ICES WGBEC Report 2007

ICES Marine Habitat Committee
CM 2007/MHC:03
Ref. ACME

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

19–23 March 2007

Alessandria, Italy

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

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

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

Recommended format for purposes of citation:

ICES. 2007. Report of the Working Group on Biological Effects of Contaminants (WGBEC), 19-23 March 2007, Alessandria, Italy. CM 2007/MHC:03. 129 pp.

For permission to reproduce material from this publication, please apply to the General Secretary.

The document is a report of an Expert Group under the auspices of the International Council for the Exploration of the Sea and does not necessarily represent the views of the Council.

© 2007 International Council for the Exploration of the Sea

Contents

Executive Summary

The Working Group on the Biological Effects of Contaminants [WGBEC] (Chair, John Thain, UK) met at the University of Alessandria, Italy, from the 19–23 March 2007. A summary of the key outcomes in respect of the Terms of Reference is described below.

- The outcomes of several activities (e.g. OSPAR WKIMON) relating to the use and application of biological effects techniques in monitoring programmes and assessment of results were reviewed and progressed.
- Advice was given in support of requests from OSPAR relating to the adequacy of OECD protocols for measuring endocrine disruption, reviewing background biological effects documents and how changes in the distribution of marine species may be affected by contaminants.
- The use of biological effects methods for OSPAR, MEDPOL, HELCOM and WFD monitoring purposes were reviewed and areas of commonality for harmonisation explored

Evaluate the report from WKIMON third workshop and the intersessional work undertaken by WGBEC members

Several members of WGBEC had attended the WKIMON III meeting in Copenhagen in January 2007 and contributed to the preparation of background documents and provided data sets for assessment purposes. The group were pleased with the initial progress being made with assessment criteria but emphasised that the values were only preliminary and there was still much work to do in finalising and validating these values and in developing tools for conducting chemical-biological effects integrated assessment. Progress with an international workshop (ICON) to assess North Sea Health and to include a programme to demonstrate the WKIMON "integrated approach was presented and well received by WGBEC.

Evaluate documents prepared intersessionally for "background" biological effects responses

OSPAR/ICES WKIMON had produced a number of "background" documents on biological effects responses, and these included; EROD, PAH-bile metabolites, DNA adducts, fish diseases including histopathology (covered by ICES WGDPMO), VTG, ALA-D, metallothionein, reproductive success in fish, water bioassays, sediment bioassays, lysosomal stability and Scope For Growth in mussels. The group felt that these were complete except that minor amendments were required to the document on Scope for Growth.

Explore the possibilities to organize a joint OSPAR-MEDPOL Workshop on the use and application of integrated chemical-biological methods

Presentations were given on how biological effects methods are applied for OSPAR, MEDPOL, HELCOM and WFD monitoring purposes. Areas of commonality were identified and it was agreed that it was important to pursue these over the coming year and included; linking in with the activities of the ICON demonstration programme and BSRP workshop, looking for a platform to harmonise methodology, AQC and intercalibration exercises and to organise shared workshops and exchange knowledge.

Review the "recommended list" of biological effect techniques

The WGBEC discussed and amended the 'promising' and 'recommended' monitoring techniques that had been last updated in 2004. The objective of preparing this list was to provide information on the status of methods to assess contaminant effects in marine ecosystems and to ensure that the methods are up-to-date and aligned with the current developments within the JAMP and the ICON demonstration programme.

Receive and evaluate reports prepared intersessionally by WGBEC on oxidative stress, cellular energy allocation and aromatase

Reports prepared intersessionally on cellular energy allocation and aromatase were evaluated. The current methodology on cellular energy allocation shows good promise. For aromatase activity/inhibition biomarkers in fish and invertebrate, although proven useful in experimental and mechanistic studies, this is not the case for application in biological effect monitoring. Therefore WGBEC recommended that aromatase activity should be removed from the list of promising methods. An evaluation of oxidative stress was deferred to 2008.

Report on progress with the assessment of imposex data in the ICES database

This item was a continuation of work initiated between WGBEC and WGSAEM at a joint session at the 2006 meeting. Recent intersessional progress made with statistical assessment of imposex data in the ICES database was presented. The group welcomed the recent improvements to the assessment methodology and was encouraged by the number of time series available in the ICES database for assessment. Information on imposex monitoring in the Basque Region of Spain was also presented along with information on an amendment to the OSPAR Guideline for imposex monitoring.

Consider progress with activities such as BEQUALM, HELCOM area/BSRP project, Prestige Oil Spill and biological effects monitoring programmes in MEDPOL

BEQUALM provides important AQC information for biological effects methods used within the JAMP CEMP. An update on progress with BEQULAM activities was given and it is clear that there are still problems with the uptake of biomarker and benthic community AQC procedures by contracting monitoring authorities within OSPAR. This has been reported to OSPAR SIME. Presentations were given on the current work within the HELCOM area / BSRP project, Prestige Oil Spill and MEDPOL. A wide range of techniques are being deployed and it is of considerable use to WGBEC in understanding the design of programmes, the application of the techniques for different scenarios and to see how data is being assessed and interpreted.

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

The current state of commissioned documents and those pending was discussed and updated. The requirement for any new method documents was discussed in relation to the core set of methods being proposed for WKIMON. The micronucleus method was identified as the only core method being proposed requiring a supporting TIMES publication.

Identify and report on changes in the distribution, population abundance and condition of marine species in the OSPAR maritime area that are driven by contaminants

Presently, WGBEC is not in the position to provide the data needed to perform an in-depth assessment concerning the role of contaminants as additional drivers for observed changes in species distributions for the entire OSPAR area in retrospect (e.g. covering the past 50 years or even the last 10-15 years with an appropriate spatial coverage). However, sufficient data should be available to test the effect of temperature increase during the past 20 years relative to fish diseases (WGPDMO), imposex in whelks and EROD activity in dab (North Sea) and perch (Baltic Sea). WGBEC believes that changes in climate variables are also likely to alter the transport, transfer, deposition and fate of contaminants. Bioavailability, metabolism and toxicity will also be affected.

Request from OSPAR regarding endocrine disruptors: - consider the adequacy of the OECD test protocols and specify proposals for a test programme for marine species if appropriate

A response to these two requests had been prepared intersessionally and evaluated and reviewed at the meeting. In response to the considering the adequacy of the OECD test protocols and their applicability for marine species – WGBEC felt that to date, there exists too much uncertainty on the extrapolation of fresh water EDC ecotoxicity data to salt water species to simply justify the applicability of OECD test protocols for protection of the marine environment. Therefore WGBEC recommend that a first assessment should be done using the data available on fish and invertebrate species, with the caution that this data has been produced for the testing of chemicals specifically for regulatory purposes.

In response to the request to specify proposals for a test programme for marine species in case OECD freshwater model species on scientific grounds are deemed not suitable for testing the effect of endocrine disrupting compounds in the marine environment – WGBEC proposed that in the short term continue to work with stickleback, but consideration should be given to other marine fish models. With the current lack of EDC specific endpoints, invertebrate models should primarily focus on full life cycle tests with endpoints such as growth, development and reproduction.

1 Opening of the meeting

The Chair, John Thain (UK), opened the meeting at 10:00 on Monday 19 March 2007, and thanked Professor Aldo Viarengo (IT), for hosting the meeting at the University of Alessandria and for organising the travel arrangements and hotel accommodation, etc. The Chair then invited the participants to introduce themselves and their affiliations and describe their area of interest and field of expertise. The list of attendees is given in Annex 1.

2 Adoption of the agenda

The Chair then invited participants to examine the Terms of Reference (ToR) and went through the agenda explaining the priority and background to the Agenda Items and also those items that had recently been added to the ToR as requests from ICES. The ToR for the meeting can be found in Annex 2. A draft agenda was adopted by the meeting and a tentative timetable agreed, Annex 3 and 4 respectively. It was noted that Agenda Items 5, 18, 19 and 20 were requests from OSPAR and Agenda Item 21c was a recent request from ICES. Two items were agreed to be included under any other business, a discussion on the publication of material in ICES Working Group Reports (21a) and presentations on risk assessment in relation to biological effects techniques (21c).

3 Appointment of Rapporteurs

Principle Rapporteurs were appointed for the Agenda Items and are given in Annex 5.

4 Evaluate the report from WKIMON third workshop and the intersessional work undertaken by WGBEC members, including a demonstration program (ToR b)

4.1 Progress with WKIMON

The ICES/OSPAR WKIMON III report had been circulated prior to the meeting and eight members of WGBEC had attended the WKIMON III meeting held in January 2007, in Copenhagen. Ketil Hylland (NO) gave a presentation on WKIMON III, which included the purpose of the workshop, the main objectives and achievements. Several members of WGBEC had contributed to the background documents prepared for each of the JAMP biological effect techniques and members had also provided data sets at the meeting to allow the derivation of background concentration / responses. John Thain (UK) and Ketil Hylland (NO) had also written a position paper on the use of biological effects techniques for integrated chemical – biological effects monitoring which was presented to OSPAR SIME.

There was concern in the group that it did not come across clearly from the report that assessment criteria produced during WKIMON III were very preliminary, basically an exercise to indicate the direction in which the work was going. The preliminary assessment criteria need to be revised and augmented with a level to differentiate moderately and highly contaminated areas. In some cases only tentative background responses were derived as shown in Table 4a below.

Table 4a. Summary of preliminary assessment criteria from WKIMON III.

BIOLOGICAL EFFECT	QUALIFYING COMMENTS	BACKGROUND RESPONSE RANGE	ELEVATED RESPONSE RANGE	HIGH AND CAUSE FOR CONCERN RESPONSE
VTG in plasma; µg/l	Cod	LOD to 2		
	Flounder	LOD – 2		
Reproduction in Eel pout; mean frequency (%)	Malformed larvae	0 – 1	> 1 – 2	> 2
	Late dead larvae	0 – 2	> 2 – 3	> 3
	Growth / retarded larvae	0 – 4	> 2 – 6	> 6
EROD; pmol/mg protein	Cod	≤ 80		
	Dab	≤ 40		
	Flounder	≤ 10		
Bile metabolites; 1-OH pyrene (µg/ml; 341/383 nm fluorescence)	Dab	≤ 220		
	Cod	≤ 0.95		
DNA adducts; nm adducts / mol DNA	Dab	≤ 7.86		
	Haddock	≤ 6.84		
	Saithe	≤ 7.90		
Bioassays; % mortality	Sediment Corophium	0 – 30	> 30 – < 100	100
	Sediment Arenicola	0 – 10	> 10 – < 100	100
	Water bivalve embryo	0 – 20	> 20 – < 100	100
	Water copepod	0 – 10	> 10 – < 100	100
	Water Echinoderm	0 – 10	> 10 – < 100	100
Lysosomal stability; minutes	Cytochemical; all species	> 20	≤ 20 – ≥ 10	< 10
	Neutral Red Retention: all species	> 120	≤ 120 – ≥ 50	< 50

Response levels above background for EROD, bile metabolites, DNA adducts and VTG have yet to be determined.

Members of the group had comments to the WKIMON III report.

Aldo Viarengo (IT) pointed out that it will be important to develop standard cultivated strains for species to be used in sediment toxicity testing. He also indicated that the method stress on stress should be included as a core method in the WKIMON guideline, a point of view that was supported by the rest of the group. It was noted that some techniques such as scope for growth and DNA adducts are not widely used, but they were needed due to their usefulness. International intercalibration has indicated that such techniques can be performed within acceptable limits. The specificity of AChE inhibition in different species was discussed; whereas AChE inhibition in fish can be seen as specific for carbamate/organophosphate exposure, it is a more general measure of stress in mussels.

It was further noted by Dick Vethaak (NL) that passive samplers should be included in the guideline (used with bioassays), partly as a link to WFD. This was accepted by the group. There were discussions in the group concerning a possible inclusion of microarray techniques (seen as premature) and oxidative stress methods (reviewed earlier and not viewed as appropriate at this time).

Recommendations from WKIMON III were considered. There were some errors (e.g. units for vtg in µg/l rather than µg/ml) and the group again emphasised that all assessment criteria must be seen as provisional until final consideration (see recommendations). Provisional assessment criteria for PAH metabolites were further seen as uncertain due to differences in standardisation procedures and DNA adducts needed further work due to different units (a limited dataset was used for the derivation of the current values).

As indicated in the action points from WKIMON III there is a need to develop a methodology for integrated assessment. An overview of the revised strategy for the WKIMON guideline can be found at Annex 6. Work on this framework needs to consider whether partial assessment can be acceptable (i.e. only sediment-related or only mussels, not fish).

The group underscored the need for quality assurance procedures to be put in place for the activities as it is to be expected that more than one group will be analysing for any given technique. WGBEC suggests that this be handled through BEQUALM in contact with MEDPOL.

Following WKIMON III and considerations at OSPAR SIME there is a need for an update of assessment criteria for all methods. In addition, brief background documents and technical documentation (protocols) will also be needed for some methods. Criteria will have to be developed for appropriate species. Wherever possible, mechanisms to correct for confounding factors should be included.

Fish species to be considered include dab (*Limanda limanda*), flounder (*Platichthys flesus*) and cod (*Gadus morhua*). For the demonstration programme, haddock (*Melanogrammus aeglefinus*) will replace cod and long rough dab (*Hippoglossoides platessoides*) will be included (to cover deep waters in the North Sea).

4.2 The ICON demonstration programme

The 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. This will be achieved through a step-wise process involving an international expert meeting with prospective project participants (15–16 May 2007) and field studies carried out in representative North Sea areas and in reference areas outside the North Sea in 2008-2009. The field studies will include methods put forward through the guidelines for integrated monitoring and assessment of contaminants and their effects developed at OSPAR/ICES WKIMON I-III. New methods will be developed and applied in an integrated risk assessment framework to indicate ecosystem health status with respect to hazardous substances. The results from a programme such as that indicated here, i.e. the use of integrated chemical and

biological effect methods to develop ecosystem indicators, will be important to OSPAR CEMP activities, OSPAR QSR 2010 and should be useful for the forthcoming EU marine strategy.

WGBEC considered the selection of locations put forward by the steering committee. There was general concern that too many locations were included and this was reduced. A revised prospectus (distributed to interested parties) can be found at Annex 7.

Recommendations

Assessment criteria are needed for WKIMON biological effects framework methods. This needs to be available by 1 October 2007.

WGBEC members agreed to prepare documents and criteria as follows:

For mussels:

- scope for growth (assessment criteria only) – John Thain, Jim Readman;
- condition index (protocol, assessment criteria) – John Thain;
- histopathology (background, protocol, assessment criteria) – Steve Feist;
- lysosomal stability (WKIMON III) – established;
- micronucleus formation (protocol, assessment criteria) – Aldo Viarengo (Claudia Bolognesi);
- AChE inhibition (assessment criteria) – Thierry Burgeot;
- stress on stress (background, protocol, assessment criteria) – Aldo Viarengo.

For fish (dab, flounder, haddock, long rough dab):

- GSI, LSI, condition (protocol, assessment criteria) – Ketil Hylland;
- Fish disease index (FDI; protocol, assessment criteria) – Thomas Lang, Steve Feist;
- PAH metabolites (assessment criteria) – Dick Vethaak, Matt Gubbins, Ketil Hylland;
- EROD (assessment criteria) – communicate with OSPAR secretariat (Ulrike Kammann);
- vitellogenin (assessment criteria) – John Thain;
- lysosomal stability – established;
- DNA adduct (assessment criteria) – Brett Lyons;
- AChE inhibition (assessment criteria) – Thierry Burgeot, Doris Schiedek.

There is also a need for modification of the existing integrated assessment systems (i.e. Fullmonti), which should similarly need to be available by 1 October 2007. John Thain, Dick Vethaak and Ketil Hylland will do this task. The expert system developed by Aldo Viarengo will need to be considered in this process.

A protocol is needed for the methods where such are not available at the moment, including mussel histopathology (Steve Feist (UK)) and stress on stress in mussels (used in MEDPOL, Aldo Viarengo).

ACME is asked to take note of and support the ICON demonstration programme for an integrated chemical and biological monitoring guideline.

5 Follow-up a third OSPAR/ICES workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-sea Areas (WKIMON III) (ToR o)

Midway through the WGBEC meeting a draft copy of the OPSAR SIME report was made available from the OSPAR Secretariat.

WGBEC noted the response from OSPAR SIME on WKIMON III and biological effects methods under CEMP.

As part of the follow-up work in relation to WKIMON, members of WGBEC will develop assessment criteria for whole sediment bioassays, sediment pore water bioassays, sediment sea water elutriates, water bioassays, reproductive success, DNA adducts and vitellogenin as well as for other methods (see section 4 of this report). The development of assessment criteria for EROD will be done in communication between WGBEC members, co-ordinated by Ulrike Kammann (DE), and the OSPAR Secretariat.

WGBEC acknowledges the wish from OSPAR that data from the ICON demonstration programme will be made available for QSR 2010.

WGBEC noted requests from SIME concerning the need to consider biological effects techniques currently under CEMP. The response from the group is shown below (Table 5a).

Table 5a. Status for planned updates for CEMP biological effects methods.

INVERTEBRATES	PLANNED ACTION
Whole sediment bioassays	Additional organisms will be considered and existing assessment criteria evaluated.
Sediment pore water bioassays	Additional organisms will be considered and existing assessment criteria evaluated.
Sediment sea water elutriates	Additional organisms will be considered and existing assessment criteria evaluated.
Water bioassays (OEB/Tisbe)	Additional organisms will be considered and existing assessment criteria evaluated.
Fish	
Lysosomal stability	No action.
Liver histopathology	To be included in fish disease index; no action.
Macroscopic liver neoplasms	To be included in fish disease index; no action.
Externally visible fish diseases	To be included in fish disease index; no action.
Reproductive success	Background document to be produced, existing criteria will be evaluated.
Metallothionein	No action.
ALA-D	No action.

6 Evaluate documents prepared intersessionally for "background" biological effects responses (ToR c)

OSPAR/ICES WKIMON had produced a number of "background" documents on biological effects responses. These had been prepared to provide information on:

- an assessment of the applicability of the biological effect technique across the OSPAR area;
- a review of the environmental variables that influence the biological effect;
- an assessment of the thresholds when the response of a biological effect technique can be considered to be of concern and/or require a response;

- proposals for assessment criteria;
- status of quality assurance techniques.

The biological effect techniques included: EROD, PAH-bile metabolites, DNA adducts, fish diseases including histopathology (covered by ICES WGDPMO), VTG, ALA-D, metallothionein, reproductive success in fish, water bioassays, sediment bioassays, lysosomal stability and Scope For Growth in mussels. Several members of WGBEC had been involved in producing, editing and reviewing these documents. The group felt that it could concur with the recommendation in the draft OSPAR SIME report that the documents were at a stage where they could be published by OSPAR. It was noted that the assessment criteria for Scope For Growth needed to be revised, and this was completed at the meeting, and the Chair, John Thain agreed to convey this to the OSPAR secretariat.

Recommendation

The background documents on biological effect techniques produced by WKIMON were sufficiently complete for publication by OSPAR.

That a process needs to be put in place to allow a regular review and update of these documents.

Action

John Thain to inform the OSPAR Secretariat of an amendment to the Scope for Growth background document.

7 Explore the possibilities to organize a joint OSPAR-MEDPOL Workshop on the use and application of integrated chemical-biological methods (ToR e)

To set the scene, several presentations were given to clarify the different approaches on integrated chemical-biological effect monitoring and assessment.

OSPAR

John Thain (UK) explained the OSPAR organization and the links with ICES and the WGBEC. The current strategy for using biological effects techniques within the OSPAR Maritime Area follows the OSPAR Joint Assessment Monitoring Programme (JAMP, 1998 a & b) guidelines and the OSPAR Co-ordinated Environmental Monitoring Programme (CEMP, 2000).

The OSPAR JAMP guidelines recommend different types of biological effects monitoring tools for different objectives. There are guidelines for General Quality Assessment, Local Impact Assessment and Contaminant-specific Monitoring. These guidelines should be used by Contracting Parties to address specific JAMP issues i.e. are there any problems emerging related to the presence of hazardous substances in the marine environment? In particular, are any unintended/unacceptable biological responses, or unintended/unacceptable levels of such responses, being caused by exposure to hazardous substances?

The CEMP is those elements of the OSPAR JAMP where guidelines and QA procedures have developed to such an extent that monitoring can commence on a Convention wide basis. Therefore, the OSPAR CEMP should be regarded as one of the main drivers for biological effects monitoring in the NE Atlantic. In 1998, OSPAR agreed that, in respect of the implementation of the CEMP, all components of the JAMP matrix should be considered mandatory. CEMP-rated biological effects issues relate to PAHs, metals and organotins. In this respect Contracting Parties should give a high priority to the implementation of the following JAMP biological effects techniques:

- cytochrome P4501A;
- DNA adducts;
- PAH metabolites;
- liver pathology;
- metallothionein,
- ALA-D;
- oxidative stress;
- imposex and intersex.

These techniques are currently rated Category II by OSPAR with the exception of liver pathology and imposex, which are rated at Category I. Category I guidelines are those for which Quality Assurance (QA) procedures are in place. Category I guidelines may be used for monitoring and the data are appropriate for Convention-wide use. Category II guidelines are those for which QA procedures are not yet in place. Category II guidelines may be used for monitoring although caution should be used when making comparisons of the data obtained between different Contracting Parties. Quality Assurance procedures are provided by BEQUALM (see Agenda Item 13) and in the case of imposex via QUASIMEME.

In addition to CEMP there are further monitoring requirements for the use of biological effects techniques. These include water and whole sediment bioassays and reproductive success in viviparous blenny. OSPAR through WKIMON (see Agenda Items 4, 5 and 6 above) is now in the process of reviewing the JAMP biological effects guidelines and how they should be implemented in integrated chemical – biological effects monitoring. New techniques such as VTG concentration in cod and flounder, lysosomal stability and Scope for Growth in mussels will be included in this revision. In addition, considerable progress has been made on the development of assessment criteria for biological effect techniques and in particular the derivation of background responses. Further work needs to be carried in this area to establish robust assessment criteria for all the techniques to enable them to be used for the OSPAR 2010 Quality Status Report.

All Contracting Parties within OPSAR are encouraged to report their data to the ICES database, along with corresponding QA information. This is important and allows OSPAR to assess biological data across the whole of the NE Atlantic OSPAR Maritime Area.

MEDPOL

Gabriel Gabrielides (GR) outlined the organization of the UN Mediterranean Action Plan (MAP) governed through the Barcelona Convention, and the MEDPOL monitoring programme. The MEDPOL, designed initially as the environmental assessment component of the MAP, has been operational since 1975. MEDPOL, as the scientific and technical component of MAP, provides the scientific basis for decision-making relating to marine pollution in the region with the aim to achieve sustainable development. The Contracting Parties to the Barcelona Convention have most recently decided to introduce monitoring of biological effects in the MEDPOL Phase II). This was considered to be not possible unless reliable and routine techniques were available. To address this concern, a manual on the biomarkers recommended for the MEDPOL biomonitoring programme was generated (UNEP/RAMOGE, 1999). Biomarkers in this manual comprised: Stress on stress, lysosomal membrane stability and metallothioneins in mussels, genotoxic damages in mussels and fish and EROD activity in fish. During the MEDPOL Phase III (1996–2005), biological effects techniques (only with biomarkers) were included in the monitoring programmes as a pilot activity to test the methodologies to be used as early-warning tools to detect any damage to organisms from pollutants. Since then, the reference laboratory of Professor Aldo Viarengo has organized various biomarker quality assurance exercises within the framework of the MEDPOL. The high levels of QA were noted by the WG. So far, biological effects methods

or/and biomonitoring programmes have been carried out by several Mediterranean countries (Spain, France, Italy, Croatia, Greece, Slovenia, Morocco, Syria and Tunisia). The Contracting Parties to the Barcelona Convention in their MEDPOL National Coordinators Meeting held in Barcelona, in May 2005, adopted the "STRATEGY FOR THE DEVELOPMENT OF MEDITERRANEAN MARINE POLLUTION INDICATORS" (MPIs) to be considered as the basis for the preparation of marine environment assessments in a manner which could facilitate the development of policy for the protection and conservation of the Mediterranean Sea and coastal areas and track its implementation. The MPIs techniques include:

- Acetylcholinesterase activity in mollusc cells;
- EROD activity in fish;
- Frequency of micronuclei in molluscs and fish cells;
- Lipofuscin lysosomal accumulation in molluscs and fish cells;
- Lysosomal membrane stability in molluscs and fish cells;
- Metallothionein in molluscs cells;
- Biomarker for the evaluation of DNA damage in mollusc and fish cells;
- Neutral lipid lysosomal accumulation in molluscs and fish cells;
- Peroxisome proliferation;
- Stress on stress (survival in air) in molluscs.

For monitoring of biological effects in Phase IV, a two-tier approach has been proposed which considers Lysosomal Membrane Stability (LMS), stress on stress and mortality as core biomarkers than can be easily applied by any MEDPOL laboratory. This will be supplemented by a battery of biomarkers to be analysed by competent labs in the region.

Aldo Viarengo (IT) outlined the proposed 2-tier approach for wide-scale biomonitoring in the Mediterranean using caged organisms (mussels or fish). An "early warning", highly sensitive, low-cost biomarker is employed in tier 1 (i.e. lysosomal membrane stability (LMS) and survival rate, a marker for highly polluted sites). Tier 2 (involving a battery of biomarkers) is used only for animals sampled at sites in which LMS changes are evident and where there is no mortality. This will then provide a comprehensive assessment of pollutant-induced stress. Possible approaches to integrate the biomarker data into a synthesised index were presented, along with proposals to use a recently developed Expert System. The latter system allows a correct selection of biomarkers at different levels of biological organisation (molecular/cellular/tissue/organism), taking into account trends in pollutant-induced biomarker changes (e.g. increasing, decreasing or bell-shaped) (Viarengo *et al.*, in press). In the ensuing discussion, the WG commended the high levels of QA currently carried out in MEDPOL for the various methods applied in MEDPOL.

WFD

Dick Vethaak (NL) gave an overview of the monitoring requirements under the Water Frame Work Directive. He then presented results from two pilot studies carried out by RIKZ/RIZA that explored opportunities for bioassays within the WFD (Maas *et al.*, 2005; van den Heuvel *et al.*, 2005). Under the Water Framework Directive (WFD, 2000), monitoring will be an extremely important tool to achieve "good ecological and chemical status" by 2015 in inland, transitional and coastal waters. One of the main benefits of the WFD and its monitoring programme is the use of both chemical and ecological parameters. The subsequent challenges for the monitoring programme are to integrate the chemical and ecological information into an overall insight into the quality of individual water bodies and to meet the monitoring requirements in a cost-effective and cost-efficient way. Although biological effects methods

are not prescribed in the WFD guidelines, opportunities can be seen in all three types of WFD monitoring. Two preferred applications of bioassays were proposed:

Eco-assays: the use of tests as a tool to determine the causes of below-standard ecological status of water bodies. Eco-assays can be used as part of a diagnostic system to identify or confirm chemical, ecological or hydro-morphological pressures. They can also be used to prioritize measures to be taken to improve ecological status, or to demonstrate the effectiveness of measures taken (Van den Heuvel-Greve *et al*., 2004).

Bio-analyses: the use of bioassays to partially replace chemical analyses of priority pollutants or other relevant compounds in chemical monitoring. The purpose here is to reduce monitoring costs and to generate a more comprehensive assessment of chemical water quality. The goal is not an extended analysis of water quality, but a better indication of hazard. In practice, a few selected bioassays should be sufficient to provide a sound hazard indication. In this respect, it is important that the judgement of bioassay results should be transparent, in order to prevent confusion in decision-making. Several selected bio-analyses are sensitive enough to measure effects of priority pollutants. Effects in bio-analyses were demonstrated in an artificial sample composed of the 33 priority pollutants at their maximum permissible level. The most important effects were caused by PAHs, herbicides and insecticides. Field samples containing priority pollutants in low concentrations showed the same or greater effects. The effects in the field are mainly caused by pollutants other than those on the priority list. Bio-analyses do not directly provide information on the compounds causing the measured effect. Any severe increase in toxicity identified by bio-analysis should trigger an investigation to identify the causes of toxicity (e.g. TIE or Effect-Directed Analysis (EDA)) in order to take appropriate management measures. TIE and EDA are very promising tools for the identification of organic toxicants in complex mixtures). In surveillance monitoring, currently there is no legal scope for the replacement of chemical analyses. There are, however, opportunities for bio-analysis in the:

- identification of relevant compounds;
- assessment of trends in toxicology;
- assessment of effects of other relevant compounds, such as endocrine disrupters.

The highest cost-efficiency may be achieved in operational monitoring if expensive high resolution chemical analyses can be focussed and their frequency reduced. Several scenarios can be envisaged for the partial replacement of chemical analyses. Cost reductions of 30% should be feasible. In addition to generally being less expensive than chemical analyses, bio-analyses enable the effects of compounds other than the selected priority pollutants to be monitored to identify sources, assess risk relating to incidents and establish a relationship between pollutants and ecological effects.

This approach was well received by the WG. In connection with this, the use of passive samplers was described and its advantages as a time-integrating tool to obtain extracts for applying low volume bioassays, were identified. One drawback relates to the physico-chemical and sorbing compatibility of the contaminants and the selected phase. The WG acknowledged the advantages of combining the use of passive samplers and bioanalyses as an important linkage between WFD and EU Marine Strategy Directive (MSD).

Recommendation: ICES should promote to OSPAR and the European Commission the additional value of bioassays and passive samplers in WFD and their potential role as connective link between WFD and the Marine Framework Directive.

Areas of commonality in OSPAR, MEDPOL and WFD programmes

Aldo Viarengo (IT) presented common interests promoting joint activities between MEDPOL and OSPAR on the use and application of integrated chemical-biological methods. These included:

- Both OSPAR and MEDPOL use chemical and biological effects monitoring methods and assessment approaches. These overlap.
- The integrated monitoring and assessment methods used/underway to be implemented by OSPAR and the Barcelona Convention will be important in ecosystem health assessment as a whole (e.g. in the EU-MSD).
- Slightly different assessment approaches with biomarkers and assays are carried out in OSPAR and the Barcelona Convention areas. There will be advantages to unify approaches and account for EU legislation, e.g. MSD.
- BEC monitoring assessments can bridge the gap between WFD and MSD.

HELCOM

Kari Lehtonen (FI) informed the WG that ICES/BSRP, HELCOM and partner Baltic Sea countries plan to organise a joint international demonstration project in 2009 on the Ecosystem Health of the Gulf of Finland ("ICES/BSRP and HELCOM Sea-going Demonstration Project on Integrated Multidisciplinary Assessment of the Ecosystem Health of the Gulf of Finland"), co-ordinated by Kari Lehtonen (Finnish Institute of Marine Research, Helsinki, Finland [FIMR]) and Thomas Lang (Federal Research Centre for Fisheries, Institute for Fishery Ecology, Cuxhaven, Germany [BFAFI/IFÖ]). Activities carried out during the Gulf of Finland sea-going workshop are intended to be harmonised with the ICON workshop to be carried out in the North Sea in 2008–2009. Possibilities for intercalibration workshops with MEDPOL will also be examined.

Conclusion

This was followed by a discussion on the possibility to organise common activities between ICES (OSPAR) and MEDPOL (Barcelona Convention) and HELCOM. The following was proposed and agreed upon:

In relation to the North Sea ICES/OSPAR ICON WS:

- to organize a parallel activity in the Mediterranean with MEDPOL in 2008 /9 using the 2-tiered approach (to be initiated/coordinated by Gabriel Gabrielides / Aldo Viarengo to be confirmed).
- to link to the ICES/BSRP/HELCOM seagoing workshop that aims at an integrated assessment of the Gulf of Finland ecosystem (action Kari Lehtonen) and the Baltic Sea project proposal that will be submitted to the forthcoming BONUS programme.

The above-mentioned workshops/activities should serve as a European platform:

- for harmonization and intercalibration exercises of biological effect techniques that are used in all three convention areas and which will largely fall under the EU MSD (lead Aldo Viarengo /MEDPOL together with Ketil Hylland, John Thain, Kari Lehtonen and Dick Vethaak). The intercalibration of biomarkers in MEDPOL is well subscribed. This contrasts OSPAR and HELCOM where the uptake of BEC methods in BEQUALM is poor or nonexistent. These intercalibrations could be combined and support each other. In the first instance, the members will be invited to the ICON WS, and subsequent exercises proposed at this WS will be discussed at OSPAR, MEDPOL and HELCOM to take them forward.
- to organize shared workshops to exchange knowledge (e.g. methods applied, new techniques, etc) and assessment approaches (learning by sharing) and to discuss

intercalibrations. These workshops should be held in 2008–2010 under the umbrella of OSPAR, MEDPOL and HELCOM. They should ideally be initiated through the ICON Workshop scientific group in collaboration with MEDPOL and HELCOM representatives. In order to achieve this it is proposed that Aldo Viarengo and Kari Lehtonen will be included in the ICON steering group (action Ketil Hylland)

- to obtain funding from organizations such as the EU through 7th FW Programme and training networks, UNEP, BONUS (Baltic Sea), etc (action by Dick Vethaak, Kevin Thomas and others).

Recommendation

ICES should advise OSPAR and the European Commission to take notice and make advantage of the existing integrated chemical-biological effect methods and integrated assessment approaches developed by OSPAR (in JAMP and CEMP) with reference to their potential value in the monitoring and assessment strategy for the MSD.

Justification

The EC had missed the opportunities to include (at that time available) BEC methods in the WFD. Since the MSD aims at an assessment of the ecosystem as a whole, integrated chemical biological effect techniques will be instrumental in assessing the impact of hazardous substances on ecosystem health.

CEMP. 2000. OSPAR Coordinated Environment Monitoring Programme (CEMP). Reference No: 1999-01.

References

Maas, J. L., and van den Heuvel-Greve, M. J., with contributions from Rotteveel, S., Roex, E., Gerritsen, A., Ferdinandy, M., Vethaak, D., Klamer, H., Bakker, J., and Schipper, C. 2005. Opportunities for bio-analysis in WFD chemical monitoring using bioassays. RIZA working document: 2005.053X.

Van den Heuvel-Greve, J. L., Maas, and Vethaak, A. D. 2004. Eco-assay verkenning; de mogelijke toepassing van eco-assays binnen de Kader Richtlijn Water. Internal report national Institute for Coastal and Marine Management/RIKZ, The Hague. RIKZ/OS/2004.827X.

Viarengo, A., Lowe, D., Bolognesi, C., Fabbri, E., and Koehler, A. 2007.The use of biomarkers in biomonitoring: a 2-tiered approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. Comp Biochem Phys. Accepted.

8 Review the use of in vitro and in vivo biological effects techniques for monitoring purposes and WFD activities (ToR h)

The WG noted that the item was insufficiently prepared for this years meeting and it was decided to reschedule it for 2008 with a focus on the EU Water Framework Directive (WFD) and the EU Marine Strategy Directive (MSD).

Action

ICES WGBEC to review the potential use and opportunities for biomarkers and bioassays in relation to monitoring and risk assessment frame works of the WFD and the MSD.

9 Review the "recommended list" of biological effect techniques (ToR d)

Review criteria and update tables for recommended and promising methods for biological effects monitoring.

The WGBEC discussed the 'promising' and 'recommended' monitoring techniques that had been last updated in 2004. The objective of preparing Tables 9.1–9.4 is to provide information on the status of methods to assess contaminant effects in marine ecosystems.

During the 2007 meeting the WGBEC confirmed that recommended methods for monitoring programmes should conform to the following criteria:

- A recommended method needs to be an established technique that is available as a published method in the TIMES series or elsewhere.
- A recommended method (or combination of methods) should have been shown to respond to contaminant exposure in the field.
- A recommended method (or combination of methods) should be able to differentiate the effects of contaminant from natural background variability.

The WGBEC also confirmed that updated descriptions of recommended methods should be published in the TIMES.

Changes to the Tables

Three methods 1) Apoptosis in fish cells, 2) AChe inhibition in other invertebrates and 3) Delayed reproduction / gonadal maturation were considered by the group to be no longer promising for use in the ICES maritime area and were removed from the lists. They were placed into Table 9.4 (Biological effects methods that would require further development/application to be considered promising for use in the ICES area) alongside oncogenes in fish and DNA adducts by ELISA. Shell thickening in oysters was completely removed from all lists as being redundant for the ICES / OSPAR areas. .

The YES and YAS reporter gene assays were promoted from promising (Table 9.3. Promising biological effects monitoring methods that require further research before they can be recommended for monitoring - Bioassays and methods for specific matrices) to the recommended table (Table 9.1c. Recommended techniques for biological monitoring programmes at the national or international level - Bioassays and methods for specific matrices).

Two new methods were introduced to the promising list (Table 9.2. Promising biological effects monitoring methods that require further research before they can be recommended for monitoring, both fish and invertebrates). These were alkylphenol bile metabolites and cellular energy allocation.

Ampelisca brevicornis was added along side *Corophium* and *Arenicola* as a recommended species for use in whole sediment bioassays in Table 9.1c (Recommended techniques for biological monitoring programmes at the national or international level - Bioassays and methods for specific matrices).

The prefixes DR, ER and AR were dropped from the CALUX assays to avoid the assumption that you had to deploy the patented and trademarked versions of the assay. The type of assay to be used (e.g. Oestrogen receptor-active compounds) is now described in the "Issues addressed" column of the table.

Where appropriate references supporting recommended and promising techniques have been updated to reflect current literature.

