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

3–7 March 2014

ICES Headquarters, Copenhagen, Denmark



**ICES**  
**CIEM**

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## **International Council for the Exploration of the Sea Conseil International pour l'Exploration de la Mer**

H. C. Andersens Boulevard 44–46  
DK-1553 Copenhagen V  
Denmark  
Telephone (+45) 33 38 67 00  
Telefax (+45) 33 93 42 15  
[www.ices.dk](http://www.ices.dk)  
[info@ices.dk](mailto:info@ices.dk)

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## Executive Summary

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The Working Group on the Biological Effects of Contaminants (WGBEC), chaired by Bjørn Einar Grøsvik (NO) and Ketil Hylland (NO), met at ICES HQ, Copenhagen, Denmark, 3–7 March 2014. There were 15 attendees through the week representing 10 countries.

This is the second year of the 3-year Terms of Reference for WGBEC. There were eight items on the agenda. Presentations and discussions took place in plenary, with rapporteur responsibility shared by members of the group. Some sessions were organised with MCWG and WGMS. All items on the agenda were completed and are reported.

**Respond to requests for advice from the Regional Seas Conventions.** WGBEC had received no official requests for advice from OSPAR via the ICES secretariat. WGBEC is aware of and welcomed the increased consideration of biological effects within OSPAR MIME and HASEC. Although widely used in European countries, most of the biological effects data has not been reported to the ICES database. The main reason is the complex reporting requirements and format, ensuring that only some institutions in each country has the capability to report. Future implementation of biological effects methods in OSPAR monitoring frameworks requires data in the database for assessment. WGBEC members will endeavour to submit as much data as possible before the autumn 2014 when the next assessment is due. The Chairs will facilitate this process.

WGBEC had been in contact with other expert groups, WGEEL, WGPDMO, MCWG and WGMS in relation to taking forward areas of common interest. As mentioned above, WGBEC, WGMS and MCWG met concurrently at ICES HQ in 2014. The joint sessions focused on ocean acidification, marine litter and passive samplers/dosing. A suggestion was forwarded to WGEEL for WGBEC members to meet with them back-to-back with a planned meeting for that group in January 2015.

**Consider emerging issues of scientific merit and address knowledge gaps (in relation to the ICES Science Plan).** In addition to issues discussed in joint sessions with MCWG and WGMS, WGBEC received presentations on oil toxicity to early life stages of fish, risk assessments, toxicity studies on nanoparticles and studies of metal pollution after mining.

**Review status of publications and consider requirements for new publications.** WGBEC reviewed status with ICES TIMES manuscripts and revised deadlines as appropriate. New and partially finalised manuscripts were available as draft for the meeting to consider. One of the Chairs will liaise with the WGBEC TIMES editor Ricardo Beiras and ICES Secretariat on progress with the manuscripts in the pipeline.

**AQC activities for biological effect methods including harmonisation activities initiated from WGBEC and within OSPAR, HELCOM and MEDPOL maritime areas.** It was noted that an EROD and a PAH bile metabolite intercalibration exercise is in progress, organised by Cefas, UK (analyses performed in 2013). The results were not sufficiently processed to be assessed by the group, but results expect to be available by the autumn 2014. The group discussed the necessity of keeping such exercises running on a regular basis and one of the Chairs (Ketil Hylland) assumed responsibility to follow up on this in 2014. No information was available concerning activities in the HELCOM area. A LMS neutral red exercise was held in Sweden with predominantly Nordic participants, but also WGBEC member Concepcion Martinez-Gomez.

**Respond to requests for advice from the ICES Data Centre.** Requests from the data centre were addressed during the meeting. There is a continuing need for updating fields and data reporting requirements. WGBEC members were identified as responsible for the different methods.

**National monitoring programmes.** Presentations and reports were provided by Juan Bellas (ES), Jakob Strand (DK), Ginevra Moltedo (IT), Thomas Maes (UK), Ulrike Kammann (GE), Thierry Burgeot (FR), Jonny Beyer (NO), Lisa Devriese (BE) and Lars Förlin (SE). An overview paper will be prepared for the 2015 meeting

**Development and harmonisation of methodologies for marine monitoring.** WGBEC received a brief report concerning the ICON integrated assessment demonstration programme from Ketil Hylland (NO). The programme is currently in the process of final publication. Integrated approaches were further discussed following presentations on different strategies in Italy, France, Spain and Sweden.

Assessment Criteria for biological effect techniques within the OSPAR SGIMC approach were reviewed and revised where deemed necessary.

**Novel and emerging compounds (e.g.) pharmaceuticals, recreational drugs, biocides and discharges from mining.** Ongoing research projects of common interest were identified for collaboration. A review on pharmaceuticals in marine ecosystems involving former WGBEC members is due in 2014. Reviews on early life stage toxicity from oil on fish and on immunotoxicological methods suitable for monitoring will be prepared prior to the 2015 meeting. Other issues that will be followed up in 2015 are toxicity of nanoparticles, as well as neurodevelopmental and behavioural effects.

**To evaluate the results of marine litter monitoring and research activities, especially microparticles (plastic/non plastic) and associated chemicals.** The group were presented with many of the research initiatives and programmes going on in the EU and elsewhere in this topic area, many of which were in their infancy. Members of WGBEC are actively involved in this work area and will contribute to the delivery of this ToR until 2015, taking account of the possible establishment of a new working group on marine litter.

## **1 Opening of the meeting**

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The ICES WGBEC was hosted this year by ICES, assisted by Maria Lifentseva, and held at ICES HQ, Copenhagen, Denmark. The meeting opened at 10:00 on Monday 3rd March, 2014. There followed a 'tour de table' to introduce group members, their affiliations background and science interests. 15 participants were present at the meeting through the week, representing 10 countries: Belgium, France, Germany, Italy, Norway, Spain, Sweden, Denmark, USA and the UK. The list of attendees is given in Annex 1.

## **2 Adoption of the agenda**

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The provisional agenda for the 2014 meeting was approved by the group (Annex 2).

Joint meetings with WGMS and WGMC were held at different times during the week on the topics ocean acidification (Monday afternoon), passive sampling/dosing (Thursday morning) and marine litter/microplastics (Thursday afternoon). WGBEC was also invited by MCWG to attend a plenary presentation on munitions in the Baltic.

## **3 Appointment of rapporteurs and ICES matters**

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Rapporteurs for the agenda items were selected.

## **4 Respond to requests for advice from the Regional Seas Conventions (e.g. OSPAR) and other EGs as required (ToR a)**

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### **4.1 OSPAR MIME**

WGBEC is of a strong opinion that reduction of the SGIMC suite of methods will reduce the possibility to detect effects of contaminants in marine ecosystems. WGBEC is also concerned about the process that has resulted in the selection of biological effects methods as 'Common Indicators'. The methods selected are not considered to represent a relevant and robust set of methods to detect effects of contaminants. Any reduction in the number of techniques used for monitoring purposes either within the SGIMC integrated approach or as choice indicator techniques should be evidence-based with clear rationale. In this respect WGBEC would like to offer its expert advice in any decision process for reducing the set of biological effect techniques for monitoring purposes.

In addition, there is an important need for OSPAR to determine a monitoring design strategy i.e. the application of the integrated approach, deployment of any techniques, with regard to targeted application, frequency of monitoring, statistical aspects of designing a monitoring programme and techniques for combining assessments across regional scales. Again, WGBEC would like to offer its expert advice in this area should the need arise.

Although widely used for monitoring purposes in European countries, a large proportion of the available biological effects data has not been reported to the ICES database. The main reason is the complex reporting requirements and format. Future implementation of biological effects methods in OSPAR monitoring frameworks requires an availability of data for assessment. WGBEC members will endeavour to

submit as much data as possible before the autumn 2014 when the next assessment is due. The Chairs will facilitate this process.

**Action:** WGBEC members to submit biological effects data to the ICES database before autumn 2014.

## 4.2 Response to other expert groups

### 4.2.1 SGOA – This request was discussed in plenary with MCWG and WGMS

Evin McGovern, co-chair of OSPAR-ICES Study Group on Ocean Acidification (SGOA), provided an update of progress of SGOA in addressing its ToR as defined by OSPAR. SGOA has a three-meeting cycle and the conclusions and products of SGOA will be incorporated in a report by the final meeting in October 2014.

SGOA is drafting an OA monitoring strategy that is well developed for physico-chemical monitoring of OA conditions although OA-specific biological impact indicators are less mature. Shell morphology of *Thecosomata* pteropods is a potentially sensitive indicator and SGOA suggests that a specimen repository would be a useful facility to enable retrospective monitoring for evidence of OA impacts once suitable indicator metrics are developed. SGOA 2013 discussed sensitivity of cold-water corals to perturbations in the carbon cycle and also new information on species responses and ecosystem interactions across CO<sub>2</sub> gradients at volcanic CO<sub>2</sub> vent sites as proxies of future OA conditions.

Guidelines for chemical monitoring have been submitted to OSPAR and data reporting formats and checks for OA-data from discrete samples to the ICES DOME database have been defined and tested by MCWG and SGOA. SGOA identified some tasks to be addressed by MCWG, specifically some outstanding reporting queries and to progress a workshop on Quality Assurance of OA measurements to support monitoring. These items were included in MCWG 2014 agenda point 5.7. Other biological expert groups have a role in ongoing development of appropriate impact metrics and in providing new information on ecosystem responses to OA. SGOA noted that WGBEC members had expressed an interest in contributing to this work and WGBEC members are invited to participate in SGOA.

### Reference

ICES (2013). Report of the Joint OSPAR/ICES Ocean Acidification Study Group (SGOA), 7-10 October 2013, Copenhagen, Denmark. ICES CM 2013/ACOM:31. 82 pp.

### 4.2.2 WGEEL – Area of mutual interest for WGBEC and WGEEL: are contaminants in eels contributing to their decline?

WGBEC and WGEEL in their 2012 reports identified the possibility to collaborate inter sessionally in 2013 and to this end propose to address the following over-reaching questions as a ToR.

WGBEC identified the following over-reaching questions:

- a) What are the concentrations of contaminants in eel populations and have they changed in recent years? To include “traditional” and/or “emerging” contaminants.
- b) Are these contaminants at concentrations likely to cause harm and contribute to decreasing eel populations via impacts on reproduction and quality of offspring including endocrine disruption?



- c) Are contaminants in conjunction with other factors (e.g. lipid metabolism) impairing the survival, fitness and reproductive capability of eels?
- d) Are there tools that can be developed to measure the effects of contaminants in a non-destructive manner?
- e) Can experiences / data from other species stand as a model for what goes on in the eel?

WGBEC have identified a sub-group who are prepared to contribute to this collaboration:

John Thain (UK), James Readman (UK), Dick Vethaak (NL), Ulrike Kammann (DE), Katja Broeg (DE) and Jakob Strand (DK).

