method requires 2 pumps for the derivatization and a reactor. This makes it a bit more expensive but this should not be considered too important. The main (still unsolved) inconvenience is that samples are usually very dirty, a fact that considerably shortens the mean life of the columns (each column costs between 450 and 600€). One column will last for 100–200 samples. We are working to overcome this problem in order to extend the mean life of the columns.

10 Term of Reference G

10.1 Review the ICES special session on HABs held during the Annual Science Conference in Nantes, France, 20–24 September 2010 (La Cité des Congrès)

Oceanography and ecology of HABs: physical/biological interactions, climate change, and other current issues

Conveners: Donald M. Anderson (USA), Geneviève Lacroix (Belgium), and Patrick Gentien† (France) Theme Session - ICES Annual Science Meeting

21 September 2010

A theme session focusing on HABs was held at the ICES Annual Science Meeting in Nantes, France in September, 2010. This concept was developed during joint meetings of two ICES working groups – the Working Group on Harmful Algal Bloom dynamics, and the Working Group on Physical Biological Interactions.

The session began with a dedication in memory of our friend and colleague Patrick Gentien, co-convenor of the session who sadly passed away prior to the meeting.

The session covered a wide range of model formulations and HAB species. Some of the models were empirical, including approaches such as utilizing sustained wind from specific directions to generate a "wind index" that had a predictive value for *Dinophysis* and *Karenia* blooms in southwest Ireland (R. Raine). Other models focused

on small-scale behaviour and physics such as the scales of turbulence that affect HABs (E. Berdalet), and a population health model of the distribution of Alexandrium cells infected by the parasite Amoebophrya (M. Sourisseau). This study demonstrated that it is realistic to have spatial separation of infected cells from healthy cells with the appropriate behaviour. Larger regional-scale models were also presented including a complex ecosystem model that simulated cyanobacterial blooms in the Baltic given different nutritional initial conditions (U. Daewel). Another large-scale model was a coupled physical-biological model of Alexandrium bloom dynamics in the Gulf of Maine (D. Anderson). That model captured the regional dynamics of this species with some skill and is now being used in short-term (days) to seasonal (months) forecasts. In this instance the abundance of resting cysts is a strong determinant of the magnitude of the resulting bloom, though results in 2010 demonstrated how large cyst germination can still fail to provide a significant bloom if growth conditions are not favorable in the water column. The session included regional reports on the monitoring of toxic phytoplankton from three Icelandic fjords (H. Gudfinnsson), biogeochemistry of cyanobacterial blooms in the Baltic Sea (O. Savchuk), population dynamics of Dinophysis acuminata in the Ría de Pontevedra in Northwest Spain (L. Velo-Suárez) and transport of Dinophysis blooms along the south coast of Ireland (R. Raine). Looking to the future, a new project entitled "ASIMUTH" was introduced (J. Silke); this will integrate Earth Observation data, models and in situ data to provide regular HAB bulletins in six locations along the western European Atlantic coast. A series of poster presentations complemented the oral session. Topics included climate change and the impact of storms on HABs (S. Aleksandrov), the rate of domoic acid production in cultures with different forms of nitrate (G. Calu), and the detection of domoic acid by using Solid Phase Adsorption (G. Hermann). Regional presentations included the summer phytoplankton in the Baltic (E. Lange) and monitoring programme in the southern Caspian Sea (M. Monshizadeh). A model of the life cycle of dinoflagellates demonstrated the role of life cycle transitions in regulating bloom dynamics (A. Kroll). Overall the breadth of model types presented for different HABs and different management needs were impressive. It is encouraging that this aspect of the HAB field is progressing at a productive pace.

All abstracts from the session are on the ICES website. In summarizing the theme session it was felt by the group that it was not very rewarding, considering the excellent work presented compared to the low number of attendees. No critical mass of HAB scientists was attending ASC. In the future we would think twice about to do it or not depending on location of the ASC. It is desirable to attract non-HAB people but people tend to attend the sessions in their own field of interest.

11 Term of Reference H

11.1 Draft aims and propose a structure for a joint meeting with the ICES Working Group on Physical–Biological Interactions (WGPBI)

It was proposed that this interaction with the Working Group on Physical-Biological interactions was a very useful linkage and that the joint theme session in 2010 ASC was a worthwhile outcome of this linkage. While the linkage will be maintained, it was appreciated that both groups have a heavy schedule and a further joint meeting would be deferred to a later date.

12 Term of Reference I

12.1 Report on new findings in the area of harmful algal bloom dynamics

12.1.1 The impact of algal toxins on key copepods in the North East of Scotland

Cook K. and Bresnan E.

Since 1997, Marine Scotland Science have operated a long term monitoring programme 5 km offshore from the Stonehaven coast in the North East of Scotland (56° 57.8′ N, 02 ° 06.2′ W). Samples for phytoplankton and zooplankton community analysis as well as temperature, salinity and nutrients are collected weekly. Further information about this monitoring programme can be found at (http://www.scotland.gov.uk/Topics/marine/science/MSInteractive/Themes/Coastal/S tonehaven).

Alexandrium, Dinophysis and *Pseudo-nitzschia*, the genera responsible for the production of toxins associated with paralytic, diarrhetic and amnesic shellfish poisoning (PSP, DSP and ASP) are observed as part of the phytoplankton community at this site on an annual basis. In the marine food web, copepods are a critical link between phytoplankton and higher trophic levels and many harmful algal toxins can affect copepod feeding, mortality and recruitment rates. There are two primary mechanisms by which algae can transmit their toxins to copepods: by active release into the surrounding medium (dissolved toxins), or by passive release during cell damage through grazing (ingested toxins). To investigate the impact of algal toxins on the copepods found at the Stonehaven monitoring site in the western North Sea a study was performed to investigate the impact of naturally occurring algal toxins on the key species (*Acartia* spp., *Calanus* spp., *Centropages* spp., *Pseudocalanus elongatus* and *Temora longicornis*). This study examined two both mecahanisms with the potential to impact zooplankton, exposure to dissolved toxins and feeding on toxin producing phytoplankton cells.

Direct exposure to dissolved toxins responsible for saxiton (PSP), okadaic acid (DSP) and domoic acid (ASP) at ecologically relevant concentrations had little impact on the copepod species studied. Copepod feeding behaviour over 24 hours on toxin (Group I) and non toxin producing (Group III) strains of *Alexandrium tamarense* was species specific: Centropages spp., Pseudocalanus elongatus and Temora longicornis fed on both strains of A. tamarense regardless of toxicity, whilst Acartia spp. and Calanus spp. did not feed on the toxin producing Group I strain. All copepods accumulated PSP toxins even if they did not feed on toxin producing A. tamarense. A further five day feeding experiment was performed with female *Calanus* spp. supplied mixed diets of A. *tamarense* ranging from 0%–100% Group I or Group III cells. There was no significant mortality during the experiment, and egg production rates were low even in Calanus spp. fed only non-toxic algae suggesting that other factors were limiting egg production in this instance. There was a significant decrease in the total number of cells that Calanus spp. ingested as the proportion of the Group I strain in their food mixture increased. This was particularly noticeable when 50% or more of the food mixture was comprised of the Group I strain cell. There appeared to be no selection for Group I or Group III cells in the food mixture (i.e. if the copepod was presented with a mixture containing 25% Group I cells then 25% of their food intake was the Group I cells). This result appeared inconsistent with that of the 24 hour feeding experiment. However, the Calanus spp. used in the 24 hour feeding experiment were collected from the field during a period of peak chlorophyll concentration and *Calanus* spp.

abundance whilst those used in the 5 day experiment were collected after field chlorophyll concentration and *Calanus* spp. abundance had declined. This could indicate that preference of *Calanus* spp. for feeding on toxic *A. tamarense* strains may be influenced by physiological condition and that more toxin producing cells are consumed during sub-optimal periods.

12.1.2 Trends and patterns in phytoplankton populations from monitoring in the Bay of Fundy (eastern Canada)

Jennifer L. Martin and Murielle M. LeGresley

St. Andrews Biological Station, 531 Brandy Cove Road, St. Andrews, NB, Canada E5B 2L9

A phytoplankton monitoring programme was initiated in the southwest New Brunswick portion of the Bay of Fundy, eastern Canada in 1987. Four stations have been sampled since the beginning with an additional site (station #25) added in 1999 to better represent the Passamaquoddy Bay region (Figure 1).