Methods for consideration at the next meeting

The working group decided that review documents for the comet assay (Brett Lyons, UK), the micronucleus assay (Aldo Viarengo, Italy) and intersex in fish (Dick Vethaak, The Netherlands) should be prepared intersessionally and presented at the 2008 meeting to allow an assessment of their suitability for recommendation to be made. In addition, a technical document describing the methods for measuring HSI and GSI in fish will be drafted for the 2008 meeting. This will supplement the document (Annex 10, 2004 WGBEC report) previously submitted my Lennart Balk (Sweden).

Actions

Following the 2008 meeting it is proposed that background documents should be drafted for abnormal embryos, online monitoring, MDR and immunosuppression.

Other points arising

The group discussed the potential application and use of genomics techniques in marine monitoring programmes. At present multi-gene microarrays are being developed for European flounder (*The Natural Environment Research Council (NERC) Post Genomics and Proteomics (PG&P) research programme*) and Mytilus sp. Furthermore, it is known that similar platforms are planned for other species relevant to the OSPAR maritime area, including the viviparous blenny (also known as the eelpout). The field of genomics as a whole is moving towards standardised methods for collecting and reporting data. Peer reviewed journals are now requesting that all studies follow guidelines and strategy detailed in the MIAME (Minimum Information About a Microarray Experiment) database (http://www.ebi.ac.uk/miamexpress/). At present the group acknowledges that genomic platforms (as well as developments in the field of proteomics and metabolomics) represent an extremely powerful and promising tool set for biological effects research. The WGBEC will continue to keep a watching brief on developments within the field and will review the area further following the publication of the NERC thematic funded European flounder microarray project in 2008–2009.

Table 9.1a. Recommended techniques for biological monitoring programmes at the national or international level - methods for fish.

METHOD	ORGANISM	INTERCALIBRATION	ISSUES ADDRESSED	BIOLOGICAL SIGNIFICANCE	REFERENCES
Bulky DNA adduct formation	Fish	B	PAHs; other synthetic organics, e.g., nitro-organics, amino triazine pesticides (triazines)	Measures genotoxic effects. Possible predictor of pathology through mechanistic links. Sensitive indicator of past and present exposure.	1–6
AChE inhibition	Fish	O	Organophosphates and carbamates or similar molecules	Measures exposure.	7–10
Metallothionein induction	Fish	B	Measures induction of metallothionein protein by certain metals (e.g., Zn, Cu, Cd, Hg)	Measures exposure and disturbance of copper and zinc metabolism.	11–15

METHOD	ORGANISM	INTERCALIBRATION	ISSUES ADDRESSED	BIOLOGICAL SIGNIFICANCE	REFERENCES
EROD or P4501A induction	Fish	B	Measures induction of enzymes which metabolize planar organic contaminants (e.g., PAHs, planar PCBs, dioxins)	Possible predictor of pathology through mechanistic links. Sensitive indicator of past and present exposure.	16–23
ALA-D inhibition	Fish	B	Lead	Index of exposure.	24–25
PAH bile metabolites	Fish	Q	PAHs	Measures exposure to and metabolism of PAHs.	26–27
Lysosomal stability	Fish	B	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.	28–31
Externally visible diseases	*Limanda limanda, Platichthys flesus, Gadus morhua*	B	Responds to a wide variety of environmental contaminants and non-specific stressors	Integrative response; measures general fish health; elevated prevalence may indicate exposure to contaminants.	43–44
Macroscopic liver neoplasms	*Limanda limanda, Platichthys flesus*	B	Effects of carcinogenic substances	Indicative of contaminant-associated liver carcinogenesis	152–153
Liver histopathology	*Limanda limanda, Platichthys flesus*	B	Effects of carcinogenic and non-carcinogenic contaminants	Indicative of non-specific and specific contaminant effects at cellular or tissue level	153–154
Vitellogenin induction	Male and juvenile fish	B	Oestrogenic substances	Measures feminization of male fish and reproductive impairment.	45–48
Intersex	Male flounder		Oestrogenic substances	Measures feminization of male fish and reproductive impairment.	49–50
Reproductive success in *Zoarces viviparus*	*Zoarces viviparous*			Measures reproductive output and survival of eggs and fry in relation to contaminants. Restricted to period when young are carried by female viviparous fish.	51

B: BEQUALM; Q: QUASIMEME

Table 9.1b. Recommended techniques for biological monitoring programmes at the national or international level - methods for invertebrates.

METHOD	ORGANISM	QA	ISSUE ADDRESSED	BIOLOGICAL SIGNIFICANCE	REFERENCES
AChE inhibition	Molluscs and crustaceans	O	Organophosphates and carbamates or similar molecules Possibly algal toxins	Measures exposure to a wide range of compounds and a marker of stress.	52–53
Metallothionein induction	Mytilus	O	Measures induction of metallothionein protein by certain metals (e.g., Zn, Cu, Cd, Hg)	Measures exposure and disturbance of copper and zinc metabolism.	54–55
Lysosomal stability (including NRR)	Mytilus. Oyster	O/B/U	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.	56–70
Scope for growth	Bivalve molluscs, e.g.,Mytilus spp. and oysters	O	Responds to a wide variety of contaminants	Integrative response, a sensitive sub-lethal measure of energy available for growth.	71–72
Imposex	Neogastropod molluscs (Nucella lapillus, Buccinum undatum, Hinia reticulata, Neptunea antiqua)	Q	Specific to organotins	Reproductive interference Estuarine and coastal littoral waters (Nucella) and offshore waters (Buccinum).	73–82
Intersex	Littorina littorea	B	Specific to reproductive effects of organotins	Reproductive interference in coastal (littoral) waters.	83
Induction/inhibition of Multidrug/multixenobiotic resistance (MDR/MXR)	Mytilus edulis		Multiple contaminants (organics and metals)	Adaptation/inhibition in response to xenobiotic stress.	84–89
Histopathology	Blue mussels		Not contaminant-specific	General responses	90–91
Embryo aberrations in field-collected amphipod crustaceans	Amphipods		Contaminant-specific	Measures frequency of different types of lethal embryo aberrations; allows for separating effects of contaminants and environmental climate variables	92–96

B: BEQUALM; Q: QUASIMEME; U: UNEP MEDPOL

Table 9.1c. Recommended techniques for biological monitoring programmes at the national or international level - Bioassays and methods for specific matrices.

METHOD	ORGANISM	QA	ISSUE ADDRESSED	BIOLOGICAL SIGNIFICANCE	REFERENCES
Benthic community analysis	Macro-, meio-, and epibenthos	B	Responds to a wide variety of contaminants, particularly those resulting in organic enrichment	Ecosystem level. Retrospective. Particularly useful for point sources. Most appropriate for deployment when other monitoring methods indicate that a problem may exist.	97–102
Whole sediment bioassays	*Corophium Arenicola, Ampelisca brevicornis*	B	Not contaminant-specific, will respond to a wide range of environmental contaminants in sediments	Acute/lethal and acute/sub-lethal toxicity only at present. May enable retrospective interpretation of community changes	103, 142–143
Bioassays of sediment pore waters, sea water elutriates, sea water samples	Bivalve embryo *Acartia*		Will respond to a wide range of environmental contaminants, Useful for dredge spoils, sediments liable to re-suspension	Acute and sub-lethal toxicity, including genotoxicity, etc. Toxicity of hydrophobic contaminants might be underestimated in pore water assays.	104
CALUX	Reporter gene assay		Ah receptor-active compounds	Predictor of dioxin like toxicity	105
YES	Reporter gene assay (yeast)		Oestrogen receptor-active compounds	Potential endocrine disruption	135–136
YAS	Reporter gene assay (yeast)		Androgen receptor-active compounds	Potential endocrine disruption	137–138

B: BEQUALM; Q: QUASIMEME

Table 9.2. Promising biological effects monitoring methods that require further research before they can be recommended for monitoring (both fish, and invertebrates).

METHOD	ORGANISM	ISSUE ADDRESSED	BIOLOGICAL SIGNIFICANCE	REFERENCES
Pre-neoplastic and neoplastic liver lesions by NADPH-producing enzymes	Fish	PAHs, other synthetic organics, e.g., nitro-organics, amino triazine pesticides (triazines)	Diagnosis of pathological changes and enzymatic markers of carcinogenesis associated with exposure to genotoxic and non-genotoxic carcinogens.	32–42
DNA strand breaks including Comet assay	Fish, mussels, cells	Not contaminant-specific, will respond to a wide range of environmental contaminants	Measures genotoxic effects, but is also extremely sensitive to other environmental parameters.	106–108, 145
BaP Hydroxylase -like enzymes	Invertebrates	Induced enzyme response to PAHs, planar PCBs, dioxins and/or furans	Measures exposure to organic contaminants.	109–110
Induction/inhibition of Multidrug/multixenobiotic resistance (MDR/MXR)	Fish and invertebrates other than Mytilus	Multiple contaminants (organics and metals)	Adaptation/inhibition in response to xenobiotic stress.	110–116
Glutathion-S-transferase(s) (GST)	Fish, molluscs	Predominantly organic xenobiotics	Measures exposure and the capacity of the major group of phase II enzymes. Considered most promising for isoenzyme-specific measurements	117–119, 144
Oxidative stress	Fish, invertebrates	Not contaminant-specific, will respond to a wide range of environmental contaminants	Measures the presence of free radicals.	120–123, 144
Immunocompetence	Fish, invertebrates	Not contaminant-specific, will respond to a wide range of environmental contaminants	Measures factors that influence susceptibility to disease.	124

METHOD	ORGANISM	ISSUE ADDRESSED	BIOLOGICAL SIGNIFICANCE	REFERENCES
On-line monitoring	Mussels and crabs	Not contaminant-specific, will respond to a wide range of environmental contaminants	Measures the effects of chemicals on heart rate using a simple and inexpensive remote biosensor. Gives an integrated response.	125
Abnormalities in wild fish embryos and larvae	Fish, including demersal and pelagic species	Not linked unequivocally to contaminants	Measures frequency of probably lethal abnormalities in fish larvae. Mutagenic, teratogenic.	126–127,
Bulky DNA adduct formation	Mussels, invertebrates	PAHs, other synthetic organics	Measures genotoxic effects	128–131
Gene arrays	Fish, mussels	Various	Combined responses from various biomarkers	132–133
Histopathology	Invertebrates (other than Mytilus)	Not contaminant-specific	General responses	Awaiting publications
Spiggin	Three-spined stickleback	Androgens	Measures environmental androgens	134
Micronuclei	Fish, bivalve molluscs	Not contaminant-specific	Exposure to aneugenic and clastogenic	150–151
Peroxisomal proliferation (enzyme assays)	Fish and invertebrates	Contaminant-specific	Potential alterations in lipid metabolism, non-genotoxic carcinogenesis	146–148
Alkylphenol- bile metabolites	Fish (cod)	Alkyl phenols	Measures exposure to and metabolism of Alkylated phenols	Awaiting publications
Cellular Energy Allocation	Invertebrates and small fish	Wide range of stressors	Changes in metabolic turnover and specific allocations will be linked to effects at higher levels of ecological organization	149

Table 9.3. Promising biological effects monitoring methods that require further research before they can be recommended for monitoring - Bioassays and methods for specific matrices.

METHOD	ORGANISM	ISSUE ADDRESSED	BIOLOGICAL SIGNIFICANCE	REFERENCES
CALUX	Reporter gene assay	Oestrogen receptor-active compounds	Potential endocrine disruption.	139
CALUX	Reporter gene assay	Androgen receptor-active compounds	Potential endocrine disruption.	
Chronic whole sediment bioassays	Invertebrates	Responds to a wide range of contaminants	Measurements such as growth and reproduction, coupled to biomarker responses, which will give a measure of the bioavailability and chronic toxicity in whole sediments.	
Pollution-induced community tolerance (PICT) water bioassay	Microalgae, bacteria	Specific contaminants can be tested	Measure of degree of adaptation to specific pollutants. Not yet widely tested; retrospective.	140–141

Table 9.4. Biological effects methods that would require further development/application to be considered promising for use in the ICES area.

METHOD	ORGANISM	ISSUE ADDRESSED	BIOLOGICAL SIGNIFICANCE
Oncogenes	Fish	PAHs Other synthetic organics, e.g., nitro-organics, amino triazine pesticides (triazines)	Activation of oncogenes (ras) or damage to tumour-suppressor genes (p53). Measures genotoxic effects leading to carcinogenesis.
ELISA for DNA adducts	Fish	Not contaminant-specific	Genotoxic effects
Apoptosis	Fish cells	Responds to a wide range of contaminants	General response.
AChE inhibition	Other invertebrates	Organophosphates and carbamates or similar molecules. Possibly algal toxins	Measures exposure
Delayed reproduction/ gonadal maturation	Fish	Not contaminant-specific	Reproductive disruption
Aromatase	Fish		In assessing the potential ecological risk of CYP19 inhibitors, in particular in the context of relating alterations in subcellular indicators of endocrine function

Recommendation

In view of the current developments within OSPAR WKIMON and the revision of the JAMP, WGBEC recommends that it revisits the "recommended list of methods" in 2009. In addition, for the 2008 meeting WGBEC recommends that review documents be prepared for comet assay, micronucleus assay, intersex in fish and technical documents be prepared describing the methods for measuring HIS and GSI in fish.

Justification

To ensure that it is up-to-date and aligned with the current developments within the JAMP and the ICON demonstration programme.

1) Dunn, B.P., Black, J.J., and Maccubbin, A. 1987.^{32}P-postlabelling analysis of aromatic DNA adducts in fish from polluted areas. Cancer Research, 47: 6543–6548.

2) Varanasi, U., Reichert, W.L., and Stein, J.E. 1989. ^{32}P-postlabelling analysis of DNA adducts in liver of wild English sole *(Parophrys vetulus)* and winter flounder *(Pseudopleuronectes americanus).* Cancer Research, 49: 1171–1177.

3) Varanasi, U., Reichert, W. L., Eberhart, B.-T., and Stein, J. E. 1989. Formation of benzo[a]pyrene-diolepoxide-DNA adducts in liver of English sole *(Parophrys vetulus).* Chemico-biological Interactions, 69: 203–216.

4) Maccubbin, A. E., and Black, J. J. 1990. ^{32}P-postlabelling detection of DNA adducts in fish from chemically contaminated waterways. Science of the Total Environment, 94: 89–104.

5) Lyons, B. P., Stentiford, G. D., Green, M., Bignell, J., Bateman, K., Feist, S. W., Goodsir, F. Reynolds, W. J., and Thain, J. E. 2004b. DNA adduct analysis and histopathological biomarkers in European flounder (*Platichthys flesus*) sampled from UK estuaries. Mutation Research, 552: 177–186.

6) Stein, J. E., Collier, T. K., Reichert, W. L., Casillas, E., Hom, T., and Varanasi, U. 1991. Bioindicators of contaminant exposure and sublethal effects: studies with benthic fish in Puget Sound, Washington. Environmental Toxicology and Chemistry, 11: 701–704.

7) Kirby, M. F., Morris, S., Hurst, M., Kirby, S. J., Neall, P., Tylor, T., and Fagg, A. 2000. The use of cholinesterase activity in flounder *(Platychthys flesus)* muscle tissue as a biomarker of neurotoxic contamination in UK estuaries. Marine Pollution Bulletin, 40(9): 780–791.

8) Finlayson, B. L., and Rudnicki, R. A. 1985. Storage and handling as a source of error in measuring fish acetylcholinesterase activity. Bulletin of Environmental Contamination and Toxicology, 35: 790–795.

9) Burgeot, T., Bocquené, G., Truquet, P., Le Dean, L., Poulard, J.C., Dorel, D., Souplet, A., and Galgani, F. 1993. The Dragonet *(Callionymus lyra),* a target species used for evaluation of the biological effects of chemical contaminants on French coasts. Marine Ecology Progress Series, 97: 309–316.

10) Galgani, F., Bocquené, G., and Cadiou, Y. 1992. Evidence of variation in cholinesterase activity in fish along a pollution gradient in the North Sea. Marine Ecology Progress Series, 91: 77–82.

11) Hogstrand, C., and Haux, C. 1990. A radioimmunoassay for perch *(Perca fluviatilis)* metallothionein. Toxicology and Applied Pharmacology, 103: 56–65.

12) Hogstrand, C., and Haux, C. 1992. Evaluation of differential pulse polarography for the quantification of metallothionein—a comparison with RIA. Analytical Biochemistry, 200: 388–392.

13) Killie, P., Kay, J., Leaver, M., and George, S. 1992. Induction of piscine metallothionein as a primary response to heavy metal pollutants: applicability of new sensitive molecular probes. Aquatic Toxicology, 22: 279–286.

14) Chan, K. M., Davidson, W. S., Hew, C. L., and Flecher, G. L. 1989. Molecular cloning of metallothionein cDNA and analysis of metallothionein gene expression in winter flounder tissues. Canadian Journal of Zoology, 67: 2520–2529.

15) Hylland, K. 1999. Biological effects of contaminants: Quantification of metallothionein (MT) in fish liver tissue. ICES Techniques in Marine Environmental Sciences, No. 26. 18 pp.

16) Burke, M. D., and Mayer, R. T. 1974. Ethoxyresorufin: Direct fluorimetric assay of a microsomal O-dealkylationwhich is preferentially inducible by 3-methylcholanthrene. Drug Metabolism and Disposition, 2: 583–588.

17) Eggens, M. L., and Galgani, F. 1992. Ethoxyresorufin-O-deethylase (EROD) activity in flatfish: Fast determination with a fluorescence plate-reader. Marine Environmental Research, 33: 213.

18) Galgani, F., and Payne, J.F. 1991. Biological effects of contaminants: Microplate method for measurement of ethoxyresorufin-O-deethylase (EROD) in fish. Techniques in Marine Environmental Sciences, No. 13. 11 pp.

19) Courtenay, S., Grunwald, C., Kraemer, G.L., Alexander, R., and Wirgin, I. 1993. Induction and clearance of cytochrome P4501A mRNA in Atlantic tomeod caged in bleached kraft mill effluent in the Miramichi River. Aquatic Toxicology, 27: 225–244.

20) Stagg, R., and McIntosh, A. 1998. Biological effects of contaminants: Determination of CYP1A-dependent mono-oxygenase activity in dab by fluorimetric measurement of EROD activity. ICES Techniques in Marine Environmental Sciences, No. 23. 16 pp.

21) Kraemer, G. L., Squibb, K., Gioelli, D., Garte, S. J., and Wirgin, I. 1991. Cytochrome P4501A1 mRNA expression in feral Hudson River tomeod. Environmental Research, 55: 64–78.

22) Belliaeff, B., and Burgeot, T. 1997. Sampling design optimization for EROD measurements in fish. Marine Ecology Progress Series, 153: 239–246.

23) Martínez-Gómez C., Campillo, J. A., Benedicto J., Fernández B., Valdés J., García, I., and Sánchez, F. 2006. Monitoring biomarkers in fish (*Lepidorhombus boscii* and *Callionymus lyra*) from the Northern Iberian shelf after the *Prestige* oil spill. Mar. Pollut. Bull. 53(5–7): 305–314.

24) Hodson, P. V. 1976. D-Aminolevulinic acid dehydratase activity of fish blood as an indicator of a harmful exposure to lead. Journal of the Fisheries Research Board of Canada, 33: 268–271.

25) Schmitt, C. J., Dwyer, F. J., and Finger, S. E. 1984. Bioavailability of Pb and Zn from mine tailings as indicated by erythrocyte D-aminolevulinic acid dehydratase (ALA-D) activity in suckers (Pisces: Catostomidae). Canadian Journal of Fisheries and Aquatic Science, 41: 1030–1040.

26) Ariese, F., Kok, S. J., Verkaik, M., Gooijer, C., Velthorst, N. H., and Hofstraat, J. W. 1993. Polycyclic aromatic compounds. In PAH: Synthesis, Properties, Analysis, Occurrence and Biological Effects, pp. 1013–1020. Ed. by P. Garrigues and M. Lamotte. Gordon and Breach, London.

27) Stein, J. E., Collier, T. K., Reichert, W. L., Casillas, E., Hom, T., and Varanasi, U. 1993. Bioindicators of contaminant exposure and sublethal effects in benthic fish from Puget Sound. Marine Environmental Research, 35(1–2): 95–100.

28) Köhler, A. 1991. Lysosomal perturbations in fish liver as indicators for toxic effects of environmental pollution. Comparative Biochemistry and Physiology, 100C(1/2): 123–127.

29) Lowe, D. M., Moore, M. N., and Evans, B. M. 1992. Contaminant impact on interactions of molecular probes with lysosomes in living hepatocytes from dab *Limanda limanda.* Marine Ecology Progress Series, 91: 135–140.

30) Broeg, K., Köhler, A., and Von Westernhagen, H. 2002. Disorder and recovery of environmental health monitored by means of lysosomal stability in liver of European flounder *(Platichthys flexus L.),* Marine Environmental Research, 54(3–5): 569–573.

31) Köhler, A., Söffker, K., and Wahl, E. 2002. Functional and morphological changes of lysosomes as prognostic biomarker of toxic injury in a marine flatfish *Platichthys flesus* (L.). Environmental Toxicology and Chemistry, 21: 2434–2444.

32) Köhler, A. 1990. Identification of contaminant-induced cellular and subcellular lesions in the liver of flounder (*Platichthys flesus*) caught at differently polluted estuaries. Aquatic Toxicology, 16: 271–294.

33) Malins, D. C., McCain, B. B., Landahl, J. T., Myers, M. S., Krahn, M. M., Brown, D. W., Chan, S. L., and Roubal, W. T. 1988. Neoplastic and other diseases in fish in relation to toxic chemicals: An overview. Aquatic Toxicology, 11: 43–67.

34) Feist, S. W., Lang, T., Stentiford, G. D., and Koehler, A. 2004. Use of liver pathology of the European flatfish dab (*Limanda limanda* L.) and flounder (*Platichthys flesus* L.) for monitoring. ICES Techniques in Marine Environmental Sciences 38. 42 pp

35) Myers, M. S., Olson, O. P., Johnson, L. L., Stehr, C. S., Hom, T., and Varanasi, U. 1992. Hepatic lesions other than neoplasms in subadult flatfish from Puget Sound, Washington: Relationships with indices of contaminant exposure Marine Environmental Research, 34: 45–51.

36) Myers, M. S., Stehr, C. S., Olson, O. P., Johnston, L. L., McCain, B. B., Chan, S. L., and Varanasi, U. 1994. Relationships between toxicopathic lesions and exposure to chemical contaminants in English sole *(Pleuronectes vetulus),* starry flounder *(Platichthys stellatus)* and white croaker *(Genyonemus lineatus)* from selected marine sites on the Pacific Coast, U.S.A. Environmental Health Perspectives, 102: 200–215.

37) Vethaak, A. D., Jol, J. G., Meijboom, A., Eggens, M. L., ap Rheinallt, T., Wester, P.W., van de Zande, T., Bergman, A., Dankers, N., Ariese, F., Baan, R. A., Everts, J. M., Opperhuizen, A., and Marquenie, J. M. 1996. Skin and liver diseases induced in flounder *(Platichthys flesus)* after longterm exposure to contaminated sediments in large-scale mesocosms. Environmental Health Perspectives, 104: 1218–1229.

38) Vethaak, A. D., and Wester, P. W. 1996. Diseases of flounder *(Platichthys flesus)* in Dutch coastal waters, with particular reference to environmental stress factors. Part 2. Liver histopathology. Diseases of Aquatic Organisms, 26: 99–116.

39) Stentiford, G. D, Longshaw, M., Lyons, B. P., Jones, G., Green, M., and Feist, S. W. 2003. Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Marine Environmental Research, 55: 137–159.

40) Köhler, A., and Van Noorden, C. J. F. 1998. Initial velocities of G6PDH and PGDH *in situ* and the expression of proliferating cell nuclear antigen (PCNA) are sensitive diagnostic markers during environmental hepatocellular carcinogenesis in marine flatfish. Aquatic Toxicology, 40: 233–252.

41) Köhler, A., and Van Noorden, C. J. F. 2003. NADPH and the higher incidence of pollution induced liver cancer in female flounder. Environmental Toxicology and Chemistry, 22: 2703–2710.

42) Köhler, A., and Pluta, H. J. 1995. Lysosomal injury and MFO activity in the liver of flounder *(Platichthys flesus*L.) in relation to histopathology of hepatic degeneration and carcinogenesis. Marine Environmental Research, 39: 255–260.

43) Bucke, D., Vethaak, D., Lang, T., and Mellergaard, S. 1996. Common diseases and parasites of fish in the North Atlantic: Training guide for identification. ICES Techniques in Marine Environmental Sciences, No. 19, 27 pp.

44) Vethaak, A. D., Bucke, D., Lang, T., Wester, P. W., Jol, J., and Carr, M. 1992. Fish disease monitoring along a pollution transect: A case study using dab *Limanda limanda* in the German Bight. Marine Ecology Progress Series, 91: 173–192.

45) Jobling, S., and Sumpter, J. P. 1993. Detergent components in sewage effluent are weakly oestrogenic to fish: in vivo study using rainbow trout *(Oncorhynchus mykiss)* hepatocytes. Aquatic Toxicology, 27: 361–372.

46) Tyler, C. R., and Sumpter, J. P. 1990. The development of a radioimmunoassay for carp, *Cyprinus carpio,* vitellogenin. Fish Physiology and Biochemistry, 8: 129–140.

47) Lazier, C. L., and MacKay, M. E. 1993. Vitellogenin gene expression in teleost fish. *In* Biochemistry and Molecular Biology of Fishes, Vol. 2, pp. 391–405. Ed. by P.W. Hochachka and T.P. Momsen. Elsevier Science Publications, Amsterdam.

48) Pelisso, C, and Sumpter, J. P. 1992. Steroids and 'steroid-like' substances in fish diets. Aquaculture, 107: 283–301.

49) Matthiessen, P., Allen, Y. T., Allchin, C. R., Feist, S. W., Kirby, M. F., Law, R. J., Scott, A. P., Thain, J. E., and Thomas, K. V. 1998. Oestrogenic endocrine disruption in flounder *(Platichthys flesus* L.) from United Kingdom estuarine and marine waters. Science Series Technical Report, No. 107, Centre for Environment, Fisheries and Aquaculture Science, Lowestoft, UK. 48 pp.

50) Gercken, J., and Sordyl, H. 2002. Intersex in feral marine and freshwater fish from northeastern Germany. Marine Environmental; Research, 54: 651–655.

51) Jacobsson, A., Neuman, E., and Thoresson, G. 1986. The viviparous blenny as an indicator of environmental effects of harmful substances. Ambio, 15: 236–238.

52) Bocquené, G., Bellanger, C, Cadiou, Y., and Galgani, F. 1995. Joint action of combinations of pollutants on the acetylcholinesterase activity of several marine species. Ecotoxicology, 4: 266–279

53) Rickwood, C. J., and Galloway, T. S. 2004. Acetylcholinesterase inhibition as a biomarker of adverse effect. A study of *Mytilus edulis* exposed to the priority pollutant chlorfenvinphos. Aquatic Toxicology, 67: 45–56.

54) Roesijadi, G., Unger, M. E., and Morris, J. E. 1988 Immunochemical quantification of metallothioneins of a marine mollusc. Canadian Journal of Fisheries and Aquatic Sciences, 45: 1257–1263.

55) Viarengo, A., Ponzano, E., Dondero, F., and Fabbri, R. 1997. A simple spectrophotometric method for metallothionein evaluation in marine organisms: an application to Mediterranean and Antarctic molluscs. Marine Environmental Research, 44: 69–84.

56) Ringwood, A. H., Conners, D. E., and Keppler, C.J. 1999. Cellular responses of oysters, *Crassostrea virginica,* to metal-contaminated sediments. Marine Environmental Research, 48: 427–437.

57) Ringwood, A.H., Conners, D.E., and Hoguet, J. 1998. Effects of natural and anthropogenic stressors on lysosomal destabilization in oysters *Crassostrea virginica.* Marine Ecology Progress Series, 166: 163–171.

58) Cajaraville, M.P., Abascal, I., Etxeberria, M., and Marigomez, I. 1995. Lysosomes as cellular markers of environmental pollution time-dependent and dose-dependent responses of the digestive lysosomal system of mussels after petroleum hydrocarbon exposure. Environmental Toxicology and Water Quality, 10: 1–8.

59) Cheung, V. V., Wedderburn, R. J., and Depledge, M. H. 1998. Molluscan lysosomal responses as a diagnostic tool for detection of a pollution gradient in Tolo Harbour, Hong Kong. Marine Environmental Research, 46: 237–241.

60) Etxeberria, M., Cajaraville, M. P., and Marigomez, I. 1995. Changes in digestive cell lysosomal structure in mussels as biomarkers of environmental stress in the Urdaibai Estuary (Biscay Coast, Iberian Peninsula). Marine Pollution Bulletin, 30: 599–603.

61) Fernley, P. W., Moore, M. N., Lowe, D. M., Donkin, P., and Evans, S. 2000. Impact of the *Sea Empress* oil spill on lysosomal stability in mussel blood cells. Marine Environmental Research, 50: 451–455.

62) Lin, S. Y., and Steichen, D. J. 1994. A method for determining the stability of lysosomal membranes in the digestive cells *of Mytilus edulis.* Marine Ecology Progress Series, 115: 237–241.

63) Lowe, D. M., and Fossato, V. U. 2000. The influence of environmental contaminants on lysosomal activity in the digestive cells of mussels *(Mytilus galloprovincialis)* from the Venice Lagoon. Aquatic Toxicology, 48: 75–85.

64) Lowe, D. M., Soverchia, C, and Moore, M. N. 1995. Lysosomal membrane responses in the blood and digestive cells of mussels experimentally exposed to fluoranthene. Aquatic Toxicology, 33: 105–112.

65) Marigomez, I., and Baybay-Villacorta, L. 2003. Pollutant-specific and general lysosomal responses in digestive cells of mussels exposed to model organic chemicals. Aquatic Toxicology, 64: 235–257.

66) Regoli, F. 1992. Lysosomal responses as a sensitive stress index in biomonitoring heavy-metal pollution. Marine Ecology Progress Series, 84: 63–69.

67) Ringwood, A. H., Conners, D. E., and Di Novo, A. 1998. The effects of copper exposures on cellular responses in oysters. Marine Environmental Research, 46: 591–595.

68) Ringwood, A. H., Connors, D. E., and Hoguet, J. 1998. Effects of natural and anthropogenic stressors on lysosomal destabilisation in oysters, *Crassostrea virginica.* Marine Ecology Progress Series, 166: 163–171.

69) Svendseb, C, and Weeks, J. M. 1995. The use of a lysosome assay for the rapid assessment of cellular stress from copper to the freshwater snail *Viviparus contectus* (Millet). Marine Pollution Bulletin, 31: 139–42.

70) Tremblay, R., Myrand, B., and Guderley, H. 1998. Temporal variation of lysosomal capacities in relation to susceptibility of mussels, *Mytilus edulis,* to summer mortality. Marine Biology, 132: 641–649.

71) Widdows, J., and Salkeld, P. 1992. Practical procedures for the measurement of scope for growth. MAP Technical Reports Series, 71: 147–172.

72) Widdows, J., and Johnson, D. 1988. Physiological energetics of *Mytilus edulis:* Scope for growth. Marine Ecology Progress Series, 46(1–3): 113–121.

73) Bryan, G. W., Gibbs, P. E., Hummerstone, L. G., and Burt, G. R. 1986. The decline of the gastropod *Nucella lapillus* around southwest England: Evidence for the effect of tributyltin from antifouling paints. Journal of the Marine Biological Association of the United Kingdom, 66: 611–640.

74) Bryan, G. W., Gibbs, P. E., Burt, G. R., and Hummerstone, L. G. 1987. The effects of tributyltin (TBT) accumulation on adult dogwhelks, *Nucella lapillus:* Long-term field and laboratory experiments (Southwest England and Isles of Scilly). Journal of the Marine Biological Association of the United Kingdom, 67: 525–544.

75) Bryan, G. W., Gibbs, P. E., and Burt, G. R. 1988. A comparison of the effectiveness of tri-n-butyltin chloride and five other organotin compounds in promoting the development of imposex in the dogwhelk. Journal of the Marine Biological Association of the United Kingdom, 68: 733–745.

76) Fiorini, P., Oehlmann, J., and Stroben, E. 1991. The pseudohermaphroditism of prosobranchs: morphological aspects. Zoologischer Anzeiger, 226: 1–26.

77) Stroben, E., Oehlmann, J., and Fioroni, P. 1992. The morphological expression of imposex in *Hinia reticulata* (Gastropoda, Buccinidae) – a potential indicator of tributyltin pollution. Marine Biology, 113(4): 625–636.

78) Stroben, E., Schulte-Oehlmann, U., Fioroni, P., and Oehlmann, J. 1995. A comparative method for easy assessment of coastal TBT pollution by the degree of imposex in prosobranch species. Haliotis, 24: 1–12.

79) Barreiro, R., González, R., Quintela, M., and Ruiz, J. M. 2001. Imposex, organotin bioaccumulation and sterility of female *Nassarius reticulatus* in polluted areas of NW Spain. Marine Ecology Progress Series, 218: 203–212.

80) Poloczanska, E. S., and Ansell, A. D. 1999. Imposex in the whelks *Buccinum undatum* and *Neptunea antiqua* from the west coast of Scotland. Marine Environmental Research, 47(2): 203–212.

81) Strand, J., and Jacobsen, J. A. 2002. Imposex in two sublittoral neogastropods from the Kattegat and Skagerrak: the common whelk *Buccinum undatum* and the red whelk *Neptunea antiqua.* Marine Ecology Progress Series, 244: 171–177.

82) OSPAR. 2002. Proposed amendments to the current OSPAR Guidelines on TBT-specific effects monitoring Working Group on Monitoring (MON 02/4/1) OSPAR Commission, London.

83) Bauer, B., Fiorini, P., Ide, I., Liebe, S., Oehlmann, J., Stroben, E., and Watermann, B. 1995. TBT effects on the female genital system of *Littorina littorea,* possible indicator of tributyltin pollution. Hydrobiologia, 309: 15–27.

84) Kurelec, B., and Pivcevic, B. 1991. Evidence for a multixenobiotic resistance mechanism in the mussel Mytilus galloprovincialis. Aquatic Toxicology, 19: 291–302.

85) Kurelec, B. 1992. The multixenobiotic resistance mechanism in aquatic organisms. Critical Reviews in Toxicology, 22(1): 23–43.

86) Luedeking, A., and Koehler, A. 2002. Identification of six mRNA sequences of genes biotransformation in Mytilus edulis. Marine Ecology Progress Series, 115–124.

87) Minier, C., Eufemia, F., and Epel, D.E. 1999. The multixenobiotic resistance phenotype as a tool to biomonitor the environment. Biomarkers, 4: 442–454.

88) Minier, C., Lelong, C., Djemel, N., Rodet, F., Tutundjian, R., Favrel, P., Mathieu, M., and Leboulenger, F. 2002. Expression and activity of a Multixenobiotic resistance systemin the Pacific oyster *Crassostrea gigas.* Marine Environmental Research, 54: 455–459.

89) Smital, T., Sauerborn, R., Pivcevic, B., Krca, S., and Kurelec B 2000. Interspecies differences in p-glycoprotein mediated activity of multixenobiotic resistance mechanism in several marine and freshwater invertebrates. Comparative Biochemistry and Physiology, 126C, 175–186.

90) Cajaraville, M. P., Díez, G., Marigómez, J. A., and Angulo, E. 1990. Responses of the basophilic cells of the digestive gland of mussels to petroleum hydrocarbon exposure. Diseases of Aquatic Organisms, 9: 221–228.

91) Cajaraville, M. P., Marigómez, J. A., Díez, G., and Angulo, E. 1992. Comparative effects of the WAF of three oils on mussels. 2. Quantitative alterations in the structure of the digestive tubules. Comparative Biochemistry and Physiology, 102C(1): 113–123.

92) Sundelin, B. 1983. Effects of cadmiun on *Pontoporeia affinis* (Crustacea, Amphipoda) in a soft-bottom microcosm. Marine Biology, 74: 203–212.

93) Elmgren, R., Hansson, S., Larsson, U., Sundelin, B., and Boeh, P. 1983. The "Thesis oil spill": Acute and long-term impact on the benthos. Marine Biology, 73: 51–65.

94) Sundelin, B., and Eriksson, A-K. 1998. Malformations in embryos of the deposit-feeding amphipod *Monoporeia afffinis* in the Baltic Sea. Marine Ecology Progress Series, 171: 165–180.

95) Sundelin, B., Ryk, C., and Malmberg, G. 2000. Effects on the sexual maturation of the sediment-living amphipod *Monoporeia affinis.* Environmental Toxicology, 15: 518–526.

96) Eriksson-Wiklund, A.-K., and Sundelin, B. 2001. Impaired reproduction of the amphipods *Monoporeia affinis* and *Pontoporeia femorata* as a result of moderate hypoxia and increased temperature. Marine Ecology Progress Series, 171: 165–180.

97) ICES. 1988. Procedures for the monitoring of benthic communities around point-source discharges. *In* Report of the ICES Advisory Committee on Marine Pollution, 1988. Cooperative Research Report, 160: 285.

98) ICES. 1989. Examples of the application of ICES guidelines for the monitoring of benthic communities around point-source discharges. *In* Report of the ICES Advisory Committee on Marine Pollution, 1989. Cooperative Research Report, 167: 150–164.

99) PARCOM. 1989. Guidelines for monitoring methods to be used in the vicinity of platforms in the North Sea. Paris Commission, London.

100)Rees, H. L., Heip, C, Vincx, M., and Parker, M. M. 1991. Benthic communities: Use in monitoring point-source discharges. Techniques in Marine Environmental Sciences, No. 16. 70 pp.