**Recommendation:** WGBEC suggest that the indicated members meet with WGEEL back-to-back with WGEEL January 2015 meeting in Brussels.

## 5 Consider emerging issues of scientific merit and address knowledge gaps (in relation to the ICES Science Plan); (ToR b)

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### 5.1 Oil toxicity to early life stages of fish

In 2013, WGBEC received a request from Tracy Collier (USA) to consider recent studies demonstrating phototoxicity of bunker fuel combined with the field work after the Cosco Busan spill. This may represent the strongest case yet for phototoxicity in a field setting. This appears to be associated with bunker fuel exposures, and not with crude oil. WGBEC was asked to address the findings that very low ppb levels of tri-cyclic PAHs, found in weathered oils, are embryotoxic and consider the implications for altering e.g. OSPAR EACs on the information available thus far, and what types of information would be useful to strengthen the case more.

Developing fish embryo and larvae are highly sensitive to different types of PAHs, and this toxicity is dependent on oil composition, weathering and photosensitization. Environmental Assessment Criteria for PAHs are at present very scarce (OSPAR Environmental Assessment Criteria, 2012) and a better resolved data set of EACs on should be elaborated for the oil toxicity to early life stages of fish based on the recent published work. WGBEC appreciate the initiative from Tracy Collier and would be interested in suggestions for EACs of PAHs in water and egg/larvae from Collier and colleagues or other researchers in this field. Such data would be highly valuable for risk assessments of oil exposures to early life stages of fish.

Considering the information provided above and discussions WGBEC decided to follow the approach below to progress this ToR before next WG meeting.

- 1) Review suitability of existing assessment criteria for hydrocarbons in light of new toxicity data to larval fish;
- 2) Identify uncertainties and knowledge gaps and place these in context of environmental risk assessment framework;
- 3) Account for photooxidation and risk factors relevant to life history and ecology of sensitive species such as exposure to surface micro-layers;
- 4) Produce a review with appropriate recommendations for environmental assessments.

Point 2 in the action list have been addressed in the published articles listed below and B. E. Grøsvik informed on these works under agenda 9b- Risk assessment.

The other points from the action list will be worked on by Bjørn Einar Grøsvik (NO), Ketil Hylland (NO), Ulrike Kamman (GE), Sonnich Meier (NO) and North American authors identified above will be invited to contribute to this process.

One of the Chairs, Bjørn Einar Grøsvik (NO), informed on ongoing research projects at IMR on effects of crude oil and PAHs on early life stages and juveniles of haddock that will be performed at IMR during spring 2014. A new project financed by the Norwegian Research Council titled “Assessment of long-term effects of oil exposure on early life stages of Atlantic haddock using state-of-the-art genomics tools in combination with fitness observations” started up in 2014. For this project haddock eggs and larvae were exposed to dispersed crude oil continuously or by pulse. Another exposure were performed on juvenile haddock in 2014, financed by Statoil titled: “Comparative DNA damage and long-term health effects in juvenile haddock after exposure to sediment or produced water associated PAHs.” For this project PAHs were given through pellets with PAH profiles similar to produced water, weathered crude oil or pyrogenic PAH. Both projects aim to give more data related to effect parameters after exposures of haddock to oil and PAH components, in addition to more data for setting environmental assessment criteria. Some of the WGBEC attendees expressed interest in collaborating on effect studies on material from the lab exposures.

**Action:** Prepare a review paper on early life stage toxicity of oil on fish.

## References

- Hauge KH, Blanchard A, Andersen G, Boland R, Grøsvik BE, Daniel Howell D, Meier S, Olsen E, Vikebø F.. 2014. Inadequate Risk Assessments – A Study on Worst-Case Scenarios Related to Petroleum Exploitation in the Lofoten Area. *Marine Policy*. 44:82-89.
- Blanchard A, Hauge KH, Gisle Andersen G, Fosså JH, Grøsvik BE, Handegard NO, Kaiser M, Meier S, Olsen E, Vikebø F. 2014. Harmful routines? Uncertainty in science and conflicting views on routine petroleum operations in Norway. *Marine Policy*, 43: 313-320.
- Vikebø FB, Rønningen P, Lien VS, Meier S, Reed M, Ådlandsvik B, Kristiansen T. 2013. Spatio-temporal overlap of oil spills and early life stages of fish. *ICES Journal of Marine Science*; doi:10.1093/icesjms/fst131.

## 5.2 Ocean Acidification

See 4.2.1 for a report of the common session with MCWG and WGMS. WGBEC developed the following strategy for the remainder of its 3-year ToR:

- 1) To review the existing literature for recommendations on suitable species / endpoints for monitoring
- 2) To focus efforts on those parameters relating to the expertise of WGBEC (end-point measurements in individuals / populations rather than eg biogeographic trends etc)
- 3) To account for in-combination effects with other climate change variables (eg carbonate chemistry changes and temperature)
- 4) To produce a written review for publication including monitoring recommendations. This will start in 2014 by a group led by Kris Cooreman (Belgium) and otherwise comprising Steve Brooks (Norway), Klaas Kaag (Netherlands), Aldo Viarengo (Italy), Matthew Sanders (UK) and Andrea Johnson (US).

**Action:** Prepare a paper on methods suitable for monitoring ocean acidification.

### 5.3 Immunotoxicity

Environmental immunotoxicology was discussed by WGBEC in 2011, 2012 and 2013, based on reviews by Tom Hutchinson (UK), Andrea Johnson (US) and a presentation by Ketil Hylland (NO), respectively.

The aim of the group over the 3-year period is to develop or identify methods by which to assess environmental immunotoxicity in marine ecosystems.

The 2013 WGBEC meeting identified Johan Aerts, Dick Vethaak and Ketil Hylland as members to be involved in the continuing work on immunotoxicology, in addition to members who have previously shown interest (Tom Hutchinson, Andrea Johnson). The 2014 WGBEC meeting discussed alternatives for developing this issue further and agreed on limiting our efforts to methods applicable for monitoring. Ketil Hylland will take the initiative in preparing a manuscript on the use of immunotoxicological methods in monitoring, liaising with the above, as well Michel Auffret (FR) and Noomi Asker (SE).

**Action:** Produce a draft manuscript by WGBEC 2015 on immunotoxicological methods suitable for monitoring.

### 5.4 Neurodevelopmental and behavioural effects

One of the Chairs, Ketil Hylland (NO), introduced the topic with a couple of papers showing the potential for early exposure to toxic substances, in this case methylmercury, to affect behaviour years later in a fish species (Fjeld *et al.*, 1998) and for a pharmaceutical to affect behaviour in perch at an environmentally relevant concentration (Brodin *et al.*, 2013).

**Action:** To be followed up in 2015.

### 5.5 Nanoparticles

Joachim Sturve (SE) introduced the topic and informed on experimental work with silver nanoparticles (AgNP) on fish compared with exposure to silver (Ag<sup>+</sup>). Responses were studied with genomic based methods like RNA expression and proteomics.

Nanomaterials and nanoparticles (NPs) are rapidly becoming an important part of new technology. NPs are today used in a variety of products, from IT to pharmaceuticals and body care products. NPs may also be part of future intelligent solutions for new environmental friendly technologies. However, very little is known about the toxicity of NPs and their effects in the aquatic environment. Compared to molecular chemicals tested, the NPs are quite complex due to their physico-chemical properties, and new integrated strategies are necessary to elucidate their toxicity. NP behaviour builds primarily on physical forces between particles and not on thermodynamic equilibria. One factor that effects the particle interaction is the salinity of the media and higher salinity leads to higher rate of agglomeration of the NPs. It has therefore been suggested by several researchers that NPs are not of major concern in marine environments, since the NP will agglomerate and end up in the sediments. However, NPs are possible sources of toxic compounds in the marine environment.

Silver NPs are of main concern regarding ecotoxicological effects due to the known toxicity of silver ions, and silver ions are toxic to several aquatic organisms such as algae and daphnia. Silver is also toxic to fish. Recent studies show that the levels of silver ions in sewage treatment effluent recipients have increased and in some case

reach levels close to LC50 for several algae species. It is believed that this increase is connected to an increase in the use of silver NPs. Silver NPs are today the most extensively used inorganic metallic nanoparticles and every fifth nano-product in the market is expected to contain Ag NPs. A large part of these products have the potential to release silver into the aquatic environment, often through sewage treatment plants.

Even though NPs are considered not to be a major threat to the marine environment it is important to follow the development of the research field. NPs might still pose a threat, especially as a source of toxic metals and/or molecules.

**Action:** to be followed up in 2015.

## 6 Review status of publications and consider requirements for new publications (ToR c)

### 6.1 ICES TIMES

WGBEC reviewed status with ICES TIMES manuscripts and revised deadlines as appropriate. Several new manuscripts were produced in draft for the meeting to review. Status of the manuscripts was deemed satisfactory. The current status of WGBEC TIMES manuscripts is given in table 6.1 below.

**Table 6.1. Current status of ICES TIMES publications.**

Resolution Ref	Deadline	Description	Comment on status
2012/1/SSGHIE08	30/04/13	The report on the COM-ET assay for fish and mussels Author/Editor: Tim Bean (UK) and Farida Akcha (France).	Formatting required prior to review.
2012/1/SSGHIE09	30/04/13	The report on the Condition Index for fish and mussels. Author/Editor: John Thain (UK), Matthew Gubbins (UK), Concepcion Martinez Gomez (ES), and Lennart Balk (SE).	Formatting and text revision required.
2012/1/SSGHIE10	30/04/13	The report on the Stress On Stress assay for mussels. Author/editor: John Thain (UK) and Concepcion Martinez Gomez (Spain).	First draft completed and preliminary review conducted at WGBEC. No change expected to delivery deadline.

2012/1/SSGHIE11	31/07/13	The report on the Lysosomal Membrane Stability in the Blue Mussel <b>Author/editor:</b> Concepcion Martinez Gomez (Spain), John Bignell (UK) and David Lowe (UK).	Reviewed version submitted to ICES.
2009/1/SCICOM08	30/11/12	The method for determining 'Reproductive Success in Eelpout' <b>Author/Editor:</b> Jakob Strand (Denmark) <b>Reviewer:</b> WGBEC/SSGHIE	Withdrawn - new request when ready.
2006/1/MHC06	08/10/12	The Protocol for Extraction Methods for Bioassays <b>Author/Editor:</b> Hans Klamer (NL), Steve Brooks (NO) and John Thain (UK) <b>Reviewer:</b> Chair of SSGHIE	Manuscript sent to ICES.
2006/1/MHC07	08/10/12	The protocol for conducting EROD determinations in flatfish <b>Author/Editor:</b> Compiled by M. Gubbins, WGBEC members <b>Reviewer:</b> Chair of SSGHIE	For publication as a minor revision to TIMES 23.
2007/1/MHC02		Blue Mussel Histopathology <b>Author/Editor:</b> John Bignell, Steve Feist, Dave Lowe and MirenCajaville	Withdrawn -new request when ready.

