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Interim Report of the ICES – IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD)

13–18 April 2015

Lisbon, Portugal

ICES International Council for the Exploration of the Sea
CIEM Conseil International pour l'Exploration de la Mer

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Executive summary

The WGHABD met on 13–18 April in Lisbon, Portugal. The meeting was hosted by Teresa Moita, Portugal and chaired by Eileen Bresnan, UK. It was attended by 19 scientists from 11 countries with three scientists from an additional two countries participating by correspondence. The group addressed 12 terms of reference (ToR) relating to harmful algal bloom dynamics during the first year of their three year reporting term.

Members presented national reports on HABs during 2014 (ToR a). Highlights include the first report of concentrations of toxins responsible for paralytic shellfish poisoning (PSP) above the EU closure limit in *Mytilus edulis* Sweden and amnesic shellfish poisoning (ASP) in *M. edulis* in England. Closures of shellfish harvesting areas as a result of high concentrations of the toxins responsible for DSP were enforced in many countries in Europe and also in the USA. Cyanobacteria blooms were recorded in Poland, Finland and Sweden. A bloom of *Concinodiscus conncinus* produced nuisance slimes in Norway. ToR (d) examined the threshold/trigger limits for cell counts of shellfish toxin producing species/genera in phytoplankton monitoring programmes which vary throughout the ICES area. ToR (e) presented new findings on biosensors (Environmental Sample Processor, Imaging Flow Cytobot) in the USA, citizen science and HABs in France, *Vicicitus globosum* in Sweden and modelling *Karenia mikimotoi* in Scotland. Tor (h) reviewed the forthcoming ICES-PICES-IOC climate change and HABs symposium in Gothenburg in May 2015 where there have been 84 registrants. ToR (i) examined molecular methods for the identification of HAB species. Methods discussed included FISH, qPCR, and metabarcoding. ToR (k) addressed the physical and chemical control of different HAB species. This year focussed on the dynamics of *Gymnodinium catenatum* in the Iberian Peninsula. The role of upwelling and cyst beds were included in discussions to explain the variability of this species in this region. ToR (j) reviewed the current status of β-N-methylamino-L-alanine (BMAA) and clarified the confusion between discrepancies in the literature.

ToRs (g) and (l) reviewed the use of HAB/nuisance species for the EU Marine Strategy Framework Directive (MSFD) and JAMP phytoplankton monitoring guidelines. The WG flagged that HABs are influenced by numerous physical, chemical and biological parameters. In some instances HABs may be influenced by anthropogenic nutrient enrichment however there are cases where other hydrographic parameters have a dominant influence. Thus the presence of HABs should not be seen as an indicator of nutrient enrichment unless the link has been proven. Comments about the JAMP guidelines promote the use of plankton net samples to assess diversity and flag the absence of methodology to address subsurface blooms. The use of *Phaeocystis* as an indicator organism throughout the ICES area was questioned as there are non-eutrophic areas within the ICES area where blooms of this genus occur.

Three ToRs contribute towards the work of the IOC Intergovernmental Panel on HABs (IP-HAB). ToR (b) will produce a review of fish killing algae in the ICES area. This manuscript will be completed during the 2015 with a focus on northern Europe. As part of ToR (c) the WG held a one day workshop focussing on the Harmful Algal Event database (HAE-DAT)(http://haedat.iode.org/) on 15 April. This data is planned to be included in a Global HAB Status Report to be produced by IP-HAB. This workshop flagged issues with historic data to be addressed by group members before the 2016 WG meeting and priori-

tised issues for computer programmers to address to facilitate data extraction. ToR (f) addressed the production of a HAB status report which could act as an ICES contribution towards the IP-HAB Global HAB Status Report as well as a supporting document for the MSFD. The requirement for intercessional work prior to the next meeting was highlighted. Formal letters requesting data and highlighting its use will be sent to data providers. Example graphics from areas where the data is readily available will be produced before the 2016 meeting.

During 2016 the WGHABD meeting will be hosted by Raeffele Siano in IFREMER, Brest, France, 19–22 April.

1 Administrative details

Working Group name

Harmful Algal Bloom Dynamics

Year of Appointment

2015

Reporting year within current cycle (1, 2 or 3)

1

Chair(s)

Eileen Bresnan, United Kingdom

Meeting venue

Lisbon, Portugal

Meeting dates

13–18 April 2015

2 Terms of Reference a) – z)

a) Deliver National Reports on harmful algal events and bloom dynamics for the years 2014, 2015, 2016

b) Finalise a review document quantifying the scale, nature and extent of the problems associated with fish killing algae in the ICES region

c) A one day Harmful Algal Event Data Workshop as part of the 2015 WGHABD Meeting (with intercessional work performed by delegates prior to WG meeting)

d) Review the methodology used for the collection of phytoplankton samples in harmful phytoplankton monitoring programmes and the abundances used as threshold levels in harmful phytoplankton monitoring programmes

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

f) Identify HAB datasets that could be used to investigate climate related changes in HAB species phenology; present the assessment of representative datasets to describe HAB initiation and temporal trends and spatial variability; review outputs using the standard WGZE and WGPME result formatting

g) Evaluate use of harmful/nuisance algae as an indicator of 'Good Ecological Status' for the Marine Strategy Framework Directive Descriptor 5 (Eutrophication). Review draft OSPAR JAMP Eutrophication Guidelines on phytoplankton species composition

h) Review progress and advice the scientific steering committee for the planned joint ICES-PICES-IOC scientific symposium on Climate change and harmful algal blooms. The symposium is planned to be arranged in 2015

i) Review progress in development and application of molecular genetic technologies for taxonomic identification, phylogenetic reconstruction, biodiversity, toxin detection and population dynamic studies of HABs

j) Review the existing knowledge and latest findings on BMAA, the amino compound ß-methylamino alanine

k) Review how physical and biological interactions control of the dynamics of relevant harmful micro-algal blooms

l) Review of draft OSPAR JAMP Eutrophication Guidelines on phytoplankton species composition ICES is requested to advise OSPAR on the revision of the OSPAR JAMP Eutrophication Guidelines which will be revised by experts from Germany, The Netherlands and Sweden. WGHABD is asked to address the identification of harmful algae species and blooms in line with MSFD Descriptor 5 and relevant monitoring and measurement techniques as mentioned in the background information

3 Summary of Work plan

Year 1	Review of OSPAR and MSFD D5 Eutrophication guidelines, review of fish killing algae, Updating and quality control of data in HAEDAT, symposium on climate change and HABs. Identify data sets and editorial team for the HAB status report, current status of BMAA. Review on HAB species *Gymnodinium catenatum*.
Year 2	Completion of HAB status report, review of sampling methodologies and threshold levels in monitoring programmes, plan workshop on molecular techniques, Contribute towards Global HAB report as required. Contribute towards MSFD as required. Review on HAB genera tbc. ToR to be decided.
Year 3	Contribute to a workshop on new/molecular genetic techniques, Review of new technologies, Review on Hab genera tbc. Contribute towards Global HAB report as required. Contribute towards MSFD as required. ToR to be decided.
Year 1-3	Work on Global HAB report , update the Harmful Algal Event Database, report new findings, physical-biological interactions – selected HAB genera

4 List of Outcomes and Achievements of the WG in this delivery period

- The WGHABD response to the OSPAR request was finalised (See Annex 10). The WG had already reviewed the topic of HABs and eutrophication (see WGHABD meeting report from 2009). Comments on the OSPAR JAMP Phytoplankton Monitoring Guidelines are presented.
- A special session about HABs '*HABs in Aquaculture and Fisheries ecosystems: prediction and societal effects'* (theme session H) was held at the ICES ASC in Spain in 2014. The main objective of this session was to review increased monitoring efforts, technological developments for in situ detection of harmful algae, and new analytical tools for toxin detection. This session comprised 27

oral and 17 poster presentations. The extended abstracts for the presentations at this special session can be found at (http://www.ices.dk/sites/pub/CM%20Doccuments/Forms/AllItems.aspx?RootFolder=%2fsites%2fpub%2fCM%20Doccuments%2fCM%2d2014%2fTheme%20Session%20H%20contributions&FolderCTID=0x0120005EBB620DAE608446B85F6C23744A8054). The ICES report about this session can be seen in Annex 11.

- Preparation of the ICES-PICES-IOC symposium on climate change and HABs to be held in Gothenburg, Sweden was ongoing during 2014. The symposium has 84 registrants and will be held from 19–22 May 2015.
- A one day workshop on the IOC Harmful Algal Event database was held during the course of the WG meeting to prioritise programming effort for improvement of data extraction. Some technical issues were identified and historic data entry was performed. A prioritised list of topics for the programmer to address is presented in Annex 4.
- Production of summary table detailing the different methodologies and threshold levels used in phytoplankton monitoring programmes across the ICES area. This will be fed into the meeting of EU Reference Laboratories for Biotoxins (EU-RL) phytoplankton monitoring meeting held in Brussels in May 2015. This table can be seen in Annex 5.
- A review of the dynamics of the PSP producing dinoflagellate *Gymnodinium catenatum* was performed. A report on this review can be found in Annex 9.

5 Progress report on ToRs and workplan

ToR a: Deliver National Reports on harmful algal events and bloom dynamics for the years 2014, 2015, 2016

National reports from 14 countries were presented during the meeting. Detailed national reports can be found in Annex 3. National reports for 2015 will be reported during the 2016 meeting.

ToR b: Finalise a review document quantifying the scale, nature and extent of the problems associated with fish killing algae in the ICES region

The working group agreed at its meeting in Oban Scotland that (a) the occurrence of fish killing algae is a serious problem in ICES member states and globally; (b) there was a need for a detailed assessment of the scale of the problem and key knowledge gaps; (c) a joint ICES/IOC/PICES meeting to quantify the scale of problems caused by fish killing algae at the global scale should help.

The WG agreed that the best way forward was for a literature review, to be published as a scientific paper, on the subject. On pragmatic grounds of information availability this was restricted to Northern Europe in the first instance with the expectation that a full ICES area review would follow any further ICES/IOC/PICES meeting. Contributions come from R. Gowen (UK), K. Davidson (UK), R. Siano (Fr), S. Lehtinen (Fi), A. McKenny (UK).

The review is currently ~ 70 % complete and is expected to be submitted for peer review during this calendar year. Expected contents: (1) Introduction (2) Mortalities of fish caused by algal blooms, (i) Diatoms, (ii) Dinoflagellates, (iii) Microflagellates, (3) Impact on Ecosystem Services (i) Fish farming, (ii) Natural fisheries (iii) Ecosystem structure and function (4) Discussion/ Conclusions.

The paper will contribute to the work of the IP-HAB Task Team on Fish Killing Algae. WG members will report on progress with this manuscript during the 2016 meeting (see section 6).

ToR c: A one day Harmful Algal Event Data Workshop as part of the 2015 WGHABD Meeting (with intercessional work performed by delegates prior to WG meeting)

A one day workshop was held to focus on the IOC-ICES-PICES Harmful Algal Event Database (HAE-DAT). HAE-DAT data has been flagged for use in the IP-HAB Global HAB Status report see (http://hab.ioc-unesco.org/index.php?option=com_oe&task=viewDocumentRecord&docID=14983). Thus there is a requirement for data in HAE-DAT to be quality controlled and up-to-date. The data output from HAEDAT was reviewed by Catherine Belin and a number of issues were flagged about the consistency of historic data and ability of database to provide data to make decadal maps. Some technical issues in extracting the data were identified. The use of the data in regional assessments was discussed and missing/non QC-ed data were highlighted. HAE-DAT will be able to provide information about the spatial distribution of harmful events and potentially the evolution of events on a decadal scale. For finer scale trends an analysis similar to that performed in the ICES WG Phytoplankton and Microbial Ecology status report will be required. Some programming amendments are required to facilitate appropriate data extraction and also to improve the ease of data QC, harmonisation and data entry. A list of issues to prioritise was produced (see Annex 4). A new programmer will begin work on the database in May 2015 who will work with the IOC HAB delegate – Henrik Enevoldsen- to address these issues. The use of HAE-DAT data to produce decadal maps of harmful algal events in the ICES area will be reviewed during the 2016 meeting (see section 6).

ToR d: Review the methodologies used for the collection of harmful phytoplankton and the abundances used as threshold levels in harmful phytoplankton monitoring programmes

Members of the WG were asked to supply information about the methodologies and threshold levels used in their areas and a summary table has been produced (see Annex 5). It is recognised that a varied approach is necessary given the diversity of both environmental conditions and phytoplankton dynamics across the ICES region, and of technical approaches to monitoring. Threshold levels may be revised as new species/genera are observed for the first time in an area or new information is discovered about their potential toxicity. Members of a new phytoplankton working group initiated by the EU-Reference Laboratory (EU-RL)for biotoxins, have also been asked to supply similar information on sampling methodologies in preparation for their first meeting on 13 May 2015. The summary table produced by WGHABD will help to inform this new group. Delegates will feed back to the WG on the outcomes of this EU-RL meeting during the 2016 WG meeting (see section 6).

ToR e: New findings

Reports on new findings included:

- A description of citizen science in France to assist in the investigation of sea-water discolorations. This initiative greatly improved the ability of the monitoring agency to target monitoring effort in areas with water discolourations. It also improved the link between science and society.
- The use of two biosensors (the Environmental Sample Processsor and the Imaging Flow Cytobot) in the monitoring of Harmful Algal Blooms. Data from these automated devices can be seen to provide an early warning of pending harmful events and can also be used in modelling studies.
- The complexity of modelling *Karenia mikimotoi* blooms in Scotland was also highlighted. Statistical modelling of the major 2006 bloom found no clear relationship between high and low risk sites and their hydrodynamic characteristics. A possible hypothesis is therefore patches of *K. mikimotoi* being advected northward, during the summer months and, periodically, being driven into sea-lochs. However, this is difficult to reconcile with the speed of the coastal or shelf edge currents in the region. This is an area of current research.
- Observations of *Vicicitus globosum* in Sweden.

Detailed reports of these new findings can be found in Annex 6. A summary of two introductory presentations from Peter Miller (UK) on remote sensing and Anke Kremp (Finland) on HABs in Finland are also presented in this Annex. New findings will continue to be reported at the 2016 meeting.

ToR f: Production of Harmful Algal Bloom Status Report

The group discussed the generation of a Harmful Algal Bloom status report similar to that produced by the WG on Zooplankton Ecology and WG on Phytoplankton and Microbial Ecology (http://wgpme.net/images/stories/status-reports/crr313-wgpme.pdf). This report could act as a contributing document from ICES to the Global HAB status report to be produced by IP-HAB and also as a supportive document for the EU Marine Strategy Framework Directive. WG delegates with available data will send these to Todd O'Brien, NOAA to produce example graphics. These will be circulated during intercessional period and will be reviewed at the WG meeting in 2016. There was concern amongst some delegates about the ability to obtain data for use in this report from the various monitoring agencies owing to resource limitations and ownership of the data. The chair of WGHABD will draft a letter for approval by the group to send to data providers explaining the purpose of the report, asking if they want to contribute to it and how the data will be used. The datasets available inclusion in the report will then be confirmed during the 2016 meeting.

ToR g: Evaluate use of harmful/nuisance algae as an indicator of 'Good Ecological Status' for the Marine Strategy Framework Directive Descriptor 5 (Eutrophication). ToR L: Review draft OSPAR JAMP Eutrophication Guidelines on phytoplankton species composition

See ToR l which duplicates this ToR.

ToR h: Provide and update on ICES–PICES–IOC symposium on Climate change and HABS

The ICES-PICES-IOC symposium on Climate Change and HABs will take place from the 19–22 May in Gothenburg, Sweden. There are currently 74 people registered to participate. A review of this symposium will be presented at the WG during 2016 (see section 6).

ToR i: Review progress in development and application of molecular genetic technologies for taxonomic identification, phylogenetic reconstruction, biodiversity, toxin detection and population dynamic studies of HABs

It is 20 years since technologies such as Fluorescent In Situ Hybridisation (FISH) and quantitative polymerase chain reaction (qPCR) were first developed. These methodologies have greatly improved the ability to monitor cryptic and difficult to identify species. More recently these assays have been further developed to target toxin producing genes to provide information about the toxicity of phytoplankton populations. More complex 'metagenomic barcoding' techniques which can produce detailed libraries on the molecular diversity of samples have been developed. Detailed reviews of the presentations can be found in Annex 7. During discussions during this ToR it was realised that many delegates have been using these techniques in routine monitoring programmes for a number of years. During 2016 this ToR will be amended to include presentations from delegates who use these techniques routinely to capture the experiences using these techniques for both monitoring and research purposes (see section 6). Detailed summaries of the presentations can be found in Annex 7.

ToR j: Review the existing knowledge and latest findings on BMAA, the amino compound, ß–N–methylamino –L–alanine (BMAA)

ß-N-methylamino –L-alanine (BMAA) is a small non-proteogenic amino acid. It was originally found in the seeds of cycad (*Cycas micronesica*) growing on the Pacific Island of Guam. Since the work published by Cox *et al.* (2003), the production of this compound by symbiotic and free-living cyanobacteria has been associated with the elevated frequency of neurodegenerative diseases among people exposed to the toxin. Recently, BMAA has also been detected in axenic strains of diatoms as well as filter feeders such as mussels and oysters. Although the presence of BMAA in aquatic organisms and food products was documented in several countries (e.g. Sweden, France, USA, China), so far, a direct link with the incidents of disease has not been proven. Methodological problems have been identified which led to high variations in the reported concentrations of BMAA. When HPLC with fluorescence detector was used, the BMAA concentrations were overestimated or even false positive results were obtained (Christensen *et al.* 2012). Currently, LC-MS/MS for the derivatized or underivetaized BMAA is recommended as the most reliable detection technique which minimizes the risk of misidentification. However, the analytical procedure should always be validated, and effects such as ion suppression or the presence of BMAA structural isomers should be considered. A more detailed review including key references on this topic can be found in Annex 8.

ToR k: Review how physical and biological interactions control of the dynamics of relevant harmful micro–algal blooms: *Gymnodinium catenatum*

A review of the dynamics of *Gymnodinium catenatum*, a PSP producing dinoflagellate which has caused problems in the Portugese and Spanish waters was presented by Tere-

sa Moita, Ana Amorim and Beatriz Reguera. This review considered the arrival and spread of these blooms, interannual variation, toxicity and strain types. Modelling studies revealed the potential role of cyst seed beds in seeding blooms, particularly in early summer. A detailed review of this species in this region can be found in Annex 9. During the 2016 WG meeting this ToR will focus on *Alexandrium minutum* (see section 6).

ToR l: Review of draft OSPAR JAMP Eutrophication Guidelines on phytoplankton species composition ICES is requested to advise OSPAR on the revision of the OSPAR JAMP Eutrophication Guidelines which will be revised by experts from Germany, The Netherlands and Sweden. WGHABD is asked to address the identification of harmful algae species and blooms in line with MSFD Descriptor 5 and relevant monitoring and measurement techniques as mentioned in the background information

The ICES Working Group on Harmful Algal Bloom (HAB) Dynamics was asked to review the draft OSPAR JAMP Eutrophication Guidelines on phytoplankton species composition. Since these guidelines were developed there has been considerable work performed on the response of the phytoplankton community to environmental and anthropogenic drivers. The OSPAR area is broad, and includes diverse regions such from North Atlantic (parts) to the Baltic Sea. Current guidelines, which are used are prepared in 1997 did require a revision and OSPAR asked NL, SW, and Ge to revise the guidelines. Mainly SW and NL collaborated on this task. The draft was produced by Bengt Karlson and Marie Johansen from Sweden together with Hans Ruiter from the Netherlands. Two comments from Peter Miller about remote sensing were included. The comment about coccolithophores is related to general monitoring.

WGHABD has previously addressed the issue of HABs and Eutrophication (see WGHABD report 2009) with members of the WG producing a review on this topic (Gowen *et al.*, 2012) and the influence of nutrient ratios on HABs (Davidson *et al.*, 2012). Both of these peer review publications provide comprehensive reviews on the physical, chemical and biological variables which influence the occurrence of HABs and the role of anthropogenic nutrient enrichment.

Case studies from Gowen *et al.*, (2012) have shown that in some coastal embayments the restricted exchange between the embayment and open coastal water can be the dominating factor which influences the nutrient-HAB association (e.g. Tolo Harbour in Hong Kong) however in other regions e.g. along the west coast of Ireland, nuisance blooms (e.g. *Karenia mikimotoi)* can occur in the absence of nutrient enrichment. For many coastal regions, attempts to relate trends in the occurrence of HABs to nutrient enrichment are confounded by increased monitoring effort and reporting of HABs, the effects of climate change (e.g. the North Atlantic Oscillation Index and the El Niño Southern Oscillation) and the introduction and transfer of HAB species. Thus the occurrence and abundance of HAB species and HABs should not be used to diagnose eutrophication unless a link to anthropogenic nutrient enrichment can be demonstrated for a specific area. Within legislation the Urban Waste Water Directive and OSPAR definitions require the demonstration of a series of cause and effects e.g. nutrient enrichment leading to accelerated growth of plants and or a disturbance of the balance of organisms which is undesirable.

Within the context of the phrasing within D5 of the Marine Strategy Framework Directive, the link between human activities and blooms/nuisance algae within a region must be proven before these can be used as an indicator of eutrophication. It should also

be noted that evidence of a link in one coastal region should not be taken as evidence of a general linkage in other coastal regions.

In general there was consensus that the JAMP guidelines are adequate for long term monitoring of the phytoplankton community. However the guidelines are not flexible enough to integrate HAB monitoring for sanitary purposes. The guidelines continue to refer to HABs as being caused by a distinct group of species that has some inherent connectivity. This is not the case. HAB species comprise a diverse range life forms/strategies. Their only unifying feature is a negative impact on the ecosystem or ecosystem services. This requires clarification in the document. The guidelines also aim to include generalized monitoring of phytoplankton which is not always suitable for focused HAB monitoring. The EU-National Reference Laboratory for Biotoxins (EU-NRL) working group on Phytoplankton Monitoring (starting May 2015) will focus on this matter specifically. WG delegates attending this EU-NRL group will report back to WGHABD during the 2016 meeting (see ToR D).

The JAMP guidelines encompass the whole phytoplankton community and the need to improve sections on spatial distribution and temporal trends was identified, particularly if a nutrient driven response is to be separated from climate change. **If OSPAR adopts these JAMP guidelines the role of ICES in the coordination and storage of data was may increase. This should be noted by ICES.**

Specific comments can be found in Annex 10.

6 Revisions to the work plan and justification

During the meeting some alterations to the ToRs were discussed. These are detailed below along with the justification for the amendment.

Table 1: Amendments to year 2 of WGHABD work plan.