101)ICES. 1994. Report of the ICES/HELCOM Workshop on Quality Assurance of Benthic Measurements in the Baltic Sea. ICES CM 1994/E:10.

102)Rumohr, H. 1999. Soft bottom macrofauna: Collection and treatment of samples. Techniques in Marine Environmental Sciences, No. 27. 18 pp.

103)PARCOM. 1993. Report of the Paris Commission sediment reworker ring test. Oslo and Paris Commissions, London

104)Thain, J. E. 1991. Biological effects of contaminants: Oyster *(Crassostrea gigas)* embryo bioassay. Techniques in Marine Environmental Sciences, No. 11. 12 pp.

105)Murk, A. J., Legler, J., Denison, M. S., Giesy, J. P., van de Guchte, C., and Brouwer, A. 1996. Chemical-activated luciferase gene expression (CALUX): a novel *in vitro* bioassay for Ah receptor active compounds in sediment and pore water. Fundamental and Applied Toxicology, 33: 149–160.

106)Kammann, U., Bunke, M., Steinhart, H., and Theobald, N. 2001 A permanent fish cell line (EPC) for genotoxicity testing of marine sediments with the comet assay. Mutatation Research, 498(1–2): 67–77.

107)Belpaeme, K., Cooreman, K., and Kirsch-Volders, M. 1998. Development and validation of the *in vivo* alkaline comet assay for detecting genomic damage in marine flatfish. Mutation Research, 415: 167–184.

108)Lacorn, M., Piechotta, G., Wosniok, W., Simat, T.J., Kammann, U., Lang, T., Müller, W. E. G., Schröder, H. C., Jenke, H.S., and Steinhart, H. 2001. Annual cycles of apoptosis, DNA strand breaks, heat shock proteins, and metallothionein isoforms in dab *(Limanda limanda):* influences of natural factors and consequences for biological effect monitoring. Biomarkers, 6(2): 108–126.

109)Livingstone, D. R. 1991. Organic xenobiotic metabolism in marine invertebrates. Advances in Comparative and Environmental Physiology, 7: 145–213.

110)Suteau, P., Daubeze, M., Miguad, M. L., and Naibonne, J. F. 1988. PAH-metabolising enzymes in whole mussels as biochemical tests for chemical pollution monitoring. Marine Ecology Progress Series, 46, 45–49.

111) Köhler, A., Lauritzen, B., Bahns, S., George, S. G., Förlin, L., and van Noorden, C. J. F. 1998. Clonal adaptation of cancer cells in flatfish to environmental contamination by changes in expression of P-gp related MXR, CYP450, GST-A and G6PDH activity. Marine Environmental Research, 46(1–5): 191–195.

112)Köhler, A., Lauritzen, B., Janssen, D., Böttcher, P., Tegoliwa, L., Krüner, G., and Broeg, K. 1998. Detection of P-glycoprotein mediated MDR/MXR in *Carcinus maenas* hepatopancreas by Immuno-Gold-Silver labeling. Marine Environmental Research, 46(1–5): 411–414.

113)Hemmer, M. J., Courtney, L. A., and Ortego, L. S. 1995. Immunohistochemical detection of P-glycoprotein in teleost tissue using mammalian polyclonal and monoclonal antibodies. Journal of Experimental Zoology, 272(1): 69–77.

114)Smital, T., Sauerborn, R., Pivcevic, B., Krca, S., and Kurelec, B. 2000. Interspecies differences in p-glycoprotein mediated activity of multixenobiotic resistance mechanism in several marine and freshwater invertebrates. Comparative Biochemistry and Physiology, 126C: 175–186.

115)Tutundjian, R., Cachot, J., Leboulenger, F. and Minier, C. 2002. Genetic and immunological characterisation of a multixenobiotic resistance system in the turbot *(Scophthalmus maximus).* Comparative Biochemistry and Physiology, 132B: 463–471.

116)Tutundjian, R., Minier, C, LeFoll, F., and Leboulenger, F. 2002. Rhodamine exclusion activity in primary cultured Turbot *(Scophthalmus maximus)* hepatocytes. Marine Environmental Research, 54: 443–447.

117)George, S.G. 1994. Biochemistry and molecular biology of phase II xenobiotic-conjugating enzymes in fish. *In* Aquatic toxicology: Molecular, biochemical and cellular perspectives, pp. 37–85. Ed. by D.C. Malins and G.K. Ostrander. Lewis Publications, Searcy, Arkansas.

118)Scott, K., Leaver, M. J., and George, S. G., 1992. Regulation of hepatic glutathione S-transferase expression in flounder. Marine Environmental Research, 233–236.

119)Gowland, B. T. G., McIntosh, A. D., Davies, I. M., and Moffat, C. 2002. Glutathione S-transferase activity in mussels, *Mytilus edulis,* exposed to discharges from an

aluminium smelter. Bulletin of Environmental Contamination and Toxicology, 69: 147–154.

120)Regoli, F. 2000. Total oxyradical scavenging capacity (TOSC) in polluted and tranlocated mussels: a predictive biomarker of oxidative stress. Aquatic Toxicology, 50: 351–361.

121)Livingstone, D. R., Garcia Martinez, P., Michel, X., Narbonne, J. F., O'Hara, S. C. M., Ribera, D., and Winston, G. W. 1990. Oxyradical production as a pollution-mediated mechanism of toxicity in the common mussel, *Mytilus edulis* L., and other molluscs. Functional Ecology, 4: 415–424.

122)Livingstone, D. R., Lemaire, P., Matthews, A., Peters, L., Bucke, D., and Law, R. J. 1993. Pro-oxidant, antioxidant and 7-ethoxyresorufin-O-deethylase (EROD) activity responses in liver of dab *(Limanda limanda)* exposed to sediment contaminated with hydrocarbons and other chemicals. Marine Pollution Bulletin, 26(11): 602–606.

123) Winston, G. W., and Di Giulio, R. T. 1991. Pro-oxidant and antioxidant mechanisms in aquatic organisms. Aquatic Toxicology, 19: 137–161.

124)Dean, J. H., Laster, M. I., and Boorman, G. A. 1982. Methods and approaches for assessing immunotoxicity: An overview. Environmental Health Perspectives, 43: 27–29.

125)Agaard, A., Andersen, B. B., and Depledge, M. H. 1991. Simultaneous monitoring of physiological and behavioural activity in marine organisms using non-invasive, computer-aided techniques. Marine Ecology Progress Series, 73 277–282.

126)Cameron, P., and Berg, J. 1992. Morphological and chromosomal aberrations during embryonic development in dab *Limanda limanda.* Marine Ecology Progress Series, 91: 163–169.

127)Klumpp, D. W., and von Westernhagen, H. 1995. Biological effects of pollutants in Australian tropical coastal waters: Embryonic malformations and chromosomal abberations in developing fish eggs. Marine Pollution Bulletin, 30(2): 158–165.

128)Ackha, F., Izuel, C., Venier, P., Budzinski, H., Burgeot, T., and Narbonne, J.F. 2000. Enzymatic biomarker measurement and study of DNA adduct formation in B[a]P-contaminated mussels, *Mytilus galloprovincialis.* Aquatic Toxicology, 49 (4): 269–287.

129)Ackha, F., Ruiz, S., Zamperon, C, Venier, P., Burgeot, T., Cadet, J., and Narbonne, J. F. 2000. Benzo[a]pyrene- induced DNA damage in *Mytilus galloprovincialis.* Measurement of bulky DNA adducts and DNA oxidative damage in term of 8-oxo-7,8-dihydro-2'-deoxyguanosine. Biomarkers, 5: 355–367.

130)Akcha, F., Burgeot, T., Venier, P., and Narbonne, J. F. 1999. Relation between kinetics of benzo[a]pyrene bioaccumulatuion and DNA binding in the mussel *Mytilus galloprovincialis.* Bulletin of Environmental Contamination and Toxicology, 62: 455–462.

131)Ackha, F., Burgeot, T., Leskowicz, A., Budzinski, H., and Narbonne, J. F. 2000. Induction and removal of bulky B[a]P-related DNA adducts and 8-oxodGuo in mussels *Mytilus galloprovincialis* exposed *in vivo* to B[a]P- contaminated feed. Marine Ecology Progress Series, 205: 195–206.

132)Gracey, A. Y., Troll, J. V., and Somero, G. N. 2001. Hypoxia-induced gene expression profiling in the euryoxic fish *Gillichthys mirabilis.* Proceedings of the National Academy of Sciences of the USA, 98: 1993–1998

133)Williams, T. D., Gensberg, K., Minchin, S. D., and Chipman, J. K. 2003. A DNA expression array to detect toxic stress response in European flounder *(Platichthys flesus).* Aquatic Toxicology, 65: 141–157.

134)Katsiadaki, I., Scott, A. P., Hurst, M. R., Matthiessen, P., and Mayer, I. 2002. Detection of environmental androgens: a novel method based on enzyme-linked immunosorbent assay of spiggin, the stickleback *(Gasterosteus aculeatus)* glue protein. Environmental Toxicology and Chemistry, 21: 1946–1954.

135)Routledge, E. J., and Sumpter, J. P. 1996. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environmental Toxicology and Chemistry, 15: 241–248.

136)Thomas, K. V., Hurst, M. R., Matthiessen, P., and Waldock, M. J. 2001. Identification of oestrogenic compounds in surface and sediment pore water samples collected from industrialised UK estuaries. Environmental Toxicology and Chemistry, 20(10): 2165–2170. 145.

137)Thomas, K. V., Hurst, M. R., Matthiessen, P., McHugh, M., Smith, A., and Waldock, M. J. 2002. An assessment of *in vitro* androgenic activity and the identification of environmental androgen in United Kingdom estuaries. Environmental Toxicology and Chemistry, 21: 1456–1461.

138)Sohoni, P. and Sumpter, J. P. 1998. Several environmental oestrogens are also anti-androgens. Journal of Endocrinology, 158: 327–39.

139)Legler, J, van den Brink, C. E., Brouwer, A., Murk, T., van der Saag, P. T., Vethaak, A.D., and van der Burg, B.1999. Development of a stably transfected estrogen receptor-mediated luciferase reporter gene assay in the human T47-D breast cancer cell line. Toxicological Sciences, 48: 55–66.

140)Blanck, H., and Wängberg, S.-Å. 1988. Validity of an ecotoxicological test system: Short-term and long-term effects of arsenate on marine periphyton communities in laboratory systems. Canadian Journal of Fisheries and Aquatic Sciences, 45: 1807–1815.

141)Molander, S., Dahl, B., Blanck, H., Jonsson, J., and Sjöström, M. 1992. Combined effects of tri-n-butyltin (TBT) and diuron (DCMU) on marine periphytoa communities detected as pollution-induced community tolerance (PICT). Archives of Environmental Contamination and Toxicology, 22: 419–427.

142)I. Riba., T. A., J. M. Forja., A. Gómez-Parra. 2003. Comparative toxicity of contaminanted sediment from a mining spill using two amphipodos species: *Corophium volutator* (Palllas, 1976) and *Ampelisca brevicornis* (A. Costa, 1853). Bull. Environ. Contam. Toxicol. 71:1061–1068

143)Beiras, R. and Saco Alvarez, L. 2006. Toxicity of seawater and sand affected by the Prestige fuel-oil using bivalve and sea urchin embryogenesis bioassays. Water, Air, and Soil Pollution. 177(1–4): 457–466.

144)Martínez-Gómez C., Campillo J. A., Benedicto J., Fernández B., Valdés J., García, I., and Sánchez F. 2006. Monitoring biomarkers in fish (*Lepidorhombus boscii* and *Callionymus lyra*) from the Northern Iberian shelf after the *Prestige* oil spill. Mar. Pollut. Bull. 53(5–7): 305–314.

145) Lee, F. R., Steinert, S. 2003. Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. Mutation Research, 544, 43–64.

146)Cajaraville M. P., Garmendia L., Orbea A., Werding R., Gómez-Mendiuka A., Izaguirre U., Soto M and Marigómez I. 2006. Signs of recovery of mussels health two years after the *Prestige* oil spill. Mar. Environ. Res. 62 Suppl:S337–341.

147)Cancio I., Ibabe A., Cajaraville M. P. 1999. Seasonal variation of peroxisomal enzyme activities and peroxisomal structure in mussels *Mytilus galloprovincialis* and its relationship with the lipid content. Comp. Biochem. Physiol. 123, 135–144.

148)Orbea A., and Cajaraville, M. P. 2006. Peroxisome proliferation and antioxidant enzymes in transplanted mussels of four Basque estuaries with different levels of polycyclic aromatic hydrocarbon and polychlorinated biphenyl pollution. Environ. Toxicol. Chem. 25, 1616–1626.

149)Smolders, R., Bervoets, L., De Coen W., and Blust, R. 2004. Changes in cellular energy allocation in zebra mussels exposed along a pollution gradient: linking cellular effects to higher levels of biological organization. Environmental Pollution 129: 99–112.

150)Al-Sabti, K., and Metcalfe, C. 1995. Fish micronuclei for assessing genotoxicity in water. Mutat Res, 343:121–135.

151)Grisolia, C. K., and Cordeiro, C. M. T. 2000. Variability in micronucleus induction with different mutagens applied to several species of fish. Genetics and Molecular Biology, 23(1):235–239.

152)Bucke, D., Vethaak, A. D., Lang, T. and Mellergaard, S. 1996. Common diseases and parasites of fish in the North Atlantic: Training guide for identification. ICES Techniques in Marine Environmental Sciences, 19, 27 pp.

153)Feist, S. W.; Lang, T., Stentiford, G. D., and Köhler, A. 2004. Biological effects of contaminants: Use of liver pathology of the European flatfish dab (*Limanda limanda* L.) and flounder (*Platichthys flesus* L.) for monitoring. ICES Techniques in Marine Environmental Sciences 38, 42 pp.

154)Lang, T.; Wosniok, W.; Barsiene, J.; Broeg, K.; Kopecka, J.; Parkkonen, J. 2006. Liver histopathology in Baltic flounder (*Platichthys flesus*) as indicator of biological effects of contaminants. Marine Pollution Bulletin 53: 488–496

10 Report on progress with intersessional activities using passive samplers (ToR f)

At the joint meeting of WGMS and WGBEC in 2006, possibilities for collaborative studies incorporating silicone rubber-based passive samplers and biological effects measurements were discussed. Although no clear way forward was identified, FRS and CEFAS (UK) agreed to try to combine the use of silicone rubber passive samplers in water with measurements of contaminants and biological effects in mussels at a small number of sites in the UK. There has been no progress on this during 2006, but the organizations will investigate the scope for undertaking this work during 2007.

ICES WGMS provided WGBEC with documents and a presentation relating to current progress with the ICES Passive Sampling Survey Trial (PSTS). Information on progress was made available to the group as background documents and the presentation was given to the group by Matt Gubbins (UK). Further information on the scheme is available on the PSTS website: http://home.tiscali.nl/fsmedes/icespsts.

WGBEC noted that good progress was being made with the use of silicone rubber-based passive samplers in the ICES region (and beyond). The suggestion from WGMS that passive samplers could be incorporated into the proposed ICON demonstration programme (see Agenda Item 4) was well received. It was felt that there was potential scope within the proposed programme to accommodate passive samplers (of a variety of types: silicone rubber, SPMD etc) and that this should be pursued. Any passive sampling element to the ICON programme will need to be coordinated and Kevin Thomas (NIVA, Norway) was proposed as a possible candidate for this role.

The details of how passive samplers could be incorporated into ICON should be discussed at the proposed workshop to be held in May. Representation at this workshop from the ICES PSTS programme would be welcomed.

Recommendation

That the potential role for passive samplers (of a variety of types) in the ICON demonstration programme be discussed at the workshop in May.

11 Receive and evaluate reports prepared intersessionally by WGBEC on oxidative stress, cellular energy allocation and aromatase (ToR k)

11.1 Oxidative stress

WGBEC were unable to conduct a comprehensive review of the use of oxidative stress measurements for environmental monitoring during the meeting. It was felt by members of the group that this was a major task, due to the wide variety of types of measurement (e.g. enzyme activities, oxyradical scavenging capacity, peroxidation products etc) and that although several

members were using some of these techniques on an *ad hoc* / research basis, a wider review on the use of these techniques for monitoring was required, perhaps by someone outside of the group (Francisco Regoli was suggested as a possibility). It was also noted that as a result of recent activities in OSPAR / WKIMON, oxidative stress has been removed from the OSPAR JAMP and the requirement for an ICES TIMES document has been dropped. It was pointed out, however, that due to the increase in use of these endpoints in studies concerning the effects of nanoparticles, their importance is likely to increase in the future and that a review was still required.

The group also noted that there have been reviews of oxidative stress in aquatic organisms by Richard DiGiulio (1995) and recently in marine bivalves by Almeida *et al*. (2007), which would aid the review process.

Action

That the use of oxidative stress measurements for marine environmental monitoring in the ICES area be reviewed intersessionally and presented at the next meeting in 2008 (to be arranged by the Chair).

References

Almeida, E. A. *et al.* 2007. Oxidative Stress in Perna perna and other bivalves as indicators of environmental stress in the Brazilian marine environment: Antioxidants, lipid peroxidation and DNA damage. Comp. Biochem. Physiol. A, 146: 588–600.

DiGiulio, R. T. 1995. Activated Oxygen Metabolism and Oxidative Stress. *In* Fundamentals of Aquatic Toxicoloy, 538– 544. Ed. by G. M. Rand. CRC Press.

11.2 Cellular energy allocation

Some of the successful types of biomarkers are linked to metabolism and energetics. Typical and well-studied environmental challenges to e.g. the energy metabolism of crustacea include environmental hypoxia, functional (internal) hypoxia, changing energetic requirements, disturbance to water balance/ion-homeostasis and changes in temperature (see review by Morris and Airriess, 1998). Additionally, exposure to toxicants also results in an energetic challenge (e.g. McKenney, 1998).

Physiological energetics provides information on key processes in the organism's energy acquisition and expenditure, possibly also elucidating the mode of action of the toxicant. For example, the allocation of specific amounts of energy to basal metabolism, growth and reproduction will vary in response to changing environmental conditions, and, theoretically, exposure to a pollutant will disturb this allocation. In addition, changes in metabolic turnover and specific allocations will be linked to effects at higher levels of ecological organization (McKenney, 1998; De Coen, 1999).

Knowledge derived from laboratory experiments

Several laboratory experiments have been conducted under standardized 24, 48, 96 and 168 hour exposure tests to single stressors using both freshwater *Daphnia magna* (stressors $HgCl_2$ and lindane (De Coen and Janssen, 1997)), $CdCl_2$, $K_2Cr_2O_7$, TBT, LAS, NAPCP and 2,4-D (De Coen and Janssen, 2003; Verslycke *et al*., 2003)), *Clarius garipinus* larvae (stressors $K_2Cr_2O_7$, $CdCl_2$, NaPCP and malathion (Nguyen, 1997)), *Chironomus riparius* (hypoxia, hyperoxia, $K_2Cr_2O_7$ and fenitrothion (Choi *et al*., 2001)) and marine organisms (*Neomysis integer* (T (temperature)=5 S (salinity)=5 DO (dissolved oxygen)=110, T=20 S=5 DO=70, T=5 S=25 DO=70, T=20 S=25 DO=70 and T=5 S=25 DO=110 (Verslycke and Janssen, 2002), TBT (Verslycke and Janssen, 2003), methoprene, nonylphenol and chlorpyrifos (Verslycke *et. al.*, 2004, Verslycke, 2004)). Muyssen and Janssen (2001) reported a

multigeneration exposure experiment (7 generations) using *Daphnia magna* exposed to Zn. Until approx. 2005, all CEA laboratory experiments have been performed using invertebrates or fish larvae. Analyses were done on whole body homogenates to provide an integrated assessment of the whole organism's energy use and availability. Whole organism measurements cannot be performed in larger organisms. In these organisms CEA should be determined on whole or parts of selected tissues, which is considered as inappropriate since lipids, proteins and carbohydrates are not evenly distributed and stored. The general trend in the CEA literature shows that toxicant stress reduces the carbohydrate and lipid levels. The trend of stressor-induced reduction of the carbohydrate and lipid levels was also observed in algae (Torres *et al.*, 2000; Kobbia *et al.*, 2001; Chaudhary and Chandra 2005), invertebrates (Surendra Nath 2003; De Luca-Abbott 2001; Ribeiro *et al.,* 2001; Smolders *et al.,* 2004) and fish (Sancho *et al.,* 1999; Handy *et al.,* 1999; Smolders *et al.,* 2003). The results on changes in the protein reserves, however, are much less straightforward. Generally, protein energy budgets increased at low levels of stress and a decrease was only observed at higher toxicant concentrations. This phenomenon has been observed in several experiments but has not received much attention. Smolders *et al.* (2003, 2004) assumed that low to intermediate levels of pollution triggers increased protein synthesis, e.g. induction of detoxification and defence proteins, when other sources of readily available energy like glycogen and lipids are still sufficiently available because their exposure experiments to effluents triggered a translocation of energy from storage (as glycogen) to maintenance (as protein) without changing the overall energy budget of zebrafish.

A recent laboratory study (Verslycke, 2004) compared the responses of *Neomysis integer* following exposure to environmentally realistic concentrations of the organophosphate pesticide chlorpyrifos using scope for growth (SFG) and cellular energy allocation (CEA). Oxygen consumption in the SFG assay was significantly correlated with cellular respiration rate in the CEA assay, and both were significantly increased by chlorpyrifos exposure. In addition, the protein, sugar, lipid and total energy content in the CEA assay and the ingestion rate in the SFG assay were significantly different in chlorpyrifos exposed mysids compared to control mysids. In contrast, absorption efficiency in the SFG assay was unaffected by pesticide exposure. Significant effects in the SFG and CEA assays were more pronounced following short (i.e. 48 h) compared to longer exposure periods (e.g. 168 h). SFG was significantly reduced at near-lethal concentrations (0.072 and 0.100 µg chlorpyrifos l-1); whereas CEA was reduced in all chlorpyrifos exposed mysids (0.038, 0.056, 0.072 and 0.100 µg chlorpyrifos l-1) although no concentration response was observed.

Cellular energy allocation (CEA) was studied in three Arctic benthic species (*Gammarus setosus*, Amphipoda), *Onisimus litoralis* (Amphipoda), and *Liocyma fluctuosa* (Bivalvia)) exposed to oil-related compounds (Olsen *et al.*, in press). Energy budget was measured in organisms subjected towater accomodated fraction (WAF) and drill cuttings (DC) to evaluate whether these compounds affect the energy metabolism of the test species. Significantly lower CEA values and higher ETS activity was observed in *G. setosus* subjected to WAF treatment compared to controls ($p= 0.03$). Higher CEA value and lower cellular respiration were observed in *O. littoralis* exposed to DC compared to controls ($p=0.02$). No difference in the energy budget of *L. fluctuosa* was observed between the treatments ($p= 0.19$). Different responses to oil related compounds between the three test species are likely the result of differences in feeding and burial behaviour and species-specific sensitivity to petroleum related compounds.

Knowledge derived from field experiments

A number of field experiments have been successfully performed to evaluate the applicability of CEA to indicate the general health of caged and wild organisms in field conditions. One of the major advantages of the use of caged organisms is that the initial energy content of the caged organisms is known and changes can be integrated over time and locations reflecting an

integrated and holistic assessment of the environmental quality. A cumulated response demonstrates the impact of all stressors present at the exposure sites and effects of different food resources can be incorporated to increase the ecological relevance of the exposure (De Kock and Kramer, 1994; Smolders *et al*., 2004). In one experiment, Smolders *et al*. (2004) caged zebra mussels (*Dreissena polymorpha*) at 5 river sites along a pollution gradient and CEA was measured after 1, 3, 7, 14, and 28 days of exposure. The pollution gradient received inputs from two different effluent sources (at locations 1 and 3). Exposure resulted in a negative CEA, indicating that the organisms lost energy during the exposure period. Only at the reference site and downstream at location 5 a positive CEA was observed. These data clearly indicated the impact of the pollution gradient on the CEA and the remediation with distance from the pollution sources.

During the BECPELAG-project, the technique was applied to caged blue mussel (*Mytilus edulis*) and cod (*Gadus morhua*) as well as to collected fish larvae and zooplankton along two transects in the North Sea, the German Bight and the Statfjord Oil Field, with different inputs of contaminants. The German Bight is mainly influenced by riverine inputs and the Statfjord Oil Field is an offshore oil production area (Smolders *et al.*, 2006). Along the Statfjord Oil Field transect, highest CEA values were generally recorded at the reference site (site 4), most remote from the oil-drilling field. The levels decreased towards the oil field area. The Statfjord transect results support the view that exposure to sub-optimal conditions is costly for the energy maintenance of the tested organisms. Along the German Bight transect, all test organisms, except blue mussels, expressed highest CEA levels at the near-coast locations (closest to the Elbe river mouth). The levels decreased further offshore and lowest values were recorded at site 4 on the Dogger Bank, which was considered the reference site. The interpretation of the Dogger Bank transect observations was not straightforward in terms of expected and measured pollution gradient levels but the similar trends in all tested organisms (mussel, cod, fish larvae and zooplankton) seem to support the cosmopolitan and integrative nature of the measurement of energy budgets as an ecotoxicological endpoint. All organisms in the German Bight responded similarly and conversely to the expected and measured contamination gradient. One possible explanation for this apparent contradiction is the natural and/or induced differences in food and prey availabilities in the examined areas. Data on time zero of exposure were unfortunately not available to allow a more time resolved approach to reveal differences in growth trends among the examined sites.

De Coen (1999) used active biomonitoring with caged *Daphnia magna* to assess the environmental impact of two effluent discharges from a metallurgic plant. Daphnids were caged during 96 hours upstream and downstream of both discharges, and also in both direct wastewater streams. Organisms exposed in both wastewater streams both had significantly lower energy budgets than organisms exposed upstream of the discharges. On the other hand, exposure in the mixing zone and further downstream increased the CEA of daphnids compared to the upstream reference population. In both zones the lipid contents were highest compared to both discharge sites while the lipid contents were severely depleted. No significant changes of the ETS activities of the exposed daphnids were noted.

As a final example of field evaluations, Verslycke *et al*. (2004) assessed the seasonal and spatial variability of CEA in caught estuarine mysids (*Neomysis integer*) along the Scheldt estuary in The Netherlands. A significant seasonal effect on CEA was observed. Mysids caught in spring allocated more energy towards their reserves than the samples from summer or winter. However, the impacts of the sampling sites were more pronounced than the seasonal influences. CEA was depressed at the two most polluted, upstream sites. While total available energy was unaffected by season and location, the energy consumption was significantly induced at the polluted, upstream sites (Verslycke *et al.*, 2004).

Towards an increased ecological value for toxicity testing

The presented results indicate that CEA is a valuable measure of stressor exposure. The technique is a rapid and sensitive early warning signal. However, experts consider its ecological relevance limited when applied as a single indicator. The added value can be found its use in combination with other higher order energy linked indicators such as condition indices, growth, Scope for Growth (SfG), reproduction and population dynamics. Most publications on CEA have reported significant correlations with higher physiological, organismal or reproductive levels. De Coen and Janssen (1997) showed that CEA in daphnids after 48h and 96h exposures were linked to the ecologically important parameters mean brood size, intrinsic rate of natural increase, net reproductive rate and adult length of daphnids. Smolders *et al.* (2004) found significant correlations between CEA levels in zebra mussels and their condition indices, filtration rates and respiration rates which are considered as endpoints at the physiological level. Comparisons between effects on SfG, condition indices and CEA during the course of exposure revealed that significant effects on CEA were observed much earlier than with SfG and condition indices: 3 to 7 days for CEA whereas changes in SfG and condition indices were firstly seen after, respectively, 14 and 28 days of exposure. This study (Verslycke *et al.*, 2004) concluded that CEA is more sensitive than SfG.

Methodological advantages and disadvantages

The current proposed methodology allows the rapid measurement of many samples by the use of multiwell plate readers. An important disadvantage is that the measurements of total sugar and lipid are based on sulphuric acid destruction. These mixtures are very corrosive for the expensive analytical apparatus. No rapid alternatives seem to be currently available.

References

Chaudhary, M. P., and Chandra, R. 2005. Toxicity assessment of heavy metals with Nostoc muscorum L. Journal of Environmental Biology 26: 129–134.

Choi, J., Roche, H., and Caquet, T. 2001. Hypoxia, hyperoxia and exposure to potassium dichromate or fenitrothion alter the energy metabolism in Chironomus riparius Mg. (Diptera: Chironomidae) larvae. Comparative Biochemistry and Physiology 130C: 11–17.

De Kock, W. C., and Kramer, K. J. M. 1994. Active biomonitoring (ABM) by translocation of bivalve molluscs. *In* Biomonitoring of Coastal Waters and Estuaries. Ed. by K.J.M. Kramer. CRC Press, Boca Raton, FL, USA.

De Coen, W. M. I. 1999. Energy metabolism, DNA damage and population dynamics of the waterflea Daphnia magna Straus under toxic stress. PhD dissertation, University of Ghent. 535 p.

De Coen, W. M., and Janssen, C. R., 1997. The use of biomarkers in *Daphnia magna* toxicity testing: IV. Cellular Energy Allocation: a new methodology to assess the energy budget of toxicant-stressed *Daphnia* populations. *J. Aquat. Ecosyst. Stress Recovery* 6, pp. 43–55.

De Coen, W. M., and Janssen, C. R. 2003. The missing biomarker link: Relationships between effects on the cellular energy allocation biomarker of toxicant-stressed Daphnia magna and corresponding population characteristics. Environmental Toxicology and Chemistry, 22: 1632–1641.

De Luca-Abbott, S., 2001. Biomarkers of sublethal stress in the soft sediment bivalve *Austrovenus stutchburyi* exposed in-situ to contaminated sediment in an urban New Zealand harbour. Marine Pollution Bulletin 41, 817–825.

Handy, R. D., Sims, D. W., Giles, A., Campbell, H. A., and Musonda, M. M. 1999. Metabolic trade-off between locomotion and detoxification for maintenance of blood chemistry and growth parameters by rainbow trout (*Oncorhynchus mykiss*) during chronic dietary exposure to copper. Aquat Toxicol, 47: 23–41.

Kobbia, I. A., Battah, M. G., Shabana, E. F., and Eladel, H. M. 2001. Chlorophyll a fluorescence and photosynthetic activity as tools for the evaluation of simazine toxicity to *Protosiphon botryoides* and *Anabaena variabilis*. Ecotoxicology and Environmental Safety, 49: 101–105.

McKenney, Jr., C. L. 1998. Physiological dysfunction in estuarine mysids and larval decapods with chronic pesticide exposure. In: Wells, P.G., Blaise, C. (Eds.), Microscale Testing in Aquatic Toxicology: Advances, Techniques, and Practice. CRC Press, Boca Raton, FL, pp. 465–476.

Morris, S., and Airriess, C. N., 1998. Integration of physiological responses of crustaceans to environmental challenge. S. Afr. J. Zool., 33: 87–106.

Muyssen, B. T. A., and Janssen, C. R. 2001. Multigeneration zinc acclimation and tolerance in Daphnia magna: implications for water-quality guidelines and ecological risk assessment. Environmental Toxicology and Chemistry, 20: 2053–2060.

Nguyen, T. H. L. 1997. Potential and limitations of early life stage toxicity tests with fish. PhD thesis, University of Ghent, 241 p.

Olsen, G. H., Sva, E., Carroll, J., Camus, L., De Coen, W., Smolders, R., Øveraas, and H., Hylland, K. Alterations in energy budget of Arctic benthic species exposed to oil-related compounds. Aquat Toxicol (in press).

Ribeiro, S., Sousa, J. P., Nogueira, A. J. A., and Soares, A. M. V. M. 2001. Effect of endosulfan and parathion on energy reserves and physiological parameters of the terrestrial isopod *Porcellio dilatatus*. Ecotoxicology and Environmental Safety, 49: 131–138.

Sancho, E., Ferrando, M. D., and Andreu, E. 1998. Effects of sublethal exposure to a pesticide on levels of energetic compounds in *Anguilla anguilla*. J Environ. Sci. Health B, 33: 411–424.

Smolders, R., Bervoets, L., De Coen W., and Blust, R. 2004. Changes in cellular energy allocation in zebra mussels exposed along a pollution gradient: linking cellular effects to higher levels of biological organization. Environmental Pollution, 129: 99–112.

Smolders, R., De Boeck, G., and Blust, R. 2003. Changes in cellular energy budget as a measure of whole effluent toxicity in zebrafish (*Danio rerio*). Environ. Toxicol. Chem., 22: 890–899.

Smolders, R., Cooreman, K., Roose, P., Blust, R., and De Coen, W. Cellular energy allocation in caged and pelagic free-living organisms along two North Sea pollution gradients. Chp 3.5 In: Biological effects of contaminants in pelagic ecosystems, Edited by Ketil Hylland, Thomas Lang, Dick Vethaak. SETAC Press, pp. 235–252.

Torres, E., Cid, A., Herrero, C., and Abalde, J. 2000. Effect of cadmium on growth, ATP content, carbon fixation and ultrastructure in the marine diatom Phaeodactylum tricornutum Bohlin. Water Air and Soin Pollution, 117: 1–14.

Surendra Nath, B. 2002. Shifts in glycogen metabolism in hemolymph and fat body of the silkworm, Bombyx mori (Lepidoptera: Bombycidae) in response to organophosphorus insecticides toxicity. Pesticide Biochemistry and Physiology, 74: 73–84.

Verslycke, T., and Janssen, C. R. 2002. Effects of a changing abiotic environment on the energy metabolism in the estuarine mysid shrimp *Neomysis integer* (Crustacea: Mysidacea). J. Exp. Mar. boil. Ecol., 279: 61–72.

Verslycke, T., Vercauteren, J., Devos, C., Moens, L, Sandra, P., and Janssen, C. R. 2003. Cellular energy allocation in the estuarine mysid shrimp Neomysis integer (Crustacea: Mysidacea) following tributyltin exposure. Journal of Experimental Marine Biology and Ecology, 288: 167–179.

Verslycke, T., Ghekiere, A., and Janssen, C. R. 2004. Seasonal and spatial patterns in cellular energy allocation in the estuarine mysid Neomysis integer (Crustacea: Mysidacea) of the

Scheldt estuary (The Netherlands). Journal of Experimental Marine Biology and Ecology, 306: 245–267.

Verslycke, T., Stephen D. Roast S. D., Widdows, J., Jones M. B., and Janssen C. R. 2004. Cellular energy allocation and scope for growth in the estuarine mysid *Neomysis integer* (Crustacea: Mysidacea) following chlorpyrifos exposure: a method comparison. J. Exp. Mar. Biol. Ecol., 306: 1–16.

Verslycke, T., Poelmans, S., De Wasch, K., De Brabander, H. F., and Janssen, C. R. 2004. Testosterone and energy metabolism in the estuarine mysid *Neomysis integer* (Crustacea: Mysidacea) following exposure to endocrine disruptors. Environmental Toxicology and Chemistry, 23: 1289–1296.

11.3 Aromatase

Cytochrome P450 aromatase (CYP19) is the enzyme that converts (aromatazable) androgens to estrogens (e.g. estradiol). This enzyme exerts multiple functions in both male and female organisms. It mainly occurs in tissues that control reproduction and reproductive behavior, such as the gonads and brain. There is increasing evidence that most teleosts (bony fish) (unlike mammals) possess two CYP19 genes: CYP19A1, which is primarily expressed in the gonads, and CYP19A2 which is mainly expressed in the brain. It has been demonstrated that CYP19A2 functions as an important factor in the reproductive endocrinology of teleosts through the brain-pituitary-gonadal axis (Kazeto and Trant, 2005; Kazeto *et al.*, 2005).

Ankley *et al* 2002 has demonstrated a range of effects in fathead minnow (Pimephales promelas) after exposure to fadrozole (a potential aromatase inhibitor). A concentration-dependent reduction in fecundity was observed in fish exposed for 21 days to water concentrations of fadrozole ranging from 2 to 50 µg/l. Consistent with the expected mechanism of action, there was a significant inhibition of brain aromatase activity in both male and female fish exposed to fadrozole. In females, this inhibition was accompanied by a concentration-dependent decrease in plasma E2 and vitellogenin concentrations. Histological assessment indicated a decrease in mature oocytes and an increase in preovulatory atretic follicles. Exposure of male fish to fadrozole significantly increased plasma concentrations of the androgens testosterone (T) and 11-ketotestosterone (KT) and resulted in a marked accumulation of sperm in the testes (Ankley *et al.*, 2002).

The well-described example of imposex/intersex in molluscs has been attributed amongst others to a possible direct inhibitory effect of organotin compounds on aromatase activity (see review by Matthiessen & Gibbs 1998). However, the precise mechanism in gastropods is still a matter of debate and there is some doubt as to whether organotin compounds function as inhibitors of enzymes that metabolize androgens in gastropods. Homologues of ER and AR have not been found in invertebrates and the nuclear family members are very different between vertebrates and invertebrates (Nakaniski, 2007).