Requirements for new TIMES manuscripts were discussed. The possibility of a method manuscript for litter monitoring was raised. This will be revisited after EU protocols have been defined.

## 6.2 Other publications

Ketil Hylland (NO) presented the ICON project publication plan as a series of peer review papers in a special edition of Marine Environmental Research. Authors have been contacted to confirm authorship, deadlines and length of publications. Final submission will be late spring 2014. An overview of contributions can be found at Annex 4.

**Action:** Chairs and TIMES editor to monitor progress of manuscripts to comply with deadlines.

## 7 Report progress on AQC activities for biological effect methods including harmonisation activities initiated from WGBEC and within OSPAR, HELCOM and MEDPOL maritime areas (ToR d)

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Any biological effect method to be used for national or international monitoring programmes should be subject to appropriate internal and external AQC, 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.

At its meeting in 2012 WGBEC discussed AQC activities for biological effect methods and agreed to initiate a low cost programme for EROD and PAH bile metabolites, organised under BEQUALM. Cefas UK, collected samples of fish liver and bile from wild caught fish and distributed these to interested laboratories, 11 in total from Norway, Denmark, France, Germany, Spain, The Netherlands and the UK. Data from this exercise were made available to WGBEC during the meeting and discussed.

WGBEC are not aware of any AQC activities underway in the HELCOM maritime area.

An intercalibration and training exercise on LMS (neutral red) in mussels took place in 2013 involving Norway, Sweden and Denmark. WGBEC member Concepcion Martinez-Gomez participated at the

**Action:** Chairs to follow-up on the performed intercalibration with a view of repeating it, including AChE and vtg in addition to EROD and PAH metabolites.

## 8 Respond to requests for advice from the ICES Data Centre (ToR e)

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Guidance was provided to the Data Centre on requirements for reporting formats for marine litter and a draft format for litter reporting was presented by Marilyn Sørensen, ICES during the litter session.

It was noted during the meeting that there is a lack of understanding of reporting format issues within WGBEC. This is caused by an absence of data submitters from the group and difficulties in comprehending the nature of the requests coming from ICES. WGBEC felt that while it was best placed to advise on issues of data quality, suitability, supporting data requirements etc., greater communication with those familiar with the reporting formats and database structure is required in order to be able to respond adequately to requests from the Data Centre. It was suggested to generate a distribution list for communications regarding biological effects reporting formats by data type/parameter. WGBEC can identify the 'experts' for advice by parameter, but would need assistance from the ICES Data Centre to identify the data submitters with the experience of the reporting formats / data base. These contacts could then be used to respond to future requests for advice, coordinated by WGBEC as appropriate.

**Recommendation:** A distribution list for communications regarding biological effects reporting formats by data type/parameter should be generated.

## 9 Development and harmonisation of methodologies for marine monitoring (ToR f)

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### 9.1 Integrated assessments

With the interim adoption of the SGIMC approach by OSPAR on a 3-year trial basis there is a request to Contracting Parties to provide evidence of application and assessment of the value of the new approach (cf OSPAR MIME 2012). WGBEC therefore collated examples of national and international case studies either completed, planned or in progress across the ICES/OSPAR regions to keep track of progress with case studies.

The use of an integrated approach was reported by Ginevra Moltedo (IT) for the environmental monitoring carried out by ISPRA for offshore industrial facilities such as oil and gas activities or a Terminal LNG and for a shipwreck (Costa Concordia). Besides chemical analysis of water and sediment samples, biological effects of contaminants were evaluated through biological assays and biomarkers analysis.

In particular, biomarker analyses were performed on mussels, clams, fishes and polychaetes. According to the species analysed specific set of biomarkers were chosen including responses at the whole organisms level (mortality, Condition index and stress on stress) and those at subcellular level such as biomarker of exposure (MT, AOX, AChE, EROD, VTG, bile metabolites), of genotoxicity (micronuclei, comet assay), of oxidative stress (CAT, GR, GSTs, GPx, totGSH level, TOSC-Assay, MDL levels) and lysosomal stability (NRRT, cytochemical).

A special emphasis was given to the need of identify bioindicator species for evaluation of sediment contamination. To that respect ISPRA highlighted the use of a benthic invertebrate species, *Hediste diversicolor*. After testing the sensibility of the species to B[a]P and to Hg in dedicated experiments, ragworms were tested with sediment samples; biological effects were evaluated after *ex situ* sediment exposure. Comparisons were performed between biological responses after exposure to sediment samples collected in the proximity of source of potential contamination and after exposure to sediment samples collected in a reference site.

Juan Bellas (ES) presented the combined use of chemical, biochemical and physiological variables in mussels for the assessment of marine pollution along the N-NW Spanish coast. Within the presentation of the activities carried out in the Spanish Monitoring Program, the calculation of a chemical pollution index (CPI) was reported. This CPI was calculated for each site, to summarize the chemical data, as the average of the ratios between the pollutant concentrations (C) in each site and the corresponding environmental quality criterion (Ccrit) for each analyte (Bellas *et al.*, 2011; Beiras *et al.*, 2012):

$$CPI = \sum_i CF_i = \sum_i [\log (C_i / C_{criti})]$$

In order to identify the most important pollutants to be used in the CPI calculation, Principal Component Analysis (PCA) was applied to the chemical database, and only those chemicals showing loadings >0.7 in the PCA were selected to calculate the CPI.

An Integrated Biomarker Response (IBR) was also calculated according to Beliaeff and Burgeot (2002), by combining the different biochemical (GST, GPx, AChE) and physiological (Respiration rate, Ingestion rate, Absorption rate) variables to a single value. IBR was calculated by means of star plots of the biomarker data. Star plots

were used to represent the scores (standardized data) of the abovementioned biomarkers for each site. IBR index is the star plot area.

In order to integrate biomarker responses and chemicals accumulated in mussels tissues a PCA was carried out. According to this analysis, Hg, Pb and  $\Sigma$ PAHs are weakly associated to GST induction, but organochlorines ( $\Sigma$ PCBs,  $\Sigma$ DDTs,  $\Sigma$ chlordanes) and  $\Sigma$ BDEs, seem to be the main responsible of the observed effects on physiological responses and on GPx induction. This finding is supported by the significant correlations obtained between organochlorines and IBR ( $\Sigma$ DDTs:  $r=0.371$ ,  $p < 0.05$ , and  $\Sigma$ HCHs:  $r=0.326$ ,  $p < 0.05$ ).

Brita Sundelin (SE) presented integrated assessment in the CORESET project. The HELCOM CORESET project started in 2010 and had the objective of developing and delivering a set of preliminary core indicators and targets to be forwarded to the national decision making processes by the end of September 2011. This HELCOM project was replaced by Coreset II project that will run from autumn 2013 to summer 2015.

The core indicators will ultimately be placed on the HELCOM web page and kept annually/bi-annually updated with new data. In order for the core indicators to be clear, concise and informative, the final core indicators and their information sheets need a uniform format. It is of utmost importance that the HELCOM core indicators covering eutrophication, hazardous substances as well as biodiversity aspects of the ecosystem comprise a uniform set of indicators with identical layout and headings.

Two assessment criteria are used to assess biological effects:

Background Assessment Criteria (BAC)

Environmental Assessment Criteria (EAC)

The assessment criteria were developed within the Oslo and Paris Commission (OSPAR) framework with scientific advice from the International Council for the Exploration of the Sea. Mean values below the BAC are said to be near background. Values below the EAC indicate no chronic effects on the organisms concerned. Full details can be found in Davies & Vethaak (2012) or OSPAR (2013).

It has been important to develop and apply tools for a science-based assessment and management with regard to the impact of anthropogenic contaminants on the Ecosystem Health of the Baltic Sea to further develop science-based guidelines, assessment and management of the impact of anthropogenic contaminants on the Ecosystem Health of the Baltic Sea.

Presently, different methodologies are available or under development to monitor and assess pollution effects and ecosystem health in marine and coastal waters. A number of integrated indices and similar approaches (e.g. Expert Systems) based on the measurement of a set of biomarkers have recently been developed and tested in the field in the North Sea/Atlantic or the Mediterranean. So far their application for the specific conditions in the Baltic Sea is still missing.

BONUS+ BEAST was invited by HELCOM to contribute to the CORESET project, the ideas and concepts briefly described above were taken into consideration into the further development and recommendations of a set of bioeffect Indicators for the future HELCOM MONAS programme. The HELCOM core indicators should primarily be used to assess the effectiveness of the implementation of the Baltic Sea Action Plan (BSAP), but also as tool for implementation of the Marine Strategy Framework Directive (MSFD). In this role, the core indicators needed to be aligned with the EU



MSFD descriptors and criteria and methodological standards of good ecological status (GES).

In accordance with the HELCOM Monitoring and Assessment strategy the core indicators will in the future be used to update the Thematic Assessments and the Holistic Assessment. Core indicators are being developed for eutrophication in the EUTRO-OPER project and for hazardous substances and biodiversity in CORESET II.

Today the imposex and bile metabolites were accepted as a core indicators, while the biological effect indicators i.e. lysosomal membrane stability (LMS), micronuclei frequency (MN), fish disease and reproductive disorders in terms of malformed embryos in eelpout and amphipods still are precore indicators. EROD, acetylcholine esterase activity (AChE) and vitellogenin induction are candidate indicators. Before being core indicators the following requirements must be fulfilled:

- Concept
- Necessary monitoring (spatiotemporal requirement and method)
- Structure for analysing data
- Assessment method and assessment area (s)
- GES-value

There is a risk that none of the biological effects methods will be updated to core indicators but instead be supplementary indicators, which means that each country is free to use them but there is no obligation.

## References

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## 9.2 Environmental risk assessment

B. E. Grøsvik (NO) presented two papers on environmental risk assessment which were also relevant to agenda point 5.1- Oil toxicity to early stages of fish. The papers are listed under 5.1 (Hauge *et al.*, 2014 and Blanchard *et al.*, 2014.).

## 9.3 Assessment criteria

### Addition of BAC-value for EROD activity in microsomal fraction for eelpout

Lars Förlin (SE) and Jakob Strand (DK) suggested criteria for EROD in eelpout liver. Swedish monitoring data for EROD activity in microsomal fraction for eelpout has been used for deriving Background Assessment Criteria at 20 pmol min<sup>-1</sup> mg protein<sup>-1</sup> based on long time trend series using sample sizes at 15 – 25 individuals per year from the reference stations Kvädojärden in the Baltic Sea and Fjällbacka in the Skagerrak (the North Sea); (Hansson *et al.* 2014).

Based on this work, a BAC-value for EROD activity in microsomal fraction at 20 pmol min<sup>-1</sup> mg protein<sup>-1</sup> for eelpout has been added to the table for assessment criteria (Table 9.1).