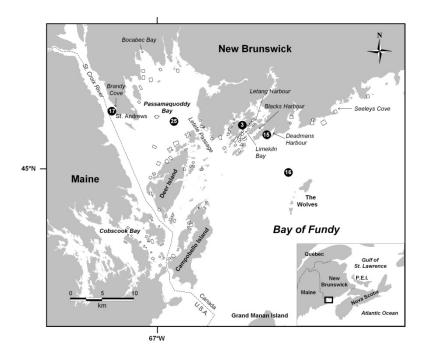


Figure 1. Locations of 5 sampling stations in the Bay of Fundy, eastern Canada.

Phytoplankton species have been enumerated microscopically for abundance and separated into 3 groups: diatoms, dinoflagellates and "other", which includes groups such as the smaller zooplankton and ciliates. When counts from all the organisms were summed, there was a trend observed indicating an increase in cell abundance since 1999. When the individual groupings were separated, this trend continued for all three groups, with even greater numbers observed with the inclusion of station #25 in 1999. Results from the total diatoms, dinoflagellates and "others" are shown on Figures 2, 3 and 4.



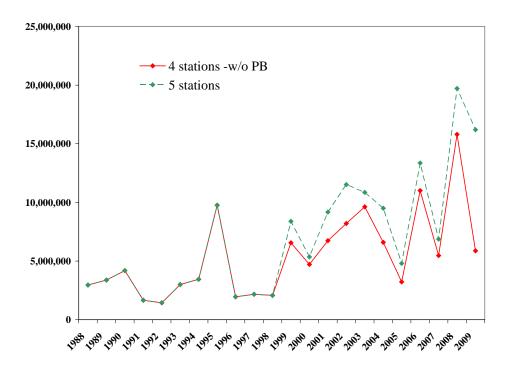


Figure 2. Total diatom abundance from a) four stations combined and b) the inclusion of the additional station in mid-Passamaquoddy Bay (#25) in 1999.

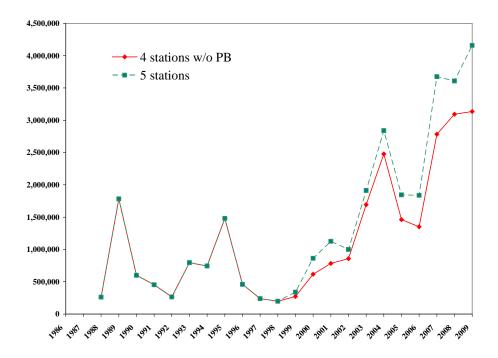


Figure 3. Total dinoflagellates counts since 1988.

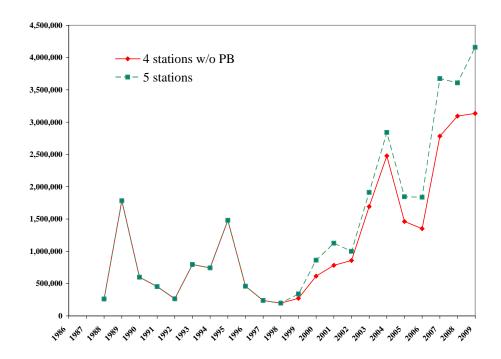


Figure 4. Total counts for "other" organisms since 1988.

Results indicated that individual species are responsible for driving the trend for increased cell concentrations. For example in the diatoms, *Pseudo-nitzscha* occurred at high concentrations; for the dinoflagellates, *Alexandrium* cell densities increased in recent years, and the ciliate *Mesodinium* influenced the high concentrations in the "other" group. In the past 10 years these three species were detected at higher concentrations. However, one must be cautious when indicating an increasing trend for increased concentrations as this may be an indication of a trend and this dataset is only from 22 years and further monitoring is required to look at patterns. For example, PSP shellfish toxicity monitoring results from the Bay of Fundy show that there appear to periods of highs and lows with increased toxicity appearing on a 20-yr cycle.

Diatom results from the Bay of Fundy monitoring programme show that the spring diatom bloom does not show as strong a signal for cell abundance as the late summer bloom. This may be as a result of the counts being done as cells/litre and chain forming organisms being counted as chains of cells/litter. The more dominant spring species tend to be the *Chaetoceros* and *Thalassiosira* species. In the fall the dominant species tend to be the smaller species such as *Pseudo-nitzschia*, which are smaller in biomass than the spring species. Therefore, a measure of biomass and the conversion of cells by bio-volume measurement might be a better indicator of abundance for comparison between datasets and measurement of the spring diatom blooms.

12.1.3 The failed forecast of a major *Alexandrium fundyense* bloom in the Gulf of Maine in 2010

Don Anderson, USA

Members of the US GOMTOX research team issued a seasonal (annual) forecast for a major *Alexandrium fundyense* bloom in the Gulf of Maine region in 2010 (see http://www.whoi.edu/page.do?pid=24039&tid=282&cid=69586). For the period 2005–2009, the abundance of resting cysts in bottom sediments from the preceding fall has

been shown to be a first-order predictor of the overall severity of spring/summer blooms of A. fundyense in the western Gulf of Maine and southern New England. Observations in the fall of 2009 indicated that cyst abundance off mid-coast Maine was significantly higher than it was preceding a major regional bloom in 2005. A seasonal ensemble forecast was computed using the fall 2009 cyst abundance and the range of forcing conditions for the period 2004–2009, suggesting a large bloom was likely in the western Gulf of Maine in 2010. Real-time forecasts of A. fundyense abundance made during the bloom season were generally within the range of variation predicted by the seasonal ensemble forecast. However, a major bloom did not materialize in 2010. Hydrographic survey data indicate that conditions in spring/summer 2010 were outside the envelope of prior observations used to construct the ensemble forecast. Water mass anomalies indicate a regional-scale change in circulation with direct influence on A. fundyense's niche (near-surface waters were warmer, fresher, more stratified, and had lower nutrients than prior years). Moreover, a weaker-thannormal coastal current lessened A. fundyense transport into the western Gulf of Maine and Massachusetts Bay. Satellite ocean colour observations indicate the 2010 spring phytoplankton bloom was more intense and crashed earlier than usual. Thus it appears that early-season nutrient depletion caused a temporal mismatch with A. fundyense's endogenous clock that regulates the timing of cyst germination. These findings highlight the difficulties of ecological forecasting in a changing oceanographic environment, and underscore the need for a sustained observational network to drive such forecasts.

Should we be able to confirm the mechanisms responsible for the lack of a bloom in the WGOM in 2010, those processes could then be included in the population dynamics model. Furthermore, we note that the water mass changes mentioned above relate to the larger scale circulation of the northwest Atlantic, and therefore are observable months in advance of the *Alexandrium* season. Therefore, it is conceivable that forecasts can be made taking into account this type of variability. It is indeed fortunate that GOMTOX cruises were scheduled for 2010, as this will allow us to understand the factors that prevented a bloom and thereby allow us to improve our model.

12.1.4 Feeding experiments and uptake of the toxins of *Azadinium spinosum* by Blue Mussels (*Mytilus edulis*) in Ireland

Joe Silke, Ireland

Azaspiracids (AZAs) are a group of lipophilic polyether compounds first detected in Ireland which have been implicated in shellfish poisoning incidents around Europe. These toxins regularly effect shellfish mariculture operations including protracted closures of shellfish harvesting areas for human consumption. The armoured dinoflagellate *Azadinium spinosum* Elbrächter et Tillmann gen. et sp. nov. (*Dinophyceae*) has been described as the *de novo* azaspiracids toxin producer; nonetheless the link between this organism and AZA toxin accumulation in shellfish has not yet been established. In August 2009, shellfish samples of blue mussel (*Mytilus edulis*) from the South west of Ireland were analysed using liquid chromatography-tandem-mass spectrometry (LC-MS/MS) and were found to be above the regulatory limit (0.16 μ g/g AZA equivalents) for AZAs. Water samples from this area were collected and one algal isolate was identified as *A. spinosum* and was shown to produce azaspiracid toxins. This is the first strain of *A. spinosum* isolated from Irish waters. The Irish *A. spinosum* is identical with the other two available *A. spinosum* strains from Scotland (3D9) and from Denmark (UTHE2) in its sequence of the D1-D2 regions of the LSU rDNA..

