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

16–20 March 2009

Weymouth Laboratory, UK

ICES International Council for the Exploration of the Sea

CIEM Conseil International pour l'Exploration de la Mer

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Contents

Executive Summary

Progress with ICES TIMES biological effects methods manuscripts was reviewed, one manuscript had been published, one had been withdrawn and eight further manuscripts have been commissioned by ICES WGBEC and have been issued with draft resolutions pertaining to their publication. These are in various stages of preparation, and there was a commitment by the authors to completing several of these very soon and certainly within the next twelve months.

A number of specific queries had been received from the ICES Data Centre concerning biological effects data submission to the ICES database. Advice and information was provided on reporting of the parameter CYP1A, provision of a mock example of a file for reporting data to the ICES database, provision of new ICES codes for new techniques, reporting of biological effect QA data to the ICES database, and comments on the amount and type of data submitted to the ICES database.

The WG considered the ICES/OSPAR SGIMC report 2009 containing a detailed forward Workplan for the SG, and three assessment products of EROD data and associated background levels prepared for OSPAR. The WG also considered the review report from ICES on the prepared OSPAR advice (document OSPAR-2-2009). Several conclusions and recommendations were made and of particular note was the need for clarification on the contribution and role WGBEC and its members to the work of SGIMC. WGBEC members were very keen to support the work programme of SGIMC.

The Marine Strategy Framework Directive is a wide-ranging framework directive, which has to put in place measures to achieve Good Environmental Status in Europe's seas by 2020. Good environmental status shall be determined at the level of the marine region or sub region, on the basis of the qualitative descriptors. The role of biological effect techniques was discussed in relation to the MSFD and also some discussion took place on the minimum requirements to be met before a biological effect can be considered suitable for use within the MSFD for defining GES.

Many countries have national monitoring programmes in place and there were presentations from the Baltic Sea (BALCOFISH - a BONUS+ project, ICES SGEH), Denmark (NOVANA programme), Spain and Ireland. There are lessons to be learnt from these programmes and it is encouraging to see an emphasis on assessment of data and the application of the "integrated chemical biological effect approach").

In order to implement the OSPAR mussel integrated approach it is essential to have in place a Technical Annex, similar to the JAMP CEMP Technical Annex for fish. A draft Technical Annex was prepared at the meeting for consideration by SGIMC.

In order to keep up to date with new and promising biological effect techniques the group identifies and reviews techniques on a regular basis to gauge the state of play and applicability of the techniques for monitoring purposes. This year reviews were conducted on omics and environmental monitoring, alkylphenol bile metabolites; intersex in gastropods, intersex in crustaceans, histopathology in mussels and passive samplers.

Reports on progress with the two chemical contaminant biological effect international workshops/programmes were presented, the ICON (Integrated Assessment of Contaminant Impacts on the North Sea) international workshop and the Baltic BONUS+ Programme BEAST project. Both activities were progressing well and should be able

to provide sufficient data to demonstrate the usefulness of an integrated strategy for monitoring and subsequent assessment of the data.

The group noted comments on possible links between contaminants, biological effects and possibly parasite, disease and other factors in the demise of the eel in Europe, made in the ICES WGEEL reports of 2007 and 2008. Some overall comments were made in respect of contaminant related effects and contact will be made with ICES WGEEL.

1 Opening of the meeting

The Chair, and meeting host, John Thain (UK) opened the meeting at 09:30 on Monday 16 March 2009, and welcomed the Working Group to the Centre Environment Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, UK. A formal welcome to the Weymouth Laboratory was then given by Mike Waldock, of the Cefas Management Board. The participants introduced themselves and their affiliations and described their area of interest and field of expertise. The list of attendees is given in Annex 1.

2 Adoption of the Agenda

The Terms of Reference (ToR) and provisional agenda had been circulated prior to the meeting. The Chair went through the agenda explaining the priority and background to the agenda items. The ToR for the meeting can be found in Annex 2. The draft agenda was adopted by the meeting and a tentative timetable agreed, Annex 3 and 4, respectively.

3 Appointment of Rapporteurs

Principle contributors and rapporteurs for the agenda were identified and are given in Annex 4. PowerPoint presentations, background documents and reporting documents were viewed and circulated using the ICES SharePoint.

4 Review progress of the publication and electronic dissemination of manuscripts on biological effects techniques in the ICES TIMES series (ToR a)

Progress with ICES TIMES biological effects methods manuscripts was reviewed by the group. It was noted that one manuscript 'The use of embryo aberrations in amphipod crustaceans for measuring effects of environmental stressors' by Sundelin, Wiklund and Ford had been published online (2006/1/MHC08). A further manuscript in-preparation concerning multi-xenobiotic resistance in mussels (2006/1/MHC09) was withdrawn in 2008 on recommendation from the proposed author due to concerns regarding the method and the fact it was not considered a priority core method for integrated assessment. ICES should withdraw the draft resolution pertaining to this manuscript.

Eight further manuscripts have been commissioned by ICES WGBEC and have been issued with draft resolutions pertaining to their publication. These are in various stages of preparation, indicated in Table 4.1 below.

Table 4.1. Current status of WGBEC TIMES methods with ICES draft resolutions.

C. Res	Publication Title/Description	ICES Deadline	Progress	Revised Deadline
2002/1E03	The report on Biological Effects of Contaminants: Oyster (Crassostrea gigas) Embryo Bioassay by J.E. Thain (UK)	August 2008	Draft MS available for review	July 2009
2006/1/MHC06	The Protocol for Extraction Methods for Bioassays. Hans Klamer and John Thain (UK)	June 2008	Some sections drafted MS in preparation	Draft by 30/04/09 action required by JT (UK)
2006/1/MHC07	The protocol for conducting EROD determinations in flatfish By M. Gubbins.	June 2008	Draft MS available for review	Draft by 30/04/09
2007/1/MHC02	Blue Mussel Histopathology, John Bignell, Steve Feist, Dave Lowe & Miren Cajaraville	September 2008	Some sections drafted. Final draft expected end Sept 09	Dec 2009
2008/1/MHC13	Protocol for measuring dioxin-like and estrogenic activity in environmental samples using CALUX assays. Dick Vethaak (Netherlands)	31/05/09	Manuscript in preparation. Some concern over IP / availability of method to be resolved.	Potentially, 1st draft in October 2009. with suggestions for Ass Cri and Background Doc.
2008/1/MHC14	Protocols for measuring micronucleus formation in cells as an indicator of toxicant induced genetic damage. Brett Lyons (UK).	31/05/09	No progress. Seeking appropriate co-authors to add scope to protocols.	April 2010
2008/1/MHC15	Protocol for measuring estrogen/androgen activity in environmental samples using YES/YAS yeast screen assays. J Thain (UK), Kevin Thomas (Norway)	31/05/09	No progress.	1st draft May 2009. To be initiated by KT.
2008/1/MHC12	The protocol for gonadal histology in flounder. S Feist *et al*.	31/05/09	Material collated. 1st draft Sept 09.	Dec 09

Following discussion with authors and WGBEC members, progress with these manuscripts was reviewed and deadlines revised. In all cases authors have indicated drafts should be available within 12 months. In the case of 2008/1/MHC13 concerning CALUX assays, concern was expressed about licensing and intellectual property rights to the assay being owned by the supplier (BDS) and this limiting access to the method for monitoring purposes. Dick Vethaak (Netherlands) agreed to pursue the issue with the supplier and report back to WGBEC TIMES coordinator (Matt Gubbins) on whether there is value in pursuing this manuscript / draft resolution.

One further TIMES manuscript has previously been identified by WGBEC as required, but has yet to be commissioned or had draft resolution agreed by ICES. This concerns a protocol for reproductive success in eelpout by Jakob Strand (Denmark). Given that reproductive success in fish is part of the draft OSPAR integrated monitoring strategy and that the author has incorporated the preparation of a standard method document as a milestone in a nationally funded project to deliver by Oct 2010, it is proposed to put this forward as a draft resolution for ICES.

During discussion on other agenda items, it was identified that two further ICES TIMES manuscripts were required and could be quickly generated by the authors. These were sea urchin embryo bioassay (Ricardo Beiras, Spain) and alkylphenol metabolites in fish bile (Jonny Beyer, Norway). Both of these methods were demonstrated during the meeting to be suitable for monitoring and in the case of urchin embryo bioassay already well used across the ICES area. The estimated time of delivery for both of these manuscripts is December 2009. Draft resolutions for both of these manuscripts should be put forward for adoption by ICES in 2009.

Recommendations

That the proposed publication of a method document for MDR/MXR be withdrawn (Draft resolution 2006/1/MHC09).

That ICES update the deadlines for draft resolutions in line with those indicated in Table 4.1.

ICES are requested to approve a draft resolutions for the publication of TIMES manuscripts concerning reproductive success in eelpout, sea urchin embryo bioassay and alkylphenol bile metabolites. These manuscript can be prepared for review by October 2010 (reproductive success in eelpout), and Dec 2009 (urchin bioassay and alkylphenol metabolites).

5 Assess the amount of biological effects data submitted to the new ICES database and answer queries / requests from the ICES Data Centre; and to consider codes for techniques now in the integrated approach – scheme (ToR b)

WGBEC received a number of specific queries concerning biological effects data submission to the ICES database from the Data Centre. These queries are detailed below, along with the WGBEC response.

a) The Data Centre has received questions concerning the reporting of the parameter "CYP1A" (Cytochrome P450 1A (non-specific)). Some submitters want to use absorbance as the measurement unit (for example, absorbance 450/mg protein) but this will cause conversion problems during assessments. Please specify what MUNIT should be used so that we can include a check for the correct unit into DATSU. Note that the parameter "EROD" is checked for MUNITs like mg/minute/milligram protein, micromole/minute/milligram protein etc.

WGBEC response: Reporters should not be submitting absorbance at 450 nm for CYP1A. This measurement is not specific to CYP1A and cannot be easily compared to other CYP1A measurements e.g. EROD activity. CYP1A should be reported as EROD activity in pmol/min/mg protein.

b) WGBEC should produce a mock example file with text (as produced by WGPDMO, see *http://www.ices.dk/env/repfor/examples/WGPDMOexampleText.txt*) to help data submitters report biological effects data such as EROD, DNA adducts, CYP1A etc. In RECO, check parameter groups B-END, B-MBA and B-TOX for a list of current biological effects parameters. The Data Centre will help with the example file if necessary.

WGBEC response: The following examples describe part a hypothetical data submission. The first number in each line denotes the record type according to the following:

File Information record

20- Sampling method record

21- Analytical method record

90- Sampling Platform record

91- Station/ sampling event record

03- Contaminant and Biological effects sample record

04- Biota Specimen Data record

10- Parameter measurement record

Data specific to specimens begins with a '91' record, which indicates geographical location and date. Commas separate data fields.

Imposex Data Submission

The following information describes the data example below. There are six pooled samples from six sampling stations. All specimens were *Buccinum undatum*.

Sample 1: 4 November 2003, Clyde Anchorage A7. Not enough sample was recorded. Pooled sample of 30 individuals. Local wild stock and mixed sex. All individuals were adult and killed. Two parameters were assessed and recorded in soft body tissue (1) Penis Classification Index (PCI) and (2) Percentage of females that have imposex (IMPF%). The mean PCI was 1.54. The IMPF% was 92.3%. Level of uncertainty was standard deviation of 1.07.

Sample 2: 4 November 2003, Clyde Anchorage A4 and A5. Not enough sample was recorded. Pooled sample of 30 individuals. Local wild stock and mixed sex. All individuals were adult and killed. Two parameters were assessed and recorded in soft body tissue (1) Penis Classification Index (PCI) and (2) Percentage of females that have imposex (IMPF%). The mean PCI was 0.70. The IMPF% was 74.1%. Level of uncertainty was standard deviation of 0.79.

Sample 3: 6 November 2003, Upper Clyde channel. Not enough sample was recorded. Pooled sample of 13 individuals. Local wild stock and mixed sex. All individuals were adult and killed. Two parameters were assessed and recorded in soft body tissue (1) Penis Classification Index (PCI) and (2) Percentage of females that have imposex (IMPF%). The mean PCI was 0.08. The IMPF% was 16.7%. Level of uncertainty was standard deviation of 0.20.

Sample 4: 7 November 2003, Mid Clyde channel. Not enough sample was recorded. Pooled sample of 20 individuals. Local wild stock and mixed sex. All individuals were adult and killed. Two parameters were assessed (1) Penis Classification Index (PCI) and (2) Percentage of females that have imposex (IMPF%). No imposex was observed.

Sample 5: 6 November 2003, Lower Clyde channel. Not enough sample was recorded. Pooled sample of 7 individuals. Local wild stock and mixed sex. All individuals were adult and killed. Two parameters were assessed (1) Penis Classification Index (PCI) and (2) Percentage of females that have imposex (IMPF%). No imposex was observed.

Sample 6: 3 November 2003, Garroch Head. Pooled sample of 100 individuals. Local wild stock and mixed sex. All individuals were adult and killed. Two parameters were assessed and recorded in soft body tissue (1) Penis Classification Index (PCI) and (2) Percentage of females that have imposex (IMPF%). The mean PCI was 0.07. The IMPF% was 9.5%. Level of uncertainty was standard deviation of 0.24.

00,ALUK,74,2003,3.2,,,,,,,,,,,,

20,ALUK,1,AGT,,,,,,,,,,,,,

21,1,ALUK,,OGTA3,AWT,,,,,GRS,,,,,,,

90,74CU,1803C,74,,,,,,,,,,,,,

91,1803C,1,5558.0459998,-00445.86200002,GPS,20031104,,,,Clyde anchorage A7,CEMP,,,,,,

03,1803C,1,CF,1,1,,,Buccinum undatum,ITLN,NS,,,,,,,

04,1803C,1,1,1,30,LW,X,AD,,K,,,,,,,

10,1803C,1,1,1,SB,,,PCI,,,1,A,,1.54,,2,1.07,SD

10,1803C,1,1,1,SB,,,IMPF%,,,1,A,,92.3,,1,,

91,1803C,2,5558.317,-00446.09900002,GPS,20031104,,,,Clyde anchorages A4 & A5,CEMP,,,,,,

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10,1803C,2,1,1,SB,,,PCI,,,1,A,,0.70,,2,0.79,SD

10,1803C,2,1,1,SB,,,IMPF%,,,1,A,,74.1,,1,,

91,1803C,3,5554.5800002,-00455.21000002,GPS,20031106,,,,Upper Clyde channel,CEMP,,,,,,

03,1803C,3,CF,1,1,,,Buccinum undatum,ITLN,NS,,,,,,,

04,1803C,3,1,1,13,LW,X,AD,,K,,,,,,,

10,1803C,3,1,1,SB,,,PCI,,,1,A,,0.08,,2,0.20,SD

10,1803C,3,1,1,SB,,,IMPF%,,,1,A,,16.7,,1,,

91,1803C,4,5550.2300002,-00456.16400002,GPS,20031107,,,,Mid Clyde channel,CEMP,,,,,,

03,1803C,4,CF,1,1,,,Buccinum undatum,ITLN,NS,,,,,,,

04,1803C,4,1,1,20,LW,X,AD,,K,,,,,,,

10,1803C,4,1,1,SB,,,PCI,,,1,A,,0.00,,2,0.00,SD

10,1803C,4,1,1,SB,,,IMPF%,,,1,A,,0.0,,1,,

91,1803C,5,5552.7059998,-00455.22500002,GPS,20031106,,,,Lower Clyde channel,CEMP,,,,,,,

03,1803C,5,CF,1,1,,,Buccinum undatum,ITLN,NS,,,,,,,,

04,1803C,5,1,1,7,LW,X,AD,,K,,,,,,,

10,1803C,5,1,1,SB,,,PCI,,,1,A,,0.00,,2,0.00,SD

10,1803C,5,1,1,SB,,,IMPF%,,,1,A,,0.0,,1,,

91,1803C,6,5540.11,-00459.89999998,GPS,20031103,,,,Garroch Head,CEMP,,,,,,,

03,1803C,6,CF,1,1,,,Buccinum undatum,ITLN,,,,,,,,,

04,1803C,6,1,1,100,LW,X,AD,,K,,,,,,,,

10,1803C,6,1,1,SB,,,PCI,,,1,A,,0.07,,2,0.24,SD

10,1803C,6,1,1,SB,,,IMPF%,,,1,A,,9.5,,1,,

EROD Data Submission

The following information describes the data example below. This data example differs from the above example in that it contains individual fish data. Six individual fish have been analysed for EROD from two sampling stations. Each station contains three individual fish.

MorayF_MoFOpenSea_fi01 contains three Plaice sampled on 8 January 1997 at 19:43. The trawl duration was 30 minutes.

Fish 1: Male, length 249 mm, EROD result 3.163 pmol/min/mg protein.

Fish 2: Male, length 222 mm, EROD result less than 3.13 pmol/min/mg protein.

Fish 3: Male, length 195 mm, EROD result less than 3.13 pmol/min/mg protein.

EScotland_EScOpenSea_fi01 contains three Dab sampled on 6th January 1997 at 08:45. The trawl duration was 30 minutes.

Fish 1: Male, length 233 mm, EROD result 6.116 pmol/min/mg protein.

Fish 2: Male, length 247 mm, EROD result 43.31 pmol/min/mg protein.

Fish 3: Male, length 200 mm, EROD result 42.57 pmol/min/mg protein.

00,BODC,74,1997,3.2,,,,,,,,,,,,,,,,,,,,,,,,

20,ALUK,1,BOT,,,,,,,,,,,,,,,,,,,,,,,,

21,1,ALUK,,,,,,SOX,,GC-ECD,,,,,,,,,,,,,,,,,,

90,74SC,0106S,MERMAN,20090318,,,,,,,,,,,,,,,,,,,,,,,,

91,0106S, 00000001,+57 58.0500,-02 54.4680,,19970108,1943,2013,,MorayF_MoFOpenSea_fi01,CEMP~NATL,,,T,,,,,,,,,,,,,,,

03,0106S, 00000001,CF,A,1,,,Pleuronectes platessa,ITLN,,,,,,,,,,,,,,,,,,

04,0106S, 00000001,A,1,1,,M,,,,,,,,,,,,,,,,,,,,

10,0106S, 00000001,A,1,WO,,,LNMEA,mm,,,,,249,,,,,,,,,,,,,,,

10,0106S, 00000001,A,1,LI,,,EROD,pmol/min/mg protein,W,8,,,3.163,,,,,,,,,,,,,,,

04,0106S, 00000001,A,2,1,,M,,,,,,,,,,,,,,,,,,,,

10,0106S, 00000001,A,2,WO,,,LNMEA,mm,,,,,222,,,,,,,,,,,,,,,

10,0106S, 00000001,A,2,LI,,,EROD,pmol/min/mg protein,W,8,,<,3.13,,,,,,,,,,,,,,,

04,0106S, 00000001,A,3,1,,M,,,,,,,,,,,,,,,,,,,,,

10,0106S, 00000001,A,3,WO,,,LNMEA,mm,,,,,195,,,,,,,,,,,,,,,

10,0106S, 00000001,A,3,LI,,,EROD,pmol/min/mg protein,W,8,,<,3.13,,,,,,,,,,,,,,,

91,0106S, 00000002,+56 30.1380,-01 23.9580,,19970106,0845,0915,,EScotland_EScOpenSea_fi01,CEMP~NATL,,,T,,,,,,,,,,,,,,,

03,0106S, 00000002,CF,A,1,,,Limanda limanda,ITLN,,,,,,,,,,,,,,,,,,,

04,0106S, 00000002,A,1,1,,M,,,,,,,,,,,,,,,,,,,,,

10,0106S, 00000002,A,1,WO,,,LNMEA,mm,,,,,233,,,,,,,,,,,,,,,

10,0106S, 00000002,A,1,LI,,,EROD,pmol/min/mg protein,W,8,,,6.116,,,,,,,,,,,,,,,

04,0106S, 00000002,A,2,1,,M,,,,,,,,,,,,,,,,,,,,,

10,0106S, 00000002,A,2,WO,,,LNMEA,mm,,,,,247,,,,,,,,,,,,,,,

10,0106S, 00000002,A,2,LI,,,EROD,pmol/min/mg protein,W,8,,,43.31,,,,,,,,,,,,,,,

04,0106S, 00000002,A,3,1,,M,,,,,,,,,,,,,,,,,,,,,

10,0106S, 00000002,A,3,WO,,,LNMEA,mm,,,,,200,,,,,,,,,,,,,,,

10,0106S, 00000002,A,3,LI,,,EROD,pmol/min/mg protein,W,8,,,42.57,,,,,,,,,,,,,,,

c) Are there any "new" techniques which require PARAM codes, METOA codes, MUNIT codes etc.? See RECO for up-to-date lists: *http://www.ices.dk/datacentre/reco/*

WGBEC response: WGBEC cross-referenced the existing biological effects techniques in the ICES database PARAM codes list against those methods detailed in the draft OSPAR integrated monitoring guidelines from WKIMON III. These 'new' methods are considered priority for monitoring purposes and where there is no provision for data entry into the ICES database, this needs to be resolved. The following biological effects techniques were identified as part of the draft integrated monitoring guidelines, but lacking data entry codes for the ICES database:

Methods from fish integrated monitoring framework:

Comet assay

This method has yet to be issued an ICES TIMES manuscript and no agreement has yet been reached on the method to be employed for monitoring in fish. In the future, PARAM codes are likely to be 'Comet', METOA 'single cell gel electrophoresis', MUNIT should be either 'olive tail moment' or '% DNA in tail'. It may be advisable to include both MUNIT possibilities for reporting of both measurements from Comet assay. ICES are advised to wait for methods to be agreed for fish before finalising codes in the database for Comet.

Reproductive Success

Denmark are progressing with an ICES TIMES publication on this method for completion in 2010. It is not anticipated that any data will be submitted prior to 2011. The recommended database codes will be developed by Denmark alongside the development of the standard method.

Intersex in fish

As above, this method is awaiting a standard method to be developed in the form of an ICES TIMES publication. Database codes cannot be set until this process is completed.

Fish condition, gonado-somatic index and hepato-somatic index are all also required for the integrated WKIMON III fsh programme, but do not have specific PARAM codes. However, these can be calculated from database entries using length and weight PARAM codes associated with different matrix codes for the same sample (e.g. whole organism, liver, gonad).

Methods from integrated mussel programme

Scope for growth in mussels

This method has a standardised procedure (TIMES) and it should be possible to enter data to ICES database. Codes do not currently exist. Recommended codes would be: PARAM 'SFG' METOA 'scope for growth' or 'physiological measurements'? MUNIT 'J/H/G' (joules per hour per gram).

Growth

This could be calculated from separate entries on length at different time periods, but specific codes for mussel growth rates would be preferable. These have yet to be determined.

Condition factor

As per condition index for fish, this can be calculated from existing PARAM codes for length and dry weight 'BMDRYWT' associated with the right matrix codes for whole animal and soft body tissues.

Comet assay

See above as for fish.

Mussel histopathology

Again, the TIMES method for this technique is in preparation and the database codes have yet to be developed. The histopathological methods for mussels will separate into reproductive markers, non-specific pathologies, digestive gland pathologies and disease conditions. The parameters to be submitted to the ICES database have yet to be determined by the authors.

Gametogenesis

There is no standard method for measuring gametogenesis, but WGBEC can recommend that the following codes to be used when data is submitted in the future: PARAM 'Gonadal stage'; METOA light microscopy 'MC-LM' MUNIT 'Stage'. There is a need to differentiate between pre- and post-spawning gonadal stages. This could be achieved by using alphanumeric values, e.g. 1–5 for pre-spawning gonadal stages and 1s–5s for post-spawning gonadal stages or by using 2 separate MUNIT codes such as pre-spawning stage and post spawning stage. ICES Data Centre are asked to advise on the most appropriate approach.

Multi-xenobiotic resistance (MXR)

There is no standard method for MXR available and it is not a mandatory method in the OSPAR integrated strategy (optional only). It is not envisaged that there will be a need to enter MXR data on the ICES database, so this is not considered a priority.

d) The Data Centre will be developing a new quality assurance database. It will allow storage of intercalibration participation results (Zscores from QUASIMEME) and expand to include other intercalibration organisations such as BEQUALM. In addition, it will store reference material standard values. These QA data will then be linked to the monitoring data in EcoSystemData to allow automated proficiency checks of analytical laboratories during assessments.

Does WGBEC have any requirements for the reporting of non-Zscore results for biological effects data? Please provide intercalibration reports and examples of participation results from biological effects intercalibrations for Data Centre consideration. We have received MERMAN documents but a brief examination did not answer our question on non-Zscore reporting.