Comparative studies on BEQUALM and other relevant samples on EROD activity in both microsomal fraction and S9-fraction of fish liver, shows that EROD activity in the microsomal fraction can be determined to be around factor 3.5 higher in average after adjustment to protein content (Förlin, pers. comm.). This factor has thereby potential for being used as conversion factor comparing EROD data for microsomal fractions and S9 fractions. Using this conversion factor will correspond to a BAC-value for S9 fraction at 5.7 pmol min<sup>-1</sup> mg protein<sup>-1</sup>, which are in the same range as the 10 pmol min<sup>-1</sup> mg protein<sup>-1</sup>, which previously has been proposed for EROD activity in S9-fraction for eelpout (WGBEC report 2012), and thereby indicate the consistency for comparison of the two BAC-values for based on microsomal fraction and S9 fractions for EROD activity in eelpout liver.

**Table 9.1. New criteria for EROD (both perch and eelpout) and vitellogenin for perch.**

Method	Species	BAC
VTG in plasma; ng/ml	Perch (M)	115
EROD; pmol/min/mg protein	Eelpout (F)	20
	Perch (F)	50

The criteria malformed embryos of amphipods is based on monitoring data during 20 years in the Bothnian Sea and Baltic proper, 1994–2011. Fourteen stations were included in the analysis. Data is based on 8600 gravid females and 230 000 embryos. A higher sample size gives less variance. Since sample size varies much between years and stations, we used repeated hazardous sampling, i.e. Bootstrapping, where we can control the sample size. A sample size of 50 gravid females (about 1500 embryos), was used. This is the recommended sample size within the National Monitoring program. The hazardous sampling of 50 females in the data set was repeated 100 000 times to obtain an even spread. The background value was 2.6 and 90e percentile (ICES cooperative Research Report no 315) 3.8 % malformed embryos, EAC >3.8. Another way to assess the effect is to analyse the proportion of females carrying malformed embryos. We used data from contaminated areas (outside industries) and monitoring data and comparison of proportion of females with more than 1 malformed embryos facilitates detection of statistically differences between pristine and contaminated areas. Hazardous repeated sampling (100 000 times) of 50 females showed that background value (BAC) of females with more than one malformed embryos is 15.4 % and 90e percentile is 22%.

#### **An assessment tool for monitoring liver cancer in marine environment– a preliminary report**

John Bignell and Allan Reese (UK) submitted a proposal for a new assessment tool for monitoring liver cancer in marine fish. The work has primarily arisen from previous concerns raised that liver cancer might simply be a surrogate for age and that contaminants may not be the sole cause. This is particular concerning when the age distribution of one of the OSPAR region's primary biomarker species, the common dab (*Limanda limanda*), is considered. Analysis of data from dab of the size range 20–24 cm, as recommended by ICES in biological effects monitoring programmes, revealed that age can vary considerably. The report is presented in Annex 5.

## References

Hansson T, Hansen W, Tjarnlund U, Balk L, Bengtsson, BE. 2014. Biomarker Investigations in Adult Female Perch (*Perca fluviatilis*) From Industrialised Areas in Northern Sweden in 2003. *Arch. Env. Contam. & Toxicol.* 66:237-247.

**Recommendation:** ICES Secretariat to inform OSPAR of revised criteria.

## 9.4 National monitoring programmes

National reports on recent monitoring and research activity were received in the form of reports and text contributions by correspondence from Denmark, France, Germany, Norway, Spain and Sweden. Details on these reports are curated on the 2014 SharePoint and it is intended to continue to build an accurate reflection of the sum of national biological effects monitoring over the last meetings and compile this information in the form of a manuscript for the final 2015 report.

Jakob Strand (Aarhus University, Department of Biosciences) presented status on the biological effects monitoring in Denmark, which are coordinated with the contaminant monitoring within the frame of the Nationwide Monitoring and Assessment Programme for the Aquatic and Terrestrial Environment (NOVANA).

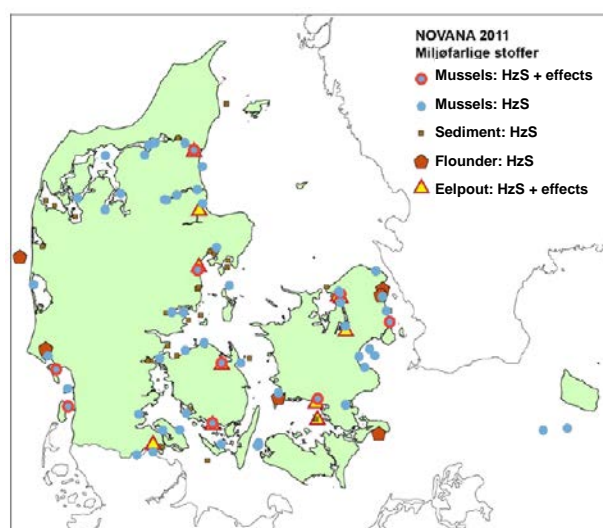


Figure 9.1. Danish monitoring stations for contaminants and biological effects measurements in mussels (mainly blue mussels), fish (eelpout and flounder) and sediments which was analysed as part of the NOVANA program 2011.

In addition to these stations TBT-specific effects (i.e. imposex or intersex) in 5 species of gastropods were also monitored covering 34 stations in 2011 (Hansen *et al.* 2012).

Following groups of hazardous substances are monitored in either in mussels (M), fish (F), and/or sediment (S) within the current NOVANA program period running from 2011 to 2015: Metals (M, F, S), organotins (M, F, S), PAHs (M, S), PFCs (M), dioxins/furans (M, F), chlorinated pesticides (F), PCBs (M, F), PBDEs (F), phthalates (S), alkylphenols (S). The list of biological effects indicators can be seen in Table 9.2.

Table 9.2. Status on monitoring activities for biological effects indicators within the current NO-VANA program period running from 2011 to 2015.

JAMP group	Biological Effect indicator	Species	Number of stations	Sampling Frequency	Start year
TBT-specific effects in gastropods	Imposex	Common whelk <i>Buccinum undatum</i>	14	Every 2 <sup>nd</sup> year	1998
		Red whelk <i>Neptunea antiqua</i>	10	Every 2 <sup>nd</sup> year	1998
		Netted whelk <i>Hinia reticulata</i>	10	Every 2 <sup>nd</sup> year	1998
		Dog whelk <i>Nucella lapillus</i>	3	Every 4 <sup>th</sup> year	1998
	Intersex	Periwinkle <i>Littorina littorea</i>	6	Every 2 <sup>nd</sup> year	1998
PAH-specific effects in fish	PAH-metabolites	Eelpout ( <i>Zoarces viviparus</i> )	12	Every year	2011
	CYP1A activity (EROD)	Eelpout ( <i>Zoarces viviparus</i> )	12	Every year	2004
General effects in mussels or fish	Lysosomal membrane stability (NRR)	Blue mussel ( <i>Mytilus edulis</i> )	10	Every year	2004
	Reproductive success in fish	Eelpout ( <i>Zoarces viviparus</i> )	12	Every year	2004

The number of biological effects stations and the monitoring frequencies has changed several times since the start in 1998 during revisions of the monitoring program in 2004, 2007 and 2011 and it will probably be revised again in 2016.

Regarding monitoring data on TBT-specific effects in gastropods, the trend clearly shows declining effect levels following the declining TBT-levels in the environment after the ban as antifouling agents in 2003, which in 2012 in average show about 10% of the TBT levels that occurred in 2003.

Similar declines has subsequently also occurred for the levels of imposex and intersex. However, the levels of imposex as well of TBT in mussels in some coastal areas are still elevated and above derived EAC-values, either in the vicinity of harbours or close to shipping lanes. In open water, only one of the more sensitive species *Neptunea antiqua* express elevated imposex levels above EAC close to international shipping lanes in Kattegat, The Sound or the Belt Sea, but not in the North Sea and Skagerrak anymore see figure 9.2.

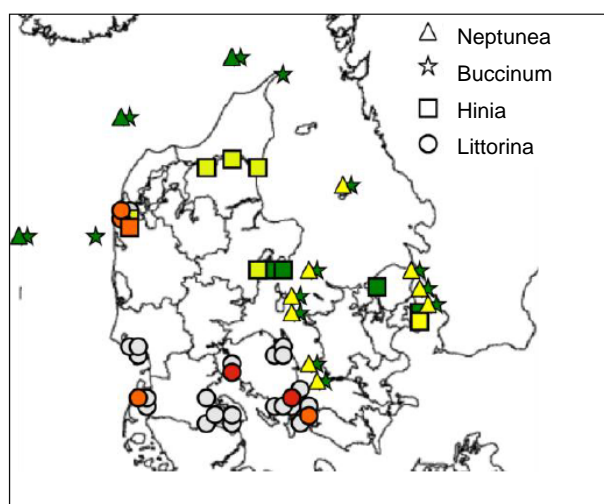


Figure 9.2. Elevated Imposex levels show that TBT in especially some coastal waters still pose a threat to the marine ecosystem in 2011 (Hansen *et al.* 2012).

Table 9.3. The levels of imposex and intersex in gastropods are assessed using the OSPARs integrated assessment scheme.

	I	II	III	IV	V
VDSI in <i>Nucella lapillus</i>	< 0.3	0.3 - < 2	2 - 4	> 4 - > 5 (sterile)	Disappeared
ISI in <i>Littorina littorea</i>	< 0.3			0.3 - 1.2	> 1.2
VDSI in <i>Neptunea antiqua</i>	< 0.3	0.3 - < 2	2 - < 4	4 - 4+	
VDSI in <i>Buccinum undatum</i>	< 0.3		0.3 - < 2	2 - < 4	4 - 4+
VDSI in <i>Hinia reticulata</i>	< 0.3		0.3 - < 2	2 - < 4	4 - 4+

For the indicators of PAH-specific effects, i.e. CYP1A/EROD and PAH-metabolites in the coastal fish eelpout, shown positive relationships to the levels of PAHs and PCBs. For instance in 2012, the use of a combined factor for PAH-metabolites (1-OHP equivalents) in bile and  $\Sigma$ PCB7 (normalised to dry weight) in muscle strengthen the correlation to CYP1A. The combined factor was determined as the sum of PAH and PCB after they both have been divided with the mean values for all stations. Compared to the developed assessment criteria, PAH-metabolites above BAC (>100 ng/ml) in all areas, whereas CYP1A/EROD is above BAC (10 pmol min<sup>-1</sup> mg protein<sup>-1</sup>) in 7 of 12 areas (Hansen *et al.* 2013)

For the general effects indicator in fish, i.e. reproductive success in eelpout, abnormal fry development occur above BAC 7 of 12 areas and among these are 3 above EAC in 2012. However, it has not until now been able to establish significant relationships to either specific contaminant groups or other environmental factors abiotic factors monitored in the sampling areas.