A 24 hour feeding trial of blue mussels (*Mytilus edulis*) using an algal suspension of the Irish *A. spinosum* culture at different cell densities demonstrated that A. *spinosum* is filtered, consumed and digested directly by mussels. Also, LC-MS/MS analysis had shown that AZAs were accumulating in the shellfish hepatopancreas. The toxins AZA1 and -2 were detected in the shellfish together with the AZA analogues AZA3, AZA6, AZA17 and -19 suggesting that AZA1 and -2 are metabolised in the shellfish within the first 24h after ingestion of the algae. The levels of AZA17 detected in the shellfish hepatopancreas (HP) were equivalent to the levels of AZA1 but in the remainder tissues the levels of AZA17 were four to five times higher than AZA1, only small quantities of AZA3 and -19 were present with negligible amounts of AZA6 detected after the 24h period. This could have implications in the future monitoring of these toxins given that at present according to EU legislation only AZA1-AZA3 is regulated for. This is the first report of blue mussels (*Mytilus edulis*) feeding on the azaspiracid producing algae *A. spinosum* from Irish waters.

13 Term of Reference J

13.1 Deliver National Reports on harmful algal events and bloom dynamics for the year 2010

13.1.1 French National Report

Catherine Belin, Ifremer, Nantes, France

Lipophilic toxins

Bio-assays have been replaced by LC-MS/MS analysis for all survey samples since 1 January 2010. They were maintained on 10 sites only, for an alert system on new and unknown toxins.

Regions which were affected by levels of lipophilic toxins (OA+DTXs+PTXs) above the European sanitary threshold (160 μ g/kg equ. OA) were quite similar to previous years: especially South Brittany with different species of shellfish, and for some scarce toxic episods, Normandy, Arcachon, and Corsica lagoons. The maximum toxin concentration was found in mussels (Mytilus edulis): 3311 µg/kg, but there was also 2621 in clams (Paphia rhomboides) and 1502 in Donax trunculus. As a great proportion of results remained unexplained when bio-assays were the official method, a comparison study was conducted in 2009 on 1034 samples, analyzed by both methods (bio-assay and LC-MS/MS). A statistical comparison, based on Kappa coefficient, showed that the global agreement between the two methods was only 70%, with many differences between sites and shellfish. In particular, some sites like Arcachon on the South Atlantic coast, but also some lagoons of the Mediterranean coast and Corsica, were particularly affected by "false positive" results (positive bio-assay, while the chemical result on the same sample was negative). For some of them, the responsible toxin is not known yet. So an alert system was maintained, with both methods applied on samples of 10 sites, once a month. These 10 sites are distributed all along the French coast. In 2010, 106 bio-assays were performed, and 17 of them gave positive results. Among these 17 results, 6 were associated to negative chemical results: these unexplained results came from the same regions than previous years.

Concerning the presence of Azaspiracids, there was only one result above the quantification limit (but below the sanitary threshold): 21 μ g/kg in oysters in Sein island (Western Brittany) in June 2010. For Yessotoxins, there was no result above the sani-

tary threshold, but 110 results above the quantification limit with the maxima (335 μ g/kg) in mussels of Groix island (South Brittany) in July/August 2010.

The distribution in time for these toxins was the following: rather in Autumn for the Channel, rather in Spring or Summer for the Atlantic coast, rather in winter for Corsica.

PSP toxins

PSP toxins were observed in only two sites of North Brittany at concentrations above sanitary thresholds: 2880 μ g/kg in cockles of Rance estuary, 2700 μ g/kg in mussels of Morlaix bay. As a matter of fact, there have not been PSP toxins (or very few) in France for several years. However, *Alexandrium* is still present in waters, but it seems that the conditions are not enough good for it to bloom.

ASP toxins

2010 was an exceptional year for ASP toxins in France: there have never been so many different contaminated species of shellfish, and the contamination has never reached such high levels: the maximum was observed in scallops (*Pecten maximus*) with 484 mg/kg equ. DA, and concentrations above 100 mg/kg were also found in clams (*Ruditapes spp.*), mussels (*Mytilus edulis*) and oysters (*Crassostrea gigas*). The affected regions were South Brittany, and for the first time the Pertuis between Loire and Gironde along the Atlantic coast. At the contrary, in 2008 and 2009, ASP toxins had affected only scallops, with concentrations which had never exceeded 60 mg/kg.

The distribution in time for these toxins showed that all toxic episods began in March or April, corresponding to the main blooms of *Pseudo-nitzschia* which always occur in April in all regions. The period of contamination in the scallops was very long (at least several months) and for some areas the toxicity is still present.

New toxins

Palytoxins have been found in sea-urchins and mussels since 2008 in Eastern Mediterranean, in summer. They were linked with increased concentrations of *Ostreopsis* every year.

Pinnatoxins were observed for the first time in mussels of a Mediterranean lagoon (Ingril) in August 2010, with a maximum of 800 µg/kg. A new species described by Nezan & Chomerat (2011, in Cryptogamie, Algologie), *Vulcanodinum rugosum*, is suspected to be the toxin producer.

Prospects for phytoplankton identification

A project in process for phytoplankton picture automatic analysis, links digitisation by FloWCAM (flux cytometry), and identification with Zoo/PhytoImage software (developed by Philippe Grosjean & Kevin Denis, Mons University, Belgium). An operational tool should be available in 2012.

13.1.2 The Netherlands National Report

Ainhoa Blanco & Marnix Poelman

In the Netherlands 2010 again (8 year in a row) was a year without any toxic events. During 2010 the shellfish production areas; North Sea, Lake Grevelingen, Wadden Sea and Oosterschelde were monitored for presence of toxic phytoplankton. This program was based on the National Shellfish Food Safety Program on a monthly (November–April) or weekly (May–October) basis. Also the MWTL (Monitoring Waterstaatkundige Toestand des Lands milieumeetnet rijkswateren) monitoring program was performed in the North Sea and Wadden Sea on a monthly basis.

Dinophysis acuminata was detected at low levels in the Wadden Sea, North Sea and Oosterschelde. These levels did not exceed the threshold limit of 100 cells/litre. In the Wadden Sea the observations were done from June through August. In the Oosterschelde the observations were between 30–40 cells per litre in July and August.

In Lake Grevelingen higher abundance of *D. acuminata* was observed. In this period cell counts ranging from 100–370 cells per litre were observed. Additionally, in lake Grevelingen *D. acuminata* counts of 120–300 cells/litre are reported in August. In some cases *D. rotundata* was reported.

All sites were also sampled for marine biotoxins, and analysed using the rat bioassay. In none of these samples toxins were reported.

High abundance of *Pseudo-nitzschia spp*. was reported in Lake Grevelingen at around 370 000 to 570 000 cells/l in August. Low levels of *Pseudo-nitzschia sp*. were observed throughout the whole year. No toxins were observed with LC-MS analyses.

13.1.3 Finland National Report 2010

Anke Kremp, Finnish Environment Institute, Marine Research Centre

Filamentous N fixing cyanobacteria are the main HAB species in Finnish waters. Blooms consisting of non-toxic *Aphanizomenon* spp., hepatotoxic *Nodularia spumigena* and microcystin producing *Dolichospermum* spp. (formerly known as *Anabaena* spp) occur every year. The blooms produce noxious surface scum, which - when washed ashore - may become a health risk.

In 2010, surface blooms developed - as predicted from relatively high post spring bloom phosphorus levels - particularly in the Gulf of Finland. Due to favourable wind conditions, the blooms were not washed ashore. However, localized surface aggregations of predominantly *Aphanizomenon* spp, occurred locally. Cyanobacterial abundances started to increase in Finnish waters later than normal due to low water temperatures at the beginning of the summer. Cold weather was also the reason for the unusually low proportion of warm water adapted *Nodularia spumigena* in the early summer community. Cell concentrations of this species remained at lower than usual levels throughout the entire bloom period. Areal coverage of cyanobacterial blooms increased in July, particularly in southern Finnish waters and prevailing warm and calm weather conditions intensified surface accumulations particularly in the open sea areas. Strong winds dispersing the surface accumulations in mid August, lead to an early end of the bloom season. No toxic events were caused by cyanobacteria in 2010.

In addition to cyanobacteria, some other toxic and harmful species may occur and even form blooms in Finnish waters. In 2010 notable concentrations of DSP producing *Dinophysis acuminata* were detected in late July in the central Gulf of Finland. A bloom of *Prorocentrum minimum* that occurred in the N. Baltic proper in late August and September extended into Finnish coastal waters, dominating the phytoplankton community here for some time but not causing any harm. *Protoceratium reticulatum*, *Chrysochromulina* spp. and *Alexandrium ostenfeldii* were occasionally encountered in open sea samples throughout the growth season.

