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Report of the Working Group on Biological Effects of Contaminants (WGBEC)

12–16 March 2012

Porto, Portugal

ICES International Council for the Exploration of the Sea

CIEM Conseil International pour l'Exploration de la Mer

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Contents

Executive Summary

The Working Group on the Biological Effects of Contaminants [WGBEC], chaired by Matt Gubbins (UK) and John Thain (UK), met at the Universidade do Porto, CIMAR, Porto, Portugal, from 12 to 16 March 2012. There were 18 attendees representing eleven countries.

A summary of the key outcomes in respect of the Terms of Reference is described below.

WGBEC includes in its membership scientists from national government institutes, academia, industry and management. The group also has a diverse membership of expertise, ranging from chemists, biologists, biochemists and environmental scientists. This year there were fourteen items on the agenda, including two items from ICES and three from OSPAR/ICES. Priority was given to the latter items. Presentations and discussions took place in plenary, with rapporteur responsibility shared by all members of the group. All items on the agenda (covering all ToRs) were completed and are reported.

Proposed change for WGBEC, reporting and ToR to move to a three year programme from and including 2013. This item was added to the agenda in order to respond to the request from the Chair of SCICOM for EGs to consider the implementation of Multi Annual Management of SCICOM Expert Groups. WGBEC considered the changes and were fully supportive of the process, and decided to implement and move to multi-annual ToRs from and including its meeting in 2013. Draft ToRs were proposed for consideration by SCICOM. WGBEC would run with the current chairperson arrangements for its 2013 meeting with new appointments for chairman to be made at the 2013 meeting.

Review of integrated monitoring and assessment. Outstanding tasks from the SGIMC report on completing two chapters were addressed, and in addition minor changes were made to some of the text for Scope For Growth, fish disease and sediment, seawater elutriate and pore water bioassays. WGBEC were aware of comments made on the SGIMC approach at the HASEC March, 2012 meeting; these were discussed and a viewpoint drafted. Since the development of the integrated contaminant and biological effect monitoring scheme by SGIMC (SGIMC, 2011), several countries have attempted to apply national monitoring data to the assessment framework with varying degrees of success. The process of trial provides valuable lessons learned as well as a demonstration of the potential utility of the integrated approach. Development of Assessment Criteria (AC) is an ongoing process as data is reviewed and new data becomes available. As agreed at SGIMC 2011, WGBEC would annually review AC and considered changes to AC for lysosomal stability, Scope For Growth, imposex and intersex in snails, sea urchin and mussel embryo bioassays, reproductive success in fish, PAH metabolites in Baltic herring and eelpout, EROD in eelpout, and AChE in eelpout, Lysosomal stability in herring and eelpout, DNA adducts in various fish and whole sediment bioassays with *Corophium*.

Review of Environmental assessment Criteria. WGBEC reviewed the Environmental Assessment Criteria (EACs) produced by the OSPAR intercessional correspondence group ICG-EAC for CEMP and pre-CEMP determinands. Six specific comments were made and three conclusions were: 1) Within EAC biota, a clear discrimination between EAC for mussels and fish is needed; 2) A further revision of the EACs, which have been identified as too low or too high, is needed before they can be applied for assessment purposes; 3) If organisms at lower trophic levels are regarded as the most

sensitive species for specific substances, EACs for biota could be derived by extrapolating from EQS for water by applying BCF values. Further published toxicity data were identified that would be useful for the further development of EACs.

Review and update Technical Annex on lysosomal stability. The OSPAR background document on lysosomal membrane stability (LMS) was reviewed and required updates to the ICES TIMES method manuscript identified in order to harmonize the use of the Neutral Red Retention (NRR) assay, in terms of monitoring and intercomparison purposes across the ICES maritime area and between Regional Seas programmes. WGBEC updated Technical Annex 6 of the OSPAR JAMP Guidelines on 'general biological effects methods' to include information on the use of the LMS method in mussel species and bring the document up to date.

MSFD – review of international progress with Descriptor 8. Brief presentations were made by some group members on progress with national approaches on initial assessments and the development of GES criteria for descriptor 8 under the MSFD. Most countries are progressing well although with differing speed. For biological effects several countries intend to develop pragmatic approaches and expressed their intentions to fit GES descriptors as much as possible to existing monitoring programmes.

Receive reports on marine monitoring activities by member states. Members of the working group were provided the opportunity to inform the rest of the group of current and future biological effects monitoring activities taking place across the ICES area.

Review of progress with publication and electronic dissemination of biological effects techniques in the ICES TIMES series. Progress with publications of ICES TIMES manuscripts was reviewed and reported. The requirement for TIMES documents in relation to the SGIMC integrated scheme was reviewed and four methods were identified where manuscripts are required. These were Condition Index (fish and mussel), COMET assay (fish and mussels), Stress on Stress and ER CALUX.

Respond to requests for advice from the ICES Data Centre. Queries received by the ICES Data Centre regarding reporting formats for biological effects parameters were addressed both intersessionally and immediately prior to the meeting. The WGBEC recommended responses to these queries are reported here. The responses recorded here improve the guidance on how biological effects data should be reported to the ICES database.

Review progress with AQC procedures for biological effect methods and include harmonisation activities. AQC activities were initiated for imposex in dogwhelks and bile and EROD in fish.

Report on developments relating to contaminant effects from litter. Recent developments relating to contaminant effects from litter/plastic particles were presented, and specifically included recent publications in the literature, monitoring activities and development of suitable indicators for the implementation of the EU MSFD Descriptor 10. This is a rapidly emerging field and WGBEC agreed that it should include this topic area in its multi-annual ToRs for 2013–2015.

Collaboration with other ICES WGs. WGBEC had reviewed its potential for collaboration with other WGs in 2011 and this year had reviewed the ToRs of these groups to identify any avenues for cross talk or liaisons. Collaboration had been identified with WGEEL in relation to contaminants in eels and the comparison of BACs and EACs and to establish if biological; effect methods could be applied. Collaboration

with MCWG and WGMS was identified in relation to the application of passive samplers and how they can be used for marine monitoring purposes. It was agreed to propose a back to back meeting of all three groups for 2014 in Copenhagen.

Consideration of issues of special scientific interest /value. Presentations were made on a number of issues identified by the group which were considered to be of special scientific interest and value to understanding the effects of contaminants in the marine environment and these included: ocean acidification, primary production, species differences in biomarker and bioassay responses, immunocompetence assays and online monitoring.

1 Opening of the meeting

The ICES WGBEC was hosted this year by Lucia Guilhermino and held at the Universidade do Porto, CIMAR, Porto, Portugal. The Chair, Matt Gubbins (UK), opened the meeting at 09:30 on Monday, 14 March 2012, and thanked Lucia Guilhermino for hosting the meeting and for organising the meeting arrangements and hotel accommodation, etc. The Chair then invited the participants to introduce themselves and their affiliations and describe their area of interest and field of expertise. There were eighteen participants present at the meeting and three corresponding members of WGBEC, representing eleven countries: Belgium, Denmark, France, Germany, Ireland, Netherlands, Norway, Portugal, Spain, UK and the USA. The list of attendees is given in Annex 1.

2 Adoption of the agenda

The ToRs and a draft agenda had been circulated prior to the meeting. The Chair invited participants to examine the Terms of Reference (ToR) and went through the agenda in detail explaining the priority and background to the agenda items and in particular those requests from ICES and OSPAR; ToR a, to review and update the Technical Annex on lysosomal stability, and ToR b, to review Environmental Assessment Criteria or equivalents. WGBEC also considered that ToR c, on integrated chemical and biological effect monitoring and assessment to be an important agenda item in relation to the final report on SGIMC and how this integrated approach could be taken forward. In addition, the chairs had been consulted over the past year and also at the ICES 2011 ASC in Gdansk on the changes proposed by ICES for Expert Groups; it was agreed that this topic should be added to the agenda for open discussion and to agree a way forward (agenda item 4).

The ToRs for the meeting can be found in Annex 2. The draft agenda was adopted by the meeting and a tentative timetable agreed, which was updated on a daily basis, Annex 3 and 4 respectively.

3 Appointment of rapporteurs

Principle rapporteurs were appointed for the agenda items and are given in Annex 4.

4 Presentation of the proposed modus operandi for ICES Expert Groups; implications for WGBEC and development of a way forward, terms of reference and chairpersons

This item was added to the agenda to enable a full discussion of the management and operational changes for Expert Groups (EGs) currently being implemented by ICES. The Chair of SCICOM had written to the chairs of all EGs to ask if they could review the implementation plans provided in the communication from the Secretariat and contribute any thoughts and ideas. Each ICES science EG has its own characteristics and SCICOM are keen ensure that every aspect of the changes are carefully considered in the drawing of final plans. A copy of the Draft Multi-Annual Management of SCICOM for Expert Groups: Implementation ICES document of 12 January 2012 was circulated at the meeting.

4.1 Background

Excerpts taken from the afore mentioned ICES Draft document on implementation:

"In 2009 ICES initiated a process of reform of its science structure and appointed a Science Committee (SCICOM) with national representation. A 'steering group' structure was established to provide scientific leadership and coordinate the work of Science Expert Groups (EGs).

Over 2010/2011 SCICOM, in consultation with the Chairs of ICES EGs, considered measures to streamline the management and reporting processes of EGs, empower EGs to plan their tasks over a longer time period, and put additional focus on the achievements and outcomes of EGs at fixed time periods. In addition, these measures were intended to better demonstrate the value of EGs to Member Countries, and maximize the uptake of EG results in advisory activities.

In September 2011, after consultation with EG Chairs, SCICOM agreed to a staggered process of introducing multi-annual Terms of Reference (ToR), with a reduced level of routine reporting and a more focused, accessible, outcome-driven EG activity. To this end a report was produced by ICES. This document explains the main changes implemented and implementation process."

*I*CES Science Committee produced a report in January 2012 on the implementation of multi-annual management of SCICOM Expert Groups.

Summary of the changes to be implemented:

CURRENT SYSTEM	NEW SYSTEM
1. SGs and W/S have fixed duration. WGs have open-ended terms.	1. EGs are appointed for an initial 3-years. They can request renewal at the end of their term. SGs disappear as a structure as they are effectively an EG with a single term. W/S are not affected by these changes.
2. ToRs for all EGs are proposed, modified and approved annually.	2. ToR for all EGs are approved at the onset for the duration of the EG (3 years for EG), although new ToRs can be considered in response to ad hoc requests.
3. EGs provide a comprehensive annual report to SCICOM via their Steering Groups.	3. WGs will provide interim, reduced, reports at the end of years 1 and 2 of their appointment, and a final, comprehensive report at the end of year 3.
4. EGs are not evaluated and continue operating as per the above guidelines as long as they are supported by the community.	4. EGs are self-assessed, through a simple questionnaire that identifies and showcases their achievements against original goals. Renewals for further terms are considered by SCICOM based on justification and self-assessments.

Timetable for implementation:

ACTIONS AND STEPS	WHEN
Consultation with Expert Groups and compilation of their feedback on the new system and related working documents	January – mid March 2012
Presentation of feedback from Expert Groups to SCICOM; SCICOM decides on document revisions and further implementation process based on feedback	End-March (SCICOM midterm meeting)
First Category 2 resolutions for multi-annual ToRs approved by SCICOM	ASC 2012

4.2 Discussion on proposed changes

WGBEC considered the changes and were fully supportive of the process. In particular, the three year reporting process was welcomed as a significant amount of time and effort is placed on producing a report at the end of each meeting and in future this can be channelled to improve debate, discussion and quality of advice. Members of the group felt that the self assessment procedure and showcasing of achievements would benefit the delivery of the ToRs and also promote buy-in from those attending, for example the potential for collaboration on work programmes and publications.

Matt Gubbins (chair) reminded the group that under the new arrangements the chairperson(s) is appointed for a three year period. No volunteers were found to take on this role immediately but it was emphasised that a new chairperson(s) must be appointed at next year's meeting. This may cause a problem for WGBEC as most working group members do not have funding and time to take on this role.

After discussion it was agreed that:

- WGBEC fully support the changes being implemented by SCICOM.
- WGBEC would implement the move to multi-annual Terms Of Reference from (and including) its meeting in 2013.
- WGBEC would run with the current chairperson arrangement for its 2013 meeting with new appointments for chairman to be made at the 2013 meeting.

4.3 Proposed ToR in relation to the ICES Science Plan

A discussion was then held on formulating a 3 year multi-annual Terms of Reference. The basis of this is described below and expanded in Annex 5.

Proposed multi-annual terms of reference; beginning 2013

1) Respond to requests for advice from Regional Seas Conventions (e.g. OSPAR, EU) as required;

2) Consider emerging issues of scientific merit and address knowledge gaps (in relation to the ICES science plan);

3) Review status of publications and consider requirements for new publications;

4) Conduct assessment of data as required;

5) Respond to requests for advice from the Data Centre;

6) Development and harmonisation of methodologies for marine monitoring and surveillance;

7) Address issues in relation to novel and emerging contaminants (e.g. pharmaceuticals, nanoparticles, toxicity of mixtures etc.);

8) Evaluate the results of monitoring and research activities on plastic litter, especially microplastics and associated chemical contaminants in the marine environment.

WGBEC had noted the importance of relating its ToRs to the ICES Science Plan. At its 2011 meeting the group had reported its initial thoughts on this matter. In proposing a multiannual ToR an attempt was made to attribute each ToR to the Science Plan, based on previous activities or perceived and potential activities (see Table 4.3.1. below).

Table 4.3.1. Multi-annual ToRs in relation to ICES Science Plan.

	Proposed multi annual term of reference.	ICES Science Plan, see codes below and Annex 6	Other Purpose / Comment
1	Respond to requests for advice from Regional Seas Conventions		Direct response e.g. OSPAR
2	Consider emerging issues of scientific merit and address knowledge gaps	112, 172, 241, 242	
3	Review status of publications and consider requirements for new publications		For OSPAR, ICES, within the group
4	Conduct assessment of data as required	123, 241, 242, 244	OSPAR
5	Respond to requests for advice from the Data Centre		Requirement from ICES data centre
6	Development and harmonisation of methodologies for marine monitoring and surveillance	241	ICES data centre, OSPAR
7	Address issues in relation to novel and emerging contaminants	123, 172, 242, 241	
8	To evaluate the results of monitoring and research activities on plastic litter	241, 243, 344	

Codes for the Science Plan High Priority Topics, see Annex 6:

11. Climate change processes and predictions of impacts

- 112 Define responses at the individual and population level to changes

12. Biodiversity and the health of marine ecosystems

- 123 Define indicators of ecosystem health: attributes of ecosystems, conditions of change, external pressures

17. Role of top predators (mammals, birds, and large pelagics) in marine ecosystems

- 172 Anthropogenic impact: removal of larger fish and increase top predators

24. Population and community level impacts of contaminants, eutrophication, and habitat changes in the coastal zone

- 241 Understanding the impacts of contaminants at the individual, population and community levels.
- 242 Estimating the cumulative impacts of contaminants, eutrophication, and changes in habitat substrate.
- 243 Synthesize knowledge on the impacts of diverse land-based and marine activities
- 244 Characterize the status of regional coastal zone ecosystems and causal relationships

34. Contributions to socio-economic understanding of ecosystem goods and services, and forecasting of the impact of human activities

- 344 Forecast the impact of human activities and evaluate mitigation options

Recommendation

In accordance with the proposed arrangements for expert groups WGBEC should move to multi-annual terms of reference from 2013.

Actions

WGBEC to produce a 3-year terms of reference. WGBEC to forward to ICES a Category 2 resolution to implement the "multi-annual approach" beginning in 2013 and to seek approval of the proposed ToRs.

WGBEC will elect a new Chairperson at its 2013 meeting.

5 Integrated monitoring and assessment (ToR c)

- Respond to SGIMC 2011 and review documentation as required;
- Application of OSPAR integrated strategy to data sets by working group members;
- Review integrated assessments from ICON and BEAST;
- Update assessment criteria in light of new data.

5.1 Respond to SGIMC 2011 and review documentation as required (agenda item 5a)

5.1.1 Outstanding tasks from SGIMC report

In the SGIMC final report, Annex 27 (Update of SGIMC Workplan from 2010/2011) it was stated that one outstanding task would be transferred to WGBEC; this was to complete the chapters on *in vitro* assays YES / YAS and ER CALUX. Both of these documents are being progressed intersessionally by WGBEC members (Kevin Thomas, Norway and Dick Vethaak, Netherlands).

The report of the SGIMC, collating the extensive advice prepared by SGIMC for OSPAR on an integrated approach to marine environmental monitoring had been approved for publication by ICES in the ICES Cooperative Report Series. The final manuscript had been submitted to ICES and was complete with the exception of some late modifications which WGBEC was asked to address:

a) A paper was presented by Spain at the recent HASEC meeting relating to changes to the assessment criteria for Scope For Growth. These were reviewed by WGBEC and it was agreed that there was an error in the original text and appropriate changes were made (see agenda item 5.4 below).

b) There were some queries concerning the chapter on fish diseases, these were being addressed by Thomas Lang in liaison with Dick Vethaak, and a revised chapter produced by WGPDMO during the meeting addresses these issues.

c) There were some minor editorial changes to the chapter on sediment seawater elutriate and pore water bioassays with early developmental changes of marine invertebrates. These were agreed and in addition evidence was presented to change the background responses for mussel embryos. New background response values should be as follows:

Text to be corrected:

Table 1. Background response for mussel embryo bioassays (mortality); data from IEO-Vigo.

AVERAGE	90-PERCENTILE	MEDIAN	10-PERCENTILE	N
14.7	29.8	13.4	3.2	65

It was noted that changes to assessment criteria in respective chapters would also require corresponding changes to be made to the summary chapter on assessment criteria.

Dick Vethaak, as joint editor of the SGIMC CRR agreed to contact ICES on these matters as the date for final editorial changes was imminent. This action was undertaken at the meeting.

5.1.2 WGBEC viewpoint on the comments made in the HASEC 2012 report in relation to biological effects monitoring and the SGIMC approach

Several members of WGBEC were aware that the OSPAR Hazardous Substances and Eutrophication Committee (HASEC) had met in Oslo on 27 February – 2 March, 2012, and discussed the approach on integrated chemical and biological effects monitoring proposed in the SGIMC final report.

Contracting Parties at HASEC 2012 gave synoptic information of how biological monitoring techniques are currently used in their national monitoring, of what development projects were on-going and what their perspective was on future use of biological effects monitoring. WGBEC reviewed the table and it was clear that in several instances there was disparity between the content in the table and what actually was conducted by the practitioners. The information in the table was duly corrected (see Table 5.1.2.a) to reflect the current use of biological effect monitoring but for some maritime areas this was not possible as representatives were not present at the meeting.

Table 5.1.2.a. Revised table from HASEC 2012 report concerning the Status of biological effects methods (from the ICES/OSPAR integrated approach) used by OSPAR Contracting Parties.

Country	Current techniques used in national programmes	Expertise available	Expertise based at	Comments
Belgium	Not completed			
Denmark	Imposex and intersex in snails LMS in mussels PAH-metabolites in bile EROD Reproductive success in fish Benthic community analysis	Intersex in fish Stress on Stress Whole sediment bioassays	Aarhus University Danish EPA	Integration with contaminants within the marine part of the nationwide monitoring programme NOVANA
France	Fish disease (externally visible) flounder/dab AChE flounder/dab/mussel	AChE fish/mussel EROD fish LMS mussel Comet fish/mussel DNA adducts fish/mussel	Ifremer LPTC University of Bordeaux ADNTox LEMA	Integrated approach being trialled in a Seine estuary with dab, flounder and mussels

Country	Current techniques used in national programmes	Expertise available	Expertise based at	Comments
	EROD flounder/dab Comet flounder/dab DNA adducts flounder/dab PAH metabolites flounder/dab VTG in plasma (flounder) Imposex *Nucella Lapilus* Oyster embryotoxicity	PAH metabolites fish Oyster embryo bioassays VTG in flounder	University of Le Havre	Imposex *Nucella Lapilus* along the Atlantic coasts
Germany	Not completed			
Ireland	Imposex Benthic community analysis	Fish: AChe ,liver histopathology,macroscopic Liver neoplasms, intersex, external fish disease Mussels: LMS, MN, histopathology/gametogenesis, SFG, SOS, MT, AChe, COMET Sediment: Corophium/Arenicola whole sediment tests	Trinity College Dublin, Marine Institute, Galway Shannon Aquatic Toxicity Laboratory, Dublin Institute of Technology.	Techniques developed as part of Marine Institute/EPA funded project and not as core techniques in national monitoring programme
The Netherlands	Imposex/intersex in snails dogwhelks/ periwinkles External fish diseases and liver nodules flounder/dab PAH metabolites flounder/dab	AChE mussels Scope for Growth Bivalve embryo bioassays (water) Copepod water bioassays Sea urchin embryo bioassays Whole sediment bioassays Sediment pore water bioassays Sediment elutriate bioassays In vitro bioassays (DR-LUC/ER-LUC) Microtox	Institute for Environmental Studies VU IMARES Rijkswaterstaat Centre for Water Management Grondmij-Aquasense	Techniques applied as part of the national JAMP/CEMP. Partial integrated assessment applied for certain monitoring data Techniques applied in one-off surveys/research projects and ICON Workshop
Norway	CEMP: imposex in dog whelks, EROD, CYP1A, bile metabolites, ALA-D in cod. Water column monitoring: blue mussel LMS, histochemistry, histology, micronuclei, condition index. Condition monitoring: bile	Oyster/Sea urchin/fish larvae bioassays, MT, AChE, stress on stress, whole sediment bioassays	NIVA IRIS IMR	Applied integration of biological responses in certain monitoring programmes.

Country	Current techniques used in national programmes	Expertise available	Expertise based at	Comments
	metabolites, CYP1A, oxidative stress, vitamin E, Vtg, DNA adducts, and fatty acid composition in fish.			
Portugal	AChE, GST, LPO in mussels PAHs metabolites, micronucleous in mussels (to be started next year) AChE, energy parameters, condition index, oxidative stress & bile PAH metabolites (to be started next year) Bioassays with the common goby, microalgae and the common prawn Metallothioneins, oxidative stress parameters in clams	EROD, AChE, PAH, GST, EROD, PAH metabolites, energetic enzymes, oxidative stress parameters, LPO, condition indexes, in fish and invertebrates GST in Fucus Oxidative stress and LPO in microalgae Bioassays with fish, invertebrates & microalgae (both native and standard species) Imposex in gasteropods LMS in mussels and clams	CIIMAR & ICBAS, University of Porto CEMA, University of Algarve	Monitoring done in the scope of research projects going on. Approaches integrating biological effects and chemical concentrations in tissues and sediments in some cases
Spain	EROD Imposex in gastropods Embryo-larval bioassays with sea-urchins Amphipods survival bioassays Scope for Growth in mussels Metallothioneins in mussels Micronucleus in mussels AChE in mussels LMS in mussels Stress on Stress in mussels	EROD Imposex in gastropods Embryo-larval bioassays with sea-urchins Amphipods survival bioassays Scope for Growth in mussels Metallothioneins in mussels Micronucleus in mussels and fish AChE in mussels and fish LMS in mussels Stress on Stress in mussels	Instituto Español de Oceanografía (IEO) Universidade de Vigo Universidade de A Coruña	Integrated approach applied within the framework of the MSFD implementation.
UK	Fish disease (externally visible) Liver histopathology EROD PAH bile metabolites Imposex in dogwhelks and periwinkles Benthic community analysis	Intersex Micronuclei fish/mussels Comet fish DNA adducts AChE mussels Scope for Growth Bivalve embryo bioassays (water) Copepod water bioassays Sea urchin embryo bioassays	Marine Scotland Science Cefas	Integrated approach being trialled in a few areas offshore (dab) and inshore (flounder and mussels)

Country	Current techniques used in national programmes	Expertise available	Expertise based at	Comments
	Comet (mussels) Mussel LMS Stress on stress Mussel histopathology VTG in plasma	Whole sediment bioassays Sediment pore water bioassays Sediment elutriate bioassays		

WGBEC were also aware that reservations were expressed at the HASEC meeting concerning the implementation of the SGIMC integrated approach, firstly on the number of techniques used and whether these could be implemented by all contracting parties, and secondly, on needing to see the integrated approach trialled such as with the ICON data or similar case studies.

WGBEC discussed the option of reducing the core set of techniques in the SGIMC approach, and agreed that this was not advisable on the grounds that:

- The SGIMC core set was based on sound science and best available methodology which was well documented.
- The core set was the minimum required to undertake an integrated approach, reducing the core set to one or two methods would be impractical and reduce the value of the assessment.
- The core set currently includes techniques that are applied at different levels of biological organisation.
- The core set includes techniques that cover a wide range of contaminant exposure, i.e. from metals, genetic damage, endocrine disruption, PAHs, neurotoxicity, organotin, and general responses to contaminants.
- Comparisons have been made between the fish core set of techniques in the HELCOM CORSET Baltic programme and the SGIMC approach. The schemes are almost identical as shown in Table 5.1.2.b.

Table 5.1.2.b. Core set of biological effect techniques used in the HELCOM CORESET scheme and the SGIMC scheme.

Technique	In HELCOM CORSET scheme	In OSPAR / ICES SGIMC scheme
PAH bile metabolite	Yes	Yes
EROD	Yes	Yes
Micronuclei	Yes	Yes
COMET	No	Yes
AChE	Yes	Yes
LMS	Yes	Optional
Intersex in fish	Yes	Yes
Fish Disease Index	Yes	Yes
Reproductive success in fish	Yes	Optional
VTG	No	Yes

In summary, WGBEC were of the opinion that the core set of techniques recommended in the SGIMC approach remained the best option for taking forward the integration and assessment of chemical contaminants with biological effects and in marine monitoring programmes. Furthermore, the techniques cover a wide range of contaminant effects, both from well known and emerging chemical compounds.

5.2 Application of OSPAR integrated strategy to data sets by working group members (agenda item 5b)

Since the development of the integrated contaminant and biological effect monitoring scheme by SGIMC (SGIMC, 2011), several countries have attempted to apply national monitoring data to the assessment framework with varying degrees of success. The process of trial provides valuable lessons learned as well as a demonstration of the potential utility of the integrated approach. Therefore WGBEC reviewed assessment work by WG members and collated the outputs below. Six presentations were reviewed by the group (Jakob Strand, Denmark; Michelle Giltrap, Ireland; Craig Robinson, UK; Juan Bellas, Spain; Lucia Guilhermino, Portugal; Conception Martinez-Gomez, Spain). Three of these presentations are summarised below.

5.2.1 Application of integrated strategy to data sets by working group members: Integration of biological effects data for eelpout (*Zoarces viviparus*) from Danish coastal waters

Jakob Strand (Aarhus University, Denmark) presented results of the shared activities between the two BONUS+ projects BalcoFish and BEAST on biological effects in eelpout (*Zoarces viviparus*). In addition to data generated within the projects, some already existing data on biological effects in eelpout has been identified by BALCOFISH partners including national and regional monitoring and research data in Sweden, Denmark and Germany. Many of these data have also been incorporated in the shared project database called BonusHaz, hosted by Aarhus University. Some of these eelpout data have also been submitted by BalcoFish to the ICES database in 2012.

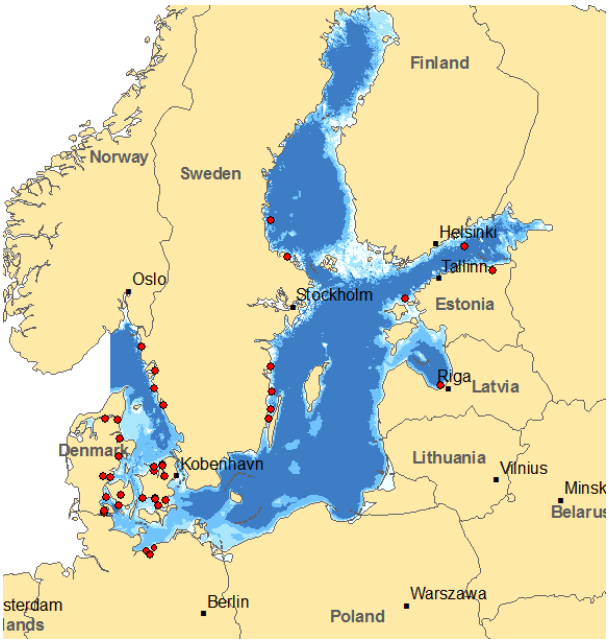

Figure 5.2.1.a. Locations on sampling stations with eelpout data in the BonusHaz database.

At the moment more than 30 000 eelpout results from the period from 2003 to 2010 have been submitted to the BonusHaz database from Swedish west coast (4 stations), Swedish east coast (4 stations), Botnian Bight (2 stations), Danish Belt Sea (20 stations), German Baltic Sea (3 stations), Gulf of Riga (2 stations); for locations see Figure 5.2.1.a. The data of biological effects measurements include both data on several contaminant-specific biomarkers, general effects biomarkers, endocrine disruption and reproductive success, i.e. both subcellular, tissue and whole organism responses. The data on contaminant levels include the following groups of important hazardous substances: dioxins, furans, coplanar PCBs, PBDEs, organotins, trace metals, phenols and PFCs.

The various results show that spatial differences occur for most biomarker responses and support the use of eelpout as an important indicator species for the assessment of pollution effects in the Baltic Sea. In addition to biological effects on subcellular responses also up to more than 50% of male fish express intersex in the gonad tissue in the western Baltic Sea and the Belt Sea. Also elevated levels of abnormal fry development (part of reproductive success) are more prevalent in some coastal areas indicating that also whole organism responses occur. Integration in line with the proposed ICES SGIMC scheme for integrated monitoring is therefore possible with these eelpout data.

For the integration of biological effects data for eelpout from Danish coastal waters, five sites among the Danish eelpout monitoring stations, were selected for more detailed studies with measurements of the biological effects methods: PAH-metabolites, EROD, lysosomal membrane stability, micronuclei, AChE, intersex and reproductive success with focus on different types of abnormal fry development.

Results show PAH-metabolites and intersex are above the proposed BAC values at most of the sites. LMS, AChE and reproductive success also exceed the EAC in some areas.

For further integration of the data, an "Integrated Biomarker Assessment Tool (IBAT)" has been developed, which allows comparisons of input data for relevant biological effect parameters with the BAC- and EAC-values. IBAT can calculate an overall Integrated Biomarker Assessment Score (IBAS), which includes the principle of using factors, i.e. weighted score values, depending on the biological response level of the respective biological effects, i.e. subcellular, tissue or whole organism responses. IBAS summarizes all weighted scores by the formula IBAS = (Σ X)/ (sqrt n), which is similar to the formula in the CHASE tool applied for integrated assessment of contaminants in the Baltic Sea (HELCOM 2011). IBAS thereby provides a score, which can be used for a traffic light assessment with a scale as e.g.: <1 (low impact), 1 - <5 (moderate impact), >5 (high impact).

If IBAT is applied on data from a single species, e.g. eelpout, it can be used for providing a kind of health index. However, potentially IBAT can be applied on data from several species, and thus provide an integrative measure for the all observed biological effects in a particular area.

Tested on a data set for eelpout at five Danish stations in the Belt Sea, IBAT shows that both areas with low, moderate and high impact in eelpout populations occur, and that it follows the expected contaminant levels in the areas, although specific compounds cannot be pointed out at the moment.

5.2.2 Irish approach to integrated monitoring (Michelle Giltrap)

Data from the Seachange (Marine Institute/EPA) funded project "Biological effects and chemical measurements in Irish marine waters" was used to create an initial integrated assessment pilot study using the SGIMC scheme for integrated monitoring. Data from this project was gathered from a two tiered approach whereby a general stress screen was used for eight sites around Ireland with a small number of biomarkers and bioassays and from these sites a more comprehensive multi-biomarker and chemical assessment was performed on only four sites. The data used for the assessment is listed in Table 5.2.2.a. below.

Table 5.2.2.a. Biological effects and chemical measurements data available from the Irish Seachange project.

	Mussel data	Fish data	Gastropod data	Water data	Sediment data
Sites 1-8 (TIER 1)	SFG, SOS, CF				WST: Corophium, Arenicola
	contaminants: metals, PCBs,PAHs,BFRs, EDCs				Porewater/elutriate tests SC, VF and TB
					metals
Sites 1-4 (TIER 2)	Histopathology, AChe, MT, ALP, LMS, COMET	Bile, EROD, AChe,VTG	Imposex	EDCs, PAHs with Passive sampling	WST: Corophium, Arenicola
		FDI, Ext disease, intersex			Porewater/elutriate tests SC, VF and TB
		metals, PCBs,BFRs, EDCs			Benthic indices
					metals
					YES assay

This was a multistep process proposed following on from assessment of contaminant data for sediment, fish and shellfish in OSPAR. Data was integrated over a number of levels including matrix, site and region with the Irish data that was available. Only contaminants/biomarkers with AC available were used for this exercise. For contaminant data, absolute values were used and normalised as per appropriate criteria and upper/lower confidence limits of ± 15% were added to allow for analytical error (with exception of dry weight data). In a situation where there was only a BAC available for a contaminant, for trial purposes, a factor of 2 was applied to the BAC to generate an upper value "EAC" for assessment. Also where EAC was not available but EC was available, EC was used and for imposex the EcoQo used. A colour application was then applied to each of the individual assessment of determinands (contaminants or effects) against defined assessment criteria i.e. application of colouring system< BAC (blue), between BAC and EAC (green) or >EAC (red). For biological effects, mean values were used to compare with AC. Following this the proportion of each category was determined across matrices for each site and with mussel data only across regions. For this exercise, Ireland was demonstrated as one region (8 sites data). Data from four sites for passive sampling, sediment and fish contaminant data, fish disease and benthic data is still in progress has yet to be included in assessment. Passive sampling shall measure "dissolved contaminants" only and therefore criteria shall be carefully selected for this. For future exercises, other sources of AC e.g. EQS, ER_L/ER_M shall be investigated and where there is no assessment criteria available, Irish AC may be developed with Irish data e.g. MT (gluthathione) and ALP in *Mytilus edulis*, sediment porewater and elutriate toxicity tests with *Tisbe battagliai*, Skeletonema costatum and *Vibrio fischeri*. Assessment criteria for other contaminants such as EDCs in fish and mussels may also be developed with Irish data. A review of the applicability of all the BACs/EACs and other standards will be completed prior to finalization. A number of complications were observed during the process including differences in numbers of assays between sites, spatial range and number of sampling stations in one location, confidence in data and missing values are not represented well. For purposes of determination of GES (MSFD) it was proposed not to adopt an approach whereby EAC failure results in failure of GES in a region. A threshold setting of 95%

of determinands <EAC shall indicate that good environmental status is achieved. This approach will be critically reviewed over time.

5.2.3 UK Integrated Contaminant and Biological Effects Monitoring programmes

Over the last 3–4 years, Cefas (England & Wales) and Marine Scotland Science have developed parallel demonstration programmes for undertaking integrated monitoring and assessment of contaminants and their effects. These programmes have been developed alongside the development of advice by the ICES/OSPAR Study Group on Integrated Monitoring of Contaminants (SGIMC) and incorporate both the mussels and fish schemes of SGIMC, alongside existing imposex and sediment contaminant monitoring. The two main aims of the UK programmes are (1) capacity building/ knowledge transfer within the partner institutes and (2) to investigate the utility and added value of the integrated approach compared to previous practise. The mussels programme in Scotland has applied the SGIMC scheme at 4 shoreline sites in each of three years, with data available for two of those. Biota contaminant data were assessed using JAMP guidelines (comparing mean±95% CI with OSPAR BACs/EACs), passive sampling data were compared against Scottish background concentrations and effects data were assessed by comparing median values with the SGIMC assessment criteria. There were some missing data, but these were not allowed for in the assessment process. Figure 5.2.3.a. shows the assessment of endpoints from the four sites in the Firth of Clyde and Figure 5.2.3.b. shows the overall assessments for the Firth of Forth and the Firth of Clyde, albeit only with respect to mussels. The SGIMC scheme proposes that GES is achieved when <5% of endpoints exceed their EAC at the regional level; this was the case for both the Firth of Clyde (5% >EAC) and the Firth of Forth (4% > EAC).