The current QA database

In brief, the current QA database for chemicals is based on RECO codes and the following measured values:

Reference material standard value fields:

CRMCO, DTYPE, PARAM, BASIS, VALUE, Deviation, Deviation type, MUNIT, and Certification type.

Intercalibration result value fields:

ICCOD, DTYPE, CRMCO, PARAM, Assigned Value, MUNIT, Mean, Zscore, Pscore, LabValue

WGBEC response: The working group was not able to answer this query during the meeting. However WG members have contacted the UK data centre (MERMAN) managers to develop a response to this query. This will be progressed after the meeting.

e) As requested, a biological effects data summary of parameters currently existing on the ICES database has been made in a separate Excel file. It does not include EROD legacy data because these data were not converted and transferred to EcoSystemData due to problems in the data. Data submitters will be contacted with questions concerning the EROD transfer this year.

WGBEC response: ICES WGBEC looked at the scope as provided in the WGBEC 2009 Biological Effects Data Summary. The majority of the provided data is still on imposex and intersex related to gastropods. It was noted that there was a substantial increase in the quantity of data on TBT effects compared to last year, which has been able to contribute to the OSPAR MON assessment for QSR 2010 (c.f. agenda item 12). However, other biological effects are still lacking in the database. Only Norway, UK, Germany and The Netherlands have reported on other biological effects such as ALA-D, EROD, PAH bile metabolites, MT and PNR (bioassay).

Action

WGBEC members are urged to submit their biological effects monitoring data (including legacy data) to the ICES database to enable future assessments to be made.

6 Evaluate national /international monitoring activities, including harmonisation initiatives / integrated assessment / and application of biological effect techniques within OSPAR / MEDPOL / WFD / HELCOM /EU MSD (ToR c)

Integrated Assessment and Application

6.1 ICES/OSPAR Study Group on Integrated Monitoring of Contaminants and Biological Effects (SGIMC)

WGBEC considered the ICES/OSPAR SGIMC report 2009 containing a detailed forward Workplan for the SG, and three assessment products of EROD data and associated background levels prepared for OSPAR. The WG also considered the review report from ICES on the prepared OSPAR advice (document OSPAR-2-2009).

Several members of WGBEC had been invited to attend SGIMC and would have made a valuable contribution to the work of the group, but were unable to attend due to the delay of the meeting (originally planned for Autumn 2008) and the short-time notice of the SG meeting in early January . However, the WG appreciates the efforts and progress made by the SG and after consideration and discussion by the WG arrived at the following conclusions and recommendations:

1) WGBEC noted that several of its tasks have direct relevance to SGIMC and will require intensive ongoing interaction between both groups. Members of WGBEC currently involved in intersessional work on developing ACs for EROD and other PAH-specific biomarkers should be pro-active and be made aware of the importance to deliver their tasks in time before the Aberdeen Workshop on ACs for EROD and other PAH-specific biomarkers, and to be encouraged to attend the Workshop.

These include the following tasks and actions:

- to contact the appropriate task person (K. Hylland) to complete intersessional work on PAH metabolites as defined by WKIMON4 (task D. Vethaak)
- to contact the appropriate tasks person (?) to complete intersessional work on DNA adducts as define by WKIMON4 (task John Thain)
- It was noted that work at FRS Aberdeen was in progress to further develop the temperature / seasonality EROD model that should enable EROD AC development at the meeting. WGBEC members should also bring or submit available data sets on EROD, PAH metabolites, DNA adducts, and DR CALUX to the Aberdeen Workshop for the development of assessment criteria (all WG members)

2) WG members should be strongly encouraged to complete intersessional work in 2009 related to the development of AC for VTG, bioassays and other core biological effects methods as outlined by SGIMC 2009 report (D. Vethaak and J. Thain to identify task leaders and progress).

3) The membership of SGIMC should be defined and the meeting agenda/details should be circulated in good time. It is recommended that invitations to the Aberdeen Study Group meeting are extended to WGBEC members.

4) ICES SCICOM/ ACOM are requested to define more clearly the tasks for WGBEC in respect to the development of biological effects monitoring and the development of assessment criteria and its role in the integrated chemical biological monitoring strategy developed by SGICM and OSPAR. Currently there is an overlap in tasks between groups and no clear short-term mechanism for interaction.

6.2 EU Marine Strategy Directive

Thomas Maes (UK) gave a presentation, updating the WG on current activities in respect of the EU MSD.

The Marine Strategy Framework Directive is a wide-ranging framework directive, which has to put in place measures to achieve Good Environmental Status in Europe's seas by 2020. Good environmental status shall be determined at the level of the marine region or sub region, on the basis of the qualitative descriptors (see DIRECTIVE 2008/56/EC below).

Member States sharing a marine region shall cooperate to ensure that the measures required to achieve this Directive are coherent and coordinated across the marine region. Member States concerned endeavour to follow a common approach:

a) preparation

b) programme of measures

This will involve:

Preparation:

1) Initial assessment by 15 July 2012 of the current environmental status of the waters concerned and the environmental impact of human activities thereon.

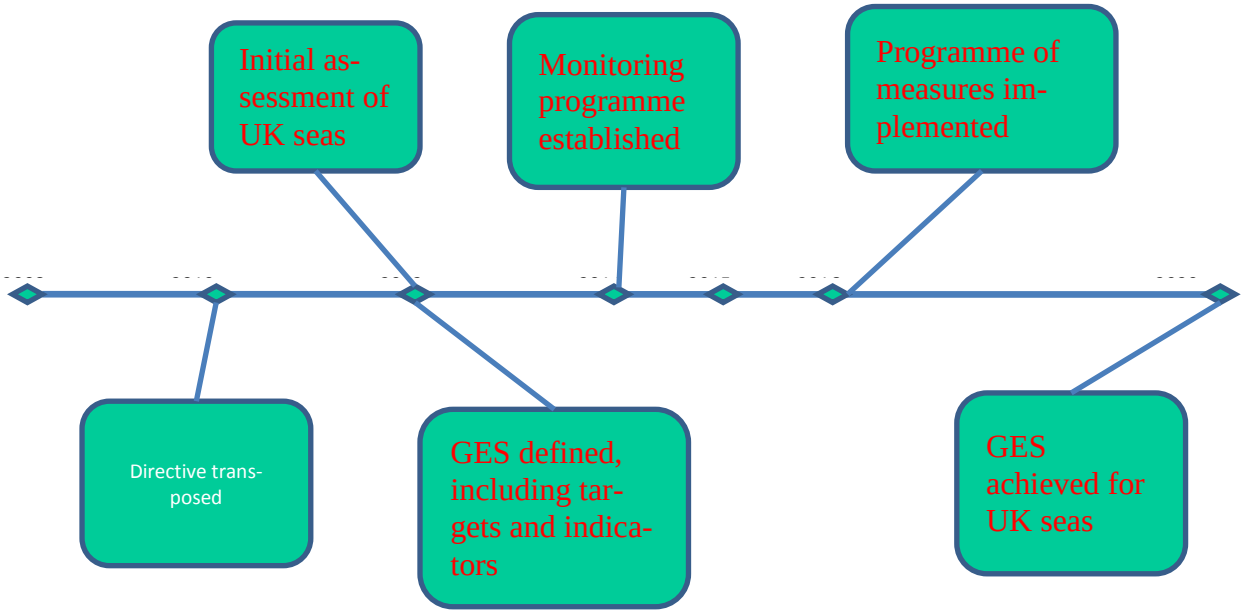
2) Determination by 15 July 2012 of good environmental status for the waters concerned.

3) Establishment by 15 July 2012 of a series of environmental targets and associated indicators

4) Establishment and implementation, by 15 July 2014 of a monitoring programme for ongoing assessment and regular updating of targets Programme of measures:

1) Development, by 2015 at the latest, of a programme of measures designed to achieve or maintain good environmental status

2) Entry into operation of the programme provided for in point (i), by 2016 at the latest.



This directive will have significant implications for future marine monitoring and assessments but at this stage it is hard to say exactly what the monitoring/assessment needs will be. A good starting point will be to look at our existing monitoring programmes to decide whether they are delivering what we need to assess GES. Common monitoring and assessment methodologies for the Directive will be developed which will apply right across the EU. This EU level work is unlikely to begin until 2011/12, once the details of GES have been determined. JRC and ICES will be working together to develop the criteria and methodological standards. The output of the JRC/ICES project will go to the Working Group on GES but interaction with this WG will be through the Commission (i.e. DG ENV). A deadline of the end of June has been set for an initial report from all of the Task Groups.

Table 6.2.1. Task Groups and Chairs as of February 2009.

Task group	Chair
TG 1: Biological diversity	Kari Nygaard
TG 2: Non-indigenous species	Sergej Olenin
TG 3: Commercially exploited fish/shellfish	Gerjan Piet
TG 4: Marine food webs	Stuart Rogers
TG 5: Eutrophication	Joao Ferreira
TG 6: Sea-floor integrity	Jake Rice
TG 7: Hydrographical conditions	Open
TG 8: Contaminants	Robin Law
TG 9: Contaminants in fish/other seafood	Frans Verstraete[1]
TG 10: Marine litter	Open
TG 11: Energy/noise	Mark Tasker

Progress report on contract ICES-JRC-MSFD Feb 2009 rev 3, 2nd March 2009. Paul Keizer (ACOM Vice-Chair) and Claus Hagebro (ICES Secretariat).

Table 6.2.2 GES Task groups Timetable.

2009													2010					
Month	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6
Steering Group	21-22/1	Informal MS meeting 5/2		Webex 16or 17/4	Progress report to WG on GES				Meeting before/ after MG	Progress report to WG on GES						(Progress report to WG on GES)		
Management Group (MG)		16-17/2 at ICES			Interim report of TG chairs	Webex 24/6			17-18/9 Meeting, Berlin					2-4/2 meeting				
Task Groups			TG Meetings (2 days)				Work by correspondence			(3-4) days meetings								
	Consultation of EGs (ICES)																	
WG on GES		Establish			15/5					X						X		
WG on Data, info. and knowledge						X												
Marine Strategic Coordination Group					15/5					X								
Marine Directors					29/5						X						X	
Deliverables			3.1: Guidance/ template				2.2: Library/ bibliography						4.1 (draft) Report on descriptors			4.1 (final) 4.2 Report on implications		

Progress report on contract ICES-JRC-MSFD Feb 2009 rev 3, 2nd March 2009, Paul Keizer (ACOM Vice-Chair) and Claus Hagebro (ICES Secretariat)

Determining the detail of what Good Environmental Status (GES) means for marine waters is a crucial step in the implementation of the directive. It will be one of the key factors in determining our future marine monitoring and assessment requirements as well as determining the management measures which we need to consider in order to achieve GES. This group strongly believes that biological effects tools can be used to define Good Environmental Status under the Marine Strategy Framework Directive.

As part of this process of determining GES and developing measures to achieve it we will need to gain a better understanding of the impacts of anthropogenic activities on the marine ecosystem. The descriptors that will be of special importance to this group (ICES WGBEC) are probably:

1) Biological diversity is maintained. The quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climatic conditions.

3) Populations of all commercially exploited fish and shellfish are within safe biological limits, exhibiting a population age and size distribution that is indicative of a healthy stock.

4) All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity.

8) Concentrations of contaminants are at levels not giving rise to pollution effects.

9) Contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards.

11) Introduction of energy, including underwater noise, is at levels that do not adversely affect the marine environment.

DIRECTIVE 2008/56/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 17 June 2008

Establishing a framework for community action in the field of marine environmental policy

ANNEX I

Qualitative descriptors for determining good environmental status

(Referred to in Articles 3(5), 9(1), 9(3) and 24)

To determine the characteristics of good environmental status in a marine region or subregion as provided for in Article 9(1), Member States shall consider each of the qualitative descriptors listed in this Annex in order to identify those descriptors which are to be used to determine good environmental status for that marine region or subregion.

When a Member State considers that it is not appropriate to use one or more of those descriptors, it shall provide the Commission with a justification in the framework of the notification made pursuant to Article 9(2).

Marine Strategy Directive

Annex I: Qualitative descriptors for determining good environmental status

The use of biological effects tools offer enormous potential to meet the challenges outlined by the Marine Strategy Framework Directive (MSFD), whereby Member States are requirement to develop a robust and set of tools for defining qualitative descriptors of Good Environmental Status (GES), such as demonstrating that contaminants in marine waters do not give rise to pollution effects. The MSFD states that the Directive should also contribute to the fulfilment and important commitments of the Community and Member States to existing international agreements, relating to the protection of the marine environment from pollution (specifically monitoring programmes run under the auspices of the Regional Conventions, such as OSPAR JAMP). Therefore, it is essential that going forward there is a ICES WGBEC agreed strategy for using biological effect tools in relation to delivering a cost effect programme for both the MSFD and Regional Council objectives (OSPAR, MEDPOL, HELCOM).

Minimum requirements to be met before a biological effect can be considered suitable for use within the MSFD for defining GES

The MSFD clearly lays out a number of key criteria, which need to be met before any tool can be used to define and assess GES

1) Provision needs to be made for the development of methodological standards for the assessment of status of the marine environment, monitoring and environmental targets along with the adoption of technical formats for the transmission and processing of data.

2) Assessment methodologies should be consistent across marine regions or sub regions.

3) The Regional Sea Conventions (e.g. OSPAR) should be consulted before any criteria and methodological standards for determining GES are proposed by Member States.

In relation to producing biological effects tools of the required standard these points are already being specifically addressed under the existing framework of the OSPAR JAMP monitoring programme and being taken forward through activities such as The Study Group on Integrated Monitoring of Contaminants and Biological Effects (SGIMC) and ICES Working Group on the Biological Effects of Contaminants (WGBEC). At present, only biological effects tools that meet the following criteria should be considered for inclusion in future monitoring programmes, which aim to deliver outputs for the MSFD.

1) An internationally recognized Quality Control Programme must be in place (e.g. BEQUALM).

2) Data collection and submission formats must be compatible with National (e.g. UK MERMAN) and international (ICES) databases.

3) Internationally accepted assessment criteria must be developed (or at an advanced stage of development) for a specific biological effect tool. Such as those developed under WIKIMON and the recently initiated SGIMC.

4) The biological effect tool should have been reviewed by ICES WGBEC and be listed in the tables of recommended techniques for a) methods for fish; b) methods for invertebrates; c) bioassays and methods for specific matrices (see ICES WGBEC 2008 report).

International Monitoring Activities

6.3 BALCOFISH – a BONUS+ project in the Baltic Sea

Jakob Strand presented the project BALCOFISH, acronym for "Integration of pollutant gene responses and fish ecology in Baltic coastal fisheries and management", which is a newly started 3-years BONUS+-project funded by Baltic Organisations Network for fUnding Science EEIG (www.bonusportal.org) with focus on contaminants and biological responses in coastal fish in the Baltic Sea. Project leader is Lars Förlin from Department of Zoology, University of Gothenburg, Sweden.

The aim is to provide science-based input to foster the development of appropriate measures in the management of the Baltic Sea environment to protect it against anthropogenic chemical pollution and other stressors.

For this purpose it is important to unravel causal links between the current pollution situation and effects observed in the field. To establish such links the BALCOFISH will develop and explore toxicogenomics approaches and integrate these with existing early effects biomarkers. These responses will be anchored to effects relevant to the sustainability of coastal fish populations with focus on the indigenous viviparous eelpout (*Zoarces viviparus*) in the Baltic Sea, such as impaired reproduction.

The research and technology development in the BALCOFISH project concern in-depth studies of pollutant impact in fish using ecotoxicogenomics. By these means the project will go into depth on the importance of the environmental stressors like contaminants and the relationships to gene responses and also link them to early warning biomarkers, reproduction, larvae development and population dynamics on coastal fish like eelpout in the Baltic Sea.

The eelpout is already an integrated part of the Swedish, Danish and German national and regional monitoring programs for temporal and spatial studies of contaminants and/or effects. This implies that information about levels and patterns of classical contaminants, natural variation over time or variation explained by factors like e.g. salinity or temperature or individual specimen variables like age, sex or measured physiological variables, geographical differences etc. is already available or readily extracted from available databases.

The benefit of using eelpout is that this species is relatively stationary during its entire life-cycle, viviparous (field studies on early life stages are feasible), common in coastal waters of the north-east Atlantic and the Baltic Sea, used in Swedish, Danish and German (and some other Baltic countries) monitoring programs for several years

and also part of OSPAR JAMP and HELCOM COMBINE manuals for marine monitoring of biological effects of contaminants.

In the BALCOFISH project the interaction between science and users are both natural and important. This approach will ensure transfer of research needs and research outcome, and thus benefit development of both environmental sciences and management, i.e. policymakers, stake holders and the public as well as with the scientific world including the ICES community.

Approaches will be explored and strategies will be tested in 6 work packages (WPs), which also include deliveries to ICES:-

WP1. Provide a data matrix on contaminant levels, effects and population descriptors in eelpout, and supporting environmental variables from Baltic coastal waters (Project database).

WP2. Develop new tools for studying effects of contaminants on eelpout in Baltic Sea (Gene arrays & population genetic markers).

WP3. Apply existing and new tools in field studies of eelpout in contaminated coastal sites in the Baltic Sea (i.e. gene arrays and population genetics together with contaminants, contaminant-specific and general biomarkers and reproductive disorders).

WP4. Confirm laboratory studies and validate extrapolations between species (Stickleback and/or zebra fish).

WP5. Link gene responses to population effects (Individual–based dynamic population model).

WP6. Bridge the gap between scientists, stakeholders and managers (incl. development of guidelines and environmental assessment tools, disseminate data, workshops and stakeholders meeting).

Some of the WP tasks have a direct reference to ICES and the expected outcome of these activities includes:-

- Development of environmental assessment tools for biological effects in eelpout will include reproductive success (ICES SGIMC 2009 recommendation: Further refinement of ACs as more data becomes available), 1 - 2 biomarkers and an integrative eelpout health index, e.g. combining/weighting different endpoints at molecular, cellular and individual level.
- Development of guideline as ICES TIMES on reproductive success in eelpout, draft expected to be submitted to ICES WGBEC in 2010.
- In addition, all relevant eelpout data to be agreed on within the project will be submitted to the ICES database in the end of 2011. This will certainly also include new ICES codes to the existing parameter lists and these codes have to be developed in agreement with ICES.

6.4 Baltic Sea Issues: Report on ICES SGEH activities

The ICES Study Group for the Development of Integrated Monitoring and Assessment of Ecosystem Health in the Baltic Sea (SGEH) met in its current formation for the first time in Warnemünde (DE) in March 2-5, 2009, just over a week prior to the WGBEC meeting. The group is chaired by Kari Lehtonen (FI), a WGBEC member.

The SGEH meeting began by reviewing the reports and and discussing the past activities of its predecessor, the "old" SGEH (Study Group on Baltic Ecosystem Health Issues in support of the BSRP [Baltic Sea Regional Programme]) that was operational between 2003-2007. A summary of the previous activities (the "legacy" of the past SGEH) was considered to be of great value as a theoretical background and practical starting point for (1) common agreement on the meaning of the term Ecosystem Health in the Baltic Sea, (2) updating the goals and outputs of the new SGEH, and (3) establishing its working methods.

It was agreed that the SGEH of now will focus its main activities on matters related to biological effects of contaminants in marine organisms in the Baltic Sea, a field with a significantly lesser research emphasis in this geographical area compared e.g. to eutrophication biodiversity and fisheries, and high and urgent needs for developlment. However, as in the previous SGEH, information on the effects of contaminants and on biodiversity are also closely followed. To achieve the target of developing assessments of Ecosystem Health in the Baltic Sea links with groups dealing with fisheries and eutrophication impacts will be established with expected participation of experts having data and information relevant to SGEH. In particular, collaboration with Working Group for Integrated Assessments in the Baltic Sea (WGIAB) was considered of utmost importance.

An important aspect was indentification of links between SGEH work related to HELCOM, OSPAR, EU (with a special reference to the Marine Strategy Framework Directive [MSFD]) and other ICES EGs. In regard to the MSFD, suggested criteria and methodological standards for the descriptors should be discussed in this group in the meeting next year. Since OSPAR is working on the Quality Status Report for 2010, SGEH should follow the outcome of this report, and it will be discussed in next year's meeting.

SGEH reviewed and updated information on the progress with national and international biological effect monitoring activities, e.g. OSPAR, HELCOM, MEDPOL, WFD, MSFD, harmonization initiatives, integrated assessment and application of biological effect techniques, and the North Sea ICON programme. Among these informations, ICES has been invited by HELCOM to advice on methods for determining effects primarily on reproduction, immunology and metabolism of marine organisms. In addition, the recommendations of OSPAR on the parameters used should be taken into considerations to harmonize the programmes and to make use of the expertise relevant for Baltic species and environment. HELCOM stresses the need for studies providing knowledge about the applicability of several of the contamination-related biomarkers in current use (e.g. EROD induction, histopathology) in Baltic Sea organisms that are potentially useful as monitoring species. In addition, the development of chronic sediment and water bioassays are considered by HELCOM useful for studies in heavily contaminated areas.

SGEH also reviewed and reported the progress on the development of assessment criteria and integrated chemical-biological effect assessment tools in the Baltic Sea region concerning environmental impacts provided in the previous SGEH reports.

The SGEH concluded that information on assessment criteria for hazardous substances and their biological effects contained in the SGEH report from its 2006 meeting still represents the current status. However, progress is expected to be made through the BONUS+ programme through the BEAST project (Biological Effects of Anthropogenic Chemical Stress: Tools for the Assessment of Ecosystem Health) and other activities in areas outside the Baltic Sea. These should be reviewed at the 2010 SGEH meeting and an update report should be prepared. It was pointed out that for many of the parameters for which assessment criteria are needed there is still a lack of baseline data from sub-regions of the Baltic Sea. Such data are required and will be obtained within the BEAST project.

SGEH reviewed progress within the BEAST project with discussions on development of especially the parts of the project related to development of integrated monitoring (WP 2) and assessment of ecosystem health (WP 3) to serve the goals of the SGEH and BSAP (see in detail WGBEC 2009 report Item 11.2.).

A great deal of SGEH meeting time was devoted to the planning of intersessional work, contributions to the implementation of HELCOM BSAP, formulation of recommendations and key topics of next year's SGEH meeting. All these are available in the SGEH 2009 meeting Final Report.

6.5 Marine monitoring in Denmark within the NOVANA programme

Jakob Strand presented the Nationwide Monitoring and Assessment Programme for the Aquatic and Terrestrial Environment in Denmark, called NOVANA, which is an integrated monitoring programme of nature and the environment including groundwater, aquatic (i.e. freshwater and marine) and terrestrial habitats, species, air quality and point sources (NERI, 2004).

The monitoring programme was initiated on 1 October 1988, and has been adjusted several times since, latest in 1998 and 2004, and it is expected to be adjusted again in 2010. The Ministry of Environment in Denmark is responsible for the programme.

The NOVANA monitoring on contaminants in the marine environment includes spatial and temporal trends of substances as PCBs and other OCs, PBDEs, organotins, PAHs, metals, dioxin, phthalates, alkylphenols in sediment, mussels and/or flounder. In addition, also screening surveys on PFCs, organomercury and HCBD are performed.

In many coastal areas elevated levels of one of more contaminants occur, like TBT, although the TBT levels have declined significantly through the last years (Figure 6.5.1a.b).

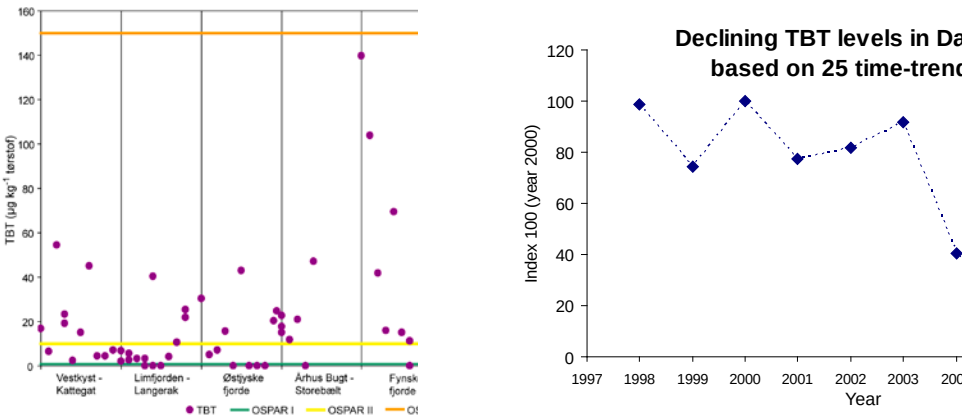


Figure 6.5.1a,b. TBT as an example of the spatial and temporal trend monitoring of contaminants within the NOVANA programme 2007. a) TBT data in mussels is assessed using OSPAR assessment criteria I - V, b) Index-based temporal trend of TBT in mussels (year 2000 = Index 100). From Larsen *et al*. (2009).