In coastal waters of the Åland Archipelago, however, *A. ostenfeldii* formed – as in previous years – dense blooms that caused visible bioluminescence events. Maximum

cell concentrations of > 200 000 cells L⁻¹ were reported in mid August from a confined coastal bloom area. PSP toxins produced by the Åland *A. ostenfeldii* bloom were found to accumulate in bivalves from the bloom site to concentrations exceeding regulatory levels: 2.2 µg PSP toxins g⁻¹ wet weight in *Macoma baltica* and > 8 µg g⁻¹ in *Cerastoderma* sp.. Due to their small size in the low salinity waters of the N Baltic Sea, these shellfish are not consumed by humans and the blooms are not a direct threat to human health.

13.1.4 Germany National Report

Allen Cembella

Along the southeastern coast of the German Bight, within the Jutland current influenced North Sea border between Germany (Schleswig-Holstein) and Denmark, there were no significant HAB developments observed in 2010. Both *Alexandrium tamarense* and *A. ostenfeldii* were recorded at Sylt but only in low cell numbers. Similarly, putative |DSP toxin-producing dinoflagellates, *Dinophysis acuminata*, *D. acuta* and *D. norvegica* were also detected but in low abundance. The raphidophytes Chattonella sp. and *Fibrocapsa japonica* were also noted occasionally, along with the dictyophyte *Pseudochattonella* sp. but never at bloom concentrations. Among the HAB diatoms, species of the genus *Pseudo-nitzschia*, especially from the *P. pungens* and *P. delicatissima* complex were frequently present, but no toxin measurements were performed and associated toxic effects remain therefore unknown. Unusually for this region, from among the prymnesiophytes no bloom of *Phaeocystis* occurred in 2010 – only small numbers of colonies were observed. As it typical for the Wadden Sea coast near Sylt, potentially harmful cyanobacteria occurred only rarely in substantial amounts and caused no apparent damage.

In 2010 bivalve shellfish samples (mussels and oysters) were analyzed from the North Sea (56 mussels, 22 oysters) and the western Baltic Sea (16 mussels) for the presence of phycotoxins (primarily ASP-, DSP-, and PSP-type). In some oysters collected near Sylt, azaspiracids (maximum 40 μ g/kg) were found, but these oysters apparently came from Ireland and were cultivated in Germany. None of the other samples showed appreciable amounts of toxins.

As is typical for the Baltic Sea, the coast of Germany is plagued in summer by the appearance of cyanobacterial blooms, especially of the potentially toxic species Nodularia spumigena. In 2010 this pattern was no exception, but the cyanobacterial blooms received special media attention – with extravagant claims made for unusually high biomass and spatial coverage in June and July ("gigantic algal carpet covers a huge part of the Baltic Sea", Der Spiegel, July 2010). Nevertheless, based upon observations from oceanographic monitoring cruises, which did not support the dramatic interpretation of the extent of the bloom, it appears that the satellite images have been completely misinterpreted, particularly for the central Mecklenburg Bight and the outer Lübeck Bight. In the Arkona Sea and Pommeranian Bight almost no cyanobacteria were observed. Only north of Bornholm (Bornholmgat) in mid-July were high concentrations of cyanobacteria found in the water column but not aggregated at the surface. Eastward of Bornholm (17°–19°E) in the southern Baltic, a huge cyanobacterial carpet was visible at the surface, but towards the Gotland Basin (northwards of 55°40') no further bloom was found. Nevertheless, Stralsund Harbour, near Ruegen Island experienced abundant cyanobacterial fouling. More unusually, in midsummer there was extensive cyanobacterial beach fouling at Willemshaven in the southern Wadden Sea, which posed a nuisance to tourism and recreational activities.

13.1.5 USA National Report

Don Anderson

2010 was basically a "normal" HAB year for most regions of the USA, with several noteworthy events.

PSP. On the US west coast, Alaska, Washington, and Oregon all recorded PSP toxicity during 2010. Several people in Alaska suffered from PSP and there were two confirmed deaths attributed to the poisoning. This is the first time anyone has died from PSP in Alaska since 1997. Washington had a very significant PSP year with many sites in North Puget Sound hitting new records for toxicity levels. Similarly the outside coast and the Strait of Juan de Fuca also set new PSP records. South Puget Sound had a very unusual PSP bloom as well. On the contrary, Oregon had a light year with a PSP bloom in September forcing the closure of recreational mussel harvesting from the Columbia River south to Cascade Head.

For the first time since 2002, no PSP toxicity was recorded in New Hampshire. Likewise no toxicity was recorded in Massachusetts (the first time since 2004 that this has happened). Maine experienced PSP toxicity but in much lower levels than previous years.

The 2010 *Alexandrium* forecast for the Gulf of Maine was not borne out by cruise observations and shellfish toxicity measurements. We predicted a large regional bloom in the WGOM that did not happen. Toxicity along the coast was low, and cruise data showed very few cells in the region from Massachusetts and Cape Cod Bays to midcoast Maine.

Two possible (and potentially related) reasons for the absence of the bloom in the WGOM are evident thus far: 1) there was a water mass change that lies outside the envelope of observations from at least the last six years, which were the basis of the ensemble forecast that led to the 2010 prediction; and 2) along-coast surface transport off southern Maine was slower than normal.

For the second year in a row, Florida experienced *Pyrodinium bahamense* blooms on both the east coast (Indian River Lagoon) and the west coast (Tampa Bay). The Tanpa Bay bloom lasted approximately 3 months but did not reach the lower levels of the Bay where harvesting is permitted. The maximum cell concentration for the west coast bloom was 600K cells/L. The Indian River lagoon bloom lasted for approximately 5.5 months with a maximum cell count of 1.1 million cells/L. Hard clam harvesting was closed in this area; pufferfish harvesting has been banned in this area since the event of 2002.

ASP. California experienced approximately 100 sea lion illnesses / mortalities due to domoic acid. Once again, Washington State had very low levels of domoic acid with no closures reported. Oregon had a fairly light ASP year with a closure for recreational razor clam harvesting due to domoic acid on the south coast. The bloom later spread north, forcing closures up to the southern end of Clatsop Beaches. DA was only detected in razor clams and not any other species. Typically, these blooms occur in summer / fall on the Oregon coast, but this year the bloom occurred in the spring.

NSP. The *Karenia brevis* bloom which began in southwest Florida in late 2009 continued until late February 2010. Texas did not experience *K. brevis* blooms in 2010.

DSP. *Dinophysis ovum* cells were detected by the Imaging Flow CytoBot in Aransas Pass, Texas. Subsequently, shellfish samples were confirmed to have okadaic acid. 13

major and minor bays from Copano Bay to Gavleston Bay were closed to shellfish harvesting. This is the second occurrence of DSP in Texas.

Brown tide. Once again, the south shore of Long Island, NY experienced a significant brown tide bloom, which began in May and ended in October. Suffolk County reported 1.4 million cells per ml in June. This was the fourth year in the row with elevated *Aureococcus* concentrations, following a decade of very low levels. Before that, there was a decade of high concentrations, beginning in 1987.

Other species. Blooms of *Cochlodinium polykrikoides* occurred in the James River in Virginia. The York River bloom reached a magnitude of 80 million cells/L and was associated with an unpleasant odour in that area. There were also fish deaths, but these may have been caused by low oxygen conditions.

13.1.6 The United Kingdom National Reports

13.1.6.1 Northern Ireland

In 2010, thirty one sites were sampled routinely on a fortnightly basis from sea loughs and coastal waters in Northern Ireland. A total of 703 samples were analysed.

Alexandrium spp. were recorded in 1.2 % of samples and the maximum cell abundance (140 cells L⁻¹) was recorded in a sample from Belfast Lough in March. PSP toxins in shellfish did not exceed the regulatory limit in 2010.

Dinophysis spp. were present in 15.5 % of water samples. Maximum abundance (560 cells L⁻¹) was recorded in a sample collected from Killough Harbour in early June. As in previous years the most abundant species was *D.acuminata* with only low numbers of *D.acuta, D.norvegica* and *D.rotundata* counted. Cells of *Prorocentrum lima* were present in 2.5% of samples with a maximum abundance of 920 cells L⁻¹ recorded in a sample from Carlingford Lough in May. Diarrhetic shellfish toxins were detected in two shellfish samples tested as part of the official control programme. On one of these occasions low levels of *Dinophysis* sp. were recorded in the water column.

Pseudo-nitzschia spp. were present in 59 % of samples and reached a maximum abundance of 164 880 cells L⁻¹ in a sample from Dundrum Bay in late August. Toxicity due to domoic acid was confined to samples of scallops (*Pecten maximus*) from Strangford Lough.