Aromatase activity was determined in brain and gonads of wild bream collected along the river Elbe, Germany, and correlated with other endocrine and reproductive endpoints such as plasma sex steroid concentrations, secondary sex characteristics (STI), plasma vitellogenin, gonad size (GSI) etc. (Karbe *et al.*, 2006; Hecker *et al.*, 2007). Furthermore, regional patterns of aromatase activity were correlated to a number of environmental factors such as exposure to environmental contaminants and parasitism. While aromatase activity was not detectable in the gonads of male and female fish with the assay used, fish of both genders revealed relatively high levels of brain enzyme activities. As for most of the endocrine and reproductive parameters, aromatase activities were significantly less in fish from a river stretch characterized by elevated exposures to organic contaminants and metals. Brain aromatase activity was positively and significantly correlated with plasma estradiol (E2) in females, and showed a similar trend with plasma 11-ketotestosterone (11KT) and STI in males. The authors hypothesized that the effects on brain aromatase activity were likely to be related to the

disruption of other reproductive parameters including sexual maturity and expression of secondary sex characteristics. Although a number of factors such as exposure to pollutants and prevalence of the tapeworm Ligula intestinalis correlated with the suppression of aromatase activity, the exact causes for the regional decrease in brain aromatase activity remain unclear due to inconsistencies of these correlations between sampling events or gender.

Conclusion

The state of the art of aromatase activity/inhibition biomarkers in fish and invertebrates and their potential use in routine monitoring has been briefly reviewed. Although proven useful in experimental and mechanistic studies, this is not the case for application in biological effect monitoring. Therefore it is recommanded that aromatase activity should be removed from the list of promising methods.

References

Ankley, G. T., Kahl, M. D., Jensen, K. M., Hornung, M. W., Korte, J., Makynen, E. A., and Leino, R. L. 2002. Evaluation of the Aromatase Inhibitor Fadrozole in a Short-Term Reproduction Assay with the Fathead Minnow (Pimephales promelas) Toxicological Sciences, 67: 121–130.

Hecker, M., Sanderson, T., and Karbe, L. 2007. Suppression of aromatase activity in populations of bream (Abramis brama) from the river Elbe, Germany. Chemosphere. 66: 542–552.

Karbe, L., Ternes, T., Wenzel, A., and Hecker, M. 2006. Estrogens, xenoestrogens, and effects on fish in German waters. *In* Chapter 15. Estrogens and Xenoestrogens in the Aquatic Environment: An Integrated Approach for Field Monitoring and Effect Assessment, pp. 365–406. Ed. by Dick Vethaak, Marca Schrap and Pim de Voogt. Society of Environmental Toxicology and Chemistry (SETAC). SETAC Press, Pensacola, FL, USA.

Kazeto, Y.. Goto-Kazeto, R., Place, A.R., and Trant, J.M. 2005. Aromatase expression in zebrafish and channel catfish brains: changes in transcript abundance associated with the reproductive cycle and exposure to endocrine disrupting chemicals. Fish Physiol. Biochem., 28: 29–32.

Kazeto, Y., and Trant, J. M. 2005. Molecular biology of channel catfish brain cytochrome P450 aromatase (CYP19A2): cloning, preovulatory induction of gene expression, hormonal gene regulation and analysis of promoter region. J. Mol. Endocrinol., 35: 571–583.

Matthiessen, P. and Gibbs. P. E. 1998. Critical appraisal of the evidence for tributyltin-mediated endocrine disruption in mollusks, Environ. Toxicol. Chem., 17: 37–43.

Nakaniski, T. 2007. Potential toxicity of organotin compounds via nuclear receptor signalling in mammals. Journal of health Science, 53: 1–9.

12 Report on progress with the assessment of imposex data in the ICES database (ToR i)

Matt Gubbins (UK) rapporteured this item and gave a presentation and progress report on the assessment of imposex data. Also reviewed under this item, were recent developments in imposex monitoring in harbours/marinas in the Basque region of Spain (presented by Maria Jeśus Belzunce (ES)) and a recent position paper from the UK submitted to OSPAR SIME (SIME 07/5/6) recommending that organo-tin residue analysis in gastropods be made a voluntary determinand in the OSPAR guideline for TBT effects monitoring (item 3).

12.1 Progress with assessment of imposex data

Matt Gubbins (UK) presented recent intersessional progress made with statistical assessment of imposex data in the ICES database conducted by Rob Fryer (FRS, Aberdeen). Progress was outlined in a working document to the group provided at Annex 8.

At WGBEC 2006, working documents describing a preliminary assessment process for imposex data were presented to a joint session of WGBEC and WGSAEM (Fryer & Gubbins 2006a, 2006b). These proposed methodologies for temporal trend assessment of imposex and also proposed a regional assessment method based on VDSI data for *Nucella* in Sullom Voe, UK. It was demonstrated how VDSI time series can be assessed by scaling the indices to lie between 0 and 1 and then modelling them using generalised linear (or additive) models assuming quasi-binomial errors. They applied the method to all VDSI time series in the ICES database with at least five years of data. The method appeared reasonable for any one time series, but problems arose when several time series were modelled simultaneously (Fryer & Gubbins, 2006b), as the mean-variance relationship implicit in the quasi-binomial distribution was shown to be inappropriate.

Following consideration of this issue at the joint Working Group session in 2006, it was recommended that the statistical methodology should be adapted to take account of the comments made and that OSPAR MON should adopt the methodology to assess imposex data in the ICES database at its meeting in 2006. It was noted that MON had been unable to achieve this in 2006, but that the method had been improved intersessionally for VDSI in *Nucella lapillus* and applied to all time series (>4 years) of VDSI data in the ICES database at ICES WGSAEM. The text below from ICES WGSAEM 2007 describes:

"Individual VDS data were obtained intersessionally, modelled using a proportional odds model, and the results used to estimate a more appropriate mean-variance relationship. This was then used to assess the time series of VDSI in *Nucella lapillus* in the ICES database. The changes in methodology provide more accurate significance levels and better model diagnostics. The number of females that contribute to each VDSI is required for the analysis and should be submitted to the ICES database. Following consideration of the methodology by WGSAEM in 2007 it was concluded that he submission of individual imposex data (e.g. the VDS class of each female) might have long term benefits and should be encouraged.

Four sets of individual VDS data in *Nucella lapillus* were modelled using a proportional odds model (McCullagh & Nelder, 1999), a generalised linear model for ordinal data, and used to estimate a mean-variance relationship appropriate for VDSI data. Figure 12.1 shows the mean-variance relationship under the assumption of quasi-binomial errors (black) and based on the individual VDS data (blue). The two curves clearly differ, the one based on individual data showing markedly lower variability when the mean VDSI is around 4. Thus, when the mean VDSI is around 4, most individual VDS classifications would be 4 and the VDSI from replicate samples would all be close to 4. Conversely, when the mean VDSI is around 2, individual VDS classifications would be more widely spread and the VDSI from replicate samples would have relatively large variability.

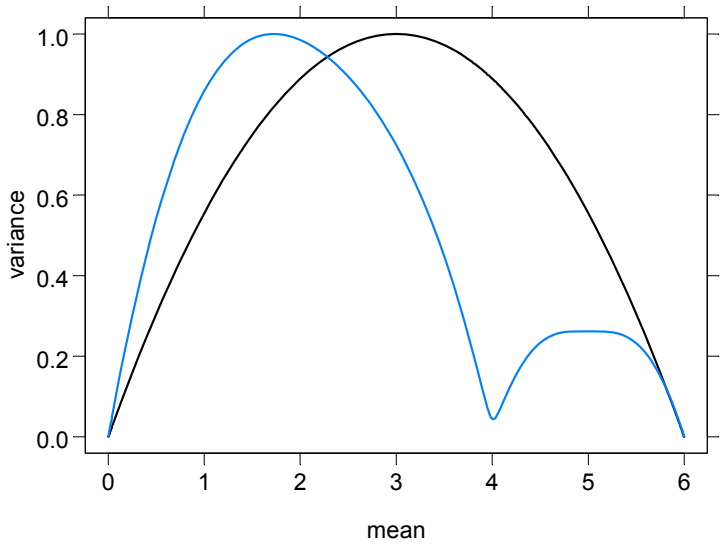

Figure 12.1. The mean-variance relationship under the assumption of quasi-binomial errors (black) and based on the individual VDS data (blue). The two relationships are scaled to have a common maximum of unity.

Generalised linear models, assuming the mean-variance relationship based on individual data, were used to assess all time series of VDSI in *Nucella lapillus* in the ICES data base with at least four years of data (Annex Y). There were 114 time series. Most of the estimated trends were downwards. There were 17 significant downward trends (at the 5% level) and only 1 significant upwards trend. There were 1, 14, 38, 58, and 3 time series in assessment classes A through E respectively. There were some data that require quality checks.

The new mean-variance relationship provides more accurate significance levels and better model diagnostics.

The assessments should weight each VDSI by the number of females in the sample, but this is not available in the ICES database. Instead, half the total number of individuals in the sample was used.

There is evidence of over-dispersion in some time series, so a between-year variance component will need to be introduced. Estimating these variance components will help inform the design of imposex monitoring programmes.

Mean-variance relationships now need to be estimated for other measures of imposex in other species (e.g. VDSI in *Neptunea antiqua* and PCI in *Buccinum undatum*).

In principle, modelling individual imposex data using e.g. the proportional odds model should be more informative than modelling summary measures such as VDSI. However, it is unclear whether, in practice, such a change would yield substantial benefits for large-scale assessments. Nevertheless, access to individual data would allow for this possibility in the future, and would help to estimate appropriate mean-variance relationship for use in VDSI assessments. Therefore, submission of individual data to the ICES database should be encouraged."

Conclusions

The group welcomed the recent improvements to the assessment methodology and was encouraged by the number of time series available in the ICES database for assessment. This was largely due to recent data submissions from France.

The method for modelling VDSI in *Nucella lapillus* should be adopted by OSPAR MON and imposex should be assessed along with other biological effects measurements in 2007.

Similar methods should be developed for other measures of imposex and other species (e.g. VDSI in *Neptunea antiqua* and PCI in *Buccinum undatum*). WGSAEM had recommended that this should be taken forward by the MON Intersessional Group (MIG). This will require the collation of data-sets containing individual rather than indexed data on these parameters. Group members were encouraged to send any appropriate data-sets they may have to FRS to help with this process.

There is still a need to make regional assessments of imposex data and the new methodology should help with that process. A process for stratifying imposex data into areas needs to be identified and the use of WFD water body areas for this purpose is one possibility.

The assessment process would benefit from the submission of individual (rather than indexed) data to the ICES database and this should be encouraged, but may be time consuming. It was pointed out that submission of indexed data along with the sex ratio of the sample would still allow a weighted assessment of indexed data.

12.2 Recent developments in the monitoring of imposex in the Basque region of Spain

MJ Belzunce (AZTI Tecnalia) presented a work on imposex, organotin bioaccumulation and sterility of gastropods in polluted areas of the Basque Country (prepared by G. Rodríguez). Regional monitoring has been carried out in harbours and marinas of the Basque coast (North Spain) in order to (1) evaluate the level of TBT contamination in sediments from harbours and marinas, (2) measure TBT concentrations in prosobranchs and (3) to evaluate the intensity of the biological effects in prosobranchs (imposex).

18 harbours and marinas are being sampled for sediments and organisms. The surface layer of sediment (first centimeter) is taken for analysis: grain size distribution, organic matter content, redox potential and TBT concentrations. Two dominant species of prosobranchs are found in the estuaries of the Basque littoral which are used in this study: *Nassarius reticulatus* and *Nassarius nitidus.*

Three indicators for imposex determination are used in this study: VDSI (vas deferens sequence index), Relative penis length index (RPLI) and Percentage of females with imposex. Some preliminary results on percentage of females with imposex and RPLI are presented here. From the figures below (Figure 12.2.a and 12.2.b) it is clear that there is a high incidence of imposex in all the studied harbours except for the outer zone of the Armintza port. The RPLI shows more differences in the imposex effect between the studied areas.

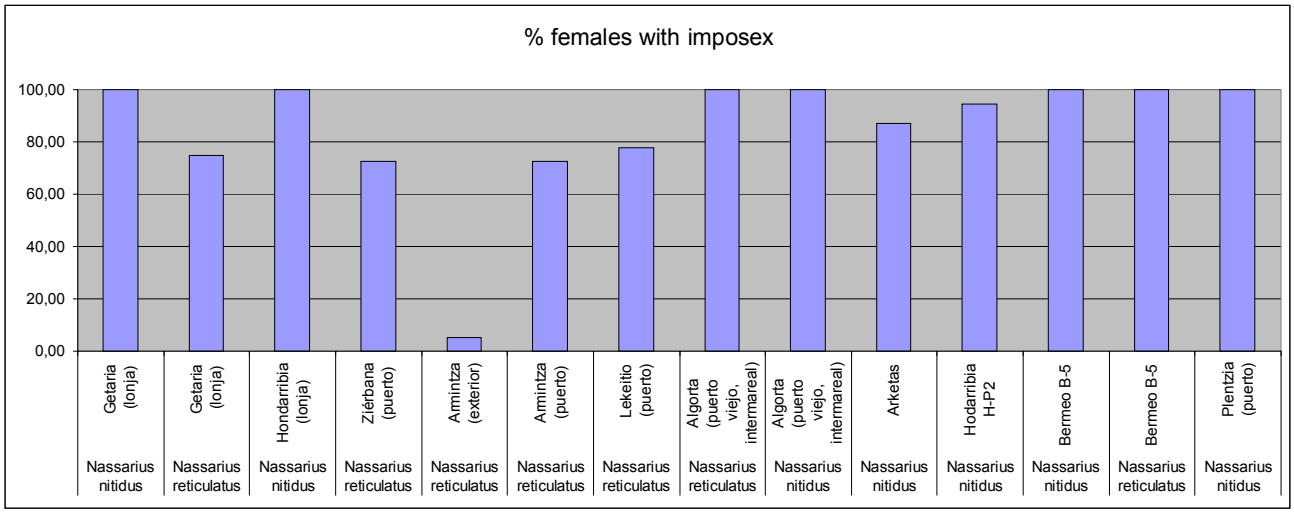

Figure 12.2.a. Percentage of female whelks (*Nassarius* sp.) with imposex studied in marinas and harbours of the Basque coast.

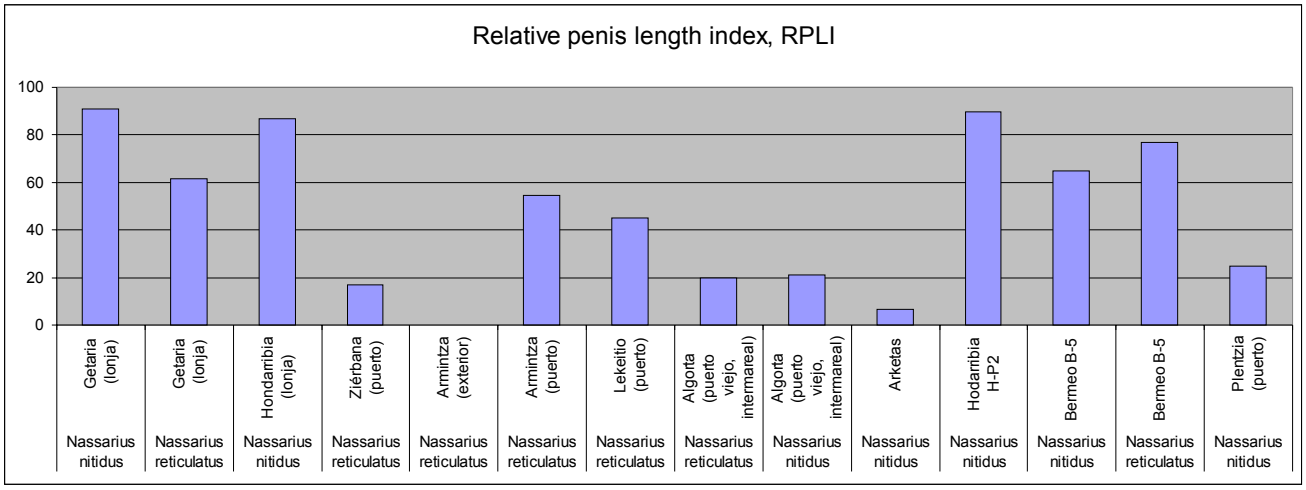

Figure 12.2.b. Imposex measurements (Relative Penis Lenngth Index) of whelks (*Nassarius* sp.) studied in marinas and harbours of the Basque coast.

12.3 OSPAR SIME position paper relating to the OSPAR Guidelines for Contaminant-specific Biological Effects Monitoring (TBT-specific biological effects monitoring)

A position paper from the UK to OSPAR SIME (SIME 07/5/6, OSPAR, 2007) proposing an amendment to Technical Annex 3 of the OSPAR Guidelines for Contaminant-specific Biological Effects Monitoring (TBT-specific biological effects monitoring) was presented to the group as a background document. This document proposed to change the role of organo-tin residue analysis in gastropods from mandatory to a voluntary supporting determinand. This was proposed by the UK following a National TBT effects monitoring survey in 2004, conducted in accordance with the guideline above, where pooled female gastropods from all stations (120) were analysed for TBT, MBT, DBT and TPhT. Residues were only detectable at relatively few sites (19, 7, 0, 0 respectively) where imposex was high and it was difficult to draw any conclusions regarding the contamination history of the affected populations on the basis of these data. Residue analysis is expensive and the cost of analysis did not justify the value added by these data to the monitoring programme.It was therefore recommended that organotin tissue residue analysis be removed as a necessary determinand from the OSPAR

Guidelines. However, it may be used by Contracting Parties as a voluntary determinand to provide ancillary information on the likely timescale of exposure. It is most likely to be of use for this purpose in heavily contaminated areas where a long time series of data can be generated.

Recommendations

OSPAR MON should adopt the new methodology for assessing VDSI in *Nucella lapillus*.

Mean-variance relationships should be established for imposex parameters in other species of gastropods to allow the assessment procedure to be applied over a wider part of the ICES area.

Members should submit individual imposex data to the ICES database, but where this is not possible, indexed data should be submitted along with sex ratio of the sample.

References

Fryer, R. J., and Gubbins, M. J. 2006a. An assessment of temporal trends in VDSI. Annex 9 to ICES WGBEC Report, 2006.

Fryer, R. J., and Gubbins, M. J. 2006b. A regional assessment of VDSI in dogwhelks from Sullom Voe and Yell Sound. Annex 10 to ICES WGBEC Report, 2006.

McCullagh, P., and Nelder, J. A. 1989. Generalized Linear Models (second edition). Chapman & Hall, London.

OSPAR. 2004. Proposal for Assessment Criteria for TBT – Specific Biological Effects. ASMO 04/3/3. OSPAR Environmental Assessment and Monitoring Committee, Stockholm, 29 March–2 April 2004.

13 Consider progress with activities such as BEQUALM, HELCOM area/BSRP project, Prestige Oil Spill and biological effects monitoring programmes in MEDPOL (ToR j)

13.1 Progress Report on BEQUALM – Biological Effects Quality Assurance in Monitoring Programmes

Background

The Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM) is a self-funding quality assurance (QA) scheme for marine biological effects monitoring techniques. BEQUALM was developed under an EU research programme; it was completed in April 2002 and in September 2004 became a self-funding scheme. One of the main purposes of BEQAULM is to provide a QA framework for biological effects techniques within the JAMP CEMP and permit the holistic assessment of data from contracting parties across the OSPAR area. In addition, data submitted to the ICES database should be accompanied by appropriate QA through BEQUALM.

This report gives progress on the BEQAULM programme.

The scheme comprises three components:

Whole Organism (bioassays and fish disease) - led by the Centre for Environment, Fisheries and Aquaculture Science (Cefas)

Biomarkers – led by the Norwegian Institute for Water Research (NIVA)

Community Analysis - led by the UK National Marine Biological Analytical Quality Control Scheme (NMBAQC).

The BEQUALM Project Office (Cefas) acts as the overall administrative and co-ordinating centre for the whole scheme.

In Year 1 of the self-funded scheme the Project Office was established using pump prime funding by Cefas, from internal resources. This was used to set up and maintain a website (www.bequalm.org) and produce the necessary legal documentation (e.g. agreements between participants and lead laboratories, special terms and conditions of sale).

In year 2, PO costs were recovered by a combination of: a) Cefas internal resources (seedcorn), b) levy on Year 1 registration fees, c) levy on assays in Year 2 and d) contributions from other lead laboratories in the scheme. In Year 1, the Whole Organism component achieved surplus income, due to greater than forecast participant numbers for some of the bioassays. This surplus was channelled into marketing and promotional activities.

The programme is currently in its third year and Project Office costs must be fully recovered from within the scheme, by contributions from lead laboratories (including Cefas) via levies on registration fees. The project office levy charged is negotiated between the PO and the Lead Laboratory and is primarily determined by the services the PO provides to the lead laboratory, and takes account of the total number of assays forecast for that work stream.

Activities in Years 1–3

Year 1

- **Whole Organism (Bioassays)**; 4 assays offered– two water column bioassays using the marine copepod *Tisbe battagliai* and the freshwater cladoceran *Daphnia magna*, two whole sediment bioassays using the amphipod *Corophium volutator* and the polychaete *Arenicola marina*. Total of 28 laboratories across all assays participated (Table 1).
- **Whole organism (fish disease)**; 6 laboratories took part in liver and external disease intercalibration exercises, using slides and photographs.
- **Biomarker**; intercalibrations conducted for EROD, CYP1A, VTG and protein analysis. Total of 32 participants across all assays.

Year 2

- **Whole Organism (bioassays)** – portfolio of assays expanded to 6, to include the PARCOM tests using the marine alga *Skeletonema costatum* and the copepod *Acartia tonsa* – perceived to be a gap in availability of external QA/QC for these regulatory tests. Total of 33 laboratories participated (Table 1) – uptake to the marine algae test was good. *Acartia* however was not taken forward due to lack of uptake (only 2 laboratories expressing an interest).
- **Whole Organism (luminescent bacteria) –** luminescent bacteria introduced to the whole organism portfolio, offered to participants in UK and Europe (outside Spain) via a new Lead Laboratory (University of Catalunya (UPC), Spain). 6 laboratories participated through BEQUALM, with a further 20+ from Spain (the latter has been running since 1994, organised by the UPC)
- **Whole organism (fish disease)**; fish disease workshop held at Cefas Weymouth Laboratory, incorporating elements of training and wash-up exercises from Year 1 activities. 25 attendees.
- **Biomarker**; no intercalibration organised, data assessment and development of more appropriate reference materials progressed.
- **Community (Phytoplankton)** - intercalibration conducted for UK/Eire participants under auspices of NMBAQC and BEQUALM, with the Marine Institute, Galway as the Lead Laboratory.

Year 3

- **Whole Organism (bioassays)** - 5 assays offered: *Daphnia*, *Tisbe*, *Skeletonema*, *Corophium*, *Arenicola* and fish disease. *Corophium* assay and luminescent bacteria only conducted due to lack of uptake for the others. 9 participants for *Corophium*.
- **Whole organism (fish disease) –** second round of intercalibrations for liver histopathology (using new "virtual slide" technology) and external disease organised. 6 participants registered.
- **Whole Organism (luminescent bacteria) –** continuing as previous year; anticipated greater number of UK/Europe participants registering via BEQUALM but same number (6) obtained.
- **Community (Phytoplankton)** – Phytoplankton assemblage analysis second intercalibration conducted. Relationship with BEQUALM strengthened through formation of agreement between MI and Cefas (CTL) for financial management of the intercalibration. 18 participants registered, wash-up workshop scheduled for early March.
- **Biomarker** – NIVA to plan to offer EROD, protein, Mt and vtg but will not progress unless there is sufficient uptake.

The number of registrations for each assay in each year are presented in Table 13.1.

Table 13.1 Number of participants for each BEQUALM component and activity Years 1–3.

COMPONENT	ACTIVITY	YEAR 1	YEAR 2	YEAR 3
Whole Organism	Corophium	10	7	9
	Arenicola	7	5	3
	Daphnia	4	8	1
	Tisbe	7	5	2
	Marine algae	__	8	2
	Acartia	__	2	__
	Fish disease	6	25	6
	Luminescent bacteria	__	6	6
Biomarker	VTG	5	__	?
	CYP1a	5	__	?
	EROD	13	__	?
	Protein	9	__	?
Community	Phytoplankton assemblage	__	16	18

Figures in red denote that the intercalibration for that assay did not proceed due to less than optimal participant numbers.

Issues

a) Declining interest in bioassays and requirement to cancel the majority of intercalibrations in Year 3.

Participant numbers between years 1 and 2 declined slightly for 3 of the assays – *Corophium*, *Arenicola* and *Tisbe* (i.e. the JAMP recommended assays); for the *Daphnia* assay numbers doubled and a good response was obtained for the newly introduced marine algae assay, which is part of the PARCOM suite of toxicity tests. The PARCOM marine copepod test using *Acartia* received a poor response and was not taken forward for intercalibration. In this third year of the scheme, response to the call for registrations has been very poor all round, despite several emails to previous participants and potential new participants. The result is that

only the *Corophium* assay is proceeding for Year 3. Reasons for declining numbers, as fed back from previous participants, are varied and include price, availability of resources and time to conduct QA/QC, lack of specific drivers requiring QA/QC and availability of other schemes for achieving external QA. The latter include such schemes as Aquacheck in the UK, which offers QA for the *Daphnia* assay at a significantly lower price than BEQUALM, and a new proficiency testing scheme for laboratories that conduct toxicity testing as part of the UK Direct Toxicity Assessment (DTA) of effluents as part of IPPC legislation. There is significant overlap between this scheme and BEQUALM in the assays that are offered, including *Daphnia*, *Tisbe* and marine algae. The scheme is being run free of charge by the UK Environment Agency. This has meant that regulatory laboratories who previously obtained their external QA/QC from BEQUALM can achieve the same outcome by participating in an alternative, more relevant and cost free scheme and have thus withdrawn from BEQUALM. BEQUALM was set up primarily to provide a framework for QA/QC for laboratories submitting monitoring data to OSPAR via ICES. Currently, only the UK labs that submit data for the National Monitoring Programme regularly participate in the relevant assays. It would seem that, outside of the UK, there is a distinct lack of uptake by equivalent laboratories.

b) Lack of response to expression of interest for new assays

In an attempt to address perceived gaps in availability of QA/QC for (a) JAMP and CEMP assays that were included in the BEQUALM research phase and (b) new GMO assays, a call for expressions of interest was put on the website and also potential participants emailed. The assays proposed were the Yeast oestrogen screen, lysosomal fragility, DNA adducts and acetylcholinesterase activity. The response to this call was nil with the exception of one positive response for lysosomal fragility. Again, this is very disappointing as it is recognised by the ICES WGBEC that there is a need within OSPAR for contracting parties to submit this type of data, particularly DNA adducts which are part of the CEMP.

c) Failure to progress with Biomarker component beyond Year 1

NIVA suffered a 5K loss in Year 1 as a result of high set-up costs with regards to dosing fish in real time and preparing homogenous tissues (i.e. reference materials). Since then NIVA have experienced internal changes and have been unable to secure funding to maintain the biomarker programme. Participant numbers for all assays, particularly the EROD and protein, were encouraging and demonstrate that the interest and need for QA/QC for these exists. An alternative approach to producing the reference material has been sought to keep Lead Laboratory costs to the minimum. The current situation is that NIVA will offer in year three, protein, EROD, and vtg.

d) Failure to extend Benthic Community analysis outside UK

The NMBAQC has continued to operate as normal within the UK but has not been able to expand the programme to the rest of Europe. In Year 1 of BEQUALM, the NMBAQC extended the UK scheme into Europe by inviting organisations to participate in 2 of the 5 components offered. Uptake from Europe has been very disappointing, with only one laboratory, from Germany, participating in the first year and one from Eire in 2005. (It should be noted that 20 laboratories throughout Europe took part in the EU BEQUALM development programme). Each year, around 20 organisations from the UK participate in this scheme. Despite the OSPAR requirement for AQC for benthic analyses across the convention, laboratories are not signing up to the scheme. The reasons for this were not clear. To help address this issue, a member of the Project Office attended the ICES STGQAB in February 2006 to give an overview of the scheme and obtain feedback on the views of European laboratories. Disappointingly there was no representative from OSPAR at this meeting, the majority of attendees being affiliated with HELCOM. Some useful feedback was received from the German laboratory that participated in Year 1. The main problems that were encountered, which could be applicable to a large proportion of European participants,

included the lack of available "own samples" for submission to the scheme for checking, unfamiliarity with some of the species that formed part of the ring test (although it was highlighted that the NMBQAC do not fail a laboratory if samples cannot be identified or are identified incorrectly; this part of the scheme is considered to be a training exercise and allows taxonomists to broaden their skills and become familiar with species that they may come across on rare occasions) and the cost of participating, not just in terms of the registration fee to be paid but the time resource required to conduct the QA. If a laboratory is participating in national, regional and international QA programmes then potentially a substantial amount of time will be taken up with these exercises and this is not sustainable. The STGQAB recommended that NMBAQC/BEQUALM further develop and take forward a questionnaire that was produced by the SGQAB in 2005, which would gather information from laboratories on their required level of participation in QA exercises and more specifically what is involved in each. This would allow NMBAQC/BEQUALM to explore ways to harmonise QA activities, in order to reduce the workload for a single laboratory and also develop mechanisms to overcome the problems of regionality.

Despite being unable to generate interest in benthos AQC outside the UK, the NMBAQC is continuing to extend its remit, taking forward AQC requirements under the WFD by organizing ring tests for juvenile fish and macro algae and planning and conducting workshops on transitional fish identification and epibiota.

Conclusion

Each Lead Laboratory has yearly fixed costs associated with organising and running each intercalibration; these costs determine the registration fee; the greater the number of participants the lower the per-participant fee. This also applies to the Project Office levy on a Component basis. The downturn in participant numbers for the JAMP bioassays, together with lack of progression with the biomarker and benthic community components is clearly a situation that cannot be considered financially sustainable. No Project Office contributions have been received from either the Biomarker or benthic community components and thus effort into taking the scheme forward in these areas has had to cease. In addition, the PO staff resource associated with the whole organism component has been significantly reduced (since numbers have declined and not increased as anticipated), to minimise the levy on bioassays and fish disease registration fees.

All biological effects data submitted to the OSPAR database should have accompanying AQC provided by BEQUALM or QUASIMEME in order to permit the holistic assessment of data across the OSPAR maritime area. ICES should note the current position that if circumstances do not change then BEQUALM may no longer be a financially viable organisation and hence QA/QC for OSPAR biological effects measurements will be in jeopardy.

Recommendation

That ICES should inform OSPAR of the current position.

WGBEC would recommend that ICES inform other appropriate ICES Working Groups of the activities of BEQUALM in relation to benthic community analysis and phytoplankton assemblages to avoid duplication of effort and improve harmonisation of AQC. WGBEC would recommend that a report of the MEDPOL funded external QA scheme be presented at its 2008 meeting.

Justification

AQC data is important to allow for the co-ordinated assessment of data across the OSPAR Maritime Area, and should accompany all data submitted to the ICES database. It is important

that AQC procedures are therefore harmonised, in place and used by Contracting Parties. Failure to use AQC schemes downgrades the use of the data for assessment purposes.

13.2 Progress with activities in the HELCOM area

Outcome of the BEEP project

In September 2006, results from the EU project BEEP Baltic Sea component were published as a special issue of Marine Pollution Bulletin (http://www.sciencedirect.com/science/journal/0025326X) that contained 12 scientific papers, including a suggested outline for a biological effects monitoring programme in the Baltic Sea. This compilation has been recognised also by HELCOM as a key source of information and guidelines in developing biological effects monitoring in the Baltic Sea region.

Activities within HELCOM and BSRP

Activities in the future implementation of biomarker methods into the HELCOM monitoring programme has been carried out both in HELCOM workshops (incl. MONPRO project for the revision of the HELCOM monitoring programme) and within the Baltic Sea Regional Project (BSRP) Study Group for Ecosystem Health (SGEH). The work within the SGEH has been focused on the development of indicators of biological effects of hazardous substances measured at different levels of biological organization. From 2007 onwards the SGEH will continue as a regular WG of ICES. Documents on the outcome of the work can be found in the reports of SGEH at the ICES website.

Emerging from SGEH activities, ICES/BSRP, HELCOM and partner Baltic Sea countries plan to organise a project in 2009 on the Ecosystem Health of the Gulf of Finland ("ICES/BSRP and HELCOM Sea-going Demonstration Project on Integrated Multidisciplinary Assessment of the Ecosystem Health of the Gulf of Finland"), co-ordinated by Kari K. Lehtonen (Finnish Institute of Marine Research, Helsinki, Finland [FIMR]) and Thomas Lang (Federal Research Centre for Fisheries, Institute for Fishery Ecology, Cuxhaven, Germany [BFAFi/IFÖ). The idea was first developed during the 2005 ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM), which provided recommendations supporting the activity (http://www.ices.dk/reports/BCC/2006/WKFDM06.pdf):

- "Baltic Sea countries harmonise the components of their national marine monitoring and assessment programmes in order to implement an integrated programme on contaminants (and other anthropogenic stressors) and their biological effects";
- "Baltic Sea countries and HELCOM investigate the potential for an internationally coordinated integrated monitoring programme in the Baltic Sea, encompassing joint sampling campaigns and the involvement of appointed expert laboratories in the Baltic countries responsible for the conduct of specific analytical measurements";
- "ICES/BSRP, HELCOM and Baltic Sea countries consider to organise an international demonstration project in 2007 or 2008 on the ecosystem health of the Gulf of Finland, providing baseline data and assessing the feasibility of coordinated sample collection and analysis."

The coming marine strategies are aiming at an ecosystem-based approach to monitoring with the goal to find suitable indicators for a healthy ecosystem and appropriate measures. The proposal is based on the implementation of the revised HELCOM monitoring programme concerning hazardous substances, with focus on their biological effects on the Baltic Sea ecosystem. The revision has been elaborated during the HELCOM MONPRO project and SGEH, with the latest update made in the HELCOM Expert Workshop aimed at the

elaboration of indicators and targets for the hazardous substances goal of the HELCOM Baltic Sea Action Plan (BSAP) (Vilnius 9/06), as well as the outcome of the EU BEEP project.

Objectives of the Gulf of Finland demonstration project are to (1) develop integrated chemical-biological monitoring of hazardous substances in the Baltic Sea, (2) enable an ecosystem-based approach of the joint Baltic Sea implementation of the future EC Marine Strategy, (3) give support to the HELCOM Baltic Sea Action Plan (BSAP) to identify the main pollution characteristics in each sub-region. The main practical aims of the project are (1) identification of suitable indicators of effects of hazardous substances at different biological levels in the Gulf of Finland (2) assessing the "ecosystem health" of the Gulf of Finland, and (3) developing a strategy for integrated environmental monitoring of the Gulf of Finland marine environment.

Although the emphasis of the GOF project is on the application of parameters directly or closely related to the effects of hazardous substances (biomarkers, histopathology, fish diseases), various studies encompassing other relevant ecosystem health indicators such as biodiversity, community structure, water quality, productivity, etc. will be studied during the cruises providing new data and insights to integrated ecosystem assessments (e.g. ICES WGIAB).

The project should be fully co-ordinated with activities of the BSRP SGEH (provided that funding for the second phase continues). In addition to BSRP funding the project must receive national support from the participating Baltic Sea countries. Field sampling is carried out in late summer–autumn 2009 as an extensive coastal–open sea sampling network in the Gulf of Finland. The research vessels involved consists of R/V Aranda (Finland; oceanographic sampling) and R/V Walther Herwig III (Germany; fish trawling). Assistance from local vessels from Estonia, Finland, and Russia is planned. After the seagoing workshop special practical workshops (e.g. biomarker techniques and intercalibration) will be arranged. The results of the workshop will be presented at an open wrap-up seminar in 2010.

Part of the project costs is expected to be covered by the BSRP (if its funding by GEF is continued in 2009). Ship costs will be covered by national budgets. Funding for other costs will be sought from various sources during 2007-2008 (e.g. BONUS programme; see below).Activities carried out during the Gulf of Finland sea-going workshop are intended to be harmonised with the ICON workshop carried out in the North Sea in 2008-2009. In addition, possibilities to arrange intercalibration exercises with institutes and programmes in the MED POL area will be examined.

The BONUS programme

A research programme under the BONUS programme (http://www.bonusportal.org) will be launched in the Baltic Sea with the first call for proposals opening in autumn 2007. A total amount of 25 M € (18 M € from the main funding agencies around the Baltic Sea + 7 M € from the EU) will be allocated to research activities covering all main themes of marine research. Selection of funded projects is expected to be based on the Baltic Sea Science Plan (2006) developed during the BONUS programme.

Build-up of a consortium to prepare a project application for the forthcoming BONUS call was initiated in early 2007, basing on the network of partners of the EU BEEP project in the Baltic Sea. The project is planned to consist of two main components: Component 1 – Research: Effects of anthropogenic contaminants on the Baltic Sea ecosystem, Component 2 – Application: Developing integrated monitoring of hazardous substances and their effects in coastal and open-sea areas of the Baltic Sea.

13.3 Progress with biological effects monitoring programmes in Spain

C. Martínez-Gómez (IEO, Spain) presented a review of the ongoing biological effects monitoring programs in Spanish Atlantic and Mediterranean waters.

Biological effects monitoring of contaminants (BEC) are usually repetitive observations of one or more biomarkers for defined purposes, according to a prearranged schedule over time and space, using comparable and standardized methods. In Spain, different monitoring programmes that include biomarkers are currently under way.

a) Biomonitoring programmes on a regional scale (OSPAR /Barcelona conventions):

Spain is a contracting party to both OSPAR and to the Barcelona Convention. Due to its geographical location, Spain contributes to both the CEMP and MEDPOL programmes.