The biological effects monitoring includes spatial and temporal trends of TBT-specific effects, i.e. imposex and intersex in the gastropod species *Buccinum undatum, Hinia reticulata, Neptunea antiqua* and *Littorina littorea*, and also more general biological effect like lysosomal stability in hemolymph from blue mussel (*Mytilus edulis*) and reproductive success and CYP1A/EROD in eelpout (*Zoarces viviparus*).

Supplementary studies on eelpout have also included other biological effects techniques like sex ratio in broods, intersex/ovotestis, CYP1A rna expression, PAH-metabolites and population genetics. The surveys have shown that both elevated biomarker responses and impaired reproduction occur in many areas.

The assessment criteria OSPAR's BAC- and EAC-values together with Norwegian environmental assessment criteria and also WFD QS for shellfish and fish for human consumption are used to assess the contaminant levels (Strand *et al*., 2008).

Similar the assessment criteria for biological effects techniques as proposed by ICES/OSPAR (WKIMON, 2008) are used to assess the effect levels of lysosomal stability in mussels, reproductive success in eelpout and imposex/intersex in gastropods (Figure 6.5.2. a and b).

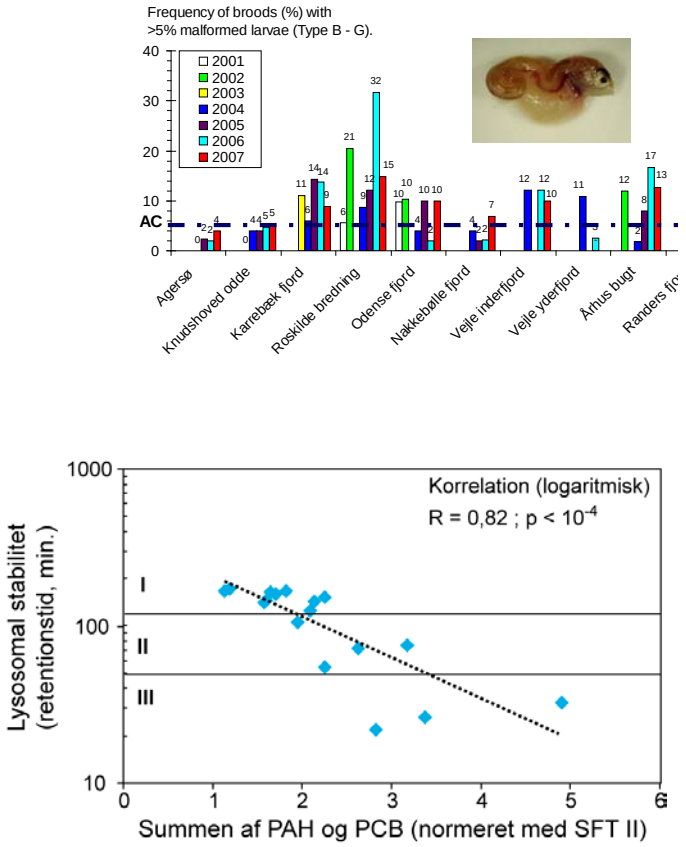


Figure 6.5.2. a,b Examples of biological effects data on a) Reproductive success (here shown as frequency of broods with elevated levels, >5%) in eelpout and compared with proposed background response (ACI) and b) Lysosomal stability in blue mussel correlated to the sum of PAH and PCB (both normalised to Norwegian Environmental assessment criteria SFT II for contaminants in mussels). In addition, also the proposed ICES/OSPAR assessment criteria 1 - III are shown. From Strand *et al*. (2009).

The biological effect techniques are in addition integrated with the chemical measurements of contaminants, for instance finding relatively good agreements between the assessments of potential anthropogenic stressors, mainly TBT, PAH and PCB, and observed effects. Potentially, both contaminants and biological effects parts can also be integrated with other physical-chemical and biological indicators monitored within the NOVANA programme.

References

NERI. 2004. NOVANA i.e. the National Monitoring and Assessment Programme for the Aquatic and Terrestrial Environment. http://www.dmu.dk/International/Monitoring/NOVANA/

Larsen, M. L., Strand, J., and Dahllöf, I. 2009. Miljøfremmede stoffer i muslinger og fisk, In: Marine områder 2007 - Tilstand og udvikling i miljø- og naturkvaliteten. NOVANA. Eds. Dahl K & Josefsson A., Scientific report from NERI, in press (In Danish). Will soon be available at www.dmu.dk.

WGBEC took note on the approaches for environmental assessments as well as integrating data on both contaminants and biological effects in the marine environment within the NOVANA programme. It was recognised that monitoring of both mussel

and fish are important for the assessment of status and trends of both contaminants and biological effects in the Danish marine environment.

References

Strand, J., Dahllöf, I., and Larsen, M. L. 2009. Biologisk effektmonitering, In: Marine områder 2007 - Tilstand og udvikling i miljø- og naturkvaliteten. NOVANA. Ed. By K. Dahl and A. Josefsson. Scientific report from NERI, in press (In Danish). Will soon be available at www.dmu.dk.

6.6 Biomonitoring activities in Spain

Presented by Concepción Martínez Gómez (Spain). A National Programme to monitor the Marine Chemical Contamination in Spanish marine waters has not been established. However, two major biomonitoring programmes, along the Northern Iberian coast and along the Iberian Mediterranean coast, have been conducted through several research projects over the past decade until 2008, by the Instituto Español de Oceanografía (IEO). From November 2005 to December 2006, both biomonitoring programmes were undertaken by the IEO, under the responsibility of the Spanish Ministry of Environment, to contribute to both CEMP and MED POL programmes. More recently, a new agreement is being assessed to continue with such biomonitoring activities during the period 2009-2011 and therefore to meet the obligations of the both conventions (OSPAR and Barcelona). However, the IEO projects associated to undertake such biomonitoring activities are actually compromised to the final assessment of the agreement.

In both programmes, measurements are performed yearly (excepting temporal trends in sediments that are conducted biannually, in the case of the Mediterranean program) and the application of both chemical and biological effect techniques (biomarkers/bioassays) is included (Table 6.6.1.).

Table 6.6.1. Biological effect techniques deployed in monitoring programmes in Spain.

BIOMONITORING IEO	SPANISH MEDITERRANEAN WATERS	SPANISH ATLANTIC WATERS
Sampling SS	Autumn (Sept-October)	Yearly
Sampling Fish (MB/MM)	Autumn (Sept-Oct) Post-spawning	Autumn
Sampling MG	Spring (May-June) Pre-spawning	Autumn (Oct-Nov) Pre-spawning
Sampling NR/NL	not	Yearly
Parameter	Matrix	Matrix
Trace metals	MG/MB/SS	MG/MM/SS
PAHs	MG/MB/SS	MG/SS
Organochlorinated Compounds	MG/MB/SS	MG/MM/SS
BFRs	not	MG/MM/SS
TBTs	not	NR/NL/SS
Imposex	not	NR/NL
SFG	not	MG
SoS	MG	not
LMS	MG	not
MT	MG/MB/SS	not
AChE	MG/MB/SS	not

MN	MG/MB/SS	not
EROD	MB	not
Genotoxicity	MB	not
Sea urchin Embryotoxicity assay	SS*	SS
Amphipod bioassay	not	SS
CI/CF	MG/MB	MG/MM
GSI	MB	not

MG: *Mytilus galloprovincialis*

MB: *Mullus barbatus*

MM: *Merluccious merluccious*

NL: *Nucella lapillus*

NR: *Nassarius reticulates*

SS: Superficial sediments

*pilot study

6.7 Marine monitoring in Ireland

Michelle Giltrap (IR) presented a project entitled "Biological Effects and Chemical Measurements for the Assessment of Pollution in Irish Marine Waters". This project was initiated in August 2008 and is a three and a half year collaborative project with four partners namely, Trinity College Dublin, Marine Institute, Shannon Aquatic Toxicity Laboratory and Dublin Institute of Technology.

At present there is limited expertise available to undertake a complete biomonitoring programme in Ireland. Through this project skills shall be developed in both chemical and biological analysis which will allow for the assessment of the impact of various contaminants on the marine environment. The overall objective of this project is to develop the methodology and techniques recommended by WKIMON which are necessary to allow for the full chemical and biological assessment for both 'traditional' and more 'novel' contaminants. This shall enable Ireland to contribute to the valuable work undertaken by ICES.

The project shall involve a two tiered approach (TIER I and II sites) for assessment of 12 sites around the coast of Ireland which are representative of a range of contaminant burdens. TIER I sites shall be screened with two biomarkers in the marine mussel, *Mytilus edulis*, namely the physiological marker scope for growth (SFG) and lysosomal membrane stability (LMS) with the neutral red retention assay. Sediment toxicity shall be evaluated at each of the TIER I sites with the marine copepod, *Tisbe battagliai* using a sediment elutriate screening assay. A comprehensive assessment of environmental quality requires a multiple biomarker/ multiphase sediment exposure testing scheme and therefore sites at which biological effects/sediment toxicity are observed in TIER I screening will be assessed in more depth in TIER II screening. The latter involves the use of multiple biomarkers in mussels and fish including SFG, LMS, metallothionein, acetylcholinesterase activity, EROD and PAH metabolites and assessment of DNA damage using the Comet assay. The effects of endocrine disruption in fish (dab/flounder), mussels (*Mytilus edulis*) and dogwhelks/periwinkles (*Nucella lapillus*/*Littorina littorea*) shall also be assessed with vitellogenin and alkali labile phosphate assays in both fish and mussels respectively and also imposex and intersex measurements in gastropods. A complete assessment of fish and mussel

histopathology and benthic monitoring shall also be conducted for TIER II sites. Caging studies shall be conducted at sites whereby resident species are absent. Chemical analysis involving the analysis of a suite of organic and inorganic pollutants in tissue, sediment and passive sampling devices will also be performed on TIER II sites. This shall include the analysis of both 'traditional' and emerging 'novel' contaminants. The integration of these biological and chemical measurements will result in the development of an overall health index per site with the use of the 'Full Monti' index method which is currently under development by OSPAR.

Activities relation to harmonisation of biological effect techniques and intercalibrations

6.8 BEQUALM EROD Intercomparison

Presented by Kevin Thomas (Norway). In 2008 NIVA successfully ran an intercomparison study for EROD which included participants from 10 institutes. The participating labs submitted EROD results for the microsomal fractions of fish liver homogenates exposed to a "high" (50 mg/kg) and "low" (5 mg/kg) dose of benzo[*a*]pyrene dissolved in corn oil, administered by intra-peritoneal injection. This inter-calibration exercise clearly showed that inter-laboratory differences exist even in samples that have been prepared identically. However, with the exception of one laboratory, there was reasonable agreement in EROD activity between laboratories for the nine microsomal fractions with all Z scores within the satisfactory criteria of ± 2. It is possible that a workshop will be run to further discuss the findings of the intercomparison study should there be sufficient interest from the participating institutes.

Recommendation

The group would like to recommend that following the success of the EROD inter comparison that it would be very beneficial to have a wash-up workshop to discuss lessons learned and to refine the AQC procedures.

Action: Kevin Thomas (Norway) to consider the feasibility of holding a workshop.

6.9 BEQUALM Fish Disease Measurement: 2008–2009 Work Programme

John Bignell (UK) presented a progress report of the BEQUALM Fish Disease Measurement programme organized by Cefas. The aim of the programme is to assess the ability of participating laboratories with international monitoring commitments to accurately identify external fish diseases and liver histopathology in accordance to BEQUALM criteria.

There were four participants in the 2008-2009 programme, which is the lowest number since the programme began in 2004. This needs to be addressed. Efforts were made to decrease costs by (a) aligning BEQUALM activities closely with existing monitoring commitments, (b) reducing in-year intercalibration exercises to a minimum and (c) placing increasing emphasis on primary ring tests. As a result, Fish Disease Measurement registration fees were reduced to 750 GBP. This was used to cover reference materials including:

- Portfolio of liver histopathology
- ICES TIMES publications for Fish Disease (T19) and Liver Histopathology (T38)

- Training CD-ROM
- Online intercalibration exercise
- External fish disease ring test
- Virtual Slide liver histopathology ring test on DVD
- Individual certificate of performance/feedback

Virtual Slide technology was again implemented for the liver histopathology ring test. The 2008–2009 work programme saw the introduction of "in-situ" results that consist of electronic files that are imported into the virtual slides. As a result, all pathology is annotated providing a higher degree of feedback to participants. This technology was demonstrated at the meeting.

It was discussed that the manipulation of data into the ICES format is a lengthily process and can take many days before submission is possible. In 2008, Cefas developed a new data entry template that allows for the automatic submission of data to the UK's monitoring database (MERMAN). Data is automatically formatted into the appropriate format in "real-time" as data is entered in the field. This allows for immediate submission upon return to the laboratory. It is anticipated that a similar data submission tool will be developed for the ICES database in the foreseeable future. This will be provided to BEQUALM participants and will hopefully attract more participation as a result. Similar tools should also be made available to enable liver histopathology data to be entered efficiently into the ICES Databank. This will allow for development of a liver histopathology index similar to the Fish Disease Index (FDI).

The problem with low participant numbers was discussed. In order to attract more participants, one recommendation is to separate Fish Disease Measurement into two programs (1) External Fish Disease programme and (2) Liver Histopathology programme. The macroscopic assessment of liver tumours would be included under an External Fish Disease programme. However, because histopathology is the confirmatory test, it may prove more difficult to separate these two aspects as initially anticipated.

Forward look

The current work programme is coming to a close. All ring test results have now been submitted and performance feedback is due to be sent to participants shortly.

The 2009–2010 work programme will commence in April 2009. This will be the first year that ring tests will be registered officially with ICES. As a result, pass and fail "flags" will be assigned to fish disease data that is submitted to the ICES International Databank. Pass and fail "cut-off points" were agreed at the ICES WGPDMO 2009 meeting and will be used for the assessment of forthcoming BEQUALM ring tests.

The lead laboratory (Cefas) for the Fish Disease Measurement programme will explore the potential for separating external fish disease and liver histopathology components.

It is anticipated that there will be 6 participants in the 2009–2010 work programme.

Recommendation

It is recommended that Cefas explore the potential for separating external fish disease and liver histopathology components and report back to WGBEC and WGDPMO.

6.10 MEDPOL harmonization / intercalibration

At the last two ICES WGBEC meetings there was very constructive discussion on the OSPAR and MEDPOL strategies and the need for more harmonisation. To reiterate "the results of the intercalibration exercises were discussed in a Workshop organised by MED POL (Alessandria, Italy, 2006), which was attended by scientists from 12 Mediterranean and 2 non-Mediterranean countries. Among other conclusions, the Workshop recognized the need for harmonization of the assessment criteria with those of the Northern European organizations and Conventions (such as OSPAR and HELCOM), including biomarker selection, standard operating protocols and data management, as well as, the development of common inter-calibration exercises, training courses and databases".

It was noted at the 2008 meeting that the MED POL intercalibration exercise could include some non-Mediterranean laboratories. However the intercalibration exercise was deferred to 2009 and a proposed workshop deferred to 2010.

The Chair, John Thain, had contacted Aldo Viarengo (IT) and Michael Angelidis (UNEP) and there was still a willingness and capacity to include other European laboratories in a mussel intecalibration exercise with the methods NRR, micronuclei frequency, lipofuscin lysosomal acculution and Mt.

Some WGBEC members expressed an interest in this initiative and the Chair agreed to pursue the feasibility with Aldo Viarengo.

7 Assess biological effects data with new assessment criteria: for VTG, Neutral Red, bioassays and EROD (ToR d)

It was noted that progress through the WKIMON 4 and SGIMC process had been slow, and as a result the new assessment criteria urgently needed for the assessment of biological effect data had not yet been developed. However, presentations were made by WG members on assessment criteria in respect of bioassays and EROD.

7.1 Assessment Criteria for the sea-urchin embryo toxicity bioassay

Presented by Riacardo Beiras (Spain). As part of the Spanish contribution to CEMP (OSPAR), biological monitoring of pollution in the Galician Rias (NW Spain) has been conducted by means of sediment elutriate bioassays using embryos of the sea-urchin *Paracentrotus lividus*, a test species widely distributed in both Atlantic and Mediterranean European waters. The method is directly applicable to other echinoid species used in ecotoxicology worldwide, such as the *Strongylocentrotus* and *Arbacia* genus. The bioassay endpoint is a quantitative, observer-independent, automatically readable response. Statistical methods and assessment criteria to classify sediment samples according to their biological quality status are developed (see Table), in line with the demands of the European Water Framework Directive. Assessment Criteria AC1 of PNR=67.8% allows division into good and moderated categories with an alpha as low as 0.01 and a beta lower than 0.05, according to MSD power analysis. Assessment Criteria AC2 of PNR=55.6% allows division into moderate and poor categories.

Sediment Quality Status	Current progress TU	PNR	WKIMON IV PNR	"traffic-light" colour code
high or good	<0.25	>0.678	>0.80	green
moderate	0.25 to 0.54	-0.678-0.572	0.80 to 0.50	amber
poor or bad	>0.54	<0.572	<0.50	red

7.2 Provisional Assessment Criteria of EROD activity for *L boscii* and *C. lyra* from the Nortern Iberian Shelf

An attempt to establish EROD assessment criteria (ACs) for the two demersal fish species used to monitor the biomarker responses after the Prestige accident (*L. boscii and C. lyra*) was presented by C. Martínez Gómez (Spain), (IEO, Spain). Only dataset obtained along the northern Iberian shelf in autumn 2004 and autumn 2005 from selected areas were used from each especies (Martínez-Gómez *et al*., 2006, 2009). ACs were calculated for those areas which were not considered unequivocally as reference sites but as those less affected by Prestige oil spill and less influenced from human and industrial activity (OSPAR, 2000). In the case of *L. boscii,* data from Basque country and Cantabria were excluded. In the case of *C. lyra,* only data from Asturias W and Asturias E were considered. EROD values were transformed logarithmically and their frequency distributions were plotted becoming bell-shaped (see Figure 7.2.1).

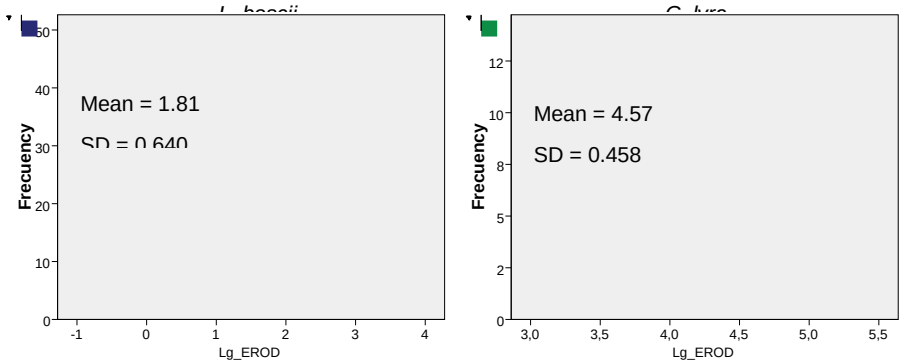


Figure 7.2.1. Frequency distributions of EROD values (log transformed) for the two species *L. boscii* and *C. lyra*.

Using the Mean and the Standard Deviation of the log-normal curve, provisional assessment criteria were categorised in three levels: Baseline Response Range (BRR), Elevated Response Range (ERR) and High Effect Range (HER), following the procedure of O'Connor (1990) for chemical pollution, though with modifications in order to be adapted to the bell shaped dose–response curves of EROD activity (Table 7.2.1. and Figure 7.2.2.).

Table 7.2.1. Provisional assessment criteria for EROD for the two species *L. boscii* and *C. lyra*.

EROD Assessment Criteria for L. boscii . Sampling season September-October Bottom water temperature range [11.7-12.7 ºC]; Size length [18-22 cm]		EROD activity (pmol/min/mg prot)
Baseline Response Range (BRR)	Ln-EROD value < Mean + 1 SD	< 11.5
Elevated Response range (EER)	Mean + 1 SD < Ln-EROD value < Mean + 2 SD	11.5 – 21.8
High Cause of Concern Range (CCR)	Ln-EROD value > Mean + 2 SD	> 21.8
EROD Assessment Criteria for C. lyra Sampling season September-October Bottom water temperature range [12.0-12.8 ºC]; Size length [15-22 cm]		EROD activity (pmol/min/mg prot)
Baseline Response Range (BRR)	Ln-EROD value < Mean + 1 SD	< 152.6
Elevated Response range (EER)	Mean + 1 SD < Ln-EROD value < Mean + 2 SD	152.6-241.3
Cause of Concern Range (CCR)	Ln-EROD value > Mean + 2 SD	>241.3

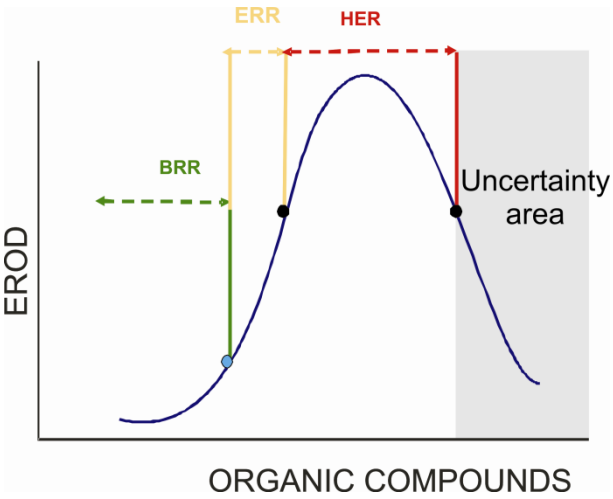


Figure 7.2.2. Assessment criteria for EROD in relation to the EROD bell-shaped response curve.

Finally, the mean values of EROD activity measured by area/year along the northern Iberian shelf during were classified according such ACs (Green= BRR; Yellow=EER; Orange=CCR), see Table 7.2.2.

Table 7.2.2. Classification of sampling sites with provisional assessment criteria between 2003 and 2005.

AREA	AUTUM 2003	AUTUM 2004	AUTUM 2005
Lepidorhombus boscii (Middle/outer shelf)			
Galicia S	19.0	4.3	6.9
Finisterre	26.1	4.6	8.0
Galicia N	22.6	6.1	9.9
Asturias W	14.8	5.8	11.1
Asturias E	18.6	4.1	10.9
Cantabria	47.7	9.2	14.7
Basque C.	32.4	15.7	16.9
Callionymus lyra (Inner shelf)			
Galicia S	n.d.	121.2	381.3
Finisterre	n.d.	162.7	427.0
Galicia N	348.5	175.6	70.6
Asturias W	223.7	135.7	71.3
Asturias E	301.7	121.5	66.3
Cantabria	248.8	157.9	70.6
Basque C.	n.d.	n.d.	n.d.

Background responses of EROD activity has been provisionally established in other fish species by using the 90 percentile of results obtained from "uncontaminated" areas (WKIMON IV). Experience suggests that an EROD value in most marine especies above 2-3 fold the upper limit of the baseline levels indicate an ecosystem influenced by planar organic contaminants. BRR values obtained by using the Mean and the Standard Deviation of the EROD frequency log-normal curve of each dataset (*L. boscii* and *C. lyra*) are close to the BRR values if they were established by using the 90 percentile (See Tables 7.2.3. below). However, the EER values fall lower than 2 folds the upper limit of the BRR. That is caused mainly because the EER range comprised Ln-EROD value < Mean + 2 EROD in order to guaranty a lower uncertainty area in HER (related with bell shaped EROD curve response). Any case, It should be notice that ACs values obtained are restricted to the sampling conditions during the mid term monitoring (Martínez-Gómez *et al.*, 2009) and the size length of the specimens used. The values of the assessment criteria must be considered as provisional and should be updated and revised when more data are available for these species. Any case, because the bell shaped dose–response curves of EROD activity, it is highly convenient a final interpretation of the EROD values considering other metric related measurements (chemical or molecular measurements), especially in those cases were justified suspicions of a significant chemical impact exist.