ToR b: Finalise a review document quantifying the scale, nature and extent of the problems associated with fish killing algae in the ICES region**.**
Amendment: Review focused on fish killing algae in Northern European waters
Justification: Scale of ICES region too large
ToR c: HAE-DAT
Amendment: During year 2 the decadal maps produced by HAE-DAT will be reviewed.
Justification: Decadal maps have been a key product from WGHABD and usually produced by hand. Using HAE-DAT to produce these will make the process more efficient and provide a product that can be used in the IP-HAB Global Status report.
ToR d: Review the methodology used for the collection of phytoplankton samples in harmful phytoplankton monitoring programmes and the abundances used as threshold levels in harmful phytoplankton monitoring programmes
Amendment: Feedback from EU-RL meeting on phytoplankton monitoring methodologies

Justification: The EU-RL will set methodologies that will become standard across Europe. The WG should be aware of developments in this area.

ToR f: Identify HAB datasets that could be used to investigate climate related changes in HAB species phenology; present the assessment of representative datasets to describe HAB initiation and temporal trends and spatial variability; review outputs using the standard WGZE and WGPME result formatting.

Amendment: Review graphics produced by Todd O'Brien for HAB status report. Finalise available data sets for inclusion in report.

Justification: Progress towards the production of a HAB status report needs consensus on the production of graphics. In order to identify potential editors of the report the final datasets for inclusion in the report will be identified. The datasets for inclusion will be based on response from official latter from WGHABD requesting the data.

ToR h: Review progress and advice the scientific steering committee for the planned joint ICES-PICES-IOC scientific symposium on climate change and harmful algal blooms. The symposium is planned to be arranged in 2015.
Amendment: This will be amended to 'Feedback to the group on ICES-PICES-IOC symposium on climate change and harmful algal blooms, Gothenburg, Sweden, May 2015'.
Justification: Not all WG delegates are in a position to attend the meeting.

ToR i: Review progress in development and application of molecular genetic technologies for taxonomic identification, phylogenetic reconstruction, biodiversity, toxin detection and population dynamic studies of HABs.
Amendment: WG members who routinely use molecular methods will present on their experiences using these techniques are part of routine monitoring programmes.
Justification: Discussion during this ToR flagged a number of WG members had experience using these techniques in routine monitoring programmes. This experience should also be documented as they may differ from experiences from research purposes.

ToR k: Review how physical and biological interactions control of the dynamics of relevant harmful micro-algal blooms
Amendment: During the 2016 meeting this ToR will focus on *Alexandrium minutum.*
Justification: The location of the 2016 WGHABD meeting in IFREMER, Brest provides access to a considerable breath of expertise with this toxin producing species which has not been previously examined by this group in any detail.

7 Next meetings

The next WGHABD meeting will take place from 19–22 April 2016 in Brest, in France. Raeffele Siano, Ifremer will be the host.

Annex 1: List of participants

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Annex 2: Agenda

DRAFT AGENDA FOR ICES WGHABD MEETING 13TH – 17TH APRIL 2015

Time	ToR	Leads	Item
Monday 13th April			
10:00		E. Bresnan T. Moita A. Silva	Opening of meeting, logistics, introductions, adoption of agenda
10:20		B. Karlson	Update on 2014 report
10.30		New Members	Introduction from new members
11:15			Health Break
11.30		New Members	Introduction from new members (ctd)
11:45	H	B. Karlson	Update on ICES/PICES climate change and HABs symposium
12:30			Lunch
13:45	J	H. Mazur	Review existing knowledge and latest findings on BMAA
14:30	A	All	National reports
15:30			Health Break
15:45	D	S. Milligan	Review sampling methodologies and thresholds for collection of phytoplankton samples in harmful phytoplankton monitoring programmes
16:45	E	All	New Findings
17:30		Meeting close	

Annex 3: ToR a: Deliver National Reports on harmful algal events and bloom dynamics for the years 2014, 2015, 2016

USA (Don Anderson)

New England experienced relatively moderate levels of PSP toxicity during 2014 extending from the Casco Bay area of western Maine through the north shore of Massachusetts. In addition, a small area of far eastern Maine experienced toxicity. Once again, the state of Maine instituted blanket closures for mussel harvesting for the entire state beginning from May 15 – October 31. However, the state lifted the closures in August when toxicity in mussels returned to below quarantine levels. There were no shellfish closures due to ASP toxin detection in this region.

In New York (Long Island), no closures were implemented due to presence of PSP toxins for the first time since 2007. Levels never exceeded 49 μg / 100 g shellfish on the south shore / eastern end of Long Island. The highest toxin level was 53 μg / 100 g shellfish at one station at one occasion in mid-May (located between the north and south forks of eastern Long Island). There were no New York Department of Environmental Conservation closures due to DSP toxins. A research program documented DSP toxin levels of 20 μg / 100g shellfish in blue mussels and soft shell clams in the Northport Bay and Cold Spring Harbor regions. This was the eighth year in a row with elevated *Aureococcus* (responsible for brown tide) concentrations (570 000 000 cells/L), following a decade of very low levels. Before that, there was a decade of high concentrations, beginning in 1987. *Cochlodinium* has bloomed in eastern Long Island since 2005. It is a persistent "red tide" former throughout the Long Island region, as well as in Buzzards Bay and other areas of southeastern Massachusetts.

In Florida *Karenia brevis* blooms occurred on the west coast of Florida as well as Cedar Key. Both blooms occurred in the fall. Fish kills (multiple species) were attributed to both blooms but no respiratory problems in humans were reported at either location. The west coast bloom reached cell densities of > 5.5 million cells /L; the Cedar Key bloom reached 2.3 million cells / L. There were no reports of *Karenia brevis* on the east coast.

Pyrodinium bahamense blooms occurred on the west coast (Tampa Bay) and in the Indian River Lagoon. The Tampa Bay bloom lasted approximately four months but did not reach the lower levels of the Bay where shellfish harvesting is permitted, thus no seafood testing was done. The maximum cell concentration was 1 153 000 cells/L. The Indian River Lagoon bloom (east coast) also lasted for approximately four months with a maximum cell count of 2 425 000 cells/L. A management closure was implemented because saxitoxins were found in hard clams (*Mercenaria mercenaria*) at 217 μg / 100 g shellfish. No human illnesses were reported. *Pyrodinium bahamense* has been a recurring issue in both Florida locations over the past decade. Before that, PSP toxicity was not known to be a problem in Florida. There were no occurrences of any brown tide organisms. A *Pseudo-nitzschia* bloom (maximum cell count of 6,783,333 cells/L) occurred in Saint Joseph Bay (west coast) in late fall. Domoic acid was detected in eastern oysters (*Crassostrea virginica*) at a low level – 2.2 mg /100 g shellfish tissue.

In Texas a *Karenia* blooms occurred in the fall, first observed at Port Aransas by the Imaging Flow Cytobot (IFCB) in early-mid-September and reach peaks > 10 000 cells / L by the end of September. No shellfish harvest closures were issued – the bloom receded in mid-October.At the beginning of March, the IFCB provided information of increasing abundances of *Dinophysis ovum*. This was the seventh successful early warning by the IFCB for the Texas coast. By mid-March, cell counts exceeded 5000 cells / L. The Texas Department of State Health Services reported elevated counts of *Dinophysis* in the Galveston region and issued a closure in mid-March. The closure was extended to the Matagorda system through Corpus Christi Bay in later March; *D. ovum* reached a maximum count of 6 700 000 cells / L. The overall closure lasted over one month.

In California *Alexandrium* was observed at sites along most coastal counties during 2014. The highest percent composition and frequency of occurrence was in July at Chimney Rock LBS (Marin County). Measurable concentrations of PSP toxins were found in 222 shellfish samples from the following coastal counties: Del Norte, Humboldt, Sonoma, Mendocino, Sonoma, Marin, San Mateo, Santa Cruz, Santa Barbara, Los Angeles, and Orange. Low levels of the PSP toxins were detected at sites in Humboldt and Del Norte County for each month between January and September. Concentrations of PSP toxins greater than or equal to the alert level (80 μg/100 g of tissue) were detected in 33 samples from the following counties: Del Norte, Mendocino, Sonoma, Marin, and Los Angeles. The highest PSP toxin concentration occurred in sentinel mussels collected at Chimney Rock (Marin County), which contained 2134 μg/100 g. California's Department of Public Health's volunteer phytoplankton observers detected significant increases in *Pseudo-nitzschia* at sites representing all coastal counties during 2014. High relative abundances of *Pseudo-nitzschia* were observed at sites between Humboldt and Los Angeles counties. The estimated percent composition of this diatom exceeded 90 percent at sites along each coastal county between Santa Cruz and Ventura. The greatest relative abundances occurred in the Monterey Bay Area in April. The magnitude of domoic acid toxicity in 2014 was similar to that observed in 2013, with moderate toxin levels occurring mostly in the spring at various locations. Measurable concentrations of domoic acid were found in 102 samples during 2014, compared to 100 samples during 2013. Domoic acid was detected in samples from the following coastal counties: Humboldt, Sonoma, Marin, San Francisco, San Mateo, Santa Cruz, Monterey, San Luis Obispo, Santa Barbara, Ventura, Los Angeles, Orange, and San Diego. Concentrations of domoic acid above the alert level (20 μg per gram of shellfish meat, or 20 parts per million (ppm)) were detected in 33 samples from the following four counties: Santa Cruz, Monterey, Santa Barbara, and Ventura. These high levels of domoic acid were detected in February near the Channel Islands, from March through June in Monterey Bay, and from September through October in Ventura County. There were no reports of DSP toxins in shellfish this year.

In Oregon PSP toxin levels reached 792 μg / 100 g shellfish in mussels, resulting in closures. A domoic acid closure along the Oregon coast began in September and continued through the end of the year; levels reached 53 ppm in razor clams.

In Washington the highest PSP toxin level recorded was 12 688 μg / 100 g shellfish in blue mussels in Central Puget Sound. This is the third year in a row with very high toxicity. There were 26 sub-tidal geoduck clam tracts and 5 general growing areas closed in 2014 due to PSP. There were 18 recreation closures, including the Pacific coast, Sequim Bay and Puget Sound. There were no commercial or recreational closures due to domoic acid

in 2014. Low levels of domoic acid were reported again this year – with the highest being 6 ppm in razor clams at Long Beach. Two commercial closures and nine recreational closures due to DSP occurred in 2014. The highest toxin level recorded was 180 μg / 100 g shellfish found in blue mussels in Sequim Bay.

Since the first toxicity and closures reported in 2011, DSP has become a serious and persistent problem for Washington state.

Heterosigma fish kills: A bloom of *Heterosigma akashiwo* in northern Puget Sound, including Port Angeles, the San Juan Islands, and Bellingham Bay caused mortalities of net penned salmon. Densities of *H. akashiwo* reached 5 000 000 cells / L. Salmon were taken off feed to help them survive the bloom. At least 345 adult summer chum salmon were killed the third week of September.

Alaska does not conduct routine monitoring except for commercially harvested shellfish. Therefore closed areas may be ignored by residents, thereby causing the possibility for exposure to toxicity. One probable case of PSP in humans occurred in Alaska in June, 2014. In December of 2013, fish inspectors in China notified the U.S. Embassy that China was tentatively suspending imports of geoducks and other "double-shell aquatic animals" such as oysters, because they found high levels of PSP in a Nov. 21 shipment of geoducks. China closed its doors to all shellfish imports from an area that stretches from northern California to Alaska. The state of Washington reports losses of as much as $600,000 per week. The ban was instituted after finding two "bad" clams – one from Alaska with high PSP levels and one from Washington state with high inorganic arsenic levels. The ban was lifted after six months but industry sectors and agencies are worried about current monitoring protocols and the potential for another export issue with China. Many believe that if there is a continued export ban by China due to PSTs in U.S. geoducks, it will be economically catastrophic to these fisheries, potentially eliminating the export fishery from the entire west coast. Ninety percent of the geoduck harvested in Washington is sold to China and Hong Kong.

Canada (Cynthia McKenzie)

On the west coast of Canada data for PSP, DSP and ASP were unavailable. There were a number of incidences of salmon mortalities. *Chrysochromulina* sp. (provisionally identified as *C. ericina* and other *Chrysochomulina* species) was first detected May 24, 2014 in Knight Inlet (ICES CA38). The bloom lasted approximately 2 weeks and affected most of Knight Inlet. Farmed salmon mortalities were seen at mostly one farm site, although 3 were affected. Maximum cell numbers were 16 million cells.L^{-1} on May 26, 2014. *Heterosigma akashiwo* was responsible for salmon mortalities in Queen Charlotte Strait (ICES code CA38) in late September when concentrations were 40 million cells.L^{-1}. *H. akashiwo* was detected in Broughton Archipelago and Queen Charlotte Strait on the west coast of Canada on September 2, 2014 prior to the mortalities.

On the east coast highest concentrations of *Alexandrium fundyense* was observed in the Bay of Fundy, southwest New Brunswick (CA 22) with highest shellfish toxicity detected in *Mytilis edulis* at 7977 μg 100 g STX equivalent on July 16, 2014. Also in the Bay of Fundy but in Nova Scotia (CA 19) 426 ug/100g STX equivalent was detected in the soft shell clam, *Mya arenaria*, also in July 2014.The Atlantic coast of Nova Scotia toxicity values did not exceed 80 μg 100g STX equiv. and no shellfish beds were closed to harvesting. No

report was received from the St. Lawrence Estuary but shellfish harvesting areas were above threshold levels and closed to harvesting for a portion of the spring/summer. Newfoundland and the Gulf of St. Lawrence did not experience any shellfish closures in 2014. A maximum value of 1.6 ug/g YTX was found in *Mytlis edulis* on the Atlantic Coast of Nova Scotia (CA 17) on November 3, 2014. Toxin was detected in mid- October (0.51 ug/g) and continued until Dec 01, 2014 (0.54 ug/g). Only one location in New Brunswick (CA 22) had high levels of domoic acid from September 25 to December 5, 2014 with a maximum value in the *Placopectin magellanicus* of 320ug/g on October 1, 2014.There were no reported salmon mortalities at aquaculture operations on the east coast associated with HABs in 2014.

Finland (Anke Kremp)

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. occur every year. The blooms produce noxious surface scums, which - when washed ashore - may become a health risk. During 2014 the cyanobacterial bloom prognosis, made by a forecast model of the Finnish Environment Institute, predicted an increased risk of blooms for the western Gulf of Finland, Archipelago Sea and Northern Baltic proper based on high surface phosphorus levels remaining after the spring bloom. The winter of 2013/14 had been unusually stormy which caused extensive mixing of nutrient rich deep water into the surface layer of these areas. The prognosis was confirmed when extensive surface blooms developed at the SW coast of Finland after mid-July 2014. Continuous surface scums of *Nodularia spumigena* covered the entire Northern Baltic proper and coastal areas of Finland and Sweden for approximately 3 weeks. Dominance of *N. spumigena* over *Aphanizomenon flos aquae* was due to exceptionally high water temperatures (>20 C), disfavoring the latter species. The summer cyanobacterial bloom of 2014 was exceptionally intense with high chl a (> 40 ug/L) surface concentrations. The Finninsh Environment Institute, regional and municipal authorities informed the media about the blooms and advised the public on health risks involved with the *Nodularia* scums. In addition to cyanobacteria, toxic dinoflagellate blooms may occur in shallow coastal waters of Finland. In August a dense bloom of *Alexandrium ostenfeldii* was detected in the Föglö archipelago of Åland. The bloom was exceptionally dense, reaching > 6 x 10^6 cells L^{-1}. PST concentrations of ca. 14 μg L^{-1} were measured in the water. Besides PSTs, Baltic *A. ostenfeldii* produces Gymnodimines. The major compound is an unidentified novel GYM with [M+H]+ at m/z 524 which is presently being characterized. Considerable amounts of this gymnodimine were measured in the Föglö bloom. After a media release on the bioluminescent *A. ostenfeldii* blooms in coastal Finnish waters, local inhabitants and vacationers reported on bioluminescence (indicating dense *A. ostenfeldii* blooms) in the Turku archipelago and other sites in Åland. No incidences of toxicity were reported.

Sweden (Bengt Karlson)

Cyanobacterial blooms were observed during the summer of 2014. In the Baltic Proper, surface accumulations were observed offshore from 4 July–12 August. The maximum area of coverage was at the end of July when the island of Oland and archipelago of Stockholm was affected. A coastal bloom was observed in the southern part of the Stockholm archipelago 19–21 September. Surface accumulations of cyanobacteria were ob-

served in the eastern part end of the bothnian sea in July. Coastal scums were recorded in this region in the summer. The first record of PSTs about the regulatory limit was recorded in *M. edulis* in April and May 2014. DSTS were also recorded in *M. edulis* in May and November.

Norway (Wenche Eikrem)

Information about harmful algae and toxic mussels in coastal areas of Norway can be found on the following web pages http://algeinfo.imr.no and http://www.matportalen.no/verktoy/blaskjellvarsel/. The monitoring is carried out by IMR (www. imr.no), SINTEF (www.sintef.no) and NIVA (www.niva.no)

Coscinodiscus conncinus. A mucus producing bloom of *Coscinodiscus conncinus* took place in March 2014 along the Southern and Western coasts of Norway. Other species were also present, but *C. conncinus* was dominating the samples and was registered in quantities not encountered before in Norwegian waters. In Flødevigen 460 cells/liter was recorded on March 26. It was suggested that the bloom had itsorigin in the North Sea and was transported to the coast by wind and currents. Fishermen had their nets and other equipment fouled by mucus produced by the *C. concinnus* bloom. The event was described in several newspapers (e.g. Bergens tidene and Sunnmørsposten) and illustrated by pictures of fishing nets full of brown slippery mucus (see picture below from Sunnmørsposten), quite unpleasant for those involved. *Vicicitus globosus.* In October/November of 2014 a bloom of *Vicicitus globosus* was observed in the Kattegat and Skagerrak. The first report was from Havstensfjorden, on the Swedish West Coast in the beginning of October. It was registered in October and November on the Southern and Western coasts of Norway and in Flødevigen the highest abundance was recorded on October 24 at 1000 cells/liter. No harmful events were registered although initially the bloom was suspected to beassociated with the death of large quantities of *Crassostrea gigas*, but was shown later to be caused by a herpes virus. In Norway we have indications that *Vicicitus globosum* is a life cycle stage of *Dictyocha fibula*. The latter occurs commonly in the fall in Norwegian waters. PSP, DSP and ASP were also detected in Norwegian mussel farms.

Poland (Justyna Kobos)

In summer 2014, the open and coastal waters of the Gulf of Gdansk experienced blooms of cyanobacteria, with temporal (July) dominance of the nodularin-producing *Nodularia spumigena* and the non-toxic *Aphanizomenon flos-aquae*. The presence of microcystin-producing *Dolichospermum lemmermannii* was also observed. In coastal waters, the highest biomass of *N. spumigena* (185.4 mg/L) and the highest concentration of nodularin (1,590 µg/L) were recorded on 8 July. In the samples collected on that day, the biomass of *Aph. flos-aquae* and *D. lemmermannii* was 11.8 mg/L and 0.4 mg/L, respectively.

Similarly to previous years, *Alexandrium ostenfeldii* was observed in Puck Bay (inner part of the Gulf of Gdansk) at the end of August. The cell number of this species in a sample collected on 28 August was 3,980 cells/L; i.e. it was four times higher than it was recorded at the same sampling station in 2013.

Poland has about 98 bathing places located on the Baltic coast. Each year thousands of tourists spend their holidays on the beaches. To be in line with the EU Bathing Water Di-

rective (2006/7/EC), the water quality and the presence of cyanobacteria at these sites are monitored. In the 2014, between 8 July and 8 August, 14 beaches were closed per 1-4 days due to the massive occurrence of cyanobacteria. All they were located in Pomeranian District: from Sopot (Gulf of Gdansk; area code PL-01) to Jastrzebia Gora (open sea waters; area code PL-02).

Denmark (Per Anderson)

DSP closures were enforced twice during 2014. Week no 28 at the East Coast of Jutland (area 63) – Mytilus edulis: AO = 280 ug OA-eq/kg, Dinophysis acuminata = 0 cells/L and in week no 34 in the outer Wadden Sea (area 144) – Spisula subtruncata: 144 ug OA-eq/kg, no algae info A number of cases (total: 8) below critical limit (37-77 ug OA-eq/kg) occurred also in the Limfjord, together with high concentrations of Dinophysis acuminata (3.000–122.800 cells/L).Only traces of domoic acid (max. conc. 7 ug DA/kg) during weeks 11-14. No PSP in 2014 – Alexandrium tamarense max. conc. 200 cells/L in week 22 in the Limfjord. During the spring (week: 11-13) a minor bloom of Pseudochattonella (probably P. farcimen) (max. conc. 20.000 cells/L) followed by a major bloom in weeks 14–17 (max. conc. 3,3 mill . cells/L) of small round Pseudochattonella/Dictocha-like cells delayed the release of trout to fish cages in the coastal waters. No fish kills at marine aquaculture sites during the spring bloom events - because no fish were in the sea yet. No reports on negative impacts on the natural stock of sea trout fishing in the bloom period. No other dead fish species observed during the bloom. No negative effects reported on birds and mammals either.

Germany (Allan Cembella)

Germany does not have an integrated national strategy for monitoring HAB events in German coastal waters; bloom information is provided from local environmental authorities and state-funded research institutes maintaining long plankton time-series. In 2014, the German coast of the Baltic Sea was subjected to typical annual cyanobacterial blooms (primarily of *Nodularia* sp.) causing beach fouling, although not to an unusual extent. At Helgoland (HAEDAT region DE-04), the potentially toxic HAB species *Dinophysis tripos* and *Pseudo-nitzschia multiseries* were identified and recorded for the first time in September 2014, but neither occurred at bloom concentrations. This toxigenic diatom may have been present before, but identity was not confirmed until now at the species level.

The Netherlands (Marnix Poelman)

In 2014 the shellfish production areas; North Sea, Lake Grevelingen, Wadden Sea, Oosterschelde and Veerse Meer were monitored for the presence of toxic phytoplankton. This program is based on the National Shellfish Food Safety Program on a monthly basis from November until April and a weekly basis from May until October. The results are used as an early warning mechanism for potential presence of toxins in the shellfish (mussels, oysters, ensis and cockles). In total 334 phytoplankton samples have been collected at a total of 13 sampling locations.

As in many previous years, toxins have not been reported above regulatory limits. However, during the period of end of July till mid-October *D. acuminata* was reported present above threshold values (100 cells/litre). The main affected areas are the Wadden sea and Lake Grevelingen. In both cases *D. acuminata* counts up to 1.600 cells/litre were reported.

The majority of the results were however reported at levels ranging from 100-800 cell/litre.

Although no high toxin levels are found, back ground levels of lipophilic toxins (OA, DTX) are reported in ensis (5 samples, 10-18 μg OA-eq/kg), mussels (4 samples, 10-15 μg OA-eq/kg), and oysters (10-17.4 μg OA-eq/kg).

Besides OA and derivatives, also Cyclic Iminines are found in 7 mussel samples (5-12.5 μg/kg 13-des Me SPX C, and 5-6.2 μg/kg GYM A), and oyster samples (5-26.5 μg/kg 13-des Me SPX C, and 5-6.6 μg/kg GYM A. The findings on GYM A are further investigated. RIKILT, Wageningen UR has been responsible for the toxin analysis.