For the general effects indicator for blue mussels, the data for lysosomal membrane stability from most years have shown significant relationship to especially PAHs, but has in some previous years also shown co-variations with PCBs. There are generally non-significant relationships with other contaminant groups, temperature and salinity. It should be noticed that LMS retention times are determined as time for desta-

bilised membranes in lysosomes and not as the last observation time for stabilised lysosomes as outlined in the revised ICES TIMES, i.e. for being comparable the LMS values should be subtracted with 30 minutes. However this will mean that all stations can be assessed to be below BAC (average retention time at 120 minutes) instead of 7 of 10 stations and 3 stations will have LMS-values below EAC.

As MSFD relevant indicators, the Danish Nature Agency have in 2012 included all the current NOVANA effect indicators in the national report to EU on preliminary environmental indicators for the definition of GES for Descriptor 8 "Concentrations of contaminants are at levels not giving rise to pollution effects" in regard MSFD relevant monitoring in Denmark. This includes "Imposex, lysosomal membrane stability and cell damage in marine snails, mussels and eelpout" together with contaminants (e.g. heavy metals, TBT and PAHs) in water, sediment and biota. They are still included in the latest draft outline from the Danish Nature Agency in 2014 on the list of environmental indicators, which will be part of the Danish MSFD monitoring.

The CEMP monitoring strategy in France was presented by Thierry Burgeot (FR). The monitoring activity is conducted under the national network (Rocch: monitoring network of chemical contamination in France) since 1974 and allows to assure the continuity of an expertise and the collection of data in long term. The list of the chemical contaminants analysed under the national network is the list recommended by OSPAR and for some of them by the Barcelona convention. The chemical contaminants are analyzed annually in oysters and mussels. Contaminants are also analyzed in the sediment every five years. The imposex is determined every year as a mandatory biomarker since 2003. The integrated approach of chemistry and biology is adopted in the CEMP monitoring and is used on a research basis in the pilot site the channel (Baie de Seine). Biomarkers (EROD, the DNA adducts, Comet, Micronuclei, Vitellogenin, intersex, Imposex, PAH metabolites, AChE, pathologies and LMS) and bioassay in the sediment (oyster embryotoxicity) are analyzed since 2008 in the Seine Bay within the ICON program. A revisited IBR index was developed as a tool of interpretation (Devin *et al.*, 2013) France was anticipating further revisions in its monitoring programme in the context of MSFD implementation and in consideration with the new JAMP integrated guidelines monitoring and assessment of contaminants. The French ministry of the ecology and energy and sustainable development published an order for the descriptor 8 of the MSFD (17 December 2012) with a list of chemical contaminants and biological effects: Lysosomal stability, genotoxicity (Micronuclei, comet assay) in fish and mussels, reprotoxicity (gonads histology in fish and oyster embryotoxicity) and fish pathologies. It is a first step towards the potentially monitoring French monitoring plan of the MSFD actually in discussion until the end of 2014.

Ulrike Kammann (Thünen institute) presented biological effects monitoring in Germany. Most of the monitoring concerning contaminants and their effects in marine fish in Germany is carried out by the Thünen Institute of Fisheries Ecology. Since 1999 an integrated monitoring is performed combining chemical measurements and biological effects measurements in the same samples as far as possible. Different organic as well as inorganic contaminants are determined in dab and flounders at 4 to 6 offshore sites from the North Sea every year. Fish diseases, parasites, liver histopathology, fish disease index (FDI) and PAH metabolites are determined once or twice a year at the same sites. In addition fish are investigated for radionuclides. Germany started to expand the suite of applied techniques by micronucleus assay and lysosomal membrane stability in fish. However this is still in a test phase. Further

on an integrated assessment of German monitoring data guided by the SGIMC recommendations is planned for the near future.

Other German institutions perform imposex monitoring regularly along German coasts. There is no consensus in Germany concerning the national implementation of biological effects techniques under the MSFD. The Thünen Institute is willing to continue its monitoring activities and will consider other recommended techniques to fulfil the goals of JAMP, MSFD and BMP.

An overview of on-going, governmental-initiated environmental monitoring programs in Norway was presented by Jonny Beyer (NIVA). The main emphasis is on the MILKYS program and the Offshore Water Column Monitoring programme, since these programs include biological effects components.

In Norway, the governmental-initiated monitoring of chemical contamination in aquatic ecosystems is performed within four national environmental monitoring programs:

- Monitoring of hazardous substances in Norwegian fjords and coastal waters (MILKYS),
- Monitoring of pollutants in urban fjords,
- Offshore Monitoring Programme (Condition monitoring & Water Column Monitoring)

Data from 2012 were shown since the data from the 2013 work are not ready for presentation yet. The MILKYS program work in 2012 included monitoring of blue mussel (23 stations), dog whelk (8 stations), common periwinkle (1 station) and cod (14 stations) along the coast of Norway. The MILKYS stations are located both in areas with known or presumed point sources of contaminants, in areas of diffuse load of contamination like city areas, and in more remote areas exposed to presumed low and diffuse pollution. The programme includes monitoring of metals, organochlorines, pesticides, dioxins, brominated flame retardants, perfluorinated compounds, as well as biological effects methods. Analyses of hexabromcyclododecanes (HBCD), short and medium chained chlorinated paraffins (SCCP, MCCP), organophosphorus flame retardants (PFRs), bisphenol-A (BPA), and tetrabrombisphenol A (TBBPA) were included in this programme for the first time. The biomarkers which presently are included in the MILKYS program are pyrene metabolites in fish bile, ALA-D in fish red blood cells, EROD & CYP1A in fish liver, and imposex in marine snail. A selection of representative MILKYS data was presented for the WGBEC meeting.

This report address data from 2012 study at the Troll C platform as the data from the 2013 study not yet are ready for presentation. At Troll C, the data of sea current, PAH-NPD body burden in caged mussels, and POCIS extracts indicated that the position of the mussel stations was suitable to capture the produced water plume from the platform. Mussels from stations located 500 m from the platform showed significantly increased concentrations of PAH and NPD. An overall reduction in PAH-NPD concentrations was found with distance from the platform. Low to background concentration of PAH-NPDs was found in mussels 2000 m from the platform. POCIS extracts measured elevated levels of APs compared to the reference station. No clear relationship between biological responses and distance from the platform was observed, the biological responses at the biochemical and physiological level indicated relatively healthy mussels at all stations, but histopathological analysis of mussel digestive gland indicated a minor stress condition in mussels caged 500 and 1000 m

from the platform. The validity of these latter data was discussed at the WGBEC meeting.

Juan Bellas presented data from the Spanish National Monitoring Program, which includes the assessment of pollution in a large region (over 2500 km of coastline) of the N-NW Spanish coast, by combining the use of biochemical (AChE, GST, GPx) and physiological (SFG) responses to pollution, with chemical analyses in wild mussel populations (*Mytilus galloprovincialis*). High levels of pollutants were found in mussel populations located close to major cities and industrialized areas and, in general, average concentrations were higher in the Cantabrian than in the Iberian Atlantic coast. AChE activities ranged between 5.8 and 27.1 nmol/min/mg prot, showing inhibition in 12 sampling sites, according to available ecotoxicological criteria. GST activities ranged between 29.5 and 112.7 nmol/min/mg prot, and extreme variability was observed in GPx, showing activities between 2.6 and 64.5 nmol/min/mg prot. Regarding SFG, only 5 sites showed 'moderate stress' (SFG value below 20 J/g/h), and most sites presented a 'high potential growth' (>35 J/g/h) corresponding to a 'healthy state'. Multivariate statistical techniques applied to the chemical and biological data identified PCBs, organochlorine pesticides and BDEs as the main responsible of the observed toxicity. However, the alteration of biological responses caused by pollutants seems to be, in general, masked by biological variables, namely age and mussel condition, which have an effect on the mussels' response to pollutant exposure.

An overview of fish monitoring work in Sweden was given by Lars Förlin (SE). It was reported that the health status of two sentinel fish species perch (*P. fluviatilis*) and the viviparous eelpout (*Z. viviparus*) have been regularly studied in four national reference Swedish coastal sites, one located in the Bay of Bothnia (Holmön), two in Baltic Proper (Kvädöfjärden and Torhamn) and one in Skagerrak (the North Sea)(Fjällbacka). In these coastal reference sites with no or small local point sources of contaminants, perch and eelpout health studies together with analytical chemistry work to measure anthropogenic chemicals and fish ecology studies, form an integrated fish monitoring program supported by the Swedish EPA. The fish health work has been run yearly for more than 25 years, and the integrated work for 15 years. Generally the fish health studies seem to indicate good status in the reference sites but an increasing number of the fish health parameters (i.e. biomarkers) clearly show significant time trends which suggest changes of concern. For example in female perch from Baltic Sea coastal sites has been observed 20-30% reduction of gonad size, and a more than five times increase of the activity of the detoxification enzyme EROD. Other significant time trends include e.g. changes in blood plasma ions i.e. chlorine and calcium contents, increase lymphocyte number, and indications of oxidative stress.

It was also presented results from a recent project focused on the reference area Kvädöfjärden. The purpose of this project was to find possible explanation for the observed time trends. It was for example reported some details about co-variation over time for different biomarkers and some pollutants as well as that different biomarkers co-varied with different environmental factors e.g. temperature, precipitation, salinity as well as benthic fauna composition. It was concluded, based on current knowledge that it is not possible to find any simple explanation/causation for the observed changes of fish health in coastal fish from Kvädöfjärden. Instead the causes the changes seen in coastal fish health is to be found in combined impact of continuous and varied exposure to mixtures of chemicals and changes over time in different environmental factors such as temperature, salinity and food availability. The outcome of the project thus showed that there still are a number of questions needed to be focused to elucidate causality. These questions are related for example



to route of transport and exposure of pollutants including bioturbation in sediment, food preference and availability, land-sea gradient of pollutants and biomarker patterns. It was also stressed that more data is needed about degradation product of pollutant, and especially OH-PBDEs were mentioned, and that knowledge gaps with respect to ecotoxicity and time trends in biota must be filled for certain compounds groups such as organophosphate esters, adipates and siloxanes.

In addition, it was also presented that the Swedish monitoring biomarker data obtained in fish from the four national coastal areas has been used to set background or reference values. All the values were based on the variation in the average values each year, and set to represent 95% of all values from the reference sites. The established interval will then represent the normal variation from a reference area. It was finally shown how these reference values can be an aid in assessing the fish health impact in monitoring for example downstream point sources (e.g. in receiving areas of industry effluents).

### References

Bellas J, Albentosa M, Vidal-Liñán L, VBesada V, M. Franco MA, Fumega J, González-Quijano A, Viñas L, Beiras R. Combined use of chemical, biochemical and physiological variables in mussels for the assessment of marine pollution along the N-NW Spanish coast. *Marine Environmental Research*, in press.

**Action:** To prepare an overview paper for the 2015 meeting.

## 10 Address issues in relation to novel and emerging contaminants (e.g. pharmaceuticals, nanoparticles, toxicity of mixtures, discharges from mining activities, etc.)