Table 7.2.3. To show that BRR values obtained by using the Mean and the Standard Deviation of the EROD frequency log-normal curve of each dataset (*L. boscii* and *C. lyra*) are close to the BRR values if they were established by using the 90 percentile.

DATASET 1: L. BOSCII

AREA/YEAR	N valids	Mean	SE Mean	Median	SD	Variance	Minimum	Maximum	Percentile 90
GS_04_LB	17	3.79	0.44	3.59	1.82	3.30	0.86	8.94	5.91
FI_04_LB	36	5.03	0.41	4.97	2.45	5.99	1.62	14.88	7.21
GN_04_LB	28	6.11	0.71	4.89	3.74	14.02	2.24	19.88	11.34
AW_04_LB	41	5.82	0.48	4.92	3.10	9.61	1.60	14.64	11.77
AE_04_LB	31	4.59	0.72	3.82	3.99	15.94	0.00	22.48	9.12
GS_05_LB	32	6.74	0.53	6.66	3.00	8.99	0.64	14.33	10.24
FI_05_LB	33	8.13	0.64	7.78	3.66	13.40	1.02	16.89	12.93
GN_05_LB	36	10.04	0.83	8.67	4.95	24.52	2.97	23.64	18.46
AW_05_LB	30	11.72	1.04	10.83	5.69	32.33	3.46	26.40	21.30
AE_05_LB	33	10.17	1.12	9.24	6.46	41.71	2.45	31.85	20.93
Median				5.81			2.71	25.02	11.55
Mean		7.21	0.69	6.54	3.89	16.98	1.69	19.39	12.92

DATASET 2: C. LYRA

AREA/YEAR	N valids	Mean	SE Mean	Median	SD	Variance	Minimum	Maximum	Percentile 90
AW_04_CL	35	134.23	6.68	125.43	39.49	1559.70	66.67	216.63	186.22
AE_04_CL	14	113.38	8.85	111.12	33.10	1095.35	70.80	170.06	166.86
AW_05_CL	16	74.76	7.01	74.96	28.03	785.40	30.53	136.86	120.52
AW_05_CL	14	66.09	6.31	63.75	23.62	557.78	29.29	108.20	102.88
Median				93.04			48.60	153.46	143.69
Mean		97.12	7.21	93.82	31.06	999.56	49.32	157.94	144.12

References

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Martínez-Gómez, C., Fernández, B., Valdés, J., Campillo, J.A., Benedicto, J., Sánchez, F., and Vethaak, A.D. 2009. Evaluation of three-year monitoring with biomarkers in fish following the Prestige oil spill (N Spain). Chemosphere, 74: 613–620.

O´Connor, T. P. 1990. Coastal Environmental Quality in the United States, 1990: Chemical Contamination in sediments and Tissues. NOAA Rockville, MD. 34 pp.

8 Review or (if not available to meeting) produce a Technical Annex for OSPAR mussel integrated strategy (ToR e)

A technical annex was not available for review at the meeting; therefore WGBEC produced a draft as follows.

Technical Annex: Mussel Integrated Monitoring

Presented and reported by John Thain (UK). In the OSPAR JAMP CEMP for fish and imposex in whelks there are technical annexes relating to sampling and biological effect techniques. These annexes provide details of the sampling procedures , methodologies, data reporting and aspects such as requirements for supporting determinands and confounding factors. In the light of the mussel integrated monitoring strategy proposed in the OSPAR / ICES WKIMON reports (see 2006 and 2007 reports) there is a need to provide a similar technical annex for mussels to assist the implementation of the mussel integrated approach. WGBEC has expertise in this area and is able to provide support and comment in taking this forward. In this respect WGBEC has initiated the technical annex below for further development by ICES/OSPAR SGIMC as appropriate.

Proposed technical annex for mussel integrated monitoring.

Technical Annex for Mussel (*Mytilus* sp.)Integrated Monitoring

Background

The basis for the technical annex is the mussel integrated monitoring strategy incorporating biological effect techniques at the sub-cellular, tissue and whole organism responses and tissue chemistry. This is outlined below in Figure 8.1:

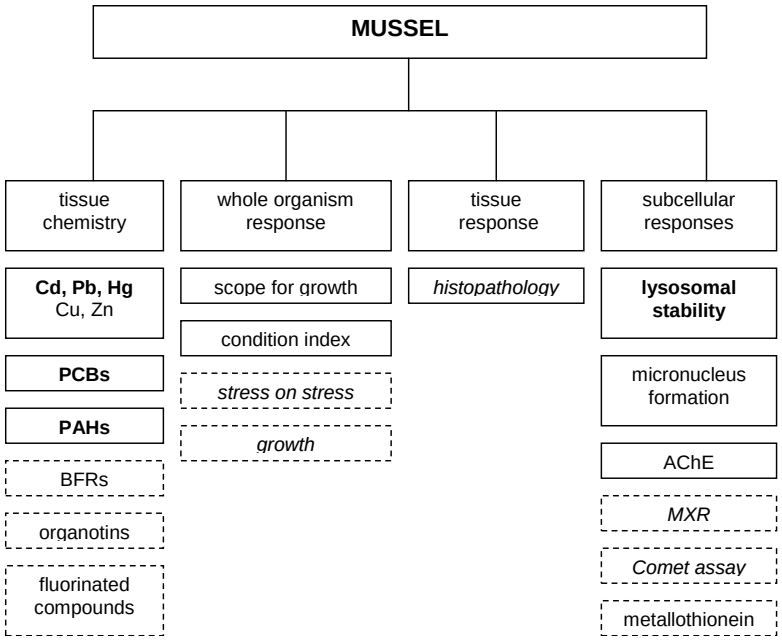


Figure 8.1. Overview of methods to be included in a programme for blue mussel; bold text – included in CEMP; italics –ICES WGBEC promising methods (all other recommended methods); solid-lined boxes – prioritised components.

WGBEC in its 2008 report indicated that the MXR biological effect technique was as yet not sufficiently developed and validated to be recommended for monitoring purposes, and therefore it should be removed from the above strategy.

In any mussel integrated monitoring programme the prioritised components as indicated should be included as a bare minimum.

Purpose of work

The integrated approach described above can be used for:

- *Status and trend monitoring*; biological effect responses are measured over geographic areas and repeated over time. The purpose here may be to compare biological effect responses between sites, to compare changes in response with time and to observe if the "health status" is improving, at a steady state or declining.
- *Investigative monitoring*; most frequently used as a screening step to assess if biological effects are occurring in relation to a suspected contaminant gradient, pollution event or if biological effects are suspected for any reason (e.g. tissue chemical residues have been observed to be high).
- *Hot spot – site specific monitoring*; usually in relation to risk assessment at pollution sites e.g. oil platform investigations

Offshore and coastal:

Mussels (*Mytilus* species) are infrequently found in the sub littoral zone. But populations do exist in shallow waters and are found on the seabed, usually close to the coastline, in general within the 12 mile limit. They may also be found offshore attached to navigation buoys, chains, and oil and gas platforms. For monitoring purposes these mussels can be used but care needs to be exercised in sampling the organisms, to ensure that they are not damaged during sampling and that the correct size range can be obtained. For offshore monitoring purposes it is usually more applicable to use *in situ* caging methods (see below). Advantages of using caged organisms are; choice of site deployment (including reference sites), selection of depth of deployment (e.g. may be critical for oil platform studies); standardisation of origin (same source/supply), size and species. Disadvantages are: cost of deployment in respect of mooring systems and ship time for deployment and retrieval; in addition some techniques require immediate sampling and analysis which may not be feasible on a research vessel offshore.

If caging is used then hydrographical conditions must be considered with special attention given to water currents and stratification.

Shoreline:

Mussels may be regarded as ubiquitous on rocky shore coastlines and therefore, ideal for monitoring purposes. Sampling sites can be selected easily, organisms collected with little cost and reference sites located without difficulty. In addition, if mussels are not present at a site of interest then organisms can be caged on the sea shore or in estuaries on piers or similar structures.

Sampling Information

Details required:

Date, time and location on the shoreline (if applicable e.g. low water) and exposure (e.g. highly exposed Atlantic rocky shore or enclosed sheltered bay).

Position in Lat. Long.

Type of site; reference, pollution gradient, status or trend

At caging sites information on water temperature, depth of deployment, time of immersion, water column depth and information on currents and stratification if available, water temperature and salinity.

Source of mussels for caging studies; for any caging study it is important that the mussels are sourced from a clean site, and that day 0 values are determined for tissue contaminant chemistry and biological effect responses.

For shoreline monitoring, ideally the mussels must be sampled in a uniform manner between sites i.e. tidal height and similar salinity profile.

Confounding factors

For *in situ* transplants/caging the mussels must be deployed for at least three weeks in order to allow sufficient time for contaminants to accumulate in the tissues and reach a state of equilibrium. Failure to do this may produce spurious data. Also of note is that in many countries there are regulations controlling the movement and deposit of shellfish and these must be observed (i.e prevention of transfer of disease).

Reproductive state and gametogenic cycle; Mussels generally spawn in early spring, with spawning occurring later in more northern populations. At spawning there is a major loss in body lipid and a subsequent fall in condition; therefore sampling in this period should be avoided for all aspects of tissue chemistry analysis and biological effect determinations.

Salinity; be aware that low salinities affect the biomarker response, of particular importance for caging work in estuaries.

Temperature: mussels on the shoreline can be subject to extremes of temperature, cold in the winter and extreme heat in the summer. Avoid sampling when extremes are likely to occur as this may compromise the biological effects response.

Parasites; mussels with severe parasite infections should not be used.

Algal blooms: in spring and late summer and autumn intense algal blooms may occur and sampling of mussels at such times should be avoided.

Species; on some coastlines mussels are solely of one species whereas at other locations they are mixed or hybrids. It is unclear whether species difference will affect interpretation of data but wherever possible attempts should be made to determine the species under observation.

In caging studies (shoreline or offshore) care should be taken in sourcing mussels from a "clean site". If rope grown mussels are chosen then particular attention must be given to transporting the mussels as they tend to have weak adductor muscles and easily gape and become stressed during transportation which may give rise to initial mortalities or erroneous biological effect responses.

Supporting measurements

Condition index; dry meat relative to wet meat weight, live weight or internal shell volume.

Gonad state; index of reproductive state

Lipid content; usually a determined and measured along with tissue chemistry and useful for interpretation of biomarker responses.

Real growth; if available measured using growth of marked intervals over time, usually months.

Water quality measurements; salinity, temperature, suspended solids or turbidity, DO, pH, and chlorophyll.

Chemical analysis of tissues; this is essential for interpretation of biological effects data and for the implementation of the integrated chemical biological effect strategy as outlined above. Prioritised contaminants are Cd, Cu, Hg, Zn, Cd, PAHs and PCBs. As a minimum 50 mussels (>45mm in length) should be collected, taken to the laboratory and held in running seawater for 24hr to eliminate gut contents (e.g. sediment etc). The tissues should then be extracted from the mussel and placed in acid washed hexane rinsed glass jars, stored at -20 C for subsequent chemical analysis using ICES or appropriate protocols.

Sampling for bio effects

For some methods the samples require immediate processing at the time of sampling whereas for other techniques processing is undertaken in the laboratory. An overview of this is shown in the table below (Table 8.1), and also includes the number of animals typically sampled for each method. Ideally the size of individual mussels for all methods is >40mm.

Table 8.1. Overview of sampling procedures for mussels.

Method and numbers of animals usually sampled per site in brackets	When analytical sampling is undertaken	Acclimation	Comments and aspects that are crucial
SFG (10)	24hr	Ca 10 hr	Crucial
Ache (10)	Immediate in field	Not applicable	Stored immediately in liquid nitrogen
Mt (10)	Any time within 24 hr on live mussel	Not applicable	Take tissue sample – freeze in liquid nitrogen
Micronuclei (20)	Within 3 days	None	Mussels can be kept out of water but cool
NRR (10)	Within 24 hr	Store if required in water for no more than 24 hr. Must be consistant in startegy	Do as quickly as possible
Lysosomal histochemical method (10)	Freeze immediately	Not applicable	In liquid nitrogen
Stress on stress (30)	Not aplicable	Transport at low temperatures for no more than 24 hr	Analysis done at 18 C
Histopathology (30-50)	Sample immediately if possible	Anything more than 6 hr delay in sampling place in water for 48 hr acclimation	Dessication must be avoided, correct dissection to incude all organs

Condition (10)	Within 24 hr	If > 24 hr place in water and do sample within 48 hrs	
Growth (25)	Within 24 hr	If > 24 hr place in water and do sample within 48 hrs	
Tissue chemistry (50)	Place in 24hr clean running sea water	Not applicable	Depuration of sediment is crucial

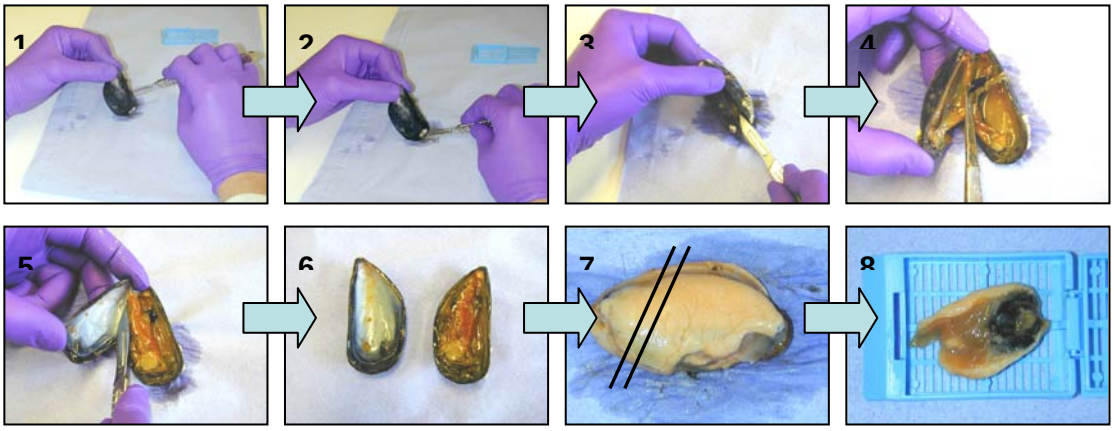
Mussels are attached to each other or to a substrate by a byssal thread. When mussels are sampled care should be used not to pull the mussels and byssal threads too vigorously as this can damage and stress the mussels. If mussels have to be transported this should be kept to a minimum and they should be kept damp and cool and if possible the temperature logged during the transport.

For some techniques such as SFG the mussels will need to be carefully cleaned. It should be noted that there are limitations of analysis for some methods e.g. for SFG and NRR where time-wise it may be difficult to process more than two samples in a single day.

For histological sampling it is essential that the dissection is conducted in a precise manner and this is described below:

The technical procedure essential for correct mussel sampling for histology (taken from draft TIMES doc. under preparation, provided by J. Bignell, UK, Cefas):

- Insert scalpel into ventral byssal cavity and move knife down so it cuts the posterior adductor muscle.
- Open shell and remove byssal thread.
- Remove mussel from one shell half. Repeat for remaining half.
- Analyse tissue for presence of parasites, pearls or other abnormalities.
- Obtain a standardised section as shown in photographs 1–8 in order to include all organs of interest in one section and place into histo-cassette.
-



- Samples should be preserved for a minimum of 24 hours in Bakers Formal Calcium.
- The correct ratio of mussels to fixative is 30 samples per 800ml (approx) of fixative. This is the recommended volume of fixative to ensure adequate fixation.

- Samples should be agitated periodically to ensure thorough fixation. A rocker plate facilitates this perfectly.

Methods to be used

These are listed in the mussel integrated strategy above. An overview of the methods is given in the table below (Table 8.2) with references to the analytical procedures.

Table 8.2. Overview of methods and reference to analytical procedure.

METHOD	ISSUE ADDRESSED	BIOLOGICAL SIGNIFICANCE	REFERENCES
AChE inhibition	Organophosphates and carbamates or similar molecules Possibly algal toxins	Measures exposure to a wide range of compounds and a marker of stress.	1–2
Metallothionein induction	Measures induction of metallothionein protein by certain metals (e.g., Zn, Cu, Cd, Hg)	Measures exposure and disturbance of copper and zinc metabolism.	3–4
Lysosomal stability (including NRR)	Not contaminant-specific, but responds to a wide variety of xenobiotic contaminants and metals	Measures cellular damage and is a good predictor of pathology. Provides a link between exposure and pathological endpoints. Possibly, a tool for immunosuppression studies in white blood cells.	5–19
Scope for growth	Responds to a wide variety of contaminants	Integrative response, a sensitive sub-lethal measure of energy available for growth.	20–21
Stress on stress	Responds to a wide variety of contaminants and other environmental conditions	Integrative response , a measurew of stress, condition, health and well being .	26
Micronuclei	Exposure to aneugenic and clastogenic	Exposure to aneugenic and clastogenic	22–23
Histopathology	Not contaminant-specific	General responses	24–25

Quality assurance

Wherever possible all analytical methods must be supported with quality assurance procedures. These should be through international intercalibration exercises where they exist and through internal quality controls.

The current position with quality assurance is:

- NRR – currently being developed across OPSAR, exists in MEDPOL, for internal QA a dual assessment with a colleague on the same samples is recommended.
- Ache – not yet developed but include internal standard

- Mt – MEDPOL have intercalibration exercises, elsewhere there have been *ad hoc* intercalibrations and additionally an internal standard should be included.
- SFG – none at present
- Stress on Stress – none at present and not a practical option.
- Histology – TIMES doc and circulation of reference material
- Lysosomal histochemical procedures – none currently available but include an internal standard.

Reporting requirements

Biological effect responses; these should be reported in-line with requirements detailed in each analytical method. When different biological effect measurements are made on the same individual mussel then the data should be identified in the reporting and data assessment.

Contaminants: reported in line with standard analytical procedures.

Supporting parameters:

Essential; date and time of sampling, Lat. Long. position, organism length, whole weight, site characterisation (e.g. position on shore, or caging, DO, salinity etc); for caged studies the source of organisms and duration of exposure.

Desirable; identification of species particularly if in a hybrid zone

Recommendation

WGBEC recommends that the draft technical annex for mussel ntegrated chemical biological effect monitoring is forwarded to ICES/OSPAR SGIMC for further development in line with the development and advancement of the integrated monitoring approach.

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9 Review new and promising biological effects techniques: gene array; alkylphenol bile metabolites; bile estrogenicity; ovotestis; intersex in gastropods and crustaceans; histopathology in mussels plus mussel disease index; and passive samplers (ToR f)

9.1 Omics and Environmental Monitoring

Tim Williams (UK), gave a prsentation on the current state of play with omics and environmental monitoring with additional contributions from Stephen George (UK), Brett Lyons (UK).

When a stimulus is applied to an organism the nature of its response may inform on a) the nature of the stimulus and b) the outcome for the organism. In environmental monitoring the responses may be used as 'biomarkers' and include alterations in the concentrations of biological molecules. However, environmentally-sampled animals are usually exposed to multiple uncharacterised stimuli, such that individual biomarkers are unlikely to summarise such a complex response. 'Omics techniques, including genomics, transcriptomics, proteomics and metabolomics, allow a comprehensive view of the molecular response.

Transcriptomics has been used to find alterations in gene expression between, for example, test and control animals, or animals sampled from reference and suspected polluted sites. Microarrays employ an array of gene-specific probes (either cDNA or oligonuceotides) organised on a solid support, with competitive hybridisation of labelled sample cDNAs. Current technologies include robotically-spotted cDNA and oligonucleotide microarrays, inkjet-synthesised oligonucleotide microarrays and 'genechip' microarrays with photlithographic on-chip oligonucleotide synthesis. These techniques can monitor the expression of thousands of genes simultaneously. cDNA arrays have been developed for a range of fish species, including European flounder (*Platichthys flesus*) and stickleback (*Gasterosteus aculeatus*), that are suitable sentinel species for the OSPAR CEMP. Although complex assays, microarray applications are now being accepted in clinical prognosis. Real-time reverse transcriptase PCR is generally a more senstive technique than microarrays, but can only be applied to a few genes at any one time, so does not have the 'open' character necessary to detect unanticipated changes. Therefore it has been used to determine expresion changes in known 'biomarker' genes and validate microarray data. PCR arrays, in 96-well or 384-well format, allow multiple reactions to be carried out simultaneously, are still not 'open' techniques, but show promise for more routine use than microarrays. Developments in DNA sequencing have led to high-throughput techniques capable of quickly generating large quantities of sequence data at comparatively low cost. These techniques can be applied to determine changes in gene expression by 'digital transcriptomics', though this approach is still in its infancy. Alternatively, they provide a convenient pathway to developing oligonucleotide microarrays for species poor in DNA sequence data, indeed this approach is currently underway for European mussel species (*Mytilus edulis* and

Mytilus galloprovincialis). The advantages and disadvantages of these technologies are shown in Table 9.1.1.

Table 9.1.1. The advantages and disadvantages of gene technologies in respect of availability, specificity, AQC and costs.

TECHNOLOGY	AVAILABILITY	PRIOR NEEDS	GENE SPECIFICITY	QUALITY CONTROL	COVERAGE	COSTS
cDNA microarray	Good - Already constructed for a variety of fish species	Sequenced physical cDNA library for species	Poor - Cross hybridization	Poor -Cross contamination -Printing errors	Fair -Proportion of transcriptome	Average
Oligonucleotide microarray	Good - Can be synthesised on demand	Sequence data for species	Good -Dependant on primer design	Average -Printing errors	Good - Dependant on sequence data	Medium-High
Inkjet Microarray (eg. Agilnet)	Good - Can be synthesised on demand	Sequence data for species	Good -Dependant on primer design	Good	Good - Dependant on sequence data	High
Gene Chip Array (eg. Affymetrix)	Poor -Only fish species = zebrafish	Sequence data for species	Good -Dependant on primer design	Good	Good -Dependant on sequence data	High
Real-time PCR	Good - Can be designed swiftly	Sequences of specific genes	Good -Dependant on primer design	Average -Cross contamination possible	Very Poor - Only 10s of genes	Low
Direct cDNA sequencing	Good	None, though genome data will aid interpretation	Good	???	Good -Dependant on depth of sequencing	High

Whichever method is used to determine changes in gene expresion, a major challenge is the interpretation of the very large data sets. There are two main paths that can be followed, and they are complementary. The first is to treat the overall pattern of gene expression as a fingerprint and use this to compare samples. Analyses include hierarchical clustering and principal components analysis (PCA). These provide a broad overview of the similarities and differences in gene expression in different samples and can be employed for categorisation. The second approach is to focus on the individual genes that statistically significantly alter in expression, some may be recognisable as individual biomarkers described by extensive prior literature (eg. vitellogenin, cytochrome P4501A, metallothionein), but often many have not previously been linked to ecotoxicology, so relating these to membership of known biological pathways is useful for discovering the processes disturbed by the stimulus.

One key need in ecotoxicology is to relate reponses in laboratory exposures to those discovered in environmental samples. In the EU-funded Genipol project (collaboration Birminham and Stirling University) we have made progress toward demonstrating such a linkage. Flounders were sampled from seven North Sea sites of different pollutant impact. By use of a genetic algorithm (Galgo) and multivariate statistics we demonstrated that gene expression profiles of fish could be classified by their site origins with reasonable accuracy. By incorporating prior knowledge of biomarker genes and results of laboratory toxicant-exposure experiments, we improved the classification by limiting the search space for the genetic algorithm. The final model of 17 genes was used to develop a PCR array that successfully classified independent flounder samples. This indicates that expression changes derived from short-term laboratory exposures can, in some cases, be used to infer longer term exposures accumulated over months or years of exposure. These gene expression changes combine recent and longer term responses to the environment. In a preliminary collaboration with the Environment Agency we found that expression of well-established biomarker genes correlated with risk categorisation of UK estuaries.

While the above has focussed on transcriptomics, metabolomics presents the advantage that its analytical technologies are not species-specific. Metabolomics employs nuclear magnetic resonance spectroscopy (NMR) or mass spectrometry (MS) to determine alterations in small-metabolite profiles and can lead to identification of individual metabolites. As, unlike microarrays, it is not dependant on a species-specific technology, it is amenable to cross-laboratory intercomparison. In an international exercise, (organised by Mark Viant) hepatic metabolites of flounder from the Tyne (polluted) estuary were compared with those from the Alde (reference) estuary by NMR. Both globally, by PCA, and at the level of individual metabolites, there was excellent consistency in the results from all seven laboratories.