UK (Sarah Swan, April McKinney, Steve Milligan)

In Northern Ireland the genus *Alexandrium* spp. was recorded in 4.8% of samples. A maximum abundance of 140 cells L^{-1} was recorded on the 6th May in a sample taken from Belfast Lough. Results from the Biotoxin Programme showed that no shellfish flesh samples, tested as part of the Official Control Programme, contained levels of PSP toxins above the regulatory limit.

Cells of *Dinophysis* spp. were recorded in 20.6% of samples although only 4.8 % of samples contained concentrations above the trigger value of ≥100 cells L^{-1}. The maximum cell abundance recorded was 2320 cells L^{-1} in a sample taken from a Belfast Lough site on 29th September. *Prorocentrum lima* was counted in only 1.8 % of samples reaching a maximum abundance of 100 cells L^{-1} in a sample taken from Carlingford Lough on 9th June. This was the only occasion when the trigger value of ≥100 cells L^{-1} was breached. Results from the Biotoxin Programme showed that no shellfish flesh samples contained DSTs above the set regulatory value.

Pseudo-nitzschia spp. were present in 69.7% of water samples received during the year. The maximum recorded cell abundance was 262 500 cells L^{-1} in a sample from Dundrum Bay on 28th April. Domoic acid was not detected above the regulatory level of 20 μg/g in shellfish samples tested as part of the Official Control Programme.

Prorocentrum cordatum was recorded on three occasions between the 28th April and the 7th July. Cell abundance reached a maximum of 46 440 cells L^{-1} on the 28th April in a sample from Carlingford Lough. The ichthyotoxic species *Karenia mikimotoi* was present in 5.5% of samples recording a maximum abundance of 120 cells L^{-1} in a water sample from Belfast Lough on the 22nd September. The haptophyte genus *Phaeocystis* was present in 2.8 % of samples with a maximum cell abundance recorded of 193 520 cells L^{-1} on the 14th May in a sample from the Dundrum Inner South site in Dundrum Bay.

During the year blooms of *Mesodinium rubrum*, *Leptocylindrus minimus* and *Prorocentrum triestinum* were also recorded. *Mesodinium rubrum* was recorded at 2.27 x 10^6 cells L^{-1} in a sample from Carlingford Lough in June. A bloom of *Leptocylindrus minimus* in Lough Foyle in July was recorded at 3.96 x 10^6 cells L^{-1} and in August a bloom of *Prorocentrum triestinum* reached a maximum abundance of 10.2 x 10^6 cells L^{-1}. No detrimental effects were reported with any of these events.

In Scotland *Pseudo-nitzschia* was observed in 96.5% of all samples analysed and present throughout the year. *Pseudo-nitzschia* counts in excess of 50 000 cells per litre (threshold level) were recorded in 9.1% of the samples, with almost 13% of the samples analysed in

June having greater than threshold cell densities. The earliest blooms were recorded around the Shetland Islands and Dornoch Firth during March. The densest *Pseudo-nitzschia* bloom was observed in Loch Sligachan (Highland: Skye & Lochalsh) on 23-Apr-14, where a maximum density of >3.1 million cells/L was recorded. This bloom was widespread in the Highland region, from Arisaig: Morar Sands (Lochaber) to Loch Eishort (Skye & Lochalsh), Loch Torridon and Loch Ewe (Ross & Cromarty), with some associated ASP toxicity. Blooms exceeding 1 million cells/L were also recorded in Luce Bay (Dumfries & Galloway) in April, and around the Shetland Islands (Vementry South: Seggi Bight, Busta Voe and Olna Firth) on 30-Jun-14, and toxin-producing *Pseudo-nitzschia* was widespread around Shetland between June and August, although the maximum permitted level of 20mg/kg ASP toxins in shellfish tissue was not exceeded.

Alexandrium was present in 42.2% of the total samples analysed. It was recorded at or above the trigger level (set at 20 cells/L until 20-Jul-14 and then at 40 cells/L from 21-Jul-14) in 39.1% of all samples. *Alexandrium* was most frequently observed during May, June and July, and was recorded at or above 40 cells/L in 48.6% of samples during July. The densest recorded *Alexandrium* bloom was observed in Loch Leven (Highland: Lochaber) on 18-Jul-14, with an abundance of 24 660 cells/L. The majority of cells were relatively small in size and although the presence of *Alexandrium minutum* was confirmed, no associated PSP toxicity in shellfish was reported at this time. However, an *Alexandrium* bloom of density 7280 cells/L recorded in Loch Striven (Argyll & Bute) on 17-Jun-14 was associated with an extensive PSP toxic event from late May until late July. PSP toxins were detected in common mussel flesh at more than 18 times the regulatory limit of 800 μg STX eq. kg^{-1}. *Alexandrium* was widespread throughout the Shetland Islands and was continuously present in Sandsound Voe for a period of 23 weeks between 05-Mar-14 and 04-Aug-14. Continuous blooms were also recorded in Braewick Voe (19 weeks from 02-Apr-14) and East of Linga (15 weeks from 30-Apr-14), with maximum densities of 1260 cells/L and 1200 cells/L observed at these sites, respectively on 30-Jul-14. PSP toxins were reported in shellfish from both of these sites and exceeded permitted levels at East of Linga. Toxin-producing *Alexandrium* was also variously recorded between March and July in Barassie (South Ayrshire), Arran: Lamlash Bay (North Ayrshire), Loch Fyne: Ardkinglas and Loch Scridain (Argyll & Bute), Arisaig: Morar Sands (Highland: Lochaber), Loch Laxford and Dornoch Firth (Highland: Sutherland), Forth Estuary: Largo Bay (Fife), and Fersness Bay (Orkney Islands).

Dinophysis was present in over 47.2% of the samples analysed throughout the year and occurred at or above threshold level (100 cells per litre) in 19.3% of all samples. It was recorded at all sites where regular monitoring took place over the summer months. The earliest blooms were observed in Argyll & Bute and North Ayrshire during late March and early April, although the majority of *Dinophysis* blooms occurred in July and August, with 43.6% of the samples at or exceeding threshold counts in July. The largest recorded *Dinophysis* bloom was observed in Loch Torridon (Highland: Ross & Cromarty) on 22-Jul-14, with an abundance of 3520 cells/L. *Dinophysis* blooms were widespread around North Ayrshire, Argyll & Bute, and the Highland region from May to late September, with associated DSP toxicity reported in shellfish. Arran: Lamlash Bay (North Ayrshire) recorded *Dinophysis* counts at or above trigger level for a continuous period of twenty-one weeks from the second week of May until the end of September. Both Loch Striven and Loch Scridain (Argyll & Bute) also had prolonged bloom periods lasting about three

months from late May into August. The blooms of *Dinophysis* that were observed around the Shetland Islands in July and August 2014 were neither as dense nor extended as those that occurred in in 2013. In general, the number of *Dinophysis* blooms at or exceeding trigger level over the reporting period was similar to the years 2010, 2011 and 2012, but not as high as in 2013.

Prorocentrum lima was present in 14.9% of all samples analysed during 2014 and occurred at or above the trigger level (set at 100 cells/L) in 1.4% of samples. It was most frequently observed in samples from Colonsay: The Strand East, Loch Fyne: Otter Ferry, and Loch Melfort (Argyll & Bute), but the densest bloom of 2014 of 920 cells/L was recorded in South Voe (Shetland Islands) on 14-Jul-14.

In England 703 inshore shellfish samples were tested for Amnesic Shellfish Poisoning (ASP) toxins using a high performance liquid chromatography (HPLC) method. ASP toxins were detected in 36 samples from 22 production areas. Twenty six of these results were recorded in the south west of England, from Poole in Dorset to the Taw/Torridge in North Devon, between April and July. One sample of mussels exceeded the maximum permitted limit (MPL) of 20 mg [domoic+*epi*-domoic acid]/kg [shellfish tissue}. This sample had been collected from the Portland production area on 13/05/2014. It is the first time that ASP toxins have exceeded the MPL in an English classified production and relaying area since 2001. ASP toxins below the MPL and *Pseudo-nitzschia* species above the re-sampling trigger level (set at 150 000 cells/L) were detected in the week prior to the 13/05/2014 in this production area.

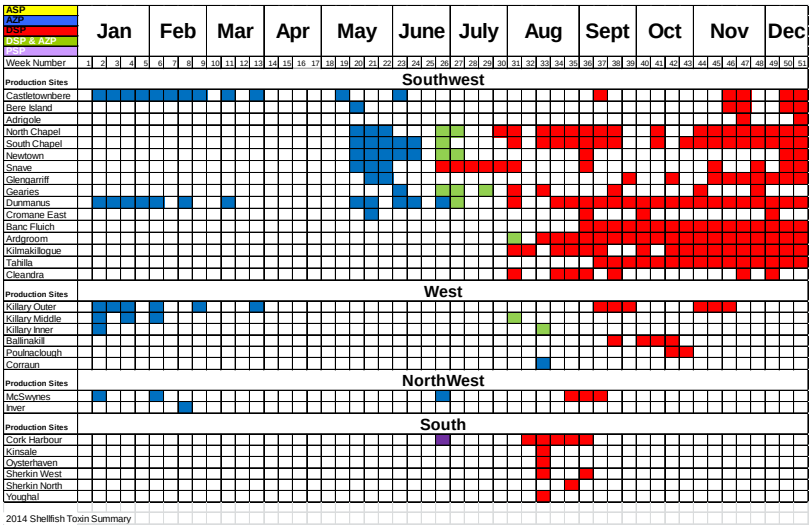
A total of 744 shellfish samples were screened for PSP toxins using a separate HPLC method. Three samples were forwarded for full quantitative analysis, with one mussel sample from the Yealm production area (collected on 25/06/2014) recording a high value of 315µg STX eq/kg. This is the lowest number of recorded PSP occurrences in England and Wales since the HPLC full quantitative method was introduced in 2008. This may be partially attributed to several sites which have traditionally recorded PSP toxins not being monitored over the period from April to September 2014, due to changes in harvesting activities (Fal, Fowey and Milford Haven).

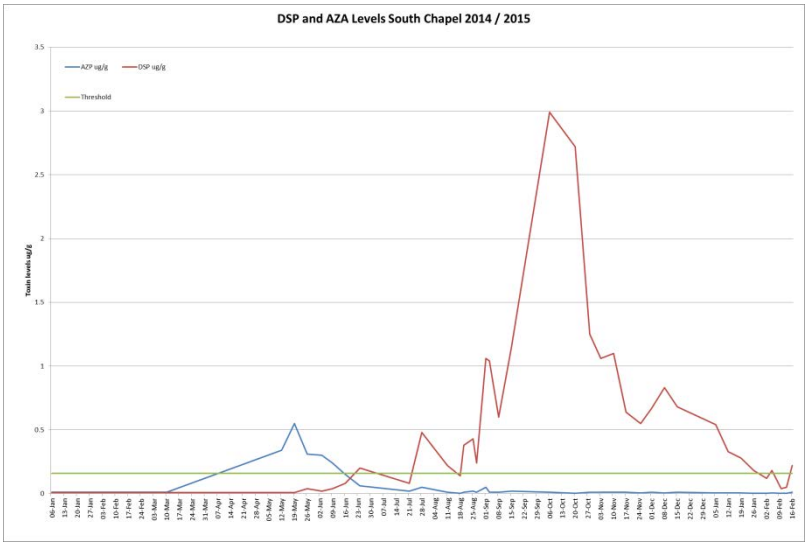
A total of 753 samples were analysed for LTs using a Liquid Chromatography with tandem mass spectrometry (LC-MS/MS) method. The lipophilic toxins are sub-divided into the three regulated groups. Yessotoxins (YTXs) were detected in one mussel sample collected from the Brixham production area on 03/06/2014. This is the first time YTXs have been detected in an English classified production/relaying area since the LCMS method was introduced in 2011. The result did not exceed the MPL (set at 3.75 mg YTX eq/kg). Azaspiracid group toxins (AZAs) were detected in one Pacific oyster sample from the Holy Island production area. The results did not exceed the MPL (set at 160 µg AZA1 eq/kg).

Ireland (Joe Silke)

HAB events in Ireland in 2014 were limited to the occurrence of 3 shellfish poison types detected in the course of the national monitoring programme. Unusually, there were no detections of ASP in rope mussels in the spring/ early summer, and the usual late summer Azaspiracid event was much earlier in May / June followed by a protracted DSP event that lasted from July through to the end of the year. This event extended into 2015

and finally cleared during the month of February coinciding with the establishment of increased diatom presence in early spring. No other HAB events were reported (red-tides or fish killing blooms).





Pseudo-nitzschia and ASP: *Pseudo-nitzschia* spp. cell counts were observed to remain at constant concentrations and distributions throughout Jan - Feb, increasing in March. The increases were observed to be predominatly in the *Pseudo-nitzschia delicatissma* group. Molecular analysis on phytoplankton samples during March from the SW was observed to contain the presence of the ASP toxin producing spieces *Pseudo-nitzschia australis*, and the non-toxic species *P. fraudalenta* & *P. delicatissma.* Typically all shellfish samples analysed (non scallops) were <LOD & <LOQ during this time frame, with some minor concentrations observed. ASP above the regulatory levels have been observed in the Gonad and remainder tissues in scallops from Bantry, Roaringwater, Dunmanus during Jan - Mar, highest concentrations observed are in the table below. ASP above the regulatory levels have been observed in the remainder tissues in scallops from Ballinakill, Mulroy, Kinsale & Valentia River. *Pseudo-nitzschia spp*. cell counts and their distribution were ob-

served to be increasing from April, in particular in the SW. The increases were observed to be predominately in the *Pseudo-nitzschia seriata* group. Molecular analysis on phytoplankton samples during March onwards from the SW was observed to contain the presence of the ASP toxin producing species *Pseudo-nitzschia australis*, and the non-toxic species *P. fraudalenta & P. delicatissma*. The other known ASP toxin producing species *Pseudo-nitzschia seriata* and *multiseries* were not detected during the ASP event in the SW. During April - May the presence of *Pseudo-nitzschia australis* was observed to increase rapidly, the presence of ASP was detected in samples of *M. edulis* in Dunmanus, Bantry, Kenmare, Castlemaine Hbr. All ASP concentrations observed were below the regulatory level. From June, typically all shellfish samples analysed (non scallops) were <LOD & <LOQ during this time frame, with some minor concentrations observed. In addition to routine ASP analysis via HPLC, from June a semi-quantitative screen for analysing the presence of Domoic Acid via LCMS was introduced to run on all non-scallop routine samples, where the majority of samples were observed to be N.D. (not detected). ASP above the regulatory levels were observed in the remainder tissues in scallops from classified production areas Rosmuc, Blasket Islands, Castletownbere, Kenmare River, Cahirciveen & Portmagee Channel, Broadwater North, Clew Bay South & North) from April – Aug. ASP above the regulatory levels have been observed in the gonad tissue from the offshore site OS-NQ-NQ (New Quay 33-E5) in Aug. For August – September typically all shellfish samples analysed (non scallops) were <LOD & <LOQ during this time frame, with some minor concentrations observed. In addition to routine ASP analysis via HPLC, from June a semi-quantitative screen for analysing the presence of Domoic Acid via LCMS was introduced to run on all non-scallop routine samples, where the majority of samples were observed to be N.D. (not detected) ASP above the regulatory levels were observed in the remainder tissues in scallops from classified production areas Castletownbere, Portmagee Channel and Clew Bay North (highest concentrations observed in table above). ASP above the regulatory levels were also observed in the gonad tissue of scallops from Portmagee Channel.

Alexandrium and PSP: Between Jan – Mar 2014, all samples analysed for PSP were typically Not Detected for PSP toxins, Alexandrium spp. have been sparodically observed in this time frame at very low population densities. *Alexandrium spp* cell counts and distribution was observed to increase nationally from June onwards, and increasing in particular in July & Aug in the West and NorthWest. Some sporadic high counts were also observed in the South & SouthWest during this time period. Quantifiable concentrations below the regulatory level were observed in samples of *C. gigas* from Cork Harbour, Kinsale & Oysterhaven. One sample of *M. edulis* was observed to be above the regulatory level during the last week of June from Cork Harbour. *Alexandrium spp* cell counts and distribution was observed to decrease nationally from September onwards. Very low quantifiable concentrations below the regulatory level were observed in samples of *C. gigas* from Cork Harbour, Kinsale & Oysterhaven and in samples of *M. edulis* from Cork Harbour. These concentrations in shellfish were observed to be decreasing throughout August – September.

Dinophysis and DSP: Quantifiable concentrations, below the regulatory level, were also observed throughout Jan – Mar 2014, mainly in the SW and Killary, in all other locations DSP concentrations were typically <LOD or <LOQ. *Dinophysis spp* cell counts were observed to increase from June onwards, initially in the SouthWest, and increasing further

during July and August in the South & SouthWest, with lower cell densities being observed in the West. *Dinophysis acuminata* was the predominant species observed in samples in June, where from the end of June onwards *Dinophysis acuta* was the predominant DSP causative organism present. DSP concentrations above the regulatory level were first observed in samples of *M. edulis* in June in Bantry & Dunmanus, followed by closures in Kenmare in July. DSP concentration were observed in increase throughout July, reaching a peak by the end of July in the majority of sites in SouthWest. During August, DSP concentrations above the regulatory level were observed in samples of *C. gigas* along the South Coast from Sherkin West, Kinsale, Oysterhaven and Youghal Bay, in samples of *M. edulis* from Cork Harbour, and also in the remainder tissues of *P. maximus* from Valentia River. Also in August, DSP concentrations above the regulatory level were observed for a short period in samples of *M. edulis* from Killary in the SouthWest, the cell no.'s of *Dinophysis acuta* (the predominant DSP causative organism present causing DTX-2 and hydrolysed DTX-2 in shellfish) was observed to continue to decrease in the SouthWest during October, with further decreases at the beginning of November. Subsequently, DSP concentrations increased to high levels in samples of *M. edulis* from Dunmanus, Bantry, Kenmare and Castlemaine Harbour, reaching a peak concentration in September / October before starting to decrease. DSP concentrations in samples of *C.edule* from Castlemaine were also above the regulatory level during October. During September – October DSP concentrations were observed above the regulatory level in samples of scallops (remainder and gonad tissues) from Valentia River (Aug – Sept) and in the remainder tissues from Castletownbere (Oct). Also in September - October on the West Coast, DSP concentrations above the regulatory level were observed in samples of *M. edulis* from Killary, Ballinakill & Ballyvaughan.

Azadinium and AZA: Toxicity from 2013 persisted above the regulatory level into 2014 in Castletownbere and Dunmanus and also in Killary, the toxicity was observed to decrease during Jan – Mar to below the regulatory level by the end of March A new AZA event occurred from May – Jun in the SW, where concentrations above the regulatory level were observed in sites in Bantry, Castlemaine and Dunmanus, and also in the NW for a short period in one sample of *M. edulis* from Bruckless. Molecular analysis conducted on phytoplankton samples from these sites showed the presence of the AZA causative species, *Azadinium spinosum*. AZA concentrations were also observed above the regulatory level during end of July and August in samples of *M. edulis* from Killary and in one sample of *C. gigas* from Achill South No further AZA concentrations were observed above the regulatory level during for the remainder of the year in samples of shellfish submitted.

France (Catherine Belin)

Three types of toxic episodes were observed in France during the year 2014: DSP, PSP and ASP.

DSP. Several species of *Dinophysis* comprise the main DSP producing species in France. They were observed over a large part of the French coast, as in most years. In 2014, the highest concentrations were observed in Normandy (80 000 cells/L) Toxic episodes, with toxin results above the sanitary threshold of 160 µg/kg for the group of OA+DTXs+PTXs, took place on many sites along the Channel and Atlantic coast, affecting diverse shellfish

as mussels, oysters, scallops, etc. The highest toxin concentration was observed in mussels (*Mytilus)* of the Seine bay (Normandy) with 4876 μg/kg.

PSP. *Alexandrium minutum* is the main PSP producing species along the coasts of Channel and Atlantic. *Alexandrium* was observed primarily along the Brittany coast. In 2014, the highest concentrations were observed in Jaudy river (North Brittany) with 708 000 cells/L, and in Brest bay (West Brittany) with 403 000 cells/L. Only one toxic episode followed these blooms, in Brest bay, affecting mussels (*Mytilus*) with a maximum of 3881 μg/kg (compared to the sanitary threshold of 800 μg/kg).

ASP. Several species of *Pseudo-nitzschia* are the ASP producing species in France. They were observed on the whole French coast at high concentrations during spring, as each year. In 2014, the highest concentrations were observed in the North of France (Cap Gris Nez and Somme bay) with a maximum of 4,780,00 cells/L. Toxic episodes, with toxin results above the sanitary threshold of 20mg/kg, took place in Normandy and West and South Brittany, affecting mainly scallops but also mussels and oysters in Brittany. The highest toxin concentration was observed in scallops (*Pecten maximus)* in Brest bay (West Brittany) with 860 mg/kg.

Spain (Margarita Fernandez-Tejedor, Luz Maman, Yolanda Pazos, Beatriz Reguera)

Highlights: Very long lasting DSP events in the whole Atlantic coast (Galicia and western Andalucia). Uncommon (once every several years) bloom of *G. catenatum* in western (Atlantic) Andalucía. At least 12 people suffered respiratory irritations during *Ostreopsis* blooms in Catalonia. HAB monitoring of the Valencia community region, carried out by IRTA, started in 2014. Galician local press reported two mild cases of ASP in consumers of mussels from areas under harvesting quarantine.

ANDALUCIA

ASP

Atlantic coast. Off Huelva, a *Pseudo-nitzschia* cf *australis* event that lasted from mid-March to the end of April (max. 69 708 cells L^{-1}) was associated with detection of domoic acid below regulatory levels (RL). The situation occurred again after a brief bloom (max. 1.6 x 10^5 cells L^{-1}) in July.

Mediterranean coast. Positive values above RL were found in shellfish from Cádiz and Málaga after a bloom developed in the whole eastern coast of Andalucia. A new bloom, not causing shellfish closures, developed in Algeciras Bay (Cádiz) in July (max 7.5 x 10^5 cells L^{-1}).

DSP

Atlantic coast. DSP events in western Andalucia (Huelva) are mainly associated with blooms of *Dinophysis acuminata.* In 2014 an early moderate bloom (max. 1.7 x 10^3 cells L^{-1}) led to positive values all along the western coast. A new major bloom started in April (max 6.7 x 10^3 cells L^{-1} in late April) that declined in late May and peaked again later (9.5 x 10^3 cells L^{-1}) lasting until September. From late July through August a bloom of *Dinophysis acuta* (max. 3.2 x 10^3 cells L^{-1}) co-occurred with *D. acuminata* in the Guadiana River estuary and adjacent areas. Positive (DSP toxins) results were recorded from April to Oc-

tober throughout the region. From October to December, low densities (≤ 500 cells L^{-1}) of *D. acuminata* persisted but only one positive result was recorded.

Mediterranean coast: Moderate blooms of *D. acuminata* off the coasts of Cádiz and Málaga in April as well as in late August – September (max. 1.2 x 10^3 cells L^{-1}) associated with some scattered positive results.