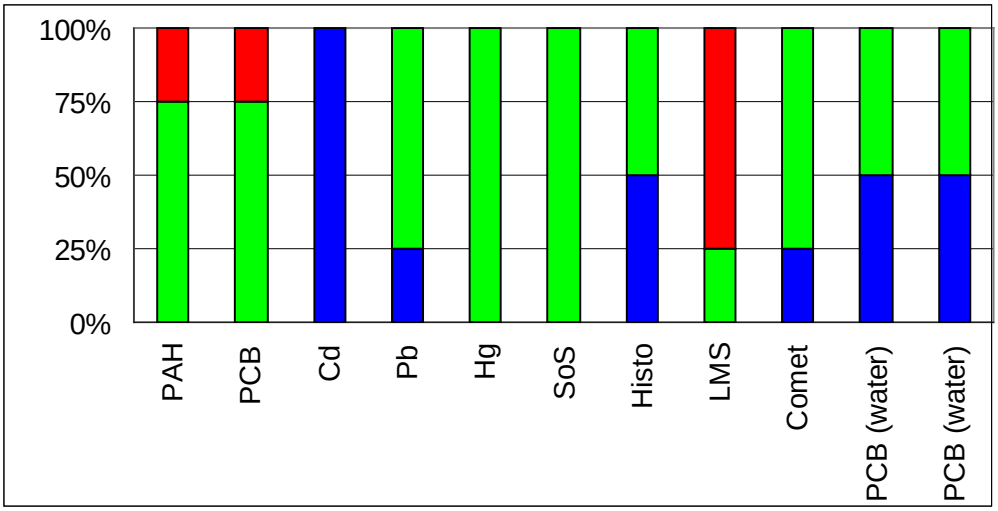

Figure 5.2.3.a. Regional assessment for the Firth of Clyde, by technique. Blue = less than Background Assessment Criteria (BAC); Green = above BAC but less than Environmental Assessment Criteria (EAC); Red = above EAC.

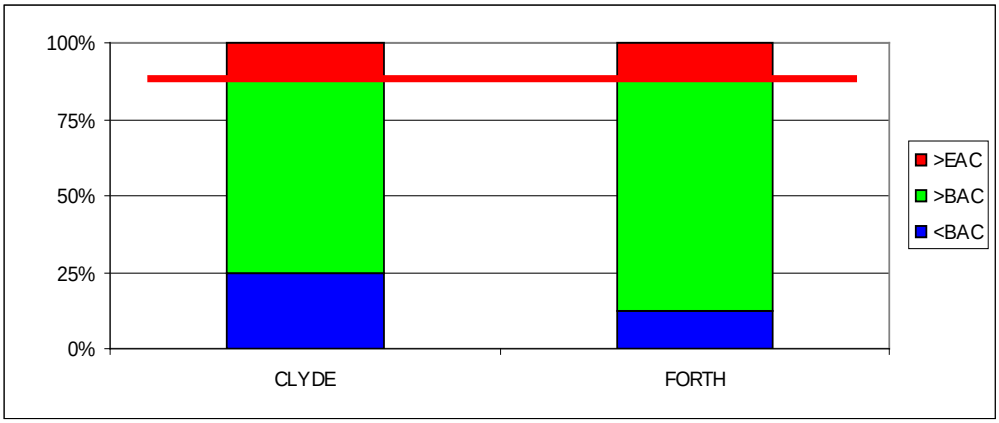

Figure 5.2.3.b. Regional assessment for mussels in two Scottish regions. The red line is the 95% value. Both regions satisfy the GES criteria of ≤5% exceeding EACs.

Scotland has also been running an extensive fish programme, involving flounder from 5 inshore sites and dab from 2 inshore and 5 offshore sites in each of three years. The dataset is not yet complete and has therefore not yet been integrated.

In England and Wales, the approach has been applied to monitoring data from the Humber-Wash region and information on contaminants and biomarkers from 6 sites and sediment contaminants from 4 sites integrated into a regional assessment. The contaminant concentrations were assessed according to the OSPAR JAMP procedure; the effects were assessed by comparing the 90th percentile of the log-normal data against the Assessment Criteria. Using this approach Figure 5.2.3.c. shows that a number of contaminants were assessed as above EAC, but the effects in fish were <EAC. The integration of all endpoints resulted in <5% of results for this region exceeding EACs and thus meeting the SGIMC GES target (Figure 5.2.3.d.).

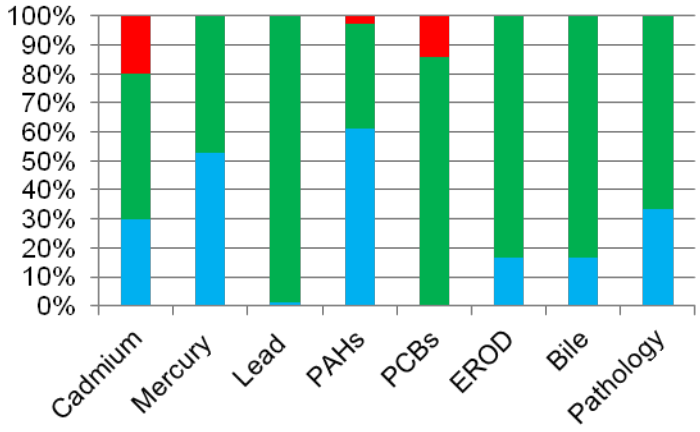

Figure 5.2.3.c. Integration of contaminants in biota, contaminants in sediment and effects in fish from the Humber-Wash region. Blue = less than Background Assessment Criteria (BAC); Green = above BAC but less than Environmental Assessment Criteria (EAC); Red = above EAC.

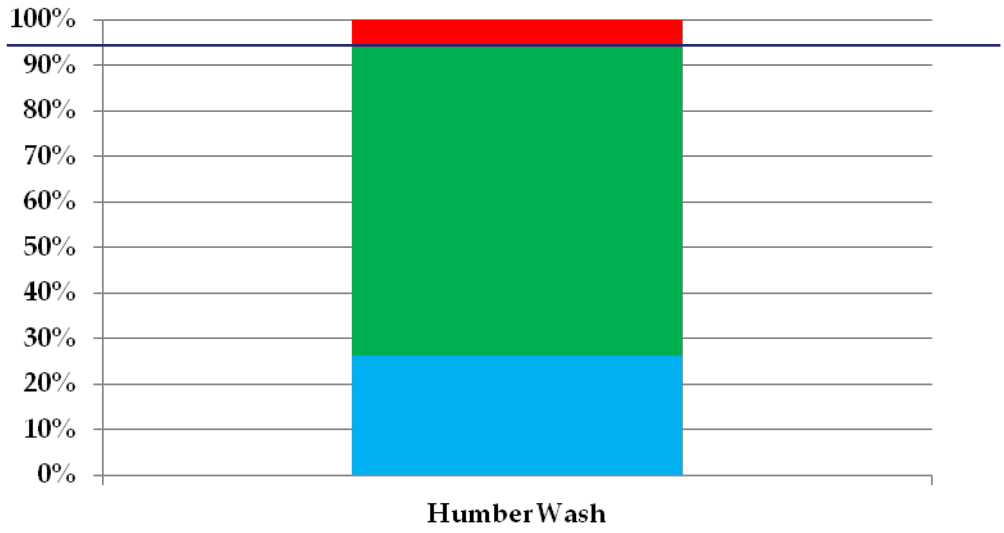

Figure 5.2.3.d. Regional integrated assessment (fish and sediments) for the Humber-Wash region. The blue line indicates the 95% value. The region satisfies the GES criteria of ≤5% exceeding EACs.

The UK is continuing with assessing its regions using the SGIMC approach. Although data are not available for all of the biological effects techniques that the SGIMC approach recommends, the scheme has been successfully trialled and the approach (or aspects of it) is likely to be used in the future to investigate the health status of wildlife in areas with known current and historic contaminant inputs.

5.3 Review of integrated assessments from ICON and BEAST

5.3.1 Integrated assessment and demonstration with ICON data

Some members of WGBEC presented (see 5.2) how they conducted the integrated approach from field studies by applying the integrated assessment framework for contaminants and biological effects developed by the SGIMC (Technical Annex 25, ICES, 2010). A number of differences related to the interpretation of the methodology for performing the integration process were identified and discussed.

The absence of detailed information in the technical annex concerning which summary statistic to use for the comparison of individual determinands (contaminants or effects) in specific matrices at individual sites against the defined assessment criteria (BACs and EACs) lead to differences in conducting the first step of the integration approach. Different approaches in the treatment of data included using the mean, median, log transformed mean value, upper level of the 95% confidence interval of the mean and percentage of individuals above the BAC for comparison to AC.

For the next level of integration (such as matrix or category), results are expressed by using tricoloured bars showing the proportions of determinands that exceed the BACs and EACs. C. Martínez-Gómez (Spain) demonstrated the use of grey to indicate the proportion of determinands for which data were missing, an approach which illustrates uncertainty in the assessment (Figure 5.3.1.a).

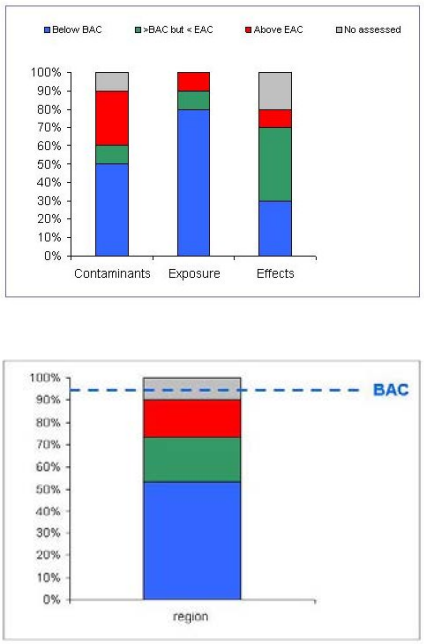

Figure 5.3.1.a. Illustration of the added benefit of including the proportion of missing data in the assessment process.

However, AC (particularly EACs) are not available for all of the determinands recommended in the Guidelines developed by SGIMC (Annex 21, ICES, 2011). As the integration of data is performed on multiple levels of aggregation, the contribution of each category plays an important role in the final result. This was evident when data from different categories (contaminant, biomarkers of exposure and biomarkers of effects) were integrated.

Given the current importance of demonstrating the value of integrated monitoring, dedicated time was allotted at the meeting to progressing the assessment of the monitoring data collected as part of the ICON project. This project involved a comprehensive sampling programme at sites around the North Sea in 2008 and the majority of data are now available for assessment (Table 5.3.1.a). The selected fish species (dab, flounder and haddock) were not found in all sites. At some sites, e.g. the Seine estuary and the Baltic, both dab and flounder were collected, and at some other sites both dab and haddock were collected. Samples were distributed to more than 20 different laboratories throughout Europe for analyses. Sampling locations, country and matrices sampled are shown in Table 5.3.1.a. below.

Table 5.3.1.a. Locations and matrices sampled.

Location	Country	Matrices sampled
Spanish Med	Spain	mussels, sediment
Wadden See	The Netherlands	flounder, mussels, sediment
Southampton	England	mussels, sediment
Alde	England	flounder, sediment
Iceland (inshore)	Iceland	flounder, mussels
Seine Bay	France	dab, flounder, mussel
German Bight	Germany	dab, sediment
Egersund bank	Norway	dab, haddock, sediment

Dogger Bank	international	dab, sediment
off Firth of Forth	Scotland	dab, haddock, sediment
Ekofisk	Norway	dab, haddock, sediment
Iceland (offshore)	Iceland	dab, haddock, sediment
Baltic	Germany	dab, flounder, sediment
Firth of Forth (gradient)	Scotland	flounder, mussel

General issues with ICON data

For the purposes this exercise (and in the time allocated), data for chemistry and biological effects were integrated on a site basis for contaminants (sediment, fish and mussels) and biological effects in mussels and fish (dab and flounder). Figure 5.3.1.b. shows the assessment of the sediment chemistry data. The assessments for the biomarkers of exposure and effect and the overall integration remain to be completed.

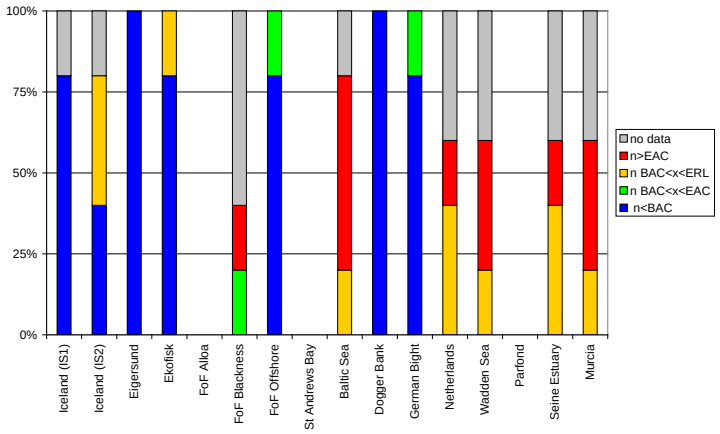

Figure 5.3.1.b. Assessment of contaminants (PAH, PCB, Cd, Hg, Pb) in sediments collected during the ICON project.

Contaminants with AC available (as used in OSPAR QSR 2010) were used for this exercise. For contaminant data, absolute dry weight values were used and normalised as per appropriate criteria. This proved problematic as contaminants were required to be normalised differently for differing Assessment Criteria. For the ICON dataset there were a limited number of observations for contaminant data at each sampling site and thus the recommended OSPAR JAMP approach (use of upper/lower–bound confidence intervals) could not be followed.

Mussel data had the most substantial dataset for one site (Iceland) and showed good relationship between a background contaminant assessment and low biological effects at this site. For the rest of the mussel data, there were incomplete datasets. Missing data was also an issue where digestive glands were analysed instead of whole tissue which made AC comparison impossible. It may be possible to use data from existing monitoring programmes in place of these data. It was observed in the mussel dataset one case where there was elevated lead but a low stress response and the opposite was observed in a second case whereby a stress effect was observed even when contaminant concentrations were <EACs. That was found as a clear example of how the inclusion of biological effects data to the system adds considerable value to the interpretation of the assessments. ICON data presented a comparison of caged (Murcia) and wild mussels in other sites.

Fish biological effect data proved to be the most incomplete dataset with a lot of missing data. Lysosomal membrane stability data was provided for two different methodologies with the same AC and therefore this needs to be checked. Also more data were missing for flounder for DNA adducts, COMET assay, Vtg and intersex prevalence. Different numbers of individuals also presented a problem with differences in datasets. Information on the Fish Disease Index was not available to WGBEC, although it is believed that this Index has been calculated elsewhere for these data.

Conclusions

From this demonstration it is evident that a complete integration of all parameters including all missing datasets is essential for future evaluation of the application of such an assessment.

It is clear that a minimum number of assays are required to perform such an integrated assessment. This approach tested the median comparison whereby if the median was determined to be above EAC but the spread of data was above and below, this value was given a red colouring and if the median was below the EAC, a green colour was applied.

Spreadsheets detailing the assessments conducted at WGBEC were generated and it is recommended that ICES establish a SharePoint site to allow update of the ICON dataset and for the assessment to be progressed. Furthermore, a workshop should be organised by the ICON Steering Group to complete the assessment in 2012.

5.3.2 Integrated assessment and demonstration with Seine estuary ICON data

Thierry Burgeot presented data and how this was assessed in relation to the Seine estuary. The general objective of the ICON project is to demonstrate the applicability of biomarkers and bioassays within the CEMP and in the descriptor 8 of the GES of the MSFD. The report of the SGIMC 2011 establishes a very efficient integrated methodological with assessment criteria and it allow us to conduct an exercise of applicability with the French ICON data obtained in the Seine estuary in 2008/2009.

This exercise is limited because of a low number of parameters but it is very illustrative because of a combination of the data from two fish, mussel and sediment. France deployed a sampling programme on the OSPAR pilot site of Seine estuary. Flounder, dab and mussels were sampled in 2008 and 2009 at two stations, Parfond and the Seine estuary. Sediment was sampled in 2008 at the Seine estuary site.

Five steps of integration recommended by the SGIMC 2011 report were conducted from the means obtained with biomarkers and the bioassays analyzed by the French laboratories and MN in Vilnius University. The results show a relevant response of the mussel between the less contaminated station in Le Moulard and the more contaminated station in the Seine estuary. The results obtained with fish are more diversified with the light color scale because they integrate two species of fishes (flounder and dab), two biomarkers of exposure (EROD, AChE) and five biomarkers of effects (DNA adduct, Comet, PAH metabolites, Vtg in flounder, MN). The results obtained with mussels are more contrasted between the two sampled stations but the light scale color of exposure was due to AChE only.

The number of biomarkers used for the assessment is critical to the overall result. The weight of every biomarker of exposure and effects as well as the parameters of integration (e.g. mean or the median) should also be specified to complete the methodology of the SGIMC 2011. Only a detailed methodological protocol will allow consistent application between countries.

5.3.3 Integrated assessment under the BONUS + BEAST project

Ulrike Kammann (Germany) presented an overview of the BONUS+ project BEAST project where monitoring data have been applied to assessment criteria developed for the CORESET methods. Some new AC values were proposed by this project and are addressed under 5.4 below.

5.4 Update assessment criteria in light of new data (5d)

5.4.1. In light of the requirement for national and international assessments of monitoring data against agreed assessment criteria. WGBEC considered that it would be best practice to maintain an up-to-date record of the current recommended AC values (BAC and EAC) arising from the SGIMC process and updated as new data arise. Changes are foreseen in the calculation of BAC values based on new monitoring data from reference sites for example. Data from new sentinel monitoring species may also arise, new toxicity data may enable the calculation of new EAC values or new techniques may be added.

The current list of AC for biological effects methods is therefore replicated here (Table 5.4.1.a) and details of changes to values proposed added beneath. Where an AC value has been updated or newly included it is given in bold in the table. A new column has been added to the table to describe the summary statistic that should be used for assessment against the AC value. This will be populated at further meetings, informed by demonstration assessments such as ICON (agenda 5c).

Table 5.4.1.a. CURRENT Assessment criteria for biological effects measurements. Values are given for both background assessment levels (BAC) and environmental assessment criteria (EAC), as available. Values in bold have been updated by WGBEC 2012.

Biological Effect	Applicable to:	BAC	EAC	Summary statistic for assessment
VTG in plasma; µg/ml	Cod	0.23		
	Flounder	0.13		
Reproductive success in fish Mean prevalence (%) of:	Eelpout, *Zoarces viviparus*			
	Malformed fry	1	2	
	Late dead fry	2	4	
	Early dead fry	**2.5**	**5**	
	Total abnormal fry	**5**	**10**	
EROD; pmol/mg protein pmol/min/ mg protein S9 * pmol/min/ mg microsomal protein	Dab (F)	178		
	Dab (M)	147		
	Dab (M/F)	680*		
	Flounder (M)	24		
	Plaice (M)	9.5		
	Cod (M/F)	145*		
	Plaice (M/F)	255*		
	Four spotted megrim (M/F)	13*		
	Dragonet (M/F)	202*		
	Red mullet (M)	208		
	Eelpout (F)	**10**		

PAHs Bile metabolites; (1) ng/ml; HPLC-F (2) pyrene-type µg/ml; synchronous scan fluorescence 341/383 nm (3) ng/g GC/MS * 1-OH pyrene ** 1-OH phenanthrene	Dab	16 (1) * 3.7 (1) ** 0.15 (2)	 22(2)	
	Cod	21 (1) * 2.7 (1) ** 1.1 (2)	483 (3) * 528 (3) ** 35 (2)	
	Flounder	16 (1) * 3.7 (1) ** 1.3 (2)	 29(2)	
	Haddock	13 (1) * 0.8 (1) ** 1.9 (2)	 35(2)	
	Eelpout	**92 (1) *** **7.9 (1) **** 	 	
	Herring	**151 (1) *** **4.5 (1) **** 	 	
DR-Luc; ng TEQ/kg dry wt, silica clean up	Sediment (extracts)	10	**40**	
DNA adducts; nm adducts mol DNA	Dab	1	**4,0**	
	Flounder	1	**4,0**	
	Long Rough Dab		**4,0**	
	Halibut		**5,8**	
	Herring and sprat		**0,39**	
	Cod	1.6	**6,7**	
	Haddock	3.0	**6,7**	
Bioassays; % mortality	Sediment, Corophium	20	60	
	Sediment, Arenicola	10	50	
	Water, copepod	10	50	
Bioassays; % abnormality	**Water, oyster embryo**	**20**	**50**	
	Water, mussel embryo	30	50	
	Water, sea urchin embryo	10	50	
Bioassay; % growth	Water, sea urchin embryo	30	50	
Lysosomal stability; minutes	Cytochemical; liver all species	20	10	
	Neutral Red Retention: all species	120	50	
Micronuclei; $^{0}/_{00}$ (frequency of	*Mytilus edulis*	2.5 [1] 2.5 [2]	 	

micronucleated cells) [1] Gill cells [2] Haemocytes [3] Erythrocytes	*Mytilus galloprovincialis*	3.9 [2]		
	Mytilus trossulus	4.5 [2]		
	Flounder	0.3 [3]		
	Dab	0.5 [3]		
	Eelpout	0.4 [3]		
	Cod	0.4 [3]		
	Red mullet	0.3 [3]		
Comet Assay; % DNA Tail	*Mytilus edulis*	10		
	Dab	5		
	Cod	5		
Stress on Stress; days	*Mytilus* sp.	10	5	
AChE activity; nmol.min^{-1} mg prot^{-1} [1] gills [2] muscle tissue [3] brain tissue * French Atlantic waters ** Portuguese Atlantic waters [+] French Mediterranean Waters [++] Spanish Mediterranean Waters [+++] Baltic sea	*Mytilus edulis*	30 [1*]	21 [1*]	
		26 [1**]	19 [1**]	
	Mytilus galloprovincialis	29[1+]	20[1+]	
		15 [1++]	10 [1++]	
	Flounder	235 [2*]	165 [2*]	
	Dab	150 [2*]	105 [2*]	
	Red mullet	155 [2+] 75 [3++]	109 [2+] 52 [3++]	
	Eelpout	**124** [2+++]	**87** [2+++]	
Externally visible diseases*** Ep,Ly,Ul Ep,Ly,Ul Ac,Ep,Fi,Hp,Le,Ly,St,Ul,Xc Ac,Ep,Fi,Hp,Le,Ly,St,Ul,Xc Ac,Ep,Hp,Le,Ly,St,Ul,Xc Ac,Ep,Hp,Le,Ly,St,Ul,Xc Italics: ungraded, bold: graded NA: Not applied	Dab	Fish Disease Index (FDI): F: *1.32*, 0.216 M: *0.96*, 0.232 F: *1.03*, 0.349 M: *1.17*, 0.342 F: *1.09*, 0.414 M*: 1.18*, 0.398 M: males F: females	Fish Disease Index (FDI): F: *NA*, 54.0 M: *NA*, 47.7 F: *50.6*, 19.2 M: *38.8*, 16.1 F: *48.3*, 21.9 M: *35.2*, 16.5	

Liver histopathology-non specific	Dab	NA	Statistically significant increase in mean FDI level in the assessment period compared to a prior observation period *or* Statistically significant upward trend in mean FDI level in the assessment period	
Liver histopathology-contaminant-specific	Dab	Mean FDI <2	Mean FDI ≥ 2 A value of FDI = 2 is, e. g., reached if the prevalence of liver tumours is 2 % (e. g., one specimen out of a sample of 50 specimens is affected by a liver tumour). Levels of FDI ≥ 2 can be reached if more fish are affected or if combinations of other toxicopathic lesions occur.	
Macroscopic liver neoplasms	Dab	Mean FDI <2	Mean FDI ≥ 2 A value of FDI = 2 is reached if the prevalence of liver tumours (benign or malignant) is 2 % (e. g., one	

			specimen out of a sample of 50 specimens is affected by a liver tumour). If more fish are affected, the value is FDI > 2.	
Intersex in fish; % prevalence	Dab Flounder Cod Red mullet Eelpout	5		
Scope for growth Joules/hr/g dry wt.	Mussel (*Mytilus* sp.) (provisional, further validation required)	**15**	**5**	
Hepatic metallothionein ìg/g (w.w.) [1] Whole animal [2]Digestive gland [3]Gills * Differential pulse polarography	*Mussel edulis*	0.6 [1*] 2.0 [2*] 0.6 [3*]		
	Mytilus galloprovincialis	2.0 [1*] 3.9[2*] 0.6 [3*]		
Histopathology in mussels	VVbas: Cell type composition of digestive gland epithelium; $\mu m^3/\mu m^3$ *(quantitative)*	0.12	0.18	
	MLR/MET: Digestive tubule epithelial atrophy and thinning; µm/µm *(quantitative)*	0.7	1.6	
	VVLYS & Lysosomal enlargement; $\mu m^3/\mu m^3$ *(quantitative)*	VvLYS 0.0002	V>0.0004	
	S/VLYS: $\mu m^2/\mu m^3$	4		
	Digestive tubule epithelial atrophy and thinning *(semi-quantitative)*	STAGE ≤1	STAGE 4	
	Inflammation *(semi-quantitative)*	STAGE ≤1	STAGE 3	
Imposex/intersex in snails VDSI	***Nucella lapillus***	**<0.3**	**<2**	VDSI

*****: Assessment criteria for the assessment of the Fish Disease Index (FDI) for externally visible diseases in common dab (*Limanda limanda*). Abbreviations used: Ac, *Acanthochondria cornuta;* Ep, Epidermal hyperplasia/papilloma; Fi, Acute/healing fin rot/erosion; Hp, Hyperpigmentation; Le, *Lepeophtheirus sp.*; Ly, Lymphocystis; St, *Stephanostomum baccatum*; Ul, Acute/healing skin ulcerations; Xc, X-cell gill disease.**

Full details of how the original assessment criteria and how they were derived can be found in the SGIMC 2010 and SGIMC 2011 and WKIMON 2009 reports on the ICES website and in the OSPAR Background Documents for individual biological effects methods.

5.4.2. WGBEC 2012 Considered amendments to the following assessment criteria:

5.4.2.1 Lysosomal membrane stability in mussels (cf agenda 7)

There were some concerns that the BAC values of 120 minute retention time were too long. Many countries reported an inability in reference site samples to achieve these long values in spite of a lack of significant contaminant exposure and adherence to protocol guidance. Given that individual mussels and some sites in pristine areas were able to achieve background values, it was decided not to amend the assessment criteria at this meeting. The inclusion of alternative weighted scores for lysosomes as proposed by David Lowe was considered. Ideally future AC would be developed using these measurements (and would be advantageous over RT) but monitoring data will have to be generated first.

Lysosomal membrane stability (NRRT) has also recently been measured in blue mussel (*Mytulis edulis*), northern shrimp (*Pandalus borealis*) and Iceland scallop (*Chlamys islandica*) (Northern shrimp and Iceland scallop are environmental indicator species in northern sea areas, e.g. the Barents Sea) after exposure to dispersed crude oil. EAC values were determined at the same oil concentrations as critical effects were seen. By this procedure the EAC value found were < 60 minutes for all the three species. This is close to the EAC value of 50 already assigned, and the above results confirm that the EAC level chosen is reasonable and that the LMS is rather transparent between species and the EAC value can be generally applicable to all.

5.4.2.2 Scope for Growth

Spain presented to OSPAR HASEC a suggestion that the SFG BAC and EAC values proposed should be substantially changed based on data acquired using a modification of the TIMES methodology to include suspended solids in the feeding mixture (to improve accuracy of measurements to calculate clearance rate). Arising from this discussion, the issue of standardisation of food conditions between methods is important.

WGBEC critically reviewed the suggestion to change the AC values. WGBEC considered that because the new values presented by Spain were arrived at by the use of a non standard, modified method, they could not be accepted as revised AC as proposed directly. The existing criteria were based on an extensive data set and the new method may have some issues concerning use of an algal culture species too small for complete ingestion. However WGBEC thanks Spain for pointing out the apparent error in the values proposed in the SGIMC/WKIMON background document by Widdows *et al*. They differ considerably from evidence in the peer reviewed literature. Following discussion, WGBEC suggests to change the values to values consistent with the peer review literature and close to those presented by Spain at HASEC. The BAC should be amended from 5 to 15. The EAC should be amended from -2 to 5.

5.4.2.3 Imposex and intersex in snails

The OSPAR assessment criteria were transposed to BAC and EAC values and added to the table. The AC table for biological effect assessment criteria for TBT (i.e. imposex and intersex in gastropods) has been amended by adding the corresponding assessment classes for the mud snail *Hydrobia ulvae*, which occurs more widely in the Baltic Sea than the other species. This was based on an interspecies correlation with *L. littorea* established by Schulte-Oehlmann *et al*. (Toxico-kinetic and -dynamic aspects of TBT-induced imposex in *Hydrobia ulvae* compared with intersex in *Littorina littorea*. Hydrobiologia 1998, 378:215–225). Potentially other gastropod species can also be included in this Table with assessment criteria for TBT effects, if interspecies correlations for sympatric populations have been determined. This can be useful for a further expansion of a harmonised assessment level for TBT effects into other sea areas, e.g. the Mediterranean Sea.

Updated Table 2. OSPAR Biological effect assessment criteria for TBT. Assessment criteria for imposex in Nucella lapillus are presented alongside equivalent VDSI / ISI values for sympatric populations of other relevant species.

Assessment	*Nucella*	*Nassarius*	*Buccinum*	*Neptunea*	*Littorina*	*Hydrobia*
class	VDSI	VDSI	VDSI	VDSI	ISI	VDSI
A (<BAC)	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
B (>BAC<EAC)	0.3-<2.0			0.3-<2.0		
C (>EAC)	2.0 < 4.0	0.3–2.0	0.3–2.0	2.0–4.0		0.3-< 1
D (>EAC)	4.0–5.0	2.0–3.5	2.0–3.5	4.0 ^	0.3-< 0.5	1-< 2
E (>EAC)	>5.0	> 3.5	> 3.5		0.5–1.2	
F (>EAC)	-				> 1.2	> 2

5.4.2.4 Sea urchin embryo bioassay

The BAC and EAC for sea urchin embryo bioassays were reviewed to include more data from Universidade de Vigo (Spain). The original values derived in a publication (Durán and Beiras 2010), the values updated in WGBEC 2011 and the new ones updated for this year is presented in Table 5.4.2.4a. The EAC is defined as 50 % of growth inhibition and the BAC corresponds to 30 % of growth inhibition (no change required to table).

Table 5.4.2.4.a. Update of the assessment criteria for the sea urchin embryo bioassay in toxic units (TU), and percentage of growth inhibition (PGI).

		Durán and Beiras (2010)		Updated 2011		Updated 2012	
		PGI (%)	TU	PGI (%)	TU	PGI (%)	TU
EAC0	Perc 50	12.1	0	12.6	0	14.6	0
EAC1	Perc 5	30.6	0.27	31.6	0.29	31	0.29
	Perc 10	29.8	0.23	30.3	0.25	30.3	0.25
EAC2	Perc 50 (LR)	49.2	0.86	48.3	0.83	47.7	0.81
EAC3	Intersc. Pob.	76	1.73				

The BAC and EAC values for bivalve bioassays were updated with data for mussel (*Mytilus galloprovincialis*), and University of Vigo recommends to separate the EAC for oyster and mussel. The database for mussel has been increased to 65 data and the new percentiles are presented in the Table 5.4.2.4.b. below. The BAC is 30 % of abnormal larvae corresponding to the 10th percentile and the EAC is 50 % of abnormal larvae.

Table 5.4.2.4.b. Percentile of control samples for mussel bioassays and percentage of abnormal larvae.

percentile	% Abnormal larvae (n=65)
mean	15.6
10	29.8
50	13.4
90	3.2
percentile	% Abnormal larvae (n=65)
mean	15.6
10	29.8
50	13.4
90	3.2

5.4.2.5 New assessment criteria produced by the BEAST project

One of the deliverables of the BONUS+ project BEAST were Baltic-specific assessment criteria for techniques in biological effects monitoring where necessary. These assessment criteria were calculated for recommended CORESET methods as well as for some CORESET candidates. Ulrike Kammann (vTI, Germany) presented a table with assessment criteria updated and newly calculated within BEAST. New BACs for PAH metabolites in herring, flounder and eelpout were calculated according to ICES recommendation using the lower 10th percentile of a bigger data set. The values can be used for assessment of the Baltic Sea exclusively and in some cases are applicable for trans-regional approaches. The BEAST assessment criteria were used to update and expand the WGBEC list of assessment criteria under a regional aspect.

The HELCOM project CORESET developed a list of core indicators for D8 containing contaminants and effects techniques. BEAST assisted CORESET in selecting the methods. The biological effects in this list are: Imposex index, PAH metabolites, Lysosomal membrane stability, Fish disease index, Micronucleus induction and Reproductive success, i.e. embryo aberrations, of eelpout and amphipods. The CORESET list is not fully adopted yet.

5.4.2.6 Reproductive success in fish

The BAC and EAC-values for the different types of abnormal fry development in broods of eelpout has been revised within the BONUS+ project BALCOFISH based on updated data from Denmark, Sweden and Germany from the Baltic Sea, Belt Sea, Kattegat and the Skagerrak. The previously derived BAC-values for mean prevalence of malformed fry and late dead fry in broods were shown to be still correct. However, new BACs were derived for early dead fry, and total abnormal fry and should replace the BACs for growth retarded fry and frequency of broods with malformed and late dead larvae. In addition, EAC-values have also been derived for reproductive success in eelpout, which are supported by a population modelling study (Bergek *et al*. From individuals to populations - Impacts of environmental pollution on natural eelpout populations. Ecotoxicology and Environmental Safety, accepted 2012). The study shows that overall, induced malformation from environmental pollution can have a large effect on natural populations, and especially in sensitive eelpout populations with lower growth rates. Consequently, even though a threshold value of 2 % malformation may seem low, it could be harmful at the population level, especially then recognised that also other types of severe abnormal fry developments can occur in the broods.

Biological Effect	Applicable to:	BAC	EAC	Summary statistic for assessment
Reproductive success in fish. Mean prevalence (%) of:	Eelpout			
	Malformed fry	1	2	
	Late dead fry	2	4	
	Early dead fry	2.5	5	
	Total abnormal fry	5	10	

5.4.2.7 PAH metabolites in Baltic herring and in Baltic eelpout

The BAC values for PAH metabolites in eelpout and herring were calculated within the BONUS+ projects BEAST using BEAST and BALCOFISH data based on fish caught in several regions from the Baltic Sea. There are no previously derived AC for these species in the list. 483 individually analyzed eelpout were used to calculate BAC based on the lower 10th percentile of all data. For herring a data set of 29 pool samples were used and also the lower 10th percentile method was applied too. Each pool represents 10–20 individual herring. Most herring pool samples (20 of 29) were related to the German coastal waters. The herring BAC should be updated when new ACs based on more samples are available.

These values are derived exclusively from Baltic fish. They should be used for regional assessment and can be replaced when values from lower contaminated regions are available.

Biological Effect	Applicable to:	BAC	EAC	Summary statistic for assessment
PAHs Bile metabolites; (1) ng/ml; HPLC-F (2) pyrene-type µg/ml; synchronous scan fluorescence 341/383 nm (3) ng/g GC/MS * 1-OH pyrene ** 1-OH phenanthrene	Eelpout	92 (1) * 7.9 (1) **		
	Herring	151 (1) * 4.5 (1) **		

5.4.2.8 EROD

Eelpout has been added to the table. BAC at 10 pmol/min mg protein for EROD in S9-fraction in liver from female eelpout (sampling Oct/Nov) has been derived within the BEAST project as the mean of 90th percentile of monitoring data from Danish coastal areas regarded as less polluted.

Biological Effect	Applicable to:	BAC	EAC	Summary statistic for assessment
EROD; pmol/mg protein pmol/min/ mg protein S9	Eelpout	10		

5.4.2.9 AChE

Eelpout has been added to the table. BAC at 124 nmol/min mg protein for AChE in muscle tissue from eelpout has been derived within the BEAST project. In addition, an EAC value at 87 nmol/min mg protein has been derived as 0.7*BAC cf. other OSPAR ACs for AChE.

Biological Effect	Applicable to:	BAC	EAC	Summary statistic for assessment
AChE activity; $nmol.min^{-1}$ mg $prot^{-1}$ 2 muscle tissue +++ Baltic sea	Eelpout	124 2+++	87 2+++	

5.4.2.10 Lysosomal stability

Herring and eelpout were considered for addition to the table with species specific BAC- and EAC- for lysosomal stability measured with the cytochemical method. BAC at 15 minutes and EAC at 8 minutes for LMS in herring and eelpout have been derived within the BEAST project. However it was not made clear at WGBEC how these values were derived or why species specific values should be derived for Baltic species only, when all other AC for LMS are considered comparable across species. A consistent approach for the definition of AC needs to be applied and this will be re-visited during WGBEC 2013. The proposed changes are given below:

Biological Effect	Applicable to:	BAC	EAC	Summary statistic for assessment
Lysosomal stability; minutes	Cytochemical; liver Herring and eelpout	15	8	
	Cytochemical; liver all other species	20	10	

5.4.2.11 DNA adducts

New EAC values for DNA adducts in various fish species were included based on new data from Norway.

Long rough dab was added as relevant species for northern sea areas. EAC = 4.0 – also made representative for other flatfishes (dab and flounder).

Dab and Flounder: Long rough dab values replace values based on preliminary values for halibut: 4.0 (for both species) instead of 6 (for both species).