To meet the obligations of the conventions, during the last decade the Spanish Institute of Oceanography, IEO (www.ieo.es), has been performing chemical pollution monitoring activities in both Spanish Atlantic and Mediterranean waters through several research projects. Since 2001, the Oceanographic Centre of Murcia (COMU; IEO) started to include selected biomarkers in the pollution monitoring activities conducted along the Iberian Mediterranean coast using wild mussels (*Mytilus galloprovincialis*) as the target organism (BIOMEJIMED Project, Annex 9). Through this, some valuable information on mussel biomarkers has been generated. Further pollution monitoring activities of the Iberian Mediterranean and North-Atlantic Spanish waters have been undertaken by the Spanish Institute of Oceanography (IEO) since November 2005, under the responsibility of the Ministry of Environment. More recently, two other research projects (MEDPOLIEO project, Annex 9) and CONOSPAR were planned and approved by the Spanish Institute of Oceanography (IEO). These projects started in January 2006 having, as main objectives, the determination of spatial distribution and temporal trends of chemical pollution in coastal and reference areas, as well as to seek evidence of detrimental biological effects. In both projects, sediment samples and wild mussels are analysed for selected pollutants. Partly because of the different requirements of the CEMP and MEDPOL programmes, some measurements relate to one but not the other programme. Concerning biological effects, only the prevalence of imposex in *Nucella sp.* and *Nassarius sp.* is included in the north-Atlantic Spanish programme (since 2005) (See Annex 9). In the Iberian Mediterranean programme, a suite of biomarkers are being monitored: Lysosomal membrane stability (LMS) is measured in wild mussels, EROD activity and genotoxic damages in feral red mullet (*Mullus barbatus),* and Metallothionein (MT) content in both species. Biological effects data obtained in both projects are stored in the OSPAR and MEDPOL databases.

b) Biomonitoring programmes on a local scale: Autonomic Communities (CCAA):

Spain is organized into different territories by the Autonomic Communities (CCAA). As far we know, only in the Basque country some biomarkers (bioassays and imposex) are included in marine pollution monitoring programmes. These are conducted by AZTI-Tecnalia (www.azti.es), which is a private foundation (see Agenda Item 12).

c) Monitoring research activities:

Though they cannot be considered in a rigorous sense as monitoring programmes, supplementary monitoring research activities on biomarker responses under field conditions have been performed along the Spanish coastal waters and continental shelf. Data obtained are of great valuable to direct future monitoring programmes.

- As a result of the Prestige oil-spill in November 2002, Marine Contamination and Biological Effects research group (MCBE, Oceanographic centre of Murcia), belonging to the Spanish Institute of Oceanography (IEO), initiated a mid-term

study in order to assess a suite of biomarker responses in two representative demersal fish species (*Lepidorhombus boscii* y *Callyonimus lyra)* from the northern Iberian shelf. Selected biomarkers (genotoxic damage, EROD, Glutathion-s-transferase, catalase and glutathione reductase activities) were determined in liver. Liver samples were collected in April 2003, October 2003, October 2004 and October 2005 at seven different geographical areas of the continental shelf. Detailed information about the sampling strategy and data availability of such activities is included in 2004-2007 WGBEC reports and also in Martínez-Gómez *et al*., 2006.

- Recently, valuable biomarker data for mussels and for red mullets in areas under Spanish jurisdiction were obtained by Spanish partners within the framework of the BEEP project (BCTA group/UPV and IIQAB-CSIC). Detailed information about the sampling strategy and data availability of such activities is included in Annex 10).
- Data of MT content and EROD activity in *Mullus barbatus* caught in six areas of the Iberian Mediterranean coast were also obtained within the framework of the BIOMARC project by the Spanish research groups of MCBE (IEO) Environmental Toxicology group of the Environmental Chemistry department (IIQAB-CSIC). Data obtained from BIOMARC project are limited to 2 samplings surveys including 10 stations for red mullets. Detailed information about the sampling strategy and data availability of such activities is included in Annex 10).
- As the same way, after the Prestige oil-spill, Cell Biology in Environmental Toxicology group (BCTA, Department of Zoology and Cell Biology), belonging to the University of the Basque Country (UPV, Spain), initiated a mid-term study in order to assess a suite of biomarker responses in wild mussels (*Mytilus galloprovincialis*) along the North-Atlantic Spanish coast. Detailed information about the sampling strategy and data availability of such activities is included in WGBEC report 2004 and 2005, and also in Annex 11.
- During the last 20 years the group of Cell Biology in Environmental Toxicology (BCTA) from the University of the Basque Country has been involved in studies about the biological effects of contaminants in both terrestrial and aquatic ecosystems, especially in estuarine and coastal ecosystems. Detailed information about the sampling strategy and data availability of such activities is also included in Annex 11.

Comparability of the biomarker data in Spain

Some Research Groups (MCBE, ET, BCTA and ECIMAT/U. Vigo) have participated in inter-comparison exercises within BEQUALM and MEDPOL Programmes framework. Data Quality Assurance has been tested only for the following Biological effect techniques:

RESEARCH GROUP	TISSUE	YEAR	PROGRAMME	BIOMARKERS
MCBE (IEO)	Liver (*Pleuronectes platessa*)	2000	BEQUALM	EROD
ET (IIQAB-CSIC)	Liver (*Pleuronectes platessa*)	2000	BEQUALM	EROD
BCTA (UPV)	Liver (Fish)	2000	BEQUALM	MT
ET (IIQAB-CSIC)	Liver (Fish)	2001	MEDPOL	EROD
BCTA (UPV)	Liver (Fish)	2001	MEDPOL	EROD
MCBE (IEO)	Digestive gland (mussel)	2001	MEDPOL	MT
MCBE (IEO)	Digestive gland (mussel)	2003	MEDPOL	MT
ET (IIQAB-CSIC)	Gills (mussels)	2003	BEEP Project	AchE
MCBE (IEO)	Digestive gland (mussel)	2004	MEDPOL	MT
MCBE (IEO)	Digestive gland (mussel)	2005	MEDPOL	MT
BCTA (UPV)	Digestive gland (mussel)	2005	MEDPOL	MT
BCTA (UPV)	Digestive gland (mussel)	2005	MEDPOL	NLLA
BCTA (UPV)	Digestive gland (mussel)	2005	MEDPOL	LL
BCTA (UPV)	Digestive gland (mussel)	2005	MEDPOL	LMS
BCTA (UPV)	Liver (Fish)	2005	MEDPOL	EROD
MCBE (IEO)	Liver (Atlantic cod)	2005	BEQUALM	EROD
CBET (UPV)	Liver (Atlantic cod)	2005	BEQUALM	EROD
ECIMAT (U. Vigo)	Amphipod bioassays	2003	BEQUALM	

Keys: LMN (Lysosomal Membrane Stability), NLLA (Neutral lipid lysosomal accumulation), LL (Lipofucsin Lysosomal).

Natural variability and fluctuations in relation to the reproductive cycle, food availability, sex and other biotic and abiotic factors make that only data obtained from the same specie, within the same length range interval and sampled in the same seasonal period can be usefully compared.

Prof. Dr. Cajaraville M. P. reported that the data produced in CBAT research group (UPV) are comparable to data produced in the reference laboratory of Prof A. Viarengo (MEDPOL) and in other Mediterranean and in general European laboratories, for the same species and same biomarker.

Dr Campillo J. A. reported that MT and EROD data produced in MCBE (IEO) research group are comparable to data produced in the reference laboratory of Prof Viarengo A. (MEDPOL) and EROD data produced in the reference Norwegian Institute for Water Research (BEQUALM), respectively. So far, currents Quality Assurance Programmes have not offered inter-comparison exercises for Neutral Red Retention Assay. Therefore, Assurance Quality for Lysosomal Membrane Stability data obtained by MCBE Group (IEO) is not guaranteed. Nevertheless, it was included in a BEQUALM Inter-comparison exercise but with only limited participation.

Quality Assurance Control (QAC) results for EROD data produced by Environmental Toxicology group (ET, IIQAB-CSIC) are unknown. As far we know, this research group participated in two inter-comparison exercises organized by the reference laboratory of Prof A. Viarengo (MEDPOL) and the reference Norwegian Institute for Water Research (BEQUALM). AchE data obtained by Environmental Toxicology group research group (ET, IIQAB-CSIC) are comparable to data produced in the European laboratories. This research group participated in an inter-comparison exercise on AchE measurements in mussels organized within the framework of BEEP project during November 2003.

Availability of adequate time series and reasonable coverage

Most of the biomarkers data available in Spain were obtained from different monitoring research activities. Important hot spots, marine reserves, urban centres and sea farm facilities were sampled along the Iberian Mediterranean coast and Basque Country coast. However there is no full coverage in the Spanish coastal waters of the Iberian Peninsula.

To date, in Spain, availability of biomarker data for future time series and reasonable coverage is only achieved for MT and LMS data obtained within BIOMEJIMED-MEDPOLIEO Projects:

- Lysosomal membrane stability in mussels: Annual survey, data from 2002 to 2006; 10–12 sampling sites along Iberian Mediterranean coast
- Metallothionein in mussels: Annual survey, data from 2001 to 2006; 10-18 sampling sites along Iberian Mediterranean coast

13.4 Progress with biological effects monitoring in projects related with the Prestige oil spill

In order to monitor research projects related to the *Prestige* oil spill, a second Symposium (http://otvm.uvigo.es/vertimar2007) is going to take place, from 5–8 July 2007 in Vigo (Galicia, Spain). This Symposium is organised by the Technical Bureau of Marine Spills and by the Scientific Coordination Commission of Strategic Action (http://otvm.uvigo.es), created by the Spanish Government for studying the effects of Prestige oil-tanker wreck. One of the topics will be discussed is "Impact on biological systems", and final results of biomarker studies will be presented.

Concerning biological effects responses related with the Prestige oil spill, some interesting articles have been recently published. Complete references are provided in Annex 12. Most of these works were focused in the assessment of different biological responses using toxicity bioassays approach.

TARGET ORGANISMS	BIOMARKER	TYPE OF STUDY	REFERENCES
Cells lines (RTL-W1, RTG-2)	EROD activity CYP1A levels	Toxicity bioassay	Casado *et al.*, 2006.
Algae (*Chorella vulgaris*, *Skeletonema costatum*)	Growth inhibition test	Toxicity bioassay	Navas *et al.*, 2006 Mariño-Balsa *et al.*, 2003
Invertebrates (*Daphnia magna*)	Acute and reproductive toxicity test	Toxicity bioassay	Navas *et al.*, 2006
Copepod (*Oithona davisae*)	Behaviour and mortality bioassay	Toxicity bioassay	Barata *et al.*, 2005
Amphipod	Microtox ® Behaviour and mortality 10-day bioassay	Toxicity bioassay	Morales-Caselles *et al.*, 2006
Sea urchin (*Paracentrotus lividus*) Bivalve (*Crassostrea gigas* and *Venerupis pullastra, Venerupis rhomboideus*)	Embryogenesis bioassays	Toxicity bioassay	Beiras and Saco-Alvarez, 2006. Fernández *et al.*, 2006. Franco *et al.*, 2006 Mariño-Balsa *et al.*, 2003
Bivalve (*Venerupis pullastra and Tapes decussates)*	Burrowing behaviour bioassays	Toxicity bioassay	Mariño-Balsa *et al.*, 2003.
Mussels (*Mytilus galloprovincialis)*	Lysosomal responses Peroxisome proliferation Acyl-CoA Oxidase activity Neutral lipids accumulation	Field study	Orbea *et al.*, 2006 Cajaraville *et al.*, 2006 Marigómez *et al.*, 2006
	Comet assay	Toxicity bioassay	Laffon *et al.*, 2006. Perez-Cadahia *et al.*, 2004
	Cellular immune parameters (i.e. Phagocytic activity)	4-months Toxicity bioassay	Ordas M.C., 2007
Fish (*Chelon labrosus*)	Genomics Proteomics Peroxisome proliferation Acyl-CoA Oxidase activity Neutral lipids accumulation Lysosomal responses	Short-term toxicity bioassays	Bilbao *et al.*, 2006 Raingeard *et al.*, 2006
Fish (*Lepidorhombus boscii* and *Callionymus lyra*)	EROD activity GST activity Catalase activity Glutathione reductase activity Genotoxic damages	Field study	Martínez-Gómez *et al.*, 2006
Fish (*Sparus aurata* and *Solea senegalensis*)	Metallothionein content EROD activity Histopathology in gills and liver	2-months sediment toxicity bioassay	Morales-Caselles *et al.*, 2006 Jiménez-Tenorio *et al.*, in press.
Fish (*Merluccius merluccius* and *Engraulis encrasicolus*)	Liver histopathology	Field study	Marigómez *et al.*, 2006

Final results of the field studies performed along the area affected by the Prestige oil-spill are not completely published. However, information about these studies is available and it is presented to the WGBEC.

Biomonitoring along the north-Iberian continental shelf using demersal fish species (four-spotted megrim and dragonet) as sentinel organisms

C. Martínez-Gómez (IEO, Spain) made a presentation showing the mid-term biomarker monitoring results (2003–2004–2005) obtained by the Oceanographic Centre of Murcia (IEO) within the framework of the "Special Actions" in 2003 and DEEP II project (Distribution, fate and effects of the fuel oil in the coastal zone affected by the *Prestige* oil spill. VEM 2003-20068; funded by the Spanish Ministry of Science and Technology).

1) Two demersal fish species (Lepidorhombus boscii and Callionymus lyra) were identified as target species for future biomonitoring purposes along the North Iberian continental shelf, though the use of Callyonimus lyra is compromised by the scarcity in the catches in the Gulf of Biscay. In both species, the assessment of a suite of enzymatic biomarker responses (EROD, GST, Catalase and Glutathione reductase activities) and genotoxic damages, have permitted to identify areas in the North Iberian shelf, in which demersal fish populations are more exposed to organic pollutants (Finisterre, Cantabria and Basque Country) than in others ones (Galicia S. and Asturias W.).

2) The followed sampling strategy (only immature male and female specimens, within the same size range) have permitted to know that differences on biomarker responses due to sex will not have a major influence on the results as a whole.

3) Though the lack of pre-Prestige oil spill (POS) biomarker data and the chronic pollution existing along the shelf did not allow to attribute the biomarker responses observed in fish populations directly and/or exclusively to the POS, generalized and significant decrease on biomarker responses has been found along the north Iberian shelf during the period studied.

4) Besides, significant positive linear correlations were found between antioxidant activities in L. boscii and tar aggregate data five months after the accident.

5) Biomarker values observed in some areas of the Northern Iberian shelf, three years after the POS, may be considered as background levels for these species. However, a strong induction of EROD activity in C. lyra (representative target species for inner Iberian shelf) has been found in Finisterre and Galicia S. areas during the last survey (October 2005).

6) An increase of genotoxic damages was also detected in both species three years after the POS. Further research would be required to establish significance of these findings and to fully determine the potential effect that POS and chronic contaminant exposure are causing on the shelf ecosystems.

B) Biomonitoring along the coast using mussels as sentinel organisms

Prof. Miren Cajaraville (University of the Basque Country/UPV, Spain; Laboratory of Cell Biology and Histology, Department of Zoology and Animal Cell Biology) contributed to this item sending to the group the results of the mid-term monitoring programs obtained within the frame work of the "Special Actions" in 2003, PRESTEPSE project in the period 2004-2006 (VEM2003-20082-CO6-01-PRESTEPSE) in the period 2004-2006 (funded by the Spanish Ministry of Science and Technology), ETORTEK actions-IMPRES, 2003-2007 (funded by the Basque Government) and by the University of the Basque Country (grant to consolidated research groups, since 2000).

Mussels were collected in April, July and September of 2003, February, April, July and October of 2004, April, July and October of 2005 and April of 2006 along the Basque coast (Muskiz, Arrigunaga, Gorliz, Bakio, Mundaka, Mutriku, Orio and Hondarribia), the Cantabrian coast (Llanes, San Vicente, Suances, Pedreña and Laredo) and the

Galician/Portuguese coast (Sao Bartolomeu do Mar, Cíes, Ons, Oia, Aguiño, Caldebarcos, Camelle, Segaño and Estaca de Bares). Thus, for most localities, a historical data series is available from April 2003 to April 2006 (in total 11 sampling campaigns).

1) The studies carried out have provided knowledge about the biology of the mussel, one of the most widely used sentinel organism for the monitoring of the biological effects of pollution. Especially, the application of innovative "-omics" techniques (genomics, proteomics) have contributed to provide new sequences of toxicological interest to international databases.

2) The analysis of the Glu-5' gene, coding for the byssus adhesive protein, and the comparative study of seasonal variability of the studied biomarkers in the localities of Oia (Galicia) and Mundaka (Basque Country) have allowed to eliminate two possible variability sources that could have interfered in the survey. Thus, it has been determined that the mussel species collected in all localities from Oia to Hondarribia is Mytilus galloprovincialis and that, in general, there are no geographical differences (among stations) either in the range of the values nor in the seasonal variation pattern for most of the biomarkers and parameters analyzed.

3) The biological impact of the Prestige spill has been detected since the first sampling campaign in April 2003. Evidences indicate that the Prestige oil spill inhibited lipid catabolism in the peroxisomes, caused significant effects in the digestive lysosomal system and in the structure and cellular composition of digestive diverticula, and provoked alterations in gamete development accompanied by abnormally high prevalences of gamete atresia in mussels from many localities. The impact was more marked in Galicia and, to a lower degree, it was also evidenced in the Basque Country, although in some Basque localities such as Arrigunaga the chronic pollution could have interfered in the measurement of the effects of the Prestige oil spill.

4) Most of the biomarkers studied indicate that the recovery process of the health of mussels started in mid 2004, while some others seem to indicate that the stress situation or health deterioration has persisted until 2005.

5) Once that the first effects of the Prestige oil spill have been attenuated, it is recommended to continue the studies of the monitoring of the biological effects of pollution in the Basque coast using mussels as sentinel organisms and the early warning biomarker approach with two main objectives: first, to obtain basal values of the biomarkers studied in order to use them as reference values for future possible accidental oil or chemical spills, and, second, to evaluate the effects of chronic pollution due to industrial and agricultural activities, urban effluents, etc.

C) Monitoring of the effects on fish of commercial value (anchovy and hake) in the platform of Biscay Gulf

The studies with fish of commercial value have been carried out with specimens provided by AZTI-TECNALIA, the group in charge of fish collection. Briefly, in the period 2003-2006 anchovies (adult males and females) were sampled in spring in two areas (namely North and South) in front of the Basque coast, except in 2006 in which due to the low captures only the samples from the North area were obtained. In the case of hake, two sampling campaigns have been carried out per year, at the end of spring and at the end of autumn, in 2004 and 2005 in a single area in front of the Basque coast (immature male and female, mature male and female). Hake samplings have not been always complete due to the difficulties in catching specimens of a desired group, for instance, no adult females were found in the autumn campaign in 2005. In spring 2006 hake samples were not obtained due to the lack of catches.

1) The studies carried out in the project have given rise to basic data about the biology of the anchovy and hake of the Biscay Gulf, increasing the previous knowledge on these two species. Especially, data on peroxisomal and biotransformation enzyme activities, structure and activity of the lysosomal

system, and prevalence of different hepatic histopathological alterations have been obtained for the first time. In addition, in hake different genes of toxicological interest have been cloned and their expression studied, being these sequences sent to international databases.

2) The obtained results do not allow to draw clear conclusions about the possible exposure of the studied anchovy and hake populations to the Prestige oil spill and about the possible impact of such exposure. This lack of concluding data is due at least to the following factors: a) lack of data on the situation previous to the Prestige oil spill, b) lack of a control or reference population, c) the difficulty to obtain the desired samples, d) the relatively short duration of the study, and e) the huge biological variability found.

3) Nevertheless, the obtained data do not indicate that the Prestige oil had provoked any long-term deleterious effect on the populations of anchovy and hake studied.

4) Although both anchovy and hake have a high commercial value, it is not recommended to continue the monitoring of these species as they are not appropriate sentinel species for the quantification of biological effects of pollution due to their scarcity, type of fatty liver in hake which hampers the analysis and lack of comparative data in other European or world regions. Instead of anchovy or hake, it is recommendable to continue the studies in mullet and turbot, or even in other species such as sticklebacks, to assess their possible usefulness as sentinels of pollution in the Gulf of Biscay.

AZTI-Tecnalia submitted to the group the results obtained in their monitoring activities with hake and anchovy species from Basque Country area after the Prestige oil spill (see Annex 12).

References

Barata, C., Calbet, A., Saiz, E., Ortiz, L., and Bayota, J. M. 2005. Predicting single and mixture toxicity of petrogenic polycyclic aromatic hydrocarbons to the copepod *Oithona davisae*. Environmental Toxicology and Chemistry, 24(11): 2992–2999.

Beiras, R., and Saco Alvarez, L. 2006. Toxicity of seawater and sand affected by the Prestige fuel-oil using bivalve and sea urchin embryogenesis bioassays. Water, Air, and Soil Pollution, 177(1–4): 457–466.

Bilbao, E., Diaz de Cerio, O., Cajaraville, M. P., and Cancio, I. 2006. Cloning and expression pattern of peroxisomal enzymes in the mussel *Mytilus galloprovincialis* and in the thicklip grey mullet *Chelon labrosus*: generation of new tools to study peroxisome proliferation. Marine Environmental Research, 62: 109–112.

Cajaraville, M. P., Garmendia L., Orbea, A., Werding, R., Gómez-Mendiuka, A., Izaguirre, U., Soto, M., and Marigómez, I. 2006. Signs of recovery of mussels health two years after the *Prestige* oil spill. Marine Environmental Research, 62: 337–341.

Casado, S., Babín, M., Tarazona, J. V., and Navas, J. M. 2006. Activation of the Aryl hydrocarbon receptor by the water soluble fraction of the Prestige fuel oil. Marine Environmental Research. 62(1): 75–76.

Fernández, N., César, A., González, M., and delValls, T. A. 2006. Level of contamination in sediments affected by the Prestige oil spill and impact on the embryo development of the sea urchin. Ciencias Marinas. 32(2B): 421–427.

Franco, M. A., Viñas, L., Soriano, J. A., de Armas, D., González, J. J., Beiras, R., Salas, N., Bayota, J. M., and Albaigés, J. 2006. Spatial distribution and ecotoxicity of petroleum hydrocarbons in sediments from the Galicia continental shelf (NW Spain) after the *Prestige* oil spill. Marine Pollution Bulletin, 53(5–7): 260–271.

Labarta, U., Fernández-Reiriz, M. J., Garrido, J. L., Babarro, J. M. F., Bayona, J. M., and Albaigés, J. 2005. Response of mussel recruits to pollution from the "Prestige" oil spill along the Galicia coast. A biochemical approach. Marine Ecology Progress Series, 302(4): 135–145.

Laffon, B., Rabade, T., Pasaro, E., and Mendez, J. 2006. Monitoring of the impact of the *Prestige* oil spill on *Mytilus galloprovincialis* from Galician coast. Environ. Int., 32(3): 342–348.

Marigómez, I., Soto, M., Cancio, I., Orbea, A., Garmendia, L., and Cajaraville, M. P. 2006. Cell and tissue biomarkers in mussel, and histopathology in hake and anchovy from Bay of Biscay after the *Prestige* oil spill (monitoring Campaign 2003). Mar. Pollut. Bull. 53(5–7): 287–304.

Mariño-Balsa, J. C., Pérez, P., Estévez-Blanco, P., Saco-Alvarez, L., Fernández, E., and Beiras, R. 2003. Assessment of the toxicity of sediment and seawater polluted by the *Prestige* fuel spill using bioassays with clams (*Venerupis pollastra*, *Tappes decussatus* and *Venerupis rhomboideus*) and the microalga *Skeletonema costatum*. Ciencias Marinas. 29(1): 115–122.

Martínez-Gómez, C., Campillo, J. A., Benedicto, J., Fernández, B., Valdés, J., García, I., and Sánchez, F. 2006. Monitoring biomarkers in fish (*Lepidorhombus boscii* and *Callionymus lyra*) from the Northern Iberian shelf after the *Prestige* oil spill. Marine Pollution Bulletin, 53(5–7): 305–314.

Morales-Caselles, C., Jiménez-Tenorio, N., de Canales, M. L., Sarasquete, C., and del Valls, T. A. 2006. Ecotoxicity of sediments contaminated by the oil spill associated with the tanker "Prestige" using juveniles of the fish *Sparus aurata*. Arch. Environ. Contam. Toxicol., 51(4): 652–660.

Morales-Caselles, C., Kalman, J., Riba, I., and del Valls, T. A. 2006. Comparing sediment quality in Spanish littoral areas affected by acute (*Prestige* 2002) and chronic (Bay of Algeciras) oil spills. Environ. Pollut., 146(1): 233–240.

Navas, J. M., Babin, M., Casado, S., Fernández, C., and Tarazona, J. V. 2006. The Prestige oil spill: a laboratory study about the toxicity of water-soluble fraction of the fuel oil. Mar. Environ. Res., 62 Suppl: S352–355.

Orbea, A., Garmendia, L., Marigómez, I., and Cajaraville, M. P. 2006. Effects of the "Prestige" oil spill on cellular biomarkers in intertidal mussels: Results of the first year studies. Marine Ecology Progress Series, 306(11): 177–189.

Ordas, M. C., Albaigés, J., Bayota, J. M., Ordás, A., and Figuera, A. 2007. Assessment of in vivo effects of the *Prestige* fuel oil spill on the Mediterranean mussel inmune system. Arch. Environ. Contam. Toxicol., 52(2): 200–206.

Pérez-Cadahia, B., Laffon, B., Pasaro, E., and Méndez, J. 2004. Evaluation of PAH bioaccumulation and DNA damage in mussels (*Mytilus galloprovincialis*) exponed to spilled Prestige crude oil. Comp. Biochem. C. Toxicol. Pharmacol., 138(4): 453–460.

Raingeard, D., Cancio, I., and Cajaraville, M. P. 2006. Cloning and expression pattern of peroxisome proliferator-activated receptor α in the thicklip grey mullet *Chelon labrosus*. Marine Environmental Research, 62: 113–117.

14 Review progress with publication and electronic dissemination of biological effects techniques in the ICES TIMES series (TORa)

The biological effects editor Matt Gubbins (UK) reviewed progress with publications during 2006/2007. During this period, one manuscript has been published by ICES following successful completion of the review process. This was "Measurement of Scope for Growth in mussels" by J. Widdows & F. Staff. This method is now available on the ICES website as TIMES document 40.

The manuscript concerning "The use of embryo aberrations in amphipod crustaceans for measuring effects of environmental stressors" by B. Sundelin, A. Wiklund & A. Ford has been submitted and reviewed but reviewers comments need to be addressed. The current deadline for submission of the amended document to ICES is 31/05/07.

Four further manuscripts have been commissioned and draft resolutions for these have been submitted to ICES. These are shown in Table 14.1 below. Revised deadlines have been agreed with ICES and are shown in the table. Documents to amend the methods for oyster embryo bioassay and EROD activity were progressing well and should meet deadlines to ICES. A first draft of the method for extraction techniques for bioassays has been produced (1st author Hans Klamer) but the deadline to ICES should be amended to 31/10/07.

14.1 Documents commissioned

METHOD	LEAD AUTHOR	ICES DRAFT RESOLUTION?	DEADLINE TO ICES
Oyster embryo bioassay	John Thain	Yes	September 2007
MDR / MXR Calcein –am efflux	Angela Kohler *et al.*	Yes	October 2007
EROD assay amendment	Matt Gubbins	Yes	30/04/07
Extraction techniques for bioassays	Hans Klamer / Dick Vethaak / John Thain / Kevin Thomas	Yes	31/05/07 (ammend to 31/10/07)

Five further TIMES documents have previously been identified as required. These are shown in Table 14.2 below. The need for these was reviewed and it was decided that the method for blue mussel histopathology should be commissioned and a draft resolution submitted in 2007. The biological effects editor will contact the authors to commission the document.

Methods for YES / YAS, CALUX and reproductive success in fish were also considered necessary and permission to commission these manuscripts should be obtained from ICES with a view to submitting draft resolutions in 2008 when potential deadlines have been discussed with the authors.

14.2 Documents pending

METHOD	LEAD AUTHOR	ICES DRAFT RESOLUTION?
Blue mussel histopathology	Steve Feist / David Lowe / Miren Cajaraville	Needed 2007
YES / YAS screen	John Thain	2008?
CALUX	Dick Vethaak / John Thain	2008?
Reproductive success in eelpout	Jakob Strand	2008?
Gonadal histology of flounder	Steve Feist	No

The requirement for any new method documents was discussed in relation to the core set of methods being proposed for WKIMON. The micronucleus method was identified as the only core method being proposed requiring a supporting TIMES publication. Aldo Viarengo and Caludia Bolognesi were identified as possible authors of a background document for this method and will be approached with a view to also producing a TIMES manuscript. Permission from ICES will also be required to commission this manuscript.

Recommendation

Permission is requested from ICES to commission methods for: Blue mussel histopathology, YES / YAS, CALUX, reproductive success in fish and the micronucleus assay. Requests for publication of method documents concerning blue mussel histopathology will be put forward as Draft Resolutions in 2007.

15 Assess the amount of biological effects data submitted to the new ICES database (ToR g)

WGBEC had received an update from Marilynn Sorensen on the amount of biological effects data submitted to the new ICES database and this is summarised in Table15.1 and Table 15.2. It was encouraging to see an increase in the amount of data particularly in respect of imposex data.

It was noted that a significant amount of data outside of that held on the database had been used in the recent WKIMON III workshop. The outcome of the WKIMON workshop also indicated the importance in having access to the data not only for deriving assessment criteria but ultimately to conduct assessments across the OSPAR Maritime Area A *tour de table* indicated that many contracting parties were in the process of submitting data and therefore this should dramatically improve the current data holding over the next year.

WGBEC would review the situation again at its meeting in 2008

Table 15.1. Summary of biological effects data held on the ICES database.

PARAMETERS	CONTRACTING PARTIES HAVING REPORTED	YEARS AVAILABLE	TOTAL NUMBER OF MEASUREMENTS IN THE ICES DATABASE
Aminolevulinic acid dehydratase	NO	1997–2005	1382
DNA adducts			0
Percent net response	UK UK NE	1990–1991 1990–1991 1990, 1993	100 water/100 sediment
Acetylcholine esterase activity			0
EROD	NO UK --- UK/GE/NE/FR	1997–2005 1998–2005 --- 1988–1996 (old parameters)	4405 --- 3450 (usable?)
% mortality (sediment bioassay)			0
Glutathionine transferase			0
Unsaturated neutral lipids			0
Lethal concentration which kills 50% of test organisms			0
Neutral red retention			0
Catalase activity			0
Super oxide dismutase			0
Lipofucin			0
PYR1OH	NO	1998–2005	2692
PA1OH	NO	2000–2005	865
BAP3OH	NO	2000–2003, 2005	700
NAP2OH	NO	2000	215
Vitellogenin			0
MT	NO	1997–2002	1110

Table 15.2 Summary of imposex data held on the ICES database.

IMPOSEX PARAMETERS	CONTRACTING PARTIES HAVING REPORTED	TIME SERIES	YEARS AVAILABLE	TOTAL NUMBER OF MEASUREMENTS IN THE ICES DATABASE
Relative Penis Size Index (%)	UK	2	1990–2003 (gaps)	947
	Norway	No	2005	
	France	1	2003–2006	
Relative Penis Length Index (%)	Denmark	No	2001	43
	Norway	No	2005	
	Spain	No	2005	
% Sterile females	UK	1	1990–2001 (gaps)	391
	Denmark	No	2001	
	Spain	No	2005	
% Females displaying imposex	UK	1	1997–2003	468
	Denmark	1	1998–2003	
	Spain	No	2005	
Length of female penis	France Norway	1	1997–2005	2660
		1	2003–2006	
Length of male penis	France Norway	1	1997–2005	2362
		1	2003–2006	
Penis Classification Index	Denmark	1	1998–2003	80
	UK	No	2003	
Imposex stage	Norway	1	1997–2005	2293
Vas Deferens Sequence Index	Denmark	1	1998–2003	1140
	France	1	2003–2006	
	Spain	No	2005	
	UK	2	1987–2004 (gaps)	

16 Provide expert knowledge and guidance to ICES Data Centre (possibly via subgroup) as requested (ToR m)

WGBEC had received a request from the Data Centre to provide guidance notes for Contracting Parties wishing to submit biological effects data to the ICES database. This is viewed as being valuable for those submitting data for the first time. Marilynn Sorensen provided WGBEC with two actual submissions with disguised country, coordinates, and lab. etc. WGBEC agreed that it could write a text to describe the sampling, number and species of fish, matrices, the parameters measured, method used etc. but that this would need to be done intersessionally as there was insufficient time to complete this task during the meeting. John Thain agreed to action this request in consultation with Matt Gubbins and the Data Centre.

A request had also been received for a conversion factor for units of DNA adduct. John Thain and Brett Lyons (UK) agreed to investigate this request.

Action

Provide Data Centre with guidance notes for an example file for data submitters.

Provide Data Centre with conversion factor for units of DNA adducts

17 Review integrated methods for assessment of effects on biota from lindane, BFRs and methodology of the COMET assay (ToR l)

17.1 Lindane

There was insufficient tome to complete this review at the meeting since the task was unexpectedly large. Rolfe Schneider (DE) communicated to WGBEC (via correspondence) that he had identified some 400 publications on lindane (gamma-HCH only) effects in aquatic organisms. In summary, it is evident that practically all biomarkers on the WGBEC list of recommended methods (except the really metal-specific ones) have in some species or stages proven to respond to lindane, from neurotoxicity (rarely AChE, more frequently Na/Ka- and Ca/Mg-ATPases or GABA-and serotonin-related, and behavioural changes), through endocrine disturbances in all aspects of hormone systems (reproduction, immuno system, physiological control of protein-, carbohydrate- and lipid metabolism) to teratogenesis, mutagenesis, carcinogenesis and histopathology. Numerous bioassays have been conducted, particularly with fish and crustaceans. In some cases the biomarkers tested responded, in others they did not. There appears to be no biomarker specific to lindane. Many of the single biomarkers reactive to lindane react to many other xenobiotics as well. It is anticipated that this review will be completed and presented at WGBEC in 2008.

17.2 BFRs

The current knowledge concerning effects of BFRs on mammals and aquatic organisms is limited. Toxicity studies with BFRs point to neurobehavioral and developmental effects in mammals and reproductive developmental toxicity in fish and invertebrates (see Kuiper, 2006). Laboratory studies have shown that a number of BFRs and BFR-metabolites can interfere with thyroid and (sex) steroid hormone function (endocrine disruption) (Hamers *et al.*, 2006). Further, there is evidence for weak ecdysteroid antagonistic activity of some pentabrominated diphenyl ethers in crustaceans (Wollenberger *et al.*, 2005). Some recent relevant studies are presented below in more detail.

Long term studies with flounder (*Platichthys flesus*) and zebrafish (*Danio rerio*) exposed to the most frequently found BFRs: tetrabromobisphenol A (TBBPA), hexabromocyclododecane (HBCD), and a brominated diphenylether mixture (PentaBDE) were carried out in the Netherlands in framework of the EU FIRE project (Wester *et al.*, in press; Kuiper, 2007; Kuiper *et al.*, in press). Juvenile flounders were exposed to TBBPA for 105 days via the water, and to HBCD and PBDE (78 and 101 days, respectively) via food and/or sediment. Zebrafish were exposed to the test substances via the water in a partial life cycle test. Exposed fish were examined macroscopically and histologically with emphasis on reproductive and endocrine organs. Plasma thyroid hormone (T3 and T4) concentrations were determined in all flounder studies and in zebrafish exposed to PentaBDE. Cytochrome P4501A (CYP1A) activity (EROD) was determined in livers from flounder as a possible indication for dioxin-like effects. Activities of the steroidogenic enzyme CYP19 (aromatase) were determined in flounder gonads, and production of vitellogenin (VTG) was determined in plasma from flounder exposed to TBBPA. Concentrations of BFRs in tissue from exposed fish were evaluated to provide a dose background for risk assessment. All exposures resulted in a linear increase of internal BFR concentrations with exposure concentrations. Whereas the concentrations in zebrafish were generally higher, the range in flounder included environmentally relevant concentrations. Exposure of flounder to TBBPA resulted in an increase in the concentration of thyroid hormone T4 in plasma and a mild increase of aromatase activity in testes. In zebrafish, egg production and juvenile survival were reduced. Significant effects occurred at internal concentrations that were at least 10 times the highest levels observed in fish in the environment. Exposure to HBCD did not result in adverse effects in either flounder or zebrafish. Exposure to a mixture of PBDEs resulted in juvenile mortality

in zebrafish at the highest PBDE concentrations, and a mild decrease of aromatase activity in ovaries and plasma T4 concentrations in flounder. In general, the studies presented in this thesis show minimal indications for endocrine effects of exposure to BFRs in fish at concentrations observed in the environment.

In the study of Ronisz *et al.*, 2004, a screening of selected biomarkers in juvenile rainbow trout (Oncorhynchus mykiss) and feral eelpout (Zoarces viviparus) was performed after exposure (i.p. injection) to HBCDD and TBBPA. Two out of four short-term experiments with HBCDD showed an increase in the activity of catalase. A 40% increase in liver somatic index (LSI) could be observed after 28 days. HBCDD did also seem to have an inhibitory effect on CYP1A's activity (EROD). HBCDD did not seem to be estrogenic or genotoxic. TBBPA increased the activity of glutathione reductase (GR) in rainbow trout suggesting a possible role of this compound in inducing oxidative stress. The compound did not seem to be estrogenic. TBBPA seemed to compete with the artificial substrate ethoxyresorufin in vitro, during the EROD assay. In eelpout, only one 5 days in vivo experiment was performed. Neither of the compounds gave rise to any effect in this fish.