PSP

Atlantic coast: Some minor blooms of *Gymnodinium catenatum* (max. 3. 4 x 10^3 cells L^{-1}) off Huelva were not associated with PSP closures.

Mediterranean coast: *G. catenatum* exhibited the same patterns throughout the whole eastern Andalucian coast, i.e., minor proliferations on January and late May and a major bloom developed from late September to November, with maxima of 5.6 x 10^3 cells L^{-1} off Cádiz and 6. 9 x 10^3 cells L^{-1}off Málaga. Positive results detected in these two provinces. Blooms were more moderate in the most northerly province, Almería, where cell densities never exceeded 10^3 cells L^{-1} and positive PSP results were only observed in January.

[1] Information provided by Laboratorio de Control de Calidad de Recursos Pesqueros

(LCCRRPP), Junta de Andalucia

https://ws128.juntadeandalucia.es/agriculturaypesca/moluweb/

CATALONIA[2]

In coastal embayments of the Ebro Delta abundances of toxic species during 2014 were low in comparison with other years; alert levels were reached only occasionally (*Dinophysis sacculus* and *Alexandrium minutum* in January).

DSP

Closures were enforced in Arenys and Masnou in June-July after positive mouse bioassay results. The presence of yessotoxins under regulatory levels was confirmed by LC-MS/MS.

PSP

Precautionary closures due to high levels of *Alexandrium minutum* (5x10^5 cells L^{-1} inside several harbours) were enforced in Arenys from February to the end of April, and in Vilanova during April and June.

Fish Killers

High densities of *Heterosigma akashiwo* found (7x10^5 cells L^{-1}) in August in Vilanova Harbor

Benthic HABs

Ostreopsis (4 x10^4 cells L^{-1}) in open waters off Vilanova at the end of July and beginning of August.

[2] Information provided by the Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Generalitat de Catalonia http://www.irta.cat/ca-ES/RIT/A/A3/Pagines/A31.aspx

CATALAN Beaches[3]

During summer 2014, microalgal species causing harmful blooms in 22 beaches of the Catalan coast were identified. The most abundant species were dinoflagellates: *Ostreopsis* spp (benthic HAB), *Alexandrium taylori* (green discolorations) and several Gymnodinioids (high biomass HAB).

Benthic HABs

O*streopsis* spp. (blooms associated with toxic aerosols) in high densities are detected in rocky shores. Exceptionally high densities of *Ostreopsis* cf. *ovata* (> 10^6 cells L^{-1}) were found in mid July in Sant Andreu de Llavaneras, a hot spot for the species in the Catalan coast were blooms are recurrent and last for several months. By the end of July-early August, at least 12 people exposed to the beach aerosols suffered from respiratory irritations and required medical attention. By late July, high densities were also detected in Sitges-Terramar ($6.6 \cdot 10^4$ cells L^{-1}) and Ribes-Rojes ($1.5 \cdot 10^4$ cells L^{-1}).

High Biomass HABs

Alexandrium taylori was detected in all the Costa Brava beaches surveyed in densities below than usual (< 10^6 cells L^{-1}). Densities above 10^5 cells L^{-1} were observed at the Muga Estuary, l'Estartit 3 and La Fosca (max. $3 \cdot 10^5$ cells L^{-1} in early August). Densities observed were unusually low in other beaches (La Gola, el Rec, el Grau i Sant Feliu).

Blooms of *A. taylori* are usually accompanied by other naked bloom forming dinoflagellates (*Gymnodinium litoralis, Levanderina fissa* (=*Gymnodinium instriatum*), *Barrufeta bravensis*,...)

During summer 2014, densities of these Gymnodinioids exceeded that of *A. taylori* with maxima of $2.9 \cdot 10^6$ cells L^{-1} by mid-July in Grau Beach and > 10^5 cells L^{-1} in l'Estartit 3 and la Gola.

Other high biomass blooms (so far not associated with harmful effects) included: *Protoperidinium quinquecorne* ($1.3 \cdot 10^5$ cells L^{-1}) in La Gola beach; nanoflagellates in La Muga estuary, Cala Montgó (max. $4.3 \cdot 10^6$ cells L^{-1}), La Fosca and Sant Feliu; a bloom of pennate diatoms in La Muga estuary ($7.4 \cdot 10^6$ cells L^{-1}).

[3] Information provided by the "Grupo de Investigación en Procesos Biológicos Litorales" from the Instituto de Ciencias del Mar (CSIC, Barcelona)
http://pbl.cmima.csic.es/es/content/portada

GALICIA[4]

ASP

Pseudo-nitzchia spp. blooms caused mussel harvesting bans for a short period from late May to early June in the middle reaches of Ria de Arousa (max. $3.5 \cdot 10^5$ cells L^{-1} *Pseudo-nitzschia australis* at Pobra H in early June). This outbreak also affected infaunal species from Arousa, Pontevedra and Vigo (Rias Baixas).

DSP

Blooms of *Dinophysis acuminata* caused mussel harvesting bans in the whole Rías Baixas region from mid April to mid July (max. $2{\cdot}10^4$ cells L^{-1}at P5, mouth Ria de in early May). Infaunal shellfish was also affected. A second outbreak lasted from August to October with record values for *D. acuminata* (81920 cell L-1) at P5 on mid August, accompanied by *D. caudata* (1960 cell L^{-1}).

PSP

Alexandrium minutum blooms did not cause closures of raft-shellfish but affected infaunal shellfsh species from the inner reaches of Ría de Ares-Betanzos (Rias Altas) in April and August. Cell maxima at L3 ($7.4{\cdot}10^3$ cells L^{-1} in April and $2.6{\cdot}{\cdot}10^3$ cells L^{-1} in August). Additional closures affected infaunal shellfish from Ría de Cedeira between August and October (max. $1.5{\cdot}10^3$ cells L^{-1} at G5 on early August) and Ría de Camariñas from May to November (max 7.6 10^4 cells L^{-1} at GD on early August).

Gynnodinium catenatum (max. 880 células L^{-1}) caused a minor two-weeks outbreak in early November affecting raft shellfish but not infaunal species from Portonovo (Ría de Pontevedra).

[4] Information provided by the "Instituto Tecnoloxico para o Control do Medio Mariño de Galicia" (INTECMAR), Xunta de Galicia (www.intecmar.org)

VALENCIA[5]

ASP

Pseudo-nitzschia was frequent and abundant along the year reaching the maximal abundance (10^6 cells/L) in Sagunto at the end of August. ASP toxins were < regulatory levels.

DSP

Dinophysis sacculus and *inophysis caudate* were present in low abundances; D. *sacculus* reached alert levels at the end of June in Burriana. *Lingulodinium polyedrum* and*Gonyaulax spinifera* were frequent and present in low abundances along the coast. There were two DSP closures in Valencia at the end of March-beginning of April.

PSP

Alexandrium minutum and *Alexandrium catenella* were present in low abundances in Valencia and Alicante respectively, PSP toxins were < regulatory levels.

Fish Killers

In Alicante, a bloom of *Gymnodinium impudicum* (non toxic but producer of mucilage) lasted from June to September. Maximal density (10^5 cells/L) found in early September was associated to fish kills in the area.

Benthic HABs

*Ostreopsis*reached 5060 cells/L in Villajoyosa in early July; this concentration is over the warning level for palytoxins used in other European countries (4000 cells/L).

[5] Information provided by the Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Generalitat de Catalonia http://www.irta.cat/ca-ES/RIT/A/A3/Pagines/A31.aspx

Portugal (A. Silva and M.A. Castelo-Branco)

The Portuguese Monitoring of HABs and phytotoxins, carried out by IPMA (Portuguese Institute for the Sea and Atmosphere, www.ipma.pt/), covers the whole coast of Portugal except Madeira and Açores archipelagos. The sampling grid covers 10 coastal areas (20 stations) and 26 estuaries+coastal lagoons (30 stations). The sampling is carried out on a weekly basis: 50 samples from shellfish harvesting areas and phytoplankton retention areas (90% of the stations are coincident for water and shellfish samples and 10% are sentinel stations for HABs initiation). During 2014, 15 events were reported in coastal areas and 14 in estuaries+coastal_lagoons. The events have lasted a minimum a month and a maximum of nine months, starting, in general, in April. From these events, 25 closures were by DSP associated toxins above the critical limit, *Dinophysis acuminata* recorded a maximum concentration of 76x103cells/L (850 µg OA equiv. Kg-1 in *Spisula solida*) in the NW coast in September (29/9/14) and 4 closures by ASP in Aveiro Ria, when 217x103cells/L of *Pseudo-nitzschia seriata* complex were observed (27 mg/Kg in *Cerastoderma edulis*) in June. The most problematic areas were the NW coast and Algarve. In these areas, events and bans are for longer periods, while for the SW coast events are normally shorter in time. The year of 2014 was warmer and rainy than 2013 and the number of events varied slightly, 29 in 2014 in relation to the 26 events in 2013 (9 DSP and 2 ASP events in coastal areas and 12 DSP and 3 ASP events in estuaries+lagoons). Compared to 2013, in 2014 events were detected earlier in the year:

- ASP events were detected in June instead of September, with a similar interval, around a month and half and mostly located at the NW coast.

- DSP events occurred in January, and again, from April until December, the longer and continuous ban recurrently observed in the NW and S coasts.

Regarding the presence of new species, *Karenia* genus, instead of single and sporadic detections, is becoming regular in monitoring water samples but in lower concentrations (<200 cells/L). *Gymnodinium aureolum* and *Takayama cladochromum* are also becoming regular presences in samples. No events were recorded for benthic *Prorocentrum* species or benthic habs (e.g. O*streopsis*, *Coolia*) In October 2014, a single and occasional event of fish mortality (natural fish) was reported in Obidos lagoon, by locals that contacted IPMA. Water samples were collected and 250x103 cells/L of *Mesodium* spp. and 800 cells/L of *Kryptoperidinium foliaceum* were observed. These species were either absent or in very low concentrations in the monitoring water samples from the weeks prior and subsequent to this event.

Annex 4: ToR c: Harmful Algal Event Data Workshop as part of the 2015 WGHABD Meeting

ToR C: A one day Harmful Algal Event Data Workshop as part of the 2015 WGHABD Meeting (with intercessional work performed by delegates prior to WG meeting)

Henrik Enevoldsen/Catherine Belin

A one day workshop was held to focus of the IOC Harmful Algal Event Database (HAEDAT). Data from HAEDAT will contribute to the generation of a global harmful algal bloom status report. The data output from HAEDAT was reviewed by Catherine Belin and a number of issues were flagged about the consistency of historic data and ability of database to provide data to make decadal maps. A number of issues to address were raised. These include**:**

1. There is a link between event and syndrome, implying that important to fill syndrome field
2. There is a link between grid and event , implying old records need a grid
3. What defines a start and end of an event?
4. Magnitude of an event?
5. What does 'event' mean over time when monitoring change over time? Is it one or two events if a species comes one week go away but comes back 4 days later? : *to be judged in each case*.
6. How to take into account when changes in legislation imply higher or lower no of events?
7. Why species names with and without author?
8. Should toxin list only include regulated toxins for which there is a method? For P Hess and his Task Team to clarify.
9. With the help of your database expert, it will be possible to make the existing records more useful for time-series analysis.
10. Update front page and add: Some browsers may not provide full functionality (e.g Firefox on Mac)

Technical issues to be address/changes to make ASAP:

11. Insert: definition of HAB event on top of input form
12. Check why CVS file does not contain all data, e.g. toxicity data, dates?
13. Can data be imported back in from a CSV file – e.g if data is corrected and completed?
14. Change: 5. 'Toxin Assay Information' to '5. Toxin Information'
15. Change: '**Assay type' to 'Analytical technique', Add LC-FD, LC-UV**
16. Change: '**Toxin assay comments': to 'Toxin technique comments':**
17. Change: **Economic losses: to Socio-Economic losses:**
18. Delete: Kit used?: If yes, kit type:

Wishes for changes and improvements in DB:

19. Wish: Associated syndrom should be mandatory if the following fields are filled : , Seafood toxins; Has any toxicity been detected
20. Wish: Make "Has the event directly affected:" mandatory (otherwise not data for HAEDAT)
21. Wish: Change of date fields:
    Date of detection of harmful effect and/or regulatory action:
    End of harmful effect and/or regulatory action:
    Start of harmful bloom:
    End of harmful bloom:
    = mandatory
22. Delete event date
23. Wish; new functionality in verification tool to indicated that a given grid year x was: no monitoring/ no events/ and if this is not done then it implies that reporting was not done.
24. Wish: Verification function - can it be a waiting room? As it is now data already in DB before verified Wish: Section 5. **Max. Concentration. Wish: Pick list: ug/kg, ug/L, ug/g, (ug/100g: only if there is a conversion script!). Wish: Script to convert all to ug/kg except for those originally indicated in ug/L. Field is at the moment not included in CVS files!**
25. Wish: When name of a monitoring programme is entered, it should be saved and later picked from pull down meny: Is this report the outcome of a monitoring programme? Can entry field be made bigger?
26. Wish: Codes for grids could they include the longer name in pull down menu so easier to recognize
    (or even show map with location on map?)
27. Wish: Map position: a choice to show center of grid or actual position of each record Wish: Pick list for shellfish/fish names. Most common + option to type in.
28. As the 'Event Date' field is being removed from the form, if that is the only date that has been entered then this will be lost, and we must ensure that the now mandatory impact start/end dates are filled for old records. So something like:

    IF ("harmful impact start date" is blank) THEN
    IF ("HAB event date" is valid) THEN
    "harmful impact start date" = "HAB event date".
    ELSE IF ("Harmful bloom start date" is valid) THEN
    "harmful impact start date" = "Harmful bloom date".
    ENDIF

    IF ("harmful impact end date" is blank) THEN
    IF ("Harmful bloom end date" is valid) THEN
    "harmful impact end date" = "Harmful bloom end date".

ELSE
“harmful impact end date” = MAX(“Harmful event date”, ”Harmful impact start date”),
ENDIF

Annex 5: ToR d: Review the methodologies used for the collection of harmful phytoplankton and the abundances used as threshold levels in harmful phytoplankton monitoring programmes

ToR d: Review of the methodologies used for the collection of harmful phytoplankton and the abundances used as threshold levels in harmful phytoplankton monitoring programmes (Steve Milligan)

Many HAB monitoring programmes are designed to provide an early warning of biotoxins being detected in shellfish flesh. In some instances threshold levels (cell abundance of a particular species) are used to take further action, such as an increasing temporal and spatial sampling of shellfish flesh and/or water, or to instigate flesh sampling. These threshold levels vary between regions and in some instances were established historically. In some areas threshold levels are constantly under revision as new species/genera are observed or new information about the toxicity of species is disseminated. The reasons for the selection of these threshold levels is often not well documented and may also require review to ascertain whether they are still appropriate.

It is also important that any HAB monitoring programme adequately samples the water in which the shellfish are feeding. It is important that the water samples collected provide representative samples of the phytoplankton community in each shellfish harvesting area. There is a lack of information regarding how these sampling methodologies vary between countries or how the methods vary from other standards (e.g. OSPAR, etc).

WGHABD will review the threshold levels used in the various HAB monitoring programmes, within the ICES community, and how these threshold levels are being used to instigate further action. It is hoped to gain an understanding of how these threshold levels were obtained and whether they are still relevant. WGHABD participants were also asked to supply information on their sampling methodologies to better understand the differences / similarities between the various sampling methods.

Members of a new phytoplankton working group initiated by the EU-RL for biotoxins, have also been asked to supply similar information on sampling methodologies in preparation for their first meeting on 13 May 2015. It is recognised that this approach is necessary given the diversity of both environmental conditions and phytoplankton dynamics across regions, and of technical approaches to monitoring. The summary table produced by WGHABD will help to inform this new WG.

Country	Species monitored	Threshold levels	Detection levels	Actions resulting from breaches of thresholds	How were thresholds determined? (If known)	Comments
England & Wales	*Alexandrium spp.*	Presence	max 40 cells/litre	Increased temporal and spatial sampling.	Not documented.	
	Dinophysiales spp.	100 cells/litre				
	Prorocentrum lima	100 cells/litre				
	Pseudo-nitzschia spp.	150,000 cells/litre				
Northern Ireland	*Alexandrium spp.*	Presence	Max 20 cells/litre	Increased temporal and spatial sampling.	Not documented.	
	Dinophysiales spp.	100 cells/litre				
	Prorocentrum lima	100 cells/litre				
	Pseudo-nitzschia spp.	150,000 cells/litre				
Scotland	*Alexandrium spp.*	Presence	Max 20 cells/litre	Increased temporal and spatial sampling.	Not documented.	
	Dinophysiales spp.	100 cells/litre				
	Prorocentrum lima	100 cells/litre				
	Pseudo-nitzschia spp.	50,000				

		cells/litre				

France	*Alexandrium spp*	between 1000 and 10 000 cells/L, depending on the zone	generally 100 cells/L, sometimes 50 cells/L	triggers shellfish sampling	from historical data	
	Dinophysis spp.	Presence	generally 100 cells/L, sometimes 50 cells/L	triggers shellfish sampling if not already done	from historical data	
	Species likely to produce some other lipophilic toxins as Gonyaulax spinifera, Lingulodinium polyedra, Protoceratium reticulatum, Prorocentrum lima	10 000 cells/L	generally 100 cells/L, sometimes 50 cells/L	triggers shellfish sampling if not already done	from historical data	
	Pseudo-nitzschia - "thin" species	300 000 cells/L	generally 100 cells/L, sometimes 50 cells/L	triggers shellfish sampling	from historical data	
	Pseudo-nitzschia - "large" species	100 000 cells/L	generally 100 cells/L, sometimes 50 cells/L	triggers shellfish sampling	from historical data	

Spain						
Catalonia	*Alexandrium minutum*	1,000 cells/L	20 cells/L	Increased temporal and spatial sampling, precautionary closures	By analysis of the time series on phytoplankton and biotoxins	
	Alexandrium catenella	1,000 cels/L				
	Dinophysiales	500 cells/L				
	Prorocentrum lima	500 cells/L				
	Pseudo-nitzschia spp.	200,000 cells/L		Increased temporal and spatial sampling.	Bibliographic references from other regions	
Andalucía * currently under review	*Alexandrium minutum*	1,500 cells/L	40 cells/litre atlantic; 20 cells/L mediterranean	Precautionary closures and increased temporal sampling	By analysis of the time series on phytoplankton and biotoxins	
	Gymnodinium catenatum	500 cells/L *				
	Dinophysis acuta	500 cells/L*				
	Dinophysis acuminata group	500 cells/L*				
	Dinophysis fortii	500 cells/L*				
	Prorocentrum lima	2,000 cells/L				
	Other DSP producers	2,000 cells/L				
	Pseudo-nitzschia cf australis *	50,000 cells/litre *				
Canada	*Alexandrium fundyense*	presence	N/A	Notification to CFIA and increased shellfish monitoring	200 c/l indicate rising PSP levels in flesh. 200 - 500 c/l shellfish toxicity usual-	Salmon farmers can continue to feed fish until A. fundyense reaches 200,000

					ly above threshold levels. From historical knowledge.	c/l. Only programme is in the Bay of Fundy with a reduced programme in the St Lawrence estuary.
	Pseudo-nitzschia multiseries	100,000 cells/L	N/A	Increased shellfish monitoring		

Poland

Netherlands	*Alexandrium spp.*	1000 cells/litre	Max. 25 cells/litre	Administrative consequences, Quarantine until originating area is tested for toxins. Sample intensification.	Product Board of Fish, NVWA (FSA)	Research Institutes advise on the scientific basis of the threshold values, mainly based on literature, and historical background.
	Dinophysis spp.	100 cells/litre	Max. 25 cells/litre	Notification to CFIA and increased shellfish monitoring	200 c/l indicate rising PSP levels in flesh. 200 - 500 c/l shellfish toxicity usually above threshold levels. From historical knowledge.	Salmon farmers can continue to feed fish until A. fundyense reaches 200,000 c/l. Only programme is in the Bay of Fundy with a reduced programme in the St Lawrence estuary.
	Pseudo-nitzschia spp.	500000 cells/litre	Max. 150 cells/litre	Increased shellfish monitoring		

Sweden	*Alexandrium minutum/tamarense*	200 cells per		Note that these are not used		

		litre		alone for closing harvesting areas except for Alexandrium spp. Cell numbers on the other taxa are used together with toxin data in shellfish for closures.		
	Alexandrium spp. is evaluated					
	Dinophysis acuminata	1 500 cells per litre		Administrative consequences, Quarantine until originating area is tested for toxins. Sample intensification.		
	Dinophysis acuta	200 cells per litre or 100 cells per litre three weeks in row				
	Dinophysis norvegica	4 000 cells per litre				
	Lingulodinium polyedrum	no warning limit				
	Protoceratium reticulatum	1 000 cells per litre		Note that these are not used alone for closing harvesting areas except for Alexandrium spp. Cell numbers on the other taxa are used together with toxin data in shellfish for closures.		
	Azadinium spinosum	no warning limit				
	Pseudo-nitzschia spp.	100 000 cells per litre				

Portugal	*Dinophysis spp.*	200	Min 20 cells/litre	Sampling is intensified; Focus on conditions (SST,Chla, circulation, wind…); Particle tracking model; Biotoxins can be detected below DL	From historic data	
	Alexandrium spp.	500				
	Gymnodinium catenatum	500				
	Pseudo-nitzschia seriata complex	1336				
	Pseudo-nitzschia delicatissima complex	1336				

Annex 6: ToR e: New Findings

(1) Citizen participation in monitoring phytoplankton seawater discolorations

Raffaele Siano (Ifremer – Brest)

Citizen science is a nexus between science and society, being both a new opportunity for scientists to acquire new data and for the public to interact with science. Although already used in the past (Miller-Rushing *et al.* 2012) the engagement of non-professional participants in scientific investigations has increasingly developed in recent times, especially for environmental sciences (astronomy, ornithology, paleontology, marine and terrestrial biodiversity) (e.g. Bodilis *et al.* 2014), which require large amounts of data from heterogenic sources (Kelling *et al.* 2009). Citizen sciences have been lauded for i) the education of voluntary participants to environmental issues, ii) providing low-cost data, iii) empowering citizens to participate more actively in local conservation and management decisions, iv) engaging stakeholders in conservation planning (Dickinson *et al.* 2010; Jansujwicz *et al.* 2013). Marine citizen science monitoring programs are well established for the detection and identification of a) marine macrofauna easily observed at the sea surface (i.e. marine mammals, sharks, jellyfishes) and b) some marine organisms, which can be observed by 'citizen' SCUBA divers, (Goffredo *et al.* 2010; Holt *et al.* 2013). However, the use of a citizen science approach for the detection of phenomena related to phytoplankton blooms has rarely been implemented. Volunteer HAB monitoring programs have been launched along the west coast of United States in the early 1990's (Anderson *et al.* 2001) and recently along Canada's west coast (McIntyre *et al.* 2013) in response to the onset of human poisoning (ASP and DSP) events. In 2001, the Phytoplankton Monitoring Network (PMN) (http://products.coastalscience.noaa.gov/pmn/) was established by the National Oceanographic and Atmospheric Administration of the United States department of Commerce (NOAA), initially as part of South Carolina's Pfiesteria HAB surveillance program. The scientific objectives of all these programs concern the distribution, phenology and managing of HAB species. Other citizen-monitoring programs, which main goal is the detection of changes in seawater color, are ongoing (i.e. the Citclops project http://www.citclops.eu, or Plymouth University's Secchi App - http://www1.plymouth.ac.uk/marine/secchidisk/Pages/default.aspx), but to the best of our knowledge no existing European program is specifically focused on water discolorations caused by phytoplankton.