UK and international workshops and regulators have made recommendations regarding the uptake of 'omics in regulatory toxicology and environmental monitoring. An overview of outcomes is shown. These include the US EPA Interim Genomics Policy (courtesy of Bill Benson, US EPA), SETAC Pellston meetings, NERC Knowledge Transfer workshop 'Molecules and the Environment' and NERC Aveiro and Vancouver workshops 'Fish toxicogenomics – Advancing practical implementation'.

The US EPA encourages 'omics research for understanding the molecular bases of toxicity and biomarker discovery. Genomics data alone are not sufficient for risk assessment in regulatory toxicology, but will be useful in a weight-of-evidence approach for both human and ecological risk assessment. Key needs are linking genomic changes to adverse outcomes and interpretation of data in a risk-assessment context. The SETAC Pellston meeting identified needs to formalise 'omics protocols for standard test species, and to generate libraries of responses to well characterised chemicals. Longer term needs included the generation of sequence data for environmentally relevant species and advancing linkage of molecular responses to adverse biological outcomes.

The NERC workshops identified potential UK end-users for genomic data and provided a framework for the application of transcriptomics in ecotoxicogenomics. To some extent this has been superseded by technological advances shown above, that have facilitated 'omics in environmentally-relevant non-model species. The identification of road-blocks to progress in ecotoxicogenomics was one objective, this was felt to be a too-pessimistic formulation and instead 'hurdles' were defined that will need to be, or are currently being, overcome. Progress has been made in the relating of mo-

lecular changes to ecological outcome, with examples of such relationships being established for endocrine disruption in fish and metal contamination in earthworms. As an outcome of the workshops, a collaborative project is in progress to focus on the differences between adaptive and toxic changes in gene expression, using zebrafish as a model organism. Echoing the US EPA conclusions, it was felt unlikely that fish toxicogenomics assays will advance to full validation soon. However, as a fully validated procedure is not necessary to contribute to regulatory decision-making, realistic applications of 'omics techniques may include use in pre-screening chemicals and mixtures for prioritization in further tests, or in environmental terms, pre-screening animals from sites of concern.

Therefore, in summary, 'omics techniques provide a multi-level biological approach to safety assessment. Combining molecular-, cellular-, tissue-, individual-, and population-level data represents a powerful new approach involving different disciplines. Present applications include-

Key advantages for developing a fit for purpose genomics programme are as follows:

- Microarray technology offers an enormous power to dissect out gene expression patterns most intimately associated with physiological stress and contaminant toxicity. Therefore ideally suited to both **investigative monitoring** and **site-specific risk assessment**.
- Genomic tools can produce "expression fingerprints" specifically related to environmental pressures and can be used to identify the genetic factors that determine susceptibility and resistance of individual fish and populations in polluted environments, as such they may be a key supporting parameter in defining qualitative descriptors of **Good Environmental Status**.
- DNA microarrays are particularly suited to the study of organism response following exposure to **mixtures** of environmental contaminants.
- As an open system (i.e. no prior knowledge of the mechanistic action of the contaminant is required) they are ideally suited to the study of novel or **emerging/novel contaminants** (e.g. nanomaterials), **elucidating modes of toxicant action**, and **biomarker discovery**.

9.2 Alkylphenol metabolites in fish bile:

Jonny Beyer(NO) gave a presentation on analysis and assessment of alkylphenol contamination in fish based on work by Jonny Beyer [a,b], Rolf C. Sundt [a], Sonnich Meier [d], Steinar Sanni [a] & Grete Jonsson [c] ([a] *IRIS - International Research Institute of Stavanger, P.O. Box 8046, N-4068, Stavanger, Norway;* [b] *University of Stavanger, N-4036 Stavanger, Norway Stavanger University Hospital, P.O. Box 8100, 4068 Stavanger, Norwa;* [c] *Institute of Marine Research, P.O. Box 1870, N-5817, Bergen, Norway*): Entiltled - Recommendation of analysis method for assessment and monitoring of alkylphenol contamination in fish caused by offshore produced water discharges

Alkylphenol (AP) metabolites in fish bile have in recent studies been demonstrated as sensitive exposure markers for assessing AP contamination in aquatic environments. Produced water (PW) discharges occurring at multiple oil production locations in the North Sea represent large and continuous sources of AP contamination. This is a concern because of the endocrine disruption potency of some APs, and because of the need for protecting the fish resources present in the downcurrent area. APs are readily taken up by fish, preferably over the gill epithelium, and are also readily excreted along the liver-bile elimination pathway (Sundt *et al*., 2009). For the detection of AP

specimens typical for PW, i.e. APs having alkyl chains with a limited number of carbons (C_1-C_7), our studies indicate that the measurement of deconjugated APs in bile offers a two order of magnitude greater sensitivity as exposure markers in comparison to detecting the same PW relevant APs in liver tissue. In studies comparing the performance of different analytical procedures, gas chromatography mass spectrometry was found to have best analytical performance towards APs that are common in PW. The recommended procedure consist of a deconjugation pre-treatment of bile followed by solid-phase analytical derivatization with bis(trimethylsilyl)trifluoroacetamide and then separation and quantification of the derivatized APs using GC-MS in the electron ionization mode (GC–EI-MS) (Jonsson *et al*., 2008a). In comparison, the analysis using high performance liquid chromatography connected to a fluorescence detector (HPLC-F) was found to perform well in high-concentration mechanistic studies but not for detection of PW relevant APs in more complex mixtures (Jonsson *et al*., 2008b). The GC-MS method is already in use within the Norwegian water column monitoring program for assessing AP contamination in fish (caged and feral) at offshore oil fields. The results and conclusions from the performed AP metabolite studies and the experience from the recent field studies in the North Sea were presented to WGBEC. Based on the presented results, the possible implication of the bile AP method in connection with future PW monitoring in the North Sea was discussed. WGBEC considered that the methodology was sufficiently robust to request that the authors develop a full SOP for the GC-MS method and submit it for online publication in the ICES-TIMES series. Subsequently, a ring test exercise with the procedure addressing PW relevant AP metabolites in fish bile should be organised.

Recommendation

WGBEC to request ICES to publish the full GC-MS method for bile AP analysis and for the method to be intercalibrated with interested parties within WGBEC and beyond.

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9.3 Review of intersex in gastropods and other molluscs

Jakob Strand (Denmark) presented an extensive review of intersex in gastropods and other molluscs.

Various studies have demonstrated that the presence of imposex, i.e. an imposition of a penis and/or a vas deferens in addition to the normal reproductive tracts in females of gonochoristics prosobranch gastropod species, is a common phenomenon in the marine environment today. The imposex phenomenon has been related to irreversible effects of endocrine disruptions induced specifically by exposure to the trisubstituted organotin compounds tributyltin (TBT) and triphenyltin (TPhT), which

have been widely used as antifouling agents in marine paints for ship hulls and stationary constructions or as fungicides in agriculture.

However, also other signs of endocrine disruption causing sex reversal, characterized as intersex, have been reported for marine molluscs. Intersex can more generally be defined as disturbance of the phenotypic sex characters and different types of intersex have been recorded for both female and male molluscs. This includes examples like;

- Transformation of the reproductive and genital tracts in gastropods and cephalopods e.g. masculinisation in periwinkle *Littorina littorea* or feminisation in squids (e.g. Stroben *et al*., 1992; Bauer, 1995; Hoving *et al*., 2006).
- Spermatogenesis in the female ovary, e.g. masculinisation of neogastropods and abalone gastropods (e.g. Gibbs *et al*., 1988; Horiguschi *et al*., 2000).
- Oocyte development in male testis (ovo-testis), i.e. feminisation of the clam *Scrobicularia plana* (e.g. Chesman and Langston, 2006).

Imposex in gastropods have been reported worldwide in at least 259 different species (Strand, unpubl.) indicating a widespread pollution of TBT in the marine environment. High levels of imposex have even been reported in some neogastropod species from polar areas, including the high Artic (e.g. Brick and Bolte, 1994; Strand *et al*., 2006) and recently also on Antarctica and it is thereby one of first clear signs of endocrine disruptions occurring in wildlife on the South polar continent (Strand *et al*., in prep.).

However, it has been argued that a low, but natural, background level of imposex can occur in gonochoristic prosobranch gastropods. Some studies have shown that up to 16% of female neogastropods with imposex, but only in mild stages, has been found in some (but not all) museum collections from pre-TBT historic times, i.e. before 1960 (Kantor, 1984; Graventa *et al*., 2006).

Imposex in marine gastropods is today an established TBT-specific biomarker in many marine monitoring programmes and is also part of OSPAR CEMP and JAMP (OSPAR 2008). Environmental assessment criteria for imposex in four different neogastropod species have been developed by OSPAR/ICES. International laboratory performance studies have in Europe been performed yearly since 1998 and have been undertaken by the QUASIMEME (e.g. Davies *et al*., 1999, 2002).

Intersex in gastropods. In addition to what normally is regarded as imposex characters, i.e. imposex according to the VDS-scheme for imposex stages 1 - 6 used in for instance OSPAR/ICES guideline (OSPAR JAMP, annex 10), also some further alternations of the sexual characters has been described for female gastropods with severe imposex stages, where they are regarded as sterile. Actually, the VDS-stage 5a involves a beginning transformation of the pallial oviduct into a prostate gland, and VDS-stage 5c involves an open capsule gland (Stroben *et al*., 1992).

Other alternations of the female characters, for instance the oviduct into a curled oviduct similar to the male seminal vesicle, which can block the transport of oocytes from the ovary to the capsule gland and thereby causing sterility (Figure 9.3.1), have been reported for gastropods with severe stages of imposex (Fioroni *et al*., 1991, Strand and Jacobsen, 2002).

These further alternations observed in females with severe stages of imposex can actually also be described as a disturbance of the phenotypic sex characters and subsequently also be characterised as intersex.

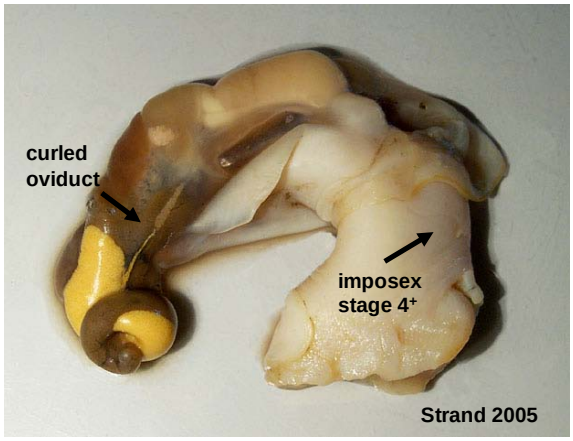

Figure 9.3.1. A further alternation of the female characters in form of a curled oviduct blocking the transport of oocytes from the ovary to the capsule gland causing sterility in the red whelk (*Neptunea antiqua*) from the Inner Danish waters 2005. (*Photo: J. Strand).*

These further alternations is actually much like the well-known intersex phenomenon in the periwinkle (*Littorina littorea*), where the pallial oviduct in females (Figure 9.3.2.) is gradually (defined four stages) is supplanted by a male-like prostate gland (Bauer 1995, 1997, Sunderman *et al*., 1998).

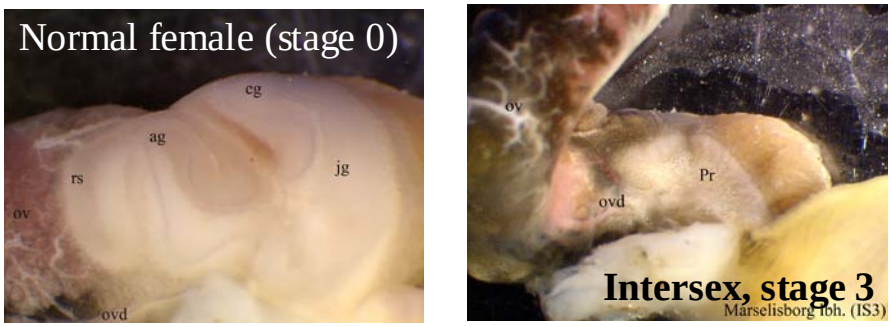

Figure 9.3.2. Intersex stage 3 in *Littorina littorea*. The pallial oviduct is supplanted by a male-like prostate gland. cg: Capsule gland, jg: jelly gland, ag: albumin gland, ov: ovarium, ovd: oviduct, Pr: Prostate. Photos: C.A. Jensen).

Intersex in *Littorina littorea* have been used as an established TBT-specific biomarker in several North-Atlantic countries (see Table 9.3.1.) and intersex in *L. littorea* is also part of OSPAR JAMP (OSPAR 2008). An ICES TIMES has also been published (Oehlmann 2004). Environmental assessment criteria have been developed by OSPAR/ICES in line with the assessment criteria for imposex in neogastropods.

International laboratory performance studies have in Europe been performed yearly since 1998 and have been undertaken by the QUASIMEME (e.g. Davies *et al*., 1999, 2002).

Table 9.3.1. Examples of intersex studies with *Littorina littorea* in different North Atlantic countries.

Germany (Bauer *et al*. 1995,1997)	USA (Evans *et al*. 2001)
Ireland (Minchin *et al*. 1996,1997)	Sweden (Sveder 2002)
Denmark (Strand 1999, Jensen 2002, Strand 2003)	England (Birchenough *et al*. 2002, Galloway *et al*. 2004)
Norway (Green *et al*. 1999)	Portugal (Barroso *et al*. 2003)
The Netherlands (Wolf *et al*., 2001,2004; Van den Broeck *et al*., 2007, Schipper *et al*. 2008)	Scotland (Miller & Boyle 2003)
	Canada (Coray & Bard 2007)

Intersex in *Littorina littorea* has been recorded in all studies, but is not such a sensitive TBT biomarker compared to imposex in many other prosobranch gastropod species. It is assessed that intersex is induced at TBT-concentrations >15 ng/l (Bauer, 1997), whereas imposex can be induced for instance in *Nucella lapillus* at TBT-concentrations 1 ng/l (Gibbs *et al*. 1988).

Subsequently, elevated intersex levels in *L. littorea* have mainly been observed inside or in the close vicinity of TBT-polluted harbours and marinas, as for instance shown in Figure 9.3.3.

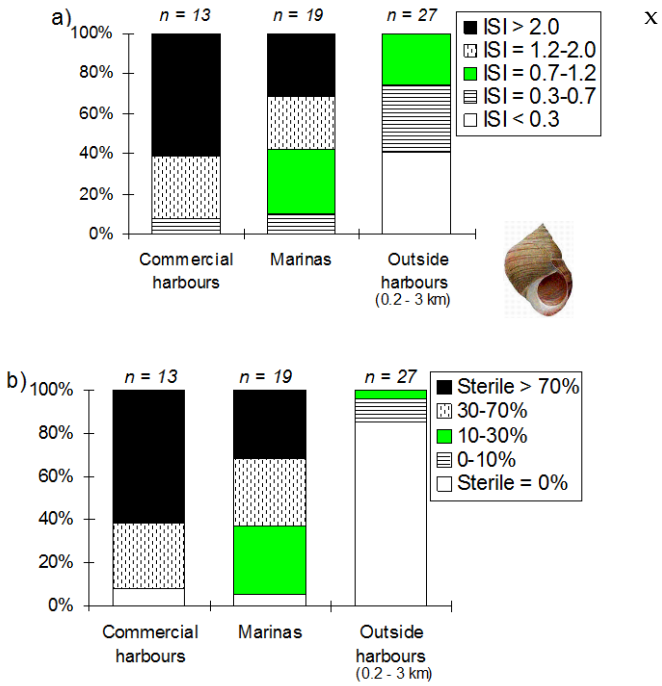

Figure 9.3 3. Example: The distribution of intersex severity in populations of L. littorea as a) ISI and b) % sterile females in 13 harbours and 19 marinas compared to 27 stations outside harbours in Danish coastal waters (Strand, 2003).

Also other Littorina species have been examined for intersex in the literature. However, intersex has not been found in species, like *L. saxatilis*, *L. brevicula* and *L. mandshurica*, even when sampled inside TBT-contaminated harbour areas(Strand & Asmund 2003; Syasina and Shcheblykina 2007).

An additional effect often observed in *Littorina littorea* sampled in the close vicinity of harbour areas (Figure 9.3.4) is a reduced number of mamilliform penial glands (PG) on the male penis, which also has been described by Bauer *et al*. (1997). The mechanism for this is unknown, but it is thought also to be related to endocrine disruptions

caused by TBT, although it might be more reversible effect than the intersex development in females, because male periwinkles are developing a new penis each year.

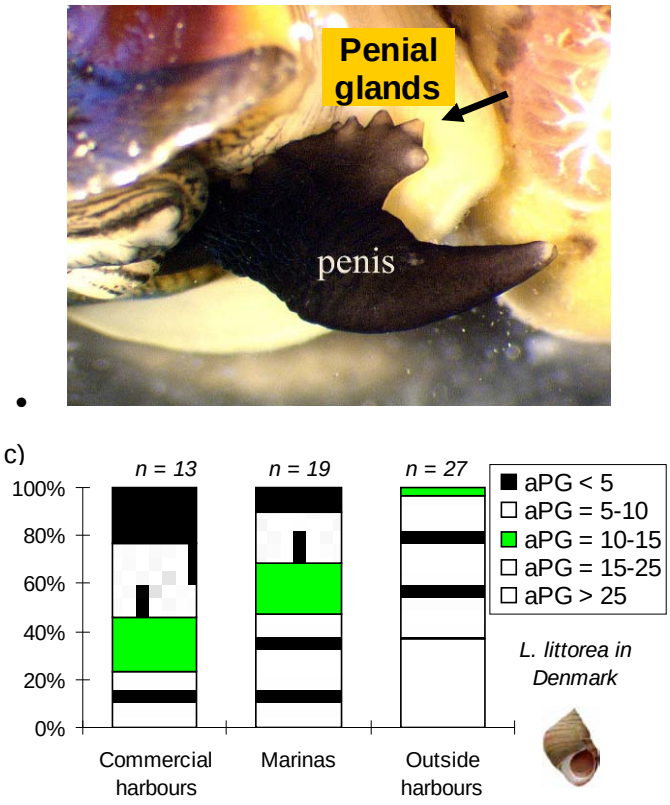

Figure 9.3.4.a and b. Reduced number of penial glands on the male penises in populations of L. littorea can occur in the close vicinity of harbours and marinas, showing a similar pattern as for the intersex severity in females (see Figure9.3.3.). aPG: average number of penial glands on male penises. Danish data from 59 stations (Strand, 2003).

Histological studies have found that spermatogenesis also can occur in female ovaries of marine neogastropods like dogwhelk *Nucella lapillus* (Gibbs *et al*., 1988) and ivory shell, *Babylonia japonica* (Horiguchi *et al*., 2006), which has developed severe stages of imposex, indicating that these effects also are related to TBT. Such kind disturbance of the gonadal sex characters, can be characterised as intersex, and is in its expression similar to environmental signs of endocrine disruption described for vertebrate species like fish and amphibians.

Similar kind of intersex has also been described in the edible giant abalone (*Haliotis gigantea*), where spermatogenesis occurs in about 20% female abalone ovaries at some TBT-polluted sites in Japan, although this species is not expressing imposex development at all (Horiguchi *et al*. 2000, 2005). Similar kind of intersex has also been found in 0–50% of female Roe's abalone (*Haliotis roei*) in Western Australia (Sloan and Gagnon, 2004).

Intersex in bivalves. Some histological studies have also shown signs of feminisation of male bivalves, for instance between 0 and 100% of the male clams belonging to *Scrobicularia plana* have developed oocytes in the testis in different sites of UK coastal waters indicating a widespread oestrogenic effect, although also seasonal changes

occurred in the prevalence of intersex males (Chesman & Langston 2006, Langston *et al*. 2007).

Intersex in cephalopods. Few studies have also described the occurrence of intersex in cephalopods, like squids and octopuses. For instance, Hoving *et al*. (2006) has found that 7 of 16 sexually mature *Ancistrocheirus lesueurii* male squids from southern African waters had female nidamental glands in the mantle cavity in addition to a normally developed male reproductive system.

A rare case of intersex has also been described in the Patagonian octopus, *Enteroctopus megalocyathus,* which had developed a penis, in addition to the ovary, but no testis. Also other sexual characters were abnormal (Ortiz and Ré, 2006).

Other promising techniques on endocrine disruptions in molluscs. Various studies have included other biological effects techniques for assessing if effects of endocrine disruptions other than the described intersex and imposex phenomena occur in mollusc populations sampled in the field or in laboratory studies using environmental relevant exposure levels. Many of these techniques seem promising for both descriptions of the mechanisms for the endocrine effects and for assessment of biological effects in the environment, for instance;

- Vitellogenin-like proteins (measured as alkali-labile phosphate) in male bivalves (e.g. Blaise *et al*. 1999; Matozzo and Marin, 2007).
- Altered sex steroid balance in bivalves and gastropods (e.g. Bettin *et al*., 1996; Pellerin *et al*., 2003).
- Inhibited aromatase activity in bivalves and gastropods (e.g. Morcillo *et al*., 1999; Santos *et al*., 2002)
- Delayed gametogenesis in bivalves (e.g. Gauthier-Clerc *et al*., 2002; Siah *et al*., 2003).
- Reduced or increased fertility/fecundity of gastropods (e.g. Schulte-Oehlmann *et al*., 2000; Tillmann *et al*., 2001; Wagner and Oehlmann, 2009).
- Skewed sex ratio in bivalves (e.g. Gagné *et al*. 2003; Hellou *et al*. 2003).

Many of these signs on endocrine disruptions, which are related to masculinization of female molluscs, are primarily discussed in relation to TBT pollution in the marine environment, although some studies have also indicated that effects of oestrogen-like compounds, causing feminisation of molluscs should.

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9.4 Review of intersex in crustacean.

Alex Ford (UK), as an invitee to WGBEC presented an extensive review of intersexuality in crustacea and whether it posed an environmental issue.

This review aimed to give an overview of current understanding about intersexuality in crustaceans and assess gaps in our knowledge over whether it could/should be an issue of environmental concern. Intersexuality in wildlife has received considerable attention due to the issues raised by endocrine disrupting chemicals (Colburn *et al*., 1996). Many crustacean species are hermaphroditic with several examples of organisms starting as one particular sex and changing sex with age (Yaldwyn, 1966), others can be simultaneous hermaphrodites displaying both male and females characteristics at the same time (Baeza *et al*., 2009). Consequently, there have been confusion as to whether accounts of intersexuality in the literature are correct, or whether these specimens were at the time unknown sequential and/or simultaneous hermaphrodites.

The first known published accounts of intersex in a crustacean was a report given Royal Society of 'a hermaphrodite lobster' in 1729 (Fisher, 1729) which outlined a specimen which was male on one side and female on the other. This was undoubtedly an example of gynandromorphism and not hermaphroditism and has been reported in many crustaceans since. Sexual gynandromorphism (a particular form of intersexuality) is a condition which is thought to arise when genes (e.g. governing sex determination) are switched on/off during the bilateral developmental of an embryo resulting in one side appearing male and the other female.

Intersexuality in the context of this report is the appearance of male and female characteristics occurring simultaneously either externally with secondary sexual characteristics, or internally within the reproductive organs (e.g. ovitestes). It has been suggested that intersexuality in crustaceans may arise may arise through different mechanisms (Ford, 2008). Bilateral sexual gymnandromorphism may arise through disruption in sex determining hormones whereas intersexuality may also arise through perturbations of androgenic gland hormone activity. Intersexuality is certainly not a new phenomenon. An intersex specimen of a crab has been reported in a

fossil from the upper cretaceous dating back about 70 million years ago (Bishop, 1973). Despite the outlined confusion, intersexuality has been reported within the literature amongst a wide variety of Orders including Decapods (Yaldwyn, 1966), Isopods (Rigaud and Juchault, 1993), Copepods (Moore and Stevenson, 1991) Amphipods (Ford and Fernandes, 2005a), Mysidae (Mees *et al*., 1995), Anomurans (Turra, 2004), Artemians (Bowen and Hanson, 1961) and Daphnids (Mitchell, 2001).

The causes of intersex are multifaceted and can occur through a number of mechanisms including parasitism (Bulnheim, 1975; Rodgers-Grey *et al*., 2004), abnormal environmental sex determination (Dunn *et al*., 1993), genetic abnormalities (Parnes *et al*., 2003) and pollution being implicated in an increasing number of cases (Moore and Stevenson, 1991, 1994; Takahashi *et al*., 2000; Ford *et al*., 2004; Barbeau and Grecian, 2003; Vandenbergh *et al*., 2003; Jungmann *et al* 2004; Ayaki *et al*., 2005). Further studies have highlighted how pollution might increase the prevalence of feminising parasites, thus causing a form of indirect endocrine disruption (Ford *et al*. 2006).