A large bloom of *Eutreptiella* sp. was recorded in Carlingford Lough during July. The maximum cell abundance recorded was ~ 3 million cells L⁻¹ on 27 July.

13.1.6.2 England and Wales

From the 1 June 2005, the Food Standards Agency (FSA) funded a comprehensive phytoplankton monitoring programme for England and Wales when all commercial shellfish harvesting areas in England and Wales were included in the phytoplankton monitoring programme for the first time. In 2010, a total of 1088 samples were collected from 55 production areas.

Alexandrium spp. (PSP) occurred slightly more frequently than in 2009, being recorded from 24 of the 55 sampled areas, and in 104 of the 1088 samples collected. Highest concentrations were all found in the South West Coast of England e.g. in the Fowey where it occurred from May to September, reaching a maximum density of 3.3 million cells per litre on 9 August. Greatest frequency of *Alexandrium spp* occurred in the River Avon (Devon) where concentrations over 10 000 cells/litre occurred 10 times, and where it persisted from April to September. A maximum concentration of

292 000 cells/litre was recorded from a sample collected in June. *Alexandrium spp* were only found at three other sites at concentrations greater than 10 000 cells/litre. These were all in the south-west of England. PSP toxins breached action levels on 7 occasions, all in the Fowey Estuary in August 2010, when they coincided with the presence of *Alexandrium spp*.

Dinophysis spp. (DSP) were observed on 27 occasions, and breached action levels of 100 cellsL⁻¹ 14 times. *Dinophysis spp.* occurred most frequently in Morecambe Bay - Barrow (West Coast) where they occurred four times in April and once in June when the maximum concentration of 560 cells/litre was recorded. *Prorocentrum lima* (DSP) were found on only four occasions. It was once again found most frequently in the Fleet Lagoon, Weymouth, Dorset (South Coast) where it was found twice in June. Maximum concentrations (800 cells/litre) were found in a sample collected from Poole, Dorset on 6 May. DSP toxins were recorded from 20 samples (3 more than in 2009) and they persisted in samples of Pacific Oysters collected from the Salcombe Estuary from early March to early October

Pseudonitzchia spp. (ASP) were found in 519 samples from most of the production areas in 2010, and were again widespread and persistent as in 2009. They breached the action level (150 000 cells L⁻¹) 14 times. Peak concentration reached 1.6 million cells L⁻¹ at Blakeney, north Norfolk, in June. There were no closures of shellfish production areas in England and Wales in 2010 due to the presence of ASP toxins.

13.1.6.3 Scotland

The dinoflagellate *Alexandrium* spp. was present in over 29% of the samples analyzed. It was most frequently observed in samples between April and June, occurring in 44% of all samples analyzed during May, and was also widespread in August. The densest *Alexandrium* bloom recorded in 2010 occurred in Loch Creran (West Coast). *Alexandrium* was present for a continuous period of eleven weeks from 10 March until 19 May, reaching a maximum cell density of 3100 cells per litre on 05 May. The dominant species appeared to be the non-toxic *Alexandrium tamutum*. The distribution of *Alexandrium* around the Scottish coast was similar to previous years and while some PSP was detected in some shellfish samples there were no closures for PSP toxins above the closure limit.

The dinoflagellate *Dinophysis* spp. was present in almost 55% of the samples analyzed, and occurred above threshold level (100 cells per litre) in over 20% of the total samples. It was abundant around the west coast and in the Shetland Islands during the summer months. *Dinophysis* and DSP toxic events were widespread around Scotland in August, and there was an exceptional bloom in West Loch Tarbert (West Coast) in late August, with a *Dinophysis* cell count of 1720 cells per litre.

Pseudo-nitzschia spp. was present in almost 90% samples analyzed as part of the Scottish harmful phytoplankton monitoring programme during 2010, and occurred above threshold level (50 000 cells per litre) in more than 16% of all samples. Blooms were widespread from June through to October and occurred in most sites around the Scottish coast. The largest recorded *Pseudo-nitzschia* bloom in 2010 was seen in the Shetland Islands to the North of the Scottish mainland (Vaila Sound: East of Linga) in mid July, with a maximum density of > 3.5 million cells per litre recorded on 12 July. Analysis of samples from the Shetland Islands during this time showed the blooms to be dominated by *Pseudo-nitzschia* cf. *pseudodelicatissima*.

The dinoflagellate *Prorocentrum lima* was observed in relatively low numbers, but was present in almost 19% of the samples analyzed, exceeding threshold level (100 cells

per litre) in 3% of all samples. *Prorocentrum minimum* was observed at many of the monitoring sites from early March through to mid October, and was present in more than 41% of the samples analyzed. Similar to 2009, *Protoceratium reticulatum* was recorded at low concentrations in approximately 4% of the samples analyzed, mostly on the West Coast.

Karenia mikimotoi was frequently observed from early June through to early October along the west coast and Shetland. Blooms reached peak abundance in the Western Isles from mid to late August with the highest cell densities occurring from late August into mid September. The largest recorded *Karenia mikimotoi* bloom of 2010 was at Aith Voe Sletta: Slyde (Shetland) on 30 August with a density of >1.1 million cells per litre. No mortalities of farmed fish were reported however there were some reports of impacts on the benthos on the West Coast.

13.1.7 Spain National Report

The usual shellfish poisoning events affecting the shellfish industry were registered during 2010. Some exceptional events were: i) Report of the first ASP event in Catalonia; ii) Huge densities of *Ostreopsis* both attached to macroalgae and in floating scums in Llavaneres beach also in Catalonia; iii) Record densities of *D. acuminata* in hose samples from Ria de Pontevedra (Galician Southern Rias).

13.1.7.1 Andalucia

Atlantic coast

ASP: *Pseudo-nitzschia* spp., with different peaks and troughs, were present all year round. ASP levels above regulatory limits only in November in four Mediterranean sites.

DSP caused by *Dinophysis* cf *acuminata* off Huelva between April and June. Maximum densities of 7600 cell \cdot L⁻¹ off Doñana Natural Park.

Mediterranean coast

PSP: Moderate concentrations of *Gymnodinium catenatum* on the Mediteranean sites with the exception of a peak of 10520 cell \cdot L⁻¹ off Málaga that led to shellfish harvesting closures on the eastern end of this province.

13.1.7.2 Catalonia

ASP: In area CAT-1-09 (Cap Gros-Vilanova) there was a 3-weeks closure (23 April to 14 May) due to high densities of *Pseudo-nitzschia* spp. (>10⁶ cell \cdot L⁻¹) and ASP toxins. Blooms of *Pseudo-nitzschia* spp. were also detected between April and July in several beaches as well as in Barcelona and tarragona harbours in April, July and September. This is the first report of an ASP closure in the Catalan coast.

DSP: Three closures in the Ebro Delta bays in April, May and June and one in coastal areas in September. *Dinophysis sacculus* and *Dinophysis rotundata* were above warning levels at the time of the first closure in April, but not during the other three. Densities of 10^3 to 10^6 cell \cdot L⁻¹ of *Dinophysis sacculus* were found in several harbours from the southern half of Catalonia. During these events, harvesting in the area CAT1-05 (Fangar Bay and the Gulf of L'Ampolla) was closed after detection of DSP toxins during 2 weeks in May. Harvesting of oysters (*Crassostrea gigas*) was allowed one week after the onset of the other closures. In area CAT1-09 (Cap Gros-Vilanova) there was one closure the last week of September.

PSP: There were no PSP-closures during 2010. *Alexandrium minutum* was present attaining maximum cell abundance of 4×10^{3} cell \cdot L⁻¹ in April; PSP concentrations (380 µg eq STX diHCl \cdot kg⁻¹) were below regulatory levels.

Very localized blooms of *Alexandrium minutum* (up to $2.3 \cdot 10^6$ cell \cdot L⁻¹) occurred in several harbours (Palamós, Arenys de Mar, Port Olímpic, Barcelona and Cambrils). In addition, blooms of *A. catenella* (up to $1.8 \cdot 10^5$ cell \cdot L⁻¹) occurred in Tarragona harbour. These blooms occur in areas not dedicated to aquaculture but cause water discoloration and social alarm.

Ichthyotoxic events: *Karlodinium* spp. was present at the Ebro Delta bays attaining maximum cell densities of 2×10^4 cell \cdot L⁻¹, which is below warning level; there were no fish mortalities associated with this bloom.