A citizen monitoring program of water discoloration observations (Phenomer) caused by phytoplankton proliferations was launched throughout the coastal waters of Brittany (France) in 2013 in parallel to the ongoing phytoplankton and biotoxin monitoring network (REPHY). Beyond communication and outreach objectives, this project aims to explore the possibility to acquire scientifically valuable data on Harmful Algal Blooms (HABs) through extending the survey area of coastal waters by means of citizen alerts. A theoretically infinite number of sampling points (public observations) could contribute to identify i) HAB frequency and recurrence; ii) the distribution and extension of water discolorations; iii) the biogeography of causative taxa.

During the first two years of project implementation, citizens had heard about the Phenomer project mostly through articles in the regional or national press and through the

internet website. They were relatively well informed and interested about the aims of the project. Their interest augmented with time as proved by the increase in number of calls (from 40 to 75) during the two years of project implementation. This proves that the transfers of scientific information towards the general public was relatively successful and encouraging, since it suggests that the outcomes of Phenomer will likely progress with time, after more than two years of project implementation, as in other citizen science programs. Citizens will to help scientists in understanding triggering factors of water discolorations was particularly noticeable, clearly demonstrating general public sensitivity and interest towards understanding environmental abnormal phenomena

In 2013 and 2014 14 and 32 out of respectively 40 and 75 observations corresponded to HAB events. Citizen observations contributed to evaluate the extension and duration of water discolorations phenomena. In 2013, *Noctiluca scintillans* red discolorations were observed at ca. 180 km of distance, and in 2014, *Lepidodinium chlorophorum* created impressive green discolorations, lasting over one month, being at times associated to massive fish mortalities. New harmful algal bloom risks were identified for the first time in Brittany. A bivalve mortality event, not detected by the REPHY network, coincided with a dark-brown phytoplankton bloom characterized by the dominance of the toxic raphidophytes *Heterosigma akashiwo* and the dictyochophyte *Pseudochattonella verruculosa*.

Results obtained on the West Atlantic coast of France within the frame on the first two years (2013 and 2014) of deployment of the Phenomer project show that our citizen science approach was very helpful to: 1) signal water discolorations and HAB phenomena not detected by the national monitoring network; 2) assess the spatial extension of water discolorations, and particularly of those caused by *N. scintillans* across southern Brittany; 3) evaluate the temporal duration of discoloration phenomena, in particular of green ones caused by *L. chlorophorum* in the Vilaine Bay-Loire output area. Beyond regional interests, these data contribute to the study of global biogeography of harmful species and HAB events. Moreover our approach has shown the potential of citizen science in research on phytoplankton ecology and harmful event detections. Structured and scheduled phytoplankton monitoring networks can profit from the potentially infinite number of observation points of phytoplankton discolorations, which can certainly improve coastal management and research activity in areas frequently affected by these phenomena. Citizen science related to research on phytoplankton ecology and biodiversity cannot answer specific questions on the phenology of harmful species, which requires species sampling, samples and data, but can add precious information regarding target species and areas to sample. Furthermore, this citizen science approach has the great unquantifiable value of creating a link between science and society, disseminating knowledge on microscopic organisms (phytoplankton species) hardly attainable otherwise, and educating voluntary participants to environmental issues connected to phytoplankton such as harmful blooms.

References

Anderson DM, Andersen P, Bricelj VM, Cullen JJ, Rensel JE (2001) Monitoring and Management Strategies for Harmful Algal Blooms in Coastal Waters, APEC #201-MR-01.1, Asia Pacific Economic Program, Singapore, and Intergovernmental Oceanographic Commission Technical Series No. 59, Paris

Bodilis P, Louisy P, Draman M, Arceo HO, Francour P (2014) Can citizen science survey non-indigenous fish species in the eastern Mediterranean Sea? Environ Manage53:172-80

Dickinson JL, Zuckerberg B, Bonter DN (2010) Citizen Science as an Ecological Research Tool: Challenges and Benefits. Annu Rev Ecol Evol Syst 41: 149-172

Goffredo S, Pensa F, Neri P, Orlandi A, Gagliardi MS, Velardi A, Piccinetti C, Zaccanti F (2010) Unite research with what citizens do for fun: "recreational monitoring" of marine biodiversity. Ecol Appl 20: 2170-2187

Holt BG, Rioja-Nieto R, Aaron MacNeil M, Lupton J, Rahbek C (2013) Comparing diversity data collected using a protocol designed for volunteers with results from a professional alternative.

Jansujwicz JS, Calhoun AJK, Lilieholm RJ (2013) The Maine Vernal Pool Mapping and Assessment Program: Engaging Municipal Officials and Private Landowners in Community-Based Citizen Science. Environ Manage 52: 1369-1385

Kelling S, Hochachka WM, Fink D, Rierewald M, Caruana R, Ballard G, Hooker G (2009) Data-Intensive Science: A New Paradigm for Biodiversity Studies. BioScience 59: 613-620

McIntyre L, Cassis D, Haigh N (2013) Formation of a Volunteer Harmful Algal Bloom Network in British Columbia, Canada, Following an Outbreak of Diarrhetic Shellfish Poisoning. Mar Drugs 11: 4144-4157

Methods Ecol Evol 4: 383–392

Miller-Rushing A, Primack R, Bonney R (2012) The history of public participation in ecological research. Front Ecol Environ 10: 285–290

(2) Use of two biosensors in studies of *Alexandrium fundyense* in the New England region: (Donald M. Anderson USA)

This report covered recent developments in the use of two biosensors in the study of toxic *Alexandrium fundyense* blooms in the New England region.

The Environmental Sample Processor (ESP).

The only instrument currently capable of measuring both HAB cell abundance and toxicity is the Environmental Sample Processor (ESP; Scholin *et al.*, 2009). The ESP is a submersible, robotic device that collects discrete water samples, concentrates microorganisms and other particles in the samples, extracts target molecules, and delivers processed extracts to assay modules. Real-time detection chemistries rely on DNA probes for cell detection (Greenfield *et al.*, 2008) and protein (antibody) arrays to detect target molecules such as HAB toxins (Doucette *et al.*, 2009), with data transmitted immediately to shore-based locations. The ESP is commercially available from McLane Research Laboratories Inc.

The Anderson laboratory has deployed multiple ESPs for the last few years, with three deployed in 2014 along the coast of the Gulf of Maine. These instruments estimated *A. fundyense* cell concentrations 5 days per week for 6 weeks. An example of the data from one of those ESPs is given in Figure 1, along with weekly shellfish flesh-testing results obtained by the Maine Department of Marine Resources (DMR) at a nearby station. The patchiness of the *Alexandrium* population is evident in the ESP data, and a general

agreement is seen between the patterns in cell abundance and the toxicity measured in shellfish.

In a novel application of the ESP data, we converted the *Alexandrium* cell counts into estimates of toxicity in shellfish using a simple one-compartment model. (Note that these toxin estimates are not related to the direct measurement of saxitoxins on the ESP – that capability has yet to be proven in field deployments. The toxin estimates discussed here are calculations based on ESP-based cell counts.)

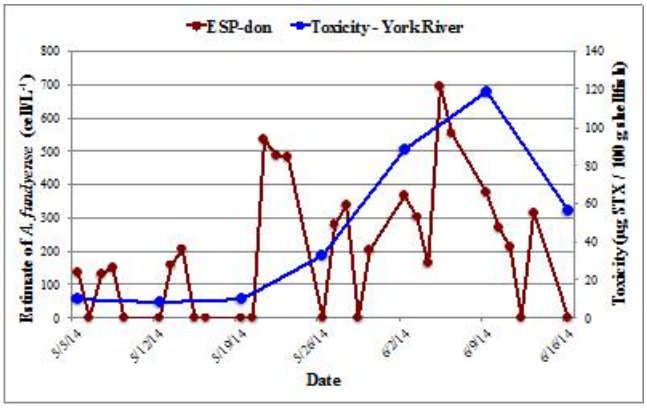


Figure 1. High-frequency ESP estimates of *A. fundyense* cell concentrations (red) and weekly shellfish flesh testing results (blue) at the nearby York River DMR station. (D. M. Anderson, unpub. data)

A simple one-compartment toxicity model:

Let *T(t)* be toxicity at a station at time *t* and *C(t)* be the *Alexandrium* cell concentration at time *t* at the nearest "downstream" ESP instrument. A simple model relating toxicity to cell concentration is:

$$T(t+1) = T(t) + \beta C(t) - \gamma T(t)$$

The model parameters were estimated using data from the 3 ESPs (n > 100) and the downstream shellfish flesh testing monitoring data for each instrument (n = 21) , assuming a common value of β and γ. Parameter values were estimated by minimizing the sum of the squared differences between modelled and observed toxicity.

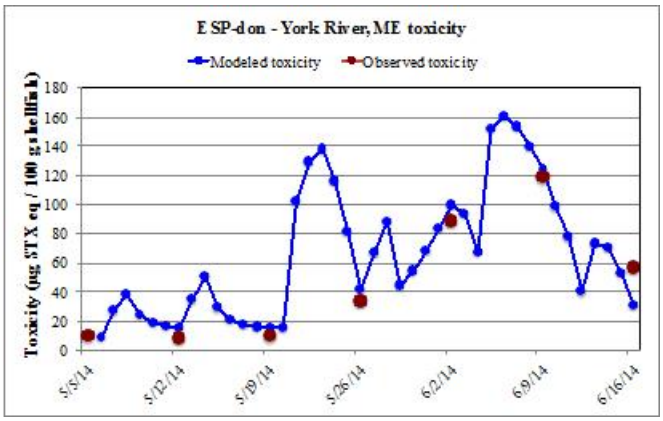


Figure 2. Estimated shellfish toxicity from ESP cell counts, versus direct flesh-testing measurements at the nearest DMR station. (D. M. Anderson, unpub. data)

Figure 2 shows the ESP estimates of shellfish toxicity, and the measured toxicity at the nearest DMR station. The agreement is remarkable, and is similar to that obtained with the other 2 ESPs, each related to its nearest DMR shellfish station. A regression of all ESP shellfish toxicity estimates versus the flesh testing data from the DMR stations was highly significant ($p<0.001$). Of course, these results need to be verified at other stations, under different environmental conditions, but if confirmed, ESP data have the potential be used to provide information of more direct use to managers – shellfish toxicity estimates.

It is likely that the good correlations observed in the Gulf of Maine reflects the nature of the PSP problem in that region, namely the lack of non-toxic *A. fundyense* strains, or a wide range in toxin phenotypes that can lead to large variations in toxicity per cell. Consistencies in the coastal circulation (e.g., the existence of a persistent coastal current) also contribute to the observed correlation.

The Imaging FlowCytobot (IFCB)

The IFCB is a submersible imaging flow cytometer that uses a combination of flow cytometric and video technology to capture high resolution (1 µm) images of suspended particles (Olson and Sosik, 2007). Extended unattended deployments (6-9 months) are possible because IFCB's automated operation includes antifouling procedures and periodic standard analysis to monitor instrument performance. Long term continuous plankton imaging by an IFCB deployed at Port Aransas, TX has already provided early warnings of six HAB events (Campbell *et al.* 2010; Campbell *et al.* 2013). The instrument has also been used to obtain an unprecedented level of detail and resolution of life history transitions in *Alexandrium fundyense* blooms (Brosnahan *et al.*, in press). This has been accomplished with a second-generation research prototype, which has now advanced to commercial production through license to McLane Research Laboratories, Inc.

Briefly, the results of the studies of Brosnahan *et al.* (in press) are summarized here. An IFCB an Imaging FlowCytobot (IFCB), was deployed in Salt Pond (Eastham, Massachusetts), a small, tidally flushed kettle pond that hosts near annual, localized *A. fundyense* blooms. Machine-based image classifiers differentiating *A. fundyense* life cycle stages, including parasitized cells, were developed. The level of resolution and detail of *Alexandrium* bloom dynamics was provided by the IFCB were remarkable – allowing the discrimination of singlet cells, dividing cells, gametes, fusing gametes, planozygotes, and

infected cells. The development phase of sustained vegetative division lasted approximately three weeks and was followed by a rapid and near complete conversion to small, gamete cells. The gametic period (~3 days) coincided with a spike in the frequency of fusing gametes (up to 5% of *A. fundyense* images) and was followed by a zygotic phase (~4 days) during which cell sizes returned to their normal range but cell division and diel vertical migration ceased. Cell division during bloom development was strongly phased, enabling estimation of daily rates of division that were more than twice those predicted from batch cultures grown at similar temperatures in replete medium. Data from the Salt Pond IFCB deployment provide the first continuous record of an *A. fundyense* population through its complete bloom cycle and demonstrate growth and sexual induction rates much higher than are typically observed in culture. Details are provided in Brosnahan *et al.* (in press).

References

Brosnahan, M. L., Velo-Suarez, L., Ralston, D. K., Fox, S. E., Sehein, T. R., Shalapyonok, A., Sosik, H. M., Olson, R. J., & Anderson, D. M. (In press). Rapid growth and concerted sexual transitions by a bloom of the harmful dinoflagellate *Alexandrium fundyense* (Dinophyceae). *Limnology and Oceanography*.

Campbell, L., Olson, R. J., Sosik, H. M., Abraham, A., Henrichs, D. W., Hyatt, C. J., & Buskey, E. J. (2010). First harmful *Dinophysis* (Dinophyceae, Dinophysiales) bloom in the U.S. is revealed by automated imaging flow cytometry. *Journal of Phycology*, *46*(1), 66-75.

Campbell, L., Henrichs, D. W., Olson, R. J., & Sosik, H. M. (2013). Continuous automated imaging-in-flow cytometry for detection and early warning of Karenia brevis blooms in the Gulf of Mexico. *Environmental Science and Pollution Research*, *20*(10), 6896-6902.

Olson, R. J., & Sosik, H. M. (2007). A submersible imaging-in-flow instrument to analyze nano-and microplankton: Imaging FlowCytobot. *Limnology and Oceanography: Methods*, *5*(6), 195-203.

Doucette, G.J., C.M. Mikulski, K.L. Jones, K.L. King, D.I. Greenfield, R. Marin III, S. Jensen, B. Roman, C.T. Elliott, and C.A. Scholin. 2009. Remote, subsurface detection of the algal toxin domoic acid onboard the Environmental Sample Processor: assay development and field trials. Harmful Algae 8(6): 880-888. doi:10.1016/j.hal.2009.04.006.

Greenfield, D.I., R. Marin III, G.J. Doucette, C.M. Mikulski, K.L. Jones, S. Jensen, B. Roman, N. Alvarado, J. Feldman, and C.A. Scholin. 2008. Field applications of the second-generation Environmental Sample Processor (ESP) for remote detection of harmful algae: 2006-2007. Limnol. Oceanogr: Methods 6: 667-679.

Scholin, C., G. Doucette, S. Jensen, B. Roman, D. Pargett, R. Marin III, C. Preston, W. Jones, J. Feldman, C. Everlove, A. Harris, N. Avarado, E. Massion, J. Birch, D. Greenfield, K. Wheeler, R. Vrijenhoek, C. Mikulski, and K. Jones. 2009. Remote detection of marine microbes, small invertebrates, harmful algae and biotoxins using the Environmental Sample Processor (ESP). Oceanogr. 22:158-167.

(3) Temporal and spatial trends of *Karenia mikimotoi* in Scottish waters: Keith Davidson (UK)

K. mikimotoi common member of background flora in Scottish waters. Historically blooms were very infrequent. A major bloom occurred in 2006, and relatively frequent blooms have occurred subsequently.

K. mikimotoi has been monitored in programmes funded by the UK Food standards Agency and the UK Crown Estate programmes since 2006. The composite dataset generated therefore includes contains all *K. mikimotoi* counts (including zero values) from a total of 8408 sampling events covering 114 unique sites. The data set was analysed to investigate the spatial/temporal distributions of blooms and their potential environmental drivers

K mikimotoi blooms were found to occur in the water column in Scottish waters from May to October only. Elevated cell densities (taken be in excess of a threshold of 1000 cells l^{-1} or greater) occurred in all years and in all sampling areas. However, the frequency of such events was only about 5%.

1 million cells l^{-1} was exceeded in four of the eight summers studied, and a value just below this threshold occurred in one further year.

On an individual site basis "high incidence" sites (at which that frequency of above threshold counts was 10% or greater) were relatively few, and only four in number. These sites were geographically dispersed and have no clear common hydrodynamics characteristics.

Six sites did not experience above threshold *K. mikimotoi* events over the sampling period. Some geographical commonality exists between these sites, all being on the North or East Coast or in the Orkney or Shetland Islands. However, once more there were no clear common hydrodynamic characteristics between these low incidence sites.

In general terms, there was a northward shift in the development of *K. mikimotoi* blooms in Scottish over the period May to October.

Statistical modelling found no clear relationship between high and low risk sites and their hydrodynamic characteristics. There was some evidence that the location and magnitude of blooms are linked to environmental forcing, with an association with higher irradiance or collinear predictors such as low rain or light winds. However, despite the comprehensive (large) data set the models did not indicate any statistically significant relationship between the development of *K. mikimotoi* blooms and the tested environmental drivers.

Modelling indicated there was no evidence of local production in sea-lochs, at least related to rainfall and runoff and/or flushing, as there was substantial variability in *K. mikimotoi* counts between pods that could not be accounted for by the fixed effects in the model.

A possible hypothesis is therefore patches of *K. mikimotoi* being advected northward, during the summer months and, periodically, being driven into sea-lochs. However, this is difficult to reconcile with the speed of the coastal or shelf edge currents in the region. This is an area of current research.

(4)Ocean colour discrimination of HABs

Peter Miller, Plymouth Marine Laboratory

Peter Miller, from Plymouth Marine Laboratory, UK, has joined the WGHABD to represent the potential usage of satellite ocean colour data for monitoring of harmful algal blooms (HABs), continuing on from the earlier membership of Prof Jim Aiken, who has since retired. Peter has expertise in satellite Earth observation (EO) tools for monitoring

water quality for finfish and shellfish aquaculture, in particular his team has developed ocean colour algorithms for discriminating certain high-biomass HAB species, shown to be more effective than just a chlorophyll estimate (Kurekin *et al.*, 2014, Figure 1).

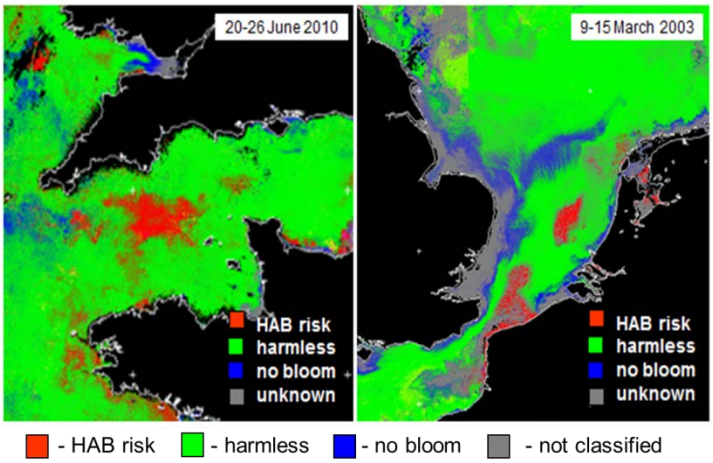


Figure 1. Harmful algal bloom risk maps using EO ocean colour discrimination: *Karenia mikimotoi* bloom in the Western English Channel in summer 2010, and *Phaeocystis globosa* bloom in the Southern North Sea in spring 2003.

He described his background in EO monitoring of HABs through various EC and UK projects, highlighting three projects of particulalr relevance: *Scottish Karenia HAB Watch* (weekly monitoring for salmon farming industry since 2008); *AQUA-USERS* (tools for management decisions and site selection for aquaculture industry, EC FP7, 2014-2016); and *ShellEye* (HAB and bacterial warning bulletins for shellfish farms, UK BBSRC, 2015-2017). He finished with a brief introduction to his other research strand on EO detection of oceanic fronts, and their linkages with marine megafauna, applied towards marine conservation policy (Miller and Christodoulou, 2014).

(5) HAB monitoring and research at the Finnish Environment Institute (SYKE)

Anke Kremp, Marine Research Centre, SYKE, Helsinki

The Marine Research Centre (MRC) at the Finnish Environment Institute coordinates the national phytoplankton monitoring which is a duty of every member state of HELCOM and serves the assessment of the status of the Baltic Sea. MRC is also responsible for the monitoring of cyanobacterial summer blooms, which are a major HAB problem in Finnish coastal waters. Every spring, a prognosis for summer cyanobacterial blooms is provided by scientists of MRC. The forecast is obtained from a Baltic ecosystem model based on probability estimates derived from winter time phosphorus levels in surface waters and the intensity of the spring bloom.

During the bloom season the routine phytoplankton monitoring is extended by weekly cyanobacteria monitoring at selected stations to assess bloom intensity and potential risks. At high cell concentrations and dominance of hepatotoxic *Nodularia*, the media is informed to advise the public on risks. Additionally, MRC co-ordinates an Algae-Watch program collecting regular reports by local environmental agencies and citizens on the

cyanobacterial situation at selected coastal sites. A smartphone application, Järvi-wiki, has been developed for this purpose. Phycotoxin monitoring is included in the program for hazardous substances and carried out by another department of MRC. Fixed stations are sampled during the annual summer cruise of the HELCOM monitoring program and screened for Nodularin and Microcystins in plankton. Total peptides are analyzed from the water phase. During autumn, herring samples are collected from trawls in coastal Finnish waters and analyzed for total concentrations of hepatotoxins.

Monitoring data generated by SYKE on cyanobacteria and other phytoplankton, covering approx. 40 years, is available in SYKE and ICES databases. SYKE scientists have started to analyze phytoplankton data for trends in relation to changing environmental conditions. A recent study by Suikkanen *et al.* (2013) showed for example that cyanobacterial abundances have increased significantly since the 1980s and that this trend is related to decreases in salinities and increases in inorganic nutrient concentrations.

Besides cyanobacteria, several other toxic and potentially harmful phytoplankton species occur in Finnish coastal waters. However, due to the lack of a shellfish industry, no dedicated monitoring efforts are made by the Finnish authorities for abundances and toxins. *Dinophysis* spp. (with demonstrated DTX, PTX and OA production and accumulation in benthic biota) is common in the summer phytoplankton community as well as yessotoxin producing *Protoceratium reticulatum*. *Prorocentrum minimum* and *Prymnesium polylepis* may form dense blooms, but harmful effects have not been observed so far. Recently, research activities not related to the governmental monitoring program have confirmed the presence of haemolytic *Karlodinium veneficum* in shallow coastal waters.