Halibut value was updated and species added as relevant species for northern sea areas. EAC = 5.8 instead of 6 – Based on experiments with Atlantic halibut - Might be considered used also for Greenland halibut, which is more available to catch for monitoring in northern sea areas (The previous value was only included in the previous table as EAC for Dab and Flounder).

Herring and sprat added as relevant species for northern seas and Baltic areas EAC = 0.39 - based on DNA adduct values in sprat corresponding to critical fitness value in herring.

New EAC value for cod 6,7 instead of 6 – previous value was preliminary, related to a preliminary critical fitness value.

New EAC value for haddock – still represented by value for cod (new) = 6.7.

DNA adducts; nm adducts mol DNA	Dab	1	4.0
	Flounder	1	4.0
	Long Rough Dab		4.0
	Halibut		5.8
	Herring and sprat		0.39
	Cod	1.6	6.7
	Haddock	3.0	6.7

5.4.2.12 Updates to PAH Bile metabolite EAC presented by Norway

The following changes were presented by Steinar Sanni (Norway) after the meeting. Due to some concern over consistency of units used, these values need to be formally reviewed by WGBEC (2013) before they can be accepted into the live assessment criteria table.

Pyrene-type (μg/g bile):

Long rough dab was added as relevant species for northern sea areas. EAC = 12 – also made representative for other flatfishes (dab and flounder).

Dab and Flounder: Long rough dab values replace EAC values based on cod and turbot: 12 (for both species) instead of 22 and 29 (respectively for dab and flounder).

Halibut added as relevant species for northern sea areas. EAC = 19 – Based on experiments with Atlantic halibut - Might be considered used also for Greenland halibut, which is more available to catch for monitoring in northern sea areas.

Herring and sprat added as relevant species for northern seas and Baltic areas EAC = 4.1 - based on bile metabolite values in sprat corresponding to critical fitness value in herring.

New EAC value for cod 23 instead of 35 – previous value was preliminary, related to a preliminary critical fitness value.

New EAC value for haddock – still represented by value for cod (new) EAC = 23.

New EAC value for turbot 11 instead of 29 – previous value was preliminary, related to a preliminary critical fitness value based on another species. (The previous value was included in the table as EAC for Flounder. This new value is not entered into the table, since turbot is not considered a common monitoring species, and Flounder is represented by Long Rough Dab value).

NB The Pyrene-type metabolite EACs given in the existing table are most probably Fixed Fluorescence values (at wavelength pair 341/383 nm) – not synchronous-scan values! The new values are FF Pyr-met at 341/383 nm.

1-OH pyrene and 1-OH phenanthrene metabolites (ng/g bile; measured by GC/MS):

Long rough dab added as relevant species for northern sea areas. EAC = 320 for 1-OH pyrene and 251 for 1-OH phenanthrene – also made representative for other flatfishes (dab and flounder).

Dab and Flounder: Long rough dab values may represent EAC for dab and flounder: 546 for 1-OH pyrene and 228 for 1-OH phenanthrene (both species) as for pyrene type metabolites (above).

Herring and sprat added as relevant species for northern seas and Baltic areas EAC = 207 for 1-OH pyrene and 67 for 1-OH phenanthrene (for both species) - based on bile metabolite values in sprat corresponding to critical fitness value in herring.

Halibut added as relevant species for northern sea areas. EAC = 320 for 1-OH pyrene and 251 for 1-OH phenanthrene – Based on experiments with Atlantic halibut - Might be considered used also for Greenland halibut, which is more available to catch for monitoring in northern sea areas.

New EAC values for cod 318 instead of 483 for 1-OH pyrene and 605 instead of 528 for 1-OH phenanthrene_– previous values were preliminary, related to a preliminary critical fitness value.

EAC values for haddock – can be represented by values for cod (new), as it was done for Pyrene-type metabolites (above); = 318 for 1-OH pyrene and 605 for1-OH phenanthrene.

PAHs Bile metabolites; (1) ng/ml; HPLC-F (2) pyrene-type µg/ml; synchronous scan fluorescence 341/383 nm (3) ng/g GC/MS * 1-OH pyrene ** 1-OH phenanthrene	Dab	16 (1) * 3.7 (1) ** 0.15 (2)	320 (3) * 251 (3) ** 12(2)	
	Long Rough Dab		320 (3) * 251 (3) ** 12(2)	
	Halibut		546 (3) * 228 (3) ** 19(2)	
	Herring and sprat		207 (3) * 67 (3) ** 1.4(2)	
	Cod	21 (1) * 2.7 (1) ** 1.1 (2)	318 (3) * 605 (3) ** 23 (2)	
	Flounder	16 (1) * 3.7 (1) ** 1.3 (2)	320 (3) * 251 (3) ** 12(2)	
	Haddock	13 (1) * 0.8 (1) ** 1.9 (2)	318 (3) * 605 (3) ** 23 (2)	

PAH metabolites related to oil based discharges

The above values based on HPLC-F and 1-OH pyrene and 1-OH phenanthrene by GC/MS are not useful to assess petrogenic PAH bile metabolites. In the table below is shown a typical composition of petrogenic PAH metabolites originating from crude oil based discharges (in bile from sprat exposed to dispersed crude oil with TPAH concentration 0.85 ppb).

Compound	ng PAH / g bile
1-OH-Naphthalene	*< (21)
2-OH-Naphthalene	38
C1-OH-Naphthalene	1 744
C2-OH-Naphthalene	9 320
C3-OH-Naphthalene	17 511
1-OH-Phenanthrene	146
C1-OH-Phenanthrene	5 832
C2-OH-Phenanthrene	5 327
1-OH-Pyrene	403
TPAH met (ng/g)	40 321

As can be seen the two GC/MS analysed compounds 1-OH pyrene and 1-OH phenanthrene typically constitute less than 1% of the total petrogenic PAH metabolites. It is the alkylated forms of OH-Naphthalene and OH-Phenanthrene that dominates, which are only reflected in the GC/MS based analysis. The table shows the nine PAH metabolite compounds usually analysed by GC/MS, and the sum of these nine to provide the total PAH metabolites (TPAH met, usually expressed in µg/g bile).

The pyrene-type analysed by fluorescence at wavelength pair 341/383 nm will often correlate well with TPAH met, however the naphthalene-type metabolites analysed at 290/334 nm will usually provide higher values. Therefore EAC values for Naphthalene-type and TPAH met (GC/MS) are given for assessment of petrogenic PAH metabolites in the following:

Naphthalene-type (µg/g bile) and TPAH met GC/MS (µg/g bile) measured by GC/MS):

Long rough dab added as relevant species for northern sea areas. EAC = 115 for Naphthalene-type and 111 for TPAH met (GC/MS) – also made representative for other flatfishes (dab and flounder).

Dab and Flounder: Long rough dab values may represent EAC values for dab and flounder: 115 for Naphthalene-type and 111 for TPAH met (GC/MS) (for both species) as done for pyrene type metabolites (above).

Herring and sprat added as relevant species for northern seas and Baltic areas EAC = 31 for Naphthalene-type and 23 for TPAH met (GC/MS) (for both species) - based on bile metabolite values in sprat corresponding to critical fitness value in herring.

Halibut added as relevant species for northern sea areas. EAC = 90 for Naphthalene-type and 62 for TPAH met (GC/MS) – Based on experiments with Atlantic halibut - Might be considered used also for Greenland halibut, which is more available to catch for monitoring in northern sea areas.

Added EAC values for cod 115 for Naphthalene-type and 83 for TPAH met (GC/MS).

EAC Values for haddock – can be represented by values for cod, as it was done for Pyrene-type metabolites (above); EAC = 115 for Naphthalene-type and 83 for TPAH met (GC/MS).

(4) PAHs Bile metabolites petrogenic = (related to oil based discharges) ***naphthalene-type µg/g; fluorescence 290/334 nm **** µg/g GC/MS	Dab		115 (4) *** 111 (4) ****	
	Long Rough Dab		115 (4) *** 111 (4) ****	
	Halibut		90 (4) *** 62 (4) ****	
	Herring and sprat		31 (4) *** 23 (4) ****	
	Cod		115 (4) *** 83 (4) ****	
	Flounder		115 (4) *** 111 (4) ****	
	Haddock		115 (4) *** 83 (4) ****	

5.4.2.13 Whole sediment bioassays – Corophium

Klaas Klaag presented data from 87 contaminated sediment samples in the Netherlands that suggested revision of BAC value to 20%. This was agreed by the group.

References

SGIMC 2011. Report of the Joint ICES/OSPAR Study Group on Integrated Monitoring of Contaminants and Biological Effects (SGIMC). ICES CM 2011/ACOM:30. ICES Copenhagen, Denmark. Available under reports at **www.ices.dk**

Durán I. and Beiras R. (2010). Assessment criteria for using the sea-urchin embryo test with sediment elutriates as a tool to classify the ecotoxicological status of marine water bodies. *Environmental Toxicology and Chemistry*, 29 (5): 1192–1198.

Recommendations

- Secretariat to advise OSPAR MIME to take note of the WGBEC review of the SGIMC advice and that it is considered fit for purpose in its current form and is suitable for application for MSFD Descriptor 8 Indicator 8.2.2.
- ICES Secretariat should advise OSPAR MIME that should they require further advice on this matter for their 2012 deliberations on integrated assessment, WGBEC would be willing to provide such intersessionally.
- Secretariat to inform OSPAR MIME that after trial applications, WGBEC considers there to be an important gap in the application of the integrated approach, with regard to targeted application, frequency of monitoring, statistical aspects of designing a monitoring programme and techniques for combining assessments across regional scales.
- Secretariat to advise OSPAR MIME to take note of the national trials of the integrated assessment scheme that have been applied with some success.
- That further assessment of the ICON database is brought under the auspices of ICES by hosting a 2 day assessment workshop at ICES HQ in 2012, and a SharePoint site is created to host the data being assessed. (Secretariat).
- Secretariat to advise OSPAR to take note that WGBEC is maintaining a live document of updated biological effects assessment criteria from the ICES CRR publication. This updated document can be made available for update of the OSPAR biological effects assessment criteria on request.

Actions

- WGBEC to maintain a current document on uncertainties and problems / solutions encountered during trials of the integrated approach and consider using to inform a future publication on integrated assessment methodology.
- ICON participants to compile missing data (intersessionally) onto the assessment spreadsheets created at WGBEC 2012.
- That WGBEC maintain a live document on biological effects assessment criteria.

6 Review of Environmental Assessment Criteria or equivalents (ToR b): Review scientific robustness and update, as necessary, EACs or equivalent effects levels calculated for CEMP and pre-CEMP determinands (OSPAR request 2012/2)

WGBEC was requested to review the revised list of Environmental Assessment Criteria (EACs) produced by ICES intersessional correspondence group ICG-EAC. Craig Robinson (UK) introduced the ICG-EAC work and gave an overview of the process for the development of the proposed EACs. One of the outcomes of ICG-EAC work

(after having tried other approaches) was a decision tree for the production of EAC values by ICG-EAC. Following this tree, if an EQS value was available - including existing and proposed EQS values which was higher than the Background Assessment Concentration (BAC) and considered fit for purpose; this was used as the EAC. If an EQS was available, including proposed EQS values, but less than the BAC then either the BAC needed to be reconsidered, or an EAC would need to be calculated. If no EQS value was available, EACs would have to be calculated. This had the effect that the WFD-EQS for sediments (derived for protection of benthic communities) and biota (derived for protection of predators for secondary poisoning or humans from uptake from marine food), if they exist, were brought forward as the new EAC-values. For several of the substances (e.g. the PCBs, dioxins/Furans, PBDEs, PAHs and PFOS) EACs were based on the EQS derived in the latest drafts of the WFD EQS dossiers for priority substances from 2011.

WGBEC recognises the importance of developing scientifically robust EAC-values for relevant monitoring matrices such as sediment and biota, which can be applied for assessing if contaminant levels are of concern for causing pollution effects in marine organisms. In addition to a forthcoming OSPAR QSR, this will also be highly important for the monitoring strategies and assessments in relation to Descriptor 8 in MSFD, including for the integrated approach with biological effects indicators.

WGBEC went through the list of suggestions for EACs for OSPAR priority substances in sediments and biota. The suitability of the EACs seems to be highly variable. Some values seem reasonable, but others are either much too high, or too low (even below the derived BAC-values as also noticed by MCWG). There also remain a number of substances for which no EACs are available. However, it was difficult for WGBEC to assess most values without insight into the latest versions of the WFD EQS dossier and the data and TGD derivation of EQSs within.

The following, more specific comments and suggestions for improvements to the proposed values or process for the derivation were made:

1) It should be stated clearly in the table that the proposed EACs for biota are derived for contaminants in mussels (and not in fish). This means that the corresponding EACs for fish are still missing. This discrimination between mussel and fish is also necessary, because many of the EACs for biota are based on EQS for humans (i.e. for food stuff), where different thresholds often are derived for shellfish and fish.

2) It does not seem reasonable that EACs for biota are based on EQSs derived for protection of predators and humans for substances for which the lower trophic levels are regarded as the most sensitive species in marine ecosystems. This concerns substances like 2–3 rings PAHs, TBT and maybe PFOS. It was suggested that the ICG-EAC decision tree be modified, so that WFD-EQS values for specific substances in biota only were adopted if predators at higher trophic levels or humans were regarded as the more sensitive species in the ecosystem (such as for mercury, >4 rings PAHs, HCHs, PCBs and dioxins). Otherwise, if organisms at lower trophic levels are regarded as most sensitive, EACs for biota could be derived by extrapolating the EQS for water by applying BCF values before adequate environmental protection levels can be met (i.e. as in previous OSPAR approaches for deriving EACs).

3) The proposed EQS for PBDEs seems very low, and it seems not reasonable that they are regarded as even more toxic than the dioxins and furans. Al-

though a similar EQS value at 0.0425 μg/kg dw, it should be noted here that the proposed value for dioxins and furans is converted into μg WHO98-TEQ/kg dw, whereas the EAC for PBDEs is based solely on the nominal concentration in μg/kg dw.

4) WGBEC is aware that some additional toxicity data for PAHs and alkylated PAHs are available in the literature, (see e.g. references below). It is suggested that these can be included in the consideration of derivation of EACs for sediments.

5) Concerning the EACs for sediment, it should be noted that in WFD EQS dossiers, the EQS for sediment for organic pollutants mainly are derived based on equilibrium principle and extrapolation from EQS for water, and therefore often regarded as tentative values, because of the uncertainty recognised in this approach.

6) WGBEC also notes that other relevant toxicity data exist, which can be useful for derivation of some of the missing EACs, for instance where lower trophic levels are regarded as the more sensitive species. Given the paucity of available toxicity data to support the calculation of new EACs, any additional available data will be useful in further consideration of deriving EAC values. Laboratorio de Ecoloxía Mariña (Universidade de Vigo, Spain) and CIIMAR, University of Porto, Portugal has produced toxicity datasets, which will be of value and have been made available in Annex 7 and 8.

Conclusions

1) Within EAC biota, a clear discrimination between EAC for mussels and fish is needed.

2) A further revision of the EACs, which have been identified as too low or too high, is needed before they can be applied for assessment purposes.

3) If organisms at lower trophic levels are regarded as the most sensitive species for specific substances, EACs for biota could be derived by extrapolating from EQS for water by applying BCF values.

References (toxicity of alkylated PAHs).

Altin *et al*. 2008. Approaches for Derivation of Environmental Quality Criteria for Substances Applied in Risk Assessment of Discharges from Offshore Drilling Operations. Integrated Environmental Assessment and Management 4(2): 204–214

Fisher T.T. *et al*. 2011. Towards a scheme of toxic equivalency factors (TEFs) for the acute toxicity of PAHs in sediment. Ecotoxicology and Environmental Safety 74, 2245–2251.

Olsen *et al*. 2011. Arctic versus temperate comparison of risk assessment metrics for 2-methylnaphthalene. Marine Environmental Research 72: 179–187.

Smit MGD *et al*. 2011. Achievements of risk-based produced water management on the Norwegian continental shelf (2002–2008). Integrated Environmental Assessment and Management 7(4): 668–77.

US EPA 2003. Toxicological review of 2-methylnaphthalene (CAS No. 91–57–6). In Support of Summary Information on the Integrated Risk Information System (IRIS). December 2003, EPA 635/R-03/010, www.epa.gov/iris.

Recommendations

- Secretariat to advise OSPAR that WGBEC considers that further toxicity data are available that should be used to assist in the derivation of EACs for some contaminants.
- Secretariat to advise OSPAR that WGBEC considers that WFD EQS values derived for protection of predators and secondary consumers are not considered suitable substitutes for calculation of EACs for some substances, which have been identified as more toxic to lower trophic levels.

7 Review and update of the Technical Annex on lysosomal stability (ToR a): Review and update, as necessary, the Technical Annex 6 (lysosomal stability) to the JAMP Guidelines for general biological effects monitoring. This should build on the latest developments through the Workshop on Lysosomal Stability Data Quality and Interpretation and WGBEC (OSPAR request 2012/1)

Several ICES and OSPAR documents form the basis of background information on lysosomal membrane stability as a technique for marine environmental monitoring in the ICES / OSPAR areas. These are:

1) An OSPAR background document (OSPAR, 2007). (This has been reproduced with minor edits as Annex 5 to the 2010 advice from the Study Group on Integrated Monitoring of Contaminants (SGIMC) and section 9 in the current ICES CRR / OSPAR guidelines on integrated monitoring).

2) A Technical Annex (6) to the OSPAR Joint Assessment and Monitoring Programme (JAMP) on general biological effects monitoring (OSPAR, 2007a).

3) An ICES TIMES manuscript describing the method for neutral red retention in mussels (Moore *et al.*, 2004).

The current OSPAR request was to update 2) The JAMP TA6. At its 2011, WGBEC reviewed the ICES/OSPAR WKLYS report on the quality and interpretation of lysosomal stability data and assessment criteria for LMS using NRR assay. It was noted that there were inconsistencies in operational and analytical procedures through the ICES/OSPAR and MEDPOL area. Furthermore, refinement and agreement of assessment criteria would be desirable as the technique is now used widely in national monitoring programmes. WGBEC agreed that it was important to address outstanding issues before reviewing Technical Annex 6 to the JAMP guidelines, and this is detailed below.

7.1 Review and update of the procedures for LMS using NRR (Rap.: C. Martínez-Gómez (SP))

The OSPAR background document on lysosomal stability as a general health status indicator used for biomonitoring (Annex 5 of the Report of the Joint ICES/OSPAR Study Group on Integrated Monitoring of Contaminants and Biological Effects 2010) was reviewed by WGBEC and updated, with particular reference to the latest developments through the ICES/OSPAR Workshop on Lysosomal Stability (WKLYS: ICES, 2010). During the ICES\OSPAR WKLYS (ICES, 2010) a number of uncertainties surrounding the methods being used and the assessment criteria proposed for ICES /OSPAR SGIMC were identified. All these aspects were discussed and reported below. They are relevant to harmonize the use of the Neutral Red Retention (NRR) as-

say, in terms of monitoring and intercomparison purposes across the ICES maritime area and between Regional Seas programmes.

7.1.1 Assessment Criteria of LMS by using NRR assay

It was identified during WKLYS and during the WGBEC meeting that much data (median values) from 'reference' sites do not achieve 120 mins. Lysosomal membrane stability NRR times for reference sites in the ICES area which were available were collected (Table 7.1.1a). It was decided not to amend the ACs on the grounds that some reference sites clearly do achieve retention times of >120 mins (and may be truly representative of background values).

Table 7.1.1.a. Lysosomal Membrane Stability Neutral Red Retention Times for Reference Sites in ICES Area.

Country	Median	Highest median value	90th / 10th percentile (if available)	Comments	Source of raw data
UK	<120	90	120 (90)	Individuals >120 min, never medians	Craig Robinson (MSS) John Bignell (Cefas)
Iceland	>120	180	180 (90)	Individuals NRRT ranging from 90 to 180	John Thain (Cefas)
Norway	<120	180	Range (90–180)	Based on 10 datasets from west coast and Barents Sea in blue mussels	Steve Brooks (Niva)
Norway, Barents Sea; N.Norw. coast (sub-Arctic)	>120	150	180 / 90	Iceland scallop, Sub-arctic, Barents Sea; North coast of Norway	Steinar Sanni (IRIS)
Norway, W.Svalbard (high Arctic)	=120	120	120 / 72	Iceland scallop, High-Arctic; West coast of Svalbard	Steinar Sanni (IRIS)
Spain Mediterranean	<120	105	159 (90)	Individuals >120 min, median values use to range from 70–100	C. Martinez-Gomez (IEO)
Spain Atlantic	<120	75		Individuals >120 min, never medians	C. Martinez-Gomez (IEO)
Denmark	>120	165		Individuals up to 180 min, Medians often above 120 min	Jakob Strand (Aarhus University)
Ireland	120	120	150	Median value in reference station along the year range from 30 to 120 min	Michelle Gilltrap

7.1.2 Review of the methodology of NRR assay

During 2011/2012, C. Martínez-Gómez (Spain) and M. Gubbins (Scotland) contacted the authors of the original TIMES Nº 36 manuscript (Moore *et al*., 2004) and received feedback.

In agreement with the authors, it was decided that NRR assay described in TIMES Nº 36 document should be amended and improved to make it more informative and robust, particularly concerning the following aspects:

- Correct the size of the needle to be used (21 gauge) for haemolymph extraction;
- Incorporate the step of tipping off the dye and replacing it with seawater as recommended in MED POL protocol;
- Suggest the use of use of physiological saline adjusted to the equivalent ionic strength of the ambient water or use ambient filtered seawater from the sampling sites;
- Change the wording for the determination of endpoint to improve clarity;
- Photographs on the original manuscript to be updated.

7.1.3 Scoring method to establish LMS in mussels using NRR assay

Additionally, David Lowe made available during the meeting a new scoring method to record data of LMS using NRR assay, not only based on neutral red retention time but also that takes into account the observed changes that occur in the lysosomes during this assay. C. Martínez-Gómez (Spain) presented this to the group. She made available also some pictures that illustrated the different pathologies described by D. Lowe. During the course of the meeting D. Lowe reviewed and agreed that the images chosen (see Figure 7.1.3.a) to represent the different lysosomal alteration types were appropriate.

Briefly, samples are analysed under the microscope and scored at 15, 30, 60, 90 and 120 minutes incubation for evidence of 50% or greater of the cells exhibiting the pathologies below which are listed in increasing severity of effect.

Pathology	Score
No effect	0
Enlargement but no leakage	1
Leakage but no enlargement	2
Leakage and enlargement	3
Leakage and enlarged but colourless lysosomes	4
Rounded up fragmenting cells	5

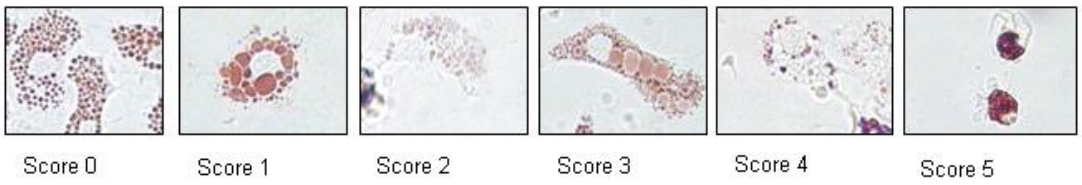

Figure 7.1.3.a. Illustrations of granulocytes (M. galloprovincialis) exhibiting different pathologies and the associated score established: Score 0= No effects; Score 1= Enlargement but no leakage; Score 2= Leakage but no enlargement; Score 3= Leakage and enlargement; Score 4= Leakage and enlarged but colourless lysosomes; Score 5= Rounded up fragmenting cells.

In calculating the total final score for the lysosomal condition, the points in time at which they exhibited one of the 5 conditions above are coded 1, 2, 3, 4 or 5 and the individual scores are multiplied by these weighting factors. Weighted score is calculated by multiplying score by weighting factor: % stability = (1-(weighted score/75))*100.

Examples of monitoring data using these new scoring approaches are presented below in Tables 7.1.3.a. and 7.1.3.b.

Table 7.1.3.a. Example of recording data using lysosomal damage with weighted scores.

Time Period / Weighting factor	15 / 1		30 / 2		60 / 3		90 / 4		120 / 5		Sum of Weighted scores	% Stability
Slide Nr	Score	Wtd score	Score	Wtd score	Score	Wtd score	Score	Wtd score	Score	Wtd score		
1	0	0	0	0	0	0	0	0	0	0	0	100
2	5	5	5	10	5	15	5	20	5	25	75	0
3	1	1	1	2	3	9	3	12	4	20	44	41.3
4	2	2	2	4	3	9	3	12	4	20	47	37.3
5	2	2	4	8	4	12	5	20	5	25		
6	3	3	3	6	3	9	4	16	4	20		
7	0	0	0	0	1	3	1	4	1	5		
8												
9												
10												

Table 7.1.3.b. Example of results using lysosomal damage with weighted scores.

Dartmouth	Avon	Fal	Helford	Looe	Fowey	Yealm	Exmouth	Teignmouth	Town Quay
45.3	74.7	36.0	20.0	68.0	25.3	60.0	86.7	44.0	48.0
62.7	62.7	60.0	22.7	49.3	33.3	44.0	81.3	50.7	42.7
76.0	76.0	44.0	22.7	40.0	40.0	53.3	54.7	65.3	56.0
100.0	93.3	48.0	41.3	42.7	40.0	20.0	93.3	60.0	48.0
93.9	80.0	22.7	53.3	36.0	41.3	60.0	57.3	52.0	56.0
68.0	20.0	42.7	49.3	60.0	62.7	46.7	42.7	69.3	60.0
68.0	68.0	56.0	62.7	40.0	44.0	60.0	48.0	62.7	53.3
48.0	68.0		53.3	48.0	33.3		49.3	56.0	28.0

Under the existing system (recording only NRR time) two samples can be considered as being the same status, even when they display different severity level of pathology. C. Martínez-Gómez pointed out that this fact is one of the main reasons for the high variability observed in NRR results between individuals from same sampling site and between laboratories, as interpretation of observations are sometimes not completely clear (i.e. when lysosomes are swollen but not leaking dye). Whilst the general pattern response is the same using the two systems of recording data, the differences between sites is less extreme using weighted scores and the inter animal variability is reduced (see figure 7.1.3.b.).

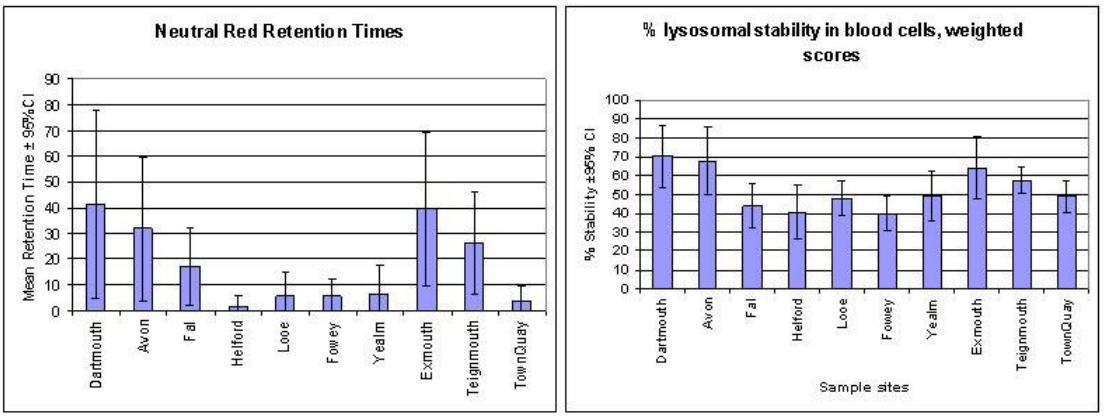

Figure 7.1.3.b. Lysosomal membrane stability in blood mussel cells expressed as NRR time (left) and as % of lysosomal stability (right).

Using the score generated by the scoring method it is possible to also determine the endpoint that would have been ascribed by the existing endpoints/criteria and thereby make a comparison between the two approaches. WGBEC agreed that this new approach is a big improvement on the original methodology and one that has the potential to provide a better understanding of how different classes of contaminants affect lysosomal membrane stability and how this is manifested. Therefore, it was agreed by WGBEC that it would be beneficial to also include details of the new lysosomal scoring system proposed by D. Lowe in the amended TIMES manuscript, so that this new improved approach can be disseminated and hopefully weighted score data generated alongside retention time data, which will hopefully lead to the generation of new assessment criteria.

There are Data Centre report format implications resulting from the change of methodology proposed. If weighted scores are reported as % LMS alongside retention time (mins) a new parameter code may be required.

7.2 Review and update, as necessary, the Technical Annex 6 (lysosomal stability) to the JAMP Guidelines for general biological effects monitoring

WGBEC updated Technical Annex 6 include information on the use of the LMS method in mussel species and the information concerning assessment criteria. The existing information on the determination of LMS in fish (using the cytochemical method was also updated with information on QA activities). Reference is also made to the new OSPAR background document produced during SGIMC and adopted by OSPAR on lysosomal membrane stability as a global health status indicator in biomonitoring. This document contains the most comprehensive and up to date summary of the methods, their use and assessment criteria for marine monitoring purposes. The updated JAMP Technical Annex 6 is provided at Annex 9 of this report.

References

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OSPAR Commission, 2007. Assessment and Monitoring Series. Background Document on Biological Effects Monitoring Techniques Chapter 4. Lysosomal Membrane Stability as a global health status indicator in biomonitoring pg 20. ISBN 978–1-905859–72–6 Publication Number: 333/2007.

OSPAR 2007a. JAMP Guidelines for General Biological Effects Monitoring (OSPAR Agreement 1997–7). Technical Annex 6 Lysosomal stability. P9.

Recommendations

- Secretariat to supply the revised TA6 and associated guidance on issues identified by WKLYS to ADGLYSAC and subsequently OSPAR.
- A draft resolution is requested to amend the ICES TIMES manuscript 36 as identified above.

Actions

WGBEC to progress amendment of TIMES 36 on Lysosomal stability (CMG)
WGBEC chairs to draft a draft resolution for TIMES 36.

8 MSFD – review initial assessments for Descriptor 8 and advise as required on implementation of monitoring programmes for GES Commission indicator 8.2.1 (ToR d)

During 2011 WGBEC had a round table discussion on this subject and this year the opportunity was taken to review the ongoing national approaches on initial assessments and the development of GES criteria for descriptor 8 under the MSFD (Rap K. Coorman (BE)).

The developments on initial assessments, GES and environmental targets are progressing well in most countries although with differing speed. German, Sweden, Belgium and Denmark have a draft ready for public consultation. Other countries are on schedule: UK, Belgium, the Netherlands, Norway, Denmark, Spain, and France. In Portugal, The discussions on MSFD D8 are ongoing but there is at the moment no information available. Ireland has just started the progress and is substantially delayed.

Short summaries on the state of progress of CPs on MSFD Descriptor 8 are given below:

Denmark is currently working on the initial assessment, largely based on information and data of the National Monitoring Programme for Aquatic and Terrestrial Environment. This also includes information of existing monitoring data on levels of contaminants and pollution effect indicators like PAH-metabolites, CYP1A, intersex and reproductive success in fish and LMS and imposex in molluscs from three sub regions North Sea/Skagerrak, Kattegat and the Baltic Sea/Belt Sea, which the Danish waters have been divided into. Reference has also been made to list of proposed pollution effects indicators proposed by HELCOM CORESET. The draft IA is currently being reviewed within the political system and is expected to be available for public hearing in the last week of March.

Spain - Spanish territorial waters are divided in five marine sub regions, so-called demarcations. A marine strategy is being elaborated for each demarcation. The coor-

dination of the implementation of the MSFD in Spain is done by the Ministry of Agriculture, Food and the Environment (MAGRAMA), whilst the Instituto Español de Oceanografía (IEO) is the scientific organisation which advices MAGRAMA in relation to MSFD, and is in charge of the initial assessment and GES definition, including the development of the eleven descriptors of Good Environmental Status. The 2012 road-map in Spain for the MSFD implementation includes several national coordination meetings in order to harmonize the MSFD documents, and to involve other units from several ministries related to the different descriptors, and trilateral meetings (FR/PT/ES and IT/ES/FR) in order to compare, coordinate and harmonize the initial assessments and GES definitions with neighbour countries. The public consultation process for MSFD documents will start in mid-April, and a workshop will take place in June in order to incorporate changes and comments to the documents.

The approach followed for GES definition in descriptor 8 was the integrative approach proposed by SGIMC, although due to data gaps it was not possible to reach the final level of integration and only step 3 was reached. The biological responses that were used for the initial assessment and GES definition varied among marine demarcations, depending on data availability. Embryo-larval bioassays with sea-urchins and amphipods survival bioassays, Scope for Growth in mussels, imposex in gastropods and EROD activity in fish, were used in the North Atlantic demarcation. Embryo-larval bioassays with sea-urchins and amphipods survival bioassays, were used in the South Atlantic demarcation. EROD activity in fish, metallothioneins, micronucleus analysis, AChE activity, LMS and Stress on Stress on mussels, were used in the Levantine-Balearic and in the Gibraltar Strait and Alboran Sea demarcations. Only imposex data was available in the Canary Islands demarcation.

For scientific work related to the MSFD carried out in the Basque coast, south-eastern part of the Bay of Biscay, since 2006 AZTI-Tecnalia has included three stations located in (in the west, north and east) of offshore waters for monitoring activities. These activities comprise the analyses of hydromorphological, physico-chemical and biological quality elements (phytoplankton, macro algae, benthos and fishes). However, as information is lacking for some qualitative descriptors of the MSFD (i.e. contaminants in seafood, litter or noise) (Borja *et al*., 2011), in the last two years specific campaigns have been carried out. With regard to descriptor 8, four fish species, dogfish (*Scyliorhinus canicula*), hake (*Merluccius merluccius*), thickback sole (*Microchirus variegatus*) and common sole (*Solea solea*) were selected according to their abundance, habitat and commercial value. The muscle and liver tissues were dissected for the determination of metals (Cd, Hg, Pb, Cu and Zn) and organic compounds (HCHs and PCBs). Additionally, liver and gonad tissues were used for histopathology and blood smears were prepared for micronuclei frequency. The main results of the pilot study indicate that in general there are no significant differences among the three stations distributed along the Basque coast (150 km length) suggesting that for future monitoring programmes, a pressure and impact gradient should be followed from estuarine to offshore waters. In this respect, the most appropriate species seems to be the common sole since this flatfish appears either in estuarine or in offshore waters. Nevertheless, the conclusions addressed indicate that it is difficult to attribute changes in biological effects to contaminants exposure. Therefore, data collection is necessary to study the variability of biological effect responses and establish background values.

In **France**, the measures and monitoring plans have not yet been constructed or decided upon. Yet, the orientations followed by France take into account the scientific state of the art regarding assessments of indicators of Good Environmental Status

(GES), and the ability to translate into a resource-effective monitoring programme the measures that might be taken.

For Descriptor 8, the chosen indicators of GES were the concentrations of selected hazardous substances, and selected biological effects observed in biota that is induced physically or chemically.

The selected substances comprise nine classes (PBDE's; PCB's/PCDD's/F's; PAH's; HBCD's; PFOS; 3 metals and their species; OC pesticides; TBT; pharmaceuticals), augmented by the mandatory compounds (e.g., listed by the WFD or other conventions) and the ones accidentally spilled and which have a physical effect on the ecosystem. The biological effects selected are of 4 classes (general stress; fish pathologies, genotoxicity; reprotoxicity). They are to be used with biota from either the coastal environment for hot-spot identification/delimitation, or from off-shore (reprotoxicity).

The scales of variability of the marine environment will guide the definition of the appropriate geographical and temporal scales. As of now, three broad classes are foreseen: coastal, surface + offshore, and deep + offshore. Focus zones include contamination hotspots and pristine zones.

The observed values of levels and effects will be compared to the existing criteria (EAC's, EQS'), or to newly developed ones. For the latter, France favours Background Assessment Criteria (BAC's).

France adheres to the characterization of GES drawn by OSPAR's HASEC 2012 meeting.

Ireland. Reports on initial assessments and decisions on targets/indicators for MSFD in Ireland are currently in progress. There is a delay and working groups are only starting on this and therefore there is no information to provide as of yet for Ireland.

Norway. Monitoring of environmental quality status in Norway is performed along the Norwegian coast in the CEMP programme by Niva and in open Seas by IMR. Monitoring food quality is performed by the National Institute of Nutrition and Seafood Research (NIFES) in collaboration with IMR. In addition discharges from the oil and gas industry are monitored by the Water Column Effect Monitoring programme and the Condition Monitoring.

The CEMP programme analyses contaminant levels in blue mussels, mercury and imposex in dog whelk, contaminant levels in Atlantic cod and flatfish, and bile metabolites, ALA-D, EROD and CYP1A in Atlantic cod.