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- Pharmaceuticals and recreational drugs in the marine environment
- Nanoparticles in the marine environment
- Discharges from mining activities

Joachim Sturve (SE) showed results with monitoring and effects studies of pharmaceuticals and nanoparticles in the marine environment (Ref Chapter 5). Ketil Hylland (NO) introduced to discussions on pharmaceuticals and discharges of mining. Jacob Strand (DK), presented results from monitoring discharges of mining activities in Greenland.

Following discussion it was decided to follow the subsequent strategy for delivery of this ToR

- 5) Continue to receive updates on inputs, concentrations and effects of emerging contaminants including: biocides, pharmaceuticals, nanoparticles and recreational drugs and in-combination effects
- 6) Consider the above in the context of environmental risk assessment
- 7) Produce a review document for each of these issues by 2015 highlighting advances made, continued knowledge gaps and recommendations for environmental monitoring.

A review on pharmaceuticals in marine ecosystems involving former WGBEC members is due in 2014. Other issues that will be followed up in 2015 are toxicity of nanoparticles, discharges from mining activities as well as neurodevelopmental and behavioural effects.

## 11 Evaluate the results of marine litter monitoring and research activities, especially microparticles (plastic/non plastic) and associated chemicals (ToR g)

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This agenda point was addressed in a joint session with MCWG and WGMS, see also <http://www.ices.dk/news-and-events/news-archive/news/Pages/43-scientists,-three-expert-groups,-one-overriding-theme.aspx>

The joint session was chaired by WGBEC who also provided the majority of presentations on marine litter and microplastics, see section 5.4.1. Furthermore, Marilynn Sørensen of the ICES Data Centre presented the Data Centre's work on a draft format for litter reporting, as further described in section 5.5.1.

Thomas Maes (CEFAS, UK) presented a comprehensive review of several aspects of the marine litter issue.

The term "Marine Litter" has been introduced to describe "any persistent, manufactured or processed solid material discarded, disposed of, or abandoned in the marine and coastal environment". It consists of articles that have been made or used by people and, subsequently, deliberately discarded or accidentally lost. They originate from ocean-based or land-based sources and can be found in marine environments around the globe. Most sources of marine pollution are land based. Marine litter, mainly plastic, poses a serious environmental threat to marine organisms, as well as a series of economic and social problems. The majority of marine debris is comprised of plastic materials (60–80% overall and 90% of floating debris).

All marine litter particles smaller than 5 mm are considered microparticles. Most microparticles consist of microplastics, although other types exist. The abundance and global distribution of microplastics in the oceans has steadily increased over the decades to around the year 2000 following the rising plastic consumption worldwide since the 1940s. However, there has been a decrease in the average size of plastic litter over this time.

Primary microplastics are produced either for direct use, such as for exfoliants, cosmetics, industrial abrasives or for indirect use as precursors (nurdles or virgin resin pellets) for the production of multiple plastic consumer products

Secondary: Microplastics formed in the environment as a consequence of the breakdown of larger plastic material, especially marine debris, into smaller and smaller fragments (so called "secondary microplastics"). The breakdown is caused by mechanical forces (e.g. waves) and/or photochemical processes triggered by sunlight (especially UVB)

Other types of microparticles are synthetic fibres shedding of textiles by domestic clothes washing, rubber fragments from tires rubbing tarmac, fly ash fine particles that rise with the flue gases after combustion.

The potential impacts of litter span both economic and ecological dimensions. The following section highlights the different aspects that are considered relevant.

Economic:

- Losses to fishing and shipping industry
- Clean up costs on beaches
- Loss of tourist revenues

- Aesthetic disturbance

Ecological:

- Ingestion
- Entanglement
- Introduction of invasive species
- Bioavailability and transfer due to sorbing/leaching
- Smothering
- Disturbance

Marine litter comes from a variety of land-based and sea-based sources and is essentially a consequence of poor waste management. However, the main sources can be grouped as follows:

The main land-based sources of marine litter:

- Discharge of untreated municipal sewage, including storm water discharges and overflows
- Tourism (recreational visitors to the coast; beach-goers)
- Riverine transport of waste from landfills or other sources along rivers and other inland waterways and canals
- Industrial facilities: Solid waste from landfills, and untreated waste water
- Municipal landfills (waste dumps) located on the coast or inland
- Direct littering

The main sea/ocean-based sources of marine litter:

- Fishing vessels
- Merchant shipping, ferries and cruise liners
- Military fleets and research vessels
- Pleasure craft
- Offshore oil and gas platforms
- Fish farming installations

The MSFD requires member states to manage their seas to achieve Good Environmental Status (GES) by 2020. MSFD Descriptor 10 requires litter to be at levels where the *'properties and quantities of marine litter do not cause harm to the coastal and marine environments'*.

MSFD criteria and indicators require understanding and monitoring of (JRC, 2013):

The characteristics of litter in the marine and coastal environment – including:

- Trends in the amount of litter washed ashore and/or deposited on coastlines, including analysis of its composition, spatial distribution and, where possible.
- Trends in the amount of litter in the water column (including floating at the surface) and deposited on the sea-floor, including analysis of its composition, spatial distribution and, where possible.
- Trends in the amount, distribution and, where possible, composition of micro-particles (in particular micro-plastics).

The impacts of litter on marine life – trends in the amount and composition of litter ingested by marine animals.

Cefas is involved in several national and international marine litter projects. Thomas Maes (Cefas) focused on two EU projects MICRO and MARLISCO:

The Micro EU Interreg project is monitoring microplastics (MP) within the 2 Seas Region and will provide a risk assessment based on field observations, lab experiments and mathematical models. MICRO is a cross border cooperation to prevent environmental, technological and human risks attributed to MP. Furthermore the project will contribute to establish common strategies for environmental risk assessment by modelling the potential impacts on the environment, and by proposing follow-up tools and mitigation measures. The three main pillars of the project are:

Scientific: a risk assessment of the current situation by combining distribution data, modelling and biological effect measurements with socio-economic endpoints.

Educational/knowledge exchange: establishing good practices for adequate monitoring or impact determination across Europe.

Public/scientific awareness: increase awareness of human behaviour in relation to waste production and management by creating co-responsibility among the different actors.

The EU FP7 MARLISCO project activities take place in the four European Regional Seas: North-East Atlantic, Baltic, Mediterranean and Black Sea, by a consortium with members located in 15 coastal countries. MARLISCO's overarching goal is to raise public awareness, facilitate dialogue and promote co-responsibility among the different actors towards a joint vision for the sustainable management of marine litter across all European seas. It will do this by developing innovative mechanisms and tools. MARLISCO aims to effectively engage, inform and empower society, reaching the widest possible audience.

Bavo de Witte and Lisa Devries (both ILVO, Belgium) presented recent results of their research framed by the European projects MICRO and CleanSea into the associations between litter and contaminants in terms of the following two presentations:

A quantitative GC-MS screening was performed on marine litter, present within benthos beam trawl nets during fishing activities. No clear indication of chemical contamination was found on blue synthetic rope. None of the OSPAR -7 indicator PCBs were found at concentrations > 0.1 ng/g. The origin of determined PAHs, alkylated PAHs, alkanes, alkenes and alkylated aromatic compounds may be pyrogenic/petrogenic pollution as well as plastic production. Phenols and specific antioxidants and UV-absorbers can also be related to plastic production.

Little data is available on the role of microplastics as a vector for PCBs through the marine trophic levels and impact studies are required under controlled conditions. Benthic marine organisms such as the common shore crab and Norway lobster were exposed to PCB loaded microplastics under controlled laboratory conditions. In these experiments, 500–600 µm diameter polyethylene or polystyrene spheres were loaded with PCBs. The microspheres will pass the digestive tract without accumulation in the organism and egestion of the spheres was observed within two days after uptake. Within this research, it was shown that PCBs could desorb from the microspheres during the short period in the digestive system, but only a very small uptake of PCBs was observed by Norway lobster. No additional effect caused by the microspheres could be observed.

Work on plastic litter as a vector for bacteria had been carried out by Lisa Devriese, Caroline de Tender and Sara Maes (all at ILVO, BE). The possibility for microplastics and litter to act as a vector for bacteria and pathogens was suggested based on a bacterial screening on beach pellets, marine plastic litter and plastic beach litter. Diverse methods such as Next Generation Sequencing (NGS), TOPO TA cloning, PCR-DGGE were used to identify the bacterial communities of the different types of plastic. The NGS work is performed in the framework of a new ILVO-genomics platform 'GA Genomics'.

Michiel Kotterman (NL) presented research on plastics. Next to monitoring the presence of plastics in the environment (as monitored by trawling; bottom and egg surveys), in biota (fish, fulmars and seals) the main research topic is to determine the role of microplastics with regard to contaminants. Are they a vector of contaminants, enhancing the uptake of contaminants by biota, or are they a sink for some contaminants due to their high affinity for some contaminants, lowering the exposure.

This is being investigated with lugworms under realistic conditions, micro-PS in contaminated sediments (Besseling), and models for effects of plastic ingestions have been made (Koelmans). So far, plastics can be vector as well as sink, the effects under natural conditions are, from of risk assessment perspective, generally small. More data is required for proof and to improve the models. Therefore, research will be focussed on the net effects of plastic on the uptake of contaminants under natural conditions.

Within the EU project ECsafeSEAFOOD, IMARES is involved in feeding trials of fish. Salmon will be exposed to plastics and contaminants in the feed. In one treatment plastics will be equilibrated with the contaminants before feeding, while in another treatment clean plastics will be added to contaminated food while feeding. This may add to the understanding of processes (rates especially) during the digestion.

Jakob Strand (DK) gave a presentation on the relationships between microplastic particles, sediment characteristics and contaminants in sediments from Danish waters based on a study on distribution of microplastic particles (38  $\mu\text{m}$  – 5mm) in sediment in the Danish waters from the Baltic Sea towards the North Sea. The results indicate that normalisation of microplastic abundances to adequate sediment characters can reduce the variability caused by natural heterogeneity between samples and thereby increase the power of identifying more or less affected areas. Strong relationships between the content of microplastics in sediments and both %TOC and fine sediment fraction (<6  $3\mu\text{m}$ ) were found throughout the area supporting that microplastics will accumulate in sedimentary depositional areas – i.e. with parallels to organic pollutants sorbed to organic materials. Positive correlations were also established to contaminants, especially PAHs and to lesser extent to alkylphenols and phthalates in sediments. It could be due to co-variation with sources and TOC rather than due to chemical extraction of microplastic particles. However, at least antifouling agents like TBT in paint flakes from ship lanes and harbours can be one exception.