Subchronic effects of TBBPA, tribromophenol (TBP), and four polybrominated diphenyl ethers (BDE-28, BDE-47, BDE-99, and BDE-100) on larval development of the marine copepod Acartia tonsa were studied by Wollenberger *et al.*, 2005. For TBBPA and TBP 5-d effective median concentration (EC50) values for inhibition of the larval development rate were 125 and 810 microg/L, respectively, whereas the PBDEs were much more potent with 5-d EC50 in the low microg/L range (for example 1.2 microg/L for BDE-100 and 13 microg/L for BDE-47). These concentrations were up to two orders of magnitude below the 48-h LC50 for acute adult toxicity. To distinguish between general toxicological and endocrine-mediated toxic effects, the BFRs were assessed in vitro for ecdysteroid agonistic/antagonistic activity with the ecdysteroid-responsive Drosophila melanogaster B (II)-cell line. The pentabrominated diphenyl ethers BDE-99 and BDE-100 showed weak ecdysteroid antagonistic activity. Thus, these PBDEs may be regarded as potential endocrine disrupters in invertebrates.

A full life-cycle (< or =26 days exposure) ecotoxicity test with the particle-feeding copepod Nitocra spinipes was used to study effects of BDE-47, -99 and -100 on larval development rate (LDR) and population growth rate (r (m)) (Breitholtz and Wollenberger, 2003). LDR significantly decreased in copepods exposed for 6 days to nominal concentrations > or =0.013 mg/l BDE-47 and > or =0.03 mg/l BDE-99. Large concentration ratios (< or =338) between adult acute and juvenile subchronic endpoints were observed. Exposure over the full life cycle showed that r (m) in general was a less sensitive endpoint than LDR. Still, the r (m) in copepods exposed to 0.04 mg/l BDE-47 was significantly reduced compared to the controls (***P<0.001). The findings indicate that development and reproduction in N. spinipes are sensitive to the tested PBDEs.

The objective of the study by Kallqvist *et al.* 2006 was to determine the toxic effects of 2,4,2',4'-tetrabromodiphenyl ether (BDE 47) on the growth of the marine diatom Skeletonema costatum and on the parthogenetic reproduction and filtering activity of the freshwater crustacean Daphnia magna. The results showed that BDE 47 caused growth inhibition in S. costatum (NOEC, 6.6 microg/L) and depressed the reproductive output of D. magna (NOEC, 14 microg/L). No effects were seen on the filtering rate of D. magna at any of the concentrations tested. Although sublethal toxicity was observed at low-microg/L levels, documented environmental water concentrations are many orders of magnitude lower, thus suggesting that BDE 47 is of minor risk to these organisms through direct water exposure

Conclusion: The available studies on fish indicate little risk from environmental exposure to BFRs and provide no direct clue for biomarker application and / or useful endpoints in fish. However, transcription studies underway in the UK hope to determine whether PDBE

exposure in flounder causes diagnostic changes in gene expression profiles. The combined application of in vitro assays for ecdysteroid antagonistic activity and subchronic developmental test with crustacean species may hold promise for a rapid and cost-effective tool when screening for sublethal effects of BFRs (and other chemicals), but this requires further study. In general there is a need for further studies of long-term, low-dose effects of flame retardants especially for invertebrates.

17.3 The Comet assay

Presentations on the use of the comet assay were provided by Brett Lyons (UK) and Matt Gubbins (UK). The presentations dealt with advances and application of the comet assay in the field of aquatic environmental monitoring. Details on advances in the mammalian field to standardise the assay via a series of comet assay workshops were provided. The latest of which is the 7th International Comet Assay Workshop (http://comet2007.ulster.ac.uk) due to be held at the University of Ulster, Northern Ireland 2007. Current limitations of the assay were discussed. For example, due to excessive movement when working onboard research vessels it is often not possible to standardise electrophoresis conditions. Matt Gubbins provided details of a purpose built gimballed table (essentially a displacement compensation system), which Fisheries Research Services (FRS) have successfully tested in Scottish waters to tackle the problem of running electrophoresis units at sea. Thierry Burgeot (France) provided a brief update of their success in using cryo-preservation techniques with flatfish red blood cells. This method allowed samples to be frozen and sea and taken back to the laboratory for analysis. However, it was acknowledged by several members of WGBEC that the cryo-preservation method is not universally applicable to all tissues and species and that the area required further research. A detailed background paper on the comet assay will be prepared intersessionally (Brett Lyons, UK) and presented at the 2008 meeting.

The use of the Comet assay within the OSPAR maritime area

Fisheries Research Services (FRS) Marine Laboratory Aberdeen has been investigating the potential for integrating the COMET assay with their routine biological effects monitoring programme. During surveys of the Firth of Clyde in 2005 and 2006, plaice (*Pleuronectes platessa*) sampled for contaminant analysis and biological effects measurements (EROD, bile metabolites, plasma VTG) were also used to provide erythrocytes for COMET assay. Electrophoresis of fresh samples was conducted at sea on a counter-balanced gimballed table, contained within a refrigerated (10 C) box. Preserved (in ethanol) electrophoresed cells were then returned to the laboratory for scoring using Comet Assay III software. The use of this system enabled fairly reproducible results in moderate weather conditions. The design of the gimballed table and preliminary results from 2005 were shown to the group and it was demonstrated that by using the method at sea it was possible to detect significant differences in % tail intensity between reference and control sites. The method was used more extensively during 2006 but results of cell scoring were still pending and could be shown to the group at the next meeting. FRS aims to continue the use of COMET assay at sea during biological effects monitoring cruises in the short term, and if successful may adopt the technique as part of an integrated package of methods.

FRS has also field trialled the assay for detecting DNA damage in gill cells of wild shoreline mussels (*Mytilus edulis*) from the Firth of Forth. Preliminary results suggest that high levels of background damage may be masking differences between controls and mussels from contaminated sites, however the technique is able to detect differences in DNA damage between groups of mussels under laboratory exposure conditions. Following further development work FRS may continue to apply this technique on an ad hoc basis for monitoring purposes.

References

Ronisz, D., Finne, E. F., Karlsson, H., and Forlin, L. 2004. Effects of the brominated flame retardants hexabromocyclododecane (HBCDD), and tetrabromobisphenol A (TBBPA), on hepatic enzymes and other biomarkers in juvenile rainbow trout and feral eelpout. Aquat. Toxicol., 69: 229–245.

Hamers, T., Kamstra, J. H., Sonneveld, E., Murk, A. J., Kester, M. H. A., Andersson, P.L., Legler, J., and Brouwer, A. 2006. *In vitro* profiling of the endocrine disrupting potency of brominated flame retardants. Toxicological Sciences, 92(1): 157–173.

Kuiper, R. 2007. Toxicity of brominated flame retardants in fish, with emphasis on endocrine effects and reproduction. Academic thesis. Utrecht University. http://www.igitur.uu.nl/igiturarchief/arch_works.php?author=Kuiper%2C+R.

Kuiper, R. V., Cantón, R. F., Leonards, P. E. G., Jenssen, B. M., Dubbeldam, M., Wester, P. W., van den Berg, M., Vos, J. G., and Vethaak, A. D. 2007. Long-term exposure of European flounder (*Platichthys flesus*) to the flame-retardants tetrabromobisphenol A (TBBPA) and hexabromocyclododecane (HBCD). Ecotoxicology and Environmental Safety, In Press, Available online 26 January 2007.

Wester, P. W., Kuiper, R. V., Fernandez-Cantón, R., Jensen, B. M., Leonards, P. E. G., and Vethaak, A. D. 2006. Brominated flame retardants in environmentally relevant test setup: No major endocrine effects found in fish. Organohal. Comp.Volume 68 (in press).

Breitholtz, M., and Wollenberger, L. 2003. Effects of three PBDEs on development, reproduction and population growth of the harpacticoid copepod Nitocra spinipes. Aquat. Toxicol., 64: 85–96.

Wollenberger, L., Dinan, L., and Breitholz, M. 2005. Brominated flame retardants: activities in a crustacean development test and in an ecdysteroid screening assay. Environ Toxicol Chem., 24: 400–407.

Kallqvist, T., Grung, M., and Tollefsen, K. E. 2006. Chronic toxicity of 2,4,2',4'-tetrabromodiphenyl ether on the marine alga Skeletonema costatum and the crustacean Daphnia magna. Environ. Toxicol. Chem., 25: 1657–62.

18 Together with MCWG and WGMS, review the existing technical annexes on PAHs to see whether they are adequate for monitoring of target alkylated PAHs (TORn)

WGBEC were requested to review the technical annexes on PAHs to see whether they are adequate for monitoring of target alkylated PAHs and, as appropriate, prepare advice on any revisions. Concerning the specificity of the five recommended biomarker techniques (CYP 1A activity, bulky aromatic-DNA adducts PAH metabolites in bile, liver histopathology and macroscopic liver neoplasms = liver nodules) used to describe the impact of PAH compounds on biota at the biochemical and at the cellular/tissue level to alkylated rather than the parent PAHs, WGBEC are unaware of any published data that addresses the issue. They are aware, however, of a study currently being performed to investigate the relative affects of alkylated versus parental PAHs on CYP1A in cod in Norway. We await the results of this study.

19 Identify and report on changes in the distribution, population abundance and condition of marine species in the OSPAR maritime area that are driven by contaminants or by interactions between the effects of contaminants and changes in hydrodynamics and sea temperature (ToR p)

A sub group of Doris Schiedek (DE), James Readman (UK) and Britta Sundelin (SE), reviewed the information available to WGBEC and led the discussion and rapporteured this item.

This request on 'An assessment of the changes in the distribution and abundance of marine species in the OSPAR maritime area in relation to hydrodynamics and sea temperature' is only peripherally linked to the remit of the WGBEC. The request does, however, suggest "Expert Groups should invite external expertise to deal with this request as required".

Ocean temperatures in most parts of the world are increasing and are expected to continue to rise during the 21st century. In using four of the world's longest calibrated daily time series it has been shown that trends in surface temperatures in the North and Baltic Seas now exceed those at any time since instrumented measurements began in 1861 and 1880. Temperatures in summer since 1985 have increased at nearly triple the global warming rate which is expected to occur during the 21st century and summer temperatures have risen 2-5 times faster than those in other seasons. The recent warming event is exceeding the ability of local species to adapt and is consequently leading to major changes in the structure, function and services of these ecosystems (Mackenzie and Schiedek 2007 a, b).

Regarding "changes in the distribution and condition of species that would have not occurred in the absence of direct or indirect human impacts", chemical pollution has to be taken into consideration as an additional factor imposing stress to biota (Wood and McDonald 1997, Lanning *et al.* 2006). Studies on the clam *Macoma balthica* at its southern distribution have shown that the species has been able to cope with pollution caused e.g. by trace metals (Hummel *et al.* 2000) but there is some indication that with the increasing temperatures during recent years, this species is losing its ability to survive at the uppermost limits of their southern distribution (Jansen *et al.*, 2007).

This provides a clear link between pollution and climate change, with increasing temperature, decreasing pH or salinity (as predicted for the Baltic Sea) or UV radiation possibly acting as additional or synergistic stressors. In addition, an altered composition in primary production as shown in the Baltic, might influence food availability with more serious pollutant effects. Climate related changes in fish communities might also result in a modified transfer of contaminants within the food web.

Generally, on-going and predicted future changes in SST are very much related to the physiological acclimation capacity of native biota. Differences among species concerning their thermal tolerance limits and in their capacities to adjust to these limits (Somero 2005) may determine how populations will be affected by climate change (Pörtner and Knust 2007). The ability to cope with additional stress due to pollution under a changing climate regime may thus also differ among species.

Another major area of change with respect to contaminant exposure, distribution and effects will relate to shifts in land use and agricultural distribution/practices. This, to respond to climate change, will bring about changes in chemical treatments and hence in pesticide distributions.

In the report to the European Water Directors on "Marine and Coastal Dimensions of Climate Change in Europe" (2006), it is stated that "eutrophication is, and will remain, an issue for the coastal zone because both agriculture and in particular urbanisation are the main drivers. Pollution (e.g. from toxic chemicals) is considered of relatively lesser importance although the report considers the legacy of past contaminations an important issue. As noted there, "toxic chemicals are stored in waste dumps, behind dams, in soils and are present in deposited sediments in catchments. These natural and man-made repositories are, in principle, subject to erosion and further transport in the direction of the coastal zone. Changes in the hydrological regime (e.g. through climate change) can mobilize these stored contaminants."

WGBEC does not, however, believe that contaminants are only a "legacy of the past". There is sufficient evidence that "new" contaminants have been produced and are released and we have only just started to understand the biological effects they cause.

Conclusions

Presently, WGBEC is not in the position to provide the data needed to perform an in-depth assessment concerning the role of contaminants as additional drivers for observed changes in species distributions for the entire OSPAR area in retrospect (e.g. covering the past 50 years or even the last 10-15 years with an appropriate spatial coverage).

However, sufficient data should be available to test the effect of temperature increase during the past 20 years relative to fish diseases (WGPDMO), imposex in whelks and EROD activity in dab (North Sea) and perch (Baltic Sea).

WGBEC believes that changes in climate variables are also likely to alter the transport, transfer, deposition and fate of contaminants. Bioavailability, metabolism and toxicity will also be affected.

More research is required to evaluate the interactions between climate change and contaminants to better understand and predict how on-going and future climate changes may interact with anthropogenic impacts (e.g. chemical pollution).

Experimental studies and modelling efforts are needed to test various scenarios concerning transport, transfer and cycling of chemical pollutants and to assess the counteracting effects on important species including the impact on their well-being/fitness, and the potential effects on populations/ecosystems.

WGBEC is well positioned to provide up-dates on research reported in the literature on this topic, e.g. as an outcome of the SETAC (Europe) 17th Annual Meeting in May 2007 in Porto.

References

Hummel, H., Bogaards, R. H., Bachelet, G., Caron, F., Sola, J. C., Amiard-Triquet, C. 2000. The respiratory performance and survival of the bivalve *Macoma balthica* (L.) at the southern limit of its distribution area: a translocation experiment. J. Exp.Mar. Biol. Ecol., 251: 85–102.

Jansen, J., Pronker, A. E., Wendelaar Bonga, S., and Hummel, H. 2007. Macoma balthica in Spain, a few decades back in climate history. J Exp Mar Biol Ecol, 344: 161–169.

Lanning, G., Cherkasiv, A. S., and Sokolova, I. M. 2006.Temperature-dependent effects of cadmium on mitochondrial and whole-organism bioenergetics in oysters. Mar Environ Res, 62: 79–82.

MacKenzie, B. R., and Schiedek, D. 2007. Daily ocean monitoring since the 1860s shows record warming of northern European seas. Global Change Biology, *in press*.

MacKenzie, B. R., and Schiedek, D. 2007. Long-term sea surface temperature baselines - time series, spatial covariation and implications for biological processes. J Marine System, in press.

Pörtner, H. O., and Knust, R. 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science, 315: 95–97.

Somero, G. N. 2005. Linking biogeography to physiology: evolutionary and acclimatory adjustments of thermal limits. Frontiers in Zoology, 2, doi:10.1186/1742-9994-2-1

Marine and Coastal Dimensions of Climate Change in Europe. 2006. *In* Report to the European Water Directors, pp 100. Ed by N. Hoepffner. ISBN 92-79-03747-1.

Wood, C. M., and McDonald, D. G. 1997, Global warming –Implications for freshwater and marine fish. Cambridge University Press, 441 pp.

20 Request from OSPAR regarding endocrine disruptors (TORq): - consider the adequacy of the OECD test protocols and specify proposals for a test programme for marine species if appropriate

WGBEC reviewed text on this Agenda Item prepared by John Thain, Yvonne Allen (UK) and Dick Vethaak (NL). In addition, comments were provided also by Tom Hutchinson and Peter Matthiessen (UK), Chair and Co-Chair respectively of the OECD Validation Management Group for Ecotoxicological Test Methods for Endocrine Disrupters.

In 2005, OSPAR HSC asked ICES to consider current developments within OECD/EU regarding endocrine disruptors and draft advice on whether any further work is necessary. WGBEC responded through its Working Group Report, but OSPAR HSC felt that further information, detail and clarification were needed in order to fully address the question. Subsequently, this issue has been added to the WGBEC Terms of Reference for 2007, specifically these are:

a) to consider the adequacy of the OECD test protocols and their applicability for marine species, and

b) to specify proposals for a test programme for marine species in case OECD freshwater model species on scientific grounds are deemed not suitable for testing the effect of endocrine disrupting compounds in the marine environment.

20.1 To consider the adequacy of the OECD test protocols and their applicability for marine species

Background

Information has been gathered on the test methods under preparation in the OECD and the status of their development.

The OECD Secretariat established a Validation Management Group for Ecotoxicological Test Methods for Endocrine Disrupters (VMG- Eco); with the remit to oversee the work on validation of EDC fish tests as well as tests using other taxa such as amphibians, birds, and certain invertebrates. It is important to note that the OECD tests are being developed specifically for screening and testing chemicals for endocrine disruption activity, not as bioassays for testing environmental samples. In some cases however it may be possible to adapt them for use as bioassays. The current validation status of the fish and invertebrate tests are described below:

21-day fish screening assay

A short-term (21-day) fish screening assay test guideline is being validated using three core species: Fathead minnow (*Pimephales promelas*), Japanese medaka (*Oryzias latipes*) and zebrafish (*Danio rerio*). Two phases of validation have been completed and a third is underway, with completion expected March/April 2007. Phase 1A tested a potent oestrogen (17β oestradiol) and androgen (trenbolone); phase 1B tested a weak oestrogen (4-tert pentylphenol), an aromatase inhibitor (prochloraz) and an anti-androgen (flutamide). Phase 2 addressed testing of negative substances, and potassium permanganate and n-octanol were chosen for this purpose. Phase 3 involves further testing with fathead minnow and zebrafish (on the recommendation of the EDTA) with a weak oestrogen (4-tert-octylphenol), a weak androgen (androstenedione) and a "difficult" negative (pentachlorophenol). Although initially intended as a screen to detect oestrogenic, androgenic, anti-androgenic and aromatase activity, the results from the core species thus far demonstrate that there is a lack of a relevant and reliable endpoint for anti-androgens. Furthermore, one of the proposed endpoints for

androgenic activity is a decrease in vitellogenin in females; however, the underlying mechanism is not well understood or documented as yet and therefore the biological relevance is not established. The primary objective of the screen as it stands at the moment therefore is to detect oestrogenic and androgenic agonists and aromatase inhibiting substances using two core endpoints –vitellogenin and secondary sex characteristics (nuptial tubercles in female fatheads and anal fin papillary processes in female medaka, exposed to androgens). The VMG-Eco is of the opinion that these endpoints have been sufficiently validated for the Japanese medaka and that preparations for the peer-review of the assay and Test Guideline development can proceed on these for this species. On completion of Phase 3 work with fathead minnow and zebrafish, the peer-review process for these will be initiated. A guideline could therefore be in place by 2008.

Other endpoints initially included in the assay i.e. gonad histology and fecundity have not yet been validated, due to lack of reproducibility (histopathology) and its non-specific role and interpretation in an ED screening context (fecundity). Both require further work in order to be included in a future test guideline and the VMG-Eco have recommended that guidance documents (note not guidelines as such) be prepared addressing measurement, test design, techniques and interpretation of these endpoints, as well as including information on the limitations and advantages of these and the core endpoints for detecting a range of endocrine activities. The EDTA has clarified the purpose of the assay as an endocrine disrupters screen as opposed to a reproductive screen; the latter remains a possibility for a revised or more generic Test Guideline. Some issues remain with the current protocol: (a) acceptability criteria for the endpoints (e.g. VTG level in control males and females for each species); (b) replication (2 vs 4 replicates per treatment). These are being considered further and will be reflected in the future Test Guideline.

Validation of the stickleback assay

Following initial isolation and identification of the protein by a Swedish research group (ref), research under the UK Endocrine Disruption in the Marine Environment (EDMAR) programme developed an androgen-specific biomarker using the three-spined stickleback, *Gasterosteus aculeatus*. This biomarker is spiggin, a proteinaceous glue produced by the kidneys of males in response to endogenous androgens and used for constructing nests during the breeding season. Spiggin is also produced by females exposed to exogenous androgens since they also have the androgen receptor (*i.e.* analogous to male fish producing VTG in response to exogenous estrogens). This work was advanced further by the development of a specific and sensitive biomarker of oestrogenic exposure in male stickleback *i.e.* VTG induction. Due to the fact that none of the core species are indigenous to Europe, they lack any specific endpoint for androgens and, furthermore, a suitable endpoint for anti-androgenic activity, it has been proposed to the OECD that there is now a possible additional/alternative test species which could be included in the ED fish screening assay guideline, which is ecologically relevant for European waters and, more importantly, now has appropriate endpoints for both (anti) estrogens (VTG) and (anti) androgens (spiggin). The ability to measure both endpoints potentially allows simultaneous testing for (anti) estrogenic and (anti) androgenic properties of compounds, thereby reducing the number of fish required in experiments. A proposal to consider inclusion of the stickleback in the ED screen was presented to the VMG-Eco in 2003, which agreed that a small intercalibration exercise should be carried out to evaluate the feasibility of the OECD fish screening assay, adapted for stickleback, and generate data on the reproducibility of the stickleback spiggin and VTG assays, thus bringing information on the stickleback into line with that on the core species. This was duly carried out in 2004 and biomarker endpoints of VTG in males and spiggin in females were shown to be both relevant and reproducible, being able to reliably detect the potent oestrogen E2 and the potent androgen trenbolone. The results of this first intercalibration were presented to the OECD VMG-Eco. The group approved the findings and

requested that the UK organise and lead on a second intercalibration to mimic Phase 1b, which assessed core species responses to the weak oestrogen 4-tert-pentylphenol, the anti-androgen flutamide and the aromatase inhibitor prochloraz. This is currently underway. Although 6-7 laboratories from Sweden, Finland, Canada, Netherlands, Norway and France expressed an interest in participating in this second intercalibration, only 3 laboratories took part, therefore further validation may be required to satisfy the requirements of the VMG-Eco and EDTA. VMG-Eco has expressed continuing support for these studies, given that no other protocol has shown sensitivity to anti-androgens. It is hoped that the results will continue to demonstrate that the stickleback shows potential as a test species and facilitate its inclusion in the final Test Guideline.

Validation of a fish sexual development test

As a second tier in the risk assessment of endocrine disrupting chemicals, a Scandinavian working group has developed a protocol for a fish sexual development test. The protocol is in principle an enhanced version of OECD Test Guideline 210 "Fish Early Life Stage Toxicity Test". This assay is intended to detect chemicals with androgenic or estrogenic properties as well as anti-androgenic, anti-estrogenic and aromatase inhibiting properties. The protocol is based on chemical exposure during the sex labile period in which the fish is expected to be most sensitive towards endocrine disrupting chemicals. Four core biomarker endpoints are measured as indicators of developmental or endocrine aberrations, namely: i) gross morphology (e.g. secondary sexual characteristics); ii) vitellogenin levels iii) gonadal histology and iv) sex ratios. The test is initiated with newly fertilised eggs and monitoring continues for up to 60-days post hatch (dph) and includes hatching rate, development, survival, growth (total length and body weight), sexual differentiation, secondary sex characteristics, gonadal development, gonadal histology and VTG levels. Phase 1 of the validation using fathead minnow and zebrafish is underway with results anticipated by mid 2007. The two test substances are 4-TPP (4-tert pentylphenol; weak oestrogen) and prochloraz (aromatase inhibitor). The results from one of the participating laboratories so far shows that the sex ratio becomes biased towards females on exposure to 4-TPP and towards males on exposure to prochloraz, and the thresholds for these effects are significantly lower than those found for VTG induction/reduction in the 21 day fish screening assay. Phase 2 is being planned, testing a weak androgen and a negative substance. There is scope for deploying the stickleback in this test procedure, but no OECD-sponsored work is currently underway with this species.

In addition, Germany, the US and Japan are conducting pre-validation studies with 1- and 2-generation full life cycle test protocols, using medaka and zebrafish for application in higher tier risk assessments.

Validation of invertebrate tests

At the moment there are no internationally harmonised chronic toxicity test methods for marine invertebrates therefore the development and validation of a test assessing reproduction and development of marine copepods has been added to the work plan of the OECD Test Guideline programme. The copepod life-cycle test is urgently needed in several multi-national regulatory programmes (e.g. REACH) and will facilitate harmonisation of hazard and risk assessments. Two common and regularly used species in toxicity tests were originally proposed – *Nitocra spinipes* and *Tisbe battagliai*. A further harpacticoid copepod species *Amphiascus tenuiremis* and the calanoid copepod *Acartia tonsa* were included. It should be noted that the endocrine system for copepods is completely different from vertebrates. The knowledge about crustacean endocrine systems is lower than for fish but this group is comparatively well studied due to the close relationship to insects where the information is needed and used to control noxious insects. However, it is not known if male crustaceans induce vtg, but the most useful endpoint seems to be ecdysteroid concentrations (the hormone

that regulates reproduction and growth). Of the methods available HPLC techniques would appear to be the most promising and sensitive. The proposed guidelines (one for harpacticoids and one for the calanoids listed above) are intended for evaluation of adverse long-term effects of various types of chemicals e.g. industrial chemicals, pesticides and pharmaceuticals as well as compounds used in the offshore oil and gas industry. Although these guidelines are intended to include endocrine disruptive effects it is currently not possible to definitively attribute observed effects to this mechanism of toxicity, due to the paucity of knowledge on the endocrine systems of these species and the "non-specific" nature of the endpoints i.e. unlike VTG induction in fish they are not diagnostic and can be attributed to other modes of action. Ring-tests with *Nitocra*, *Amphiascus* and *Acartia* took place in 2006, using 3,5-dichlorophenol as the single reference substance. Results in terms of developmental and reproductive endpoints were consistent among species. Recommendations made for future validation work were: (a) if all species are to be included in a multi species testing system then a partial life cycle test, focussing on the developmental stages, would be a better alternative if data comparability across all species is critical; (b) if however the OECD considers that there is a greater regulatory need for a full life cycle test, then *Amphiascus* should be used as a single test species, owing to the significantly shorter test duration (25 opposed to 40 days) and better reproductive output and success resulting in greater statistical power; (c) other reference chemicals should be used; (d) more laboratories should participate to strengthen the dataset and minimise random errors etc.

An enhanced *Daphnia* reproduction test is also being ring-tested, based on OECD 202, using an additional endpoint of offspring sex ratio to detect juvenile hormone agonists. The results of this have yet to be fully analysed.

Some limited progress is also being made with a variant of the *Daphnia* 3-week reproduction assay, and with an *Americamysis bahia* life cycle assay, but inevitably it will be some years before these have been properly evaluated.

Recommendation

To date, there exists too much uncertainty on the extrapolation of fresh water EDC ecotoxicity data to salt water species to simply justify the applicability of OECD test protocols for protection of the marine environment. Therefore we recommend that a first assessment should be done using the data available on fish and invertebrate species, with the caution that this data has been produced for the testing of chemicals specifically for regulatory purposes.

Justification to support this recommendation

There are generally less toxicity data available for saltwater species than for freshwater ones, especially in relation to organic compounds and several organic endocrine disrupting chemicals, such as alkylphenols, phthalates and brominated flame retardants. Although phylogenetically there is reason to assume that freshwater fish respond similarly to marine fish, and that the distributions of the sensitivities of the two groups of species are identical, these assumptions remain largely untested (and have led to proposals to add an additional safety factor of 10 to marine risk assessments based upon freshwater data (see paper by Wheeler *et al.*, 2002))

Uncertainty exists in the area of bioavailability and possibly differential uptake of EDCs by marine and euryhaline species - in marine and especially estuarine conditions - as compared to fresh water species. As such, endocrine disrupting chemicals in freshwater may have effects in fish other than estuarine or marine ones and tests with freshwater species will be inadequate to fully protect the marine environment. The same may be true for invertebrates including phyla exclusive to the marine environment. To justify the use of marine tests an assessment of comparable data on relevant EDC sensitivity distributions between freshwater and marine animals should be made. If sufficient evidence exists and sensitivity between marine and fresh

water animals exceeds one order of magnitude, testing of additional estuarine or marine species may be then required. Therefore we recommend that a first assessment should be done using the data available on species such as stickleback (androgen-associated) and flounder (estrogen associated). A similar assessment should be done for invertebrates There is a however a general need for more high quality data on the effects of a wide range of EDCs to both freshwater and saltwater organisms

In addition to the sensitivity and extrapolation issue (above) another argument relates to the representativity in terms of taxa/phyla. Will freshwater vertebrate and invertebrate models including marine copepods and crustaceans adequately protect the marine environment? The current lack of marine invertebrate ED tests generally is mainly due to lack of knowledge and the problems to differentiate between general/reproductive toxicity and the modulation of complex endocrine mechanisms of biological regulation. Most invertebrates rely on a totally different set of hormones than those commonly studied in vertebrate models. Most of the available invertebrate tests are not designed to identify an endocrine disruptor, but they merely yield apical endpoints such as reproduction, development and offspring sex ratios that included influences on the hormone systems and can be used in an environmental risk assessment (see special issue on EDC in invertebrates in ecotoxicology 2007). The justification to include more marine invertebrate species in OECD EDC screening and test development and regulatory frame works is of a complex nature and requires more studies and time. However, it is important to include invertebrates to increase the possibility to detect all kinds of EDC. For the near future OECD should focus on the development of full life cycle tests representing a variety of invertebrate phyla exclusive to the marine environment such as Echinoderms that are widely distributed in all coastal waters. This phylum deserves much more attention. For example, reproductive and regenerative phenomena of echinoderms can be considered possible models for studies on EDC effects. Some studies confirm that these compounds interfere with fundamental physiological processes including growth, development and reproductive competence. Other promising species could include nematodes, cnidaria and tunicates but only if it could be demonstrated that they are more sensitive to new types of endocrine action, or if they provide a substitute for oestrogen/androgen-sensitive vertebrates (for ethical reasons).

20.2 To specify proposals for a test programme for marine species in case OECD freshwater model species on scientific grounds are deemed not suitable for testing the effect of endocrine disrupting compounds in the marine environment

Fish protocols

The fish screening assay focuses at the moment on freshwater systems and via exposure in the water column. None of the core species are adaptable to salt water. Although the stickleback validation work described above has also been carried out in a freshwater test system, it does have the advantage of significant potential to be used in a marine screening system, whereby a "parallel" guideline could be developed for screening ED effects in the marine environment. If however, there is scientific evidence to support the proposition that freshwater ED effects data are related to saltwater ED effects in a systematic and predictable way, the former can be used to predict the latter and thus nullifying the need for a marine screen. This potential for extrapolation from fresh to salt water has been investigated for acute toxicity, using species sensitivity distributions; the degree of similarity between responses in freshwater and marine test organisms was dependent upon the chemical studied (*i.e.* the chemical behaved differently between the two systems and therefore mode of action/bioavailability was different) and the parity and representativeness of the species in the dataset. Due to a paucity of comparative data, both in terms of fate of EDCs and ED endpoints, it cannot be concluded with any certainty that the ED effects thresholds (and thus "safe" levels in the form of PNECS) in freshwater fish tests can be extrapolated to marine fish. It may be the case that freshwater

PNECS would not be adequately protective for marine species, or conversely, be over-protective. It should also be noted that there are substantial species differences even between freshwater species. It is therefore not straightforward to extrapolate between species, be freshwater or marine.

Before any decisions are made on proposing estuarine/marine ED fish tests, it would be useful to conduct a comparative exercise on the small dataset that does exist exposing freshwater and marine fish to a range of known endocrine disrupters in the laboratory. Certainly some studies have been conducted exposing stickleback to the same EDC in fresh and salt water (studies at Cefas, UK using estrogen and methyltestosterone) and it would be interesting to compare the responses. The same can be done using available UK and Dutch field data of euryhaline species such as flounder

If it is decided that a marine fish screen needs to be developed, several species have the potential to be the test organism of choice. Flounder (*Platichthys flesus*), Atlantic cod (*Gadus morhua*), sand goby (*Pomatoschistus minutus*), viviparous blenny (*Zoarces viviparus*) and 3-spined stickleback have all been used in laboratory tests with EDCs and have RIA/ELISAs or mRNA probe for VTG. Other species, such as dab (*Limanda limanda*), may also be relevant if a VTG assay is developed. The stickleback, however, has several advantages over other marine species: (a) it is already some way through the validation process as a freshwater species, although further work with more laboratories is essential, thus a guideline could be available in a much shorter time frame than any other screen which would have to start from scratch; (b) a screen for this species would be equally applicable for freshwater and marine water thus allowing direct comparison of threshold effects; (c) currently, the stickleback is the only species that can be used in marine waters which has validated endpoints for (anti) oestrogens and (anti) androgens. During the first phase of validation of the fish screen, the assay changed from being a straightforward adult non-spawning assay, to a screen where spawning status has to be demonstrated (albeit only for quality assurance purposes and not as an ED endpoint). Whilst this was not a particular problem for stickleback, the test design being amended to allow the complex behaviours to take place which are essential for pre-spawning, it would pose a problem if this species were put forward for an equivalent marine screen, since although gonad maturation takes place in salt water, it is not clear if the pre-spawning behaviours would be induced. Demonstrating spawning in any other species e.g. cod, flounder etc may be even more problematic. If, on the other hand, this QA endpoint were waived then the stickleback would again be the favoured candidate.

The fish sexual development test draft protocol could include stickleback and although initially intended as an additional freshwater species, there is significant scope for the protocol to be adapted (as a separate guideline) for exposure in salt water. There may also be scope for including other marine fish species in this type of protocol, such as goby and viviparous blenny. All would need to begin the validation from scratch (although the UK were very interested in taking part in the FSDT intercalibration using stickleback, no funds were available and there was a lack of uptake to this species from other OECD member countries); therefore a useable guideline would not be available for several years.

Invertebrate protocols

The protocols being validated for the marine copepods are not ED specific and therefore if OSPAR requires invertebrate tests that are designed to screen and test for this class of compounds, then alternatives must be developed with species for which endpoints that are diagnostic of an endocrine mediated effect exist.

Recommendation

In the short term we propose to continue work with stickleback, but consideration should be given to other marine fish models. A current UK protocol of the stickleback test exists and can

be elaborated for endocrine effects. With the current lack of EDC specific endpoints, invertebrate models should primarily focus on full life cycle tests with endpoints such as growth, development and reproduction. For certain invertebrate groups with particular relevance for the marine environment, especially echinoderms, possibly polychaetes or gastropods, we propose to stimulate specific research as these may hold promise for future test development.

Justification for Recommendation

Fish

Although several species may be used for this purpose, stickleback and goby have the advantages of being relatively small species with relatively short life spans and are culturable in the lab. The androgen stickleback bioassay can be easily adapted as an androgen and estrogen screen. The same species might be good candidates to screen or test for thyroid hormone disruptive chemical by including suitable biomarkers (f.e. plasma T3/T4 levels) in these species, but this will require further work.

Invertebrates:

Work on development and (sexual) reproduction of aquatic invertebrates by OECD is currently underway. However so far they include few marine invertebrate species, i.e. potentially only harpacticoid and mysid crustaceans. Chronic test developments with marine copepods and Mysids (see text above) should be further implemented/encouraged. Testing protocols published by Verslycke *et al.* 2007 on the use of estuarine Mysid crustaceans as standard models for the screening and testing of endocrine disrupting chemicals are very much in the focus for guideline development.

Further consideration should be given to a standard (marine) molluscan test. The fresh water model described by Duft *et al*. (2006) should be compared to available marine molluscan models for the same reason as given for fish (see above; speciation and bioavailability of TBT in marine waters/organisms differ from those in fresh water/organisms). Small molluscan species such as Nassarius and Bittium could be good candidates and deserve more attention. Much more research is however needed to conclude this. OSPAR/OECD should consider the strategic advantages of testing animals different from vertebrates, whose employment is often restricted by ethical and practical reasons. There is increasing hope that prosobranch snails can be used as possible surrogates for fish. Several animal groups can be valuable and useful model species for ecotoxicological tests assessing the effects of EDCs. However development of such test models will require considerable research and time.

References

Gourmelon, A., and Ahtiainen, Æ. J. 2007. Developing Test Guidelines on invertebrate development and reproduction for the assessment of chemicals, including potential endocrine active substances—The OECD perspective Ecotoxicology (2007), 16: 161–167.

deFur P, Crane, M., Ingersoll, C., and Tattersfield, L .1999. Endocrine disruption in invertebrates: endocrinology, testing and assessment. SETAC Pensacola, FL.

Duft, M., Schmitt, C., Bachmann, J., Brandelik, C., Schulte-Oehlmann, U., Oehlmann, J. 2006. Prosobranch snails as test organisms for the assessment of endocrine active chemicals—an overview and a guideline proposal for a reproduction test with the freshwater mudsnail *Potamopyrgus antipodarum*. Ecotoxicology DOI 10.1007/s10646-006-0106-0.

Hutchinson, T. H. 2002. Reproductive and developmental effects of endocrine disrupters in invertebrates: *in vitro* and *in vivo* approaches. Toxicol Lett, 131: 75–81.

Kusk, K. O., and Wollenberger, L. 2006. Towards an internationally harmonised test method for reproductive and developmental effects of endocrine disrupters in marine copepods. Ecotoxicology DOI 10.1007/s10646-006-0112-2.

Verslycke, T., An Ghekiere, E., Raimondo, S., and Janssen, C. 2007. Mysid crustaceans as standard models for the screeningand testing of endocrine-disrupting chemicals. Ecotoxicology, 16: 205–219.