Currently the only certain cases of intersexuality caused by chemical contamination are studies conducted on Daphnia. Olmstead and LeBlanc (2002) found that terpenoid hormone methyl farnesoate (MF) is a sex-determining factor in *Daphnia* sp. Specimens exposed to elevated concentrations of MF or MF synthetic analogues resulted in all male broods, which are considered highly unusual in otherwise parthenogenic species. Recently, the same authors also demonstrated that exposing *Daphnia* to MF in the laboratory can induce sexual gynandromorphism (a form of intersexuality) (Olmstead and LeBlanc, 2007). However, studies by Mitchell (2001) have also implicated temperature changes as also causing intersex in *Daphnia magna*. Vandenbergh *et al*. (2003) in a study exposing amphipods to 17α-ethinylestradiol found first generation males had reduced gnathopods, intersex testes and disrupted spermatogenesis, although the potential role of parasites in the observed results was unclear (Ford and Fernandes, 2005b). Jungmann *et al*. (2004) interestingly found when amphipods collected from streams with a low prevalence of intersexuality were caged in streams with high levels of intersex, or kept under laboratory conditions in water from high-intersex streams, a greater proportion became intersexed than if kept in 'low-intersex' stream water. Again, the role of parasites could not confidently be ruled out or any specific chemical contaminant identified.

Sexual differentiation and secondary male characteristics are under the control of the androgenic gland (AG; Sagi *et al* 1997). Reduction in androgenic gland hormone (AGH) from the AG results in de-masculinisation, cessation of spermatogenesis and in some circumstances ovarian development.

Numerous studies around the world have developed VTG assays for crustacean species similar to those used successfully as fish biomarkers, however despite this investment none have been able to prove feminisation by chemical contamination (Ford, 2008). Studies under the EDMAR program (Endocrine Disruption in the Marine Environment; Allen *et al* 2002) found no levels VTG induced in male common shore crabs from polluted estuaries. Ford (2008) recently suggested that is essentially quite difficult to feminise a crustacean without some prior de-masculinisation (due to the over-riding effects of androgenic gland; see paper for detailed argument) and concluded '*in determining whether endocrine disrupting chemicals maybe impacting the sexual chemistry of crustacean hormones it might be more fruitful, especially when attempting to design early-warning biomarkers of exposure (and effect), to address the question of de-masculinisation, rather than feminisation*'.

There have been a couple of studies indicating de-masculinisation of crustaceans as a possible impact of environmental contamination however, so far, comprehensive studies of this type have been few and far between. Ford *et al*. (2004) observed reduced gnathopod (claw) sizes in normal male amphipods collected from a field site categorised as contaminated. The gnathopods are a secondary male characteristic under the control of the AG and suppressed AGH has been shown in intersex male *Echinogammarus marinus* (Ford *et al*., 2005). Yang *et al*. (2008) recently observed reduced sperm counts (20%) in normal males collected from the same contaminated sites when compared to reference sites. Yang *et al*. (2008) also observed internal signs of intersexuality (testes with developing oviduct) in amphipods collected from an industrially impacted field site. Interestingly, specimens previously showing no external signs of intersexuality have been found to be negative for parasite infection (Ford *et al*., 2006). Allen *et al*. (2002) reported that crabs collected from polluted locations had more broadened abdomens (i.e. more female-like) when compared to reference locations. Although, in the more widespread survey, Brian (2005) found it difficult to correlate changes in morphology between clean and polluted sites from background population variability, she did, however, observe a correlation between the degree of contaminant exposure and the sizes of the crab's front claws. Li (2002) similarly found a correlation between the degree of fluctuating asymmetry and pollution in Taiwanese crabs. Interestingly, however in this particular study the incidence of crab intersexuality decreased with contamination (Li, 2002).

Despite many studies on the effects of EDCs on crustaceans, very few have focussed on wild populations; rather many laboratory studies have been conducted on a wide range of crustacean species attempting to assess biomarkers of feminisation (e.g. VTG induction) found in vertebrate species (reviewed in Zou and Fingerman, 2003; Zou, 2003; Oetken *et al*., 2004; Rodrıguez *et al*., 2007; LeBlanc, 2007). This is surprising as many of the seminal papers on endocrine disruption focussed on effects found in the wild (e.g. feminisation of fish; reproductive abnormalities in alligators and frogs) which were subsequently investigated in detail through a series of monitoring and laboratory experiments. Possibly for this reason, it is currently unclear whether reproductive abnormalities such as intersexuality are an environmental issue within the Crustacea as no comprehensive monitoring projects have been conducted. Furthermore, biomarker development has been hampered by paucity of genomic and endocrine knowledge of many crustacean model species. The advent of cheaper affordable genomic techniques (e.g. high-throughput sequencing) should help to address the balance. The Daphnia genome has been completed (http://wfleabase.org/) and high throughput sequencing (Roche 454) has been applied to compare transcriptomes of normal and intersex crustaceans (Ford *et al*, in prep). These (and similar studies) should aid greatly the future development of sex/endocrine specific biomarkers.

The following recommendations are made:

1) Firstly, field based monitoring studies are required to assess whether any developmental abnormalities and/or abnormal sex ratios are occurring in wild populations of crustaceans. These monitoring studies should take into account whether the species is commercially harvested and if so whether sexes are differentially selected.

2) Based on arguments that is physiologically difficult to feminise a crustacean (Ford, 2008), both laboratory and field studies should concentrate less

on feminisation markers (e.g. VTG) and those associated with de-masculinisation and 'other' endocrine associated effects (e.g. moulting).

3) Many crustaceans have environmental sex determination which may make them susceptible to changes in their sex ratios or developmental abnormities such as gynandromorphism.

4) Sexual gynandromorphism and intersex forms (such as ovitestes) should be carefully and separately recorded as they may originate via different developmental aberrations.

5) Studies should be alert to the potential of parasites to confound observations. Currently, studies have shown parasites can increase (Microsporidia in amphipods) and decrease (Rhizocephala in decapods) the incidence of intersexuality under polluted conditions.

6) Greater emphasis should be made on understanding the genetics of sex determination and sexual differentiation of crustaceans, along with a better understanding of their general endocrinology.

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9.5 Histopathology in mussels plus mussel histopathology index

John Bignell (UK) gave a presentation on mussel histopathology and the approach adopted in the UK by Cefas for monitoring purposes. This will form part of the Cefas contribution to the ICES TIMES publication currently being written, *'Histopathology of mussels Mytilus sp. for health assessment in biological effects monitoring'*.

Histopathology is a valuable tool for the overall health assessment of animals in the aquatic environment. Not only does it demonstrate abnormal changes in relation to disease, but environment-induced changes such as chronic exposure to contaminants. As such it lends itself well to biological effects monitoring programmes that utilise mussels. Mussel histopathology also compliments other biological effects techniques. It can be used to rule out the effects of disease and associated stress when using generic stress techniques such as the Neutral Red Retention (NRR) assay. Furthermore, histopathology offers a great deal in the application of genomic and proteomics in future biological effects programmes. Used correctly, it can inform scientists about the potentially contaminating effects of pathogens thus enabling high quality sample selection for downstream analysis.

Mussels are assessed for a number of health index parameters that are split into four major categories:

1) Reproductive
   - Adipogranular tissue index
   - Gonadal status
   - Atresia
   - Apoptosis
   - Intersex / Hermaphrodite

2) Non-specific pathology
- Inflammation
- Granulocytomas
- Pearl formation
- Kidney lipofuschin

3) Digestive diverticula pathology
- Atrophy of digestive diverticula
- Degeneration
- Lysosomal pathology

4) Disease conditions
- Neoplasia
- Rickettsia/Chlamydia-like organisms
- Viruses
- Parasites

In the context of histopathology, quality assurance is often a term associated with the accurate diagnosis of pathological and disease conditions. However, it is important to consider quality assurance at all stages of the process including field sampling, histological processing and pathological interpretation. One such aspect that warrants further investigation is the issue of air exposure and the effect on histopathology and other biological effects. Biological effects programmes that utilise mussels often require specimens to be sent long distances to the laboratory for analysis. As such transportation times can be overnight or more resulting in samples being "exposed" for extended periods of time. Previous studies have shown that air exposure can result in a decrease in neutral red retention time indicating reduced lysosomal stability in Blue mussel, Pacific oyster and Blacklip Abalone. (Harding, 2004; Zhang *et al.*, 2006; Song *et al.*, 2007). Cefas are currently investigating air exposure effects to prove or discount a similar effect with respect to mussel histopathology. As a result, appropriate sampling procedures will be recommended in the forthcoming TIMES publications.

Standardised dissection techniques should also be employed to ensure that all major tissues and organs are incorporated in a single histological section and standard orientation of tissues and organs is achieved. These important aspects enable the pathologist to appreciate any pathology observed in the correct context of other tissues in addition to dramatically decreasing the time taken to evaluate a sample.

Recommendations

Grading indices have been developed for the assessment of reproductive status (Seed) and adipogranular (ADG) tissue (Bignell *et al.*, 2008), however, more work is required in the development of indices for those conditions deemed as appropriate. This will play a vital role in ensuring that assessment tools are established in the future. Although some initial work on this area has already been initiated at Cefas, it was suggested that a central steering group under the BEQUALM framework develop this further. A series of mussel histopathology workshops would facilitate this

process. It is also recognised that in order to successfully develop assessment tools, quality assured data from multiple sources must be made available.

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9.6 Biomarkers in marine mammals

Veronika Hellwig (DE) gave a presentation about an *in vitro* approach to study pollution impact on marine mammals. Marine mammals as top predators in the marine food web are considered as bioindicators of middle- and long-term changes as well as of effects of environmental contamination in the marine ecosystem. In the Wadden Sea, harbour seals (*Phoca vitulina*) are considered as indicator organisms in the Trilateral Monitoring and Assessment Program (TMAP). Developments in the harbour seals population is monitored by aerial surveys (Reijnders *et al*., 2008). For Schleswig-Holstein, Germany long-term field and pathological investigations on their health conditions is performed by U. Siebert and co-workers at the FTZ Research and Technology Centre in Büsum (University of Kiel) (Siebert *et al*., 2007). To monitor the health status of marine mammals as indicator organisms, reliable bio(chemical) markers are important for an early diagnosis of disorders caused by anthropogenic pollutants (Reijnders *et al*., 2007).

Bioanalytical methods for the detection of biological effects of contaminants and changes in the health condition are developed at the GKSS Research Centre Geesthacht in close cooperation with the FTZ. I*n vitro* toxicoproteomic studies aim at identification of a (species) specific set of proteins with pollutant-induced protein expression, which should preferably be measured in blood and tissue samples in a non-invasive monitoring strategy on harbour seals. For isolating primary hepatocytes from wild-ranging harbour seals (*Phoca vitulina*), two procedures were developed using tissue samples from freshly dead animals: a biopsy perfusion method digesting cannulated liver pieces in a perfusion apparatus and a non-perfusion method digesting manually minced liver tissue (Hellwig *et al*., 2007, Wargel *et al*., 2009). The protein expression levels in cells incubated with contaminants (e.g. Aroclor mixture, PFOS) in three concentration levels are compared with those in internal reference controls from the same individual. Hereby, all modifications in the protein pattern, other than those induced by the targeted dosage of the contaminants, should be eliminated during data interpretation (Behr *et al*. 2008).

Action

In previous years, although not recently, WGBEC has been given TOR relating to the effects of contaminants on whales and seabirds and the group felt that it should review the current state of knowledge in this area at next year's meeting, particularly with a view to a more integrated approach (i.e. all components and all levels) to contaminants, and their effects in marine ecosystems.

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9.7 Passive samplers

A review on the use of passive samplers as a promising technique within the biological effects field was presented by Norway and the UK. Passive samplers are by design devices that passively collect contaminants and provide an integrated signal of contaminant loading. The advantages of passive samplers are that they are:

1) Non-mechanical; require no maintenance, power or other energy supply.

2) Can be deployed in a wide range of environments including remote regions with little infrastructure and highly polluted sites where biomonitoring may be impossible.

3) Laboratory analysis of passive samplers can be faster and less expensive than many conventional water, sediment or tissue analysis methods

4) Passive samplers do not breed/ die/ get hungry or suffer from natural biological variation and are available year round.

5) Variation between individual samplers is normally much less than that seen in organisms.

6) Spot water samples reflect residue composition only at the moment of sampling and may fail to detect/ overestimate episodic contamination

7) Quality control and physical difficulties - large volumes of water necessary for quantifying and assessing trace/ ultra trace organic contaminants.

8) Standard low volume (< 5 L) techniques often fail to detect such low, but ecologically relevant, levels of contaminants, (e.g. TBT).

9) Concentrations of truly dissolved contaminants are not accurately measured by most conventional approaches. (e.g. liquid extraction)

A wide number of passive samplers are available and a there have been a large number of peer review publication on passive samplers since the mid 1980's (Figure 9.7.1). A comprehensive review is available (Vrana *et al*., 2005). The most common passive samplers frequently used for monitoring are:

1) Semi-permeable membrane devices (SPMDs)

2) Polar organic contaminant integrative sampler (POCIS)

3) Silicon rubber

4) Chemcatcher

5) Low density polyethelene (LDPE)

6) Diffuse gradient thin films (DGTs)

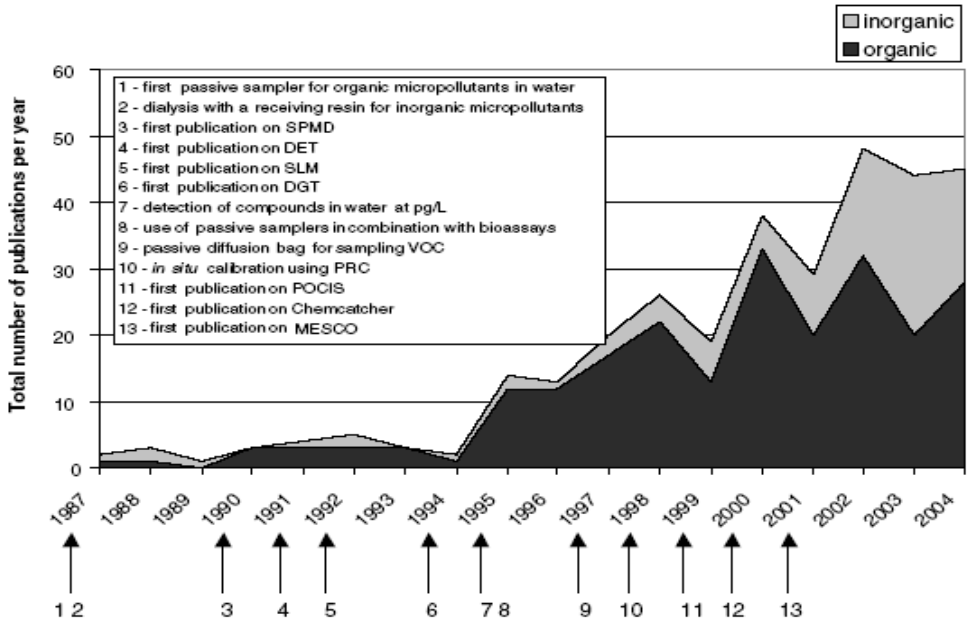

Figure 9.7.1. Overview of passive sampler publications (Vrana *et al*., 2005).

These passive samplers can be used for organic contaminants of differing hydrophobicity depending on the type of passive sampler used (Figure 9.7.2). DGTs are only suitable for the sampling of metals.

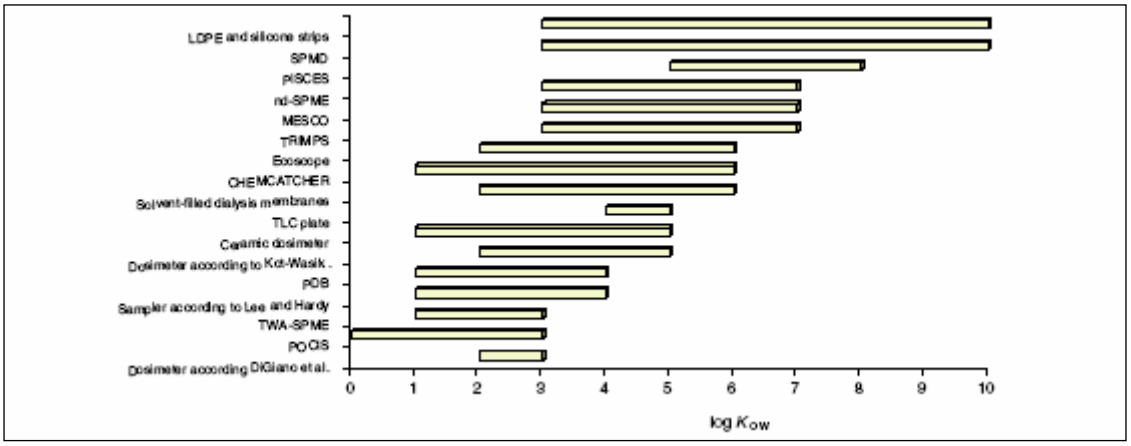

Figure 9.7.2. Typical hydrophobicity range of organic compounds sampled by passive sampling devices (Vrana *et al.*, 2005).

An ICES intercomparison study for silicon was recently performed demonstrating the usefulness of these techniques in monitoring studies. A summary of the results is presented in Figure 9.7.3. What has been demonstrated is that passive samplers for quantitative applications need to use performance reference compounds (PRCs) which allow the uptake of contaminants to be compared with the rates of release of these substances from the sampler. PRCs have been shown to be essential especially in the marine environment where a high amount of fouling can influence contaminant uptake. Other studies have shown that the uptake of contaminants such as PAHs can be correlated with the concentration of PAH in the bile of fish (Harman *et al.*, 2009). Indeed passive samplers are often used alongside blue mussels in monitoring studies and therefore show promise as proxies for both fish and mussels.

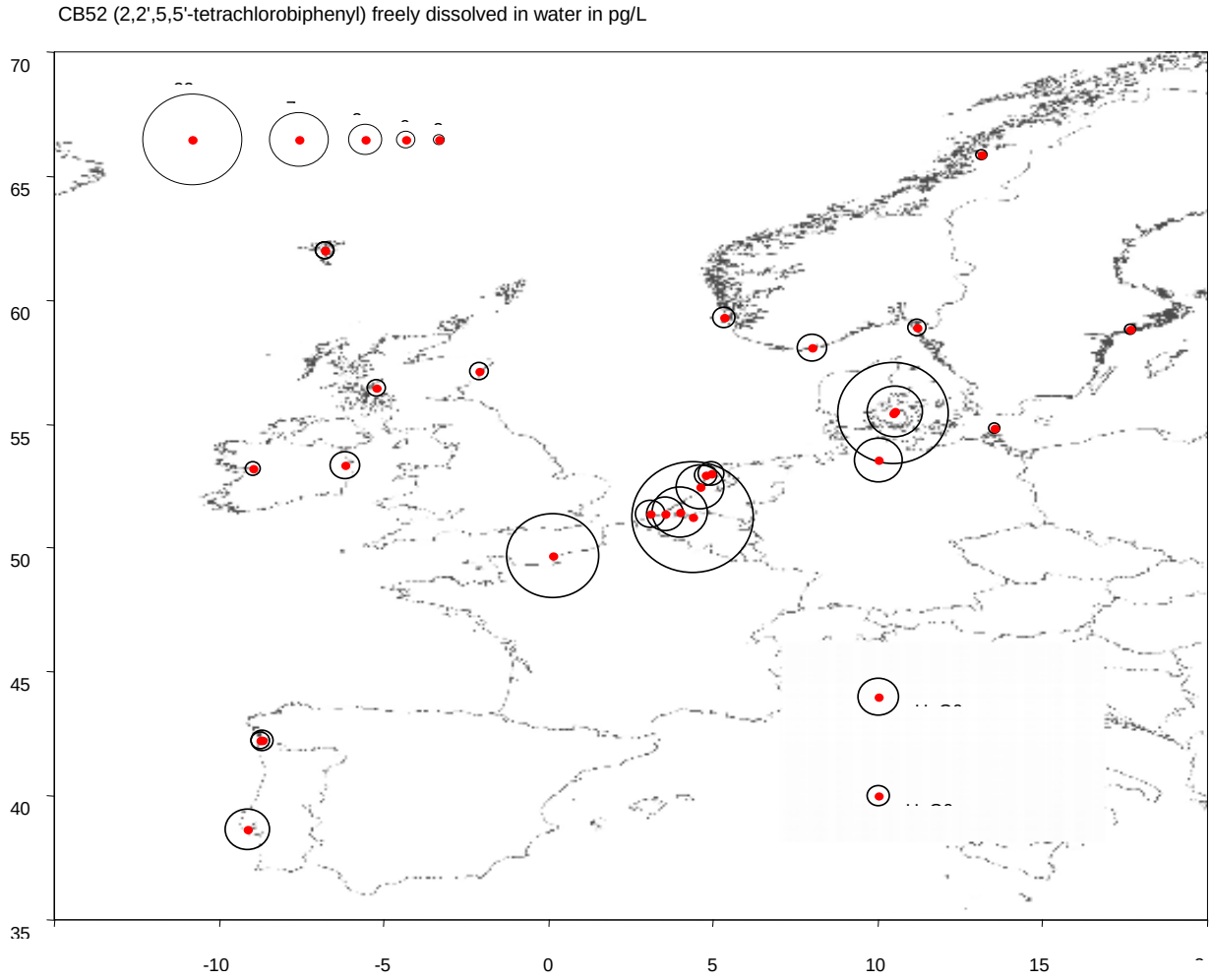

Figure 9.7.3. Concentration of CB52 determined by silicon passive sampling deployed across Europe.

In conclusion:

- There are a wide range of samplers available
- QA/QC is in place for a number of samplers
- PRCs are essential for quantitative work
  - Not suitable for bioassay work but can be run alongside
- Fouling not an issue if PRCs are used
- Good correlation with bile metabolites

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9.8 Biosensors

Steinar Sanni (Norway) gave a presentation on new initiatives for on-line monitoring methods to measure biological effects. At the ICES WGBEC meeting 2007 it was discussed to remove on-line monitoring methods from the list of new and promising biological effect methods for review, since there had been little activity related to it in recent years. Due to knowledge of new initiatives with on-line monitoring methods it was decided to await a review on these. Several terms for on-line methods is presently in use, among them real time biomarkers, biosensors, bioelectronic systems and instrumented (sentinel) animals.

Two new, application driven on-line initiatives were presented in the 2009 meeting: Firstly - Safeguarding drinking water quality conducted by St. Petersburg Research Centre for Ecological Safety and secondly - off-shore monitoring by IRIS–Biomiljø and BiotaGuard environmental monitoring technology company in Stavanger, Norway.

The background for the first mentioned initiative is that immediate responses are needed if unwanted chemicals enter drinking water supplies, and real time monitoring is therefore required. The method is based on cardiac activity measurements in the freshwater crayfish *Pontastacus leptodactilus*. The method is a further development of a method developed at the Univeristy of Plymouth (Bamber & Depledge, 1997), and it was presented in more detail at ICES Annual conference Sept. 2007, Helsinki (Kholodkevich *et al*. 2007, 2008). Stress responses are observed based on "pulsometric" analysis. Responses measured in test situations demonstrate that the method can be regarded as a general, real-time, stress indicator. The method is currently applied industrially in several water supply stations in St. Petersburg.

The background for the off-shore monitoring initiative is that there are increasing offshore operations in remote areas as well as sub-sea and unmanned operations. It is a need to bring biomonitoring into the Control Room of Integrated Operations (IO) for which real time monitoring is required. To facilitate this, a real time environmental effect monitoring system called "BiotaGuard" has been developed. It is based on known methods of cardiac activity and valve gape behaviour in blue mussels (*Mytilus edulis*). Conventional chemical/physical sensors and passive samplers are integrated in the system. "Passive" (not instrumented) mussels are also exposed and can be collected as needed as basis for more detailed laboratory analysis of mussel health conditions. The system can also accommodate other biosensors to represent specific marine conditions in other regions and water depths where such organisms are present. Further development of the system for application in Arctic oil fields is presently being initiated, financed by oil companies and the Research Council of Norway.