Bjørn Einar Grøsvik (NO) presented a collaboration project with the Polar Research Institute of Marine Fisheries and Oceanography (PINRO) in Russia. Co-workers on this study were Elena Eriksen (IMR, Norway) and Tatiana Prokhorova and Pavel Krivosheya (both PINRO, Russia). Since 2004 these institutes have collaborated on ecosystem based surveys in the Barents Sea. From 2010 registration of marine litter has been a part of this collaboration. Surface investigations and trawl catches have demonstrated highest occurrence of litter in the areas of intensive fishery and naviga-

tion. Plastic prevailed among observed litter. Other types of litter (metal, paper, rubber, textile, glass) were sporadically observed.

Taking into account the presentations given during the marine litter session as well as the available literature, the groups remark that there is currently insufficient information to assume that the uptake of chemical contaminants by marine biota through digestion of microplastics is significant. In some cases, enhanced uptake of plastic additives can occur, if these are not yet in equilibrium with the surrounding environment. More plastic uptake might also occur at locations where marine litter accumulates by marine gyres. Major problems of marine plastic pollution seem to be related to obstruction by and/or uptake of large amounts of plastics.

WGBEC as well as MCWG stress their interest to work further on the field of marine litter as well as microplastics. Both groups would be particularly be interested in further information on desorption studies in gastrointestinal tracts and work on uptake of chemical contaminants by organisms from marine litter.

MCWG recommended to WGBEC to share new information with MCWG identifying plastics as a vector of enhanced contaminant transfer to biota.

The large amount of information provided through the presentations did not leave enough time to work on a comprehensive problem description. Activities in the field of marine litter have increased significantly, including a number of national and EU research projects and work in several fora towards marine litter monitoring in relation to MSFD. As described above, a separate ICES working group dedicated to marine litter has been proposed by members of WGBEC.

The ICES data centre has set up new litter record to include litter information in Environmental Reporting Format 3.2. In the framework of the MSFD (descriptor 10) the task group marine litter at the ICES Data Centre defined different litter categories in 2013. It was, however, too complex, to include the variability of types and sizes within the existing framework, and it was decided to set up a separate litter record. This includes the following information: depths min/max, litter size, litter reference list, parameters/unit/value, litter source, litter use, number of entangled biota, state of litter, polymer type, and attached organisms (non-microbial). ERF3.2.5 is available on the WGBEC sharepoint and comments to the new record can be given to [mari-lynn.sorensen@ices.dk](mailto:mari-lynn.sorensen@ices.dk) by the 1 April 2014.

It was suggested that one person of each group should give suggestions and that the database should be kept as lean as possible since this will lead to more people who will fill in the database.

## References

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[www.marlisco.eu](http://www.marlisco.eu)

[www.ilvo.vlaanderen.be/micro](http://www.ilvo.vlaanderen.be/micro)

<http://www.cleansea-project.eu/drupal/index.php>

<http://www.ecsafeseafood.eu/>

## 12 Any other business

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At the 2013 meeting, WGBEC had received a request from ex-member Lennart Balk (Sweden) to consider the emerging issue of thiamine deficiency in marine wildlife which has been linked to population-level effects in sea birds and marine fish. The issue may be linked to contaminant effects and would be of considerable interest to the group. Accordingly WGBEC would like to invite Lennart Balk to introduce the issue to the group at its meeting in 2015. Jacob Strand suggested to extend this session to other vitamin research like effects on the Vitamin A system.

**Action:** Chairs to invite Lennart Balk for a specific time slot during the 2015 meeting and to include research on other vitamins like vitamin A for this session. The ToR for 2015 is amended accordingly below at Annex 3.

## Annex 1: List of participants

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Name	Institute	Email
Juan Bellas Bereijo	Instituto Español de Oceanografía Centro Oceanográfico de Vigo Cabo Estai - Canido P.O. Box 1552 36200 Vigo (Pontevedra) Spain	juan.bellas@vi.ieo.es
Jonny Beyer	Norwegian Institute For Water Research Gaustadalléen 21 NO-0349 Oslo Norway	jonny.beyer@niva.no
Thierry Burgeot	Ifremer Nantes Centre Rue de l'île d'Yeu P.O. Box 21105 44311 Nantes Cédex 03 France	tburgeot@ifremer.fr
Barbara Catalano	National Institute for Environmental Protection and Research - ISPRA via di Castel Romano 100 00128 Rome Italy	barbara.catalano@isprambiente.it
Lisa Devriese	Institute for Agricultural and Fisheries Research (ILVO) Ankerstraat 1 8400 Oostende Belgium	Lisa.devriese@ilvo.vlaanderen.be
Lars Förlin	University of Gothenburg Department of Biological and Environmental Sciences P.O. Box 463 SE-405 30 Gothenburg Sweden	lars.forlin@gu.se
Bjørn Einar Grøsvik (Co-Chair)	Institute of Marine Research Nordnes P.O. Box 1870 5817 Bergen Norway	bjoern.einar.groesvik@imr.no
Ketil Hylland (Co-Chair)	University of Oslo Department of Biosciences Blindern P.O. Box 1066 N-0316 Oslo Norway	ketil.hylland@bio.uio.no
Andrea Johnson	University of Maryland Department of Natural Sciences LMRCSC, Carver Hall Eastern Shore, Princess Anne, 21853 MD United States	akjohnson@umes.edu



Ulrike Kammann	Thünen Institute Institute for Fisheries Ecology Palmaille 9 22767 Hamburg Germany	Ulrike.kammann@ti.bund.de
Thomas Maes	Centre for Environment, Fisheries and Aquaculture Science (Cefas) Pakefield Road NR33 0HT Lowestoft Suffolk United Kingdom	thomas.maes@cefas.co.uk
Ginevra Moltedo	National Institute for Environmental Protection and Research - ISPRA via di Castel Romano 100 00128 Rome Italy	ginevra.moltedo@isprambiente.it
Daniel Proefrock	Helmholtz-Zentrum Geesthacht, Center for Material and Coastal Research Max-Planck-Straße 1 21502 Geesthacht Germany	daniel.proefrock@hzg.de
Jakob Strand	Aarhus University Department of Bioscience Frederiksborgvej 399 P.O.Box 358 DK-4000 Roskilde Denmark	jak@dmu.dk
Joachim Sturve	University of Gothenburg Department of Biological and Environmental Sciences P.O. Box 463 SE-405 30 Gothenburg Sweden	joachim.sturve@bioenv.gu.se
Brita Sundelin	University of Stockholm Applied Environmental Science Svante Arrhenius väg 8 11418 Stockholm Sweden	brita.sundelin@itm.su.se

## Annex 2: Agenda

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1. Opening of the meeting;
2. Adoption of the agenda;
3. Timetable and appointment of rapporteurs;
4. Respond to requests for advice from Regional Seas Conventions as required;
5. Consider emerging issues of scientific merit and address knowledge gaps;
  - a. Oil toxicity to early life stages of fish
  - b. Ocean Acidification
  - c. Immunotoxicity
  - d. Neurodevelopmental and behavioural toxicity
  - e. Novel monitoring techniques
6. Review status of publications and consider requirements for new publications;
  - a. ICES TIMES
  - b. Other ICES publications
  - c. Peer review publications
7. Conduct assessment of data as required;
  - a. Quality assurance data from method intercomparison trials
  - b. Integrated assessment of monitoring data
8. Respond to requests for advice from the Data Centre;
9. Development and harmonisation of methodologies for marine monitoring and surveillance including:
  - a. Integrated assessments
  - b. Environmental risk assessment
  - c. Review and develop assessment criteria for biological effects methods
  - d. Report on national monitoring programmes for biological effects
10. Address issues in relation to novel and emerging contaminants (e.g. pharmaceuticals, nanoparticles, toxicity of mixtures etc)
  - a. Pharmaceuticals and recreational drugs in the marine environment
  - b. Biocides in the marine environment
  - c. Nanoparticles in the marine environment

11. 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:
  - a. Status on development of tools to quantify and qualify (micro) plastics in marine organisms, e.g. fish, turtles, crustaceans, marine mammals, and sea birds
  - b. Results of impact assessment surveys and research projects of microplastics and non-plastic microparticles in marine organisms from all trophic levels
  - c. Evidence of bioaccumulation, toxicity and of 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
  - d. Evidence of microplastics and associated contaminants to transfer through marine food chains
12. Any other business;
13. Recommendations and action list;
14. Adoption of the report and closure of the meeting

WGBEC will report on the activities of 2014 (the second year) by 30 April 2014 to SSGHIE.

### Annex 3: WGBEC Terms of reference 2014–2015

The **Working Group on Biological Effect of Contaminants** (WGBEC), chaired by Bjørn Einar Grøsvik, (NO), and Ketil Hylland, (NO), will meet in Bergen, Norway, 9–13 March 2015, to work on ToRs and generate deliverables as listed in the Table below.

WGBEC will report on the activities of 2014 (the second year) by 30 April 2014 to SSGHIE.

#### ToR descriptors

ToR	DESCRIPTION	BACKGROUND	SCIENCE TOPICS ADDRESSED	PLAN DURATION	EXPECTED DELIVERABLES
a	Respond to requests for advice from Regional Conventions (OSPAR, EU) as required.	<i>Advisory requirement.</i> 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.	Advice to ICES	Annual 2012-2015	Each year advice is reported to ICES secretariat for onward transmission e.g. to OSPAR
b	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 -Immunotoxicity - Novel monitoring techniques (eg 'omics technology)  - Thiamine deficiency and how pollutants may effect effects on other vitamins like vitamin A in marine wildlife (2014 only)	<i>Science and advisory requirement</i> In reviews over the past three years WGBEC has considered emerging special scientific issues in relation to biological effects and contaminants and also in relation to the ICES Science Plan. These topics have been selected as of current concern.	112, 172, 241, 242	2012-2015	Review paper published in the peer review literature (2015)

c	<p>Review status of publications and consider requirements for new publications</p> <ul style="list-style-type: none"> <li>- ICES TIMES</li> <li>- Other ICES publications</li> <li>- peer review publications</li> </ul>	<p><i>Science and advisory requirement.</i></p> <p>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.</p>	<p>Advice to ICES Annual 2012-2015</p>	<p>Publication of ICES TIMES methods for marine monitoring purposes</p>
d	<p>Conduct assessment of data as required</p> <ul style="list-style-type: none"> <li>-Quality assurance data from method intercomparison trials</li> <li>- Integrated assessment of monitoring data (and advise on procedures to other groups as appropriate)</li> </ul>	<p><i>Science and advisory requirement</i></p> <p>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.</p>	<p>123, 241, 242, 244, 2012-2015</p>	<p>Report each year via ICES secretariat to OSPAR on progress with AQC initiatives / schemes for biological effect methods.</p> <p>Report to ICES data centre on current AQC programmes.</p>
e	<p>Respond to requests for advice from the Data Centre</p>	<p><i>Advisory requirement</i></p> <p>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.</p>	<p>Advice to ICES Annual 2012-2015</p>	<p>Provide support and information to ICES data centre that can be used to facilitate submission of biological effects data to the ICES data base</p>
f	<p>Development and harmonisation of methodologies for marine monitoring and surveillance including:</p> <ul style="list-style-type: none"> <li>- Integrated assessments</li> <li>-Environmental risk assessment</li> <li>-Review and develop assessment criteria for biological effects</li> </ul>	<p><i>Science and advisory requirement</i></p> <p>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 /</p>	<p>241 Annual 2012-2015</p>	<p>Report via ICES secretariat to OSPAR on annual review of assessment criteria for JAMP biological effects and progress with the application of the OSPAR SGIMC integrated approach.</p> <p>Report to ICES data centre on</p>