The Water Column Effect Monitoring programme is based on annual caging exercises in oil fields of the different regions on the Norwegian Continental Shelf (NCS). The BECPELAG workshop has been a basic template for how it is conducted. Blue mussels and Atlantic cod have frequently been used, and the analyses include chemical and biological methods. An important driver for the programme is the "zero harmful discharge" regulation (a definition of GES for the NCS), which has motivated for the mandatory measurement of biological effects of contaminants. The core set of methods are mostly among the suite of biomarkers proposed by SGIMC/WGBEC: condition index, lysosomal membrane stability, micronuclei frequency, pyrene hydroxylase, histochemistry and histopathology in the mussel and condition index, CYP1A, GST, VTG, bile metabolites and DNA adducts in cod. In addition the programme allows testing of new methods and relevant support or side studies based mainly on proposals from the participating scientific institutions (IRIS and NIVA).

The SGIMC assessment criteria have been used preliminary in the last two years as part of the evaluation of the results. Each year the content of the programme is proposed based on joint input from NIVA & IRIS by the Norwegian Oil Industry Association to the environmental authorities (Klif) for approval.

In the Condition Monitoring, which is performed every third year, possible effects in wild caught fish from regions in the Norwegians Seas are measured, with emphasis on the Tampen region in the North Sea, where the highest amounts of produced water are discharged. Hydrocarbon components in fish are measured, in addition to selected biomarkers as bile metabolites, CYP1A, oxidative stress biomarkers, vitamin E, VTG, DNA adducts, and fatty acid composition. The content of the programme has been proposed by IMR in discussion with the Norwegian Oil Industry Association and the environmental authorities (Klif) for approval.

It is not clear how Norway dealt with the Initial Assessment and no information is currently available on the implementation of the MSFD Descriptor 8.

(Extract from MCWG; Patrick Roose did not reply) **Belgium** is on schedule both with the IA and the development of GES Descriptors. The draft IA will be available for public consultation in March. Equally, the draft GES Descriptors are being finalised this month. Belgium has opted for a pragmatic, quantifiable approach for its Descriptors, relying as much as possible on existing legislation and approaches (e.g. OSPAR - EcoQOs). For Descriptor 8 ("Contamination"), Belgium will use existing WFD EQS values (in water and biota) for its marine waters and OSPAR EACs (even though they are preliminary) when there are no EQS values available (biota and sediment). For bird eggs, the OSPAR EcoQO will be applied. The contaminants monitored will be the WFD priority substances in the 12-mile zone and OSPAR (JAMP and the Seabird EcoQO) substances in the remaining continental shelf area. For bird eggs, an additional indicator "no difference is measured between Hg concentrations in bird eggs from estuarine and non-industrial zones" has also been defined. Effects measurement has so far been limited to:

- Biota and oil: the average proportion of oiled common guillemots (*Uria aalge*) is below 20 % of the total number found dead or dying on the beaches (OSPAR EcoQO);
- Effects: the average level of imposex is consistent with an exposure to TBT concentration less than the EAC (OSPAR EcoQO).

It is also worthwhile to note that:

For Descriptor 5 ("Eutrophication") the environmental targets and associated indicators are based on Commission Decision 2008/915/EC for chlorophyll a and Phaecystis cells. Nutrient DIN and DIP are based on the OSPAR Common Procedure.

Descriptors 1, 4 and 6 are dealt with together, due to the strong link and overlap between these Descriptors.

For Descriptor 9 ("Contaminants in seafood for human consumption"), Belgium intends to check if all measured contaminants in fish and shellfish for human consumption have concentrations below regulatory levels (Commission Regulation 1881/2006 and Directive 2006/113/EC).

The Netherlands are also developing a pragmatic approach similar to Belgium and will fit this as much as possible in existing programmes. The existing long-term programmes contain the following BE techniques: fish diseases, PAH metabolites, im-

posex and will probably be expanded with LMS in mussel and DR-Calux in sediment.

Portugal: - the biological effects of environmental contaminants have been investigated in several estuaries (e.g. Minho, Lima, Cávado, Douro, Ria de Aveiro, Sado, Tagus, Ria Formosa) and in coastal areas (e.g. NW coast, Algarve), in several cases combining also levels of contaminants in sediments and/or tissues of sentinel species, including bivalves (e.g. *Mytilus galloprovincialis*), fish (e.g. *Pomatoschistus microps*, *Dicentrarchus labrax*, *Anguilla anguilla*). As far as we know, a considerable part of these monitoring programmes have been conducted in the scope of research projects. Biological parameters that have been analysed include: acetylcholinesterase activity, several oxidative stress parameters (e.g. catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase, and lipid peroxidation levels), micronuclei, energetic enzymes, biotransformation enzymes (MFO, GST), condition indexes (e.g. Fulton condition index and hepatosomatic index in fish), and metallothioneins, among several others. Chemical parameters include several metals (e.g. Hg, Cu, Ni, Cd, Cr), PAHs, several pharmaceuticals, PFOs, PCBs, etc.

In several cases, monitoring programmes in wild populations are complemented with in situ assays (e.g. with microalgae, *Hedistes diversicolor*, *Carcinus maenas*) and laboratory bioassays with native (e.g. mussels and several other invertebrates, several fish) and standard species.

United Kingdom - UK territorial waters fall into two MSFD sub-regions (North Sea, Celtic Seas) and have been divided into 8 sub-divisions for the initial assessment. This was produced last year (Charting Progress 2) and is available on the Department of Food and Rural Affairs website. The D8 assessment was undertaken in an integrated manner using the traffic-light approach.

Targets have been suggested as follows:

- For Indicator 8.1.1 (Concentrations); Concentrations of substances identified within relevant legislation and international obligations are below those at which adverse effects are likely to occur (e.g. are less than EQSs applied within WFD; EACs applied within OSPAR).
- For Indicator 8.2.1 (Effects); The intensity of biological or ecological effects, due to contaminants, is below the toxicologically-based standards agreed by OSPAR as appropriate for MSFD purposes.
- For Indicator 8.2.2 (Acute Events); The UK is likely to adopt the recent OSPAR target on oil spills.

It is not yet clear exactly what effects measurements will be used to address the target under Indicator 8.2.1. Imposex will be included as it is required for the OSPAR CEMP, although the intensity of sampling will reflect known trends and pressures. It is possible that the core part of the SGIMC scheme will be used to assess status, but the number of stations and the frequency of sampling will be decided following a risk-based analysis of known pressures. This implies a primary focus on coastal waters, with less intensive sampling in areas with fewer pressures. How the overlap between WFD (chemical status) and MSFD (effects) will be reconciled for coastal waters is not yet clear.

In conclusion

Several countries intend to develop pragmatic approaches and expressed their intentions to fit their GES descriptors as much as possible in existing monitoring programmes.

Based on the intentions and comments of CPs, the ICES WGBEC concluded that the MSFD GES and OSPAR CEMP should be connected, harmonized and compatible, taken into account that the Integrated Assessment Framework, developed during OSPAR/ICES Workshops on Integrated Monitoring of Contaminants should form the backbone of the preferably combined programmes.

WGBEC noted that there were two notable science conferences concerning MSFD in 2012. The first being the EU conference Marine Strategy 2012 in Copenhagen, Denmark hosted by the Danish Presidency on 14–16 May. In addition a theme session (G) at the 2012 ICES ASC in Bergen 17–21 Sept will focus on 'Implementation of the European Union Marine Strategy Framework Directive (EU MSFD): Implications for science and policy'.

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9 Receive reports on marine monitoring activities being undertaken by member states (ToR f)

Within this agenda item the members of the working group were provided the opportunity to inform the rest of the group of current and future biological effects monitoring activities taking place across the ICES area. During the meeting several work programmes were presented and summary text has been provided below. Due to time restraints, not all members of the group were able to present their respective activities, in some cases the summary of these programmes not presented have been included in the text below.

Summary of presentations given:

9.1 Germany

Heike Helmholz presented 'Integrative sampling techniques for the determination of contaminants and their effects' (Heike Helmholz; Daniel Pröfrock, Department Marine Bianalytical Chemistry, Helmholtz-Zentrum Geesthacht. Germany).

The research plan on the development and application of integrative sampling techniques for the determination of contaminants and their effects was introduced. Integrative sampling devices (passive and active *M. edulis*) were implemented as fixed stations at Cuxhaven and at Helgoland. In order to identify pollutants as harmful substances proteomic-tools and -techniques are used to identify adequate indicators for the effects caused by harmful substances at molecular and cellular levels in mussels. The focus is laid on metal binding and metal-containing proteins acting as potential indicator of exposure and effect. Quantification of emerging pollutants will be done by a set of state-of-the-art methods such as multielemental analysis (ICP-MS;

TXRF) and by coupled systems with element specific (GC-ICP-MS; LC-ICP-MS) and molecule specific (GC-MS/MS; LC-MS/MS; MALDI-MS/MS) detection methods.

9.2 International

Toxicity profiling of the major EU transported oil types (TOXPROF). The EU funded project, co-ordinated by Kevin Thomas (Norway) with participants from members of the working group from UK, Spain, and France was presented by Thierry Burgeot (France). A summary of TOXPROF is provided.

Toxprof evaluated the toxicity profiles of six oils and one HNS (styrene monomer) as being representative of the different oils that are transported through EU waters. The oils were comprehensively characterized using the most advanced techniques available prior to toxicity profiling with bioassays and biomarkers. Bioassay profiling of the water accommodated fractions (WAF) of the oils provided a toxicity ranking. The WAFs were analysed for common oil components and is invaluable when interpreting oil toxicity. Bioassay profiling was performed on Atlantic cod (*Gadus morhua*), two-spot goby (*Gobiusculus flavescens*) and blue mussel (*Mytilus edulis*) following three weeks exposure to three weathered oils – Arabian light, Ekofisk and ship diesel - in a flow-through system. Chemical analyses showed that both concentration and composition of oil components changed over the three weeks, as expected. This was also reflected in the responses observed in organisms, but different persistence of responses resulted in somewhat different fingerprints for each oil, the two levels and one, two and three weeks. Results for blue mussels was somewhat surprising as the most obvious responses were observed in groups exposed to the low concentration of the three oils, both after 7 and 21 days. The reason for this is not clear, but it could be due to overt toxicity causing narcosis. For Atlantic cod, there was a clear relationship between responses thought to relate to exposure to carcinogenic PAHs, i.e. cytochrome P4501A activity (EROD) in gill and liver. The data further indicated that smaller PAHs may increase lipid peroxidation in gills. Whereas all oils affected gill EROD in cod, there was no obvious response in two-spot goby gill EROD, highlighting the need to target species with comprehensive baseline knowledge in post-spill monitoring. Effect-directed analysis was used to characterise the arylhydrocarbon receptor (AhR) agonists and estrogen receptor (ER) antagonists present in the oils. AhR agonists and general bioassay toxicity was greatest from the aromatic compounds present in the oil, whereas ER antagonism was caused by polar oil components. Overall, the suite of biomarkers and bioassays used proved to be very useful for the post-incident monitoring of oil spills, whilst providing valuable data for the wider risk assessment of oil components. The combined and integrated application of both biological effects and advanced analytical tools is a powerful approach in identify both the effects and environmental residues of oils in the marine environment.

9.3 France

Thierry Burgeot presented 'Application of the alkaline comet assay in different marine organisms in situ and lab studies'; F. Akcha, N. Wessel, J. Rouxel, D. Menard, C. Spagnol, X. Caisey, F. Quiniou, G. Arzul, T. Burgeot. Ifremer, Department of Biogeochemistry & Ecotoxicology, Rue de l'Ile d'Yeu, 44311 Nantes Cedex 03, France.

The alkaline comet assay has been utilised in our laboratory to study the genotoxicity of environmental pollutants on marine organisms since 2000. *In situ*, dab (*Limanda limanda*) caught from the Eastern English Channel (France) was studied in order to ascertain the relationship between chemical organic contamination and genotoxic and carcinogenic effects. Significant effects of biotic (age, sex) and abiotic (site, season)

factors on the level of DNA strand breaks in dab erythrocytes was demonstrated. Positive linear correlations between the level of DNA damage and the polychlorinated biphenyl (PCB) and polychlorinated dibenzo-*p*-dioxin (PCDD)/ polychlorinated dibenzofuran (PCDF) concentrations in dab tissue were observed. Chemical contamination is expected to play a role in the genetic alterations. Highest level of correlation was obtained between the DNA strand break and highly bio transformed PCB . This result confirms that PCBs are a good tracer of chemical contamination. The PCBs like other highly bioaccumulated compounds (PBDE) could be used as a good indirect tracer in relation with the DNA damage.

In the laboratory, the comet assay was also applied to obtain insight into the consequences of genotoxicity at population level. The embryotoxicity and the genotoxicity of model pollutants, benzo[a]pyrene (BaP), 17α-ethynylestradiol (EE2), and endosulfan (ES), for oyster embryos were studied in parallel. Except for EE2, BaP and ES displayed both developmental toxicity and DNA damage. A significant correlation was demonstrated between genotoxic and embryotoxic parameters. As previously suggested, genotoxicity could have an indirect effect on oyster recruitment. More recently, the alkaline comet assay was successfully applied in oyster sperm to study the impact of genotoxicant exposure on reproduction as illustrated by the results obtained following a 1hr exposure to environmental concentrations of diuron.

In the laboratory, the comet assay was also used to get an insight into the consequences of genotoxicity on population levels. Phytoplankton is a model of choice for population studies because of its short life cycle and its situation at the bottom of the trophic chain. The comet assay was used on these microscopic organisms to evaluate pesticide toxicity. For the dinoflagellate, *Karenia mikimotoi*, the comet assay was validated and demonstrated the genotoxicity of different pesticides. These results give us the possibility to study the trans-generational effects of genotoxicant exposure from the unicellular organisms to the community level of organization.

9.4 Norway

Norwegian water Column Monitoring programme of 2011; Steven Brooks[1], Rolf Sundt[2], Daniela Pampanin[2], Christopher Harman[1] ([1] NIVA, Oslo; Norway [2] IRIS, Stavanger, Norway).

The Water Column Monitoring (WCM) programme performs investigations into the potential biological effects of offshore oil and gas activity on the biota living within the water column of the Norwegian sector of the North Sea. Oil companies in the Norwegian sector with produced water discharges, are obliged by the Norwegian authorities to perform water column monitoring offshore. The work has been performed at various fields within the Norwegian sector over the last 20 years. The methods used are considered to be the best available technology for the assessment of biological effects monitoring, measuring chemical bioaccumulation of oil related compounds in mussels and passive sampling devices as well as a suite of biomarker responses in mussels. Integration of the chemical and biological effects data enables a comprehensive assessment of the effects of the produced water on organism health.

The Water Column Monitoring survey 2011 was performed in collaboration between NIVA and IRIS. The objective of the survey was to assess the extent to which produced water (PW) discharged from Gullfaks C platform affects organisms living in the water column. The study was designed to monitor bioaccumulation and biomarker responses in mussels held in cages in the vicinity of the water discharge point, with supporting information from passive sampling devices. Significantly

greater bioaccumulation of PAH and NPD compounds was found in mussels from the two stations positioned 500 m from the platform, with concentrations significantly higher in mussels from one of the 500 m stations (i.e. station 2). All other mussel stations positioned 1000 m and 2000 m from the platform had PAH-NPD bioaccumulation typical of offshore background concentrations. There was very good agreement between the biomarker responses and the chemical concentration data. The calculated integrated biological response (IBR/n) was markedly higher in mussels from station 2, indicating poorer health. The IBR/n was also slightly raised in mussels from station 3 (1000 m), which was considered to be due to other chemicals within the PW. Alkyl phenols and naphthenic acids were detected in all POCIS placed at selected mussel stations from 500 to 2000 m, with mussel station 2 (500 m) and 3 (1000 m) showing highest concentrations of these compounds. PAH metabolites were detected in wild caught whiting (*Merlangius merlangus*) and tusk (*Brosme brosme*). The measured PAH metabolites in both fish species were indicative of weathered PW chemicals. Overall chemical bioaccumulation and impaired health to caged mussels was observed in mussels exposed to the PW plume located 500 m downstream from the platform.

9.5 Norway

PAH and biomarker measurements in fish from condition monitoring in Norwegian waters; Bjørn Einar Grøsvik, Sonnich Meier, Jarle Klungsøyr. Institute of Marine Research, Bergen, Norway.

Condition monitoring in fish from open seas are performed in Norway every third year. The objectives have been to investigate whether fish from Norwegian seas contain elevated levels of components that originate from discharges from offshore oil and gas production. Focus has been on the Tampen region, as this is the region with highest discharges of produced water. In 2010, 128 mill ton produced water were discharged in the Norwegian sector of the North Sea, and 59 % (76 mill ton) were discharged at the Tampen region. Condition monitoring in 2002 demonstrated changed levels of several parameters in haddock from Tampen, compared with haddock from the Egersund Bank, including 2–4 ring PAH metabolites in bile, DNA adducts in liver, and the ratio of n-3/n-6 in muscle (Balk *et al*., 2011).

These results were followed up in 2005 and 2008. The main focus has been the North Sea (Tampen and the Egersund Bank), but samples from the Norwegian Sea and the Barents Sea (reference area) were also analysed for comparison. NPD and PAH measured in fish muscle and liver from cod and haddock in 2005 and 2008 were found to be below LOQ for all regions. The main contributor to sum PAH metabolites in bile at Tampen and at the Egersund bank measured in 2008 was 1-hydroxy phenanthrene. Levels of alkylphenols in bile were below LOQ. Levels of Vtg in blood of male cod were generally low from all regions. DNA adducts in haddock liver were significantly higher at Tampen compared with Egersund Bank in 2005 and 2008, but to a lesser extent (2-fold in 2005 and 2008, compared to 5-fold in 2002). Lipid content in the liver was significantly reduced in haddock from Tampen in 2008. Fatty acid profiles showed that haddock from Tampen had relatively high levels of arachidonic acid, and the ratio between omega-3 and omega-6 (n-3)/(n-6) poly unsaturated fatty acids were significantly lower in the lipid classes in haddock from the other regions. Cod and haddock were also collected in 2011 and analyses are under process.

Reference

Balk L *et al*. 2011. PLoS ONE, Volume: 6(5), article no: e19735.

9.6 Spain

Progress in the Spanish programme for monitoring marine pollution in the Atlantic coast; Juan Bellas (Instituto Español de Oceanografía, IEO).

Recent progress in the Spanish marine pollution Monitoring Program for the Atlantic Coast, conducted by *Instituto Español de Oceanografía* (IEO), includes the adoption of an integrative approach that includes CEMP chemical methods and pre-CEMP biological methods. In order to establish clear relationships between results of chemical monitoring of pollution and the pollutant concentrations that may cause ecological damage, the following actions have been carried out: (i) a study on the biological effects of sediment elutriates by using the sea urchin embryo-larval bioassay; (ii) a study on the toxicity of sediments by using the amphipod survival bioassay; (iii) a study on the biological effects of chemical pollutants on molecular responses in mussels (GST, GPx and AChE); (iv) a study of the biological effects of chemical pollutants on the physiology of mussels.

A full explanation of the methodology and work plan is given in Annex 10.

10 Review progress with publication and electronic dissemination of biological effects techniques in the ICES TIMES series (ToR h)

An update on the progress with publication of ICES TIMES manuscripts was provided for the meeting by Ricardo Beiras (WGBEC TIMES editor) and provided at Table 10.1 below.

Table 10.1 Current state of progress with ICES TIMES manuscripts commissioned by WGBEC.

Method	C. Res	Updated Status	Action
The report on Biological Effects of Contaminants: Oyster (*Crassostrea gigas*) Embryo Bioassay by J.E. Thain (UK)	2002/1E03	Completed and reviewed by WGBEC, sent to ICES TIMES editor and ready for publication	Not required
Alkylphenol bile metabolites. Jonny Beyer	2011	Revised manuscript reviewed by WGBEC during the 2011 meeting. Draft resolution assigned in 2011. Reviewed version sent to ICES TIMES editor and ready for publication. (Published May 2012)	Not required
Sea urchin embryo bioassay. Ricardo Beiras	2011	Manuscript has been externally peer reviewed and approved by the group. Draft resolution assigned in 2011. Reviewed version sent to ICES TIMES editor and ready for publication.	Not required
The Protocol for Extraction Methods for Bioassays. Hans Klamer and John Thain (UK)	2006/1/MHC06	Produced during WGBEC 2011. Reviewed by group, needs editing for TIMES structure and checking against MHC13	External review required
The protocol for conducting EROD determinations in flatfish By M. Gubbins	2006/1/MHC07	Completed and reviewed by WGBEC, ready for publication	Updated MS requested to author
Protocol for measuring dioxin-like activity in environmental samples using CALUX assays. Dick Vethaak (Netherlands) Protocol for measuring dioxin-like activity in environmental samples using LUC assays. Dick Vethaak (Netherlands)	2008/1/MHC13	Estrogenic receptor method has been removed from manuscript. In preparation by author. Deadline revised with ICES. Produced for the meeting. Reviewed by the group with minor edits suggested. External review conducted. Waiting for author's reply.	Sent for external peer review
Blue Mussel Histopathology, John Bignell, Steve Feist & Miren Cajaraville	2007/1/MHC02	David Lowe is no longer an author of this MS. Main author is awaiting input from co-authors on specific pathologies. In preparation.	Pending on author's action

		Initial draft produced for WGBEC 2011. Reviewed at the meeting. Several sections still missing. WGBEC chair to contact co-authors and request action.	
Protocols for measuring micronucleus formation in cells as an indicator of toxicant induced genetic damage. Brett Lyons & Awadesh Jha (UK).	2008/1/MHC14	Manuscript will be based on recent background document. New co-authors identified. Revised deadline reported to ICES. There has been no action by authors. WGBEC editor to contact Janina Barseine to consider producing.	Pending on author's action
Protocol for measuring estrogen/androgen activity in environmental samples using YES/YAS yeast screen assays. J Thain (UK), Kevin Thomas (Norway)	2008/1/MHC15	No update on progress from the author. WGBEC to chase up and provide progress report. No further progress by authors.	Pending on author's action
The protocol for gonadal histology in flounder. S Feist *et al*.	2008/1/MHC12	Progress by author. 1st draft expected by the end of the month. No further progress by authors.	Pending on author's action
Reproductive success in eelpout. Jakob Strand		In preparation. In preparation. Expected late 2011. Draft resolution required. No further progress by authors.	Pending on author's action

The group reviewed those outstanding manuscripts and made the following observations:

2007/1/MHC02 Mussel histopathology. John Thain was identified to contact the lead author to determine how to progress the missing sections.

2008/1/MHC14 Micronucleus. Chair and WGBEC TIMES editor to contact Janina Barsiene to see if she would be willing to author the manuscript.

2008/1/MHC15 YES/YAS. Kevin Thomas (Norway) is in process of producing first draft of the manuscript.

2008/1/MHC12 Gonadal histology in flounder. There has been no progress with this manuscript for some time and concern that it may not be produced. It was noted that an existing Aquatic Toxicology paper describes the method quite well and could be used e.g. as the source key for the ICES database. It was therefore decided to reprioritise this publication and consider Jens Gercken as an alternative author (having worked on intersex in eelpout). John Thain was identified to progress through discussion with Cefas authors.

Reproductive success in eelpout. Jakob Strand indicated that a preliminary draft of the manuscript was already ready but that a further 3 months was required to finalise. Draft expected mid-June 2012.

WGBEC also considered the requirement for further TIMES manuscripts by assessing availability of manuscripts against the methods included in the SGIMC approach. A table was produced 10.2. below.

Table 10.2. ICES TIMES docs in relation to SGIMC integrated approach: biological effect.

SGIMC technique	C = core A = addit.	TIMES doc available; No and date	SGIMC Bgd doc available
FISH			
PAH metab	C	39 : 2005	Y
EROD	C	23 : 1998 also see 13 : 1991 In revision	Y
VTG	C	31 : 2002	Y
AChE	C	22 : 1998	Y
COMET	C		Y
DNA adducts	A	25 : 1999	Y
Lysosomal stability	A		Y
Micronuclei	A	Have resolution but no progress at last meet.	Y
Liver Histopathology	C	38 : 2004	
Macroscopic liver neoplasm	C	38 : 2004	
Intersex	C	In flounder requested	Y
Ext Vis Fish Disease	C	19 : 1996	Y
Reproductive success	A	In prep	Y
MUSSELS			
Lysosomal stability	C		Y
AChE	C	22 : 1998	Y
Micronuclei	C	Have resolution but no progress at last meet.	
Mt	A	26 : 1999 Note its for fish not mussels	Y
COMET	A		Y
Histopathology / Gametogenesis	C	In draft	Y
Stress On Stress	C		Y
SFG	A	40 : 2006	Y
WATER			
Oyster embryo	A	11 : 1991 In revision	Y
Sea urchin embryo	A	In prep and draft	Y
Copepods	A		Y
SEDIMENT			
Whole sed. Bioassay	A	29 : 2001 and 28 : 2001	Y
Pore water bioassays	A		Y
Elutriate bioassays	A		Y
DR-LUC	A	In draft	Y
Benthic community indices	A		Not available

GASTROPOD			
Imposex in dogwhelks	C	24 : 1999	OSPAR
Imposex in Buccinum	A		OSPAR
Intersex in Littorina	A	37 : 2004	OSPAR

As a result of this process it was identified that TIMES manuscripts are required for: Condition Index (fish and mussels), COMET assay (fish and mussels), stress on stress (mussels) and ER CALUX. It was also considered whether to draft a TIMES manuscript on integrated assessment, but a separate publication route for that (peer review literature) was decided on.

Possible authors for the COMET assay publication were identified as Farida Akcha (France) and Tim Bean (UK). Conception Martinex-Gomez (Spain) had already drafted a manuscript for stress on stress that should be forwarded to the TIMES editor for consideration. Dick Vethaak (Netherlands) was already working on the draft ER-Calux manuscript. Draft resolutions are required for the publication of these four manuscripts. In addition under 7 above, a revision to the existing TIMES 36 on lysosomal membrane stability is required.

Recommendation

Draft resolutions are requested for publication of TIMES methods on stress on stress, ER CALUX, COMET assay, condition index (and revision of NRR, see 7 above).

Action

WGBEC to consider publication of the integrated assessment strategy as a peer review publication.

11 Review progress from the ICES database subgroup and report advice to the ICES Data Centre (ToR i)

Several queries relating to reporting format rules and inclusion of new parameters for biological effects were received from the ICES Data Centre. These were addressed by WGBEC both intersessionally and during the meeting. The key decisions are recorded below. Queries can be grouped into 3 Categories: 1) ad hoc enquires relating to lack of clarity or unexpected difficulties experienced when entering biological effects data. 2) Residual issues relating to the inclusion of techniques required for application of the SGIMC integrated assessment approach. 3) New techniques required for the Baltic Sea 'BEAST' and 'BALCOFISH' project monitoring data storage.

11.1

11.1.1. An error was identified causing data entry confusion in the *JAMP Guidelines for Contaminant-Specific Biological Effects (OSPAR Agreement 2008–09)*. Pg 28 should state: Determination of imposex...

d. for *Nassarius reticulatus* the Relative Penis Length Index (RPLI) should be calculated as:

RPLI = (Average length female penis) / (Average length of male penis) x 100

11.1.2. Changes and additions were identified as required for the following DATSU checks:

- For all population level parameters for TBT effects (i.e., when MUNIT = 'index' and SEXCO = "X") a warning should be triggered if the condition "NOINP >39" is not met for parameters VDSI, INTSI, IMPSI and PCI. (this modifies a WGBEC 2010 decision).
- Following WGBEC 2010, for parameters EROD and CYP1A, if the condition "NOINP > 19"was not met, a warning would be triggered. This warning should now be removed as it was causing problems for data submitters and hindering population of the database.
- When reporting liver histopathology parameters, a warning should be added: "Report age when the data are available". This is to start encouraging the inclusion of fish age data to aid the interpretation of disease and effect data. Age is considered more relevant to disease and some effect progressions than size which for sentinel species is now known not to be a good proxy for age.

11.1.3. New Parameter Groups: Genotoxicity and Cytotoxicity parameters should be added to the parameter group B-MBA after expanding the definition to molecular/biochemical/cellular/assays.

11.2. New Parameters and DATSU checks required for SGIMC

11.2.1. DR-LUC (still in progress at the time of writing)

In the method record (21), the pre-treatment field (21:METPT), the method of analysis field (21:METOA), the test cell line field (21: VIVIT) and the reference document field (21:REFSK) must be filled in when a parameter (10:PARAM) for DR-LUC TEQ is reported. It will be a critical error check in DATSU, i.e. the data cannot enter the database unless all information is reported.

21:METPT must equal "Silica column" or "PL-Gel GPC column with dichloromethane"

21:METOA must be "measurement of light production by cell line"

21:VIVIT must not be blank

21:REFSK must not be blank

10:PARAM must be "DR-LUC TEQ" in Parameter group B-MBA. In the future when the method is ready, "ER-LUC TEQ" will be a new parameter which also fits in the above model.

10:MUNIT must be "pg/g"

10:BASIS must be "D"

10:MATRX can only be SEDtot", "SED2000" or "SED63"

11.2.2. COMET assay

There was some uncertainty over different pH values of lysis buffers used to run the analysis and how to distinguish between the different techniques when reporting data to the Data Centre. WGBEC advised that the following options should be available under METPT (method of pre-treatment).

- Alkaline lysis pH12.1
- Alkaline lysis pH >13
- Neutral lysis pH 7–10

11.2.3. Stress on stress response (mussels)

As a physiological response biomarker this should be included in the B-BIO parameter group. These data should be reported to the database on an individual basis as 'survival time' in days with a DATSU range check of 30 days as a maximum. The population / sample level metrics LT50 and TMM are best calculated from teh individual data in the database due to statistical requirements for data assessment.

11.2.4. Mussel condition index

This should apply to the following 3 species of *Mytilus : edulis, trossulus, galloprovincialis*. The appropriate matrix is whole organism (WO). When reporting for individuals, condition index is calculated from length and weight measurements (LMNEA on matrix WO for length). Weight is required for soft boby (Matrix SB) for some condition index calculations.

11.2.5. Mussel histopathology

Should be added to parameter group B-HST. For the parameter: 'Apidogranular caells in vesicular connective tissue' matrix should be gonad (GO). Examples of data submissions for all mussel histopathology parameters are to be provided for use as examples of good practice. John Bignell of Cefas UK was identified to provide.

11.3. New parameters required for BEAST / BALCOFISH

In order to include data for 'reproductive success in fish' the stage of embryo development needs to be reported. Discussions relolved around how to achieve this without disturbing existing fish egg development 'STAGE' codes.

11.3.1. A new field should not be added in ERF3.2 for "Reproductive stage/maturity" which was requested for the BEAST/BALCO data. Use ERF3.2 field "CONES" for deterioration, abnormalities and reproductive conditions.

11.3.2. WGBEC has no objection to adding the new Eggs and Larvae stages to RECO list «STAGE».

11.3.3. A new parameter group should be added for Reproductive Success "B-REP".

11.3.4. BEAST/BALCO parameters can now be added to RECO since reproductive stage and parameter group issues have been resolved.

Action

- WGBEC to maintain a working document on interactions with the data centre to record evidence of decision making with data centre queries.
- WGBEC to provide Data Centre with examples of data entry spreadsheets for mussel histopathology parameters.

12 Report progress from AQC subgroup and develop AQC procedures for biological effect methods including harmonisation activities initiated from WGBEC and within OSAPR, HELCOM and MEDPOL maritime areas (ToR j)

As has been reported in previous years there has again in 2011 been slow progress and activity in implementing AQC procedures for biological effect methods. This is becoming more of a problem as national monitoring organisations cut costs and reduce their monitoring effort and programmes. However the need for Quality Assurance for all methods still remains. Any method to be used for national or

international monitoring programmes must be AQC compliant, particularly as this is a requirement for submitting data to the ICES database. It is likely that the role of AQC will take on an even greater importance with the use of biological effect methods for monitoring GES (Descriptor 8) in the EU MSFD.

Current situation

The HELCOM BEAST programme ran an AQC exercise across methods and laboratories during 2009/2010, but with the completion of the data gathering this initiative ceased and no plans are in place to develop further AQC procedures. Countries involved in the MEDPOL programme have run intercalibrations on Lysosomal Membrane Stability (LMS) and Metalothionein, and although this does not take place annually it is an on-going process. In addition, in 2010 a joint ICES/OSPAR/MEDPOL LMS workshop was held in Alessandria, Italy. QUASIMEME have been unable to run imposex and bile metabolite intercalibration exercises over the past two years due to an insufficient interest and take-up from laboratories. In 2011, BEQUALM ran AQC programmes for bacteria (Microtox), fish disease, *Corophium*, phytoplankton and benthic community analysis, but because of a lack of uptake nothing on biomarkers.

Critical components of any AQC programme are regularity (annual or biannual) and cost. Following discussions in the Working Group in 2011 it was agreed to launch a low-cost programme for methods included in the integrated monitoring framework. In this respect Cefas UK had collected samples of liver and bile from wild caught fish and agreed to send these to interested parties for an intercalibration. Members of the group were asked if they were interested to receive samples for analysis. The interest was as follows:

EROD, 8 expressions of interest; 2 x Nr, Dk, Nd, Be, Es, 2 x UK

Bile, synchronous scanning, 5 expressions of interest; 2 x UK, Dk, Nr, Fr

Bile, HPLC, 4 expressions of interest; Nd, Nr, Ge, Nd

It was agreed that there was sufficient take up to send out the samples and Cefas would endeavour to action this in the summer of 2012. WGBEC would review the data on this exercise at its meeting in 2013.

The lack of uptake for the imposex AQC programme run by QUASIMEME was surprising and of concern. A round the table show of hands indicated that there was interest in taking part in another imposex AQC round, there were 7 expressions of interest, 2 x UK, Nr, Ir, Fr, Nd, Dk. Klass Kaag agreed to contact QUASIMEME to ask if they would reconsider running another round in 2012, pointing out the interest from members of WGBEC. If QUASIMEME were unable to run the intercalibration exercise then Klass Kaag agreed to run the intercalibration exercise whereby participants agreed to "courier collect" the test specimens from his laboratory. WGBEC would then review the data at its meeting in 2013.

Actions

Agenda item 12. WGBEC to conduct method intercomparison exercises during 2012/13 for EROD and bile.

Agenda item 12. Klaas Kaag to contact QUASIMEME with the information that several WG laboratories would commit to sign up for a ring trial on dogwhelk imposex. Should QUASIMEME not offer an imposex ring trial, WGBEC will conduct a small scale sample exchange.

13 Review recent developments relating to contaminant effects from litter /plastic particles (ToR k)

Dick Vethaak presented recent developments relating to contaminant effects from litter/plastic particles. The presentation was largely based on a recent review made by Deltares and the Institute for Environmental Studies (IVM) of the VU University Amsterdam (Leslie *et al*., 2011), updated with recent literature data and additional information provided by Thomas Maes (CEFAS). The review by Deltares and IVM was carried out for the Dutch Ministry of Infrastructure and the Environment in 2011 and focused on the occurrence and impact of microplastics in the wider North Sea and the development of suitable indicators for the implementation of the EU Marine Strategy Framework Directive. Litter including plastics is one of the MSFD descriptors of GES (Descriptor 10).

13.1 Background and developments

Plastic litter is major and persistent problem for the marine environment. In the UNEP Year Book (2011), plastic debris in the ocean is recognized as one of the three most pressing emerging issues for the global environment. In addition to the EU MSFD, there are several recent global actions/declarations for the prevention, reduction, and management of marine debris (e.g. ÙNEP Workshops, Marine Honolulu strategy and Manilla declaration). Plastic emissions from cities, landfills, factories and agricultural areas enter the sea via rivers and wind. A recent discovery is that microplastics (synthetic textile fibres) from washing machine waste water are polluting the open sea and beaches (Browne *et al*., 2011). Recently, synthetic fibres have been discovered also in Dutch sewage effluents (Leslie *et al*. in prep.). Marine waters also receive wastes directly from offshore activities, shipping and coastal tourism. Once in the marine environment, plastics are expected to gradually fragment into smaller pieces but will take centuries to completely degrade. This means since plastic production began early last century, all the plastic material that has entered the sea has not yet completely broken down. The current understanding is that this persistence leads to an accumulation trend of this type of marine litter. The general public is becoming familiar with the unsightly images of the plastic 'soup', seabirds dying with plastic debris in their stomachs, turtles and other marine life entangled in plastic debris.

The polymers in plastics are almost never pure. Plastics contain a cocktail of chemical compounds, such as plastic additives, which may reach out to the ambient environment or when ingested (Figure 13.1.). Additives give the plastic product a variety of desirable properties. Additives include plasticizers that make plastics flexible and durable, flame retardants, surfactants additives that enhance resistance to UV radiation and high temperature. These additives in common consumer products however contain complex mixtures of endocrine disrupting chemicals and other toxic compounds (Dodson *et al*., 2012). In addition, contaminants from other sources, such as PCB or pesticides, tend to absorb to plastics: the more hydrophobic a chemical, the greater its affinity for plastics. The above findings and facts implicate that chemical additives need to be considered as part of the potential ecological impact of macro and microplastics.