Benthic HABs: Proliferation of *Ostreopsis* spp. in Sant Andreu de Llavaneres during summer (August-September). Maximum densities were found by the end of August: $9.9 \cdot 10^6$ cell \cdot L⁻¹ in the water column and $27.8 \cdot 10^6$ cell \cdot g⁻¹ fresh macroalgae. The Catalan Water Agency warned the public health department and the Llavaneres council to take necessary measures. Brownish mucilaginous discolorations with macroalgal debris observed at the Marjal d'Alcanar beach on 30 August contained 5.1 $\cdot 10^6$ cell \cdot L⁻¹ of *Ostreopsis* spp.

13.1.7.3 Galicia

ASP: Raft mussels harvesting closures due to blooms of *Pseudo-nitzschia australis* leading to ASP toxins accumulation above regulatory levels during the second half of June in some areas (Portonovo) from Ría de Pontevedra (Southern Rias).

Two weeks shellfish closures due to *Pseudo-nitzschia australis* also in the Northern Rias: Viveiro and O Vicedo in June; Corcubión-Fisterra in August; O Burgo in September.

DSP: A late (July) proliferation of *Dinophysis acuminata* in the Southern Rias reached record levels (> $7 \cdot 10^4$ cell \cdot L⁻¹ in hose samples at 10–15m). Mussel harvesting closures in some areas lasted from July to November. In addition, there were closures of infaunal shellfish in some of the Northern Rías in July-August (Ribadeo, Foz, O Burgo, Baldaio, Corme) and September (the Southern (Pontevedra and Vigo) Rías. (Southern Rias) and in September (Camariñas and Corcubión).

PSP: Harvesting closures of infaunal shellfish species in the Northern Rias of Ares (June) and Camariñas (July) and the Southern Rías (Baiona in Ria de Vigo) (July to September) caused by *Alexandrium minutum*. There were no *Gymnodinium catenatum* events in 2010.

13.1.7.4 Basque Country

In 2010, the analysis of DSP, ASP and PSP toxins were conducted in wild mussels and oysters, once a week during October, November and December in the Butroi Estuary and from October to March in the Oka estuary. This monitoring strategy coincides with the sites and seasons allowed by the local authorities for shellfish harvesting activities. Biotoxins were below the detection limits in all the samples.

In addition, phytoplankton species composition and abundance in coastal waters and offshore, as well as in 12 estuaries were analyzed within the framework of the "Littoral Water Quality Monitoring and Control Network" (LQM) of the Basque Country. Samples for quantitative analyses (Utermöhl method) were collected during four seasonal (spring, summer, autumn and winter) cruises. Several potentially harmful phytoplankton taxa were observed in coastal and estuarine waters.

Regarding the potentially toxic dinoflagellates, *Dinophysis* spp., *Alexandrium* spp., *Phalacroma* (*Dinophysis*) *rotundatum*, *Gonyaulax* sp. and *Karenia* spp. were more frequently observed in coastal waters than in estuaries. Their abundances were always below 11 000 cells/L. *Prorocentrum minium* was detected in several coastal waters and estuaries with maximum abundances of the order of 10⁴ cells/L; cf. *Pfiesteria* was observed in 4 estuaries, with abundances ranging 10⁴–10⁶ cells/L. The potentially toxic diatom *Pseudo-nitzschia* spp. was identified in all of the coastal sampling stations. A maximum was observed in offshore waters in spring (May), with 2·10⁶ cells/L. In estuaries, the highest concentration was observed also in spring (1·10⁶ cells/L at the mouth of the Oka estuary).

Phytoplankton taxa that may cause fish mortality (due to physical damage, anoxia, toxic effects, etc.) were also detected. These included haptophytes (*Chrysochromulina* spp., *Phaeocystis globosa*), diatoms (*Chaetoceros socialis, Thalassiosira* spp. *Leptocylindrus minimus, Rhizosolenia* spp.), dinoflagellates (*Ceratium furca, C. fusus, C. tripos, Gyrodinium* spp., *Prorocentrum* spp., *Kryptoperidinium foliaceum*) and raphydophyceans (*Heterosigma akashiwo*). Bloom densities (>10⁶ cells/L) were only observed for the potentially harmful diatoms and for the dinoflagellate *Kryptoperidinium foliaceum* in some estuaries.

¹Data from the LQM (funded by the Water Agency of the Basque Government), provided by AZTI-Tecnalia and the University of the Basque Country

13.1.8 Sweden National Report

Bengt Karlson

Background

Harmful Algal Blooms (HAB:s) are recurrent phenomena in the waters surrounding Sweden. Most are likely to be of natural origin. The HAB-problems for the waters surrounding Sweden are very different for the Baltic Sea and the Skagerrak-Kattegat areas. In the brackish water of the Baltic Sea blooms of cyanobacteria, e.g. the toxic species *Nodularia spumigena*, is the major problem while in the waters with higher salinities in the Skagerrak and the Kattegat fish killing species and species that produce toxins that accumulate in filter feeders (e.g. mussels) is the major concern. However, both fish killing species and species causing shellfish poisoning occur in the Baltic Sea as well. Commercial farming and harvesting of wild mussels and oysters for human consumption is ongoing only along the Swedish coast of the Skagerrak at present.

No major harmful algal blooms occurred in the area in 2010.

13.1.8.1 The Bothnian bay and the Bothnian Sea

Blooms of cyanobacteria were observed along the Swedish coast of the Bothnian Sea in the summer 2010. This caused concern among the public but no harmful effects were observed. Surface accumulations of cyanobacteria were observed in the off shore part of the Bothnian sea in August 2010. These off shore blooms did not reach the Swedish coast. It was confirmed that the toxic species *Nodularia spumigena* was part of the bloom.

13.1.8.2 The Baltic proper

In July 2010 surface accumulations of cyanobacteria were observed in several parts of the Baltic proper. Due to wind conditions the blooms did not reach the coast of Sweden in large amounts. A few observations were made on the beaches of the island of Gotland. The toxin producing species *Nodularia spumigena* was found in the water together with non toxic *Aphanizomenon* sp. and *Dolichosperum* sp. (synonym *Anabaena* sp.).

13.1.8.3 The Skagerrak and the Kattegat

Closures of harvesting of shellfish (mainly blue mussels, *Mytilus edulis*) due to accumulation of algal toxins did occur on the Skagerrak coast but not for long periods. The main reason was levels of yessotoxins above the regulatory level. This toxin is produced e.g. by the dinoflagellate *Protoceratium reticulatum*. In May 2010 mouse bioassays indicated Paralytic Shellfish Toxins (PST) in blue mussels on two occasions.

In spring 2010 the harmful flagellate *Pseudochattonella farcimen* (Dictyochophyceae) was observed in the Skagerrak and the Kattegat. The diatom *Pseudo-nitzschia* cf. *delicatissima*, potentially a producer of Amnesic Shellfish Toxin, was observed on several occasions in 2010. The highest abundance was 1 000 000 cells per litre in Danafjord near Gothenburg in January 2010. The diatom *Chaetoceros concavicornis*, known to affect the gills of fish negatively, was observed in 2010. Other potentially harmful algae observed include *Akashiwo sanguinea*, *Karenia mikimotoi*, *Prorocentrum minimum* and *Karlodinium veneficium*.

Abundances of algae producing toxins accumulating in shellfish were below the warning level most of the time in the data set from the monitoring program for bivalve harvesting (National Food Administration). The species of interest are mainly *Alexandrium* spp. (Paralytic Shellfish Toxin, PST-producers), *Dinophysis* spp. (Diarrhetic Shellfish Toxin, DST producers), *Protoceratium reticulatum* (Yessotoxin, YTX producer), *Azadinium spinosum* (Azaspiracidic Shellfish Poisoning, AZT-producer) and *Pseudo-nitzschia* spp. (Amnesic Shellfish Toxins, AST producers).

In 2010 PST, DST, AZT and AST were not recorded at levels above the regulatory limits in blue mussels (*Mytilus edulis*), oysters (*Ostrea edule*), Pacific oysters (*Crassostera gigas*) or in cockles (*Cerastoderma edule*). One exception was the indication for PST in mouse bioassays which showed PST in blue mussels on two occasions in May 2010. Yessotoxins above the regulatory limit of 1 mg kg⁻¹ mussel meat were observed in May–September 2010 in blue mussels.