Hutchinson, T. H. 2007. Small is useful in endocrine disrupter assessment—four key recommendations for aquatic invertebrate research Ecotoxicology, 16: 231–238.

21 Any other business

21.1 Publication of papers

During January and February of this year several members of WGBEC had contacted the Chair John Thain to inform of a paper "Biomarkers and Integrated Environmental Risk Assessment: Are There More Questions Than Answers?" by Haggar, J.A., Jones, M.B., Leonard, P., Owen, R. and Galloway, T.S., published in Integrated Environmental Assessment and Management, 2006, Vol 2, No 4, p 312–329, in which tables (constituting ca six pages in the paper) plus ca 150 references were copied directly from the 2004 WGBEC report. It appeared to be a "cut and paste job," it was not data that had been used and interpreted and gave its citation as ACME. John Thain as Chair had written to ICES to ask for clarification on this issue but no response was received in time for the WGBEC meeting.

WGBEC were unhappy with the publication of this paper and several options for action were raised e.g. formally writing to the authors, the editor or a letter to the Journal. However, it was concluded that the group should wait for clarification on the issue from ICES HQ to discuss an appropriate response.

Action

John Thain to seek advice from ICES HQ on the publication of material from WG reports and an appropriate response in respect of this publication. This would be circulated to WG members when available.

21.2 A request for comment on the STGQAB report had been received from ICES just prior to the meeting of WGBEC, vis-à-vis.

"STGQAB recommends a general revision of part D of the HELCOM COMBINE Manual (Agenda Item 8.1 and 8.2, ANNEX 13)". The request has been directed to HELCOM MONAS, all members of STGAQB, STGQAC, MCWG, WGBEC and WGDIM.

Response from WGBEC

The request was not fully clear for us, however, when going through Part D of the HELCOM COMBINE Manual (present version available on the HELCOM website) there are some topics that we would like to comment on.

Regarding the revised part D of the HELCOM COMBINE Manual and especially its chapter D11 "Biological effects monitoring" it appears not to be in line with the recommendations given in HELCOM MON-PRO meeting documents as well as in the ICES/HELCOM/BSRP Study Group for Ecosystem Health (SGEH) during 2005 and 2006. Documents containing reports and recommendations given by these groups are e.g. the following:

- Report of the "ICES/BSRP/HELCOM/UNEP Regional Seas Workshop on Baltic Sea Ecosystem Health Indicators", 30 March - 1 April 2005, Sopot, Poland. EH Workshop Report, ICES Baltic Committee, ICES CM 2005/H:01 Addendum.

- Minutes of the Third Meeting of Project to Review HELCOM Monitoring and Assessment Programmes (HELCOM MON-PRO). 2005.
- Report of the Study Group on Baltic Ecosystem Health Issues in Support of BSRP (SGEH), 9-11 November 2006, Kaliningrad, Russia. ICES SGEH Report 2006: ICES Baltic Fisheries Committee, ICES CM 2006/BCC:01, Ref. ACE.

In all these documents, the basic idea of establishing a biological effects monitoring programme that includes all main levels of biological organisation has been underlined. The current Part D of the revised HELCOM COMBINE Programme on monitoring of contaminants and their effects is lacking early-warning indicators (molecular, biochemical and physiological levels) in the monitoring set-up.

In the current revision of the HELCOM COMBINE part D, acetylcholinesterase (AChE) inhibition has been recommended as a biomarker to indicate exposure of marine organisms to organophosphate and carbamate pesticides in the Baltic Sea area. However, AChE is well known to be – in addition of having a specific response to the compounds mentioned above – affected by a large variety of other compound groups as well (e.g. heavy metals, detergents, algal toxins) and is therefore rather an indicator of non-specific (neurotoxic) stress. Furthermore, organophosphate and carbamate pesticides are an insignificant group of pollutants in most parts of the Baltic Sea. Moreover and most importantly, in the documents listed above, the use of a battery of indicators is clearly recommended instead of picking up just one common early-warning parameter for the whole sea area.

A substantial number of biomarker methods have been in use in the OSPAR and MEDPOL areas, and have been positively evaluated by WGBEC. Their inclusion in HELCOM monitoring programmes has been extensively discussed in recent years (e.g. MON-PRO, SGEH). In the HELCOM COMBINE document the sole mentioning of AChE activity as an early-warning indicator parameter does not reflect these recent developments and the potential application of biomarker methods in the Baltic Sea area. Information given in the document on the use of other biomarkers in the Baltic Sea area (EROD, histopathology) does not represent the current knowledge on the applicability of a large suite of biomarker methods, recently evaluated in the EU BEEP Project Baltic Sea component papers (*Marine Pollution Bulletin* 53, 2006), including the following overview paper:

- Lehtonen, K. K., Schiedek, D. Köhler, A., Lang, T., Vuorinen, P.J., Förlin, L., Baršiene, J., Pempkowiak, J., and Gercken, J. 2006. The BEEP project in the Baltic Sea: overview of results and outline for a regional biological effects monitoring strategy. Marine Pollution Bulletin 53: 523–537.

In this paper it is also suggested that a suite of effect parameters should be applied according to the specific features of each sub-region of the Baltic Sea, characterised by highly differing biotic and abiotic features (e.g. salinity). Furthermore, basin-scale comparisons could be made by developing a synthetic "pollution index" composed of different indicators of contaminants and their concentrations in environmental matrices.

For the general revision of part D, as recommended by STGQAB we think that changes to the content of chapter D11 of the guidelines should be considered according to the documents produced during the work of HELCOM MON-PRO and BSRP SGEH, and also as pointed out above.

21.3 Use of biological effects techniques in Risk Assessment

The group considered recent progress in the application of biological effects techniques in environmental risk assessment.

Steinar Sanni (Norway) was invited to present work from Norway describing linking biomarker results with risk assessment procedures for produced water discharges in the North

Sea. Environmental management has been adopted by oil and gas industry in Norway, and it is based on predictions of possible effects and on monitoring of actual impacts in the field. The impact monitoring is often based on biomarkers measured in individual animals of different species. Tools have been developed for both prediction and monitoring purposes.

It would be an important improvement in the basis for environmental control to be able to compare the risk predictions with the field measurements. In order to do so, common parameters that both can predict and be measured in the field are needed. These common parameters could with advantage be closely related to biological effects, due to political and regulatory focus on biological effects, e.g. in the North Sea. The type of parameters that seem to fulfil these requirements the best are the biomarkers. Therefore we have tried to integrate biomarkers with environmental prediction procedures. The biomarkers bridge the gap between those methods used for predictions and those used for measurements in the field. (Thus, in this context they form "biomarker bridges"). Based on data from laboratory studies, a "biomarker bridge" model has been developed to connect risk assessment and monitoring of produced water discharges to the sea. A skeleton of a "biomarker bridge" model has been incorporated into the existing risk and impact assessment software tool called "DREAM".

The "biomarker bridge" model seems to be a useful way to link biomonitoring with risk assessment and it is therefore interesting to gain enough data to make the tool ready for practical use in relation to discharges of produced water from the oil and gas industry offshore. A four years project starting in 2007 has been granted by the Research Council of Norway to carry out this work. The "biomarker bridge" model can be applied to different kinds of discharges provided that proper experimental data exist.

It was noted by Aldo Viarengo (Italy) that the approach provides a method for the interpretation of data but the extrapolation of biomarker data in the field to the population level is very difficult.

MJ Belzunce (Spain) presented a work on the use of integrative methods for marine sediment risk assessment in the estuaries of the North of Spain. The objective of this study was to develop an integrative protocol for the assessment and management of contaminated sediments from estuaries and harbours. It consists in tiered framework which proceeds through a series of sequential steps and it is based on three lines of evidence (LOE): chemical contamination, toxicity and *in situ* alteration. At the last step of the framework the data obtained in the previous stages are integrated. Among the different methods for data integration (multivariate analysis, weight of evidence approach, triangles representation), in this study a decision-matrix is developed by ranking data from the three LOE. The final decision integrates the data collected from chemistry, toxicity and benthic communities analysis in a weight of evidence approach (WoE) for sediment risk assessment. It is not a sum of the components, but each component is considered relative to one another. A scheme of the protocol is illustrated in Figure 21.3.1.

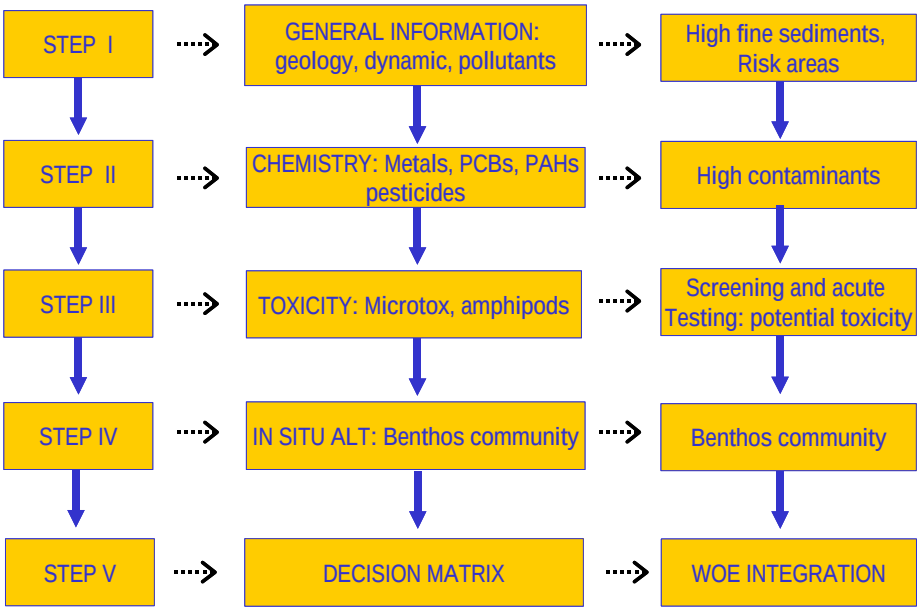

Figure 21.3.1. Scheme of the proceeding protocol for the assessment and management of contaminated sediments from estuaries and harbours.

This protocol has been applied in estuaries and harbours of the Basque littoral and the results from the Pasaia estuary were presented. From the series of data obtained from the studied estuaries it is inferred that the integrative protocol applied provides an (a robust and applicable) overall risk assessment for the marine environment protection.

The toxicity of the sediment samples is evaluated through a battery of bioassays consisting in a screening test Microtox), marine amphipods (*Corophium* sp) acute test and an embryogenesis test with sea urchins (*Paracentrotus lividus*). Two species of *Corophium, C. urdaibaiense* and *C. multisetosum*, which are dominant in the estuaries of Northern Spain are used for whole sediment bioassays.

Besides the risk assessment studies, the ecology and the distribution of the species of *Corophium* have been studied in Basque estuaries. A new species have been identified as *Corophium urdaibaiense* (Marquiegui and Perez, 2006). The population of species *Corophium urdaibaiense* has been studied for a year in the Urdaibai estuary and temporal data series of density, biomass, breeding periods, size-frequency and factors influencing the sensitivity of the species have been obtained (Perez *et al.*, 2006). In a *Paracentrotus lividus* population from a Basque estuary the seasonal variation of the percentage of mature individuals in their natural environment along one year has been evaluated and a series of data on gonadal index, spawning ratio and fertility success have been obtained. Parallel, experiments of maturity induction under lab conditions have been carried on with the objective of supplying gametes for toxicity tests.

In future work, similar studies with a sea urchin population from other estuary of the Basque littoral will be performed. Besides, the maintenance under lab conditions of mature individuals. Bioassays with sea urchins will be also perform for waters and algae toxicity evaluation.

The group noted that Norway, the UK and the Netherlands have been active over the past years in developing approaches for the ecological risk assessment of sediment whilst WGBEC reviewed the topic of dredge material assessment w in 2004. It was suggested that it may be worth reviewing progress in this area at WGBEC 2008. Cor Schipper (Netherlands) provided

the group with an update on the status of dredge material assessment (using biological effects techniques) in the Netherlands. In 2006 an integrated risk assessment was evaluated and the benefits (e.g. economic) for stakeholders assessed. The biological effect techniques used were:

- DR-CALUX
- Corophium volutator
- Vibrio fischeri

The outcome of this assessment is that it has been recommended that mechanistic assays are to be used in the future in order to assess the presence of specific groups of compounds (e.g. DR-CALUX). In addition the Netherlands will use bioassays in monitoring rather than in legislation with the net result that they will no longer be included in legislative testing. Their use in monitoring fits into a three-pronged approach consisting of:

1) Prevention (Action levels);
2) Monitoring (In vivo and in vitro biological effects techniques);
3) Remediation (Sediment remediation).

With regards biological effects techniques, harbour authorities in the Netherlands will be able to use them for monitoring the quality of the whole harbour area (holistic). The benefits of which were reported to be the ability to establish which sources are the cause and what is happening upstream for an integrated environmental improvement (ecological health status). During discussion it was noted that each country that uses bioassay uses its own suite with Corphium being the only common test organism.

Recommendation

It was decided that further evaluation of the topic and that the use of biological effects in risk assessments with respect to the EU Water Framework Directive (WFD) and forthcoming Marine Strategy Directive (MSD) will be further reviewed at the WGBEC Meeting 2008.

References

Marquiegui, M. A., and Pérez, V. 2006. Corophium urdaibaiense (Amphipoda: Corophiidae: Corophiinae: Corophiini) a new species from the Cantabrian Sea (Bay of Biscay, north-east Atlantic). J. Mar. Biol. Ass. U.K. 86: 729–736.

Pérez, V., Marquiegui, A., and Belzunce, M. J. 2006. Life history and production of Corophium urdaibaiense (Crustacea: Amphipoda) in the Urdaibai estuary (NE Spain). Marine Biology.

22 Recommendations and action list

22.1 Recommendations

1 (Agenda Item 4)

a) WGBEC recommends that the assessment criteria that are needed for WKIMON biological effects framework methods need to be made available by 1.October 2007.

WGBEC members agreed to prepare documents and criteria as follows:

For mussels:

- scope for growth (assessment criteria only) – John Thain, Jim Readman
- condition index (protocol, assessment criteria) – John Thain
- histopathology (background, protocol, assessment criteria) – Steve Feist
- lysosomal stability (WKIMON III) – established

- micronucleus formation (protocol, assessment criteria) – Aldo Viarengo (Claudia Bolognesi)
- AChE inhibition (assessment criteria) – Thierry Burgeot
- stress on stress (background, protocol, assessment criteria) – Aldo Viarengo

For fish (dab, flounder, haddock, long rough dab):

- GSI, LSI, condition (protocol, assessment criteria) – Ketil Hylland
- Fish disease index (FDI; protocol, assessment criteria) – Thomas Lang, Steve Feist
- PAH metabolites (assessment criteria) – Dick Vethaak, Matt Gubbins, Ketil Hylland
- EROD (assessment criteria) – communicate with OSPAR secretariat (Ulrike Kammann)
- vitellogenin (assessment criteria) – John Thain
- lysosomal stability - established
- DNA adduct (assessment criteria) – Brett Lyons
- AChE inhibition (assessment criteria) – Thierry Burgeot , Doris Schiedek

b) There is also a need for modification of the existing integrated assessment systems (i.e. Fullmonti), which should similarly need to be available by 1. October 2007. John Thain, Dick Vethaak and Ketil Hylland will do this task. The expert system developed by Aldo Viarengo will need to be considered in this process.

c) A protocol is needed for the methods where such are not available at the moment, including mussel histopathology (Steve Feist) and stress on stress in mussels (used in MEDPOL, Aldo Viarengo).

d) ACME is asked to take note of and support the ICON demonstration programme for an integrated chemical and biological monitoring guideline.

2 (Agenda Item 6)

a) WGBEC recommends that the background documents on biological effect techniques produced by WKIMON were sufficiently complete for publication by OSPAR.

b) Secondly that a process be put in place to allow a regular review and update of these documents.

3 (Agenda Item 7)

a) ICES should promote to OSPAR and the European Commission the additional value of bioassays and passive samplers in WFD and their potential role as connective link between WFD and the Marine Strategy Directive (MSD).

b) ICES should advise OSPAR and the European Commission to take notice and make advantage of the existing integrated chemical-biological effect methods and integrated assessment approaches developed by OSPAR (in JAMP and CEMP) with reference to their potential value in the monitoring and assessment strategy for the MSD.

Justification

EC had missed the opportunities to include (at that time available) BEC methods in the WFD. Since the MSD aims at an assessment of the ecosystem as a whole, integrated chemical biological effect techniques will be instrumental in assessing the impact of hazardous substances on ecosystem health.

4 (Agenda Item 9)

a) In view of the current developments within OSPAR WKIMON and the revision of the JAMP, WGBEC recommends that it revisit the "recommended list of methods" in 2009

Justification

To ensure that it is up-to-date and aligned with the current developments within the JAMP and the ICON demonstration programme.

5 (Agenda Item 10)

That the potential role for passive samplers (of a variety of types) in the ICON demonstration programme be discussed at the workshop in May.

6 (Agenda Item 12)

OSPAR MON should adopt the new methodology for assessing VDSI in *Nucella lapillus*.

Mean-variance relationships should be established for imposex parameters in other species of gastropods to allow the assessment procedure to be applied over a wider part of the ICES area.

Members should submit individual imposex data to the ICES database, but where this is not possible, indexed data should be submitted along with sex ratio of the sample.

7 (Agenda Item 13)

a) WGBEC would recommend that ICES inform OSPAR of the current position on AQC uptake for biological effect techniques within BEQUALM. Secondly that ICES informs other appropriate ICES Working Groups of the activities of BEQUALM in relation to benthic community analysis and phytoplankton assemblages to avoid duplication of effort and improve harmonisation of AQC.

Justification

AQC data is important to allow for the co-ordinated assessment of data across the OSPAR Maritime Area, and should accompany all data submitted to the ICES database. It is important that AQC procedures are therefore harmonised, in place and used by Contracting Parties. Failure to use AQC schemes downgrades the use of the data for assessment purposes.

8 (Agenda Item 14)

a) Permission is requested from ICES to commission methods for: Blue mussel histopathology, YES / YAS, CALUX, reproductive success in fish and the micronucleus assay. Requests for publication of method documents concerning blue mussel histopathology will be put forward as a Draft Resolution in 2007.

9 (Agenda Item 20)

a) To date, there exists too much uncertainty on the extrapolation of fresh water EDC ecotoxicity data to salt water species to simply justify the applicability of OECD test protocols for protection of the marine environment. Therefore we recommend that a first assessment should be done using the data available on fish and invertebrate species, with the caution that this data has been produced for the testing of chemicals specifically for regulatory purposes.

Justification to support this recommendation

There are generally less toxicity data available for saltwater species than for freshwater ones, especially in relation to organic compounds and several organic endocrine disrupting chemicals, such as alkylphenols, phthalates and brominated flame retardants. Although

phylogenetically there is reason to assume that freshwater fish respond similarly to marine fish, and that the distributions of the sensitivities of the two groups of species are identical, these assumptions remain largely untested (and have led to proposals to add an additional safety factor of 10 to marine risk assessments based upon freshwater data (see paper by Wheeler *et al.*, 2002))

Uncertainty exists in the area of bioavailability and possibly differential uptake of EDCs by marine and euryhaline species - in marine and especially estuarine conditions - as compared to fresh water species. As such, endocrine disrupting chemicals in freshwater may have effects in fish other than estuarine or marine ones and tests with freshwater species will be inadequate to fully protect the marine environment. The same may be true for invertebrates including phyla exclusive to the marine environment. To justify the use of marine tests an assessment of comparable data on relevant EDC sensitivity distributions between freshwater and marine animals should be made. If sufficient evidence exists and sensitivity between marine and fresh water animals exceeds one order of magnitude, testing of additional estuarine or marine species may be then required. Therefore we recommend that a first assessment should be done using the data available on species such as stickleback (androgen-associated) and flounder (estrogen associated). A similar assessment should be done for invertebrates There is a however a general need for more high quality data on the effects of a wide range of EDCs to both freshwater and saltwater organisms

In addition to the sensitivity and extrapolation issue (above) another argument relates to the representativity in terms of taxa/phyla. Will freshwater vertebrate and invertebrate models including marine copepods and crustaceans adequately protect the marine environment? The current lack of marine invertebrate ED tests generally is mainly due to lack of knowledge and the problems to differentiate between general/reproductive toxicity and the modulation of complex endocrine mechanisms of biological regulation. Most invertebrates rely on a totally different set of hormones than those commonly studied in vertebrate models. Most of the available invertebrate tests are not designed to identify an endocrine disruptor, but they merely yield apical endpoints such as reproduction, development and offspring sex ratios that included influences on the hormone systems and can be used in an environmental risk assessment (see special issue on EDC in invertebrates in ecotoxicology 2007). The justification to include more marine invertebrate species in OECD EDC screening and test development and regulatory frame works is of a complex nature and requires more studies and time. However, it is important to include invertebrates to increase the possibility to detect all kinds of EDC. For the near future OECD should focus on the development of full life cycle tests representing a variety of invertebrate phyla exclusive to the marine environment such as Echinoderms that are widely distributed in all coastal waters. This phylum deserves much more attention. For example, reproductive and regenerative phenomena of echinoderms can be considered possible models for studies on EDC effects. Some studies confirm that these compounds interfere with fundamental physiological processes including growth, development and reproductive competence. Other promising species could include nematodes, cnidaria and tunicates but only if it could be demonstrated that they are more sensitive to new types of endocrine action, or if they provide a substitute for oestrogen/androgen-sensitive vertebrates (for ethical reasons).

b) In the short term we propose to continue work with stickleback, but consideration should be given to other marine fish models. A current UK protocol of the stickleback test exists and can be elaborated for endocrine effects. With the current lack of EDC specific endpoints, invertebrate models should primarily focus on full life cycle tests with endpoints such as growth, development and reproduction. For certain invertebrate groups with particular relevance for the marine environment, especially echinoderms, possibly gastropods, we propose to stimulate specific research as these may hold promise for future test development.

Justification

Fish

Although several species may be used for this purpose, stickleback and goby have the advantages of being relatively small species with relatively short life spans and are culturable in the lab. The androgen stickleback bioassay can be easily adapted as an androgen and estrogen screen. The same species might be good candidates to screen or test for thyroid hormone disruptive chemical by including suitable biomarkers (f.e. plasma T3/T4 levels) in these species, but this will require further work.

Invertebrates:

Work on development and (sexual) reproduction of aquatic invertebrates by OECD is currently underway. However so far they include few marine invertebrate species, e.g. potentially only harpacticoid and mysid crustaceans. Chronic test developments with marine copepods and Mysids (see text above) should be further implemented/encouraged. Testing protocols published by Verslycke *et al.* (2007) on the use of estuarine Mysid crustaceans as standard models for the screening and testing of endocrine disrupting chemicals are very much in the focus for guideline development.

Further consideration should be given to a standard (marine) molluscan test. The fresh water model described by Duft *et al*. (2006) should be compared to available marine molluscan models for the same reason as given for fish (see above; speciation and bioavailability of TBT in marine waters/organisms differ from those in fresh water/organisms). Small molluscan species such as Nassarius and Bittium could be good candidates and deserve more attention. Much more research is however needed to conclude this. OSPAR/OECD should consider the strategic advantages of testing animals different from vertebrates, whose employment is often restricted by ethical and practical reasons. There is increasing hope that prosobranch snails can be used as possible surrogates for fish. Several animal groups can be valuable and useful model species for ecotoxicological tests assessing the effects of EDCs. However development of such test models will require considerable research and time.

10 (Agenda Item 21.3)

a) WGBEC felt that it should further evaluate the use of biological effects in risk assessments with respect to the EU Water Framework Directive (WFD) and would like to review this at its meeting 2008.

22.2 Action List

1 (Agenda Item 6)

John Thain to inform the OSPAR Secretariat of an amendment to the Scope For Growth background document

2 (Agenda Item 7)

Ketil Hylland to invite representatives of MEDPOL (Aldo Viarengo) and HELCOM (Kari Lehtonen) to be involved in the ICON programme.

3 (Agenda Item 8)

ICES WGBEC to review the potential use and opportunities for biomarkers and bioassays in relation to monitoring and risk assessment frame works of the WFD and the MSD.

4 (Agenda Item 11)

That the use of oxidative stress measurements for marine environmental monitoring in the ICES area be reviewed intersessionally and presented at the next meeting in 2008.

5 (Agenda Item 21.1)

John Thain to seek advice from ICES HQ on the publication of material from WG reports and an appropriate response in respect of this publication. This would be circulated to WG members when available.

23 Adoption of the report and closure of the meeting

The draft text of the meeting report was prepared and circulated for comment via the ICES WGBEC Sharepoint. Corrections and amendments were made as appropriate. The Chair agreed to incorporate these in a full draft report, which would be circulated by email at a later date.

Members of the group felt that the ICES Sharepoint facility was an excellent development; WGBEC had uploaded approximately 90 documents during its meeting. The Chair agreed to convey this message back to ICES.

The Chair thanked members of the WG for their contributions. Members of the WG expressed their thanks to Aldo Viarengo and the University of Alessandria for their hospitality and for hosting the meeting.

The group considered a venue for the 2008 meeting. Thierry Burgeot (FR) offered the IFREMER Institute in Sete in France for the week beginning 31March 2008. The group felt that this offered a real opportunity to improve, strengthen and build upon the OSPAR / MEDPOL links and initiatives taken at this meeting; it was unanimously agreed.

The Chair closed the meeting at 14:00 hrs on 23 March 2007.

Annex 1: List of Participants

NAME	ADDRESS	PHONE/FAX	EMAIL
Maria Jesús Belzunce Segarra	AZTI Herrera *K*aia, Portualdea z/g 20110 Pasajes Spain	Tel.: +34 943 004800, ext: 826 Fax: +34 943 004801	jbelzunce@pas.azti.es
Thierry Burgeot	IFREMER rue de l'Ile d'Yeu B.P. 21105 F-44311 Nantes Cédex 03 France	Tel: +33 240374051 Fax: +33 240374075	tburgeot@ifremer.fr
Kris Cooreman	ILVO – Fisheries Ankerstraat 1 8400 Oostende Belgium	Tel. +3259569820 Fax: +3259330629	kris.cooreman@ ilvo.vlaanderen.be
Gabriel Gabrielides	United Nations Environment Programme Mediterranean Action Plan 48, Vass. Konstantinou Ave (2nd floor) P.O.Box 18019 11610 Athens Greece	Tel: +30 2107273132 Fax: +30 2107253196 or 97	gabriel@unepmap.gr
Matt J. Gubbins	Fisheries Research Services Marine Laboratory P.O. Box 101 375 Victoria Road Aberdeen AB11 9DB United Kingdom	Tel: +44(0)122 429 5681	m.gubbins@marlab.ac.uk
Ketil Hylland	Norwegian Institute for Water Research (NIVA) CIENS Gaustadalléen 21, N-0349 Oslo Norway	Tel: +4741451694 FAX: +47 22185200	ketilhy@bio.uio.no
Ulrike Kammann	Institut f. Fischereiökologie BFA f. Fischerei Palmaille 9 22767 Hamburg Germany	Tel: +49 4038905198	Ulrike.kammann@ifo.bfa-fisch.de

NAME	ADDRESS	PHONE/FAX	EMAIL
Kari Lehtonen	Finnish Institute of Marine Research P.O. Box 2 FIN-00 561 Helsinki Finland	Tel: +358 9613 94566 Fax: +358 9323 2970	lehtonen@fimr.fi
Brett Lyons	CEFAS Weymouth Laboratory Barrack Road, The Nothe Weymouth, Dorset DT4 8UB United Kingdom	Tel: +44 1305 206600 Fax: +44 1305 206601	brett.lyons@cefas.co.uk
Concepción Martínez	Instituto Español de Oceanografía Centro Oceanográfico de Murcia Varadero 1, Lo Pagán 30740 San Pedro del Pinatar (Murcia) Spain	Tel: +34 968180500 Fax: +34 968184441	cmartinez@mu.ieo.es
James Readman	Plymouth Marine Laboratory Prospect Place The Hoe Plymouth PL1 3DH UK	Tel: +44 1752 633460 Fax: +44 1752 633101	jwre@pml.ac.uk
Steinar Sanni	IRIS Biomiljø	Tel: +47 5187 5504 Fax: +47 5187 5540	Steinar.Sanni@iris.no
Doris Schiedek	Institut für OstseeforschungSeestrasse 15 D-18119 Rostock Germany	Tel: +49 3815197205 Fax: +49 3815197211	doris.schiedek@ io-warnemuende.de
Cor Schipper	RIKZ P.O. Box 20907 2500 EX The Hague The Netherlands	Tel: +31 703114219 Fax: +31 703114200	cor.schipper@rws.nl
Britta Sundelin	Department of Applied Environmental Science ITM Stockholms Universitet 106 91 Stockholm	Tel: +46 867 47235	brita.sundelin@itm.su.se
John Thain (Chair)	CEFAS Burham-on-Crouch Laboratory Remembrance Avenue Burnham-on-Crouch UK-Burnham-on-Crouch CM0 8AH United Kingdom	Tel: +44 (0)1621787239 Fax: +44(0)1621784989	j.e.thain@cefas.co.uk

NAME	ADDRESS	PHONE/FAX	EMAIL
Kevin Thomas	Norwegian Institute for Water Research (NIVA) CIENS Gaustadalléen 21, N-0349 Oslo Norway	Tel: +4792265694 Fax: +47 22185200	kvt@niva.no
Dick Vethaak	RIKZ P.O. Box 20907 2500 EX The Hague The Netherlands	Tel: +31 703114219 Fax: +31 703114200	a.d.vethaak@rikz.rws.minvenw.nl
Aldo Viarengo	University of Eastern Piedmont, Via Bellini, 25G 15100 Alessandria, Italy	Tel: +39 0131 360 370 Fax: +39 33 357182439	viarengo@unipmn.it

Annex 2: WGBEC Terms of Reference 2006

2006/2/MHC03 The **Working Group on Biological Effects of Contaminants** [WGBEC] (Chair: John Thain, CEFAS, UK) will meet in Alessandria, Italy, from 15–19 March 2007 to:

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

b) evaluate the report from WKIMON third workshop and the intersessional work undertaken by WGBEC members, including the development of assessment criteria, integrated monitoring and the development of a field demonstration programme;

c) evaluate documents prepared intersessionally for "background" biological effects responses;

d) review the "recommended list" of biological effect techniques;

e) explore the possibilities to organize a practical ICES/OSPAR-MEDPOL Workshop on the use and application of integrated chemical-biological methods in monitoring the quality of the marine environment with special reference to EU-directives;

f) report on progress with intersessional activities using passive samplers;

g) assess the amount of biological effects data submitted to the new ICES database;

h) review the use of in vitro and in vivo biological effects techniques for monitoring purposes and WFD activities;

i) report on progress with the assessment of imposex data;

j) consider progress with activities such as BEQUALM, HELCOM area/BSRP project, Prestige oil spill, biological effects monitoring programmes in MEDPOL, (USA, Canada and other relevant national and international projects);

k) evaluate reports prepared intersessionally by WGBEC on oxidative stress, cellular energy allocation and aromatase;

l) review integrated methods for assessment of effects on biota from lindane, BFRs and methodology of the COMET assay;

m) Provide expert knowledge and guidance to ICES Data Centre (possibly via sub-group) as requested;

n) together with MCWG and WGMS, review the existing technical annexes on PAHs to see whether they are adequate for monitoring of target alkylated PAHs and, as appropriate, prepare advice on any revisions that are necessary;

o) follow–up a third OSPAR/ICES workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-sea Areas (WKIMON III) through review and, as appropriate, finalisation of proposals for assessment criteria for the following biological effects techniques included, or considered for inclusion, under the OSPAR Co-ordinated Environmental Monitoring Programme (CEMP):

i) metallothionein

ii) ALA-D

iii) cytochrome P4501A

iv) DNA adducts

v) PAH metabolites

vi) whole sediment bioassays

vii) sediment pore-water bioassays

viii) sediment sea water elutriates

ix) water bioassays

x) lysosomal stability

xi) liver pathology including neoplasia/hyperplasia

xii) liver nodules

xiii) externally visible fish diseases

xiv) reproductive success in fish

xv) vitellogenin

xvi) scope for growth

xvii) lysosomal stability.

c) identify and report on changes in the distribution, population abundance and condition of marine species in the OSPAR maritime area that are driven by contaminants or by interactions between the effects of contaminants and changes in hydrodynamics and sea temperature. Further details on the interpretation and handling of this ToR will be provided by ACE;

WGBEC will report by 2 April 2007 for the attention of the Marine Habitat Committee and ACME.

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)** The output from WKIMON III will influence the uptake of biological effects techniques across the OSPAR area and WGBEC should be allowed to evaluate the resulting guidelines. **c)** "Background" biological responses were discussed at WGBEC 2006. However, further work needs to be undertaken on this topic. **d)** WGBEC keeps a watching brief on the progress and development of biological effect techniques and reviews its list every three years for reporting back to ICES and OSPAR. **e)** Both OSPAR and MEDPOL currently develop and run their monitoring programs with considerable overlap in chemical and biological methods applied. These programs will be instrumental to the EU Marine Strategy and are also relevant to the EU WFD. WGBEC's meeting in MEDPOL domain (Italy) offers a unique opportunity to explore the need for a practical workshop to exchange monitoring strategies and test integrated methods (such as currently under development by ICES/OSPAR in WKIMON) in both the NW Atlantic andn Mediterranean. **f)** Investigations were initiated on the use of passive samplers during the joint meeting with WGSMin 2006 and these activities need to be reported back to WGBEC. **g)** Biological effects data can be entered into the revamped ICES database and WGBEC needs to monitor the data reporting acitivities. **h)** *in vitro* and *in vivo* methods methods are now being more widely used in monitoring programmes and WGBEC needs to evaluate progress and application of these techniques. **i)** Continue the work already undertaken at WGBEC 2006 (with WGSAEM) on the assessment of imposex data and any activities in this respect undertaken by OSPAR MON. **j)** AQC for biological effects methods has been identified as critical by international organisations; following commercialisation of BEQUALM; it is important that WGBEC

	keeps track of developments with this programme. There is an ongoing biological effects monitoring programme following the Prestige oil spill; as in previous years WGBEC wishes to be kept informed of strategies and results.In addition it is important that WGBEC keeps track and evaluates other national and international programmes that can influence the uptake of biological effect techniques, e.g., BSRP. **k)** WKIMON have requested that WGBEC assess the applicability of these techniques for inclusion in the OSPAR JAMP CEMP. **l)** In response to a request from WKIMON a review will be carried out intersessionally for WGBEC to evaluate in 2007. **m)** This is in direct response to a request by the ICES Data Centre. **n)** This is a response to an OSPAR request (OSPAR 2007/2(b)). **o)** This is a response to an OSPAR request (OSPAR 2007/5). **p)**This is in support of a request from OSPAR.
Resource requirement:	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:	There are no obvious linkages with other advisory committees.
Linkages to other Committees or Groups:	There are linkages with WGSAEM, MCWG, WGMS and WGPDMO.
Linkages to other Organisations:	None identified.

Annex 3: WGBEC Agenda

Alessandria, Italy, from 19th – 23rd March 2007

1) Opening of the meeting;

2) Adoption of the agenda;

3) Appointment of rapporteurs;

4) Evaluate the report from WKIMON third workshop and the intersessional work undertaken by WGBEC members, including a demonstration program (TORb);

5) Follow-up a third OSPAR/ICES workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-sea Areas (WKIMON III) (TORo):

6) Evaluate documents prepared intersessionally for "background" biological effects responses (TORc);

7) Explore the possibilities to organize a joint OSPAR-MEDPOL Workshop on the use and application of integrated chemical-biological methods (TORe).

8) Review the use of *in vitro* and *in vivo* biological effects techniques for monitoring purposes and WFD activities (TORh).

9) Review the "recommended list" of biological effect techniques (TORd);

10) Report on progress with intersessional activities using passive samplers (TORf):

11) Receive and evaluate reports prepared intersessionally by WGBEC on oxidative stress, cellular energy allocation and aromatase (TORk);

12) Report on progress with the assessment of imposex data (TORi);

13) Consider progress with activities such as BEQUALM, HELCOM area/BSRP project, Prestige Oil Spill and biological effects monitoring programmes in MEDPOL (TORj):

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

15) Assess the amount of biological effects data submitted to the new ICES database (TORg);

16) Provide expert knowledge and guidance to ICES Data Centre (possibly via sub-group) as requested (TORm);

17) Reiew integrated methods for assessment of effects on biota from lindane, BFRs and methodology of the COMET assay (TORl);

18) Together with MCWG and WGMS, review the existing technical annexes on PAHs to see whether they are adequate for monitoring of target alkylated PAHs (TORn);

19) Identify and report on changes in the distribution, population abundance and condition of marine species in the OSPAR maritime area that are driven by contaminants or by interactions between the effects of contaminants and changes in hydrodynamics and sea temperature. Further details on the interpretation and handling of this ToR will be provided by ACE (TORp);

20) Request from OSPAR regarding endocrine disruptors TORq): - consider the adequacy of the OECD test protocols and specify proposals for a test programme for marine species if appropriate.

21) Any other business;

   a. Publication of papers
   b. ICES request for comment on STGQAB report
   c. Risk assessment in relation to biological effects

22) Recommendations and action list;

23) Adoption of the report and closure of the meeting

WGBEC will report by 10 April 2007 for the attention of the Marine Habitat Committee and ACME, i.e. 18 days.