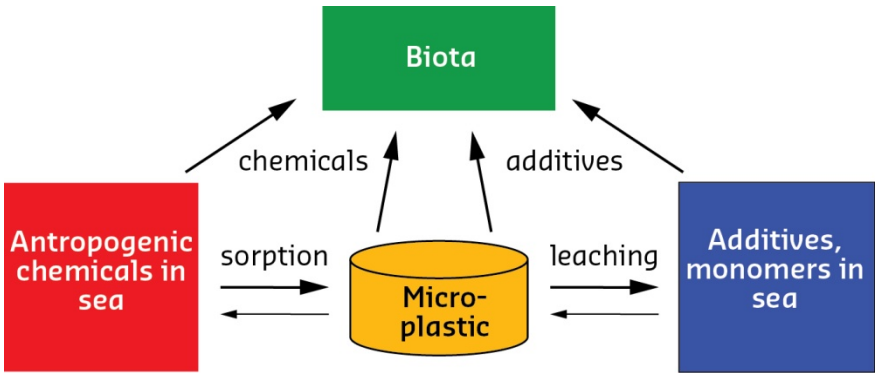

Figure 13.1. The partition of chemicals between (micro) plastic and biota and seawater (from Leslie *et al.*, 2011).

The sorption of POPs to plastic pellets have been suggested as a plausible explanation for the elevated levels of well-known toxic chemicals such as polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar polychlorinated biphenyls (PCBs) detected in albatross from remote areas of the Pacific Ocean (Tanabe *et al*. 2004) and in other seabirds (Ryan *et al*. 1988; Takada *et al*. 2006). Fries and Zahl (2011) recently showed that diffusion coefficients for PAHs are different for LDPE (Low Density PolyEthylene) and HDPE (High Density PolyEthylene): the lower the density the higher diffusivity. The authors concluded that considering the variety of polymer types, the polymer density should be taken into account when assessing the hazard of PAHs in marine environments. This study also demonstrates that lower density polymers shorten the equilibrium time and thus are more suitable for passive sampling.

A recent paper on biodegradation of plastics in sea turtle gastrointestinal fluids, experiments with three types of bags was performed over 49 days: standard, degradable and biodegradable bags (Muller *et al*., 2012). Biodegradable bags showed mass losses between 3 and 9%, while the degradation of the standard and the degradable plastic bags was statistically insignificant. The digestibility rate for the biodegradable bag (3–9%) is much less than that recorded for equivalent sized turtles consuming sponge (51–53%), implying that the breakdown rate may not be biologically significant enough to prevent a gut impaction.

Increasing attention on microplastics

In recent years there has been an increasing attention and growing concern about the tiny plastic fragments known as microplastics (particles < 5 mm in diameter including the micro-sized and much smaller nano-sized particles). Microplastics are created either by the weathering and fragmentation of mass-produced macro-sized plastic litter or are directly industrially produced as pellets and powders, polymer particles in personal care products and medicines, etc. The potential ecological and human health risks of microplastics are a new area of scientific research, and there is currently a large degree of uncertainty surrounding this question. Evaluating these risks requires knowledge both of exposure levels (i.e. the quantities of microplastics detected in the environment, including in living organisms) and of hazard (i.e. the toxicity of microplastics or their ability to cause adverse effects).

Investigations using current detection methods have so far identified microplastics contamination in North Sea sediments (offshore, harbours, beaches), North Sea water

(surface and 10 m depth) and North Sea marine life (Northern fulmars, crustaceans, fish etc.). Summary tables of field studies showing microplastic occurrence in water, sediment in the North Sea and on North Sea beaches are given in Tables 13.1–13.3.

Table 13.1. Microplastics in North Sea water. North Sea sediments and (from Leslie *et al.*, 2011, updated).

Sampling mesh size	Occurrence	Location	Reference
127 mm2 aperture in the CPR, scrolling 280 µm-mesh silkscreen	microplastic in CPR records increased since 1960, peak: 0.04 - 0.05 fibres/m3 (1980s)	Samples collected at 10 m over 40 years, shipping routes UK	Thompson *et al*. 2004
80 µm	150–2400 particles/m3	harbour and ferry locations in Sweden, depth 0–0.3 m	Norén 2008
450 µm	0.01 to 0.04 particles/m3	harbour and ferry locations in Sweden, depth of 0–0.3 m	Norén 2008
0.5–2 mm	102 000 polyethylene particles/m3	Harbour near polyethylene plant	Norén 2008
10–500 µm	Microplastic fibres in samples same concentration as control (0.2 to 1 particle/L)	Skaggerak, Norwegian S coast	Norén & Naustoll 2011
Continuous Plankton Recorder studies	microplastic widely detected, esp. in Southern North Sea	UK coastal areas, North Sea, N Atlantic	Edwards *et al*. 2011
Manta trawl surveys	Higher MP concentrations near estuaries	UK coastal waters, North Sea	CEFAS, unpublished

Table 13.2. Microplastics in North Sea sediments (from Leslie *et al.*, 2011).

Sampling mesh size	Occurrence	Location	Reference
Eckman grab, supernatant of saturated NaCl solution	Polymers detected in 23 of the 30 samples. Ca. 2.5 particles/50 ml sediment (estuarine) and ca. 5.5 (subtidal). Most plastic fragments were fibrous, colourful 20 µm in diameter.	subtidal, estuarine areas of the UK	Thompson *et al*. 2004
Eckman; supernatant of saturated NaCl solution; 80 µm mesh size	Between 2 and 332 ('hotspot') plastic particles were found per 100 ml.	3 Swedish coastal sites: Stenungsund, Tjuvkils huvud harbours (industrial)	Norén 2008
Sediment samples collected at strandlines, top 3 cm.	Between 1 and 8 particles per 50 ml sediment; higher density polymers more represented in samples than lower density	Tamar Estuary	Browne *et al*. 2010
Van Veen grab (0.1 m2 sampling surface)	Concentrations 124, 186 and up to 390 particles/kg dry harbour sediment (15–50 times higher than other similar areas). Offshore: 71–269/kg dw	Belgian harbours, sea stations	Claessens *et al*. 2011

Table 13.3. Microplastics on North Sea beaches (from Leslie *et al.*, 2011).

Sampling mesh size	Occurrence	Location	Reference
Sediment samples collected with small trowel (strandline)	• Polymers detected in 23 of the 30 samples. Ca. 0.5 particles/50 ml sediment. fibrous, 20 μm	17 UK beaches (some Irish Sea, some North Sea)	Thompson *et al*. 2004
Sediment samples collected at strandlines, top 3 cm.	1 to 8 particles/50 ml sediment; more higher density polymers than lower	Tamar Estuary (UK South coast)	Browne *et al*. 2010
Beach sediments, top 1 cm, 50 ml subsamples filtered	31 fibres /250 ml sediment (polyester>acrylic> PP> PE> polyamide)	UK (SW coast)	Browne *et al*. 2011
sediment cores (high water line, inter tidal, subtidal)	e.g. High water line (highest) 1.05 mg/kg dw 156.2/kg dw 0.46 95.9 0.49 124.2	3 Belgian beaches	Claessens *et al*. 2011

Microplastics in North Sea biota: field observations

The presence of macroplastics in wild seabirds, sea turtles, mammals and hundreds of other marine animals has been well documented and reviewed by a.o. Derraik 2002 and Thompson *et al*. 2009. Reports of microplastics in biota sampled in the field are rarer. 'Plastics' listed as prey item in UK marine fish have been identified in stomach content analysis n=22 cases since 1990 (Pinnegar and Platts 2011). Small plastic fragments have been found in 1% of 500 individual herrings from the Northern North Sea, in a pilot study by IMARES, 2010/2011. In another study, 83% of *Nephrops norvegicus* (n=120) from Clyde Sea, Scotland (W) had microplastic in their stomach (mainly filaments (Murray & Cowie 2011).

Hazards and effects of microplastics

From the above it is clear that marine animals in the wider North Sea and other areas are exposed to microplastics. Hazards of microplastics are more difficult to characterize because of: i) a worldwide lack of dedicated studies; ii) the fact that particle toxicity is size- and shape-dependent; iii) the fact that toxicity is also dependent on the specific chemical make-up of the microplastic particle (polymer, monomer, additives, sorbed contaminants); iv) the sheer diversity of possible types of microplastics in any given environmental matrix; v) the diversity of uptake routes and accumulation patterns in vastly different marine life forms and; vi) the challenges of studying the diversity of potential ecological effects (e.g. vectors for viruses and invasive species; food chain transfer; biogeochemical cycle effects, etc); (Leslie *et al*., 2011).

Several studies of the fate and pathology of ultrafine plastic particles in animal models and human cells, and human placental perfusion studies (to investigate transfer from mother to foetus) have provided particle toxicity data which is useful when assessing the hazards posed by microplastics (see Leslie *et al*., 2011). Living organisms are exposed to microplastics in the marine environment via various routes. Field and laboratory research has shown that microplastics are ingested and retained by marine organisms, after which size-dependent absorption into certain tissues may take place; food chain transfer of microplastics from prey to predator has already been demon-

strated in a field study. Many possible effects of exposure to microplastics have been postulated but these hypotheses must be tested with scientific rigour. The potential impacts of microplastics and their contaminant load (sorbed chemicals, monomers additives – which may constitute from ca. 4 up to 80% of the polymer end product) in the food chain, as well as the implications for ecosystems and human consumers, are a major concern.

Laboratory studies (for complete overview and references, see Leslie *et al*., 2011) are now also showing that microplastics are taken up by invertebrates, e.g. lugworms, amphipods and barnacles, mussels and sea cucumbers. Marine mussels – a species also used for human consumption - were exposed to seawater containing microplastics accumulated plastic particles in the hemolymph; once the particles were filtered out of the water column and ingested they were able to move from the gut to the circulatory system and be retained in the tissues (Browne *et al*. 2008). Graham & Thompson (2009) showed that benthic-dwelling sea cucumbers ingest a variety of shapes and sizes of microplastics. Sediments collected from the natural habitat of these animals contained 105–214 plastic fragments/L sediment (US Atlantic coastal zone), and preliminary chemical analysis showed the plastic particles were contaminated with PCBs. Another recent laboratory study by Teuten *et al*. (2007) has shown that plastics may be important agents in the transport of hydrophobic contaminants to benthic organisms such as lugworms. It is not yet known to what extent microplastics may be absorbed by plankton, although Bhattacharya *et al*. (2010) presented results of nano-sized plastic particles (20 nm) sorbing to phytoplankton.

Little data was found in the scientific literature on the occurrence of microplastics in marine mammals, with the exception of a study of fur seals by Eriksson & Burton (2003). Various species of fur seals on Macquarie Island consume the pelagic fish *Electrona subaspera* as a major prey species. Microplastics were observed in association with otoliths of these fish in the scat of various fur seal species, which the authors suggest would indicate a trophic transfer of these materials. Microplastics may potentially also be mistaken for food by large mammalian planktivores such as the blue whale. Once chemicals enter food chains, the top predators are often at extra risk because of the biomagnification and trophic magnification effects of some chemicals. If plastics and their associated contaminants enter food chains, humans may ultimately be at risk too (Talsness *et al*. 2009).

Reports of effects caused by microplastics or nanoplastics in marine taxa are as yet extremely rare (see Table 13.4). The marine mussel *Mytilus edulis* was exposed to microplastics between 1 and 80 ìm, which was absorbed by digestive gland vacuoles and various effects were observed, including granulocytoma formation (inflammation), an increase in haemocytes and a decrease in lysosome stability (Koehler & Von Moos, in Bowmer & Kershaw, 2010).

Bhattacharya *et al*. (2010) worked with nano-sized plastic beads and two species of algae (one freshwater and one marine/freshwater species) and found that sorption of nanoplastics to algae hindered algal photosynthesis and appeared to induce oxidative stress.

The stomach contents of wild Norway lobster contained microplastics that had formed tangled balls of filaments (most probably from the fisheries industry); (Murray & Cowie 2011). Galgani *et al*. (2010) suggest that polymer mass in the stomach 'unavoidably has mechanical and chemical consequences that affect their body condition with negative consequences for individual survival and capacity to repro-

duce'. Recent evidence indicates that food chain transport of polystyrene nanoparticles affects behaviour and fat metabolism of fish (Cedervall *et al*., 2012).

Table 13.4. The observed effects of exposed marine animals (from Leslie *et al*., 2011, updated).

Marine species	Microplastic exposure and effect	Reference
Mytilus edulis (marine mussel)	digestive gland vacuoles absorbed 1–80 μm microplastic with associated: granulocytoma formation (inflammation) increase in SB haemocytes after 48h decrease in lysosome stability after 48h	Koehler & von Moos in: Bowmer & Kershaw 2010
fresh/saltwater *Scenedesmus*	Nano-sized plastic beads; adsorption of nano plastics hindered algal photosynthesis and promotion of algal ROS (Reactive Oxygen Species) production is indicative of oxidative stress	Bhattacharya *et al*., 2010
Fulmarus glacialis (Northern Fulmar)	Sublethal or lethal effects of plastic in stomach were not tested but suggestions were made for potential endocrine disruptive effects	• Van Franeker *et al*., 2011ab
Halobates sericeus (pelagic insect)	Analyzed 90 samples from four cruises. Found strong positive relationship between abundance of *H. sericeus* and plastic debris in the North Pacific Central Gyre in 2009 but no causal relationship or ecological effects could be tested within the study design.	http://amnh.com/nationalcenter/youngnaturalistawards/2011/marci.html
Carassius carassius (Crucian carp) and other species	Food chain transport of nanoparticles affects behaviour and fat metabolism	Cedervall *et al.,* PLoS ONE 7(12) in press

Conclusions

It can be concluded from the above that:

(1) The current state of knowledge on microplastic abundance/distribution in the North Sea is (very) limited;

(2) Marine organisms are exposed to microplastics, but the biological effects are insufficiently studied.

MSFD GES 10 context

Vethaak presented the conclusions and recommendations and research priorities in support to MSFD Descriptor 10 of the Technical Subgroup GES 10 2012 report (MSFD TSG GES 10, 2012) to WGBEC. Descriptor 10 of the MSFD states that "Amount, distribution and composition of litter including microplastics do not cause harm to the coastal and marine environment.

The Technical Subgroup on GES 10 (TSG GES 10, 2012) published recommendations for sampling, analysis and monitoring of litter, e.g. including macro and microplastics. The distribution of litter is highly variable, which needs to be taken into consideration for monitoring programmes. It is necessary to identify the activity to which it is linked including, where possible, its origin. There is still a need for further development of several indicators, notably those relating to biological impacts and to micro-particles, as well as for the enhanced assessment of their potential toxicity. Indicators and tools for GES 10 proposed by the Technical Subgroup in their 2012 report are:

- Litter washed ashore/discarded on coastlines;

- Litter in the water column or deposited on the sea floor;
- Litter ingested by stranded and dead marine mammals and turtles (stranded) and by fish (currently insufficient data to assess impacts). Tools: Fulmar, Shearwater, Sea turtle (available); fishes, seals and crustaceans (to be developed);
- Microparticles (only little data available). Tools: MP in water (CPR); MP in subtidal and intertidal sediment (to be developed).

For the implementation of the above indicators several sampling and analytical methods exist, but further development is required, i.p. for microplastics and impact indicators. Methods used for identification and qualification of microplastics in the marine environment have also very recently been reviewed by Leslie *et al*., 2011 and Hidalgo-Ruz *et al*., 2012.

13.2 WGBEC discussion

The review was well received by the WG. Research into micro- and nanoplastics as environmental pollutants is a rapidly emerging field and represents a new, major, complex global environmental problem and it was agreed by WGBEC that this could have adverse effects on the environment and on humans. The field was considered relevant to the work of WGBEC especially the environmental consequences of marine litter related chemicals, the impact and risks of microplastics. However WGBEC should focus on both the chemical and physical effects of macro and microplastics in marine organisms including those on turtles, marine birds and marine mammals in their future meetings. Therefore it was proposed that this item must remain high on the WGBEC agenda for the coming years, revisited on a yearly basis. Lastly, it was recommended that existing monitoring programmes/samples could be of great use for effect monitoring of microplastics especially when carried out along lines of the framework of the integrated monitoring framework being considered for adoption by OSPAR.

Jacob Strand mentioned the potential importance of other non-plastic litter. In relation to the discussion of sources and potential impact of other types of anthropogenic particles like soot and other black carbon particles together with PAHs, attention was also put to the support of the IMO (International Maritime Organisation) for the implementation of the scrubber method for reducing atmospheric emissions from commercial shipping. However, some concern was raised that this will lead to an increased levels of marine discharges of black carbon particles and PAH, especially in relation to shipping lanes and coastal areas with dense shipping activities.

Klaas Kaag reported on plastic research by IMARES. For several years now Jan Andries van Franeker coordinates the research on plastic as cause of death in Fulmars found along North Sea shores. Fulmars are especially vulnerable for floating plastic particles, as they pick their prey from the sea surface. Recently three other projects have been started by Edwin Foekema, focussing on much smaller micro- and nanoplastics. In 2010, stomach contents of more than 1500 fishes from the North Sea were analysed for the presence of plastic particles. In the pilot, fish were taken from discards samples brought in by Dutch fishers. For the final research fish were collected during an 8 week survey on the North Sea, roughly covering Northern, Central and Southern parts of the North Sea. The full results of this research will be published during 2012. In cooperation with prof. A.A. Koelmans of Wageningen University, an experimental study was conducted on the response of lugworms to the presence of plastic particles in the size range of the sediment in the Wadden Sea. Parameters were

not only feeding rate, growth and mortality, but also the accumulation/elimination of PCB's in the sediment. In another experiment, the influence of nanoplastics on the feeding rate and efficiency of mussels is investigated. Although the size of these particles is assumed to be too small to be collected by mussels, it appeared that the mussels were able to remove the particles from the water.

The results of these studies are currently being analysed, and are expected to be published before the end of 2012.

WGBEC concluded that more field research is necessary to identify the nature and scale of the problem in the North Sea, including attention to sediments, the latter of which are suspected to be sinks. Finally, the rapidly emerging field of microplastics will be of relevance to other ICES WGs (WGMS, WGMS) and WGBEC through ICES HQ should make them aware of this.

Relevance to WGBEC

The relevance of litter, esp. plastics and microplastics (MPs) to WGBEC can be summarised as follows:

- Several WGBEC members are actively involved in plastic pollution monitoring and research (FP7, INTERREG, national initiatives).
- Plastic litter and environmental consequences of litter related chemicals is a field of increasing concern.
- Especially micro-sized particles have the potential to enter biological membranes and food chains and may have implications for ecosystems and human health (through consumption of fish and shellfish).
- MPs are clearly persistent, bioaccumulate to various degrees in living organisms and are potentially intrinsically toxic and can be transported over long distances. Therefore MPs should be taken into account when assessing the hazard and risk of chemical contaminants in marine environments.
- MPs represent a new and topical niche for WGBEC but also attention should be given to higher-level marine organisms, e.g. turtles, birds and seals.
- There is a partial overlap with the environmental issue of nanoparticles.

WGBEC proposed the following multi annual ToR for future meetings:

- WGBEC should annually evaluate the results of monitoring and research activities on plastic litter in the North Sea and the marine environment abroad in regard to:
    - Status on development of tools to measure (micro)plastics in marine organisms;
    - Results of impact assessment surveys and research projects;
    - Status on biodegradation processes of plastic litter and environmental consequences of litter related chemicals;
    - Evidence of bioaccumulation and adverse physical and chemical effects of microplastics and associated contaminants on marine organisms, populations and communities;
    - Evidence of microplastics and associated contaminants to transfer through marine food chains;
    - Hazard and risk assessment approaches for (micro) plastic litter and associated contaminants.

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13.3 Recommendations/Actions

- ICES secretariat, in alliance with SG on Marine Litter should report to OSPAR on the opportunities and benefits to incorporate the monitoring of microplastics in sediment and biota (i.e. fish, crustaceans) in existing JAMP/CEMP chemical and biological effects monitoring programmes. Apart from being cost-effective, this would allow a direct (integrated) assessment of their potential physical and chemical effects and ecosystem health consequences.
- ICES HQ should encourage Member States to submit data on type and concentrations of microplastics and associated chemical contaminant con-

centrations to the ICES Data Centre. The ICES Data Centre in alliance with SG on marine Litter, WGMS and WGMC should prepare the entrance of microplastic data in the Environmental Data Base.

- ICES HQ should bring the agenda item of microplastics and associated chemical contaminants forward to WGMC and WGMS.

Multi annual ToR WGBEC

- WGBEC should annually evaluate the results of monitoring and research activities on plastic litter in the North Sea and the marine environment abroad in regard to:
    - Status on development of tools to measure (micro)plastics in marine organisms;
    - Results of impact assessment surveys and research projects;
    - Status on biodegradation processes of plastic litter and environmental consequences of litter related chemicals;
    - Evidence of bioaccumulation and adverse physical and chemical effects of microplastics and associated contaminants on marine organisms, populations and communities;
    - Evidence of microplastics and associated contaminants to transfer through marine food chains;
    - Hazard and risk assessment approaches for (micro) plastic litter and associated contaminants.

Recommendations

- ICES Secretariat should report to OSPAR on the opportunities and benefits to incorporate the monitoring of microplastics in sediment and biota (i.e. fish, crustaceans) in existing JAMP/CEMP chemical and biological effects monitoring programmes. Apart from being cost-effective, this would allow a direct (integrated) assessment of their potential physical and chemical effects and ecosystem health consequences.
- The ICES Data Centre together with WGBEC, WGMS and MCWG should prepare the entrance of litter and microplastic and associated contaminants data in the Environmental Data Base, to prepare for likely future requirements for assessment across the ICES region and reporting under MSFD Descriptor 10.
- ICES Secretariat should bring the agenda item of microplastics and associated chemical contaminants forward to MCWG and WGMS.
- That a Theme Session on litter and microplastics in the marine environment be proposed for 2013.

Action

Prepare a Theme Session proposal on litter and microplastics at the ASC 2013 (Dick Vethaak and others to be identified).

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

WGBEC had reviewed its potential for collaboration with other WGs in 2010 and 2011 and produced a table of possible liaisons as shown below in Table 14.1.

After the announcement of the 2012 ToRs for each WG the Chairs of WGBEC intersessionally reviewed these to identify possible areas of interest and collaboration.

Table 14.1. ICES WGs identified as potential for collaboration and availability of ToR for review.

	2012 ToR	Worked before?	Interested in joint activity?	Joint meeting?
WGPDMO	Reviewed	Yes	Yes	Yes
MCWG	Reviewed	Yes	Yes	Potential
MSWG	Reviewed	Yes	Yes	Potential
ICZM	Is this WGMPCZM below?	No	Potential	No
SGONS	Not found	No	No	No
WGMASC	Reviewed	No	No	No
WGEIM	Reviewed	No	Yes	Potential
WGHABD	Reviewed	No	Potential	No
WGEXT	Not found	No	No	No
WGFCCIFS	Not found	No	No	No
WGAGFM	Reviewed	Yes	Yes	Potential
WGEEL	Reviewed	No	Yes	Potential
WGMME	Reviewed	No	Yes	No
SGIMC	Dissolved	Yes	Yes	No
SGEH	Dissolved	No	Yes	Potential
MEDPOL	elsewhere	Yes	Yes	Yes
BEWG	Reviewed	No	Potential	No

WGBEC had liaised with Francois Galgani in relation to the ICES litter group but this was a one off meeting and should this be reformed into an EG then WGBEC would re-establish collaborative links.

Review of ToRs from other WGs and in relation to potential for collaboration

ToR of interest to WGBEC is listed and comment added for possible areas of interest.

WGEEL – Joint EIFAC/ICES Working Group on Eels ToRs 2011

(Note this is ACOM)

2011/2/ACOM19 The **Joint EIFAC/ICES Working Group on Eels** (WGEEL), chaired by Russell Poole, Ireland and Cedric Briand, France will meet in September 2012, to (ToRs to be updated):

c) Develop methods for the assessment of the status of local eel populations, the impact of fisheries and other anthropogenic impacts, and of implemented management measures; test data scenarios at the local level;

d) Provide practical advice on the establishment of international databases on eel stock, fisheries and other anthropogenic impacts, as well as habitat and eel quality related data, and review data quality issues and develop recommendations on their inclusion, including the impact of the implementation of the eel recovery plan on time-series data and on stock assessment methods;

Comment: good potential here to collaborate. We have reviewed this subject at previous WGBEC meetings. Note this group will not meet until Sept 2012.

WGMME – Working Group on Marine Mammal Ecology

(Note this is ACOM)

2011/2/ACOM29 The **Working Group on Marine Mammal Ecology** [WGMME] (Chair: Eunice Pinn, UK) will meet at ICES headquarters in Copenhagen, Denmark from 5–8 March 2012 to:

a) Review and report on any new information on population sizes, population/stock structure and management frameworks for marine mammals;

Comment: WGBEC could suggest that we collaborate to look at contaminants in marine mammals and any associated bio effects – WGBEC did something similar in the past in Canada and also more recently with biomarkers in mammals.

Working Group on Pathogens and Diseases of Marine Organisms (WGPDMO)

2011/2/SSGHIE03 The **Working Group on Pathogens and Diseases of Marine Organisms** (WGPDMO), chaired by Simon Jones, Canada, will meet in Lisbon, Portugal, 31 January–4 February 2012 to:

b) Provide a review on disease interactions between farmed and wild marine finfish species with emphasis on potential threats;

e) Provide a progress report on the Fish Disease Index (FDI) in relation to 1, its implementation in marine monitoring and assessment programmes; 2, the application and further development of assessment criteria; and 3, results of FDI assessments carried out intersessionally addressing diseases of flounder and Baltic cod and data on liver histopathology and macroscopic liver lesions in the common dab;

Comment: For b) WGBEC could suggest looking at risks associated with chemical used in aquaculture as a threat and potential for bio effect and also for organisms close to where these chemicals around fish farms are used. For e) WGBEC has interest in the progress of the use of FDI in relation to contaminants and the SGIMC integrated approach for contaminants and biological effect.

Marine Chemistry Working Group (MCWG)

2011/2/SSGHIE05 The **Marine Chemistry Working Group** (MCWG), chaired by Katrin Vorkamp, Denmark, will meet in Southampton, UK, 20–24 February 2012 to:

b) Review of Environmental Assessment Criteria or equivalents (OSPAR request 2012/2):

> i) Review scientific robustness and update, as necessary, EACs or equivalent effects levels calculated for CEMP and pre-CEMP determinands.

e) Water Framework Directive and Marine Strategy Framework Directive:

i) Report on the developments in Water Framework Directive monitoring programmes, including statistical methods for compliance checking of Environmental Quality Standards;

ii) Report on developments under the Marine Strategy Framework Directive, including information on initial assessments in member states;

i) Chemical oceanography, with focus on ocean acidification:

j) Contribute, as may be required, to ICES activities on integrated chemical and biological effects monitoring and review new information on effect directed chemical analysis;

k) Emerging contaminants:

i) Report on new information regarding emerging contaminants in the marine environment;

ii) Discuss the role of atmospheric transport and deposition for the assessment of inputs of PFOS and other PFCs to the marine environment;

m) Report on new information on passive sampling of contaminants in the marine environment;

Comment: For b) with the re write of the JAMP as an integrated approach there be will be a need to ensure chemistry is strongly linked to biological effect in terms of sampling design, field sampling, data collection and reporting. e) ii) EUMSFD Descriptor 8 on contaminants and their effects and developing GES is common to both MCWG and WGBEC. i) acidification has been a previous agenda item for WGBEC. j) aspects of the SGIMC scheme we could consider liaison would be effect directed methods that may be used i.e. bioassay , YES Calux etc . WGBEC has written TIMES and Bgd docs on these subjects. k) emerging contaminants is frequently on WGBEC agenda, possibly collaborate in terms of associated effect methods. m) WGBEC has a great interest in this work area in relation to bioavailability, use of caged mussels for biological effect measurements alongside passive sampling.

Working Group on Marine Sediments in Relation to Pollution (WGMS)

2011/2/SSGHIE06 The **Working Group on Marine Sediments in Relation to Pollution** (WGMS), chaired by Patrick Roose, Belgium, and Lucía Viñas, Spain, will meet in Lisbon, Portugal, 12–16 March 2012 to:

Passive Sampling

g) Start work on a review of the use of passive sampling for measurements in sediments and approaches to the estimation of pore water concentrations;

h) To continue the work on passive sampling as a proxy for partition coefficients for organic contaminants in sediments;

i) To report on ongoing and new projects involving passive sampling.

Comment: For g) h) and i) WGBEC has interest in the use of passive samplers in relation to sediment biological effect techniques

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

2011/2/SSGHIE09 The **ICES-IOC Working Group on Harmful Algal Bloom Dynamics** (WGHABD), chaired by Bengt Karlson, Sweden, will meet in Oban, Scotland, UK, 24–27 April 2012 to:

Comment: No ToR identified. A potential area of interest could be the use of biological effect tools / markers to investigate changes in water quality associated with algal blooms

Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM)

2011/2/SSGHIE12 The **Working Group on the Application of Genetics in Fisheries and Mariculture** (WGAGFM), chaired by Dorte Bekkevold, Denmark, will meet in Bilbao, Spain, 2–4 May 2012 to:

Comment: No ToR identified for collaboration.

SSGHIE draft resolutions to be submitted for intersessional approval by SCICOM at next mid-term meeting

- Working Group on Environmental Interactions of Mariculture (WGEIM)
- Working Group on Marine Shellfish Culture (WGMASC)

Comment: ToRs not available

Working Group for Marine Planning and Coastal Zone Management (WGMPCZM)

2011/2/SSGHIE07 The **Working Group for Marine Planning and Coastal Zone Management** (WGMPCZM), chaired by Andreas Kannen, Germany, will meet at ICES Headquarters, Copenhagen, Denmark, 20–23 March 2012 to:

Comment: No ToR identified for collaboration.

Benthos Ecology Working Group (BEWG) Suggest look at benthos in Integrated Scheme

2011/2/SSGEF07 The **Benthos Ecology Working Group** (BEWG), chaired by Steven Degraer, Belgium, will meet in Sandgerdi, Iceland, 7–11 May 2012 to:

Comment: No ToR identified for collaboration. However WGBEC would be interested to collaborate and investigate how benthic ecology could be applied to the SGIMC integrated approach.

Collaboration and links

Following this review positive links were made with the Chairs of some working groups and these are listed below with suggested actions, but these still require confirmation:

A common ToR for MCWG, WGMS and WGBEC is passive sampling. Matt Gubbins had been in touch with Katrin Vortkamp (Chair of MCWG) and a concurrent meeting, also to include WGMS, has been suggested in 2014 at ICES, Copenhagen, to review the state of development and application of passive samplers, how they can be used for marine monitoring purposes, how they fit in with the MSFD, the SGIMC integrated approach and the development of assessment criteria for passive sampler data. In addition, there is also a common interest in discussing contaminants and effects associated with marine litter.

WGEEL conduct a review of contaminants each year and produce an eel quality report. Matt Gubbins had been in contact with Russell Poole (Chair of WGEEL) and a common area of interest proposed was the comparison of BACs and EACs and to establish if biological effect methods could be applied. WGEEL has identified a person to look at this and collaborate with WGBEC; WGBEC identified Dick Vethaak

and Jim Readman (to be confirmed) as collaborators. This collaboration is to take place intersessionally.

WGBEC noted that an ICES/OSPAR group on ocean acidification has been proposed and it was felt that WGBEC should contact them once they are established.

WGBEC noted that WGDPMO was in the process of changing aspects of the Fish Disease Index (FDI). Although no direct contact had been made with WGDPMO it was agreed that members of WGBEC would look at the changes intersessionally and contact WGDPMO as appropriate.

In the past WGBEC had often collaborated with the WG on statistics, but this group was disbanded. Members of WGBEC were of the opinion that such a group was still necessary and could play an important role in the development and application of the SGIMC integrated approach and the development of sampling design and monitoring strategies for the revision of the JAMP.

Recommendation

Agenda item 14/15. That MCWG, WGMS and WGBEC hold a concurrent meeting in 2014 with a full day joint plenary to address common areas of interest:

a) To define the role of passive sampling in integrated monitoring and assessment (sampling strategy, assessment criteria, deployment alongside bioindicator species) and use of toxicity tests on passive sampler extracts in monitoring programmes.
b) Microplastics

Actions

That WG members DV, UK, JT liaise with WGEEL and MCWG members on the eel quality status report intersessionally and report to WGEEL 2012 and WGBEC 2013.

WGBEC chair to liaise with WGMME chair to discuss possible joint working on assessment of risk to marine mammals from contaminant burdens in fish and possible techniques for assessing effects of contaminants in mammals.

15 Report on collaboration with other WGs as identified at 2011 meeting and any intersessional activity/representation (WGEEL, WKMAL, WGMMAL, MCWG etc.) - (ToR e)

This agenda item appears as a duplicate in the ToR for WGBEC, i.e. same as ToR l) and is reported above (see agenda item 14).

16 Consideration of issues of special scientific interest/ value (ToR g)

16.1 Acidification in marine waters in relation to contaminants and biomarker response

At the 2011 meeting it was recommended that the group should identify suitable methods for determining the effects of acidification on marine organisms. Two presentations addressed this subject.

Klaas Kaag (NL) described the EU-project RISCS on the risks of subsurface CO_2-storage and the results of the 2011 mesocosm experiment conducted by IMARES. RISCS is a European project which aims to improve our understanding of the possible environmental impacts of geological storage of CO2. There are 24 organisations participating in RISCS including research institutions, industry environmental asso-

ciations and the International Energy Agency Greenhouse Gas R & D Programme. The project is designed to study a wide range of potential impacts, thus providing tools for developing appropriate legislation and helping to ensure the safe management of CO2 storage sites. More information can be found on http://www.riscs-co2.eu/. The marine part consists of field monitoring (University of Rome, geology; OGS Rome, physiological effects, biomarkers), laboratory research (Plymouth Marine Laboratory UK, physiological biomarkers, benthic cores), experimental mesocosms (IMARES, NL), field experiments (PML, NIVA Norway).

Summary of experimental mesocosm study at IMARES: All experimental ecosystems were created by adding 150 cm sea water layer onto 20cm marine sand. Larger animals were added. The experiment consisted of 2 control systems and 3 replicated treatments. All systems had a continuous air flow and 3 fixed CO2-flows were created, resulting in 3 different pH levels. Exposure lasted for over two months. In all CO2 treatments, primary production was stimulated. In the high CO2 exposure, the pH remained below 7.5 throughout the whole period. In these systems the most obvious negative effect were seen. Most remarkable was the fact that the shells of periwinkles and cockles showed clear effects of dissolving. Population development of some smaller species was clearly reduced.

In the other two treatments, the effects were less clear. Some species seemed negatively impacted, especially on the medium CO_2 flow, but others showed a positive reaction. This is probably due to increased food availability and maybe also by competitive interactions. In 2012, a small mesocosm experiment will be initiated aimed at fluctuating exposure. The idea is to simulate a plume moving back and forth due to tidal movements.

Steinar Sanni (N) described on-going research at IRIS on the interaction between acidification and oil pollution. In one series of experiments larvae of the sea urchin *Strongylocentrotus droebachiensis* were either exposed to acidified sea water (pH 7.6), 0.5 mg/l oil, or both. It appeared that acidification at this level had no significant effect on the parameters assessed, but oil exposure had. The effect of oil exposure was not modified by acidification. In a follow-up experiment the shrimp *Pandalus borealis* will be exposed to acidified water, oil and slightly increased temperature, in order to assess multiple interactions.

Lucia Guilhermino (P) remarked that experiments have been started on the effects of pyrene on several biomarkers of the common gobby (*Pomatoschistus microps*) in relation to pH changes in laboratory bioassays. Fish were exposed to pyrene for 96h to different concentrations of pyrene, using filtered seawater as test media, at different pH. Relatively to fish maintained at pH 8.15, those maintained at 7.75 had increased LPO levels (control treatments in both cases). Increased LPO levels were also found in fish exposed at 0.25 mg/l of pyrene at pH of 7.75 relatively to those exposed at the same concentration of pyrene at water pH of 8.15.

The results shown so far, indicate that significant ecological effects of acidification may only occur when the pH is reduced to below 7.5. Some biomarkers may, however, show a response at higher pH levels. Furthermore it was suggested that compared to oceanic organisms, the coastal species used may be more resilient with regards to reduced pH levels because they are adapted to living in a more fluctuating and challenging environment. On the other hand, none of the group members present is aware of studies showing clear effects at the levels of ocean acidification expected. There may be a lot of speculation.