13.1.9 Canada National Report

Jennifer Martin

Fish Kills (west coast)

Atlantic salmon mortalities occurred as a result of HABs at aquaculture operations at the following locations:

- Heterosigma akashiwo
 - o (2 000 000 cells/litre) Quatsina Sound 16 March
 - (39 000 000 cells/litre) Bedwell Sound, Clayoquot Sound 15 July
 - (1 000 000 cells/litre) Klemtu, Finlayson Sound 4 August
 - (6 000 000 cells/litre) Clayoquot Sound mid-September
 - (26 000 000 cells/litre) Klemtu, Finlayson Channel 3 September
 - o (25 000 000 cells/litre) Sechult Inlet 30 September

- *Chaetoceros concavicorne* (15,000 cells/liter) Clayoquot Sound 16 March
- Pseudochatonella cf verruculosa (30 000 cells/liter) Nootka Sound 20 August

There were no reported cases of salmon mortalities associated with HABs on Canada's east coast during 2010.

PSP

West coast:

Major shellfish harvesting areas were closed to harvesting for a portion of the year during 2010.

East coast:

The St. Lawrence Estuary (Quebec) and Bay of Fundy (New Brunswick and Nova Scotia) experienced closures to harvesting. The greatest value observed in soft-shelled clams in the Bay of Fundy was 4921 ug/100 g STX equiv.

DSP

East coast:

During late October, 2010 harvesting of blue mussels was prohibited in Ship Harbour, Nova Scotia due to unacceptable levels of DSP toxins. The area remained closed until mid-March 2011.

13.1.10 Ireland National Report

Following a short outbreak of ASP in mussels in the southwest in response to the spring diatom bloom (*P. australis* were present), a period of DSP dominated the south west for the summer months and this persisted through the autumn and into the winter in some areas. Azaspiracid was not present at alert levels in the south west as would normally be expected, although there was a outbreak of AZA in the west of the country in August and extended into September in some areas.

ASP

Domoic Acid conc.'s observed to increase from end of Mar/during Apr in samples of *M.edulis* from SW coast to a max conc. of $133\mu g/g$ TT⁻¹. Conc.'s > regulatory level also observed for a short period in samples of *C.gigas* and *M.edulis* from the West coast during Apr/May

DSP

Conc.'s > regulatory level observed from May onwards in samples of *M.edulis* from the SW coast, where during May & June, Okadaic Acid and Okadaic Acid Esters were the predominant toxins present, from July onwardsDTX-2 was the predominant toxin observed. The highest recorded conc.'s were >ULQ (>3.53 μ g/g TT ⁻¹, with conc.'s decreasing from Sept onwards

AZP

AZA conc. carryover from 2009 in samples of *M.edulis* from SW during January–February.

AZA conc.'s observed from Jul (peaking in Aug & Sept) mainly along the West coast, but also along NW and SW coasts, max conc. observed was $0.93\mu g/g$ TT⁻¹, in samples of *M.edulis*, *C.gigas*, and *S. solida*.

PSP

No quantifiable PSP toxins observed.

14 Term of Reference K

14.1 Evaluate potential for collaboration with other EGs in relation to the ICES Science Plan and report on how such cooperation has been achieved in practical terms (e.g. joint meetings, back-to-back meetings, communication between EG chairs, having representatives from own EG attend other EG meetings)

A discussion was held on how collaborations between our group and other working groups are achieved. In general much collaborative activities has been bilateral between groups, where specific topics of mutual interest are identified. In recent years there has been significant collaborations with groups such as Working Group on Physical and Biological Interactions (WGPPI) and the Working Group on Phytoplankton and Microbial Ecology (WGPME). Our group proposed and held a joint meeting with WGPBI in 2009 to assess and jointly explore the area of modelling of harmful blooms, and one of the outcomes of this interaction resulted in a theme session at the 2010 ASC.

Other collaborative activities have included the involvement of members of the WGHABD group in the Strategic Initiative on Climate Change (SSICC) and the ICES Workshop on harmful phytoplankton that could potentially be transported or introduced by ballast water (WKHABAL).

At the 2011 Working Group Meeting we had 2 representatives from PICES, and a potential collaborative initiative was proposed. In introducing this proposal **Mark Wells** and **Charles Trick** gave a presentation on the HAB related activities of the North Pacific Marine Science Organisation (PICES). Following this overview of the PICES HAB working group and its operational structures (5 year cycle) a discussion was held which resulted in the formulation of a proposal for a joint ICES/PICES workshop was made around the topic "HABs in a changing world" with the view to producing a publication.

It was suggested that this be held in the spring of 2012 in conjunction with the next ICES WGHABD.

The proposal received favourable comment with subsequent discussion relating to the duration (~1 week), attendee list (all members of ICES WGHABD and those members of PICES able to attend) and specific topic(s). A final decision and development of this proposed meeting will be made intersessionally.

15 Election of new Chair of the group

During the working group meeting a new chair for the working group was selected. Dr Bengt Karlson (Sweden) will hold the position of the chair of WGHABD for the 3 year period from 2012 to 2014. The group thanked the outgoing chair Joe Silke (Ireland) for holding the position from 2006 to 2011.

16 Draft Resolutions

The **ICES-IOC Working Group on Harmful Algal Bloom Dynamics** (WGHABD), chaired by Bengt Karlson, Sweden, will meet in Oban, Scotland, 25–28 April 2012 to:

- a) To report on new findings in the area of harmful algal bloom dynamics;
- b) To deliver National Reports on harmful algal events and bloom dynamics for the year 2010;
- c) 1) Quantify the occurrence of fish killing algal events in the ICES region¹;
 2) Document gaps in understanding of the processes controlling the occurrence of fish killing algae and the factors that cause fish mortality;
- d) Scope and plan a workshop focused on automated in-situ devices and imaging technology (including newer molecular methods) used for observing HABs and detecting toxins;
- e) To collate and discuss data on macroalgal blooms and their impacts in the ICES region;
- f) Report on the impacts of harmful algal blooms on marine mammals and birds relevant to the Marine Strategy Framework Directive objectives.

WGHABD will report by 15 May 2012 (via SSGHIE) for the attention of SCICOM.

Supporting information

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 th health of the ecosystem. The work of this ICES-/IOC WG is deemed high priority.
Scientific justification	Action Plan No: 1.1, 1.2, 1.5, 1.7, 1.10, 1.11, 1.12, 2.3, 2.9, 3.2, 4.11, 5.10, 5.13, 5.16, 6.1 6.2, 6.3, 6.4, 8.1, 8.2, 8.4.
	Term of Reference a)
	WGHABD is a useful forum to discuss and present new findings amongst the memebers. This is an excellent forum to promote and discuss topics of relevance. There are obvious reasons to continue this topic as an ongoing term of reference
	Term of Reference b)
	National Presentations and review occurrences of HABs in the ICES area, making use of the HADAT system.
	Term of Reference c)
	 Over the past 30 years blooms of harmful algae have resulted in mortalities of farmed fish in coastal waters throughout the ICES region. This has caused substantial financial loss, and in some coastal areas has restricted the development of fish farming. In 1992 ICES published Cooperative research report 181 (Effects of harmful algal blooms on mariculture and marine fisheries) that documented those species that have been implemented in fish kills. There has been a considerable amount of research on the dynamics of harmful algal blooms HAB dynamics but much remains unknown about the mechanisms (toxin, oxygen starvation, mechanical damage to gills etc.) that cause fish mortality. It is therefore timely to review the state of knowledge on fish killing algae in the ICES region. Term of Reference d)
	To follow the development of HAB's it is desirable to be able to sample and observe HAB-organisms at a resolution high enough to resolve natural
	variability. Sampling is often made from research vessels and cost may restric the sampling frequency. Novel technology used on moorings, docks, or ferries may at least in part alleviate the problem. The term of reference refers to a

¹ This could be linked to the ToR to look for trends in the occurrence of HABs and associated events. We might not be able to perform statistical analyses, but maps of the ices area showing when and where fish kills occurred might be interesting.

Workshop on HAB In Situ Technology (WKHIST) planned to be organized in 2013. A possible venue is the Woods Hole Oceanographic Institution in Massachusetts, USA. A preliminary scope of the workshop is that *in situ* imaging flow cytometers and *in situ* molecular biology HAB detection systems would be the focus of the workshop. The aim of the workshop would be to demonstrate the novel devices and to test them with local HAB populations. One possibility is to use the *in situ* instruments in mesocosms with a local phytoplankton community. Given the difficulty in working in natural waters (or even in mesocosms)at exactly the time that HAB species might be present, an alternative would be to use the instruments on laboratory benches, with plankton samples and cultures introduced manually. The scope of the workshop will be further discussed. Also local organisers and the members of a scientific steering committee for the workshop should be identified.