	methods	HELCOM/ MSFD)	EU	current AQC programmes.
	- Report on national monitoring programmes for biological effects			Link up with MCWG and WGMS on integrated approach and assessment of data.
g	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. -Biocides in the marine environment.	<i>Science requirement</i> 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.	123, 172, 242, 2012-2015 241	Provide report to ICES on these special scientific issues and publish in the peer reviewed literature (year 3)..
h	To evaluate the results of marine litter monitoring and research activities, especially microparticles (plastic/non plastic) and associated chemicals: -Status on monitoring protocols for marine litter in biota -Marine litter research outcomes and results of impact assessments on key marine organisms. Evidence of bioaccumulation, toxicity and adverse physical, biological and chemical effects of microplastics and associated contaminants on a range of marine organisms, populations and communities. -Evidence of transfer of microplastics and associated contaminants through marine food chains.	<i>Science and advisory requirement</i> 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.	241, 243, 344 2012-2015	Review and report to ICES on how this work area is developing and identify how this may be progressed and applied to marine monitoring programmes.  Link up with other EGs with interest in this topic i.e. MCWG and WGMS (planned for 2014)  Publish outputs in peer review literature (Year 3).

## Summary of the Work Plan

Year 3	Requests for advice from ICES, OSPAR and requests for support from data centre will be addressed each year as appropriate. Time allocation is variable depending on the task and preparation required pre the meeting and reporting post meeting. Complete and sign off 3 yr report and report on publication outputs.
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## 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. The highest priority relates to providing sound scientific advice in response to requests from international programmes e.g. OSPAR JAMP.
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 15 - 30 members.
Secretariat facilities	None.
Financial	No financial implications.
Linkages to ACOM and groups under ACOM	ACOM and SSGHIE
Linkages to other committees or groups	There are linkages to MCWG, WGMS, WGPDMO and more recently WGEEL
Linkages to other organizations	None directly although the WG has had input and links at its meetings with MEDPOL scientists

#### Annex 4: List of manuscript planned for ICON special issue

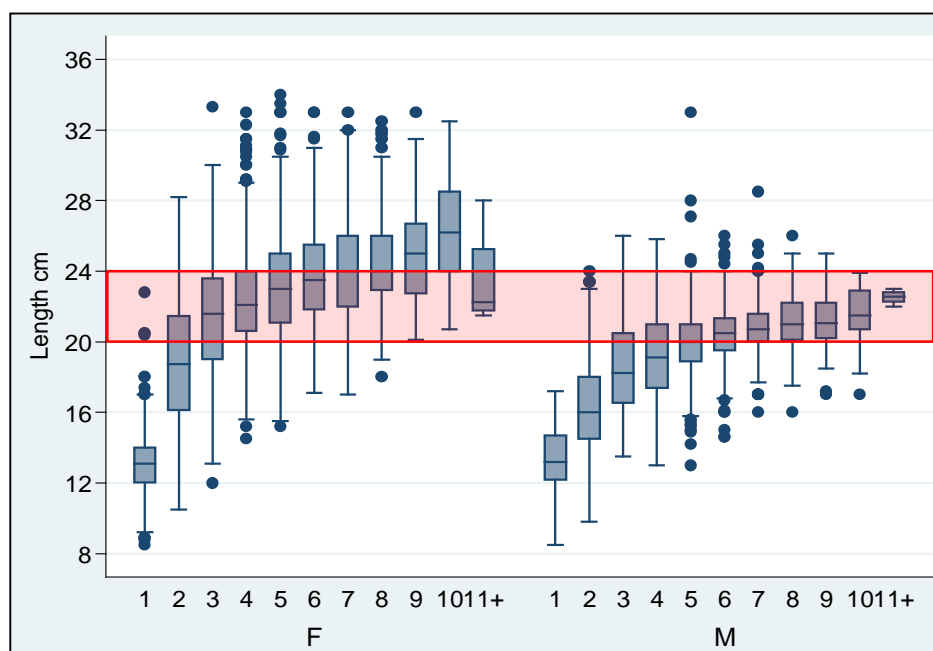
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- Hylland *et al.*, Assessing contaminant impacts in European marine ecosystems: the ICON workshop
- Robinson *et al.*, Assessment of contaminant concentrations in marine sediments, fish and mussels sampled from the North Atlantic and European regional seas within the ICON project
- Lang *et al.*, Methylmercury in dab (*Limanda limanda*) from the North Sea, Baltic Sea and Icelandic waters: relationship to host-specific variables
- Kammann *et al.*, PAH metabolites in fish bile: from the Seine Estuary to Iceland
- Vethaak *et al.*, *In vitro* and *in vivo* toxicity profiling of marine sediments from the ICON survey
- Broeg *et al.*, Lysosomal membrane stability in the liver of dab (*Limanda limanda*) – Applicability and reliability of assessment criteria under concrete contaminant-related monitoring conditions of coastal, estuarine and offshore locations
- Carney Almroth *et al.*, Is oxidative stress evident in dab (*Limanda limanda*) in the North Sea?
- Hylland *et al.*, Genotoxicity in dab (*Limanda limanda*) and haddock (*Melanogrammus aeglefinus*) from European seas
- Lang *et al.*, Diseases of dab (*Limanda limanda*): analysis and assessment of data on externally visible diseases, macroscopic liver neoplasms and liver histopathology at offshore sites in the North Sea, Baltic Sea and off Iceland
- Burgeot *et al.*, Integrated assessment of contaminant impacts in the Seine estuary
- Lyons *et al.*, Determining Good Environmental Status under the Marine Strategy Framework Directive: case study for descriptor 8 (chemical contaminants)
- Hylland *et al.*, Impacts of contaminants in European marine areas: an integrated assessment



## Annex 5: An assessment tool for monitoring liver cancer in marine environment– a preliminary report

John Bignell and Allan Reese (UK) submitted a proposal for a new assessment tool for monitoring liver cancer in marine fish. The work has primarily arisen from previous concerns raised that liver cancer might simply be a surrogate for age and that contaminants may not be the sole cause. This is particular concerning when the age distribution of one of the OSPAR region's primary biomarker species, the common dab (*Limanda limanda*), is considered. Analysis of data from dab of the size range 20–24 cm, as recommended by ICES in biological effects monitoring programmes (Feist *et al.* 2004), revealed that age can vary considerably. Box plots of length against otolith age for each sex confirms that growth is continuous at the population level but for individual fish is a poor estimator of age above 3 years (figure x). Subsequent work led by Cefas also revealed that although cancer certainly increases with age (which could be a result of continued contaminants exposure), the age of onset is accelerated at certain locations i.e. fish get cancer younger (Stentiford, *et al.* 2010). Whilst this is meaningful concerning an individual i.e. the earliest age cancer was observed at a sampling site; it does not inform us a great deal about the population as a whole. As such there is a requirement for an assessment tool which is complementary to that used elsewhere i.e. the Fish Disease Index (FDI); that is able to consider the effects of age into an assessment. Cefas are currently working on a logistic regression model that incorporates a large histopathology dataset (with corresponding age determination) from 2004–2013. In its simplest form, it provides a national liver cancer model of England and Wales for the first time. The model also allows “site to site” and “year to year” comparisons for the assessment of liver cancer.



**Figure x:** Age distribution of common dab (*Limanda limanda*) sampled between 2006 and 2013 (n= 7546) as part of the CSEMP in England and Wales.

Briefly, dab were sampled from CSEMP sampling stations in the Irish and North Sea during the summer from 2004–2013 (n=7546: currently awaiting incorporation of 2006 data). Following euthanasia, liver and gonad tissues were dissected and processed for histological analysis. Otoliths were also obtained from each fish and sectioned for age

determination. Liver was analysed using light microscopy according to ICES TIMES protocols (Feist *et al.* 2004). For assessment tool purposes, liver histopathology data concerning neoplasms were consolidated into the presence or absence of cancer (benign and malignant neoplasms). A standard logistic regression model was applied to data to produce a national model for liver cancer (figure Xa).

Logistic regression is an appropriate model to compare the cancer risks between sexes, locations, or time periods. The regression equation is a straight line on the logit scale and becomes S-shaped when transformed back to the probability (p) scale. The logistic model predicts the percentage of fish expected to have liver cancer at a specified age in England and Wales i.e. what is the risk of cancer. The model allows interrogation of the data at several hierarchy levels regarding geographical region. Initial observations demonstrate that fish from the MSFD Irish Sea region are adversely affected more than fish from the Greater North Sea MSFD region (figure Xb). This is demonstrated by the larger proportion of fish having liver cancer across the entire Irish Sea population sampled, compared to the North Sea. The model benefits from the ability to reveal which national MERMAN regions are potentially driving geographical differences i.e. hotspot areas such as Cardigan Bay (figure Xc). Furthermore, it allows geographical regions to be assessed for improvements regarding liver cancer prevalence. However, care should be exercised. For example, figure Xd appears to demonstrate that the North Sea population appears to be worsening regarding liver cancer prevalence when comparing the earliest (2004) and latest sampling events (2013). This is potentially the result of a random sampling event, although other parameters might be influencing this change i.e. sex ratios of sampled fish. Nonetheless, it is crucial that long term datasets are used to investigate trends of significance before drawing conclusions using this method.

This report describes preliminary results observed through the development of a new assessment tool for liver cancer. The sampling method and data show great promise for monitoring the health of the sea and comparing between regions and over time.

These results indicate that there is a significant but small difference in age distributions between the Western seas and the Greater North Sea. Subsequent annual samples from just one region can be compared with previous results from that region. It also suggests that year-to-year variation may be random but, if data collection continues, then subsequent years may confirm regional trends.

The power of the model can become compromised by ad hoc changes to the sampling scheme i.e. reduced frequency and numbers of samples per monitoring year. As a general principle, at least 6 or 7 years' data is required to demonstrate a trend that is gradual i.e. less than 20% annual rate change.

The next steps will be to discuss amongst experts how best to assess UK data and how this might compare to data across the OSPAR region when using the same approach. Several approaches are available including, but not limited to

- (a) Assessment of all OSPAR data to the UK logistic model due to the wide ranging prevalence of liver cancer observed.
- (b) Assessment against an OSPAR logistic model that incorporates ongoing age and cancer data from different regions i.e. Germany.
- (c) Assessment between national logistic models, although regions may not be directly comparable or
- (d) Assessment against a true reference baseline i.e. background response.

**References**

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