In light of this, WGBEC would support the establishment of a Study Group on Ocean Acidification. The group should summarize existing effects data and relate these to current and expected levels of marine pH. The group stresses the importance of interaction of ocean acidification with other chemicals as are addressed by WGBEC.

16.2 Effects of contaminants on primary production, including phytotoxicity

In the Netherlands, two related PHD studies focus on the effects of chemical stressors on marine algae. These studies are carried out at the VU University of Amsterdam (IVM) and the University of Amsterdam (IBED) in collaboration with Deltares. These are the first studies which investigate the mixture toxicity of anthropogenic compounds and natural algal toxins to marine primary producers. Toxicity of two anthropogenic compounds (Tributyltin (TBT) and Irgarol) and two natural toxins (Decadienal and microcystin) was investigated. Test were performed with three marine micro-algal species (*D. tertiolecta, P. tricornutum, T. pseudonana*) in 96 wells plates and the toxic effect was determined by Pulse Amplitude Modulation (PAM) fluorometry after 4.5 hours. After 10 minutes of incubation with actinic light the reduction in effective photosystem II (PSII) efficiency was determined as a measure for toxicity. Preliminary results showed a species, compound and mixture specific response on effective PSII efficiency which underlines the complexity of determining the toxic pressure of coastal waters on the primary producers. Other natural and anthropogenic compounds will be tested to provide a better understanding in the role of the natural toxins on the overall toxicity. Additional recovery experiments will be performed to investigate if the algae can recover after removal of the toxicant and algal viability will be tested to determine if the exposure is lethal. Future studies will include EDA analysis to identify relevant, but yet unknown, PSII-responsive contaminants and to assess community-level effects in situ experiments using flow cytometry.

The Toxprof project evaluated the toxicity profiles of six oils and one HNS (styrene monomer) as being representative of the different oils that are transported through EU waters. Bioassay profiling of the water accommodated fractions (WAF) of the oils provided a toxicity ranking. Microalgae and macroalgae bioassays were less sensitive than invertebrate animal bioassays. The results of short will be published later this year.

In the scope of the project "RAMOCS – Implementation of Risk Assessment Methodologies for Oil and Chemical Spills in the European Marine Environment" (ERAC-CT2005- 016165, within the framework of the EU ERA-Net initiative, 6th Framework Program), the effects of three PAHs (anthracene, naphthalene and phenanthrene) on the microalgae *Tetraselmischuii* were investigated in 96h laboratory bioassays carried out at two different temperatures (20 and 25ºC); (Vieira and Guilhermino, in press). Bioassays were carried out following the general OCDE guideline 201 procedure (OECD, 2006). Briefly, for each substance two independent bioassays were carried out: one at 20 and the other at 25ºC, in temperature and photoperiod (24h solar spectrum light); a control with f/2 medium and another with acetone were included in each assay; test recipients were 500ml glass beakers filled with 400 ml f72 medium; test concentrations ranges were: 0.09–5.76 mg/l for anthracene; 0.045–2.88 mg/l for naphthalene and 0.035–2.24 mg/l for phenanthrene. Population growth inhibition was used as effect criteria. The main results are indicated in table 1.

Table 1. Median effect concentrations (EC50) for population growth inhibition obtained for each PAH at both 20 and 25ºC.95% CI within brackets. (Data from Vieira and Guilhermino, in press).

	EC50s	
TESTED PAH	20ºC	25ºC
Anthracene (mg/l)	**3.326** (2.718–4.233)	**2.145** (1.734–2.743))
Naphthalene (mg/l)	**1.813** (1.571–2.136)	**0.992** (0.788–1.301)
Phenanthrene (mg/l)	**1.316** (1.032–1.729)	**0.262** (0.236–0.291)

All the PAHs tested were found to inhibit the growth of *T. chuii* at concentrations in the low ppm range, thus at concentrations considerably higher than those that have been found in marine waters. The 5ºC raise of temperature increased the toxicity of all the PAHs tested to the microalgae. Although these concentrations are higher than those expected to occur even in oil spills in the marine environment, the results of table 1 raise concern on the combined effects of temperature and PAHs on marine producers, especially in shallow waters where water temperature may show considerable fluctuations along the year.

16.3 Review of species differences in bioassay and biomarker responses, e.g. as seen in assessment criteria currently being developed – also to include sources of species for testing

Iria Durán and Ricardo Beiras (ES) presented data on comparison in sensitivity between different bioassays with marine organisms carried out at the Universidade de Vigo. Figure 16.3.1 shows the variation between species for EC50 values for several chemicals. The sensitivity depends on both the species and the chemical. A table containing toxicity values for selected compounds is attached to this document as Annex 7.

The highest amount of data included in the database corresponds to sea urchins and bivalves. Comparison between the sensitivity of both groups to a broad range of toxicants, including metals, PAHs, and different biocides, yields a correlation with $r^2=0.87$ and $p<0.01$, and a slope of the double logarithmic regression line of 1.02 (see Figure 16.3.2).

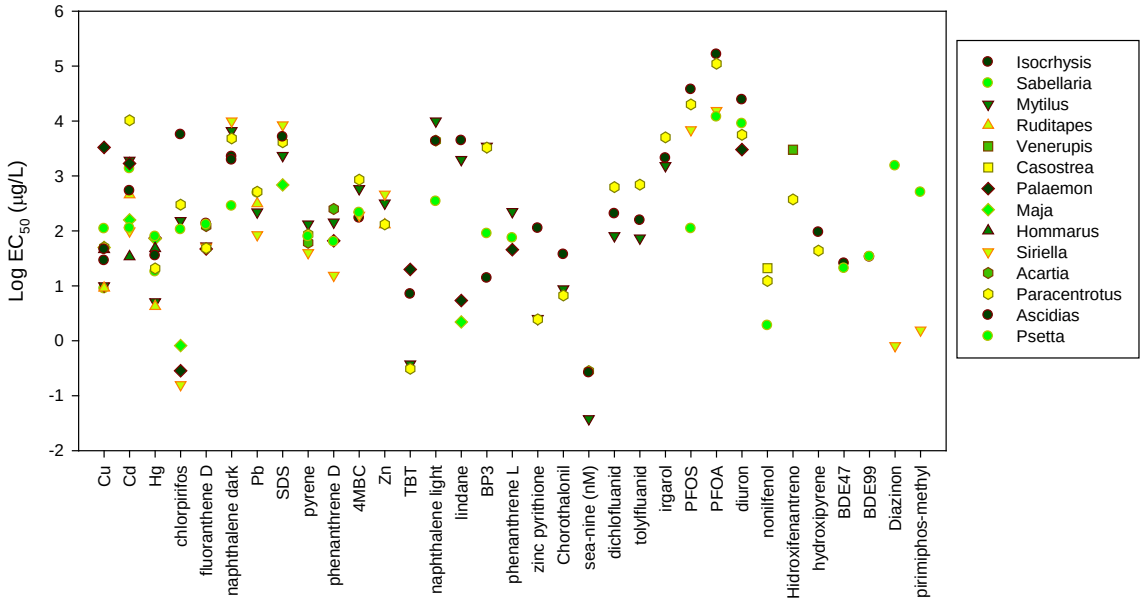

Figure 16.3.1. Variation between species for EC_{50} values for several chemicals between sea urchins and bivalves.

Steinar Sanni (N) presented data for comparison of species differences in biomarker responses. The data were taken from experiments with different marine organisms to one month oil exposures at 3–5 oil concentrations. The experiments were carried out at IRIS laboratory in Stavanger. In these data the biomarkers were divided into different groups related to: Detoxification system I (EROD and Cyp1a), PAH metabolites (Fixed Fluorescence Naph., Pyr., BaP type metabolites and TPAH metabolites GC/MS), Genotoxicity (DNA adducts, Comet assay, Alkaline Unwinding assay and Erythrocytic Nuclear Abberations), Oxidative stress (GST, Catalase and TOSC), Lysosomal Membrane Stability (NRRT and Histochemical LMS), Immunotoxicity (White blood cells, and Respiratory burst), and Histology (Gill histological changes). The comparisons for the three last mentioned groups must be regarded as tentative since less than four species were tested within each group and some of these biomarker methods were rather novel in the context used. In addition, data on larval mortality to similar oil exposures have been included as a measure of Fitness effects, and these were augmented with some literature data of similar kind.

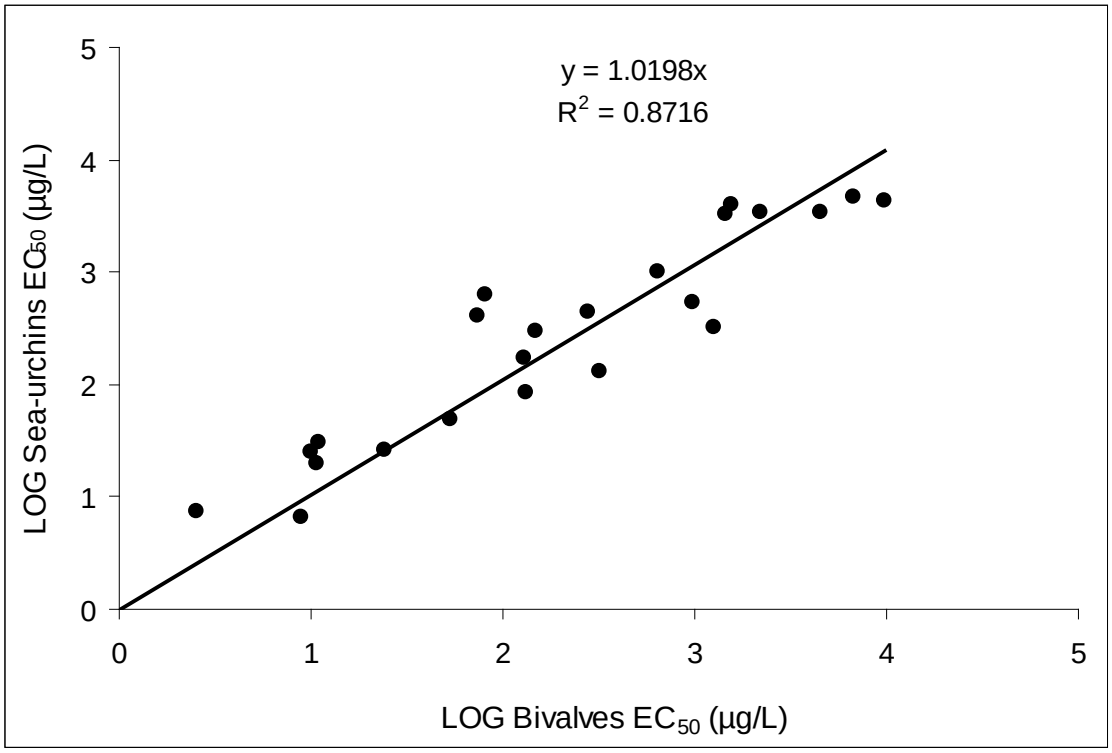

Figure 16.3.2. Comparison between the sensitivity of Bivalves and Sea urchins.

The comparisons are made on the basis of the lowest observed biomarker response concentrations ("LOBCs"), similarly determined as the lowest observed effect concentrations for the Fitness effects (LOECs). The species variation is expressed as Coefficient of variation (CV; %) within each group of Biomarkers. The results are shown in Figure 16.3.3.

It provides a quantitative indication of the expected magnitude of species variation for these different Biomarker groups. Most of the variation coefficients are within the range 50–150%. This is quite high and important to be aware of in cases where biomarker assessments are based on other species than the ones that have assigned BAC assessment criteria. These are still around three times lower than the species variation in Fitness effects. In case of EAC assessments in other species than those with assigned EAC values the species variations in Fitness effects should also be taken into consideration additively to the biomarker species variation.

The presented data were generated to establish the basis for biomarker assessment criteria based on Species Sensitivity Distributions (SSDs) for biomarker responses. In this approach, called "Biomarker Bridges" the species variations are integrated in the assessment criteria. Its principle is to translate obtained biomarker monitoring values into fitness effect values by combining the SSD functions for biomarkers and whole organism (Fitness) effects. This can also provide links to (probabilistic) risk values. This concept was described by Smit *et al*. (2009), and publication of the established Biomarker Bridge curves is under preparation (Sanni and Smit, *in prep*.). The Project was funded by the Research Council of Norway under the HAVKYST - PROOFNY program (178408).

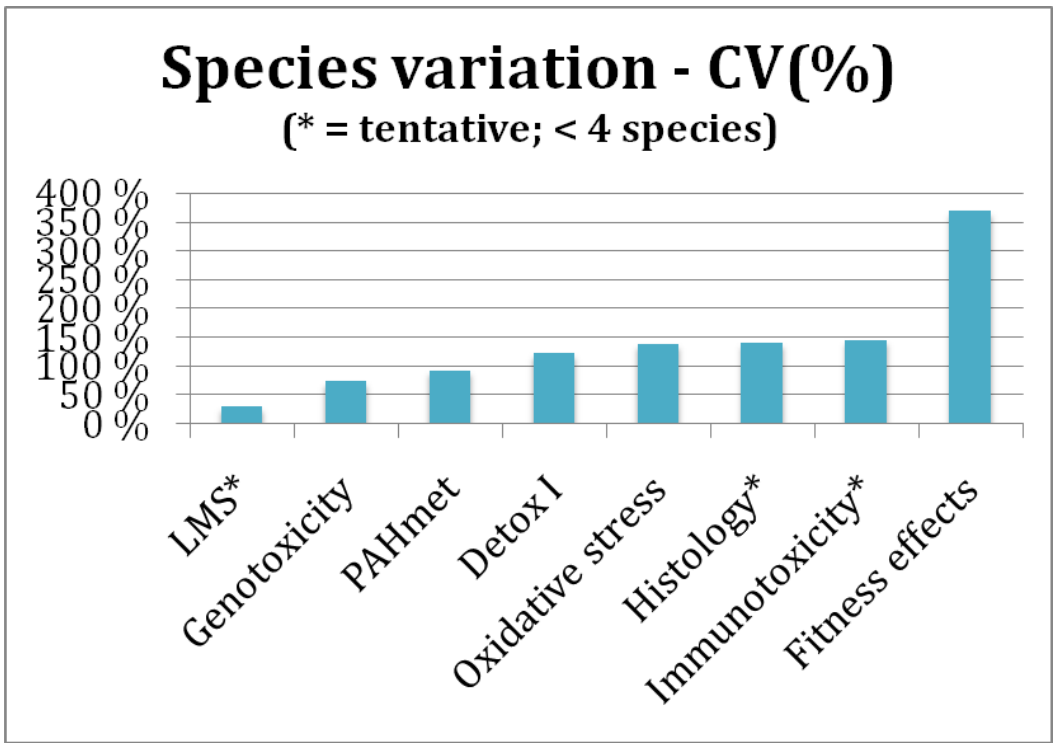

Figure 16.3.3. shows the species variation in biomarker responses to oil based exposures (see text for details).

References

Smit MGD, Bechmann RK, Hendriks AJ, Skadsheim A, Larsen BK, Baussant T, Bamber S, Sanni S. 2009. Relating biomarkers to whole-organism effects using species sensitivity distributions: a pilot study for marine species exposed to oil. Environmental Toxicology and Chemistry 28:1104–1109.

Sanni S and MGD Smit (in prep.). Building bridges with Biomarker SSDs.

16.4 On-line monitoring methods

Steinar Sanni (IRIS, Norway) presented recent development and use of on-line monitoring methods, and discussed the interest for scientific evaluation and how this can be achieved.

Considerable progression has been made the last half decade regarding on-line monitoring of biological effects of contaminants. By on-line monitoring, biomarker measurements which are non-destructive to instrumented organisms can be obtained continuously in real time from in-situ locations. This is of interest particularly to the oil and gas industry as it is presently directing more of its activities to remote offshore areas like the Arctic and deep waters, with increasing amounts of un-manned subsea installations, and with increased public focus on the industry's oil spill preparedness. Besides this interest there are several different on-line monitoring applications being made both in marine, freshwater and air recipients. The potential for technology spin-off to other sectors from the oil and gas industry seems large. With this development it seems also to be a need for a scientific evaluation of such on-line biomarker methods, and that it is within the WGBEC's domain to conduct such an evaluation. It is a question if the standard evaluation criteria used for other biomarker methods is applicable to evaluation of on-line methods, or if other or additional criteria seems necessary.

The progression made regarding on-line monitoring have many aspects. Firstly, more commercial suppliers and R&D institutions have become involved, and different industrial applications have been made. There have been increased efforts made in developing different endpoints than the original methods of valve gaping in bivalves (e.g. blue mussel) and cardiac activity in different crustaceans (e.g. crabs), and in utilizing them in different species, according to geographical regions and environmental condition of each application. The interest for this exists in different Arctic countries, including Russia, Norway, Canada and the U.S., and is also expressed in South-America related to offshore oil and gas industry. Recent advances in end-point development are focusing on parameters that can be related to bioenergetics and organism fitness (i.e. growth, feeding and excretion rates) resembling "scope for growth" related biomarkers, and if successful, they can provide environmental risk relevant in-situ monitoring information, which would be of great interest.

Instrumented organisms will for industrial applications offshore also need to be used in combination with robust deployment systems as well as data transmission and treatment systems, and also regarding these aspects much progression has been made recently. This involves development of fixed platforms and mobile sensor arrays that already incorporate, or have the possibility to incorporate on-line biomarkers as "plug and play" devices. Solutions to several of associated engineering challenges have been made, and procedures for how to handle region and site specific engineering aspects have been established. These challenges are both of technical and biological nature, and the latter seems often to require most efforts to overcome.

One of the increased opportunities that the continuous transmission of biological signals from the field opens is automated processing of received data. This holds the potential for great improvement of the "early warning" features of biomarker methods. Much progression has been made regarding this aspect by the use of bioinformatics and multivariate techniques, which have been made particularly powerful by integration with on-line information from physical and chemical sensors on the same deployment platforms as the instrumented animals. Automated data routines to sort out false positives, and technical possibilities to retrieve un-instrumented animals for manual biomarker measurements have also been important developments that aids the interpretation of the biosensor information.

Besides these new possibilities for defining assessment criteria, it has also been possible to determine threshold levels for one of the on-line methods (valve gaping) in relation to oil based discharges in laboratory experiments similar to those that have been used for determination of EACs for biomarkers in the SGIMC work. Experiments of three different species of bivalves were exposed to dispersed oil at five different and successfully increased oil concentrations. In these three species (*Mytilus edulis*, *Modiolus modiolus* and *Arctica islandica*) the threshold levels were identified within the range 60–125 ppb dispersed oil (nominally) (S. Bamber, unpubl. data).

As with manual biomarkers ("off-line" methods), the establishment of assessment criteria is reasonably one element of an evaluation of the on-line biological methods. The data mentioned above seems a valid basis for such an evaluation provided it is published in a peer reviewed scientific paper. In addition, the basic method must be described in the literature (e.g. in ICES TIMES series), and have been subject to successful field validation and inter-calibration exercise. Finally, elements that are particular for on-line monitoring methods compared to "off-line" methods might be considered included in the evaluation. The most relevant of such elements might be the technical platforms (incl. data handling systems), and the bioinformatic proce-

dures developed for closer data examinations than the ordinary SGIMC type of assessment criteria. Evaluation of all or parts of the technical platforms of on-line sensor systems might alternatively be done as part of a technology qualification process, which is commonly done for commercial products of these kinds, while the bioinformatic procedures probably will require a cross disciplinary evaluation of biological and informatics expertise.

In conclusion, scientific evaluation of on-line biological monitoring methods for assessment of effects of contaminants are of interest, and it seems it can be done in similar fashion as how the WGBEC evaluates "off-line" biomarkers, with possible additional evaluation of some elements that are particular for on-line methods. It is therefore proposed that this is followed up at future meetings as information regarding the elements mentioned above becomes available.

16.5 Immunotoxicity endpoints suitability for monitoring

Andrea Johnson (US) prepared and presented an overview on the current status of fish immunotoxicity endpoints and their suitability for monitoring. Various immunological biomarkers have been used in both laboratory and field settings to demonstrate chemically-induced immunotoxicity in aquatic organisms. These assays include biomarkers of innate and adaptive immune systems and range from hematology (Tier 1: general) to immunoglobulin specific and host challenge assays (Tiers II and III; Table 16.5.1; Weeks *et al*. 1992). Examples of immunotoxicity endpoints that have been applied successfully in the field are included in Table 16.5.2 along with others that are less sensitive as biomarkers. Both phagocytosis and disease challenge assays seemed to produce consistent responses in fish exposed to chemical contaminants such as immune dysfunction (Table 16.5.2). A decrease in phagocytosis has been observed in various aquatic organisms exposed to oil and PAH (Barron 2011; Table 16.5.2) and PCB (Zelikoff *et al*. 2000). In addition, fish exposed to PCBs and PAHs tend to show an increase in disease susceptibility (Arkoosh and Collier, 2002; Arkoosh *et al*. 2001; Table 16.5.2). Other assays such as lymphocyte mitogenesis have provided mixed results. For example, rainbow trout (*Oncorhynchus mykiss*) leukocytes exposed to aflatoxin B1, a PAH associated with hepatic carcinogenesis, have shown variability in lymphocyte proliferation in response to stimulation with lipopolysaccharide (LPS) and immunoglobulin production (Table 16.5.2; Kaatari *et al*. 1994; Ottinger and Kaatari, 1998, 2000). In addition, some cellular immune function assays (Tier 1) have been shown to be suitable biomarkers in eelpout (*Zoarces viviparous*) from the Baltic Sea (Hedman *et al*. 2011) and blue mussel (*Mytilus edulis*) from Danish coastal waters (Hoher *et al*. 2012). Immune dysfunction in invertebrates exposed to environmental contaminants has been reviewed by Ellis *et al*. (2011).

Considerations/recommendations

Most of the immunoassays reviewed above have been conducted under controlled laboratory conditions and/or require laboratories for further processing of organisms, tissues and cells. Therefore, not all of these assays may be practical for field monitoring and processing aboard a research vessel. Some Tier 1 assays that are amenable to field monitoring and total or partial processing aboard a research vessel equipped with a laboratory are: differential leukocyte counts, hematocrit and plasma protein and chemistry. For plasma chemistry, whole blood can be centrifuged aboard the vessel and plasma frozen for later processing. Methods for these assays are published and they require minimum equipment and set-up. Some of the other endpoints have limited applicability in the field because they require laboratory facilities and equip-

ment that are not available in field settings. There is evidence that some of these immunotoxicity endpoints have been integrated successfully in field studies (Johnson *et al*. 2007; Hedman *et al*., 2011; Hoher *et al*., 2012; Table 16.5.2) and while the immune response tends to be non-specific, some immunotoxicity endpoints are sensitive to different contaminants in the field (Table 16.5.2).

The innate and adaptive immune systems are regulated by both endogenous and exogenous factors; therefore natural variability in response is an important factor to consider for field monitoring. In addition, the organism's physiology (e.g. sex, maturational status, genetics) and nutritional status should be taken into consideration as these may influence an organism's immune system. Handling of organisms and their cells may become potential stressors as well. Therefore, not all of these immunotoxicity endpoints may be sensitive indicators for monitoring the health of marine organisms. The suitability of immunotoxicity endpoints for monitoring the health of marine organisms in the field is made difficult by the complexity of the immune system and lack of assay standardization. However, immunotoxicity endpoints, when used as part of a suite of health indicators, have helped directly to determine organismal health and indirectly, environmental health and thus may be used to augment existing field monitoring programs.

Table 16.5.1. Immune function assays for use in screening or comprehensive analysis of immunomodulatory effects of chemicals (adapted from Weeks *et al*. 1992, pgs. 223–224).

TIER 1 **SCREEN**				
Test	**Immune Component**	**Fish**	**Birds**	**Mammals**
Complete blood count	General	+	+	+
Cell Differential	General	+	+	+
Hematocrit	General	+	+	+
Leukocrit	General	+	-	-
Organ weights	General	+	+	+
Histology	General	+	+	+
NK cell activity	Nonspecific	+	+	+
MQ phagocytosis	Nonspecific	+	+	+
Lysozyme activity	Humoral	+	+	+
Agglutination assay	CMI	+	+	+
Chemiluminescence	CMI	+	-	+
Melanomacrophage centers	CMI	+	-	-
TIER II **Comprehensive**				
Test	**Immune Component**	**Fish**	**Birds**	**Mammals**
Immune cell quantitation	General			
surface markers		+	+	+
Flow cytometry		+	-	+
Immunoglobulin quantitation	Humoral	+	+	+
Plaque-forming cell assay	Humoral	+	+	+
Lymphocyte blastogenesis	CMI	+	+	+
Mixed leukocyte response	CMI	+	-	+
Cytotoxic T-cell activity	CMI	+	-	+
Macrophage responses	Nonspecific	+	-	+

TIER III Host Resistance Challenge				
Test	**Immune Component**	**Fish**	**Birds**	**Mammals**
Mortality	Comprehensive	+	+	+
Bacteremia/viremia/ parasitemia/tumor quantitation and duration	Comprehensive	+	+	+
Specific antibody quantitation	Humoral	+	+	+

Table 16.5.2. Examples of immunotoxicity endpoints used in field studies of fish.

Species	**Contaminant/pathogen**	**Effects**	**Assay**	**References**
English sole	Polycyclic Aromatic Compounds (PACs)	↑T-Lymphocyte proliferation (CON A); LPS (n/s) and PWM (inconsistent↑↓)	Lymphoproliferative (LP) response of splenic leukocytes-CON A, LPS, PWM	Arkoosh *et al*. 1996
Chinook salmon	PCBs and PAHs	Increase susceptibility to vibriosis	Disease challenge w/ *V. anguillarium*	Arkoosh *et al*. 2001
Chinook salmon (*Oncorhynchus tshawytscha*)	*Vibrio anguillarum* (*Listonella anguillarium*) PCBs PAHs	↑disease susceptibility ↓2° PFC response ↑mortality in urban estuary than non-urban and hatchery fish	Disease challenge Ab production Plaque forming cell (PFC) assay	Arkoosh & Collier 2002
Chinook salmon	PBDEs	Increase susceptibility to disease	Disease challenge w/ *L. anguillarium*	Arkoosh *et al*. 2010
Flounder (*Platichthys flesus*)	bis(tri-*n*-butyltin)oxide (TBTO)	↓lymphocyte counts and %; ↓Nonspecific cytotoxic cell activity (NCC); n/s lymphocyte proliferation (PHA)	Cytotoxicity assay (NCC) Mitogenesis (PHA)	G.C.M. Grinwis *et al*. 1998
Chinook salmon	PCB mixture (Aroclor 1254; injected); *Nanophyetus salmincola* (metacercariae)	↓AK 1° PFCs and splenic 2° PFCs: trematode (*N. salmincola*) ↓↓ AK 1°PFC response: *N. salmincola* + Aroclor 1254	Disease challenge Plaque-forming cell (PFC) assay	Jacobson *et al*. 2003
Rainbow trout *Oncorhynchus mykiss*	Aflatoxin B1 (AFB1)	↓serum Ig production (2° Ab)	ELISA (enzyme linked immunosorbent assay)	Kaatari *et al*. (1994)
Rainbow trout	Aflatoxin B1	↓Lymphocyte proliferation (LPS) ↓Ig production	Mitogenesis (LPS) ELISA	Ottinger & Kaatari (1998)
Rainbow trout	Aflatoxin B1	↑lymphocyte proliferation to PWM and LPS (PBL) and PWM (Ant. Kidney) ↑Ig production (PBL)	Mitogenesis (LPS, CON A, PWM) ELISA	Ottinger & Kaatari (2000)
Smallmouth bass	PCB	↓phagocytosis	Phagocytosis assay	Zelikoff, 2000

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Recommendation

Secretariat to alert the new chairs of a possible ICES / OSPAR study group on Ocean Acidification to WGBEC deliberations on the biological effects of OA and that WGBEC could assist with the SG ToR on this subject.

Action

Assess methods identified in 2012 against criteria to determine their suitability for application in a monitoring context.

17 Any other business

17.1 ICES collaboration with PICES

On behalf of ICES, a member of WGBEC was invited to participate to the PICES 2011 Annual Meeting 'Mechanisms of Marine Ecosystem Reorganization in the North Pacific Ocean', held in Khabarovsk, Russia, 14–23 October 2011. The representative co-chaired the Workshop 3 - Trends in Marine Contaminants and their Effects in a Changing Ocean: Refining Indicator Approaches in Support of Coastal Management - and gave a presentation entitled 'Building Expert Knowledge on Integrated Science and Advice'. The presentation described the aim within the ICES realm for integrated science in support of management.

During the Workshop, a proposal to establish a Study Group on Marine Pollutants (SG-MP) was developed.

The proposed SG would operate under the following Terms of Reference:

1) Identify novel or promising approaches to operational marine pollution assessment in PICES member nations with the aim of:
    a. establishing a list of priority pollutants,
    b. identifying indicators of status, trends and effects,
    c. harmonizing methods to evaluate their impacts on biota, and
    d. describing case studies which demonstrate the effectiveness of indicators and methods to inform the success of remedial actions.
2) Illustrate the socio-economic implications of marine pollution through a series of examples.
3) Identify interactions within PICES scientific committees, Advisory Panels, working groups, and sections that will complement the SG and will be consistent with the ecosystem approach espoused by FUTURE.
4) Explore potential partnerships with other professional or multilateral organizations (e.g. NOWPAP, WESTPAC, ICES, GESAMP, SETAC) which could lead to joint activities (working group, sessions, publications), improve efficiencies and strengthen scientific outcomes.
5) Develop recommendations for a possible PICES WG on marine pollutants.

The proposal was accepted by the parent committee Marine Environmental Quality (MEQ) of PICES

ICES welcomed the creation of a Study Group on Marine Contaminants and expressed its willingness to support the activities of the Study Group. Both organizations have mutual interests in these matters and collaboration would enhance working across the northern hemisphere on issues of shared importance. Both organizations would also benefit of cost effectiveness.

In this respect, the ICES WGBEC noted that the Study Group's proposed ToRs are very in line with the work of WGBEC and also welcomed the current development at PICES. In order to enhance collaboration, WGBEC offers its expertise, chair knowledge, stimulate and/or enhance progress in developments/problem solving of the PICES Study Group's activities.

17.2 Relationships between oil spills and toxicity to fish eggs and larvae

WGBEC received a recent paper from Tracy Collier on the relationships between (bunker) oil spills and toxicity to fish eggs and larvae and an editorial on how weathered crude oil can act as cardiotoxicants. There are increasing attention on such effects and increasing body of literature reporting that very low levels of petroleum compounds lead to toxic effects to the developing larvae.

The two papers received were:

Incardona JP, Vines CA, Anulacion BF, Baldwin DH, Day HL, French BL, Labenia JS, Linbo TL, Myers MS, Olson OP, Sloan CA, Sol S, Griffin FJ, Menard K, Morgan SG, West JE, Collier TK, Ylitalo GM, Cherr GN, Scholz NL. 2012. Unexpectedly high mortality in Pacific herring embryos exposed to the 2007 Cosco Busan oil spill in San Francisco Bay. 2012. PNAS. 109(2): E51-E58.

Incardona JP, Collier TK, Scholz NL.2011. Oil spills and fish health: exposing the heart of the matter. Journal of Exposure Science and Environmental Epidemiology. 1–2. doi:10.1038/jes.2010.51

The reported findings have implications for assessment criteria and threshold levels for risk assessments to crude oil and bunker oil. Improved knowledge on mechanisms involved may lead to improved methods to measure such effects.

WGBEC would like to follow up on this subject. As Tracy Collier is a member of WGBEC, we hope he has the possibility to review recent developments on this subject for the next WGBEC meeting in 2013.

It was brought to the attention of WGBEC that during the 36th Annual Larva Fish Conference to be held outside Bergen, Norway, 2–6 July 2012, there will be a session on effects of oil and natural gas surveys, extraction activity and spills on fish early life stages.

Detailed information on this Conference was provided as indicated below:

http://wwwlarvalfishcon.org/Conf_Page.asp?ConferenceCode=36th&ContentPosition=ThemeSessions

Session title: Effects of oil and natural gas surveys, extraction activity and spills on fish early life stages

Organized by Sonnich Meier, Bjørn Einar Grøsvik and Erik Olsen.

Papers presented at this session can be submitted for publication as a themed grouping of articles in the ICES Journal of Marine Science. Contact the session organizers and/or Howard Browman for details.

Offshore oil production is associated with significant environmental risks, as demonstrated by the recent Deepwater Horizon oil spill in the Gulf of Mexico. Major accidental oil spills, due to blowouts or oil tanker incidents are, however, only one of the many sources of oil pollution in the aquatic environment. Small oil spills, from "every-day" spills, or operational discharges of water from offshore platform activity (or from oil-sand production), may also represent significant inputs of oil compounds into the ecosystem. Fish embryos and larvae are sensitive to low concentrations of dissolved oil compounds. Given the importance of fish early life stages in determining the size of high-value fish stocks, there is a need for more research on how oil and oil dispersants affect the development of fish embryos and larvae. Such information is also required for risk assessment. Therefore, this theme session will focus on how

oil compounds affect the early life stages of fish and if these effects can influence recruitment and have long term effects on fish populations.

Confirmed keynote speakers:

- John Incardona, Northwest Fisheries Science Center, Environmental Conservation Division, Ecotoxicology & Environmenatal Fish Health Program, 2725 Montlake Blvd E, Seattle, WA 98112 USA (Toxicity of oil components for fish larvae)
- Mace G. Barron, US Environmental Protection Agency, Gulf Ecology Division, Gulf Breeze, FL 32561 USA. "*The photoenhanced toxicity of oil to larval fish*"
- Peter Hodson, School of Environmental Studies, Department of Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada ("The exposure of fish embryos to spilled oil")
- Kevin Kleinow, Louisiana State University, School Veterinary Medicine, Department of Comparative Biomedical Sciences 1909 Skip Bertman Drive, Baton Rouge, LA 70803 USA ("Deep Water Horizon and fish development: A story of transport, exposure, dispersants, and gene expression")

Recommendation

Secretariat to make PICES aware that the new study group on Marine Pollutants may benefit from collaboration with WGBEC as some of their ToRs have been addressed by the group for the ICES area.

Action

WGBEC to further consider the toxicity of oil and oil components to early life stages of fish with a view to informing risk assessments and revising assessment criteria (EACs) by inclusion as a ToR for 2013.

18 Recommendations and action list

Recommendations

1) **Addressed to the ICES Secretariat. Agenda item 4.** In accordance with the proposed arrangements for expert groups WGBEC should move to multi-annual terms of reference from 2013.

2) **Agenda item 5a.** Secretariat to advise OSPAR MIME to take note of the WGBEC review of the SGIMC advice and that it is considered fit for purpose in its current form and is suitable for application for MSFD Descriptor 8 Indicator 8.2.2.

3) **Agenda item 5a.** ICES Secretariat should advise OSPAR MIME that should they require further advice on this matter for their 2012 deliberations on integrated assessment, WGBEC would be willing to provide such intersessionally.

4) **Agenda item 5a.** Secretariat to inform OSPAR MIME that after trial applications, WGBEC considers there to be an important gap in the application of the integrated approach, with regard to targeted application, frequency of monitoring, statistical aspects of designing a monitoring programme and techniques for combining assessments across regional scales.

5) **Agenda item 5b.** Secretariat to advise OSPAR MIME to take note of the national trials of the integrated assessment scheme that have been applied with some success.

6) **Agenda item 5c**. That further assessment of the ICON database is brought under the auspices of ICES by hosting a 2 day assessment workshop at ICES HQ in 2012, and a sharepoint site is created to host the data being assessed. (Secretariat).

7) **Agenda item 5d.** Secretariat to advise OSPAR to take note that WGBEC is maintaining a live document of updated biological effects assessment criteria from the ICES CRR publication. This updated document can be made available for update of the OSPAR biological effects assessment criteria on request.

8) **Agenda item 6.** Secretariat to advise OSPAR that WGBEC considers that further toxicity data are available that should be used to assist in the derivation of EACs for some contaminants.

9) **Agenda item 6.** Secretariat to advise OSPAR that WGBEC considers that WFD EQS values derived for protection of predators and secondary consumers are not considered suitable substitutes for calculation of EACs for some substances, which have been identified as more toxic to lower trophic levels.

10) **Agenda item 7.** Secretariat to supply the revised TA and associated guidance on issues identified by WKLYS to ADGLYSAC and subsequently OSPAR.

11) **Agenda item 7.** A draft resolution is requested to amend the ICES TIMES manuscript 36 as identified above.

12) **Agenda item 10.** Draft resolutions are requested for publication of TIMES methods on stress on stress, ER CALUX, COMET assay, condition index (and revision of NRR, see 7 above)

13) **Agenda item 13.** ICES Secretariat should report to OSPAR on the opportunities and benefits to incorporate the monitoring of microplastics in sediment and biota (i.e. fish, crustaceans) in existing JAMP/CEMP chemical and biological effects monitoring programmes. Apart from being cost-effective, this would allow a direct (integrated) assessment of their potential physical and chemical effects and ecosystem health consequences.