Three field tests with the mussel based prototype have already been conducted, including an off shore test in a North Sea oil field in parallel to the Water Column Monitoring program (Norwegian Oil Industry Association) which is based on biomarker measurements in caged mussels and cod. A long term field validation outside an oil refinery will be conducted this year, which may provide data for evaluation of the system. The results will be presented to ICES WGBEC in the 2010 meeting.

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10 Report on emerging and novel contaminants and their biological effects with particular reference to a) pharmaceuticals, b) veterinary medicines and c) nanoparticles (ToR g)

10.1 Update on human pharmaceuticals and veterinary medicines in the marine environment.

Presented by Kevin Thomas (Norway). An update on the current status regarding the occurrence and understanding the risks associated with human pharmaceuticals and veterinary medicines in the marine environment was provided by Norway. A review performed by Thomas & Langford in 2007 showed that very few data were available for pharmaceuticals, personal care products and veterinary medicines in the marine environment. Occurrence data are available from Norway, Germany and UK with the target compounds typically being detected at low ng/L concentrations if present. Screening reports published by the Norwegian Pollution Control Authority are available on www.sft.no and provide details of pharmaceutical concentrations in the coastal environment. In 2008 the veterinary medicines emamectin and oxilinic acid were detected at low ng/L concentrations in sediments collected close to fish farms on the west coast of Norway. In addition to pharmaceuticals and aquaculture medicines a number of sunscreens and insect repellents have also been detected in fjord waters used for recreational actitivites (Langford & Thomas, 2008). Assessing the risks associated with pharmaceuticals, personal care products and veterinary medicines to marine organisms is currently hampered by the lack of data outside of acute standard test data for freshwater lab studies for the majority of pharmaceuticals detected in the marine environment. Cunningham *et al*., (2006) report that for most pharmaceuticals acute effects are only seen below 0.1 mg/L for a few pharmaceuticals. Conventional acute/chronic ratios would therefore suggest that many of these compounds pose little or no threat to the marine environment. This should be viewed with caution since the specific effects of pharmaceuticals, personal care products may affect certain no-target organisms at low concentrations as with the contraceptive synthetic hormone 17α-ethynylestradiol (Cunningham *et al*., 2006).

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10.2 Nanoparticles

An update on the current status regarding the environmental effects of engineered nanoparticles on aquatic organisms was presented by Kevin Thomas (NO) with subsequent input from the UK. A summary of the possible release of silver nanoparticles from washing machines was presented which may result in large quantities of nano silver entering wastewater treatment works. In vitro studies using primary hepatocytes showed that nanosilver can induce cytotoxicity and that gold nanoparticles are potent inducers of reactive oxygen species (ROS). Studies with fish cells have also shown that cytotoxic and ROS induction associated with carbon nanotubes is associated to metal contaminants that are artefacts of the production process, Studies on marine species have focused on the blue mussel and Jim Readman of PML (UK) gave a quick update on the uptake and biological effects of fullerenes and carbon nanotubes. At present there are few data on the effects of nanoparticles on marine organisms. Methods to monitor the occurrence of nanoaprticles in the environment are currently under developed and are promising. Such methods include size exclusion chromatography and field flow fractionation coupled to inductively coupled plasma mass spectrometry. The characterisation of nanoparticle dispersions for ecotoxicological testing is essential in order to understand what the test organisms are being exposed to since nanoparticles can change their properties, including size (agglomeration), with changes in the chemistry of exposure medium. All of the reported effects to date are observed at exposure concentrations at low mg/L concentrations. It is recommended that the topic be reviewed again in 2-years time since this is a rapidly developing field of environmental science.

10.2.1 Emerging contaminants and nano-particles in the aquatic environment

Following on from the above Jim Readman (UK) presented information relating to emerging contaminants and nono-particles. Urban and industrial sewage effluents contain important quantities of emerging and priority pollutants (including pharmaceuticals, personal care products and phenolics). However, whilst many of these substances have broad usage, our lack of knowledge concerning quantities emitted into the environment, their environmental behaviour and long-term ecotoxicological impacts need to be addressed if we are to understand the environmental, economic and human health implications. In the environment, once these chemicals are discharged into receiving waters (rivers, estuaries and coastal waters), there are substantial analytical difficulties to detect and accurately quantify the compounds. These difficulties increase with dilution and matrix complexity.

The presentation described research undertaken at the Plymouth Marine Laboratory to investigate the input and environmental behaviour of pharmaceuticals, personal care products and endocrine disrupters in effluent, riverine, estuarine and coastal waters (including Ibuprofen, Naproxen, Ketoprofen, Diclofenac, Triclosan, synthetic musks, 2-Phenylphenol, 4-tert-Octylphenol and Bisphenol A). The presentation also described novel protocols to investigate bioavailable fractions of contaminants through the analysis of invertebrate biological fluids (Fillmann, *et al.*, 2004) and combined chemical and biological effects assessments of pollution (Galloway *et al.*, 2002,

and Lewis *et al.*, 2009). Recent experiences relating to shipping incidents were also summarised (Guitart *et al.*, 2008).

Finally, research relating to nano-particles (especially fullerenes and carbon nano-tubes), their uptake and biological effects on the marine mussel were described.

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11 Report on the progress with international workshops: ICON (NSHEALTH); BEAST programme (ToR h)

11.1 ICON (Integrated Assessment of Contaminant Impacts on the North Sea): an international workshop

The main objective of the ICON project is to assess the health of North Sea ecosystems with regards to anthropogenic contaminants and their biological effects by applying an integrated approach. The programme was initiated because data available indicate that there is reason for concern for contaminant effects in the North Sea ecosystems and secondly, there is a need to evaluate a framework (see Figure 11.1.1.) for environmental contaminant monitoring developed within the ICES/OSPAR WIKMON (now SGIMC – since 2009).

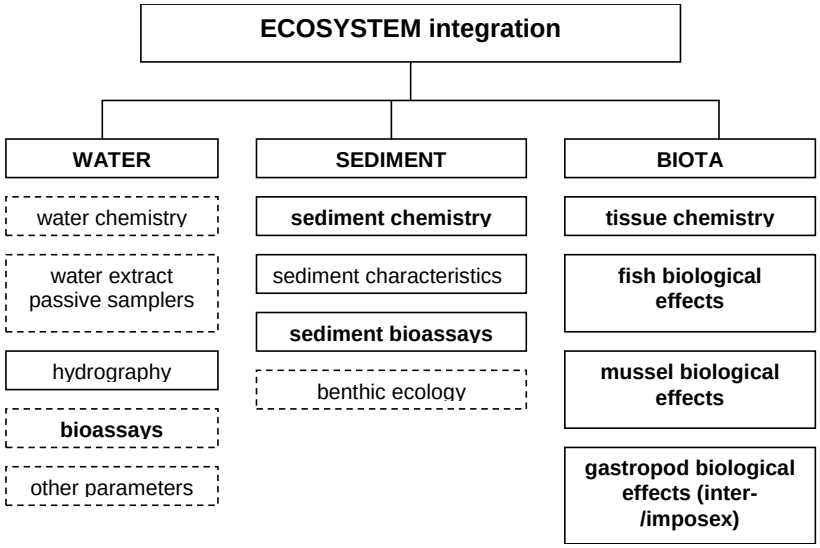

Figure 11.1.1. Overview of the components to be included in an integrated assessment of contaminant impacts in marine ecosystems; bold –part of OSPAR CEMP (Coordinated Environmental Monitoring Programme) (Thain *et al.*, 2008).

The ICON programme is based around a practical workshop involving chemical contaminant and biological monitoring in a range of North Sea habitats, two sites in Iceland and one in the Mediterranean), see Figure 12.1.

The selected species are dab(*Limanda limanda*), flounder (*Platichthys flesus*), cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) and the blue mussel (*Mytilus* sp.).

The programme involved offshore and coastal sampling and these are shown in Figure 11.1.2.

Figure 11.1.2. Locations included in the ICON workshop – approximate positions.

Field sampling was completed in the autumn of 2008 in all sites with the exception of Southern England, Firth of Forth and flounder and mussels in Iceland. These remaining sites will be sampled in the autumn of 2009. To date samples have been sent to all participating organisations for processing and assessment. A summary of the samples taken is given in Table 11.1.1.

Table 11.1.1. Summary of locations and samples taken for offshore, coastal and contaminant gradient sites.

COASTAL	SPANISH MED. COAST	MUSSELS AND SEDIMENT
	Wadden Sea	Flounder, mussels and sediment
	Southern England	Mussels and sediment (both in 2009)
	Iceland	Flounder and mussels (2009)
	Seine Bay	Dab, flounder, mussels and sediment
Offshore	German Bight	Dab and sediment
	Dogger Bank	Dab and sediment
	Off Firth of Forth	Dab haddock and sediment
	Ekofisk	Dab and sediment
	Iceland	Dab, haddock and sediment
	Baltic	Dab, flounder and sediment
Gradient	Forth of Forth	Flounder (2009), mussel (2009)

Research vessels and personnel from a range of European countries are participating in the programme:

- hydrography, nutrients: FRS, IMR, IFREMER, Deltares, vTI;
- sediment (passive samplers, bioassays, TIE): Cefas, FRS, Deltares, IEO, NIVA, IVM, Univ Le Havre;
- blue mussels (biomarkers, chemistry): IFREMER, Univ Bordeaux, IEO, Univ Vilnius, FRS, Cefas, Akvaplan-NIVA, IVM;
- fish (biomarkers, chemistry; dab, flounder, haddock): vTI, CSIC, Univ Oslo, Univ Bergen, IMR, IRIS, NIVA, Deltares, IFREMER, U Vilnius, vTI, AWI;
- SLU, Akvaplan-NIVA, Univ Bordeaux, Univ Le Havre.

A steering group consisting of Ketil Hylland (Norway; chair), Thomas Lang (Germany), Alistair McIntosh (UK), Jorundur Svavarsson (Iceland), John Thain (UK), Dick Vethaak (The Netherlands), Thierry Burgeot (France) and Concepción Martínez (Spain), meet regularly to co-ordinate the programme. This group met in CPH in January 2009.

ICON is ambitious in terms of the science and logistics involved, but not least in terms of the information it aims to extract about possible impacts of hazardous substances in the marine environment. Through the ICON programme it will be possible to evaluate and modify the integrated monitoring guideline that has been proposed through WKIMON/SGIMC prior to future implementation across the OSPAR maritime area. We will also glean more knowledge than ever before in any single activity about any impacts hazardous substances may have along our coasts and in the open waters of the North Sea. The ICON programme will be completed in 2009 and reported in 2010.

References

Thain, J. E., Vethaak, A. D., and Hylland, K. 2008. Contaminants in marine ecosystems: developing an integrated indicator framework using biological effects techniques. Journal Mar. Sci., 65: 1508–1514.

11.2 Review progress within the BONUS+ Programme BEAST project

The urgent need to develop biological effects monitoring to facilitate a reliable Ecosystem Health assessment has been indicated in the Baltic Sea Action Plan (BSAP). To contribute to the fulfilling of these aims, the BEAST project (Biological Effects of Anthropogenic Chemical Stress: Tools for the Assessment of Ecosystem Health) was launched under the new Baltic Sea BONUS+ Programme (2009–2011). Sixteen partners from all nine Baltic Sea countries are involved in the BEAST project (See Table 11.2.1.). The BEAST project has adopted the following approach:

1) research, application and evaluation of established and new biomarker techniques and other methodologies with special focus on the biological effects of selected important chemical compound groups in Baltic Sea key species in the laboratory and under field conditions;

2) generation of baseline data for regions in the Baltic Sea where few or no biological effects data exists and upgrading of data in other sub-regions, including identification of relevant target species for the highly variable Baltic Sea sub-regions;

3) determination of sub-regional reference/target/effect levels and collection of data for whole-region assessment of biological effects;

4) linking early effects and higher level effects by relating responses directly to changes in growth, reproductive output or energy utilization;

5) demonstrations of sub-regional Ecosystem Health assessments by the application of a set of techniques representing various biological processes at different levels of biological organisation in combination with contaminant measures in different sub-regions, including existing data from previous studies and monitoring activities;

6) testing and validation of integrated monitoring approaches, indices and expert systems in regard to their applicability for the Baltic Sea, taking into account the specific biotic and abiotic characteristics of the different sub-regions and different contaminant burden;

7) co-operation and co-ordination with the HELCOM Monitoring and Assessment Group (MONAS) to ensure linkage to the ongoing revision of HELCOM monitoring programmes and implementation of the BSAP.

The BEAST project consists of three thematic Work Packages (WP) and their planned activities and recent progress are described below. Research activities in the three WPs are organised under five sub-regional Tasks, i.e. field and experimental studies in the Gulf of Bothnia, G. of Finland, G. of Riga, G. of Gdansk and the Belt Sea.

WP1: Field studies and experiments in selected sub-regions of the Baltic Sea

WP1, the basic research part of the project, is targeted mainly at the validation of methods through experiments as well as testing of new and established approaches and methods and their combinations in different sub-regions. This integrated programme will be based on the requests and already existing practices of OSPAR, MEDPOL and AMAP and initiatives of ICES (WKIMON/SGIMC) to harmonise the

use of biological effects methods in monitoring and assessment programmes in European sea areas including transplantation of organisms and developing of novel techniques.

Among the major research activities carried out in 2009 is the GOF-IA (Integrated Multidisciplinary Assessment of the Ecosystem Health of the Gulf of Finland) joint research cruise of r/v Aranda (FI) and r/v Walther Herwig III (DE). The aim of the activity is to foster and execute the Ecosystem Health approach in the assessment of the state of the different sub-regions of the Baltic Sea. The collected new data will be combined, in feasible parts, with previous material. The BEAST GOF-IA will provide decisively new information especially of the biological effects of hazardous substances in this Baltic Sea subregion. Measurements and sampling will be carried out at 20–25 sampling stations in different parts of the Gulf of Finland from Finnish, Estonian and Russian waters. The study consists of measurements of several types of biological effects (mostly biomarkers) and diseases in local biota as well as contamination levels and studies on community structures. Field samplings in other study areas also start in 2009.

WP2: Application and validation of methods in monitoring and assessment in the Baltic Sea

The major focus of BEAST WP2 consists of testing and validation of monitoring and assessment methods and approaches and to provide guidelines and recommendations for integrated monitoring in the Baltic Sea. WP2 addresses questions related to the design of monitoring and assessment programmes in different sub-regions of the Baltic Sea and co-ordinates the application of methods in the sub-regions and the intercalibration of methodologies. A further tasks of WP2 includes the organisation of QA/QS activities. All BEAST partners are required to participate in the different tasks and to give their input especially in regard to developments in their territorial Baltic Sea sub-region(s).

Compilation of a handbook with guidelines & standard operating procedures (SOPs) is in progress and is, as a first step, based on guidelines developed as part of the EU funded BEEP project (2001–2004). It will further take into account guidelines established or in progress for integrated monitoring programmes in other sea areas, such as the north-eastern Atlantic (OSPAR CEMP) and the Mediterranean Sea (MEDPOL).

Recommendations for future Baltic Sea monitoring and assessment strategies in relation to hazardous substances can only be completely fulfilled once the data generated within BEAST have been analysed and assessed in an integrated way. However, basic work is already in progress and is based on experiences made in previous projects and national and international monitoring programmes.

A number of training workshops proposed for biological effects techniques applied in the BEAST project are planned, including

- fish diseases (incl. parasites, histopathology);
- biochemical biomarkers (oxidative stress and AChE);
- histochemical biomarkers (lysosomal stability, neutral lipid accumulation and macrophage activity);
- reproductive disorders in fish (eelpout), gastropods and amphipods;
- genotoxicity (micronuclei and Comet assay).

WP3: Developing tools for Ecosystem Health assessment in the Baltic Sea

Main objectives of WP3 are (1) developing and applying tools for a science-based assessment and management with regard to the impact of anthropogenic contaminants on the ecosystem health of the Baltic Sea; (2) integrated data analyses, assessment and development of tools, and integrated measures of pollution status to further develop science-based guidelines, assessment and management of the impact of anthropogenic contaminants on the Ecosystem Health of the Baltic Sea, and (3) compilation and analysis of already existing data as well as those generated in the BEAST project based on multivariate statistical analyses as well as the development, application and comparison of indices, models, and expert systems. 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.

A task of WP 3 concerns the set-up of the database needed to host all relevant data produced within the BEAST project but also other data/metadata already available concerning biological effects (e.g. BEEP data), data on chemical measurements and environmental variables measured (e.g. temperature, salinity, oxygen). The work on this database has started and a first structure should be available to all BEAST partners in June 2009.

Another WP3 activity during 2009 will be to compile and review information on existing approaches concerning integrated biomarker indices, expert systems and other assessment strategies which have been developed for other marine regions. In relation to this activity an expert workshop will be organised to be held in Jan/Feb 2010. During this workshop, BEAST partners and external experts will discuss and evaluate the compiled information, and will perform first practical exercises using Baltic Sea data. Another goal will be to give recommendations concerning the further integrated analyses of the BEAST data and other data available from other sources (e.g. BEEP).

Table 11.2.1. Institutes participating in the BEAST project, their responsibilities and specific expertise provided for the project.

PARTNER	TASKS	MAIN CONTRIBUTION TO THE PROJECT
SYKE (FI)	Co-ordination, WP1 Task 2 Co-leader	Oxidative stress biomarkers; other biomarkers; caging studies; integrated biomarker indices
ITM (SE)	WP1 Leader, WP1 Task 1 Leader	Reproductive disorders in crustaceans; biomarkers; hypoxia / contaminant effects
vTI/FOE (DE)	WP2 Leader, WP1 Task 2 Co-leader	Diseases, pathology and parasites in fish; PAH metabolites
NERI (DK)	WP3 Leader, WP1 Task 5 Leader	Reproductive disorders in invertebrates and fish; PAH metabolites; organotins; integrated indices; multivariate analyses; database
LHEI (LV)	WP1 Task 3 Leader	Sediment bioassays; selected biomarkers; haematological studies
SFI (PL)	WP1 Task 4 Leader	Fish diseases; caging studies; PAHs
AtlantNIRO (RU)	Partner	Fish diseases and parasites; histopathology

AWI (DE)	Partner	Integrated approaches; core biomarkers; novel methods
EULS (EE)	Partner	Fish olfaction and behaviour; PAH metabolites
FGFRI (FI)	Partner	PAH metabolites; oxidative stress biomarkers
IAE (DE)	Partner	Reproductive disorders; endocrine disruption; biomarkers; histopathology
IB UL (LV)	Partner	Oxidative stress (macrophytes); heavy metals
IEVU (LT)	Partner	Genotoxic and cytotoxic effects; oil-degrading bacteria in digestive tract
IOW (DE)	Partner	POPs in sediments and biota
SRCES RAS (RU)	Partner	Cardiac activity and shell movements in invertebrates
ZIN RAS (RU)	Partner	Growth, survival, fecundity, feeding and reproductive success of invertebrates

12 Evaluate the assessment of imposex (in dog whelks) data carried out by MON and receive any reports of new data / monitoring activities /Strategies (ToR h)

At the time of the WGBEC meeting the MON assessment of on imposex had been carried out but no report was available. At the meeting some draft components of the report on imposex was obtained in order for WGBEC to gain information on how the assessment was proceeding. As such no time was allocated for an evaluation and the group felt that this should be conducted by a full evaluation of interested parties when the full report becomes available. The Chair agreed to circulate the report when it is published.

Some initial comments by group members were:

In general the group agreed with the colour scheme used in the assessment but it was noted that there were no data in the "F class".

How extensive were the data sets used in the assessment as some group members thought there was missing data (i.e. from their own data sets). Was all data in the ICES database used?

AQC is important for cross country assessment but it was unclear how this was addressed in the assessment.

How will the assessment be made in the QSR since it will need to made on a regional basis?

The scale of the maps was very small and as such detail and definition was lost.

The OSPAR imposex guideline has defined types of:- harbour, hot-spot and general monitoring. It was unclear how this was taken account of in the assessment and report, or was it?

For the sediment data there were no results for the UK, but data is known to exist and been submitted to ICES database. Also for sediments was all the data AQCd?

For the sediment data assessment there were six classes listed when normally for chemical assessments three classes are used so the rationale for this needs to be explained.

Some clarification needs to be provided on how a class is assessed for status when it may have changed over the course of time between measurements.

Was there any assessment carried out for species of gastropods other than dog-whelks?

Overall the group felt that excellent progress had been made with the assessment and looked forward to seeing the full and official report when it becomes available.

Action:

The Chair to circulate the full imposex report to group members when it is available from OSPAR MON.

13 Any other business; a) Effects of contaminants on eels; b) provide background documents and draft assessment criteria on – Ache , mussel histology, micronucleus assay, COMET assay , MT, ALAD and intersex in fish; c) Biomarkers and risk assessment.

13.1 Contaminants in eels and their biological effects

The Chair of WGBEC had noted the recommendations relating to contaminants in eels in the Report of the 2007 session of the Joint EIFAC/ICES Working Group on Eels (Bordeaux, France, 3–7 September 2007) and the WGEEL report of 2008. WGBEC, as a group, felt that it should make the following comments and forward these to WGEEL.

Eels are unusual when compared to other fish in that their fat content is an order of magnitude higher. This has important implications relating to both pollutant bioconcentration and effects. Levels of lipophillic contaminants generally reflect the elevated fat content, also being one order of magnitude higher than in other fish. The elevated lipid content is important as an energy reserve and is regulated through steroidal/endocrine systems. Other characteristics that render environmental evaluation of eels difficult, include identification of gender and that eels only reproduce once in each generation. The latter impairs contaminant losses through gonadal releases. From recent research, a trend is clearly appearing indicating both a reduction in the eel populations, together with a decline in overall lipid content of individuals.

Whilst the Report of the 2007 session of the Joint EIFAC/ICES Working Group on Eels thoroughly reviews much of the available literature, we believe that some areas would benefit from further scrutiny:

- contaminants studied should be diversified (and include emerging contaminants). Focus of biological effects should include skewing of the sex ratios, reduction in lipid content or disruption of endocrine systems.
- clarification is required, of the historical changes in lipid contents and compositions. Are the analytical techniques comparable and quality assured?
- the potential impact for climate change to alter metabolism and affect lipid content and pathogenic/parasitic pressures needs to be assessed.
- the potential for contaminants that may affect the genetic pathway that regulates biochemical pathways in lipid metabolism should be evaluated.

- It would be useful to investigate how the eel decline maps against performance of other species e.g. cod, dab, flounder, plaice and eel pout.

Recommendations

- Inclusion of biological effects measurements (e.g. Guimaraes *et al*., 2009) in the data base.
- Attempt compatibility between databases holding pertinent information (both of contaminants, condition factors and biological effects).
- In Europe National Monitoring authorities should be encouraged to maintain existing chemical contaminant monitoring programmes for eels or where they do not exist to consider initiating a monitoring programme. In addition the monitoring programme should include appropriate biological effect techniques.

Reference

Guimarúes, L., Gravato,C., Santos, J., Monteiro, L.S., and Guilhermino, L. 2009. Yellow eel (*Anguilla anguilla*) development in NW portuguese estuaries with different contamination levels. Ecotoxicology, 18: 4p 385–402.

13.2 Provide background documents and draft assessment criteria on - Ache , mussel histology, micronucleus assay, COMET assay , MT, ALAD and intersex in fish;

WGBEC noted in the draft SGIMC report that it would like to task WGBEC with providing background documents and draft assessment criteria on - Ache , mussel histology, micronucleus assay, COMET assay , MT, ALAD and intersex in fish. Clearly this could not be fulfilled without prior notice and with an adequate time frame for preparation. However, comments on AChE were provided by Thierry Burgeot (FR):

WGBEC are willing to assist in the provision of these documents and the Chair will contact SGIMC to clarify what is needed and in what time frame.

The laboratory of ecotoxicology of Ifremer in Nantes will prepare a review on the enzymatic biomarker acethylcholinesterase (AChE) for the background document on biological effects monitoring techniques: assessment and monitoring series (2007). AChE enzymatic activity is a biomarker of neurotoxicity considered for inclusion in the CEMP after his validation in the ICON programme . AChE can be also studied as a model for an integration of enzymatic biomarkers in the methodological concept WFD (Water Framework Directive) and MSD (Marine strategy directive). Biomarkers are not included as ecological quality elements in the monitoring for the WFD, but there is a synergism between the CEMP and the WFD approaches as evocated in the background document on biological effects monitoring techniques: assessment and monitoring series (2007). Indeed, some initiatives as already be initiated in order to study the synergism between the CEMP and WFD with bioassays in Netherland and Spain but not with biomarkers.