Since the early 2000s dense blooms of low salinity adapted *Alexandrium ostenfeldii* have become a recurrent phenomenon in coastal waters of the Finnish archipelago (Kremp *et al.* 2009). Research projects at MRC lead by A. Kremp have investigated the dynamics and toxicity of these blooms as well as potential causes behind the recent expansion. The efforts revealed that blooms are related to significant PST concentrations in the water (Hakanen *et al.* 2012) and that these toxins accumulate in benthic biota and fish at concentrations partly exceeding regulatory limits (Setälä *et al.* 2014). rDNA analyses initially suggested that the blooms might be the result of a recent introduction, since 28S and ITS sequences were practically identical with isolates from US East coast estuaries and brackish creeks in the Netherlands (Kremp *et al.* 2014). However, results of population genetic analyses revealed significant differentiation among local Baltic populations and hierarchic genetic structure, suggesting long standing establishment of this species in the Balti Sea (Tahvanainen *et al.* 2012). Experimental work has shown a relationship between growth enhancement, increased toxicity and temperature (Kremp *et al.* 2012) and it cannot be excluded that the expansion of the species is a result of changing climatic conditions. The work of the group also includes studies on novel toxic compounds found in Baltic *A. ostenfeldii*. Presently, a new gymnodimine is characterized from Baltic isolates (Harju *et al.*, in prep).

Annex 7: ToR i: Review progress in development and application of molecular genetic technologies for taxonomic identification, phylogenetic reconstruction, biodiversity, toxin detection and population dynamic studies of HABs

(Raeffele Siano and Allan Cembella)

(1)Molecular probes for characterization and enumeration of harmful algae

Allan Cembella (AWI – Bremerhaven)

Rapid advances in high-throughput genomic sequencing, DNA bar-coding, bioinformatic platforms and transcriptomic approaches – all part of the *'omics* revolution - have increasingly been adopted to study phylogeny, population and bloom dynamics and biodiversity of HABs over the past decade. Molecular approaches can verify or contradict classical taxonomy and phyogenetic reconstruction. Nevertheless, for reasons of cost, complexity or impracticability, most of these technologies have not yet been incorporated into routine HAB monitoring programmes. Basic knowledge requirements for routine monitoring of HAB taxa are rather straightforward: 1) Which discretely identifiable HAB taxa are present?; and 2) How many cells (or how much biomass) of each putative HAB taxon are present, and for how long do they persist? To answer these questions, molecular probe technology has much to offer as a complement to traditional morphological analysis and cell enumeration by microscopy.

Unlike most biooptical methods for HAB detection, molecular probes are effective in identification and discrimination of HAB taxa even within low biomass blooms and where the target species have low dominance within complex assemblages, as well as for dispersed blooms. The proliferation of newly described HAB species, in many cases created and based upon morphological features at the limit of resolution of the light microscope, pose increasing challenges for monitoring by traditional microscopy. Such problems are most acute within genera such as the diatom *Pseudo-nitzschia*, but also for well-studied dinoflagellate genera, including *Alexandrium*, and for small-celled taxa, e.g., *Azadinium*/*Amphidoma* spp. Associated with azaspiracid shellfish poisoning (AZP). Fortunately, in these cases (and for many other HAB taxa) there exists a rather comprehensive array of molecular probes for detection and discrimination. Most common molecular approaches are targeted DNA probes, a short piece of DNA (oligonucleotide) that is homologous to a certain sequence and binds specifically, or rRNA probes, designed from an rRNA sequence, usually the small- [18s] or large- [28S] subunit of the ribosomes, or occasionally the internal transcribed spacer (ITS). rRNA probes are particularly attractive for HAB species detection in routine monitoring programmes because the RNA sequences are universally found, present in high target numbers per cell, and large number of comparative sequences are available from computer databases. Such probes can also be "stacked" and applied simultaneously as hierarchical probes for biodiversity studies by detecting variable and conserved regions at various taxonomic levels. Specific rRNA probes are capable of discrimination among microalgal groups, from higher levels down to species and even amongs strains and population in some cases.

One common way to apply these probes of routine analysis is to couple them to a fluorescent marker (FISH, *fluorescence in situ hybridization*), whereby target taxa can be ob-

served and counted by epifluorescence microscopy. This approach is cost effective in both time and financial outlay for equipment and operational aspects and the probes can be effectively applied without advanced training.

An alternative approach for identification and enumeration involves quantitative (or real-time) polymerase chain reaction (qPCR), whereby a targetted DNA molecule is simultaneously amplified and detected or quantified. For HAB taxa, this qPCR method has been routinely employed for amplification of toxin-specific genes (e.g., the STX gene cluster of *Alexandrium*) and for rDNA. Amplification of the latter gene has been successfully used to estimate cell concentrations via a calibration curve.

Practical application of these probe methods has been comparatively evaluated with respect to HAB taxa (Godhe *et al.* 2007; Karlson *et al.* 2010). More recent developments have led to the development of advanced platforms for automated and/or high throughput molecular analysis based upon flow cytometry, DNA chips, solid phase cytometry, and even field deployable systems (e.g., ESP, Monterrey Bay Aquarium), but these are not in routine monitoring application.

Godhe, A., C. Cusack, J. Pedersen, P. Andersen, D. M. Anderson *et al.*, 2007. Intercalibration of classical and molecular techniques for identification of *Alexandrium fundyense* (Dinophyceae) and estimation of cell densities. Harmful Algae 6: 56-72.

Karlson, B., Cusack, C. and Bresnan, E. (editors). 2010. Microscopic and molecular methods for quantitative phytoplankton analysis. Paris, UNESCO. (IOC Manuals and Guides, no. 55.) (IOC/2010/MG/55)

(2)qPCR assay to detect saxitoxin genes or transcripts in environmental samples

Raffaele Siano (Ifremer – Brest)

Core genes putatively involved in the saxitoxin (STX) biosynthesis pathway in *Alexandrium fundyense*, *A. minutum*, *A. catenella*, *A. tamarense* and *Gymnodinium catenatum* have been recently identified and characterized (Stüken *et al.*, 2011). This has suggested that *in situ* detection of these genes may be possible. Several of the core STX genes, including the unique core gene *sxtA*, were most closely related to a clade including producing cyanobacterial *sxtA* genes. This gene has four catalytic domains in all STX producing cyanobacterial species and the domain *sxtA4* appears to be necessary for STX biosynthesis in cyanobacteria (Kellmann *et al.*, 2008). As for dinoflagellates, the primary sequences of *sxtA4* domains from *Alexandrium* species and *Gymnodinium catenatum* appeared to be relatively conserved (Stüken *et al.*, 2011). This suggested the potential to develop genetic methods that may allow us to detect *sxtA4* in environmental samples. A novel method for detecting and quantifying the potential for STX production in marine environmental samples has been first developed by Murray *et al.* (2011) using qPCR essay to detect *sxtA4* copy number in environmental samples. The primers designed in this study were found to amplify a fragment of the correct size in all tested STX-producing dinoflagellates (*A. minutum*, *A. catenella*, *A. fundyense*, *A. tamarense*, and *Gymnodinium catenatu*) and did not amplify DNA from the non- STX-producing related gonyaulacalean species *(A. andersonii*, *A. affine*, *Gambierdiscus australes*, *Ostreopsis ovata*, or *Ostreopsis siamensis*). The mean copy number of *sxtA4* in the 3 cultured strains of a temperate Asian clade of *A. catenella* had a range of 178 to 280 cell-1. In the environmental samples containing *A.*

catenella the copy numbers of *sxtA4* were estimated to be 226 and 376 cell-1 in the Georges River and Wagonga Inlet (Australia) samples. These data allowed obtaining a positive relationship between cell number as estimated from microscopy, cell number as estimated from LSU rDNA, and the *sxtA4* copy number observed in both sets of environmental samples (Fig. 3 in Murray *et al.*, 2011).

A reliable detection limit of 110 cells liter^{-1} of *A. catenella* is achievable using the qPCR method described in Murray *et al.* (2011), and shows the advantage of qPCR-based enumeration methods over the currently microscope counts practiced in the majority of monitoring programs which have a reliable detection limit of 1,000 cells liter-1 (LeGresley *et al.*, 2010). However concentrations of *Alexandrium* species as low as 200 cells liter^{-1} have been associated with STX uptake in shellfish (Todd *et al.*, 2001), thus the reliable detection limit of species at low cell abundances is an important topic for sanitary survey. The methods proposed by Murray *et al.*, 2011 is based on genomic DNA, and it has not been tested on environmental samples with lower (< 100 cells liter^{-1}) *Alexandrium* cell numbers, nor has it been validated in other regions of the world or on field samples containing other PST-producing species than *A. catenella*.

Stüken *et al.* (2013) tested Murray *et al.* essay with spiked- and field samples from Oslofjorden, (Southern Norway), but found it not to be sufficiently specific to detect *stxA4* transcripts in samples where PST (Paralytic Shellfish Toxin)-producing algae were not dominant. Stüken *et al.* (2013) then developed a new, more sensitive *stxA* assay that could be an early warning system for dinoflagellate PST producers across different genera, at low concentration and in different regions. New primers were designed and qPCR essays were performed on RNA extracts of both cultures and environmental samples of Norwegian (Oslofjorden) samples.

Stüken *et al.* (2013) essay reliably detected *sxtA4* transcripts from 50 *Alexandrium fundyense* cells L-1 in environmental samples. The qPCR essay yielded five positive results for the Oslofjorden sample series, even though cells of putatively PST producing species were only microscopically detected in three samples. The explanation proposed is that toxins or *stxA4* transcripts seem to persist in the water column longer than cells (Fig. 3 in Stüken *et al.* (2013)). Therefore Stüken *et al.* (2013) qPCR assay is suitable for the early detection of toxic events, but similarly to cell counts or microarrays, cannot be used to determine when shellfish farms can be reopened after a toxic event. One important advantage of this *sxtA*-specific assay over available ribosomal gene-based assays (Murray *et al.*, 2011) is that it detects a highly conserved region of a transcript essential for PST synthesis rather than genus-, species-, or ribotype-specific sequences. The ribosomal regions are more variable and the risk of missing a strain or species with an unknown sequence is higher. On the other hand, since this assay detects sxtA4 transcripts across species and genus borders, one limitation is that it does not allow us to determine, which species the sxtA4 transcripts came from, and whether they originated from the same species throughout the study period, from different species, or a mixture of species (Stüken *et al.* 2013).

Since these initial results, qPCR essays on sxtA4 transcripts need to be further investigated, and it is crucial to expand our current understanding of PST genes and transcripts in dinoflagellates. Are PST genes actually transcriptionally regulated, or do they belong to the majority of dinoflagellate genes that are not? Does a higher number of a gene or transcripts present in the environment actually translate into higher PST synthesis? Does the

PST gene or transcript number per cell vary between species or even different strains of the same species? What is the PST gene and transcript sequence variation in different species and strains? These questions need to be thoroughly explored before we can judge if PST gene- or transcript-based molecular assays can be used quantitatively, or if they are ''only'' a fast and reliable method to detect potentially PST-producing dinoflagellates in environmental samples (Stüken *et al.* 2013).

References

Kellmann, R., *et al.* 2008. Biosynthetic intermediate analysis and functional homology reveal a saxitoxin gene cluster in cyanobacteria. Appl. Environ. Microbiol. 74:4044.

LeGresley, M., and G. McDermott. 2010. Counting chamber methods for quantitative phytoplankton analysis—haemocytometer, Palmer-Maloney cell and Sedgewick-Rafter cell, p. 25–30. In B. Karlson, C. Cusack, and E. Bresnan (ed.), Microscopic and molecular methods for quantitative phytoplankton analysis. UNESCO, Paris, France.

Murray, S.A., *et al.*,2011. SxtA-based quantitative molecular assay to identify saxitoxin-producing harmful algal blooms in marine waters. Applied and Environmental Microbiology 77, 7050–7057.

Stüken, A., *et al.* 2011. Discovery of nuclear-encoded genes for the neurotoxin saxitoxin in dinoflagellates. PLoS One 6:e20096.

Stüken, A., *et al.* 2013. Novel hydrolysis-probe based qPCR assay to detect saxitoxin transcripts of dinoflagellates in environmental samples. Harmful Algae 28: 108-117.

Todd, K. 2001. Australian marine biotoxin management plan for shellfish farming. Report no. 645. Cawthron Institute, Nelson, New Zealand.

(3) Metabarcording approach to the study of biodiversity, biogeography and dynamics of protists and toxic microalgal species

Raffaele Siano (Ifremer – Brest)

Marine protists (single celled eukaryotic species) are major actors of the coastal ecosystem dynamics. They belong to different taxonomic lineages and the study of their biodiversity is crucial for the understanding of the tree of life and the evolution of functional and physiological traits (Keeling *et al.*, 2005; Burki, 2014). Separate groups of protists can contribute differently to the energy budget of the marine ecosystems since they can be primary producers, predators (heterotrophs, mixotrophs), and symbionts (mutualistic and parasites). They can also have an impact on the human health and economic activities since many protists, belonging to different systematic groups, can be toxigenic or harmful for coastal economic activities (aquaculture, tourism).

The biodiversity of protist community can be shaped by different environmental forcings of the coastal ecosystem; therefore the identification of the different species appeared essential since the first studies on the ecology of microalgae. Traditionally these studies are based on data acquired in optical microscopy, but since the development of new methodologies of diversity analyses of marine protists (electron microscopy and cytometry) and especially of the molecular biology at the end of the '80s, it appears clear that the optical microscopy cannot provide an exhaustive picture of protist biodiversity. With a classical optical microscope: I) it is hard to identify pico-nanoplakton (<20μm) species, II)

some groups are distinguishable at the level of order or of group (e.g. naked dinoflagellates), III) cryptic species (identifiable only with molecular tools) and intraspecific diversity cannot be studied, IV) heterotrophic and parasitic species are not classifiable, V) the rare biosphere is not taken into account. Yet, i) the dominance of the pico-nanoplakton in the marine ecosystems has been acknowledged since many years (Li *et al.*, 1994), ii), it is evident that single species, within the same genus, have different phenologies and occupy separate ecological niches (Feheling *et al.*, 2006), iii) intraspecific diversity corresponds to different ecological signals (Foulon *et al.*, 2008), iv) heterotrophic species and parasites in particular are extremely abundant in the global ocean (de Vargas *et al.*, in press), v) rare species can play an important role as a consequence of ecosystem change (Pedros-Alio 2006, Logares *et al.*, 2014). It is evident that a clear understanding of protist ecology pends on methods that provide an exhaustive picture of species biodiversity.

Biodivesity studies based on the use of clone libraries during the last 30 years allowed the characterization of new phylogenetic clades (Díez *et al.*, 2001; López-García *et al.*, 2001; Moon-van der Staay *et al.*, 2001) and contributed to the construction of a reference database of the diversity of marine protists (Guillou *et al.*, 2013). However these analyses are limited by the number of clones analyzable, thus the diversity picture obtained is restricted to the most abundant species present in the samples. This limit has been recently overpassed by recent advances in high-throughput sequencing (Next Generation Sequencing (NGS) technologies) of hypervariable genetic regions of the DNA used as a *barcode* to identify species. Thanks to new technologies such as the *llluminaMISeq* it is possible to obtain millions of sequences of a length (450-500bp) adapted to deep, taxonomical satisfying, biodiversity studies of an environmental community (*metabarcoding*). These techniques can provides millions of sequences per sample, which theoretically would allow the saturation of the biodiversity of an environmental sample.

This approach to the study of protist biodiversity is objective, it avoids species identification errors due to the personal taxonomic knowledge of an operator at optical microscopy. The relative importance of each taxon can be evaluated in terms of the abundance of ribosomal DNA copies of the barcode region of the single taxon. Considering that the number of copies of rDNA barcode region varies across the species, this estimation of the biodiversity is hardly comparable to common quantitative plankton data (number of cells/l). The costs of the application of this technology and the expertises (molecular biology, bioinformatics, ecology, statistics) needed to analyze the data do not allow for the moment the application of the metabarcoding approach in common routine network monitoring systems of toxic species. However this technique can be interesting to analyze risk connected to harmful species in still unexplored, not monitored areas, where data of the biodiversity of toxic species are still scanty. Thanks to a metabarcoding survey at a reasonable time scale, all potential toxic species present in a given areas could be detected in order to manage potential risks connected to their development.

References

de Vargas C., Audic S., Henry N., Decelle J., Mahé F., Logares R., Lara E., Berney C., Le Bescot N., Probert I., Carmichael M., Poulain J., Romac R, Colin S., Aury J.M., Bittner L., Chaffron S., Dunthorn M., Engelen S., Flegontova O., Guidi L., Horak A., Jaillon O., Lukes J., Malviya S., Morard R., Mulot M., Scalco E., **Siano R.**, Vincent F., Zingone A., Dimier C., Picheral M., Searson S., Kandels-Lewis S., Acinas S.G., Bork P., Bowler C., Gorsky G., Grimsley N., Hingamp P., Iudicone D., Not F., Ogata H., Pesant S., Raes J., Sieracki M., Speich S., Stemman L., Sunagawa

S., Weissenbach J., Wincker P., Karsenti E. and Tara Oceans Expedition, Tara Oceans Coordinators (2015). Sea change in eukaryotic plankton diversity. *Science*, in press

Díez B, Pedrós-Alió C, Massana R. (2001). Study of genetic diversity of eukaryotic picoplankton in different oceanic regions by small-subunit rRNA gene cloning and sequencing. Appl Environ Microbiol 67: 2932-2941.

Fehling, J., Davidson, K., Bolch, C., Tett, P. (2006). Seasonality of *Pseudo-nitzschia* spp. (Bacillariophyceae) in western Scottish waters. Marine Ecology Progress Series, 323, 91–105.

Foulon E., Not F., Jalabert F., Cariou T., Massana R., Simon N. (2008). Ecological niche partitioning in the picoplanktonic green alga Micromonaspusilla: evidence from environmental surveys using phylogenetic probes. Environ Microb 10, 2433–2443.

Guillou L., Bachar D., Audic S., Bass D., Berney C., Bittner L., Boutte C., Burgaud G., de Vargas C., Decelle J., del Campo J., Dolan J. R., Dunthorn M., Edvardsen B., Holzmann M., Kooistra W.H.C.F., Lara E., Lebescot N., Logares R., Mahé F., Massana R., Montresor M., Morard R., Not F., Pawlowski J., Probert I., Sauvadet A.-L., **Siano R**., Stoeck T., Vaulot D., Zimmermann P., Christen R. The protest Ribosomal reference database (PR^2): a catalog of unicellular eukaryote small subunit rRNA sequences with curated taxonomy (2013). *Nucleic Acids Research* 41: D597-D604

Li W.K.W., (1994). Primary production of prochlorophytes, cyanobacteria, and eukaryotic ultraphytoplankton: measurements from flow cytometric sorting. Limnol Oceanogr 39, 169-175.

Annex 8: ToR j: Review existing knowledge and latest findings on BMAA

(Hanne Mazur)

ß-N-methylamino –L-alanine (BMAA) is a small non-proteogenic amino acid. It was originally found in the seeds of cycad (*Cycas micronesica*) growing on the Pacific Island of Guam. Since the work published by Cox *et al.* (2003), the production of this compound by symbiotic and free-living cyanobacteria has been associated with the elevated frequency of neurodegenerative diseases among people exposed to the toxin. Recently, BMAA has also been detected in axenic strains of diatoms (*Achnanthes* sp., *Navicula pelliculosa*, *Skeletonema marinoi*, *Skeletonema marinoi*, *Thalassiosira* sp., *Proboscia inermis*) and dinoflagellates (*Heterocapsa triquetra*) (Jiang *et al.* 2014a,b).

The accumulation of BMAA in aquatic organisms, especially in filter feeders like mussels and oysters, (e.g. Jonasson *et al.* 2010, Christensen *et al.* 2012) and the presence of the toxin in seafood sold on the metropolitan markets have been documented. In blue mussels, oysters and shrimps the concentrations were 0.01-0.90 μg/g w.w., while in fish no BMAA or only traces of the compound were detected (Jiang *et al.* 2014c). These findings led to suggestions that general population can be exposed to BMAA through marine food web. In Hérault district, south France – a main area of shellfish production on the French Mediterranean coast (Thau lagoon) - a significant and large ALS cluster was identified (Masseret *et al.* 2013). Apart from one report, the presence of BMAA in food supplements produced from cyanobacterial material was not confirmed. Although the presence of BMAA in aquatic organisms and food products was documented in several countries (e.g. Sweden, France, USA, China), so far, a direct link with the incidents of ALS has not been proven.

There were also reports on the presence of BMAA in human brains, but as the toxin was found both in ALS (Amyotrophic Lateral Sclerosis) patients (Murch *et al.* 2004) and in non-ALS patients (Bernzon *et al.* 2015), there is not enough evidence that BMAA is involved in human neurological diseases. *In vitro* studies performed with the application of neuronal cells showed that BMAA induced oxidative stress and excitotoxicity, but the effects were observed at mM concentrations of the compound, while excitotoxins usually act at μgM range. There were few *in vivo* studies with BMAA orally administered to vertebrates. Again, if any neurotoxic effects were revealed (Parkinsonian features in *Macaca fascicularis* and behavioural anomalies), they were only observed at very high doses of the toxin (100-315 mg/kg b.w./day, for 5-17 weeks, Spancer *et al.* (1987)).

There were also some methodological problems identified which led to high variations in the reported concentrations of BMAA. When HPLC with fluorescence detector was used, the BMAA concentrations were overestimated or even false positive results were obtained (Christensen *et al.* 2012). Currently, LC-MS/MS for the derivatized or underivetaized BMAA is recommended as the most reliable detection technique which minimizes the risk of misidentification. However, the analytical procedure should always be validated, and effects such as ion suppression or the presence of BMAA structural isomers should be considered.

Berntzon L., Ronnevi L.O., Bergman B., Eriksson J., 2015. Detection of BMAA in central nervous system. Neuroscience **292**: 137-147.

Christensen S. J., Hemscheidt T. K., Trapido-Rosenthal H., Laws E. A., Bidigare R. R. 2012. Detection and quantification of β-methylamino-L-alanine in aquatic invertebrates. Limnol. Oceanogr. Methods **10**: 891–898.

Cox P., Banack S., Murch S. 2003. Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. *Proc. Natl. Acad. Sci. USA* **100**: 13380-13383.

Jiang L, Mehine M, Ilag LL, Eriksson J, Lage S, Jonasson S, Rasmussen U, Shams S. 2014a. Diatoms: A Novel Source for the Neurotoxin BMAA in Aquatic Environments. *PLoS One* **9**, e84578.

Jiang L. and Ilag L.L. 2014b. Detection of endogenous BMAA in dinoflagellate (*Heterocapsa triquetra*) hints at evolutionary conservation and environmental concern. *PubRaw Science* **1** (2): 1-8.