14) **Agenda item 13.** The ICES Data Centre together with WGBEC, WGMS and MCWG should prepare the entrance of litter and microplastic and associated contaminants data in the Environmental Data Base, to prepare for likely future requirements for assessment across the ICES region and reporting under MSFD Descriptor 10

15) **Agenda item 13.** ICES Secretariat should bring the agenda item of microplastics and associated chemical contaminants forward to MCWG and WGMS.

16) **Agenda item 13.** That a Theme Session on litter and microplastics in the marine environment be proposed for 2013.

17) **Agenda item 14/15.** That MCWG, WGMS and WGBEC hold a concurrent meeting in 2014 with a full day joint plenary to address common areas of interest:

a) To define the role of passive sampling in integrated monitoring and assessment (sampling strategy, assessment criteria, deployment alongside bioindicator species) and use of toxicity tests on passive sampler extracts in monitoring programmes.
b) Microplastics

18) **Agenda item 16a.** Secretariat to alert the new chairs of a possible ICES / OSPAR study group on Ocean Acidification to WGBEC deliberations on the biological effects of OA and that WGBEC could assist with the SG ToR on this subject.

19) **Agenda item 17.** Secretariat to make PICES aware that the new study group on Marine Pollutants may benefit from collaboration with WGBEC as some of their ToRs have been addressed by the group for the ICES area.

Actions

1) **Agenda item 4.** Produce 3 year terms of reference

2) **Agenda item 4.** Elect new chairman for 2014.

3) **Agenda item 5b.** WGBEC to maintain a current document on uncertainties and problems / solutions encountered during trials of the integrated approach and consider using to inform a future publication on integrated assessment methodology.

4) **Agenda item 5c.** ICON participants to compile missing data (intersessionally) onto the assessment spreadsheets created at WGBEC 2012.

5) **Agenda item 5d.** That WGBEC maintain a live document on biological effects assessment criteria.

6) **Agenda item 7.** WGBEC chairs to draft a draft resolution for TIMES 36

7) **Agenda item 7.** WGBEC to progress amendment of TIMES 36 on Lysosomal stability (CMG)

8) **Agenda item 10.** WGBEC to consider publication of the integrated assessment strategy as a peer review publication.

9) **Agenda item 11.** WGBEC to maintain the working document on interactions with the data centre to record evidence of decision making with data centre queries.

10) **Agenda item 11.** WGBEC to provide Data Centre with examples of data entry spreadsheets for mussel histopathology parameters.

11) **Agenda item 12.** WGBEC to conduct method intercomparison exercises during 2012/13 for EROD and bile.

12) **Agenda item 12.** Klaas Klaag to contact QUASIMEME with the information that several WG laboratories would commit to sign up for a ring trial on dogwhelk imposex. Should QUASIMEME not offer an imposex ring trial, WGBEC will conduct a small scale sample exchange.

13) **Agenda item 13.** Prepare a Theme Session proposal on litter and microplastics at the ASC 2013 (Dick Vethaak and others to be identified)

14) **Agenda item 14/15.** Those WG members DV, UK, JT liaise with WGEEL and MCWG members on the eel quality status report intersessionally and report to WGEEL 2012 and WGBEC 2013.

15) **Agenda item 14/15.** WGBEC chair to liaise with WGMME chair to discuss possible joint working on assessment of risk to marine mammals from con-

taminant burdens in fish and possible techniques for assessing effects of contaminants in mammals.

16) **Agenda item 16e.** Assess methods identified in 2012 against criteria to determine their suitability for application in a monitoring context.

17) **Agenda item 17.** WGBEC to further consider the toxicity of oil and oil components to early life stages of fish with a view to informing risk assessments and revising assessment criteria (EACs) by inclusion as a ToR for 2013.

19 Adoption of the report and closure of the meeting

The meeting closed at 14:30 on Friday, 16 March. Some aspects and text of the report remained to be completed post the meeting via the SharePoint. All recommendations and actions were agreed before then closure of the meeting. The chairman thanked all those present for their contribution and a special thanks was given to Professor Lucia Guilhermino for hosting the meeting at the University of Porto, CIMAR.

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Annex 2: WGBEC Terms of Reference 2011

The **Working Group on Biological Effects of Contaminants** (WGBEC), chaired by Matthew Gubbins, UK, and John Thain, UK, will meet in Porto, Portugal, 12–16 March 2012 to:

a) Review and update of the Technical Annex on lysosomal stability:

To review and update, as necessary, the Technical Annex 6 (lysosomal stability) to the JAMP Guidelines for general biological effects monitoring. This should build on the latest developments through the Workshop on Lysosomal Stability Data Quality and Interpretation and WGBEC. (OSPAR request 2012/1)

b) Review of Environmental Assessment Criteria or equivalents:

To review scientific robustness and update, as necessary, EACs or equivalent effects levels calculated for CEMP and pre-CEMP determinands. (OSPAR request 2012/2)

c) Integrated monitoring and assessment:

1. Respond to SGIMC 2011 and review documentation as required;
2. Application of OSPAR integrated strategy to data sets by working group members;
3. Review integrated assessments from ICON and BEAST;
4. Update assessment criteria in light of new data.

d) MSFD – review initial assessments for Descriptor 8 and advise as required on implementation of monitoring programmes for GES Commission indicator 8.2.1;

e) Report on collaboration with other WGs as identified at 2011 meeting and any intersessional activity/representation (WGEEL, WKMAL, WGMMAL, MCWG etc.);

f) Receive reports on marine monitoring activities being undertaken by member states;

g) Consideration of issues of special scientific interest / value:

1. Acidification in marine waters in relation to contaminants and biomarker response;
2. Effects of contaminants on primary production, including phytotoxicity;
3. Relationship of genetic markers to biomarkers. BECOMES AC DEVELOPMENT;
4. Review of species differences in bioassay and biomarker responses e.g. as seen in assessment criteria currently being developed – also to include sources of species for testing;
5. Immunotoxicity end points – suitability for monitoring;
6. Online monitoring.

h) Review progress with publication and electronic dissemination of biological effects techniques in the ICES TIMES series;

i) Review progress from the ICES database subgroup and report advice to the ICES Data Centre;

j) Report progress from AQC subgroup and develop AQC procedures for biological effect methods including harmonisation activities initiated from WGBEC and within OSAPR, HELCOM and MEDPOL maritime areas;

k) Review recent developments relating to contaminant effects from litter /plastic particles;

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

WGBEC will report by 15 April 2012 (via SSGHIE) for the attention of SCICOM.

Supporting information

Priority	The activities of this group will enable ICES to advise on issues relating to the design, implementation and execution of regional research and monitoring programmes pertaining to hazardous substances in the marine environment. To develop procedure for quality assurance of biological effects data and to improve assessments of data relating to the biological effects of contaminants in the marine environment. To develop cross links and collaboration with other ICES Expert Groups in order to take forward and contribute to the implementation of the ICES Science Plan.
Scientific justification	Term of Reference a) and b) OSPAR requests. ToR c) In 2011 SGIMC completed its task in developing an integrated contaminant and biological effects monitoring framework. There are still some outstanding tasks for WGBEC to complete, which may include updating and reviewing assessment criteria. In addition, it is important to review how countries are using and applying the new integrated framework and this will include the ICON and BEAST programmes. Term of Reference d) In 2012 initial assessments may be available for MSFD Descriptor 8. It is important that WGBEC reviews and advise as required on implementation of monitoring programmes for GES Commission indicator 8.2.1. Term of Reference e) WGBEC has contacted several Expert Groups within SSGHIE and areas for cross linking and collaboration have been identified. It is important that progress with collaborative activities and intersessional contacts are reviewed and reported back to SSGHIE. Term of Reference f) WGBEC has found it of value to discuss, feedback and support national monitoring programmes across the maritime areas and this is a valuable opportunity to improve and harmonise programme designs and assessment of data (e.g. OSPAR / MEDPOL / WFD / HELCOM/ EU MSFD). Term of Reference g) There are a number of issues identified by WGBEC that are of value and special scientific interest to understanding the effects of contaminants in the marine environment e.g. acidification, primary production, genetic markers, immunocompetence and online monitoring. It is important that these are reviewed/assessed and taken forward, in relation to the wider aspects of environmental management and secondly in the development and application of techniques for assessment purposes. Term of Reference h) It is important for WGBEC to keep track of publication progress with biological effects methods it has sponsored. Protocols are needed for nationa and international programmes as well as the OSPAR programmes and EU MSFD. Term of Reference i) Biological effect data is increasingly being submitted to the ICES database and technical queries arise and WGBEC can assist with answering queries from the ICES Data Centre. The subgroup set up

	to work intersessipnally on any data base issues will report on its activities. Term of Reference j) AQC is vital to support, report and assess data, particularly for cross maritime areas and developments and harmonisation in this area need to be taken forward in a coordinated manner. Term of Reference k) There has been considerable interest over the past two years on the biological effects of plastic particles, particularly in relation to contaminats associated with plastic particles. It is important that this work area is reviewed and any reports and feed back fro other Expert Groups is discussed at WGBEC.
Resource requirements	The main input to this group is from National experts. Each attendee is self-funded from their own / organisation / institute resources.
Participants	The Group is normally attended by ca. 16 members and guests.
Secretariat facilities	None required.
Financial	No financial implications.
Linkages to advisory committees	ACOM
Linkages to other committees or groups	There are direct linkages with WGSAEM, MCWG, WGMS and WGPDMO and several other linkages have recently been identified and are being persued via SSGHIE collaboration initiative.
Linkages to other organizations	None identified

Annex 3: Agenda

The Working Group on Biological Effects of Contaminants [WGBEC]

Porto, Portugal, from 12th – 16th March, 2012

Host: Lucia Guilhermino, Univeristy of Prto.

Chairpersons: Matt Gubbins and John Thain

1. Opening of the meeting;
2. Adoption of the agenda;
3. Timetable and appointment of rapporteurs;
4. Presentation of the proposed modus operandi for ICES Expert Groups; implications for WGBEC and development of a way forward: terms of reference and chairpersons.
5. Integrated monitoring and assessment: (ToR c)

    a. Respond to SGIMC 2011 and review documentation as required;
    b. Application of OSPAR integrated strategy to data sets by working group members;
    c. Review integrated assessments from ICON and BEAST;
    d. Update assessment criteria in light of new data.
6. Review of Environmental Assessment Criteria or equivalents (ToR b)

    To review scientific robustness and update, as necessary, EACs or equivalent effects levels calculated for CEMP and pre-CEMP determinands. (OSPAR request 2012/2)
7. Review and update of the Technical Annex on lysosomal stability (ToR a)

    To review and update, as necessary, the Technical Annex 6 (lysosomal stability) to the JAMP Guidelines for general biological effects monitoring. This should build on the latest developments through the Workshop on Lysosomal Stability Data Quality and Interpretation and WGBEC. (OSPAR request 2012/1)
8. MSFD – review initial assessments for Descriptor 8 and advise as required on implementation of monitoring programmes for GES Commission indicator 8.2.1; (ToR d)
9. Receive reports on marine monitoring activities being undertaken by member states; (ToR f)
10. Review progress with publication and electronic dissemination of biological effects techniques in the ICES TIMES series; (ToR h)
11. Review progress from the ICES database subgroup and report advice to the ICES Data Centre; (ToR i)
12. Report progress from AQC subgroup and develop AQC procedures for biological effect methods including harmonisation activities initiated from WGBEC and within OSAPR, HELCOM and MEDPOL maritime areas; (ToR j)
13. Review recent developments relating to contaminant effects from litter /plastic particles; (ToR k)
14. Evaluate potential for collaboration with other EGs and other ICES initiatives in relation to the ICES Science Plan and report on how such cooperation has been achieved in practical terms (e.g. joint meetings, back-to-back meetings, commu-

nication between EG chairs, having representatives from own EG attend other EG meetings). (ToR l)

15. Report on collaboration with other WGs as identified at 2011 meeting and any intersessional activity/representation (WGEEL, WKMAL, WGMMAL, MCWG etc.); (ToR e)
16. Consideration of issues of special scientific interest / value: (ToR j)

    a. Acidification in marine waters in relation to contaminants and biomarker response;
    b. Effects of contaminants on primary production, including phytotoxicity;
    c. Relationship of genetic markers to biomarkers. BECOMES AC DEVELOPMENT;
    d. Review of species differences in bioassay and biomarker responses eg as seen in assessment criteria currently being developed – also to include sources of species for testing;
    e. Immunotoxicity end points – suitability for monitoring;
    f. Online monitoring.

17. Any other business;
18. Recommendations and action list;
19. Adoption of the report and closure of the meeting

Annex 4: Timetable and Rapporteurs

DATE	APPROX. TIME	AGENDA ITEM	RAPPORTEURS CONTRIBUTORS	ISSUE
Monday 12th March	09:30	1	MG	Introduction by Chairperson and Lucia Guilhermino housekeeping issues, *tour de table.*
	10:00	2	MG	Adoption of agenda, tabling of documents
	10:15	3	MG	Appointment of rapporteurs.
		5	JS, KT, SB, DV,	**Integrated Monitoring and Assessment**
			JT, CR, UK, All	
				b) application of SGIMC integrated scheme by WG members
	12:30			Lunch
	13:30	5		**Contd**....b) application of SGIMC integrated scheme by WG members
	15:30			
	17/18:00			**Close of business**.
Tuesday 13th March	09:00	5		b) Contd (Ireland, Norway)
	10:00			c) BEAST presentation
	10:30			c) ICON breakout group and a) Integrated approach review
	11:00			
	11:45			Facility visit
	12:30			Lunch
	13:30	7	CM	Lysosomal stability: Review and update Technical Annex
	14:30	5		d) update assessment criteria in light of new data
	15:30			
	18:00			**Close of business.**
Wednesday14thMarch	09:00	5		d) contd
	10:00	8	KC, All	Review from any WG member any initial assessments for Descriptor 8 under MSFD
	12:00	6, 11	JS, UK, SS, CR, MG	ICG EAC review sub group and ICES data sub-group
		4	JT	ICES issues MSFD / ASC
	12:45			Lunch
	13:30	9	All	Receive reports on marine monitoring and related activities
	14:30	12	JT, MG	Report progress with AQC procedures for biological effect methods and include harmonisation activities within OSPAR, HELCOM and MEDPOL maritime areas.
	15:30	10	RB, JT, MG, JS, CM, RB	ICES TIMES
	17:00			**Drinks by river and dinner!**
Thursday 15th March	09:00	14/15	MG, JT	Collaboration with other EGs
	09:30	13	DV, TM	Litter and microplastics
	10:00	16		**Consideration of issues of special scientific interest**
			KK, SS	a) Acidification in marine waters
			DV	b) Effects of contaminants on primary production
	12:30			Lunch
	13:30		SS, RB	d) Review of species differences in bioassays
			AJ	e) Immunotoxicity endpoints
			SS	f) Online monitoring
	15:00	17	All	Any other business.
	15:30			Report writing!!!!!
	18:00			**Close of business.**
Friday 16th March	09:00	18	MG, JT	Recommendations and action list.
	10:30	19		Adoption of the report.
	12:30			Lunch
	14:00			**Closure of the meeting.**

Annex 5: WGBEC draft ToRs for the next meeting

1. Respond to requests for advice from Regional Seas Conventions (e.g. OSPAR, EU) as required.

WGBEC has a history in its ToR of responding to requests from OSPAR and these have always been considered as a priority and importance by the EG. In addition there is a wide breadth of knowledge and expertise which allows the EG to respond in an informed manner to these requests.

2. Consider emerging issues of scientific merit and address knowledge gaps (in relation to the ICES science plan).

-Oil toxicity to early life stages of fish

-Ocean Acidification (BEG, SS, KK, ICES / OSPAR study group)

-Immunotoxicity

In reviews over the past three years WGBEC has considered emerging special scientific issues in relation to biological effects and contaminats and also in relation to the ICES Science Plan These topics have been selected as of current concern..

3. Review status of publications and consider requirements for new publications

- ICES TIMES
- Other ICES publications
- peer review publications

It is important for WGBEC to keep track of publication progress with biological effects methods it has considered useful for monitoring. Protocols are needed for national and international programmes as well as monitoring to met OSPAR and EU MSFD obligations.

4. Conduct assessment of data as required

- Quality assurance data from method intercomparison trials
- Integrated assessment of monitoring data

AQC is vital to support, report and assess data, particularly for cross maritime areas and developments and harmonisation in this area need to be taken forward in a coordinated manner.

5. Respond to requests for advice from the Data Centre

Biological effect data are increasingly being submitted to the ICES database and technical queries arise. WGBEC can assist with answering queries from the ICES Data Centre.

6. Development and harmonisation of methodologies for marine monitoring and surveillance including:

- Integrated assessments
- Quality assurance of biological effects techniques
- Environmental risk assessment
- Review and develop assessment criteria for biological effects methods
- Report on national monitoring programmes for biological effects

WGBEC has found it of value to discuss, feedback and support national monitoring programmes across the maritime areas and this is a valuable opportunity to improve and harmonise programme designs and assessment of data (e.g. OSPAR / MEDPOL / WFD / HELCOM/ EU MSFD).

7. Address issues in relation to novel and emerging contaminants (e.g. pharmaceuticals, nanoparticles, toxicity of mixtures etc)

-Pharmaceuticals and recreational drugs in the marine environment (KT, CMG)

-Biocides in the marine environment(LG, KK, JB)

These are two issues identified by WGBEC that are of value and special scientific interest to understanding the effects of contaminants in the marine environment. Information on environmental impacts is currently lacking.

8. To evaluate the results of monitoring and research activities on plastic litter, especially microplastics and associated chemical contaminants in the marine environment abroad in regard to:

-Status on development of tools to quantify and qualify (micro)plastics in marine organisms, e.g. fish, turtles, crustaceans, marine mammals, and sea birds.

-Results of impact assessment surveys and research projects of microplastics and non-plastic micro particles in marine organisms from all trophic levels

-Evidence of bioaccumulation, toxicity of an adverse physical and chemical effects of microplastics and associated contaminants on marine organisms, populations and communities. This would include the full range of marine organisms from bacteria to turtles, marine mammals and sea birds.

-Evidence of microplastics and associated contaminants to transfer through marine food chains.

There has been considerable interest over the past two years on the biological effects of plastic particles, particularly in relation to contaminants associated with plastic particles. It is important that this work area is reviewed and any reports and feedback from other Expert Groups considered by WGBEC.

Annex 6: Codes for the Science Plan High Priority Topics

The SciencePlan has been converted into bullet points, each with a code number. These could be used for matching the Theme Sessions and Expert Group Terms of Reference and allow to track the scientific activity as well as ease cross-cutting syntheses. The coding presented here was performed within SSGEF.

Topic 1 : Understanding Ecosystem Functioning

11 Climate change processes and predictions of impacts

- 111 ICES niche: ecosystem responses to selected physical oceanographic scenarios
- 112 Define responses at the individual and population level to changes
- 113 Changes in distributional patterns at the species and community levels
- 114 Prediction of responses to selected climate change future scenarios (IPCC)
- 115 Responses based on physical-biological interactions and using long-term ICES data

12 Biodiversity and the health of marine ecosystems

- 121 Biodiversity and scale in ecosystems: genetic, population, species, community levels
- 122 Relate biodiversity to resilience and plasticity of ecosystems
- 123 Define indicators of ecosystem health: attributes of ecosystems, conditions of change, external pressures
- 124 Comparative analyses to study of resilience of shelf seas exploited ecosystems

13 The role of coastal zone habitat in population dynamics of exploited species

- 131 Coastal zone: essential nursery grounds and home of invertebrates, critical to mariculture. These habitats are threatened by human activities.
- 132 Focus on processes linking habitat to spatial patterns at the population and community levels.
- 133 Ecosystem-based marine spatial planning
- 134 Sustaining ecosystem goods and services

14 Fish life history information in support of EAM

- 141 Relate population variability, vulnerability, viability to external and ecosystem drivers.
- 142 Make use of spatial contexts and in particular operational oceanographic products
- 143 Monitor the status of populations and ecosystems with indicators
- 144 Predict population distributions, connectivities, and recruitment
- 145 Relate growth, reproduction, and feeding to the quality of habitats
- 146 Increase knowledge on fish physiology and behaviour, and their genetic basis
- 147 Processes underlying connectivity between populations: larval transport, fish movements

15 Sensitive ecosystems (deep-sea, seamounts, arctic) and data-poor species

- 151 Map habitats for conservation and management: develop habitat classification systems and mapping tools
- 152 Basic studies on the biology and ecology of these species and ecosystems in relation to water circulation, productivity, and climate change
- 153 Vulnerability to fishing: unfished deep-sea habitats, long-lived slow growing species
- 154 Rare species: genuinely rare, apparently rare to sampling
- 155 New species that are as yet unknown to science in these special environments

16 Integration of surveys and observational technologies into operational ecosystem surveys

- 161 Develop an ecosystem monitoring programme with: existing time-series, emerging survey methodologies, enhanced coordination (plankton nets, acoustics, optics, trawling) and a network of fixed stations.
- 162 Aim of providing indicators in support of advisory needs of integrated management and ecosystem status reporting

17 Role of top predators (mammals, birds, and large pelagics) in marine ecosystems

- 171 Role in the functioning of marine ecosystems: "top-down" controlled systems
- 172 Anthropogenic impact: removal of larger fish and increase top predators
- 173 Comparative analyses of ecosystem dynamics in response to changes in abundance and relative composition of top predators

Topic 2 : Understanding of Interactions of Human Activities with Ecosystems

21 Impacts of fishing on marine ecosystems

- 211 Understand the impacts of fishing on all components of the ecosystem.
- 212 Gather information on biota of all types (landings, discards at sea, subject to increased mortality through unobserved interaction with fishing gear) and on habitat.
- 213 Focus on technical challenges associated with collecting and interpreting the data required to assess fishing impacts
- 214 Modify, develop, and implement fishing gears designed to minimize fishing impacts.
- 215 Strategies to reduce the costs of fishing.

22 Carrying capacity and ecosystem interactions associated with mariculture

- 221 Define carrying capacity for cultured species within diverse coastal environments where there is an increasing competition for space.
- 222 Mitigation of the impacts of aquaculture through the development of multi-trophic aquaculture systems (e.g. kelp, salmon and mussel).

- 223 Interactions between wild and "farmed" species, contaminants associated with disease control and feeds, and escapement impacts.

23 Influence of development of renewable energy resources (e.g. wind, hydropower, tidal and waves) on marine habitat and biota

- 231 Impacts on ecosystem structure and function: structural habitat features, influence on ocean circulation and mixing
- 232 Evaluate risk of potential impacts, identify mitigation options
- 233 Coordinate multi-disciplinary research to augment existing knowledge base

24 Population and community level impacts of contaminants, eutrophication, and habitat changes in the coastal zone

- 241 Understanding the impacts of contaminants at the individual, population and community levels.
- 242 Estimating the cumulative impacts of contaminants, eutrophication, and changes in habitat substrate.
- 243 Synthesize knowledge on the impacts of diverse land-based and marine activities
- 244 Characterize the status of regional coastal zone ecosystems and causal relationships
- 245 Synthesize ecological understanding, identify gaps in knowledge and monitoring needs, based on the rich data sets for the coastal zone

25 Introduced and invasive species, their impacts on ecosystems and interactions with climate change processes

- 251 Processes that facilitate intentional and accidental introductions of species in the North Atlantic and their drivers (e.g., role of climate change).
- 252 Impact on the distribution and abundance of native biota through niche displacement, ecosystem structure (e.g. biodiversity) and function (e.g. food chain processes).
- 253 Risk assessment modelling for evaluation of management options
- 254 Support the development of regulatory frameworks and implementation of management measures through member countries and IMO, OSPAR, and HELCOM.

Topic 3 : Development of Options for Sustainable Use of Ecosystems

31 Marine living resource management tools

- 311 Development of indicator-based evaluations of species and habitats at different spatial scales, with reference points.
- 312 Exploration of management options under the "ecosystem approach"
- 313 Address issues associated with integrated management and conservation objectives.

- 314 Operating needs of the EAM: spatial extent of management areas, strategies to meet conservation objectives and report on ecosystem characteristics.

32 Operational modelling combining oceanography, ecosystem and population processes

- 321 Facilitate the availability and dissemination of long-term data
- 322 Give a reliable description of the actual marine conditions including physical and ecosystem variables, using analyses, forecasts, and model-based products
- 323 Evaluate the accuracy of the predictions as well as limits to forecasting.
- 334 Operational models to support the specific needs for the advisory process.
- 335 Forecasting of trends in recruitment as a function of oceanographic variables
- 336 Prediction of spatial pattern in populations and community properties due to changes in the environment.
- 337 Operational models to predict the development and spreading of harmful algal blooms, and environmental effects in the event of oil spills in the sea.

33 Marine spatial planning, effectiveness of management practices (e.g. MPAs), and its role in the conservation of biodiversity

- 331 Develop and evaluate integrated management procedures of the multiple uses of the oceans, in particular spatial planning tools.
- 332 Predict benthic habitat spatial patterns based on a combination of geomorphological and oceanographic properties.
- 333 Utility of MPAs (with a range of sizes and spatial patterns) for diverse conservation objectives under Integrated Management.
- 334 Sensitivity of benthic habitats to disturbance and reference points on the limits to disturbance for a range of anthropogenic impacts.
- 335 Evaluate GIS methods with respect to the specific needs of marine spatial planning.

34 Contributions to socio-economic understanding of ecosystem goods and services, and forecasting of the impact of human activities

- 341 Behavioural responses/strategies of the users of ocean ecosystems.
- 342 Social and economic motivations of ocean industries
- 343 How ecosystem goods and services are turned into socio-economic values.
- 344 Forecast the impact of human activities and evaluate mitigation options
- 345 Assessment of the resilience properties of marine ecosystems
- 346 Role of biodiversity at the species and genetic levels in ecosystem functioning

Annex 7: Toxicity dataset produced in the "LABORATORIO DE ECOLOXÍA MARIÑA" (UNIVERSIDADE DE VIGO)

(µg/L)	Group	Organism	Response	NOEC	LOEC	EC10 or LC10	EC50 or LC50	ref.
Hg	Bivalve	*Mytilus galloprovincialis*	Larval growth (8 d)		2			Beiras and His, 1995
			Embryo development (48 h)			2	5.1	Beiras and Albentosa, 2004
		Ruditapes decussatus	Embryo development (48 h)			2.9	4.2	Beiras and Albentosa, 2004
	Crustacean	*Palaemon serratus*	Larval survival (72 h)				74	Mariño-Balsa et al. 2000
		Maja squinado	Larval survival (72 h)				72	Mariño-Balsa et al. 2000
		Homarus gammarus	Larval survival (48 h)				48	Mariño-Balsa et al. 2000
	Equinoderms	*Paracentrotus lividus*	Embryo development (48 h)			14.3	20	Fernández 2002 (PhD Thesis)
			Early larval growth (48 h)		8	9.6	22	Fernández and Beiras 2001
	Ascidians	*Ciona intestinalis*	Embryo development (20 h)			24	54	Bellas et al. 2001
			Larval attachment (20 h)				36	Bellas et al. 2001
			Embryo development (20 h)	23	32		45	Bellas et al. 2003
			Larval attachment (48 h)	64	128		78	Bellas et al. 2003
	Fish	*Psetta maxima*	larval survival (ligth) (48 h)	9	27	26.4	79	Mhadhbi et al. 2010
Cd	Bivalve	*Mytilus galloprovincialis*	Embryo development (48 h)			500	1925	Beiras and Albentosa, 2004
		Ruditapes decussatus	Embryo development (48 h)			265	454	Beiras and Albentosa, 2004
	Crustacean	*Siriella armata*	Mortality (96h)	20	40	62.8	99	Pérez e Beiras, 2010
		Daphnia magna	Mortality (48h)	162	322	269.6	572	Pérez e Beiras, 2010
		Palaemon serratus	Larval survival (72 h)				1686	Mariño-Balsa et al. 2000
		Maja squinado	Larval survival (72 h)				158	Mariño-Balsa et al. 2000
		Homarus gammarus	Larval survival (48 h)				34	Mariño-Balsa et al. 2000
	Equinoderms	*Paracentrotus lividus*	Embryo development (48 h)			8104	8628	Fernández 2002 (PhD Thesis)
			Early larval growth (48 h)			2377	12972	Fernández 2002 (PhD Thesis)
			Early larval growth (48 h)		50		9240	Fernández and Beiras 2001
	Ascidians	*Ciona intestinalis*	Embryo development (20 h)			432	839	Bellas et al. 2001
			Larval attachment (20 h)				>146	Bellas et al. 2001
			Embryo development (20 h)	<512	512		721	Bellas et al. 2003
			Larval attachment (48 h)	1024	2048		752	Bellas et al. 2003
	Fish	*Psetta maxima*	larval survival (ligth) (48 h)	5	10	55.4	112	Mhadhbi et al. 2010
Pb	Bivalve	*Mytilus galloprovincialis*	Embryo development (48 h)			50	221	Beiras and Albentosa, 2004
		Ruditapes decussatus	Embryo development (48 h)		156	156	156-312	Beiras and Albentosa, 2004
	Equinoderms	*Paracentrotus lividus*	Embryo development (48 h)			357.2	521	Fernández 2002 (PhD Thesis)
			Early larval growth (48 h)		250	179.4	510	Fernández and Beiras, 2001

(µg/L)	Group	Organism	Response	NOEC	LOEC	EC10 or LC10	EC50 or LC50	ref.
Nap	Microalgae	*Isochysis galbana*	Inhibition of growth (48 h)			755	2244	P.Pérez 2010 (phD Thesis)
	Bivalve	*Mytilus galloprovincialis*	Early larval growth (48 h) dark		2061	4057	6659	Bellas et al. 2008
			Early larval growth (48 h) ligth		8501	8282	9969	Bellas et al. 2008
	Equinoderms	*Paracentrotus lividus*	Early larval growth (48 h) dark		954	652	4804	Bellas et al. 2008
			Early larval growth (48 h) ligth		954	744	4379	Bellas et al. 2008
	Ascidians	*Ciona intestinalis*	Early larval growth (48 h) dark		3297	613	1958	Bellas et al. 2008
			Early larval growth (48 h) ligth		13189	3040	4302	Bellas et al. 2008
	Fish	*Psetta maxima*	larval survival (dark) (48 h)	15.8	32	121	284	Mhadhbi et al. 2010
			larval survival (ligth) (48 h)	15.8	32	152	345	Mhadhbi et al. 2010
Phe	Microalgae	*Isochysis galbana*	Inhibition of growth (48 h)			232		P.Pérez 2010 (phD Thesis)
	Bivalve	*Mytilus galloprovincialis*	Early larval growth (48 h) dark		214	29	144	Bellas et al. 2008
			Early larval growth (48 h) ligth		214	53	224	Bellas et al. 2008
	Crustacean	*Acartia tonsa*	Egg production			109	22	Bellas and Thor, 2007
			Hatching			165		Bellas and Thor, 2007
			Recruitment			69	180	Bellas and Thor, 2007
			Survival (24h)			321	381	Bellas and Thor, 2007
			Survival (48h)			316	422	Bellas and Thor, 2007
	Equinoderms	*Paracentrotus lividus*	Early larval growth (48 h) dark		1279	460		Bellas et al. 2008
			Early larval growth (48 h) ligth		321	105		Bellas et al. 2008
	Ascidians	*Ciona intestinalis*	Early larval growth (48 h) dark					Bellas et al. 2008
			Early larval growth (48 h) ligth					Bellas et al. 2008
	Fish	*Psetta maxima*	larval survival (48 h) dark	6.3	13	43	64	Mhadhbi et al. 2010
			larval survival (48 h) ligth	6.3	13	103	75	Mhadhbi et al. 2010

(µg/L)	Group	Organism	Response	NOEC	LOEC	EC10 or LC10	EC50 or LC50	ref.
PFOS	Microalgas	*Isochysis galbana*	Inhibition of growth (72 h)	7500	15000	12200	37500	Mhadhbi et al. 2012
	Crustacean	*Siriella armata*	Mortality (96h)	1250	2500	3200	6900	Mhadhbi et al. 2012
		Daphnia magna	Mortality (48h)	50000	75000	44200	86500	Mhadhbi et al. 2012
	Equinodermos	*Paracentrotus lividus*	Inhibition of growth (48 h)	1000	2000	2600	20000	Mhadhbi et al. 2012
	Peces	*Psetta maxima*	larval survival (48 h)	15	30	20	110	Mhadhbi et al. 2012
PFOA	Microalgas	*Isochysis galbana*	Inhibition of growth (72h)	25000	50000	41600	163600	Mhadhbi et al. 2012
	Crustacean	*Siriella armata*	Mortality (96h)	5000	10000	7800	15500	Mhadhbi et al. 2012
		Daphnia magna	Mortality (48h)	300000	350000	330400	404300	Mhadhbi et al. 2012
	Equinodermos	*Paracentrotus lividus*	Early larval growth (48 h)	10000	20000	30700	110000	Mhadhbi et al. 2012
	Peces	*Psetta maxima*	larval survival (48 h)	1500	3000	3900	11900	Mhadhbi et al. 2012
BDE-47	Fish	*Psetta maxima*	hatching success (48h)	2.03	4.07	4.87	27.35	Mhadihbi et al. 2011
			larval survival (96h)	0.49	1.63	2.31	14.13	Mhadihbi et al. 2011
BDE-99	Fish	*Psetta maxima*	hatching success (48h)	3.22	5.16	7.17	38.28	Mhadihbi et al. 2011
			larval survival (96h)	1.61	3.22	5.55	29.64	Mhadihbi et al. 2011
Lindane	Bivalve	*Mytilus galloprovincialis*	Embryo development (48 h)		2005	1413	1992.00	Beiras and Bellas 2008
	Crustacean	*Palaemon serratus*	Larval survival (24 h)	0.5	0.1		5.20	Bellas et al. 2004
			Larval survival (48 h)	0.1	0.5		5.59	Bellas et al. 2004
		Maja squinado	Larval survival (24 h)	0.8	4		2.23	Bellas et al. 2004
			Larval survival (48 h)	0.8	4		2.18	Bellas et al. 2004
	Equinoderms	*Paracentrotus lividus*	Early larval growth (48 h)		750		>91000	Bellas et al. 2004
	Ascidians	*Ciona intestinalis*	Embryo development (20 h)	1600	3200		4412.00	Bellas et al. 2004
TBT	Bivalve	*Mytilus galloprovincialis*	Embryo development (48 h)		0.2	0.161	0.38	Beiras and Bellas 2008
	Crustacean	*Palaemon serratus*	Larval survival (24 h)	12.5	62.5		22.30	Bellas et al. 2004
			Larval survival (48 h)	12.5	62.5		17.52	Bellas et al. 2004
	Equinoderms	*Paracentrotus lividus*	Early larval growth (48 h)	0.1	0.2		0.31	Bellas et al. 2004
	Ascidians	*Ciona intestinalis*	Embryo development (20 h)	2	4		7.10	Bellas et al. 2004

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Annex 8: Data on comparison in sensitivity between different bioassays with marine organisms carried out at the Universidade de Vigo

The table shows the variation between species for EC50 values for PAH.

BIOASSAYS WITH DIFFERENT SPECIES, OIL COMPONENTS (PAHs)

SUBSTANCE	SPECIES	EXPOSURE TIME	TEMP (ºC)	ENDPOINT	NOEC (mg/l)	LOEC (mg/l)	EC50 (mg/l)	REFERENCE
PYRENE	*Palaemon serratus*	96h (medium change at each 12h)	20	Swimming behaviour	0.2	0.4	-	Luis & Guilhermino, 2012 (in press)
	Dicentrarchus labrax	96h(medium change at each 12h)	18	Swimming behaviour	<0.07	0.07	-	Almeida *et al.*, 2012, in press
	Pomatoschistus microps	96h(medium change at each 12h)	20	Swimming behaviour	<0.125	0.125	-	Oliveira *et al.*, 2012, in press
NAPHTHALENE	*Tetraselmis chuii*	96h	20	Population growth inhibition	-	-	1.8 (1.5–2.1)	Vieira & Guilhermino, 2012 8in press)
	Palaemon serratus	96h	20	Swimming behaviour	1.0	2.0	1.7 (1.26–2.34)	Luis & Guilhermino, 2012 (in press)

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Annex 9: Updated Technical Annex 6 of the OSPAR JAMP Guidelines for General Biological Effects Monitoring

Application in monitoring:

Lysosomal membrane stability (LMS) is a sub-cellular general stress response, known to be responsive to contaminant exposure, that can be monitored in marine organisms collected from the field. Two types of techniques for the measurement of LMS are recommended for monitoring purposes. A cytochemical method for use on preserved tissues / organs of marine biota and an *in vivo*, Neutral Red Retention (NRR) method that can be applied to live cells (mussel haemocytes) sampled *in vivo*. The cytochemical method can be applied to a range of species / tissue matrices, most often fish liver and mussel digestive gland. The NRR method is most suitable for use on mussel haemocytes (in a haemolymph sample). The results of the analysis are expressed in minutes, either as a lysosomal labilisation period for the cytochemical method or neutral red retention time for the NRR method. Full details on the background to this effect measurement are given in the OSPAR background document "Lysosomal stability as a global health status indicator in biomonitoring" (OSPAR, 2007)

Target organ/organism:

Fish

Liver in dab, accepting that other species (preferably those already used for contaminants monitoring) may need to be used beyond the normal geographical range of dab.