Term of Reference e)

Blooms of macroalgae (seaweeds) can be harmful, especially to seagrass and coral reef ecosystems and the food-webs dependent on those habitats. Nuisance seaweed species replace indigenous macroalgae in the benthos and microscopic phytoplankton in the water column. They thus modify benthic habitats, affect microbial and macrofaunal foodwebs, and alter key biogeochemical features of coastal ecosystems. Because seaweeds are generally benthic organisms and inhabit inshore coastal waters that mark the interface between land and sea, they are often the first primary producers to be implicated in many harmful seaweed blooms. The causes and effects of macroalgal blooms are thus similar in many ways to those associated with harmful microscopic phytoplankton species. Some countries (e.g., the U.S.) include macroalgal blooms in their national HAB research programs.

Term of Reference f)

The Marine Strategy Framework Directive (MSFD) requires an assessment of the marine food web in the determination of 'Good Environmental Status' (GES). In some instances the numbers and breeding success of higher trophic levels (mammals, birds, large fish) in the marine food chain have been suggested as possible indicators of the state of the food web. ICES group members have previously reported impacts of harmful algal blooms on marine mammals and birds. The toxin groups and impacts on these higher levels of the food web in the ICES region will be identified.

	8
Resource Requirements	The research programmes which provide the main input to this group are already underway, and resources already committed. The additional resource required to undertake additional activities in the framework of this group is negligible.
Participants	The Group is normally attended by some 20–25 members and guests
Secretariat Facilities	None
Financial	No financial implications
Linkages to Advisory Committees	There are no obvious direct linkages with the advisory committees
Linkages to other committees or groups	WGHABD interacts with WGZE, WGPME, WGPBI.
Linkages to other organisations	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.

17 Recommendations

17.1 Cooperative research report on HABs in the ICES Area

The proposed CRR on HABs and phytoplankton toxins in the ICES area is still being drafted. Following the meeting a rescheduled deadline for November was agreed with the ICES Executive Editor. The WG was in agreement that this will be a useful document that would be of value to scientists and agencies responsible for the implementation of monitoring programmes. The report continues to be drafted intersessionally under the direction of the chapter lead authors. The nominated authors are working closely with the Editor Richard Gowen report will be assembled and edited. Chapter headings and responsible persons are listed below:

Proposed Chapters of Cooperative research report:

1.	Introduction and definitions	(Richard Gowen, UK)	
2.	Harmful species	(Beatriz Reguera, Spain)	
3.	Monitoring and Management: country by count Denmark)	ry basis (Per Anderson,	
4.	Predicting occurrence	(Keith Davidson, Scotland)	
5.	Detection and quantification of algal toxins	(Allan Cembella, Germany)	

17.2 Summary of proposed ICES/IOC/PICES workshop on "HABs in a Changing World"

The process of climate change already may be causing shifts in coastal and oceanic phytoplankton community composition, and there are fears that these changes may increase the frequency and intensity of HAB events. There is a strong need to identify key gaps in our understanding about the environmental conditions that favour HAB initiation and maintenance in terms of projected climate driven changes in coastal waters. This critical assessment will serve as a springboard to focus attention on the research issues of greatest importance.

We propose to conduct a Joint ICES/IOC/PICES 3 day workshop to bring together HAB experts who undertake this synthesis. The workshop would immediately follow a shortened ICES WGHABD in 2012. Participants would prepare and submit an extended abstract of their current research 1 month prior to the workshop to help establish a conceptual framework of HAB/environmental interactions. After short (~10 min) presentations of this work, participants would split into working groups to identify the central unresolved problems that stymie significant advances in understanding how projected climate changes may influence HAB events. Tentative topics would include 1) the impacts of projected changes in macro- and micro-nutrient distributions on HAB organisms, 2) climate effects on the ecology of HAB species and trophic interactions, 3) and climate effects on the physiology/toxicity of HAB organisms. Participants will decide on the final topic areas after the initial discussions. Working group findings would be presented and discussed in a series of plenary sessions (1 day), which would be used to develop a refined list of research themes and key environmental and physiological parameters for future study. Participants also would consider likely candidate sites for establishing coastal HAB time series stations that would provide essential observation sites for evaluating climate/oceanography/HAB hypotheses. The specific outcome of the workshop would be a participant-authored journal article communicating the synthesis of primary research directions to the broader HAB research community.

Workshop planning will be done by co-chairs Mark Wells (PICES HAB-S) and Bengt Karlson (ICES WGHABD).

18 Closing of the meeting

The Chair thanked the host from SMHI, and congratulated him on behalf of the working group in his forthcoming term as Chair. The local liaison team were also thanked for their hospitality and generosity. He also thanked the participants for their input especially the 2 rapporteurs and closed the meeting on Friday, 13:00 hours.

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Annex 2: Agenda

ICES - IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD) 2011

Venue and dates: Gothenburg/ Sweden, 5-8 April 2011 Chair: Joe Silke, Ireland Professional secretary: Adi Kellermann Support secretary: Maria Lifentseva

Tuesday, 5 April 2011

Time	TOR	Lead	Item
09:30-10:00		J.Silke	Opening of Meeting, Logistics, Introductions, Adoption of the Agenda
10:00-11:00	TOR B	R.Gowen	CRR Report
11:00-11:30			Health Break
11:30-12:30			Break Out Chapter Sessions
12:30-13:30			Lunch
13:30-15:00			Interactions with Bill Anthony re CRR
15:00-15:30			Health Break
15:30-17:00			Assembling requirements of Report

Wednesday, 6 April 2011

Time	TOR	Lead	Item
09:30-	TOR I	All Participants	Report on new findings in the area of harmful
11:00			algal bloom dynamics;
11.00			
11:00- 11:30			Health Break
	TODI		
11:30- 12:30	TOR J	All Participants	Deliver National Reports on harmful algal events and bloom dynamics for the year 2010;
12:30- 13:30			Lunch
13:30- 14:00	TOR A	N.Holdsworth	ICES database manager to give a presentation to WGHABD and Discussion
14:00- 14:30			IT platforms used in ICES Expert Groups: Ouestionnaire
			~
14:30- 15:00	TOR A	H.Enevoldsen	Review progress in entering data onto the database;
15.00			Status for upload of all records 2002-2010;
			Status of quality assurance of HAEDAT records
			prior to 2002;
			New decadal maps based on HAEDAT data
			·····
15:00-			Health Break
15:30			
15:30-			Discussions with PICES members regarding
16:30			collaboration and integration of our two
			working groups
16:30-			Theme Session on HABs in the Baltic at 2011
17:00			ASC. Planning.

Time T	OR	Lead	Item
Time	TOR	Lead	Item
09:30-10:30	TOR C	B.Karlson	Review developments in automated HAB sampling devices in FerryBox systems and on other platforms;
10:30-11:00	TOR D		Discuss the need for and logistics of a demonstration workshop on automated in situ techniques for quantitative harmful algal bloom species analysis;
11:00-11:30			Health Break
11:30-12:00	TOR E	J.Silke	Review output from the intersessional meeting of members from WG HABD and WG BOSV to generate a list of HAB species with the potential to become invasive through transportation in ship's ballast and other vectors;
12:00-12:30	TOR F	A.Cembella	Review the pre-column oxidation technique for routine analysis of PSP toxins in shellfish (Lawrence method);
12:30-13:30			Lunch
13:30-14:00	TOR G	D.Anderson	Review the ICES special session on HABs that will be held during the Annual Science Meeting from 20–24 September 2010 in Nantes, France (La Cité des Congrès);
14:00-15:00	TOR K	J.Silke	Evaluate potential for collaboration with other EGs in relation to the ICES Science Plan and report on how such cooperation has been achieved in practical terms (e.g. joint meetings, back-to-back meetings, communication between EG chairs, having representatives from own EG attend other EG meetings).
			Draft aims and propose a structure for a joint meeting with the ICES Working Group on Physical–Biological Interactions (WGPBI) during 2012;
	TOR H		
15:00-15:30			Health Break
15:30			Excursion

Friday, 8 April 2011

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Time	TOR	Lead	Item
09:30-			Select New Chair of Group
10:30			Decide on 2012 Meeting Location
10:30-			Draft 2011 Resolutions / ToRs
11:30			
11:00-			Health Break
11:30			

11:30-		Complete Report Writing
12:00		
12:00	End of Meeting	