Jiang L., Kiselova N., Rosén J., Ilag, L. L.2014c. Quantification of neurotoxin BMAA ([bgr]-N-methylamino-L-alanine) in seafood from Swedish markets. *Scientific reports* **4**: 6931 | DOI: 10.1038/srep06931

Jonasson S., Eriksson J., Berntzon L., Spacil Z., Ilag L.L., Ronnevi L., Rasmussen U., Bergman B. 2010. Transfer of a cyanobacterial neurotoxin within a temperate aquatic ecosystem suggests pathways for human exposure. *Proc. Natl. Acad. Sci. USA* **107**:9252-9257.

Lobner D., Pian, P. M. T., Salou, A. K., Peoples R. W. 2007. β-N-methylamino-l-alanine enhances neurotoxicity through multiple mechanisms. *Neurobiol. Dis.* **25**: 360-366.

Masseret E., Banack S., Boumédiène F., Abadie E., Brient L., Pernet F., Juntas-Morales R., Pageot N., Metcalf J., Cox P. 2013. Dietary BMAA Exposure in an Amyotrophic Lateral Sclerosis Cluster from Southern France. *PloS one* **8**, e83406.

Murch S. J., Cox P. A., Banack S. A., Steele J. C., Sacks O. 2004. W. Occurrence of β-methylamino-L-alanine (BMAA) in ALS/PDC patients from Guam. *Acta Neurol. Scand.* **110:** 267-269.

Spencer P.S., Nunn P.B., Hugon J., Ludolph A.C., Ross S.M., Roy D.N., Robertson R.C. 1987. Guam amyotrophic lateral sclerosis-parkinsonism-dementia linked to a plant excitant neurotoxin. *Science* **237**: 517-522 (1987).

Annex 9: ToR k: Dynamics of Gynodinium catenatum

(Teresa Moita, Ana Amorim and Beatriz Reguera)

G.catenatum dynamics off Iberia

Species general characteristcs and specificities in western Iberian waters

Gymnodinium catenatum is a meroplanktonic chain forming dinoflagellate, the only naked species known to be responsible for the human syndrome known as paralytic shellfish poisoning (PSP). Among the characteristics that strongly influence the species dynamics are the strong motility of the vegetative colonies and the life-cycle including a benthic resting stage (cyst) with a short dormancy period (<2 weeks) (Fraga *et al.*, 1989; Bravo and Anderson, 1994). The cyst is fossilizable and has a very characteristic microreticulate wall.

Since its first description, there have been several publications where *G. catenatum* has been misidentified and confused with other species. The planktonic stage has been confused with *Gymnodinium impudicum* (Fraga & Bravo) G. Hansen & Moestrup and *Gymnodinium nolleri* Ellegaard & Moestrup (Fraga *et al.*, 1995; Ellegaard and Moestrup, 1999) while the cyst stage has been confused with *G. nolleri* and *G. microreticulatum* Bolch & Hallegraeff (Bravo and Ramilo, 1999, Dale and Nordberg, 1993, Ellegaard and Moestrup, 1999). It is imperative to ensure that reported bloom events or cyst distribution records correctly refer to *G. catenatum*, particularly in areas where more than one species occurs (Hallegraeff *et al.*, 2012). On the Iberian Atlantic coast reports from cyst data, germination experiments and molecular data, suggest that the 3 different species co-occur (Bravo and Ramilo, 1999; Amorim *et al.* 2002; Ribeiro *et al.* 2012). *G.catenatum* has been reported with increasing frequency in the late 1970s and throughout the 1980s and 1990s, and is known to date from 23 countries (Halleagraeff *et al.*, 2012).

In their review on global population relationships, Halleagraeff *et al.* (2012) highlighted that this species has distinct bloom patterns in different geographic regions. Thus, there are ecophysiological differences between tropical (21–32 ºC) and warm-temperate ecotypes (12–18 ºC), the second including the Iberian populations. Nevertheless, Ordás *et al.* (2003) found these differences appeared unrelated either to ITS genotypes or PST toxin phenotypes. These authors emphasized there is a higher level of intra-population and regional variation in the presence and amount of STX derivatives and, in particular, NE Atlantic Iberian strains are characterized by a higher B1 and B2 (GTX5 and GTX6) proportion and include C1+2 and C3+4 toxins (Negri *et al.*, 2007). Recent results, from cultures obtained by isolation of vegetative cells and by laboratory germination of cysts, indicate that in Portuguese populations the origin of the inoculum is more relevant in determining the toxin profile, than the time of isolation or geographical origin (Silva *et al.*, submitted).

Antecedents of *Gymnodinium catenatum* in Iberian waters: arrival and expansion

Studies of *G. catenatum* resting cysts from sediment cores following a latitudinal gradient along the Portuguese shelf suggest that this species was introduced in Iberian waters in the late 19th century, following a south-north progression (Amorim and Dale, 2006; Ribei-

ro *et al.*, 2012). However, in the plankton this species was first detected in the region in 1976 when a PSP outbreak affecting nearly 200 mussel consumers in Europe was traced and confirmed to be caused by shellfish from the Galician Rias Baixas (Estrada, 1984). Demonstration of the toxic nature (PSP toxin producer) and description of the reticulate cyst of *G. catenatum* followed (Anderson *et al.* 1988, 1989). The species was detected again in low numbers in 1981and 1987, respectively in Galicia (Fraga *et al.* 1984) and in the SW coast of Portugal (Sampayo, pers.commun.). In 1986 (off Málaga, SE Spain) and 1987 (Algeria) the species was reported for the first time in the Mediterranean Sea (Bravo *et al.*, 1990), and later, in 1999, in central Mediterranean waters (Gomez and Hervé, 2001). Off Portugal, the first reported high density blooms (>10^3 cells L^{-1}) of *G. catenatum* occurred in 1985 along the NW coast (Moita, 1993). For five years blooms were recurrent during summer/autumn, but confined to this region and never being observed to the north of cape Finisterre. In 1992, *G. catenatum* blooms spread into the SW coast of Portugal (Moita *et al.*, 1998). In 1994 and 1995 the blooms reached the southwest coast of Iberia (Moita *et al.*, 1998; Mamán *et al.* 2000; Jaén *et al.* 2003) and were reported for the first time on the northern Moroccan Atlantic and Mediterranean coasts in late 1994 (Tahri Joutei, 1998). Since then, blooms occurred regularly in the Alboran Sea and their eastwards expansion along the North African coast to Algerian waters, was related to the Almeria-Oran jet (Illoul *et al.*, 2005). Meanwhile, in the Atlantic coast of Iberia there was a ten years gap (1996-2004) in *G. catenatum* blooms. In 2005, blooms reappeared in the area with an epicentre located in Lisbon Bay and blooms were reported for the first time in the Galician Rías Altas, north of Cape Finisterre (Pazos *et al.* 2006). In the following years, *G. Catenatum* was observed further south, with large blooms off Lisbon and Setúbal Bay in 2008, and in 2009 in the Algarve coast (south Portugal) where they were annually recurrent until 2014, but gradually decreasing in event intensity and length.

Gymnodinium catenatum bloom dynamics

Gymnodinium catenatum blooms in the Iberian upwelling system are highly variable in space and at the decadal, annual and event scales.*G. catenatum* blooms usually occur during the autumn upwelling–downwelling transition, their maxima detected in convergence zones (Moita *et al.*, 1998). Especially prominent are the blooms of these species in the Galician Rías Baixas, where retention is enhanced through cross-shelf transport (Fraga *et al.*, 1988; Figueiras *et al.*, 1996). *G.catenatum* populations are distributed in and follow the oscillations of the wind driven sea surface layer. *G. catenatum* is a vigorous swimmer and has the ability to vertically migrate and counteract the downwelling forces, maintaining itself in the water column (Fraga *et al.*, 1989; Fermin *et al.*, 1996; Moita and Silva, 2001). Intense blooms of *G. catenatum* have been observed along the whole western Iberian shelf, their maxima displaces to mid-shelf waters due to Ekman transport under upwelling conditions (Moita *et al.*, 1998).

Different hypotheses were proposed for the initiation of blooms along the Northwestern Iberia shelf and Galician rías: (i) in situ germination of cysts, particularly inside the Galician rías (Figueiras and Pazos, 1991); (ii) inshore concentration of shelf populations during upwelling relaxation or downwelling (Fermin *et al.*, 1996; Moita *et al.*, 1998); (iii) concentration of the population at the lee of upwelling filaments extending eastward or southward in association with pro-eminent capes (e.g. Cape Roca and Cape Carvoeiro) (Moita *et al.*, 2003). In the last case, blooms are initiated inshore without being advected

away, suggesting that flow fields associated with upwelling plumes must play an important role on the dynamics of *G. catenatum* populations on the central coast of western Iberia. This mechanism probably develops in other areas and helps to explain patches of *G. catenatum* observed elsewhere on the Iberian coast. Retention areas as the ones described above may also favour sexual reproduction by increasing the probability of gamete encounter, eventually leading to cyst production. In this case, cysts could be produced even during the absence of recorded blooms. The accumulation of *G. catenatum* by the physical mechanisms described reinforce the idea that the species may reach the threshold concentrations for bloom initiation from an innoculum in the water column and support the hypothesis that blooms may originate from a "pelagic seed bank" (Dale & Amorim, 2000; Moita & Amorim, 2002; Smayda, 2002). Curiously, sediments cyst surveys from 1996-1999 along the coast of Portugal showed that *G. catenatum* resting cysts presented a maximum in the central region of the W coast (95-100% of the cysts were already empty), reflecting the areas of bloom maximum intensity in autumn 1994 and 1995 (Amorim *et al.*, 1992; Moita *et al.*, 1998).

According to Slobodkin's (1953) model, once initiated, bloom development and maintenance depend on a threshold concentration of cells in the water column, that in the case of *G.catenatum* appears to be 10^3 cells L^{-1}. After reaching these threshold and develop in shelf waters, *G.catenatum* blooms often persist and can later be observed suddenly in other areas after being transported alongshore by inner-shelf currents (Sordo *et al.,* 2001), or advected into the rías, estuaries and coastal lagoons during upwelling relaxation and downwelling events. There is evidence that in 2005 *G. catenatum* blooms were progressively transported northwards from Lisbon bay in late August to the Galician Rías Bajas in late October, reaching the Rías Altas in November (Pitcher *et al.*, 2010). However, minor blooms have also been observed developing in situ in in late summer in the Galician shelf and in the southern coast of Portugal, off Faro (unplished data). Blooms of *G.catenatum* are rarely recorded on the southwest coast of Spain, between cape Gibraltar and the Portuguese border in the Guadiana estuary (Trainer *et al.*, 2010), but little is known about the hydroclimatic conditions favouring years of bloom occurrence in this region and their absence in western Iberia.

Revisiting the cyst bed-hypothesis

In the Iberian upwelling system, bloom dynamics of *G. catenatum* is usually interpreted as being more dependent on constraints in the planktonic stage than on the build-up of a benthic seed population (e.g. Figueroa *et al.* 2008). This hypothesis is based both in field and laboratory results, namely: (i) very low concentration of viable cysts at any given time in coastal and shelf sediments; (ii) very short mandatory dormancy period (4-11 days); (iii) no environmental pre-conditioning; (iv) high cyst viability. But, the question remains, where does the seeding inoculum come from?

After investigating the presence of *G. catenatum* cysts in sediments from the Iberian shelf and their viability and physiology under laboratory conditions, a 3D Lagrangian model configured to realistically reproduce shelf circulation off the study region (NW Portuguese shelf, 40-41ºN) was used to test if cysts re-suspended near the bottom could be transported to pelagic conditions compatible with their germination (Amorim *et al.,* 2014). The model was constraint to consider environmental thresholds known to influ-

ence cyst germination, namely light, temperature and persistence of favourable germination conditions. Results showed that cysts released at mid-shelf are frequently transported to the photic zone in less than 10 days contrasting with periods when this probability is null. Early-summer conditions are the most favourable for seeding of pelagic blooms. These preliminary results look promising and suggest that in western Iberia, the "cyst bed hypothesis" for the initiation of *G. catenatum* blooms cannot be discarded yet.

References:

Amorim A. , Moita M.T ., Oliveira P. (2002). Dinoflagellate blooms related to coastal upwelling plumes off Portugal. Harmful Algae, 89-91. In: *Harmful Algae 2002*, Steidinger, K., J.H. Landsberg, C. R. Thomas and G.A. Vargo (Eds.), Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography and IOC of UNESCO, pp- 89-91.

Amorim A. and Dale B. (2006). Historical cyst record as evidence for the recent introduction of the dinoflagellate *Gymnodinium catenatum* in the north-eastern Atlantic. African Journal Marine Science, 28: 193-197

Amorim A., Nolasco R., Oliveira P.B., Silva T., Silva A., Domingues B., Brotas V., Dubert J. and Moita M.T. (2014). Seeding of *Gymnodinium catenatum* blooms in Iberian shelf waters. ICES CM 2014/H: 20.

Bravo I., Reguera, B., Martínez, A., Fraga, S. (1990). First report of *Gymnodinium catenatum* on the Spanish Mediterranean coast. In: *Toxic Marine Phytoplankton*. Granéli, E., Anderson, D.M., Edler, L., Sundström, B. (Eds.). Elsevier, New York, pp. 449-452.

Bravo, I., Anderson, D.M., 1994. The effects of temperature, growth medium and darkness on excystment and growth of the toxic dinoflagellate *Gymnodinium catenatum* from northwest Spain. J. Plankton Res. 16: 513–525.

Bravo I. and Ramilo I. (1999). Distribution of microreticulate dinoflagellate cysts from the Galician and Portuguese coast. Scientia Marina 63: 45-50.

Dale B. and Nordberg K. 1993. Possible environmental factors regulating prehistoric and historic "blooms" of the toxic dinoflagellate *Gymnodinium catenatum* in the Kattegat-Skagerrak region of Scandinavia. In: Toxic Phytoplankton Blooms in the Sea (Ed. by T.J. Smayda & Y. Shimizu), pp. 53-57. Elsevier, Amsterdam.

Dale and Amorim, 2000. Dinoflagellate resting cysts as seed beds for Harmful Algal Blooms. Abstracts of the 9th International Conference on HABs, Tasmania, Australia, p.40.

Ellegaard, M., Moestrup, Ø. (1999). Fine structure of the flagellar apparatus and morphological details of *Gymnodinium nolleri* sp. nov. (Dinophyceae), an unarmored dinoflagellate producing a microreticulate cyst. Phycologia 38: 289–300.

Estrada, M., Sánchez, F.J., Fraga, S. (1984). *Gymnodinium catenatum* Graham en las rías gallegas (NO de España). Invest.Pesq. 48: 31-40

Figueiras, F.G., Pazos, Y., 1991. Hydrography and phytoplankton of the Ria de Vigo before and during a red tide of *Gymnodinium catenatum* Graham. Journal of Plankton Research 13: 589–608.

Figueiras, F.G., Gomez, E., Nogueira, E., Villarino, M.L., 1996. Selection of *Gymnodinium catenatum* under downwelling conditions in the Ria de Vigo. In: Yasumoto, T., Oshima, Y., Fukuyo, Y. (Eds.), Harmful and Toxic Algal Blooms. Intergovernmental Oceanographic Commission of UNESCO, pp. 215–218.

Figueroa, R. I. Bravo, I. , Ramilo, I., Pazos, Y., Moroño, A. 2008. New life-cycle stages of Gymnodinium catenatum(Dinophyceae): laboratory and field observations. Aquatic Microbial Ecology, 52: 13–23.

Fraga, S., Mariño, J., Bravo, I., Miranda, A., Campos, M.J., Sánchez, F.J., Costas, E., Cabanas, J.M., Blanco, J. 1984. Red tides and shellfish poisoning in Galicia (NW Spain). ICES 1984 Special meeting on the causes, dynamics and effects of exceptional Marine Blooms and related events, C:5, 10 pp..

Fraga, S., Anderson, D.M., Bravo, I., Reguera, B., Steidinger, K.A., Yentsch, C.M., 1988. Influence of upwelling relaxation on dinoflagellates and shellfish toxicity in Ria de Vigo, Spain. Estuarine, Coastal and Shelf Science 27: 349–361.

Fraga, S., Gallager, S.M., Anderson, D.M. (1989). Chain-Forming dinoflagellates: an adaptation to Red-Tides. In: Okaichi T., Anderson D.M., Nemoto T. (eds.), Red Tides: Biology, Environmental Science, and Toxicology. Elsevier, New York, pp. 281 – 284.

Fraga, S., Bravo, I., Delgado, M., Franco, J.M., Zapata, M., 1995. *Gyrodinium impudicum* sp. nov. (Dinophyceae), a non toxic, chain-forming, red tide dinoflagellate. Phycologia 34: 514–521.

Gómez F. and Hervé C. (2001). Spreading of *Gymnodinium catenatum* Graham in the western Mediterranean Sea. Harmful Algal News, 22:1-3

Illoul H. *et al.* (2005). Detection of toxic *Gymnodinium catenatum* (Graham, 1943) in Algerian waters (SW Mediterranean Sea). Harmful Algal News, 29: 10-12.

Jaén, D., Fernández, R., Mamán, L., Rivera, M.C. (2003). Seguimiento de fitoplancton tóxico en la costa andaluza durante los años 1999 y 2000. VII Reunión Ibérica sobre Fitoplancton Tóxico y Biotoxinas. Conselleria de Agricultura, Pesca y Alimentacion. Generalitat Valenciana, Valencia, pp. 175–188.

Mamán, L., Fernández, L., Ocaña, A., Marco, J., Morales, J., Carballos, M., Márquez, I., Aguilar, M. (2000). Seguimiento de fitoplancton en la costa de Andalucía. Incidencias durante los años 1997 y 1998. VI Reunión Ibérica sobre Fitoplancton

Tóxico y Biotoxinas. Junta de Andalucía, Sevilla, pp. 41–49.

Moita M.T. (1993). In: Toxic Phytoplankton Blooms in the Sea, Smayda T.J. & Shimizu Y. (Eds.), 299-304 pp.

Moita, M.T., Vilarinho, M.G., Palma, A.S. (1998). On the variability of *Gymnodinium catenatum* Graham blooms in Portuguese waters. In: Reguera, B., Blanco, J., Fernández, M.L., Wyatt, T. (Eds.), Harmful Algae. Xunta de Galicia and IOC of UNESCO, Santiago de Compostela, Spain, pp. 118–121.

Moita, M.T., Amorim, A. (2002). The relevance of *Gymnodinium catenatum* (Dinophyceae) overwintering planktonic population vs. cysts as seedbeds for the local development of toxic blooms off Western Iberia. In: LIFEHAB - Life history of microalgal species causing harmful blooms, E. Garcés, A. Zingone, M. Montresor, B. Reguera & B. Dale (Eds.), European Comission EUR 20361, pp. 87-89.

Moita, M.T., P.B. Oliveira, J. C. Mendes & A. S. Palma (2003). Distribution of chlorophyll a and Gymnodinium catenatum associated with coastal upwelling plumes off central Portugal. Acta Oecologica 24: S125-S132.

Negri, A.P., Bolch, C.J.S., Geier, S., Green, D.H., Park, T.-G., Blackburn, S.I. (2007). Widespread presence of hydrophobic paralytic shellfish toxins in *Gymnodinium catenatum*. Harmful Algae 6: 774–780.

Ordás M. C., Fraga S., Franco J.M., Ordás A. and Figueras A. (2003). Toxin and molecular analysis of *Gymnodinium catenatum* (Dinophyceae) strains from Galicia (NW Spain) and Andalucía (S Spain). J.Plankton Res, 26: 341-349.

Pazos, Y., Moroño, A., Triñanes, J., Doval, M., Montero, P., Vilarinho, M.G., Moita, M. (2006). Early detection and intensive monitoring during an unusual toxic bloom of *Gymnodinium catenatum* advected into the Galician Rías (NW, Spain). Abstracts of the 12th International Conference on Harmful Algae, Copenhagen 4-8 September 2006, p. 259.

Pitcher G.C.,F.G. Figueiras, B.M. Hickey & M.T. Moita (2010). The physical oceanography of upwelling systems and the development of harmful algal blooms. Progress in Oceanography, 85: 5–32.

Ribeiro S., Amorim A., Andersen T.J., Abrantes F. and Ellegaard M. (2012). Reconstructing the history of an invasion: the toxic phytoplankton species *Gymnodinium catenatum* in the Northeast Atlantic. Biological Invasions, 14: 969-985

Silva T., Caeiro M.F., Costa P.R. and Amorim A.. *Gymnodinium catenatum* strains isolated from the Portuguese coast: growth, toxin content and genetic characterization. Harmful Algae (submitted).

Smayda, T.J., 2002. Turbulence, watermass stratification and harmful algal blooms: an alternative view and frontal zones as pelagic seed banks. Harmful Algae 1: 95–112.

Sordo I., Barton E.D., Cotos J.M., Pazos Y. (2001). An inshore poleward current in the NW of the Iberian Peninsula detected from satellite images, and its relation with *G. catenatum* and *D. acuminata* blooms in the Galician Rias. Estuarine, Coastal and Shelf Science 53: 787–799.

Slobodkin L.B. 1953. A possible initial condition for red tides on the coast of Florida. Journal of Marine Research 12: 148-155.

Tahri Joutei, L. (1998). In: Harmful Algal Blooms. Reguera, B. *et al.* (Eds): 66-67.

Trainer, V.L., Pitcher, G.C., Reguera, B., Smayda, T.J. (2010). The distribution and impacts of harmful algal bloom species in Eastern Boundary upwelling systems. Progress in Oceanography, 85: 35-52

Annex 10: ToR l (and g): Review draft OSPAR JAMP Eutrophication Guidelines on phytoplankton species composition

Evaluate use of harmful/nuisance algae as an indicator of 'Good Ecological Status' for the Marine Strategy Framework Directive Descriptor 5 (Eutrophication).

The ICES Working Group on Harmful Algal Bloom (HAB) Dynamics was asked to review the draft OSPAR JAMP Eutrophication Guidelines on phytoplankton species composition. Since these guidelines were developed there has been considerable work performed on the response of the phytoplankton community to environmental and anthropogenic drivers. The OSPAR area is broad, and includes diverse regions such from North Atlantic (parts) to the Baltic Sea. Current guidelines, which are used are prepared in 1997 did require a revision and OSPAR asked NL, SW, and Ge to revise the guidelines. Mainly SW and NL collaborated on this task. The draft was produced by Bengt Karlson and Marie Johansen from Sweden together with Hans Ruiter from the Netherlands.

WGHABD has previously addressed the issue of HABs and Eutrophication (see WGHABD report 2009) with members of the WG producing a review on this topic (Gowen *et al.*, 2012) and the influence of nutrient ratios on HABs (Davidson *et al.*, 2012). Both of these peer review publications provide comprehensive reviews on the physical, chemical and biological variables which influence the occurrence of HABs and the role of anthropogenic nutrient enrichment.