Bivalves

Haemolymph and/or digestive gland in mussels (*Mytilus* sp.), accepting that other species of bivalves (preferably those already used for contaminants monitoring) may need to be used beyond mussel´s geographical range.

Effect measured:

Subcellular cohesion of lysosomes. A background document is available on lysosomal membrane stability as a global health status indicator in biomonitoring and describes this effect measurement in more detail (OSPAR, 2007).

Means of interpretation:

For assessment purposes, neutral red retetion time (min) or lysosomal labilisation period (min) should be assessed against the BAC and EAC assessment criteria developed for the technique (OSPAR, 2007). Retention times or labilisation periods shorter than the EAC level suggest the marine organisms sampled are severely stressed and probably exhibiting pathology. Dysfunction of lysosomal processes has been mechanistically linked with many aspects of pathology associated with toxicity and degenerative diseases (Cuervo, 2004; Köhler *et al*., 2002; Moore *et al*., 2006). Retention times or labilisation periods shorter than the BAC level but longer than the EAC level are considered to represent stressed but compensating organisms.

Fish

Reduced LMS in cells from fish liver has been shown to relate to impaired liver function. It is therefore important to have an assessment of the disease status (incidence of external disease and liver pathologies) of each individual fish sampled. LMS provides useful supporting information for other physiological and molecular biomarkers in fish taken as part of an integrated contaminant and biological effect monitoring programme.

Bivalves

Reduced LMS in bivalves is known to impact on digestive gland function, immune response and capability to effectively up-regulate proteins involved in protection from oxidative stress. This can be a significant factor contributing to the ability of organisms

to tolerate stressful and polluted environments.

Additional biological effects measurements can aid the interpretation of the significance of destabilisation of lysosomal membranes in bivalves. These include:

Stress on Stress, Scope for Growth (measurement of physiological status) and an assessment of the disease status of mussel sampled (histopathology).

Methodology: Sampling and sample handling

Where monitoring is being conducted for the purposes of integrated assessment of contaminants and biological effects, sampling should be conducted according to the integrated monitoring guidelines (SGIMC, 2011). For other purposes the guidance in the ICES TIMES method manuscript (Moore *et al.*, 2004) and summarised below should be followed.

Fish

Flatfish should be caught in short (30 min) hauls and transferred to aerated flow-through holding tanks to minimize handling stress. Individual fish should be measured, weighed, dissected and sexed. The livers of 25 fish (gender according to the monitoring programme) are removed and cut into pieces 5mm x 5mm x 5mm and rapidly placed on a cooled (4˚C) chuck. These are then quenched in n-hexane at -70˚C and prepared and stored as described by Köhler *et al.* (1992).

Bivalves

Sampling should be avoided during the main spawning period. A minimum sample of 10 individuals from the same size class (small) should be taken from the sub-littoral (to avoid fluctuations due to aerial exposure) by cutting bissus threads to avoid damaging the internal organs of the mussel. Transporation should avoid rough handling and mussels should be packed in insulated containers containing absorbent material soaked in sea water. Transportation times should be kept to a minimum and for journeys of >4 hours ice packs should be added to the insulated boxes. For the *in vivo* NRR method no sample preservation is required and haemolymph should be removed from the mussels as described by Moore *et al*. (2004). For the cytochemical method digestive glands should be removed by dissection and cut transversely into 3 equal portions. The middle portion is used for analysis and the other portions are available for histopathology. Immediately after dissection this portion should be placed on a cooled chuck as described for fish liver and prepared and stored according to Moore *et al.* (1988).

Methodology:

Analysis

Samples should be analysed by either the cytochemical method or neutral red retention assay according to Moore *et al.* (2004). At the time of writing (2012) this manuscript is under revision to improve clarity of the NRR method section.

Cytochemical method

Method is described by Moore *et al.* (2004). Protocols also exist for national programmes (e.g. Germany) (Moore, 1990; Köhler, 1991, Lowe *et al*., 1992) and for cooperative studies in the Mediterranean and the Baltic Sea.

Neutral red retention method

The analytical method is described in the ICES TIMES Series document No. 36 (Moore *et al.*, 2004) which is currently in the process of being amended in light of methodological improvements identified during the ICES/OSPAR Workshop on Lysosomal Stability Data Quality and Interpretation (WKLYS) in 2010.

Quality assurance/control:

Various activities can and have been used to conduct inter-laboratory QA exercises, including workshops and ring trials. For the cytochemical technique frozen tissue samples can be used both for internal QA as use as Laboratory Reference Materials (LRM) and distributed between laboratories for external QA purposes. For the NRR technique, live mussels from the same sources can be distributed for external QA, or workshops involving multiple participants conducted to provide external QA data on the same samples. Examples of such activities are provided below:

Cytochemical method

Intercalibration exercises for lysosomal stability techniques (in fish) have been carried out in the ICES/UNESCO-IOC-GEEP Bremerhaven Research Workshop (1990) and UNEP-MEDPOL programme. A workshop was also held at the Plymouth Marine Laboratory in 1996 (organiser: Dr M Moore) and again at Bremerhaven with the aim of harmonising methodology between participants in 2008.

Neutral red retention method

Intercalibration for the Neutral Red Retention method was carried out for mussels in the GEF Black Sea Environment Programme (Köhler *et al*., 1992; Lowe *et al*., 1992; Moore *et al*., 1997; 1998a, b; Viarengo *et al*., 2000). An intercomparison exercise on NRR in mussels was also conducted in the BEQUALM programme during 2001. The first laboratory intercalibration exercise using NRR assay combining MEDPOL and ICES was carried out in 2009, and 16 laboratories participated. Results were presented at the Consultation Meeting to review MEDPOL in 2011 (UNEP/MAP, 2012). An ICES/OSPAR workshop on the quality and interpretation of lysosomal stability data (WKLYS) was conducted in 2010.

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Annex 10: Progress in the Spanish programme for monitoring marine pollution in the Atlantic coast

PROGRESS IN THE SPANISH PROGRAMME FOR MONITORING MARINE POLLUTION IN THE ATLANTIC COAST

Rapporteur/Contributor: Juan Bellas (Instituto Español de Oceanografía, IEO).

1. BACKGROUND

Recent progress in the Spanish marine pollution Monitoring Program for the Atlantic Coast, conducted by *Instituto Español de Oceanografía* (IEO), includes the adoption of an integrative approach that includes CEMP chemical methods and pre-CEMP biological methods. In order to establish clear relationships between results of chemical monitoring of pollution and the pollutant concentrations that may cause ecological damage, the following actions have been carried out: (i) a study on the biological effects of sediment elutriates by using the sea.urchin embryo-larval bioassay; (ii) a study on the toxicity of sediments by using the amphipod survival bioassay; (iii) a study on the biological effects of chemical pollutants on molecular responses in mussels (GST, GPx and AChE); (iv) a study of the biological effects of chemical pollutants on the physiology of mussels.

2. METHODOLOGY AND WORKING PLAN

2.1. Sampling

Sediment samples were taken with a box-corer dredge and the surface layer (2 cm) was collected, placed into sealed polyethylene bags, carried to the laboratory and stored at 4ºC in the dark. Organic matter content and percentage of fine particles (<63 microns) was determined (Figure 1).

Sediment elutriates intended for embryo-larval bioassays were obtained following Beiras (2002) by rotatory mixing of 100 g of sediment and 500 ml of control FSW at 60 rpm for 30 min

in airtight polypropylene flasks with no head space. After overnight decantation at 20ºC in the dark, the liquid phase (elutriate) was siphoned into a separate beaker and then aerated for 10 min to discard any potential toxicity caused by H_2S.

Intertidal wild mussels (*Mytilus galloprovincialis*) were collected by hand during the low tides, in the prespawning season at this area (October-November), in order to minimize seasonal variations in the enzymatic activity levels (Figure 2). Mussels were transported in a portable ice-box to the laboratory.

2.2. Sea-urchin embryo-larval bioassay

PreCEMP COMPONENT: Effects of marine pollution in invertebrate embryos and larvae.

OBJECTIVES: Biological monitoring of pollution. Implementation of an integrative monitoring in the Surveillance Programmes (recommended by OSPAR/ICES).

MATRIX: Sediments.

The toxicity of sediment elutriates was measured by using the sea-urchin (*Paracentrotus lividus*) embryo-larval bioassay. The experimental basis of these bioassays consist in the exposure of fertilized eggs to the sediment elutriates and, after an incubation period in controlled conditions, an ecologically relevant biological response is registered. About 20-40 fertilized eggs were delivered into 4 ml polypropylene vials with the elutriate dilutions. Experimental vials were incubated for 48 h at 20ºC in the dark, in culture chambers. After the incubation, samples were fixed with a few drops of 40% formalin.

The toxicity study of approximately 40 sediment samples concurrently with chemical data from the sediments is part of the integrative monitoring program.

2.3. Amphipod survival bioassay

PreCEMP COMPONENT: Amphipod survival study in sediment samples.

OBJECTIVES: Biological monitoring of pollution. Implementation of an integrative monitoring in the Surveillance Programmes (recommended by OSPAR/ICES).

MATRIX: Sediments

The amphipod (*Corophium* sp.) survival bioassay was used to evaluate the toxicity of sediments, as a complement of the sea-urchin embryo-larval bioassay. The biological response measured is the survival of amphipods during a 10 day exposure to sampled sediments at 20ºC and 12:12 h day:light cycle. Organisms are placed in 1 L beakers with the sampled sediments with 3 replicates per site and 5 replicates in the control sediment treatment. During the experiment temperature, salinity, pH and dissolved oxygen were controlled. After 10 days exposure, each beaker was sieved through 2 mm and the number of surviving individuals was recorded.

The toxicity study of approximately 25 sediment samples concurrently with the study of the

associated biota and the chemical data from the sediments is part of the integrative monitoring program.

2.4. Biomarkers in mussels

OBJECTIVES: Biological monitoring of pollution. Implementation of an integrative monitoring in the Surveillance Programmes (recommended by OSPAR/ICES).

MATRIX: Biota

The GST enzymatic activity in mussel gills (*Mytilus galloprovincialis*) was determined following the method of Habig et al. (1974) adapted to microplate. The enzymatic activity was determined by measuring the increase in absorbance at 340 nm every 20 seconds for 5 minutes. GPx activity was measured according to Halliwell and Gutteridge (1999). The decrease of absorbance was monitored at 340 nm. AChE was determined according to Bocquené and Galgani (1998) and adapted to microplate, by measuring the increase in absorbance of the sample at 412 nm for 3 min.

The toxicity study of approximately 40 mussel samples concurrently with the study of the chemical data in mussel tissues is part of the integrative monitoring program.

2.5. Scope for Growth in mussels

OBJECTIVES: Biological monitoring of pollution. Implementation of an integrative monitoring in the Surveillance Programmes (recommended by OSPAR/ICES).

MATRIX: Biota

The Scope for Growth of mussels (*Mytilus galloprovincialis*) was determined according to de Widdows & Staff (2006), and standardized to a 1 g dry weight.

The toxicity study of approximately 40 mussel samples concurrently with the study of the chemical data in mussel tissues is part of the integrative monitoring program.

2.6. Imposex in gastropods

OBJECTIVES: Biological monitoring of pollution. Implementation of an integrative monitoring in the Surveillance Programmes (recommended by OSPAR/ICES).

MATRIX: Biota

The penis length of *Nassarius reticulatus* and *Nucella lapillus* was measured under the microscope with a 0.01 mm resolution digital caliper. The vas deferens sequence (VDS) was determined for each female following the recommendations of OSPAR Commission (2008).

3. REFERENCES

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Figure 1. Sampling sites for the study of the spatial distribution of sediment toxicity in the Atlantic coast of Spain using sea-urchin and amphipod bioasays.

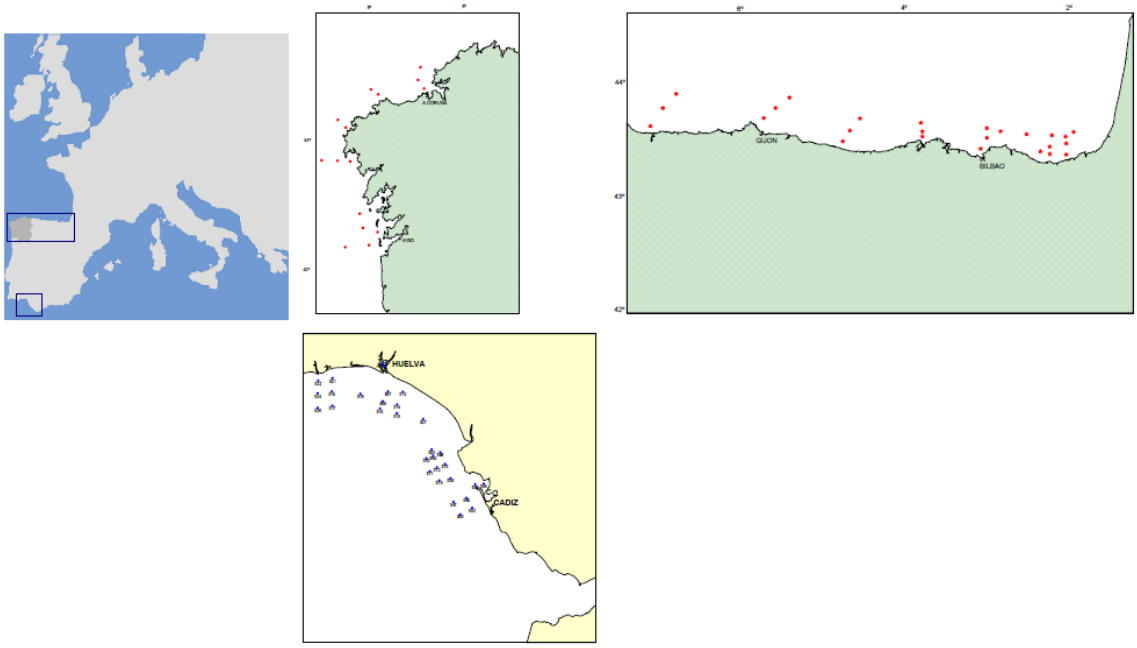
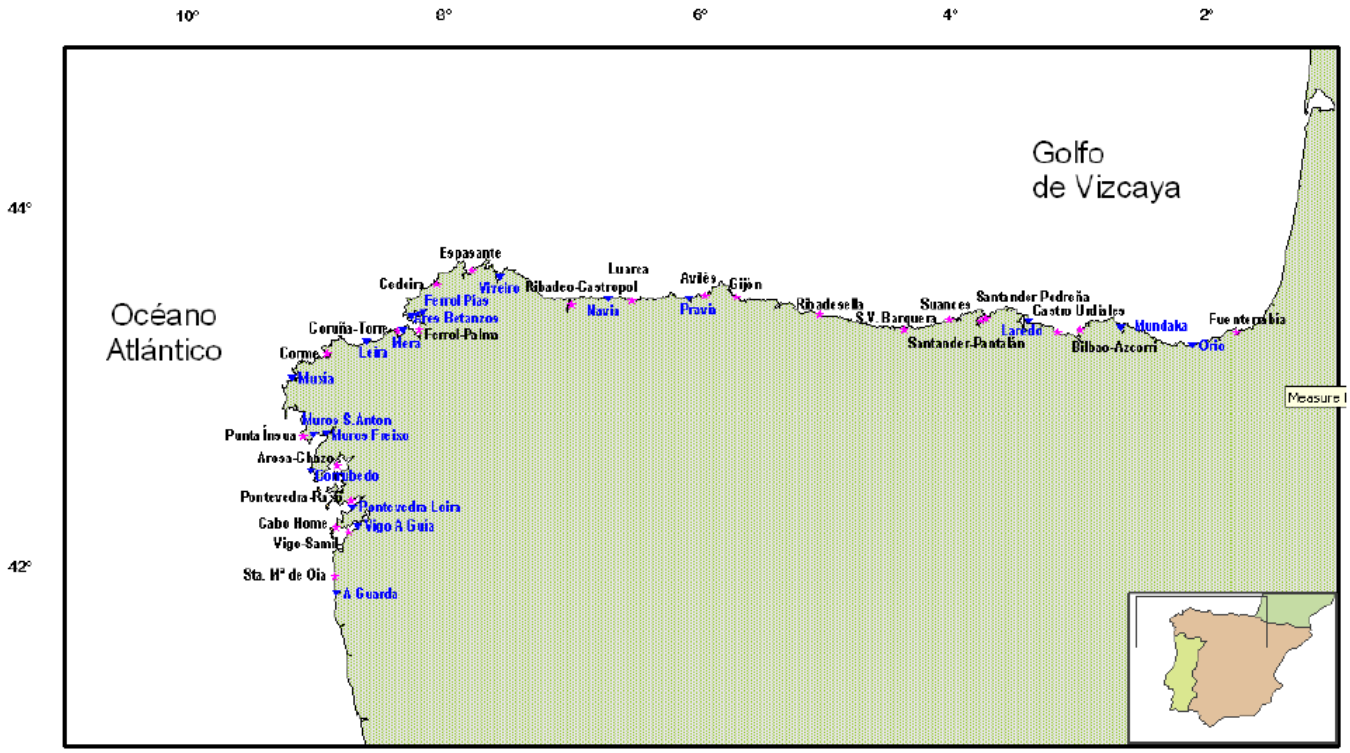


Figure 2. Sampling sites for the study of mussel (*M. galloprovincialis*) biomarkers (GST, GPx and AChE) and Scope for Growth in the Atlantic coast of Spain.



Annex 11: Technical minutes by RGLYSAC

Ketil Hylland (Chair), Michelle Giltrap, Thomas Lang

This review mainly focused on two issues: the development of assessment criteria for lysosomal (membrane) stability (1) and EACs for marine matrices (2). In addition, the RG had comments to mechanisms for updating OSPAR guidelines (3).

(1) Lysosomal (membrane) stability

Technical annex 6 in the JAMP guidelines was updated during WGBEC 2012 and references the ICES TIMES document which is now in the process of being amended following on from the MEDPOL harmonisation workshop (WLYS). In TA6 the background document is also referenced which has been updated following on from WLYS. These two documents contain information which will reduce the amount of variation in NRR assay data.

Existing assessment criteria for LMS was questioned at WKLYS as many labs that attended this workshop were observing <120min labilisation period at the reference sites. The assessment criteria for NRR were not changed since a collation of data from reference locations from different countries in the ICES area revealed that a large percentage of labs were achieving a median value of >120min. There seems to be a high degree of variation in data from reference sites, however, the source of which need be reviewed in coming years when more data is produced following the inclusion of a weighted scoring system.

All inclusions on methodology changes for the NRR assay in the TIMES document are relevant and should be included. The scoring of different cell pathologies will lead to less error associated with results from the assay.

TA6 has already been updated with sufficient information including monitoring of bivalve sp. with NRR as well as fish liver, recent intercalibration exercises and references to TIMES and background documents listing relevant changes for harmonisation of the technique.

Please see Annex 1 for suggested revisions and updates to the review provided by WGBEC 2012, describes an updated status for the method.

Recommendation

The RG supports the input from WGBEC (2012) with some additional suggestions (see Annex 1). As for other biological effects methods, it is important to revise all guidelines including this method using the latest information available.

(2) Ecotoxicological assessment criteria

This review is based on documents from OSPAR (HASEC 2011, MIME 2010) and reports from MCWG 2012 and WGMS 2012, as well as the draft report from WGBEC 2012.

Background

The intention of ecotoxicological assessment criteria (EAC) is to predict the concentration of a chemical in the tissues of marine organisms, in water or in sediment that will cause effect in the actual organism, in organisms higher in the food chain (such as humans) or in organisms present in the relevant habitat (water, sediment). As was commented by WGBEC 15 years ago during the first efforts to develop EACs, the

concept is problematic since other factors will modulate effects in ways that cannot be easily predicted, be for EACs in biota or sediment. This means that any management system using EACs will have to take into account large uncertainties.

EACs for biota

Lipophilic substances will not exercise any effects while stored in fatty depots, but if and when they are released, e.g. during starvation or other mobilization of fat. A fraction of a substance may be associated with membrane lipids and interact directly with cellular processes, so the above is not necessarily the entire truth, but serves to illustrate the problem of attempting to relate levels of chemicals in tissues to effects. Similarly, metals in tissues may be in a form which is not readily available for tissues, e.g. as granules.

EACs for sediment

It is also problematic to predict the toxicity of chemicals in natural sediments from its concentration, as commented by WGMS 2012. As for biota there are many modulating factors that will affect the toxicity and extrapolation from lab-based studies with spiked sediments is not really appropriate (spiked sediments will be more toxic than the same concentration in natural sediments). At WGMS it was indicated that a way forward could be the use of passive samplers combined with in vitro assays or effect-directed assessment. Such approaches should clearly be investigated, but there is no data available at present.

General considerations

Conceptually, the lowest value available should be used to provide protection for both human consumers and aquatic organisms. It should always be specified which "trophic chain" has been the basis for the EAC – whether human/top predator, fish or invertebrate. It is not necessarily so that human/top predator will be the most sensitive (organotins is an example of this).

It is important not to oversimplify the calculation of EACs even if it could be tempting due to scarcity of data. As commented by WGBEC (2012) it is crucial to separate between different organisms for EACs, both due to different metabolism and trophic chains. At the very least, fish and mussel need to be separated. Another major organism group for which there will be data is crustaceans, which should also be treated separately to the other two. It is to be expected that other taxonomic groups in marine ecosystems, e.g. echinoderms and tunicates, will have different sensitivity to the above, and it should be considered whether a general application factor should be applied to make the EAC more conservative, reflecting the data availability (as is done in general risk assessment of chemicals, EU TGD).

Each estimated factor, as compared to a measurement, used to derive an EAC will clearly increase the uncertainty of the final value. Any EAC should only at most include one estimated value.

The documents are using the term "dose-effect" for the relationship between the chemical and effects on aquatic organisms, but the correct term in this context is "dose-response". In toxicology and ecotoxicology, "dose-effect" is generally used to describe the relationship between the concentration of a substance in an organism and response in a single endpoint, e.g. activity of an enzyme or respiration.

Recommendations

There are fundamental chemical and biological issues with estimating coefficients in the derivation of EACs, simply because it requires impossible assumptions on processes in the environment and within organisms. Within organisms the main issue is a lack of knowledge of internal bioavailability and of interactions with other chemicals, and for sediment a lack of knowledge of bioavailability.

In addition to ensuring that the uncertainty in any given EACs is included with the value itself, it should be accepted that there may not be sufficient data to establish values for all chemicals. It is better not to have an EAC for a chemical than a highly uncertain (and probably erroneous) EAC.

(3) Updating guidelines (biological effects)

A number of guidelines currently exist within OSPAR concerning biological effects of contaminants, all of which have been developed at different times during the last 15 years. They include JAMP Guidelines for general biological effects monitoring (1997), JAMP Guidelines for contaminant-specific biological effects monitoring (1997) and OSPAR Guidelines of offshore monitoring (2004), Background document of biological effects of contaminants (2007). In addition, recent work within ICES/OSPAR SGIMC (SGIMC, 2011) has addressed methods and assessment criteria for biological effects.

As in any other research area there has been a development of methods and techniques, as well as increased experience, with biological effects methods. There is therefore be a more or less continuous need to update and revise existing guidelines. The two JAMP guidelines from 1997 will be superseded by the framework resulting from SGIMC processes and should be made redundant. The part of the guideline on offshore monitoring concerning water column monitoring need to be updated taking the outcome from SGIMC into account.

Recommendation

Efforts should be made to find an appropriate channel for regular updates to existing guidelines. ICES WGBEC would presumably be the most appropriate forum.

Annex 1. Suggested text changes for lysosomal (membrane) stability in the WGBEC 2012 report

7. Review and update of the Technical Annex on lysosomal stability:

To review and update, as necessary, the Technical Annex 6 (lysosomal stability) to the JAMP Guidelines for general biological effects monitoring. This should build on the latest developments through the Workshop on Lysosomal Stability Data Quality and Interpretation and WGBEC (OSPAR request 2012/1)

Rapporteur: C. Martínez-Gómez (ES)

Several ICES and OSPAR documents form the basis of background information on lysosomal membrane stability (LMS) as a technique for marine environmental monitoring in the ICES/OSPAR areas. These are:

1) An OSPAR background document (OSPAR, 2007a). (This has been reproduced with minor edits as Annex 5 to the 2010 report of the ICES/OSPAR Study Group on Integrated Monitoring of Contaminants (SGIMC) (ICES, 2010a) and section 9 in the current ICES CRR/OSPAR guidelines on integrated monitoring).

2) A Technical Annex (TA6) to the OSPAR Joint Assessment and Monitoring Programme (JAMP) Guidelines on general biological effects monitoring (OSPAR, 2007b).

3) An ICES TIMES publication describing the method for neutral red retention in mussels (Moore *et al*., 2004).

The current OSPAR request was to update 2) The JAMP TA6. This was updated to include information on the use of the LMS method in mussel species (using the Neutral Red Retention assay, NRR) and the information concerning assessment criteria. The existing information on the determination of LMS in fish (using the cytochemical method) was also updated with information on QA activities. Reference was also made to the new OSPAR background document produced during SGIMC and adopted by OSPAR on lysosomal membrane stability as a global health status indicator in biomonitoring (OSPAR, 2007a). This document contains the most comprehensive and up to date summary of the methods, their use and assessment criteria for marine monitoring purposes. The revised TA6 is given as Annex 7.

The OSPAR background document on lysosomal stability as a general health status indicator used for biomonitoring (Annex 5 of the 2010 SGIMC report; ICES 2010a) was reviewed by WGBEC and updated, with particular reference to the latest developments through the 2010 ICES/OSPAR Workshop on Lysosomal Stability Data Quality and Interpretation (WKLYS) (ICES 2010b). During the ICES/OSPAR WKLYS (ICES, 2010b), a number of uncertainties surrounding the methods in use and the assessment criteria proposed by ICES/OSPAR SGIMC were identified. All these aspects were discussed and are reported below. They are relevant to harmonize the use of the Neutral Red Retention (NRR) assay, in terms of monitoring and intercomparison purposes across the ICES maritime area and between Regional Seas programmes.

7.1. Assessment Criteria of LMS by using the Neutral Red Retention (NRR) assay

It was identified during WKLYS and during the 2012 WGBEC meeting that much data (median values) from 'reference' sites have not achieved a NRR time of 120 mins used as assessment criteria (AC). Available lysosomal membrane stability NRR times measured in reference sites in the ICES area were compiled (Table 7.1). It was decided not to amend the ACs because some reference sites clearly do achieve retention times of >120 mins (and may be truly representative of background values).

Table 7.1. Lysosomal Membrane Stability Neutral Red Retention Times for Reference Sites in the ICES Area.

Country	Median	Highest median value	90^{th} / 10^{th} percentile (if available)	Comments	Source of raw data
UK	<120	90	120 / 90	Individuals >120 min, never medians	Craig Robinson (MSS) John Bignell (Cefas)
Iceland	>120	180	180 / 90	Individuals NRRT ranging from 90 to 180	John Thain (Cefas)

Norway	>120	180	Range 90–180	Based on 10 datasets from west coast and Barents Sea in blue mussels	Steve Brooks (NIVA)
Norway, Barents Sea; N. Norw. coast (sub-Arctic)	>120	150	180 / 90	Iceland scallop, Sub-arctic, Barents Sea; North coast of Norway	Steinar Sanni (IRIS)
Norway, W. Svalbard (high Arctic)	120	120	120 / 72	Iceland scallop, High-Arctic; West coast of Svalbard	Steinar Sanni (IRIS)
Spain Mediterranean	<120	105	159 / 90	Individuals >120 min, median values use to range from 70–100	C. Martinez-Gomez (IEO)
Spain Atlantic	<120	75		Individuals >120 min, never medians	C. Martinez-Gomez (IEO)
Denmark	>120	165		Individuals up to 180 min, Medians often above 120 min	Jakob Strand (Aarhus University)
Ireland	120	120	150	Median value in reference station along the year range from 30 to 120 min	Michelle Giltrap

7.2. Review of the methodology of the NRR assay

During 2011/2012, C. Martínez-Gómez (Spain) and M. Gubbins (Scotland) contacted the authors of the original TIMES Nº 36 manuscript (Moore *et al*., 2004) and received feedback.

In agreement with the authors, it was decided that the NRR assay described in TIMES Nº 36 document should be amended and improved to make it more informative and robust, particularly concerning the following aspects:

- Correct the size of the needle to be used (21 gauge) for haemolymph extraction
- To incorporate the step of tipping off the dye and replacing it with seawater as recommended in MED POL protocol
- Suggest the use of physiological saline adjusted to the equivalent ionic strength of the ambient water or use ambient filtered seawater from the sampling sites
- To change the wording for the determination of endpoint to improve clarity
- Photographs in the original manuscript to be updated
- Image analysis to be included?
- Rephrase endpoint determination in manuscript with: "The test for each slide is terminated when dye loss or lysosomal change as described above are evident in greater than 50% of the granular haemocytes and the time recorded when

this occurs. The retention time is therefore the last analysis point at which less than 50% of the cells exhibit dye loss or lysosomal change, i.e. the last point at which the dye was retained and there were no structural changes. The mean and median retention time is then calculated for each sample set." (input from Dave Lowe, PML).

7.3. Scoring method to establish LMS in mussels using the NRR assay

Additionally, David Lowe made available during the meeting a new scoring method to record data of LMS using the NRR assay, not only based on neutral red retention time but also taking into account the observed morphological/pathological changes that occur in the lysosomes during the course of the assay. C. Martínez-Gómez (Spain) presented this to the group. She made available also some pictures that illustrated the different pathologies described by D. Lowe. During the course of the meeting, D. Lowe reviewed and agreed that the images chosen (see Figure 1) to represent the different lysosomal alteration types were appropriate to describe different pathologies.

Briefly, samples are analysed under the microscope and scored at 15, 30, 60, 90 and 120 minutes incubation for evidence of 50% or greater of the cells (granulocytes) exhibiting the lysosomal pathologies below which are listed in increasing severity of effect.

Pathology	Score
No effect	0
Enlargement of lysosomes but no leakage	1
Leakage but no enlargement of lysosomes	2
Leakage and enlargement of lysosomes	3
Leakage and enlarged but colourless lysosomes	4
Rounded up fragmenting cells	5

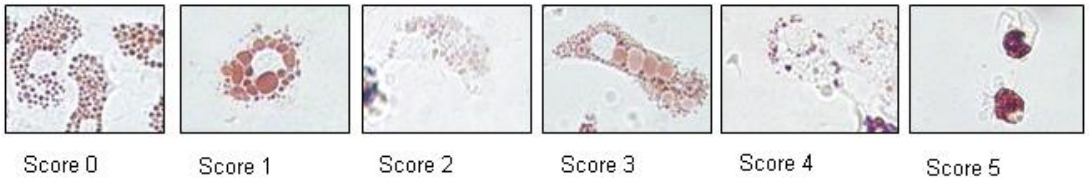


Figure 1. Illustrations of granulocytes of mussels (*M. galloprovincialis*) exhibiting different pathologies and the associated scores established: Score 0 = No effects; Score 1 = Enlargement of lysosomes but no leakage; Score 2 = Leakage but no enlargement of lysosomes; Score 3 = Leakage and enlargement of lysosomes; Score 4 = Leakage and enlarged but colourless lysosomes; Score 5 = Rounded up fragmenting cells.

In calculating the total final score for the lysosomal condition, the points in time at which they exhibited one of the 5 conditions above are coded 1, 2, 3, 4 or 5 and the individual scores[1] are multiplied by these weighting factors. Weighted scores are calculated by multiplying the scores by weighting factors: % stability = (1-(sum of the weighted scores/75))*100.

[1] Every time point for example 15, 30, 60, 90, 120 have designated weighted values (1–5). The individual score is based on the pathology of the cells at that point (0–5) and therefore these values are multiplied by the weighted score at that time point. These are then summed and % stability can be calculated.

Examples of monitoring data using these new scoring approaches are presented below in Table 1.

Table 1. Example of recording data using lysosomal damage with weighted (Wtd) scores.

Time Period	15		30		60		90		120		Sum of Weighted scores	% Stability
Weighting factor	1		2		3		4		5			
Slide Nr	Score	Wtd score	Score	Wtd score	Score	Wtd score	Score	Wtd score	Score	Wtd score		
1	0	0	0	0	0	0	0	0	0	0	0	100
2	5	5	5	10	5	15	5	20	5	25	75	0
3	1	1	1	2	3	9	3	12	4	20	44	41.3
4	2	2	2	4	3	9	3	12	4	20	47	37.3
5	2	2	4	8	4	12	5	20	5	25		
6	3	3	3	6	3	9	4	16	4	20		
7	0	0	0	0	1	3	1	4	1	5		
8												
9												
10												

Under the existing system (recording only NRR time), two samples can be considered as having the same status, even if they display different severity levels of pathology. C. Martínez-Gómez pointed out that this fact is one of the main reasons for the high variability observed in NRR results between individuals from the same sampling site and between laboratories, as interpretation of observations are sometimes not completely clear (i.e., when lysosomes are swollen but not leaking dye). Whilst the general pattern response is the same if using the two systems of recording data, the differences between sites is less extreme using weighted scores and the inter-animal variability is reduced (see Figure 2).

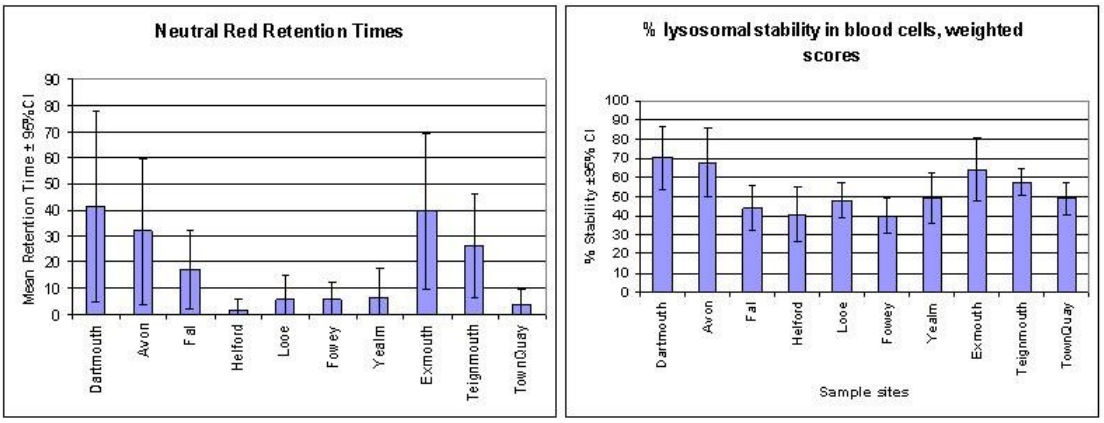


Figure 2. Lysosomal membrane stability in blood mussel cells expressed as NRR time (left) and as % of lysosomal stability (right).

Using the weighted score generated by the scoring method it is possible to also determine the endpoint that would have been ascribed by the existing endpoints/criteria and thereby make a comparison between the two approaches. WGBEC agreed that this new approach is a big improvement on the original methodology and one that has the potential to provide a better understanding of how different classes of contaminants affect lysosomal membrane stability and how this is manifested. Therefore, it was agreed by WGBEC that it would be beneficial to also include details

of the new lysosomal scoring system proposed by D. Lowe in the amended TIMES manuscript, so that this new improved approach can be disseminated and hopefully weighted score data generated alongside retention time data, which will lead to the generation of new assessment criteria.

It was pointed out that the change in methodology proposed will have implications on the ICES Environmental Data Reporting Formats. If weighted scores are reported as % LMS alongside retention time (mins) a new parameter code may be required.

Recommendation: ICES Secretariat to supply the revised TA 6 and associated guidance on resolution of issues identified by WKLYS to OSPAR.

Recommendation: A Draft Resolution to amend the publication ICES TIMES Nº 36 on measurement of Lysosomal Membrane Stability should be requested (expected publication date Dec 2012).

Action: WGBEC to follow up on the potential requirement for a change in ICES Environmental Data Reporting Format codes in the ICES Data Centre for inclusion of weighted score data.

Action: C. Martínez-Gómez to amend the existing TIMES MS 36 on neutral red retention.

References

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