As the bioassays, the biomarkers could be able to contribute to the pressure and risk assessment process which is designed to identify water bodies at risk of failing to achieve good ecological status during the classification exercise. It is a great challenge for the biomarker integration in the future MSD and it is pertinent to test now the potential application of the biomarker as a “metric” for the determination of the classification of Ecological Status according to the WFD CIS guidance (WFD methodo-

logical concept recommended by the WFD CIS Guidance document n°13: Overall Approach to the Classification of Ecological Status and Ecological Potential). The laboratory of ecotoxicology in Ifremer, will test the AChE percentile 90 as a metric parameter in fish and mussel in order to define and ecological quality scale according to the WFD CIS Guidance document n°13.

13.3 Biomarkers and Risk Assessment

Linking Biomarkers to Risk Assessments, presented by Steinar Sanni (Norway)

To obtain coherency between prognostic and diagnostic assessments it is beneficial to be able to link biomarkers to risk assessment procedures. ICES WGBEC is observing the progression of an on-going work funded by the Research Council of Norway ('Biomarker Bridges') which aims to incorporate biomarkers in risk and impact assessment models. The study includes biomarkers that are in regular use for monitoring potential effects of produced water discharged in North Sea oil fields in caged mussels and cod (Sundt *et al*., 2006). This coincides with several of the ICES recommended methods. The risk and impact system tool included in the study is the DREAM / EIF model developed to predict effects of produced water by the Norwegian oil and gas operators. The concept will also have applicability to other types of discharges.

In the two previous ICES WGBEC meetings the rationale for the concept and its applicability in particular regions (e.g. Arctic seas) and in relation to emerging pollutants were presented. The concept uses Species Sensitivity Distributions based on biomarkers for its development (Smit *et al*., 2009 *in press*). The progress in setting up the 'Biomarker Bridges' in the risk model and testing them against field data was presented in the 2009 working group meeting. Similar comparisons are planned with four available data sets. The concept is based on monitoring in more species than the two used in the North Sea produced water monitoring. Therefore, results based on multi-species caging will be used for further validation. ICES WGBEC will be updated on the further progression.

References

Smit, M. G., Bechmann, R. K., Hendriks, A. J., Skadsheim, A., Larsen, B. K. and Sanni, S. 2009 *in press*. Relating biomarkers to whole organism effects using species sensitivity distributions: a pilot study for marine species exposed to oil. Environmental Toxicology and Chemistry: In Press. DOI: 10.1897/08-464.1

Sundt, R. C., Ruus, A., Grung, M., Pampanin, D., Barsiene, J., and Skarphedinsdottir, H. 2006. Water Column Monitoring 2006. Summary report. AM 2006/013. 85 pp.

14 Recommendations and action list

Recommendations

1 (Agenda item 4).

a) For publication in the TIMES series that the proposed publication of a method document for MDR/MXR be withdrawn (Draft resolution 2006/1/MHC09).

b) That ICES update the deadlines for draft resolutions for publication of biological effect methods in the TIMES series in line with those indicated in Table 4.1.

c) WGBEC request that ICES approve the draft resolutions for the publication of TIMES manuscripts concerning reproductive success in eelpout, sea urchin embryo bioassay and alkylphenol bile metabolites. These manuscript can be prepared for review by October 2010 (reproductive success in eelpout), and Dec 2009 (urchin bioassay and alkylphenol metabolites).

2 (Agenda item 5).

a) WGBEC have responded to the queries from the ICES data base on 5 questions.

WGBEC would recommend that these response be adopted by the data centre

Responses concerned:

- Reporting of the parameter CYP!A
- A mock example of a file for reporting data to the ICEWS database
- Provision of new ICES codes for new techniques
- Reporting of biological effect QA data to the ICES database
- Comment on data submitted to the ICES database

3 (Agenda item 6.1)

In response to the SGIMC draft report a number of recommendations were made:

a) WGBEC noted that several of its tasks have direct relevance to SGIMC and will require intensive ongoing interaction between both groups. Members of WGBEC currently involved in intersessional work on developing ACs for EROD and other PAH-specific biomarkers should be pro-active and be made aware of the importance to deliver their tasks in time before the Aberdeen Workshop on ACs for EROD and other PAH-specific biomarkers, and to be encouraged to attend the Workshop.

These include the following tasks and actions:

- to contact the appropriate task person (K. Hylland) to complete intersessional work on PAH metabolites as defined by WKIMON4 (task D. Vethaak)
- to contact the appropiate tasks person (?) to complete intersessional work on DNA adducts as define by WKIMON4 (task John Thain)
- It was noted that work at FRS Aberdeen was in progress to further develop the temperature / seasonality EROD model that should enable EROD AC development at the meeting. WGBEC members should also bring or submit available data sets on EROD, PAH metabolites, DNA adducts, and DR CALUX to the Aberdeen Workshop for the development of assessment criteria (all WG members)

b) WG members should be strongly encouraged to complete intersessional work in 2009 related to the development of AC for VTG, bioassays and other core biological effects methods as outlined by SGIMC 2009 report (D. Vethaak and J. Thain to identify task leaders and progress).

c) The membership of SGIMC should be defined and the meeting agenda/details should be circulated in good time. It is recommended that invitations to the Aberdeen Study Group meeting are extended to WGBEC members.

d) ICES SCICOM/ ACOM are requested to define more clearly the tasks for WGBEC in respect to the development of biological effects monitoring and the development of assessment criteria and its role in the integrated chemical biological monitoring strat-

egy developed by SGICM and OSPAR. Currently there is an overlap in tasks between groups and no clear short-term mechanism for interaction.

4. (Agenda Item 6.8.)

In respect of BEQUALM WGBEC would like to recommend that following the success of the EROD inter comparison exercise that it would be very beneficial to have a wash-up workshop to discuss lessons learned and to refine the AQC procedures.

5. (Agenda Item 6.9.)

In order to take forward the BEQUALM fish disease intercalibrations WGBEC recommends that Cefas explore the potential for separating external fish disease and liver histopathology components and report back to WGBEC and WGDPMO.

6. (Agenda Item 8.)

WGBEC recommends that the draft technical annex for mussel integrated chemical biological effect monitoring prepared at WGBEC is forwarded to ICES/OSPAR SGIMC for further development in line with the development and advancement of the integrated monitoring and assessment approach, (currently the responsibility of SGIMC).

7. (Agenda Item 9.2.)

WGBEC to request ICES to consider publishing the full GC-MS method for bile AP analysis and for the method to be intercalibrated with interested parties within WGBEC and beyond.

8. (Agenda Item 9.4.)

Following the review of intersex in crustacean the following recommendations were made:

- Firstly, field based monitoring studies are required to assess whether any developmental abnormalities and/or abnormal sex ratios are occurring in wild populations of crustaceans. These monitoring studies should take into account whether the species is commercially harvested and if so whether sexes are differentially selected.
- Based on arguments that is physiologically difficult to feminise a crustacean, both laboratory and field studies should concentrate less on feminisation markers (e.g. VTG) and those associated with de-masculinisation and 'other' endocrine associated effects (e.g. moulting).
- Many crustaceans have environmental sex determination which may make them susceptible to changes in their sex ratios or developmental abnormities such as gynandromorphism.
- Sexual gynandromorphism and intersex forms (such as ovitestes) should be carefully and separately recorded as they may originate via different developmental aberrations.
- Studies should be alert to the potential of parasites to confound observations. Currently, studies have shown parasites can increase (Microsporidia in amphipods) and decrease (Rhizocephala in decapods) the incidence of intersexuality under polluted conditions.
- Greater emphasis should be made on understanding the genetics of sex determination and sexual differentiation of crustaceans, along with a better understanding of their general endocrinology.

9. (Agenda Item 9.5.)

In respect of taking forward the use of mussel histopathology as a biological effect tool WGBEC recommended that:-

The grading indices that have been developed for the assessment of reproductive status (Seed) and adipogranular (ADG) tissue require further development to ensure that appropriate assessment tools are established in the future. It is recommended that a central steering group under the BEQUALM framework develop this further, possibly through a specific workshop.

10. (Agenda Item 13.1)

WGBEC recommends that to assist with the integration of contaminant-related biological effect responses into the eel population investigations, as outlined in WGEEL that:

- Biological effects measurements be included in the data base.
- An attempt be made to achieve compatibility between databases holding pertinent information (both of contaminants, condition factors and biological effects).
- In Europe National Monitoring authorities should be encouraged to maintain existing chemical contaminant monitoring programmes for eels or where they do not exist to consider initiating a monitoring programme. In addition the monitoring programme should include appropriate biological effect techniques.

Actions

1. (Agenda Item 5)

WGBEC members are urged to submit their biological effects monitoring data (including legacy data) to the ICES database to enable future assessments to be made.

2. (Agenda Item 6.8.)

WGBEC recommended that following the success of the EROD inter comparison that it would be very beneficial to have a wash-up workshop to discuss lessons learned and to refine the AQC procedures. Action; Kevin Thomas (Norway) to consider the feasibility of holding a workshop.

3. (Agenda Item 6.10.)

Some WGBEC members expressed an interest in participating in the MEDPOL biological effect intercalibration exercises. Action; John Thain (UK) agreed to pursue the feasibility of this with Aldo Viarengo (IT).

4. (Agenda item 9.6.)

In previous years, although not recently, WGBEC has been given TOR relating to the effects of contaminants on whales and seabirds and the group felt that it should review the current state of knowledge in this area at next year's meeting, particularly with a view to a more integrated approach (ie all components and all levels) to contaminants, and their effects in marine ecosystems.

5. (Agenda Item 12.)

The Chair agreed to circulate the full MON imposex assessment report to group members when it is available from OSPAR MON.

6. (Agenda Item 13.2)

John Thain to contact SGIMC in respect of the provision of Background Documents and to clarify what is needed and in what time frame.

15 Adoption of the report and closure of the meeting

Draft resolutions were considered and provided in Annex 6.

Draft text of the meeting report was prepared during the meeting and at the close some text was still outstanding, to be submitted post the meeting via the WGBEC SharePoint. Text that was available was commented on at the meeting. The Chair agreed to incorporate the remaining text into the final report.

The Chair thanked members of the WG for their contributions.

The group considered a venue for the 2010 meeting. Michelle Giltrap (Ireland) offered to host the meeting at Trinity College, Dublin, in Ireland.

The Chair closed the meeting at 15:00 hrs on 20 March 2009.

Annex 1 List of participants

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Annex 2 – WGBEC Terms of Reference for 2009

2008/2/MHC04 The **Working Group on Biological Effects of Contaminants** [WGBEC] (Chair: John Thain, CEFAS, UK) will meet in Weymouth in UK from 16–20 March 2009 to:

a) review progress of the publication and electronic dissemination of four manuscripts on biological effects techniques in the ICES TIMES series;

b) assess the amount of biological effects data submitted to the new ICES database and answer queries / requests from the ICES Data Centre; and to consider codes for techniques now in the integrated approach – scheme

c) evaluate national /international monitoring activities, including harmonisation initiatives / integrated assessment / and application of biological effect techniques within OPSAR / MEDPOL / WFD / HELCOM / EU MSD.

d) assess biological effects data with new assessment criteria: for VTG, Neutral Red and bioassays.

e) review or (if not available to meeting) produce a Technical Annex for OSPAR mussel integrated strategy

f) review new and promising biological effects techniques: gene array; alkylphenol bile metabolites; bile estrogenicity; ovotestis; intersex in gastropods and crustaceans; histopathology in mussels plus mussel disease index; and passive samplers

g) report on emerging and novel contaminants and their biological effects with particular reference to a) pharmaceuticals, b) veterinary medicines and c) nanoparticles.

h) report on the progress of the ICON (NSHEALTH) programme

i) evaluate the assessment of imposex (in dog whelks) data carried out by MON and receive any reports of new data / monitoring activities / strategies.

WGBEC will report by 30 April 2009 for the attention of the SciCom and ACOM.

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.
Scientific justification and relation to action plan:	a) It is important for WGBEC to keep track of publication progress with biological effects methods it has sponsored. Protocols are needed for national and international programmes as well as the OSPAR programmes. b) Biological effects data is increasingly being entered into the ICES database and WGBEC is encouraging this and monitors this activity and assists with answering queries from the ICES Data Centre.. c) WGBEC needs to continue to explore and develop the links (OSPAR / MEDPOL / WFD / HELCOM / EU FWM) already made during its 2007 and 2008 meetings in respect harmonisation of AQC, application of techniques and interpretation of data for integrated assessment purposes d) As assessment criteria are being developed for biological effect techniques it is important that they are evaluated and VTG, bioassays

	and NRR are sufficiently developed for such an assessment. e) A mussel integrated approach has been put forward as a component in the OSPAR JAMP chemical – biological effect integrated approach; a Technical Annex is required for this and WGBEC will review this if it is written during the year or will be happy to provide a draft document to this effect. f) WGBEC needs to review the current status of development and application of these techniques in order that it can advise where a method / approach sits in the ICES list of "recommended techniques" g) As information on emerging contaminants becomes available it is important to be in a position to advise and assess their impact on biological systems and the environment and to advise on suitable monitoring techniques. h) The ICON demonstration programme was launched in March 2007 and underpins the integrated chemical – biological effects approach advocated by OSPAR WKIMON. WGBEC needs to monitor and evaluate this activity. i) WGBEC have contributed to the development of imposex (in dog whelks) as a monitoring tool and have liaised with WGSAEM on the assessment of the data. WGBEC feel that it is important to continue to be involved with this process and keep a watching brief on imposex monitoring in member states.
Resource requirements:	The main input to this group is from National experts. Each attendee is self-funded from their own / organisation / institute resources.
Participants:	The Group is normally attended by ca. 16 members and guests.
Secretariat facilities:	None required.
Financial:	No financial implications.
Linkages to advisory committees:	ACOM
Linkages to other committees or groups:	SciCOM, WGSAEM, MCWG, WGMS and WGPDMO.
Linkages to other organizations:	None identified.

Annex 3: Draft agenda

The **Working Group on Biological Effects of Contaminants** [WGBEC]

Cefas Laboratory, Weymouth UK, 16–20 March 2009

DRAFT AGENDA

1) Opening of the meeting;

2) Adoption of the agenda;

3) Appointment of rapporteurs;

4) Review progress of the publication and electronic dissemination of four manuscripts on biological effects techniques in the ICES TIMES series (To-Ra);

5) Assess the amount of biological effects data submitted to the new ICES database and answer queries / requests from the ICES Data Centre; and to consider codes for techniques now in the integrated approach – scheme ToR b);

6) Evaluate national /international monitoring activities, including harmonisation initiatives / integrated assessment / and application of biological effect techniques within OPSAR / MEDPOL / WFD / HELCOM /EU MSD (ToR c);

7) Assess biological effects data with new assessment criteria: for VTG, Neutral Red and bioassays (ToR d);

8) Review or (if not available to meeting) produce a Technical Annex for OSPAR mussel integrated strategy (ToR e);

9) Review new and promising biological effects techniques: gene array;alkylphenol bile metabolites; bile estrogenicity; ovotestis; intersex in gastropods and crustaceans; histopathology in mussels plus mussel disease index; and passive samplers (ToR f);

10) Report on emerging and novel contaminants and their biological effects with particular reference to a) pharmaceuticals, b) veterinary medicines and c) nanoparticles (ToR g);

11) Report on the progress with international workshops: ICON (NSHEALTH); BEAST programme (ToR h);

12) Evaluate the assessment of imposex (in dog whelks) data carried out by MON and receive any reports of new data / monitoring activities /Strategies (ToR h)

13) Any other business;

- 13.1) Effects of contaminants on eels
- 13.2) provide background documents and draft assessment criteria on - Ache , mussel histology, micronucleus assay, COMET assay , MT, ALAD and intersex in fish.
- 13.3) Biomarkers / biosensors and risk assessment.

14) Recommendations and action list;

15) Adoption of the report and closure of the meeting.

Annex 4: Meeting Timetable and contributors.

APPROXIMATE TIMETABLE				
Lunch times 12:30 each day for one hour				
Coffe Breaks 10:45				
Tea Breaks 15:15				

DATE	APPROX. TIME	AGENDA ITEM	ISSUE	Lead Contributors
Monday 16 March	09:30	1	Introduction by Chair, housekeeping issues, tour de table, etc	John Thain
		2	Adoption of agenda, tabling of documents.	John Thain
		3	Appointment of rapporteurs.	John Thain
		6	International Monitoring Activities, harmonisation, MSD, SGIMC, etc	
			Integrated assessment and applications	
			SGIMC – Dick – report is Doc 6.	Dick Wethaak
			Study Group for the Development of Integrated Monitoring and Assessment of Ecosystem Health in the Baltic Sea (SGEH) Kari	Kari Lethonen
			MSD activities – Brest meeting – Thomas Maes presentation + UK CP2	Thomas Maes
	12:45		Lunch	
	13:45	6	International Monitoring activities contd.	
			International monitoring activities:	

			Ireland – Michelle Giltrap	Michelle Giltrap
			Spain	Concepción Martínez
			Netherlands	Dick Vethaak
			Danish monitoring activities – Jakob - (NOVANA) on contaminants and biological effects and integration of both in Denmark	Jakob Strand
			Baltic BALCOFISH	Jakob Strand
			Norway	Kevin Thomas
			France - Thierry Tues - Weds	Thierry Burgeot
			Spain – Ricardo ….Tuesday - Weds	Ricardo Beiras
	17–18:00		Close of business.	
			Harmonisation	
			Bequalm - Fish disease – Sediment bioassay – John Thain	John Bignell
			EROD	Kevin Thomas
			EROD assessment criteria	
			MEDPOL – intercalibration – invitation from Aldo – Cefas + Concha	John Thain
	16:00	4	Review progress with publication of TIME series documents	Mat Gubbins
	12:45		Lunch	Concepción Martínez
Tuesday 17 March	09:15	8	Produce technical annex for OSPAR mussel integrated strategy	John Thain
			Sub group activity with review of TIMES doc + ms	Matt Gubbins

		5	Respond to requests from ICES data centre	Matt Gubbins John Bignell
	13:45	7	Assess biological effects data with new assessment criteria	John Thain
		13 b	b) JAMP background documents and assessment criteria.	Thierry Burgeot
			……..Provide background documents and draft assessment criteria on - Ache (Thierry + TIMES doc) , mussel histology (TIMESin prep + presentation by John B), micronucleus assay (Brett to comment), COMET assay (Brett to comment), MT (have Bgd doc but no Ass Crit), ALAD (have Bgd doc but no Assess Crit) and intersex in fish (have Bgd doc but no Assess Crit). See OSPAR Doc of compilation of Bgds on Share)	
	17–18:00		Close of business.	
Wednesday 18 March	09:15	9	Review of new and promising biological effect techniques	
			Intersex in gastropods	Jakob Strand
			Intersex in crustaceans	Alex Ford
			Histopathology in mussels and disease index	John Bignell
			Impact of contamints on mammals	Veronica Hellwig
	12:45		Lunch	
			Alkylphenol metabolites	Johnny Beyer
			Gene arrays	Tim Williams / Brett Lyons / Steve George
			Biomarkers in Marine Mammals	Veronika Hellwig
			Passive samplers	Kevin Thomas
		13 c	Real time biomarkers / biosesors and risk assessment	Steiner Sanni
	17–18:00		Close of business.	

		10	Report on emerging and novel contaminants:	
			Pharmaceuticals	Kevin Thomas / Jim Readman
			Veterinary medicines	Kevin Thomas / Jim Readman
			Nanoparticles	Kevin Thomas / Jim Readman
		13 a	a) Effects on contaminants on eels	Jakob Strand / Dick Vethaak
	12:45		Lunch	
Thursday 19 March	09:15	12	Evaluate the assessment of imposex in dogwhelks	Matt Gubbins / John Thain
		11	Report on progress with ICON	John Thain
			Report on progress with BEAST programme	Kari Lehtonen
	17/18:00		Close of business.	
Friday 20 March	09:00	14	Recommendations and action list.	John Thain
		15	Adoption of the report and closure of the meeting.	John Thain
	12:30		Lunch	
	15:00		Closure of the meeting.	John Thain

Annex 5: Recommendations for WGBEC 2010 meeting

Category 1

Recommendation for the publication of ICES TIMES papers. Permission is sought to publish:

1) Eelpout (Jakob Strand)

2) Sea urchin embryo bioassay (Ricardo Beiras)

3) Alkylphenol bile metabolites (Johnny Beyer)

These manuscript can be prepared for review by October 2010 (reproductive success in eelpout), and Dec 2009 (urchin bioassay and alkylphenol metabolites).

WGBEC would like to proceed with these topics alongside completing existing tasks as reviewed in report.

Justification

Protocols are needed for national and international programmes as well as the OSPAR programmes.

Category 2 WGBEC Draft Resolution for 2010

2009/2/SSGHIE00 The **Working Group on Biological Effects of Contaminants** (WGBEC), chaired by John Thain, CEFAS, UK, will meet in Dublin, Ireland, from 4–8 January 2009:

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

b) Assess the amount of biological effects data submitted to the ICES database and answer queries / requests from the ICES Data Centre; and to consider codes for techniques now in the integrated approach – scheme;

c) Review progress with national /international monitoring activities; to include / integrated assessment / and application of biological effect techniques within OPSAR / MEDPOL / WFD / HELCOM / EU MSD;

d) Review progress with AQC procedures for biological effect methods and include harmonisation activities within OSPAR, Baltic and MEDPOL maritime areas;

e) In close cooperation with ICES / OSPAR SGIMC conduct intersessional work for review at 2010 meeting. This could be a considerable amount of work and needs clarification; **This could be a considerable amount of work and needs clarification.**

f) Review ICES WGBEC list of recommended biological effects methods for monitoring purposes and define how this fits in for both OSPAR and EU MSFD purposes;

g) Continue to review of emerging and novel contaminants as they arise and specifically nanoparticles;

h) Review progress with the ICON (NSHEALTH) and Baltic BEAST programme;

i) Review current knowledge and research on contaminants in eel and associated biological effects;

j) Review of contaminants through the food chain to include birds and mammals.

WGBEC will report by 15 February 2010 to the attention of SCICOM and ACOM.

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.
Scientific justification and relation to action plan:	a) It is important for WGBEC to keep track of publication progress with biological effects methods it has sponsored. Protocols are needed for national and international programmes as well as the OSPAR programmes. b) Biological effects data is increasingly being entered into the ICES database and WGBEC is encouraging this and monitors this activity. In addition as more data is being submitted technical queries arise and WGBEC can assist with answering queries from the ICES Data Centre. c) WGBEC has found it of value to discuss, feedback and support national monitoring programmes across the maritime areas and this is a valuable opportunity to improve and harmonise programme designs and assessment of data (e.g. OSPAR / MEDPOL / WFD / HELCOM / EU FWM); d) 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. e) ICES / OSPAR SGIMC have a heavy work programme and WGBEC have noted that this Study Group have already identified tasks for ICES WGBEC, both intersessionally and at the WGBEC meeting. These tasks are not insignificant and WGBEC are willing to provide the support and expertise for taking this important work forward; f) WGBEC last reviewed the list of biological effect recommended and promising monitoring techniques in 2007. There has been considerable developments over three years and WGBEC feels it is necessary to conduct a major review, including the rationale for recommending techniques and how they fit in with SGIMC and EUMSFD activities; g) As information on emerging contaminants becomes available it is important to be in a position to advise and assess their impact on biological systems and the environment and to advise on suitable monitoring techniques, and nanoparticles have been identified as a fast moving research area. h) The ICON demonstration programme and the Baltic Beast programme underpins the integrated chemical – biological effects approach advocated by OSPAR and in the Baltic. WGBEC needs to monitor and evaluate these activities. i) It has been identified (see ICES WGEEL reports) that contaminants and associated biological effects may be contributing to the demise in eel populations across Europe and WGBEC will review what research there is available to support this suggestion. J) A GES descriptor in the EU MSFD involves aspects of food webs (i.e. No 4; All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity). The role of contaminats may be important in this regard.
Resource requirements:	The main input to this group is from National experts. Each attendee is self-funded from their own / organisation / institute resources.
Participants:	The Group is normally attended by ca. 16 members and guests.

Secretariat facilities:	None required.
Financial:	No financial implications.
Linkages to advisory committees:	ACOM
Linkages to other committees or groups:	There are linkages with WGSAEM, MCWG, WGMS and WGPDMO.
Linkages to other organizations:	None identified.