Case studies from Gowen *et al.*, (2012) have shown that in some coastal embayments the restricted exchange between the embayment and open coastal water can be the dominating factor which influences the nutrient-HAB association (e.g. Tolo Harbour in Hong Kong) however in other regions e.g. along the west coast of Ireland, nuisance blooms (e.g. *Karenia mikimotoi)* can occur in the absence of nutrient enrichment. For many coastal regions, attempts to relate trends in the occurrence of HABs to nutrient enrichment are confounded by increased monitoring effort and reporting of HABs, the effects of climate change (e.g. the North Atlantic Oscillation Index and the El Niño Southern Oscillation) and the introduction and transfer of HAB species. Thus the occurrence and abundance of HAB species and HABs should not be used to diagnose eutrophication unless a link to anthropogenic nutrient enrichment can be demonstrated for a specific area. Within legislation the Urban Waste Water Directive and OSPAR definitions require the demonstration of a series of cause and effects e.g. nutrient enrichment leading to accelerated growth of plants and or a disturbance of the balance of organisms which is undesirable.

Within the context of the phrasing within D5 of the Marine Strategy Framework Directive, the link between human activities and blooms/nuisance algae within a region must be proven before these can be used as an indicator of eutrophication. It should also be noted that evidence of a link in one coastal region should not be taken as evidence of a general linkage in other coastal regions.

In general there was consensus that the JAMP guidelines are adequate for long term monitoring of the phytoplankton community. However the guidelines are not flexible enough to integrate HAB monitoring for sanitary purposes. The guidelines continue to refer to HABs as being caused by a distinct group of species that has some inherent connectivity. This is not the case. HAB species comprise a diverse range life forms/strategies.

Their only unifying feature is a negative impact on the ecosystem or ecosystem services. This needs clarification in the document. The guidelines also aim to include generalized monitoring of phytoplankton which is not always suitable for focused HAB monitoring. The EU-National Reference Laboratory for Biotoxins (EU-NRL) working group on Phytoplankton Monitoring (starting May 2015) will focus on this matter specifically. WG delegates attending this EU-NRL group will report back to WGHABD during the 2016 meeting (see ToR D).

The JAMP guidelines encompass the whole phytoplankton community and the need to improve sections on spatial distribution and temporal trends was identified, particularly if a nutrient driven response is to be separated from climate change. **If OSPAR adopts these JAMP guidelines the role of ICES in the coordination and storage of data was may increase. This should be noted by ICES.**

Specific comments on the documents have been compiled in the following table. Two comments about remote sensing are included. The comment about coccolithophores is related to general monitoring.

Table 1: Comments on JAMP Phytoplankton Monitoring Guidelines.

Section	Comments	Nature
1	Toxic species that even at low concentrations....	wording.....
7.2	CEN document are not freely available. This inhibits accessibility and use.	General comment
7.3	Auto, mixo, heterotrophic not specified. It is unsure how this can be determined in many cases. If not known, not specified is included. This should be emphasised.	Adaptation required
	Many blooms are a natural part of the seasonal cycle of phytoplankton and are not HABs. Equally not all HABS occur due to human causes, HABs develop in natural systems.	General comment
8.1	ISO17025 is quite a high recommendation standard. Many countries are not accredited at this stage. Many methods used are not accredited. Laboratories should participate in *inter laboratory comparisons* rather than intercallibrations	General comment
9.4.1 Phaeocystis as a common indicator species.	Phaeocystis is not a common indicator species. Only in the Netherlands has the occurrence of *Phaeocystis* blooms been linked to nutrient enrichment. Phaeocystis blooms can occur in other regions in the ICES area and are not associated with nutrient enrichment thus	

	blooms of this phytoplankter should **not** be used as a general indicator of eutrophication. This should be flagged in the text for clarification. Other indicators are not mentioned.	
10.4	Tube samplers will not always capture rare species. A net tow should be included, not for quantitative, but for qualitative purposes when appropriate. Vertical net tow gives some indication of the presence of subsurface blooms (beneath the bottom of the tube sampler).. Include a sample method for all purposes.	
10 Additional but optional	Picoplankton analyses can be done by flow cytometer. Picoplankton is a very important plankton group within the ecosystem. This should be highlighted and certainly not in the additional options.	
Xxx	In general rapid, qualitative, or semi-quantitative methods are not included.	
Xxx	Plankton nets should be included to have an estimate of rare species during diversity studies. This would be in addition to the quantitative sampling.	
Xxx	Toxin producing phytoplankton species should not be used as an indicator for eutrophication unless the link with nutrient enrichment has been proven for a specific area. Many toxin producing species cause problems at very low cell densities and are often not the dominant species in the phytoplankton community at the time.	Clarification.
Xxx	Flow cytometry etc. should be used. Not everyone can comply, since not all labs have access to the equipment. However, to perform picoplankton monitoring, these equipment should be available.	General comment
Xxx	Eg. Subsurface blooms are not included.	
10.5	To assess the effect of	wording.
10.7	does not include EDNA (as such).	

Xxx	There should be separate methods for different purposes. This goes for sampling, as well as for analytical purposes.	
10.9	The timing of sampling should consider the state of the tide at each location. It would be preferable that sampling be conducted at the same state of the tide on each sampling occasion. For instance, in estuarine or coastal locations it might be preferable to sample at high water (+/- 1hr) to ensure that marine phytoplankton are sampled as consistently as possible.	Text suggestion
10.9	Currently reads: Also information on the distribution and frequency of blooms of coccolithophorids can be obtained. Change to (ref. Shutler, 2010) Also information on the distribution and frequency of blooms of coccolithophorids can be obtained, using robust automated techniques applied to a long time-series of ocean colour data (Shutler *et al.*, 2010).	Text suggestion
10.9	Thus the algorithms for calculating chlorophyll from satellite remote sensing data can be improved. Add: The ESA Climate Change Initiative (CCI) programme is generating a set of validated, error characterised, Essential Climate Variables (ECVs) from satellite observations. One of these 13 projects, the Ocean Colour CCI (OC-CCI) began work in 2010, and is providing ocean colour ECV data, which can be used by climate change prediction and assessment models. The dataset is created by merging MERIS, MODIS and SeaWiFS, together with per-pixel uncertainty estimates (www.esa-oceancolour-cci.org). The initial focus is on case 1 waters (where the colour is only affected by the concentration of chl-a), but the version 3 release scheduled for spring 2016 will also address case 2 waters – optically complex waters (often near coasts and in shelf-seas) which may also contain suspended sediment and coloured dissolved organic matter.	Text suggestion

Annex 1: Method for analysis for micro zooplankton.	Method for analysis for micro zooplankton, is not a good method. It is to complex (two weeks waiting time). This is not achievable for monitoring purposes. Methods based on filtration are more efficient in practice (details by BeatrizReguera, WGHABD).	

References

Davidson K., Gowen R. J., Tett P., Bresnan E., Harrison P.J., McKinney A., Milligan S., Mills D. K., Silke J., Crooks A.-M. (2012). Harmful Algal Blooms: How strong is the evidence that nutrient ratios and forms control their occurrence. *Estuarine, Coastal and Shelf Science*, 115, 399 – 413.

Gowen, R. J., Tett, P., Bresnan, E., Davidson, K., McKinney, A., Harrison, P. J., Milligan, S., Mills, D. K., Silke, J. and Crooks, A.-M.(2012). Anthropogenic nutrient enrichment and blooms of harmful phytoplankton.*Oceanography and Marine Biology: An Annual Review*,50, 65-126.

Shutler, J.D., Grant, M.G., Miller, P.I., Rushton, E. & Anderson, K. (2010) Coccolithophore bloom detection in the north east Atlantic using SeaWiFS: Algorithm description, application and sensitivity analysis. *Remote Sensing of Environment*, 114(5), 9.doi: 10.1016/j.rse.2009.12.024

Annex 11: Report of special session on HABs at ICES ASC 2014

REPORT ON ICES - ASC 2014 THEME SESSION H
Harmful Algal Blooms in Aquaculture and Fisheries ecosystems: prediction and societal effects

Convenors: Beatriz Reguera (Spain), Juan Blanco (Spain), and Bengt Karlson (Sweden)

Rapporteurs: Santiago Fraga (Spain), Joe Silke (Ireland), Lourdes Velo-Suárez (France), Teresa Moita (Portugal), José Manuel Cabanas (Spain) and Antonella Penna (Italy)

Harmful Algal Blooms (HABs) area major hazard for the exploitation of coastal resources in ICES countries. HABs include i) toxin producing microalgae, which contaminate shellfish with their toxins, cause human intoxications and lead to lengthy harvesting bans when toxins in commercial bivalves exceed regulatory levels; ii) high biomass fish killing HABs with devastating effects in areas of intensive caged-fish aquaculture; iii) emerging benthic HABs, traditionally reported from tropical areas, which cause Ciguatera Fish Poisoning or are associated with toxic sea-spray causing respiratory and skin irritations; iv) Cyanobacteria blooms, in brackish waters, associated with surface scums and mortalities of wild fauna. Improved monitoring and predictive capabilities constitute the main tools to prevent or mitigate the negative impacts of HABs for coastal ecosystem services. The main objective of this session was to review increased monitoring efforts, technological developments for in situ detection of harmful algae, and new analytical tools for toxin detection. Combined with international programmes and projects promoting species-specific research in the last two decades, these efforts have led to a considerable advance in our capabilities for early warning, detection, and for understanding of the mechanisms underlying initiation, maintenance and decay of these blooms. Additionally, the application of operational oceanography principles to forecast HAB events has improved the flow of information from research and monitoring agencies to the end-users (health and environmental authorities, shellfish growers, tourist industry). Scientists from ICES countries were invited to contribute with communications on the following topics: i) HABs and their impact on wild fisheries and shellfisheries; ii) Emerging benthic HABs and their toxins; iii) Advances in the ecology and oceanography of HABs in the ICES domain; iv) Improvements in HAB forecasting – coupled physical-biological, and toxin uptake-detoxification models; v) Advances in automated HAB observing systems, biosensors and toxin-detection methods; vi) Mitigation strategies; vii) Supporting information for the end-users.

The session, held on Thursday afternoon (15.00-19.00) and Friday (09-17.00), had a successful response and included **27 oral communications** and **17 posters**. The latter were allowed 5-min speed-talks to present their results. They were distributed in the sub-sessions described below.

HABs and their impact on wild fisheries and shellfisheries

The opening presentation (H:01) introduced the increasing number of HAB species and toxins that have been characterized since the early days (1980's) of ICES working groups, when paralytic shellfish poisoning (PSP) and some fish mortalities were practically the only known syndromes in the area. Long time series from pilot areas show that some events seem to be going through an intensification (DSP in western Europe and North America) whereas others are receding (PSP in western Europe). In addition to health impacts on humans (and the market impact), some toxic dinoflagellates, modulated by other factors, such as contaminants and parasites, affect bivalve biological processes (e.g. gametogenesis) (H:02). Diarrhetic Shellfish Poisoning (DSP) events arethe most damaging in terms of days of harvesting closures. They were dealt with from two different perspectives: practical aspects of their monitoring in Sweden (H:04) and how different food processing (steaming, canning) affect the estimates of toxicity when the official EU technique is used, thus showing the need to develope more robust ones (H:05). The latter addsfurther uncertainties to shellfish marketing.. The Curonian Lagoon, in the Baltic Sea, appeared as a hot spot of Cyanobacteria blooms causing multiple damages to the fauna and tourism (H:6, H:7). Transformation of cyanobacteria toxins in freshwater mussels were described (H:8)

Emerging benthic HABs and their toxins

Improved analytical methods (LC-MS) and the emergence of new toxins were discussed to highlight the need to improve monitoring programmes to minimize risks to human health. These included new Azaspiracid (AZP) analogs (H:09), continuing observations of ciguatoxins in the Canary Islands (H:10), Tetrodotoxins (TTX) and Spirolides (SPX) in Portugal and BMAA in Morocco (H:12), and 7 new Ovatoxins from benthic HABs (*Ostreopsis* cf. *ovata* strains collected from the Gulf of Naples) (H:13). Azaspiracids are now known to originate from several *Azadinium* and *Amphidoma* biogenic sources(10 species of *Azadinium* described). So far the toxin has been found globally in four species (*A. spinosum*, *A. poporum*, *A. dexteroporum* and *Amphidoma languida)*. There are 42 variations of the molecule described, from three structural groups, some included in legislation and others so far not regulated in the EU. Many of these analogues are very closely related, and misidentification is possible. The first cases of Ciguatera fish poisoning in the EU were detected in the Canary Islands in 2008, and 100 people have been affected between 2008 and 2013. Most cases were associated with amberjack and grouper (*Seriola* spp.). In the Islands, three species of *Gambierdiscus* (*G. excentricus*, *G. silvae*, *G. australes*) have been identified. Another emerging marine poison, tetrodotoxin, was reported in warm water gastropod species in Portugal (*Monodonta lineata*, *Gibbula umbilicalis* , and *Charonia lampas*). Spirolides were found in a range of species (Gastropods and Bivalve molluscs) in Portugal and BMAA in *Patella intermedia* and *Monodonta lineata* in Morocco. The low levels detected are felt to pose little impact to humans, but nevertheless indicate the need to be vigilant. In recent years, seven new ovatoxins have been found in *Ostreopsis* cf. *ovata* from the Mediterranean Sea using QTOF LCMS for their identification. The complementary use of positive and negative ion mode enabled the elucidation of the structural characteristics of some of these ovatoxins. The role of ICES in HAB science is recognised by the longstanding Working Group on Harmful Algal Bloom Dynamics (WGHABD). The group is a forum for ICES and UNESCO-IOC to review and discuss HAB events and to

provide advice and updates on the state of HABs in the region. It also facilitates interaction between scientists working in diverse areas of HAB science and monitoring (H:14).

Advances in the ecology and oceanography of HABs in the ICES domain/ Improvements in HAB forecasting – coupled physical-biological, and toxin uptake-detoxification models

These 2 sub-sessions described advances (some developed within the EU 7FP project ASIMUTH) from the combination of field monitoring data and other observations with modelling to improve prediction of HAB events. Contributions included a very good study that combined laboratory experiments and numerical simulations to understand *Gymnodinium catenatum* cyst dynamics off Portugal (H.20). Although the idea had earlier been dismissed, resuspended cysts might seed planktonic *G. catenatum* blooms under certain environmental conditions and the cyst inoculum should not be discarded yet. This was proposed as **best oral communication**. A time series study using monitoring data from France, found strong relationships between temperature-irradiance and *Alexandrium minutum* bloom initiation (H:21). However, no correlation between abiotic factors and bloom decline was detected. H:22, H:23 and H:25 were centered on *Dinophysis acuta* and *Dinophysis acuminata* distributions and DSP events on Iberian Atlantic shores. H:22 presented Lagragian simulations to understand *Dinophysis* transport between Aveiro lagoon and the Galician Rías. H:23 used INTECMAR (monitoring) *Dinophysis* time series and concluded that abiotic factors such as upwelling and flushingtimes in the Rías were not always good predictors for *Dinophysis* blooms in the area. The inadequacy of "*Dinophysis* concentration threshold" to predict DSP toxin detection in mussels was also stressed. H:25 suggested that longshore advection (Portugal to Galicia) of *D. acuta* and *G. catenatum* populations come from different source areas. The relationship between cyanobacterial blooms in the Curonian lagoon and physical processes related to climate change was discussed (H:24).

H:28 showed how a HAB forecasting system, based on monitoring observations and operational oceanography, developed into a successful logistic product for aquaculture farming, policy makers and stakeholders in western Ireland. This resulted in an alert system, based on tools for nowcast and forecast HABs and toxins, disseminated through a weekly bulletin including the situation in each aquaculture zone (successful in predicting 50% of *Karenia mikimotoi* and *Pseudo-nitzschia* blooms). H:29 focused on a reanalysis of a HAB event during a downwelling episode off the Galician Rias Bajas in 2005. It modelled the advection and development of toxic algae and oceanographic conditions during spring (*D. acuminata*) and autumn (*D. acuta* transport from Portugal) in 2013: A weekly HAB bulletin for the area was developed. It was highlighted that models can now simulate conditions inside the rias and are adapted to tides. An artificial neural networks (ANN) was built with monitoring data (phytoplankton and environmental conditions) collected since 1990 in the Ebro River Delta region, NE Spain (H:30). After training (misclassification, error characteristics, presence-absence events) of the ANN, these were able to explain 60% of *Pseudo-nitzschia* (ASP) and 62.5% of *Karlodinium* (fish-killer) abundance in the area. Phenological changes in the bloom season and intensification of *Pseudo-nitzschia* blooms were observed.

An Individual-based model (IBM) was used to understand biological interactions in *Alexandrium* (host) blooms infected by *Amoebophrya* (parasite) (H:31). The model balanced inputs versus outputs, focusing on *Alexandrium* mortality. It was validated with parame-

terization (data from literature and experiments) and emphasized the importance of the timing of gametogenesis and cyst infection. Results support the hypothesis of parasite-host simultaneous dormancy and the role of excystment to propagate both species. This was proposed for the **best young-scientist oral presentation.**

The following half subsession focused on the uptake and transformation of toxins within shellfish. Possible ways of uptake and elimination of toxins from the okadaic acid group (OA, DSP toxins) were discussed, in particular the cell export of toxins by passive diffusion and/or membrane transport (H:32). Mussels can accumulate free and esterified forms. Several dynamic models including different accumulation routes were used to simulate OA and DTX2 accumulation in mussels. Results suggest that esterification has an important role in reducing the accumulation of OA and DTX2 in bivalves and that the accumulation of DTX2 in mussels should be higher than that of OA. PSP toxin data from the Portuguese biotoxin monitoring programme (1996-2012) showed inter-annual and seasonal trends as well as connectivity between neighboring regions, in particular adjacent coastal and estuarine systems (H:34). Results showed absence of PSP during the period 1996-2004 in contrast with a bimodal distribution (autumn-early winter, and summer) during the period 2004-2012. The persistency of OA and spirolides in seawater is well known in Galicia. An innovative experiment was carried out on OA, spirolides and gymnodimine degradation in the water column and sediments to elucidate whetherobserved toxin levels are due to constant toxin production or to high toxin stability (H: 15),. Degradation of OA in the water is very slow; in contrast to that of gymnodimine and spirolides whose persistency (13-desmethyl SPX C) in sediments seems to be related to resistant forms of dinoflagellates (cysts). Sediment profiles of OA, spirolides and other toxins in the Galician rías were shown to be useful to study historical trends and show an increased impact of OA in recent years. This was proposed for **best young person poster presentation.**

Advances in automated HAB observing systems, biosensors and toxin-detection methods/Mitigation strategies

The notable irruption of molecular and genomic tools into HAB studies was evident in these two sub-sessions, which included twelve contributions on (i) molecular ecology, (ii) genetic and physiological basis of toxin accumulation, (iii) dinoflagellate toxin gene expression; and (iv) use of molecular tools for HABs and toxin identification in Atlantic areas.

Molecular qPCR technologies have been used for prevention and management of toxic dinoflagellate *Ostreopsis* events in Mediterranean tourist resorts (H:36), and of *Azadinium* (AZP) and *Pseudo-nitzschia* (ASP) in the eastern Atlantic (H:37). A novel study established that, using a developed parentage tool, the accumulation characteristics of mussels are inherited in a proportion that makes it possible to start selective breeding programmes to obtain low accumulating strains (H:39). *Ruditapes philippinarum* was also studied as a model for transcriptional response to exposure to microcystins (H:44). Microcystin -LR metabolism in *Ruditapes philippinarum* was investigated using this bivalve as a model for studies on biochemical degradation of microcystin (H:40). The biochemical response of mussels was investigated using the glutathione transferase proteomic response after exposure to toxic *Microcystis aeruginosa* (H:43). Meanwhile, the mussel *Mytilus galloprovin-*

cialis was used as an animal model to investigate the *in vitro* genotoxic effects of OA in haemolymph and gill cells (H:41)., Gene expression variation of saxitoxin genes (*sxtA* and *sxtG*) in the toxic Mediterranean dinoflagellate *Alexandrium minutum* under different nutritional conditions was also investigated as a preliminary molecular approach for toxin monitoring (H:42). Detection of lipophilic toxins with passive (adsorbing resins) samplers was shown to be a valuable tool for research on toxin production and transformation kinetics, but its advantage as an early warning tool, in places where toxins are detected by LC-MS, was discarded.

Supporting information for end-users

Theme session H finished with successful examples of monitoring strategies and their efficient dissemination to end users: the Galician HAB monitoring programme (www.intecmar.org, H: 45) (1992 to date), devoted to HAB and toxin monitoring to safeguard shellfish production and consumer health, and the *Baltic Algae Watch System*, monitoring cyanobacteria blooms in the Baltic Sea for the last 13 years. The recently established French network on Harmful Algal Blooms (PHYCOTOX) aims to optimize human and scientific resources devoted to HAB research and its impacts on the ecosystem through a well-coordinated national program (H:47).

Conclusions

It was shown that the number of HABs and affected areas in the ICES domain is high and that the number of harmful algal events is increasing. Also new toxins are observed. Consequently the harmful algal blooms have a large impact on fisheries and aquaculture. A new aspect of HABs is emerging – their effects on bivalves and their interaction with other processes that affect them, e.g. parasite infection. Many HAB ecological studies are currently using modelling, in many cases linked to oceanographic processes, which makes these studies more robust and prone to be of use for forecasting and therefore for mitigation of the effects of these events. The tools developed to study the past events in an area by using sediment cores are promising to evaluate the risks and to assess the impact of climate change and other environmental changes. Monitoring programs are starting to incorporate molecular tools to detect HAB events. This is a promising approach, especially for species that are difficult to identify or quantify using microscopy. In situ imaging flow cytometry is another promising approach. The methods of toxin quantification in bivalves are still not perfect. The toxins have a large impact on fisheries, aquaculture and food transforming activities. The methods should be improved to give reliable results in all situations. Some physiological and genetic studies relating to toxin accumulation in bivalves are being carried out showing good perspectives for the selection of organisms with reduced toxin accumulation. Finally, different strategies for supporting end users are being used. Most nowcasts are based on observations in situ or on analyses of collected water samples and in some cases on remote sensing. Forecasts based on observations and coupled models are being developed and are in use in a few countries.

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Annex 12: Technical minutes from RGJAMP

Review of ICES Working Group on Harmful Algal Bloom (HAB) Dynamics (WGHABD), 13–17 April 2015, regarding ToR L (and G): Review draft OSPAR JAMP Eutrophication Guidelines on phytoplankton species composition

28 May 2015

Reviewers: Harri Kuosa, Finland (chair) and Donald Boesch, USA

WGHABD Chair: Eileen Bresnan, UK

ICES Secretariat: Sebastian Valanko

The WG gives a very throughout response on JAMP Eutrophication Guidelines. They consider within D5 of the Marine Strategy Framework Directive in their review.

Considering the diverse HAB species and their occurrence, it is true that the guidelines are not flexible enough (or detailed enough) to all purposes of monitoring. Thus monitoring may have to be planned specifically as is the case of HABs.

There is a number of specific comments given as Table 1. Most of the comments show that a general guideline cannot be specific enough for all purposes. Work on more detailed guidelines for all aspects of monitoring (from sampling to reporting) probably is required to fulfil all requirements in phytoplankton monitoring. At the same time a general guideline cannot include all aspects, but also be flexible enough to allow emendations.

The last remark on the method for suggested microzooplankton analysis is relevant as the proposed one is rather complicated do not include comparison with other methods.