Annex 4: Timetable for meeting

ICES WGBEC Alessandria, 19–23 March 2007

DATE	APPROX. TIME	AGENDA ITEM	ISSUE
Monday 19 March	09:30	1	Introduction to ICES WGBEC by chairperson, housekeeping issues, *tour de table.*
	10:00	2	Adoption of agenda, tabling of documents.
	10:15	3	Appointment of rapporteurs
	10:45	4	Evaluate the report from the ICES OSPAR WKIMON III workshop including demonstration programme.
	12:30		Lunch
	13:30	5	Follow up re; third ICES / OSPAR workshop
	14:30	7	Explore possibilities on OSPAR-MEDPOL Workshop on integrated chemical-biological methods
	16:30	6	Evaluate documents prepared intersessionally on background documents
	18:00		Close of business.
Tuesday 20 March	09:15	9	Review the "recommended list" of biological effects techniques
	11:30	8	Review use of in vitro and in vivo biological effects techniques for monitoring and WFD activities
	12:30		Lunch
	13:30	18	Review existing technical annexes on PAHs to see if they are adequate for monitoring targeted alkylated PAHs
		21c	Use of biological effects techniques in Risk Assessment
	18:00		Close of business.
Wednesday 21 March	09:15	20	Request from OSPAR regarding endocrine disrupters & OECD protocols
	10:30	19	Request from OSPAR on changes in the distribution, population abundance and condition of marine species in relation to contaminants in the OSPAR area
	12:30		Lunch
	13:30	11	Receive and evaluate reports prepared intersessionally on oxidative stress, cellular energy allocation and aromatase
	16:30	12	Report on progress with the assessment of imposex data
	18:00		Close of business.
Thursday 22 March	09:15	13	Consider progress and activities with biological effects in relation to biological effects monitoring programmes; HELCOM area / BSRP, Prestige oil spill, BEQUALM, MEDPOL + +
	12:30		Lunch
	13:30	21b	ICES request regarding STGAQB report
	14:30	14	Review progress with ICES TIMES publications
	15:30	15	Assess the amount of biological effects data on the ICES database

DATE	APPROX. TIME	AGENDA ITEM	ISSUE
	16:00	16	Provide expert knowledge and guidance to ICES Data Centre as requested
	18:00		Close of business.
Friday 23 March	09:15	10	Report on progress with passive samplers
		21a	Publication of papers
			Any other business and/or items carried over from previous days proceedings
	11:15	22	Recommendations and action list.
	11:45	23	Review and adoption of the report.
	12:30		Lunch
	15:00		Closure of the meeting.

Annex 5: List of Rapporteurs

AGENDA ITEM	DESCRIPTION	LEAD / COLLATOR / RAPPORTEUR
1	Opening of meeting	John T
2	Agenda	John T
3	Rapporteurs	John T
4	Evaluate WKIMON 3 report	Ketil H & Kris C
5	Follow up WKIMON 3	Ketil H & John T
6	Evaluate background docs	John T
7	Explore OSPAR-MEDPOL workshop	Dick V, John T & Gabriel G
8	Review in vitro and in vivo bio effects tests	Dick V
9	Review recommended list	Brett L
10	Report on progress with passive samplers	Matt G
11	Receive reports on a) oxidative stress, b) cellular energy allocation & c) aromatase	Matt G Kris K Dick V
12	Progress with imposex	Matt G
13	Progress reports on a) HELCOM/BSRP, b) Monitoring in Spain & Prestige oil spill, c) MEDPOL, d) BEQUALM and other	Kari L Concha M & Maria B Gabriel G John T
14	Progress with ICES TIMES	Matt G
15	Data submission to ICES database	John T
16	Provision of guidance to ICES database	John T
17	Review integrated methods re a) Lindane, b) BFRs & c) COMET	Rolf S Dick V Brett L
18	Review technical annexes for PAHs	Jim R & Kevin T
19	Climate change request from ACE / OSPAR	Doris S, Brita S & Kari L
20	OSPAR OECD protocol request	John T & Dick V
21	AOB: a) Publication of papers, b) ICES comment on STGQAB, c) Risk assessment, other	John T Kari L & Doris S Kevin T & Maria B
22	Recommendation / Action list	John T
23	Report	John T

Annex 6: Overview of WKIMON strategy

The WKIMON strategy involves three ecosystem components: water, sediment and biota (Figure A6.1). Separate indices will be developed for the three compartments, much as outlined in the UK Fullmonti framework.

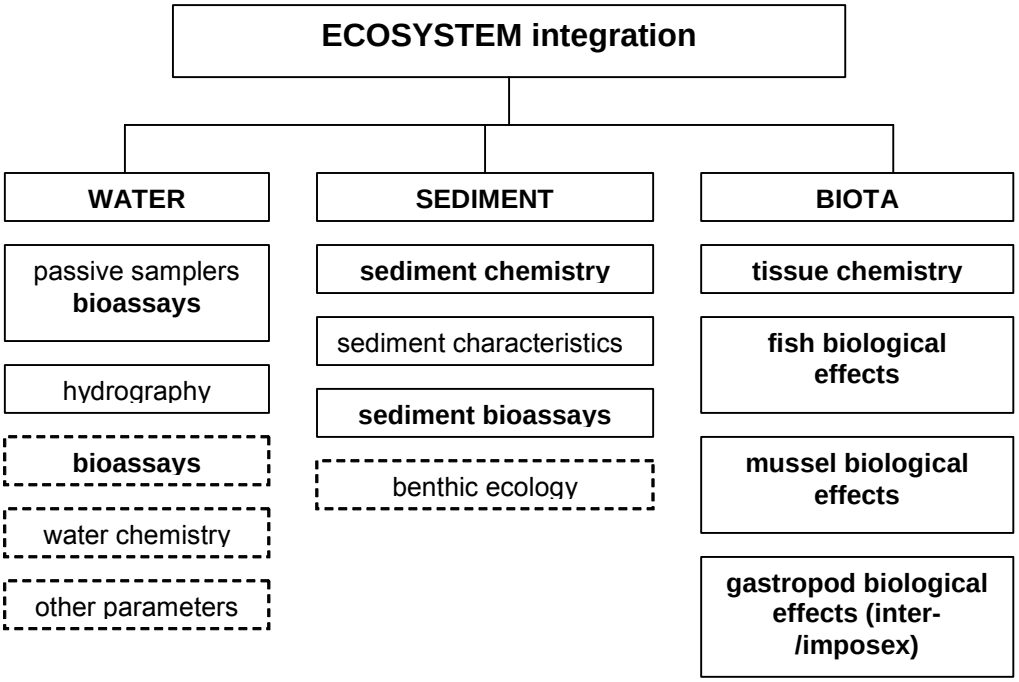

Figure A6.1. The concept of the WKIMON framework for integrated ecosystem assessment.

The component for fish includes a chemistry component, whole organism responses, tissue responses and subcellular responses (Figure A6.2.). The tissue responses will be replaced by the fish disease index (FDI) once that is finalised. Fish species to be considered include dab (*Limanda limanda*), flounder (*Platichthys flesus*), cod (*Gadus morhua*).

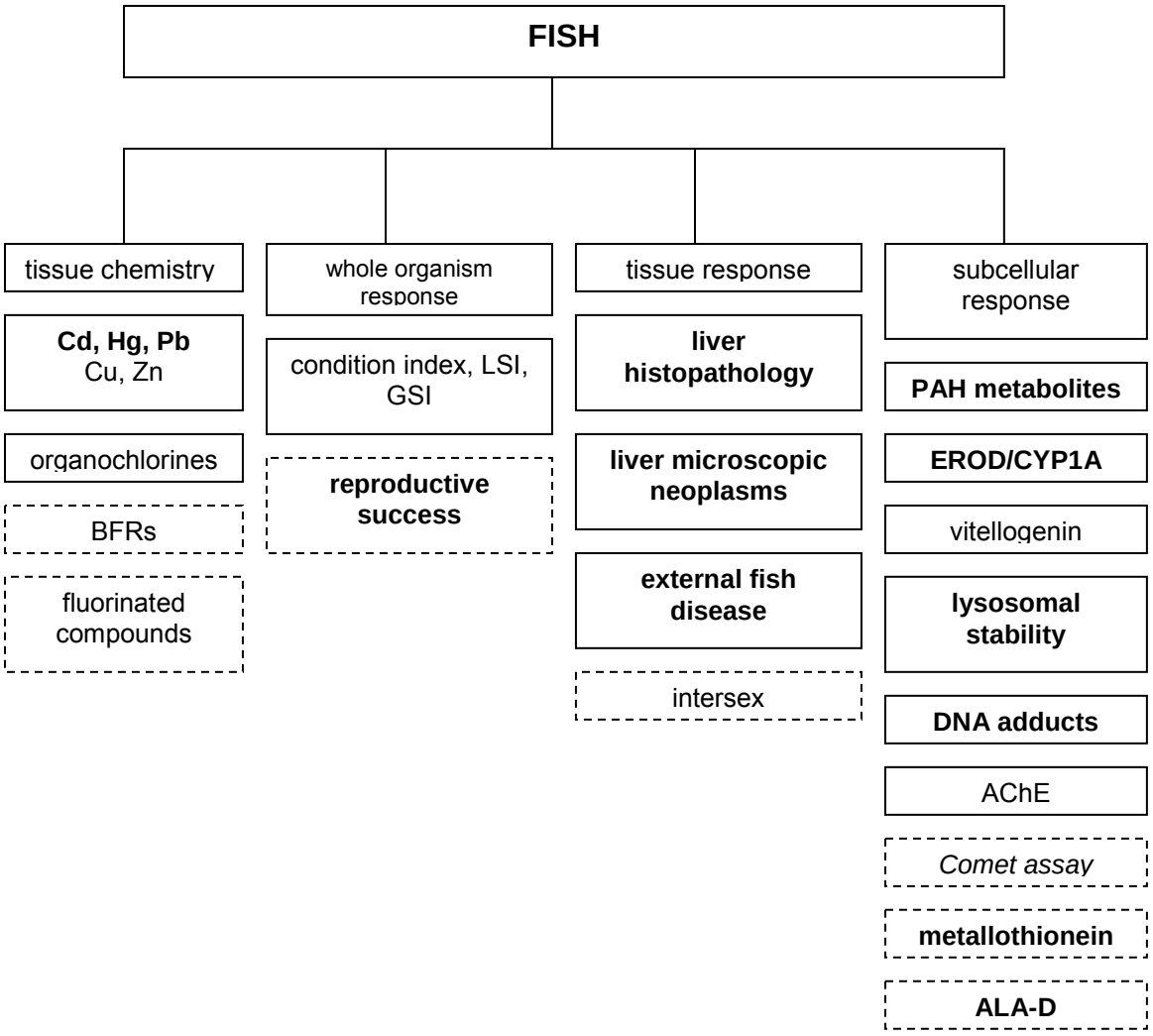

Figure A6.2. Overview of components for fish. Methods in bold are already in OSPAR CEMP, methods in italics on WGBEC list of promising methods (the rest on the list of recommended methods). Boxes with solid lines comprise the core programme, boxes with stipled lines are proposed additional methods.

As for fish, the components for mussels, either blue mussel (Mytilus edulis) or Mediterranean mussel (Mytilus galloprovincialis), comprise a chemistry component, whole organism responses, tissue responses and subcellular responses (Figure A6.3).

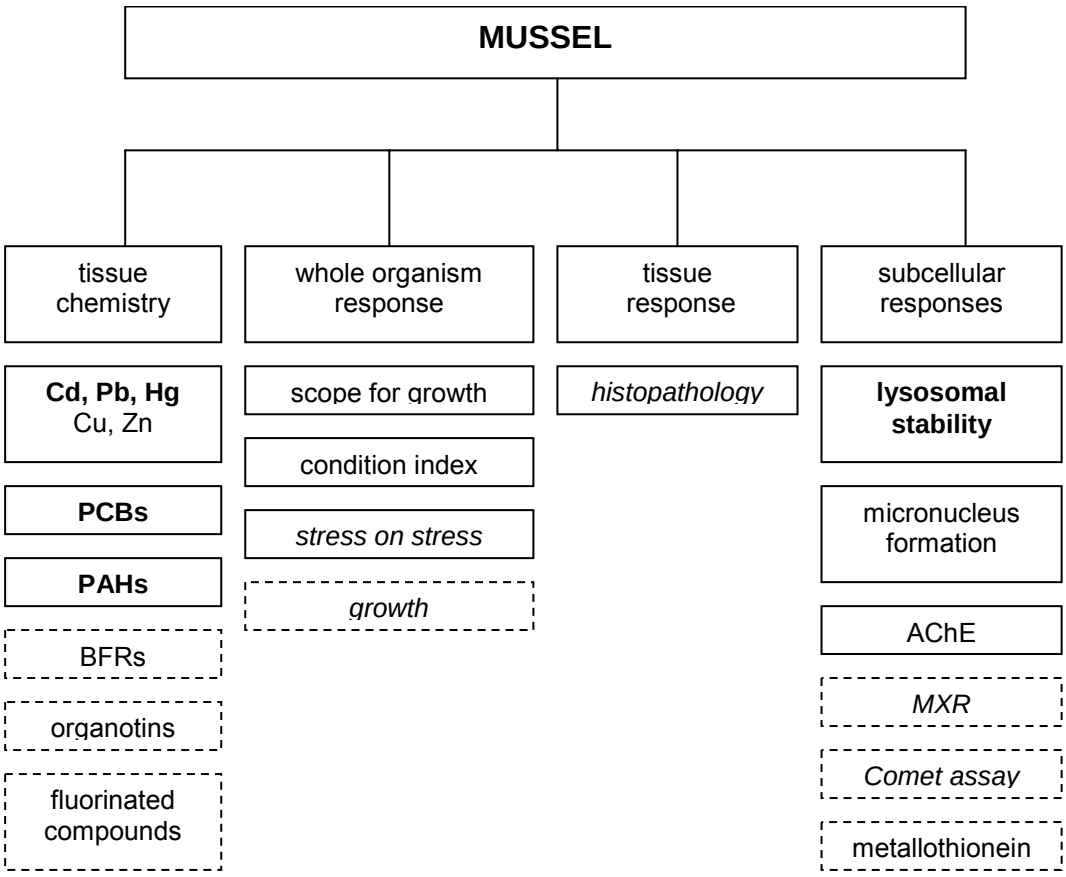

Figure A6.3. Overview of components for mussels (*Mytilus edulis* or *Mytilus galloprovincialis*). Methods in bold are already in OSPAR CEMP, methods in italics on WGBEC list of promising methods (the rest on the list of recommended methods). Boxes with solid lines comprise the core programme, boxes with stipled lines are proposed additional methods.

Annex 7: ICON prospectus

Integrated assessment of contaminant impacts on the North Sea (ICON)

Invitation to submit research proposals - deadline 1 May 2007

Background

The North Sea is a highly productive area but at the same time a repository for waste from land-based and offshore sources. North Sea ecosystems are and have been subject to intensive fishing pressure as well as contaminant inputs. The North Sea is probably the most extensively studied marine area in the world and one would perhaps think that there should now be sufficient data available, as summarised in OSPAR QSRs (http://www.ospar.org) or through the work of ICES REGNS (http://www.ices.dk). This is not true; there is in fact limited understanding of whether and how contaminant inputs actually affect marine life in this area.

-- and there is reason for concern for effects of contaminants in North Sea ecosystems: in the 1980s there were clear indications that contaminants were related to increased incidence of aberrations in fish embryos in the southern North Sea. Fish disease monitoring since the late 1970s has demonstrated decreases in some diseases and increases in others. In addition to the above, there are some indications from sublethal health-related measurements (biomarkers) that fish populations in areas with high offshore activity may be affected.

Objectives

The objective of the project is to assess the health of North Sea ecosystems through an international sea-going workshop.

Preliminary work plan

The project will comprise:

1) An introductory meeting to discuss approaches and methods. To be held in Copenhagen in May 2007; travel and accommodation will be covered for one participant from each laboratory.

2) Field studies at the selected locations and areas in 2008 and 2009; a preliminary indication of locations and fish species selected for study is indicated below. Mussels will be caged at the same locations. Project infrastructure (vessels, standard sampling gear, cages, etc.) will be provided.

Preliminary selection of areas and time intervals selected for study during ICON; C-contaminated; R-reference.

AREA NUMBER	NAME	TYPE	FISH SPECIES	TIME	RESPONSIBLE COUNTRY
1	German Bight	C	dab	Aug./Sep.	DE
2	Rhine estuary	C	flounder	Sep./Nov.	NL
3	Central North Sea (Ekofisk)	C	dab, haddock	Aug./Sep.	DE/Eng
4	Iceland	R	dab, flounder, haddock, long rough dab	Aug-Nov	to be decided
5	Firth of Forth	C	dab, flounder, haddock	Aug./Sep.	Sco
6	Tampen (oil production area)	C	long rough dab, haddock	Aug./Sep.	NO
7	Barents Sea	R	long rough dab, haddock	Aug-Nov	NO
8	Dogger Bank	C	dab	Aug./Sep.	DE/Eng

In addition to fish, mussels will be caged at the locations and gastropods sampled where appropriate (for imposex/intersex). Passive samplers will be included at selected locations. Work is in progress to include additional locations within the OSPAR area (France, Spain).

Outline proposals

The outline proposal should include a brief introduction to and rationale of the proposed project, required organism(s), required biomass/number of specimens, special sampling requirements, storage requirements, a requirement for live samples (if applicable). The proposal should not exceed two pages of A4.There will be limited space available on board the vessels, so only participants that need immediate access to samples (e.g. for *in vitro* or bioassay studies) may attend cruises in addition to the routine sampling crew. For most of the vessels involved this will require the participant to hold an offshore security certificate.

The proposals should be presented at a kick-off meeting in Copenhagen on **15-16. May 2007**; the workshop will fund travel/accommodation for one member from each research group (economy). Contact Ketil Hylland for queries (ketilhy@bio.uio.no).

Links to other activities

The workshop activity is directly linked to the development of integrated monitoring guidelines within OSPAR (WKIMON); parts of the activity will be used as a demonstration programme for this guideline. This will ensure a core set of analyses to be performed at each location. Links are being established with MEDPOL and Baltic activities.

Scientific steering committee

Ketil Hylland (Co-ordinator, NO), Jarle Klungsøyr (NO), Alistair McIntosh (Sco), Ingunn Nilssen (NO), Thomas Lang (DE), John Thain (Eng), Kevin Thomas (NO), Dick Vethaak (NL).

Supported by The Research Council of Norway (http://www.rcn.no).

Annex 8: Report on progress with the assessment of imposex

A report on the progress of modelling VDSI in *Nucella lapillus* had been prepared intersessionally by Rob Fryer (UK) and Matt Gubbins (UK), Fisheries Research Services, Aberdeen, and was presented to WGBEC by Matt Gubbins.

Introduction

An assessment of temporal trends in imposex levels, based on data held in the ICES database, was presented to WGSAEM and WGBEC in 2006 (Fryer and Gubbins, 2006a). The assessment mostly used the Vas Deferens Sequence Index (VDSI) as the measure of imposex[1]. The VDSI is based on the females in a sample of 40 individuals; each female is given a VDS classification between 0 and 6 and the VDSI is the mean of these values. In the assessment, the VDSI were scaled to lie between 0 and 1 (by dividing by 6) and then modelled as under-dispersed binomial observations using quasi-likelihood methods. The method appeared reasonable for any one time series, but problems arose when several time series were modelled simultaneously (Fryer and Gubbins, 2006b), as the mean-variance relationship implicit in the quasi-binomial distribution then appeared inappropriate. There are good biological reasons for this: VDS classes are ordinal, with no sense of distance between them, and it is 'harder' to move between some classes than others. In particular, it is 'hard' to progress to VDSI >4.0 in *Nucella lapillus*.

Here we use some individual VDS data in *Nucella lapillus* to develop an improved assessment methodology for VDSI (in *Nucella lapillus*). We model the individual data using a proportional odds model (McCullagh and Nelder, 1999), use the results to estimate a more appropriate mean-variance relationship and then reassess the data for VDSI in *Nucella lapillus* in the ICES database. This process is necessary because many historic data are only stored as VDSI and individual VDS data are unlikely to be recovered.

Individual data

We have used 5 sets of individual VDS data in *Nucella lapillus*:

- Quasimeme data (rounds 13, 14, 16 and 17). Each round provides data on about 300 individuals from a single population. Two rounds used populations with high VDSI; the other two used populations with low VDSI;
- Data from 20 stations in Sullom Voe and Yell Sound, Shetland in 2001;
- Data from 20 stations in Sullom Voe and Yell Sound, Shetland in 2004;
- Data from 47 stations around England and Wales in 2004;
- Data from 30 stations around the UK and France in 1992;

The last data set is important, as it is the only one with several individuals in VDS class 6. It also allows us to compare historic and recent classifications.

We modelled the individual data using the proportional odds model. Let

$$\gamma_j(x) = \Pr(\mathrm{VDS} \le j \mid x), \quad j = 0...5$$

be the cumulative probability that an individual VDS classification is in class j or below, where x is a vector of possible covariates. Then assume that

1 VDSI was used for *Nucella lapillus* and *Neptunea antiqua* whilst the Penis Classification Index (PCI) was used for *Buccinum undatum*. Here we only consider VDSI in *Nucella lapillus*.

$$\text{logit}(\gamma_j(x)) = \theta_j - \beta' x$$

Here, the θ_j are cut-points that measure the transition from one VDS class to the next, and the β are parameters that measure the covariate effects. (The negative sign ensures that, when the β are positive, large values of the covariates lead to a greater probability of having a high VDS classification.) The parameters θ_j, $j = 0\ldots5$, and β are estimated by maximum likelihood assuming the VDS data have a multinomial distribution.

We first modelled each data set in turn. For comparability, we combined imposex stages 5 and 6 (since only the 1992 data set has real information about stage 6), so θ_5 is no longer estimable. Quasimeme round or monitoring station were treated as categorical covariates. The estimates of θ_j, $j = 0\ldots4$ are given below in Table A8.1.

Table A8.1. Estimates of cut points from the proportional odds model.

	QUASIMEME	SHETLAND 2001	SHETLAND 2004	ENGLAND & WALES 2004	UK & FRANCE 1992	COMBINED2
θ_0	−3.6	−1.6	−1.5	−1.3	−3.3	−6.7
θ_1	−2.6	−0.6	−1.1	−0.8	−0.9	−6.0
θ_2	0.2	1.2	1.5	0.7	0.4	−4.4
θ_3	1.9	3.5	3.7	2.1	1.9	−2.9
θ_4	7.1	11.4	11.8	8.2	10.1	4.8
θ_5						7.7

Interpreting the estimates is quite tricky, because they depend on the reference value of the categorical covariate used in the fitting process. However, the important thing is that, for each data set, the estimates of θ_0, θ_1, θ_2 and θ_3 are quite close, indicating that it is 'easy' to move between VDS classes 0, 1, 2 and 3, whilst the estimates of θ_4 are much larger than the estimates of θ_3, indicating that it can be 'hard' to move out of VDS class 4.

The estimates are easier to compare if we use them to compute the mean-variance relationship of a VDSI measurement. We do this numerically by choosing a range of values for the covariate, computing the corresponding probabilities that a VDS measurement is in each class, and hence computing the mean and variance of a VDSI measurement (using the properties of the multinomial distribution). Figure A8.1 below shows the resulting mean-variance relationships, as well as the mean-variance relationship assuming quasi-binomial errors.

2 All data sets except for the Quasimeme data.

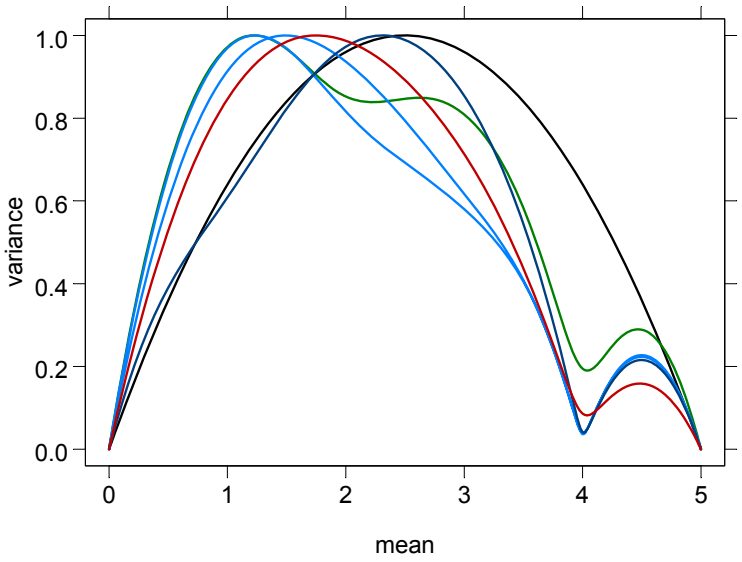

Figure A8.1. The mean-variance relationship under the assumption of quasi-binomial errors (black) and based on the individual VDS data: Quasimeme (green), Shetland 2001 (light blue), Shetland 2004 (also light blue), England & Wales 2004 (red) and UK & France 1992 (dark blue). The relationships are scaled to have a common maximum of unity.

The curves based on the individual data clearly differ from that for the quasi-binomial errors, showing markedly lower variability when the mean VDSI is around 4. The curves from Shetland, England & Wales and UK & France show pretty good agreement, although all differ statistically. The England & Wales curve shows slightly different behaviour when the mean VDSI is above about 4, perhaps because this data set only had one station with individuals in VDS class 5. There is some suggestion that the first mode in the mean-variance relationship has shifted to the left since 1992. The mean-variance relationship from the Quasimeme data is the least compatible of the relationships based on individual data. The Quasimeme data set was also the only one for which the proportional odds model showed significant lack of fit. This might be because less care was taken in choosing adults since so many individuals were required for the exercise.

We combined the individual data from Shetland, England & Wales, and UK & France and estimated a common set of cut-points for VDS classes 0 through 6 (Table A8.1). The resulting mean-variance relationship is shown below. We omitted the Quasimeme data set because it gave a rather different mean-variance relationship to the other individual data sets and because the lack of fit was disturbing.

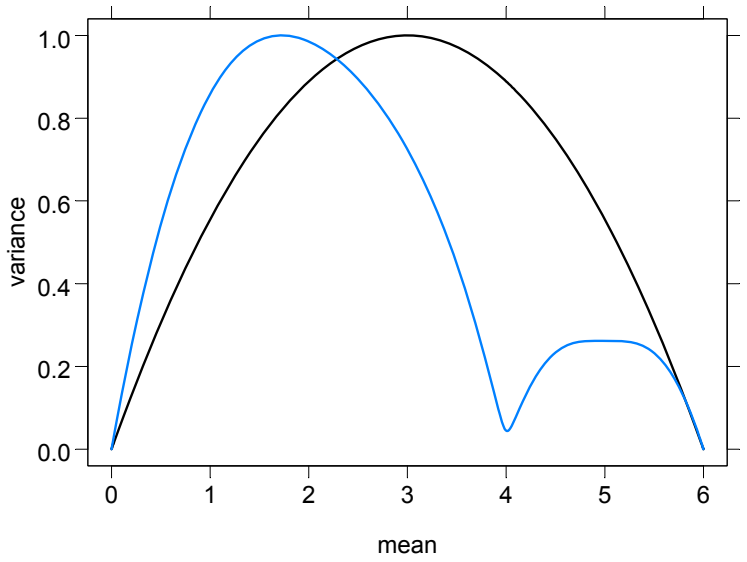

Figure A8.2. The mean-variance relationship under the assumption of quasi-binomial errors (black) and based on the combined individual VDS data (blue). The two relationships are scaled to have a common maximum of unity.

An assessment of time series of VDSI in *Nucella lapillus*

Time series of VDSI in *Nucella lapillus* in the ICES database were assessed by fitting a generalised linear model assuming the mean-variance relationship based on individual data (Figure A8.2). Only time series with 4 or more years of data were assessed. Each VDSI should be weighted by the number of females in the sample, but this is not submitted to the ICES database. Each VDSI was therefore weighted by half the total number of individuals in the sample (which is submitted).

There were 114 time series from France, Norway and the UK. The data (circles) and fitted models (solid lines) with pointwise (two-sided) 90% confidence bands (grey shaded areas) are shown in Figure A8.3. The thin dashed horizontal lines are the boundaries between the assessment classes developed for OSPAR (OSPAR, 2004). A time series lies in a particular assessment class (or better) with 95% confidence if the upper confidence limit in the final year lies below the corresponding assessment boundary. Some time series exhibited over-dispersion, and the confidence bands and significance levels have been adjusted to account for this.

Most of the estimated trends are downwards. There are 17 significant downward trends (at the 5% level) and only 1 significant upwards trend, at Scarf Stane in Shetland, UK, which may be due to a large pelagic fishing vessel that often moors close to the monitoring station. There are 1, 14, 38, 58, and 3 time series in assessment classes A through E respectively.

There are some data that require quality checks. For example, some of the Norwegian data in 2005 are suspiciously low – maybe the VDSI is based on the total number of individuals rather than the number of females? The UK data from Cultra and Carrickfergus are far more variable than any other time series. And the low VDSI from Little Roe, UK, is a known error that needs to be corrected in the ICES database.

Discussion

The new mean-variance relationship provides more accurate significance levels and better model diagnostics. The number of females that contribute to each VDSI is needed to improve things further.

There is evidence of over-dispersion in some time series, so a between-year variance component will need to be introduced. Estimating these variance components will help inform the design of imposex monitoring programmes.

Mean-variance relationships now need to be estimated for other measures of imposex in other species (e.g. VDSI in *Neptunea antiqua* and PCI in *Buccinum undatum*).

In principle, modelling individual imposex data using e.g. the proportional odds model should be more informative than modelling summary measures such as VDSI. However, it is unclear whether, in practice, such a change would yield substantial benefits for large-scale assessments. Nevertheless, access to individual data would allow for this possibility in the future, and would help to estimate appropriate mean-variance relationship for use in VDSI assessments. Therefore, submission of individual data to the ICES database should be encouraged.

Acknowledgements

Many thanks to John Thain for providing the England & Wales data.

VDSI

France

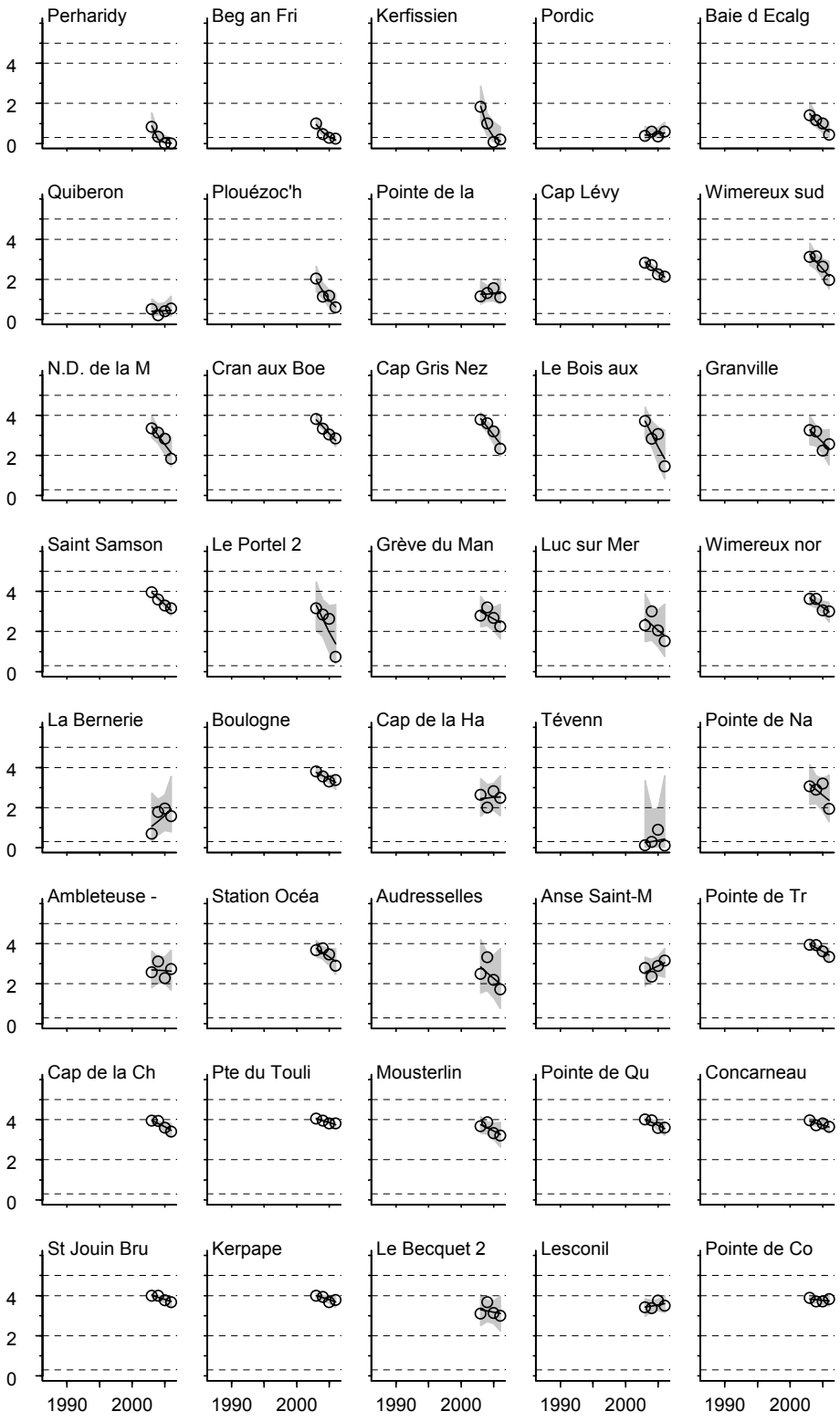

Figure A8.3. Assessment plots of VDSI in *Nucella lapillus*.

VDSI

France

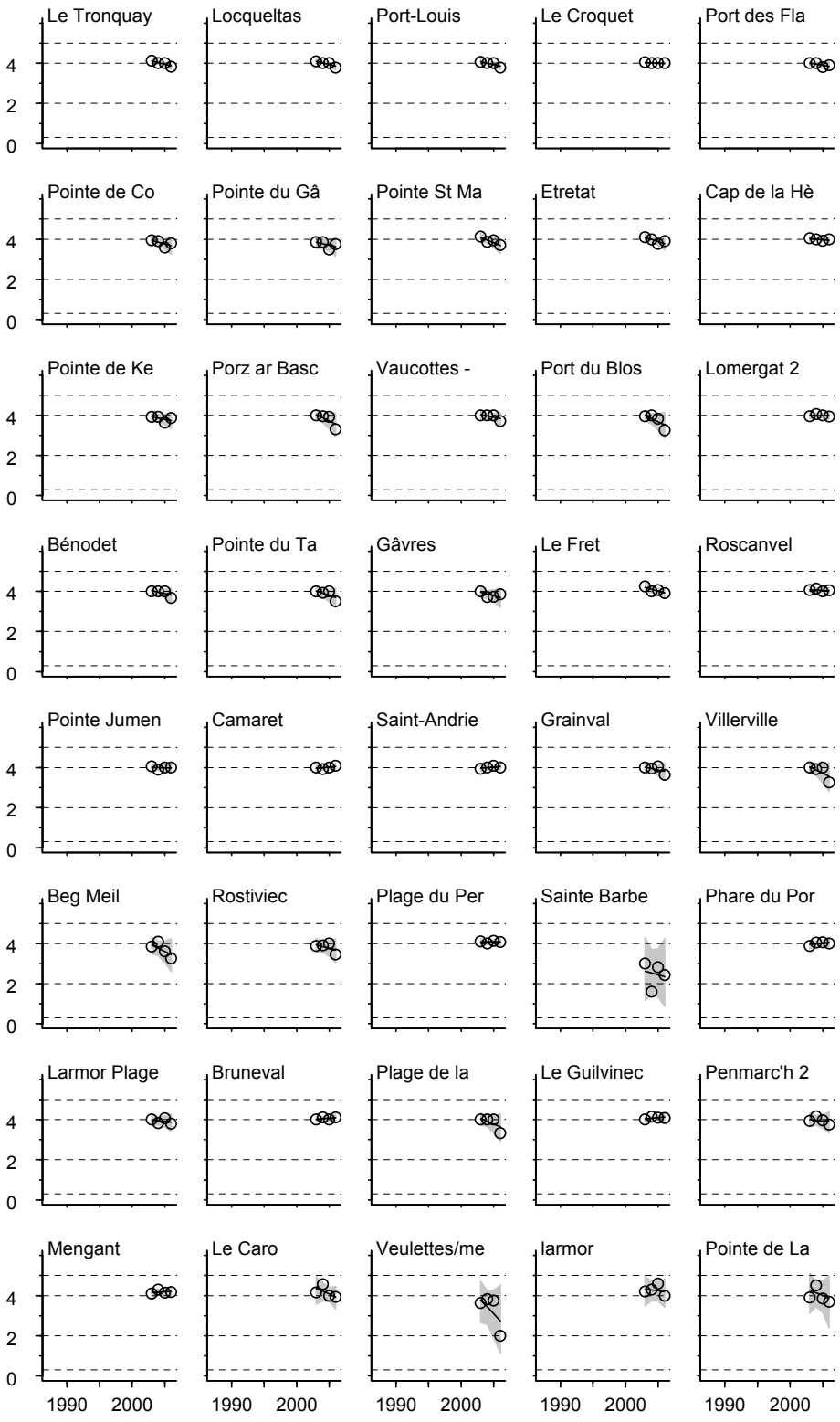

Figure A8.3. Assessment plots of VDSI in *Nucella lapillus*. Continued.

VDSI

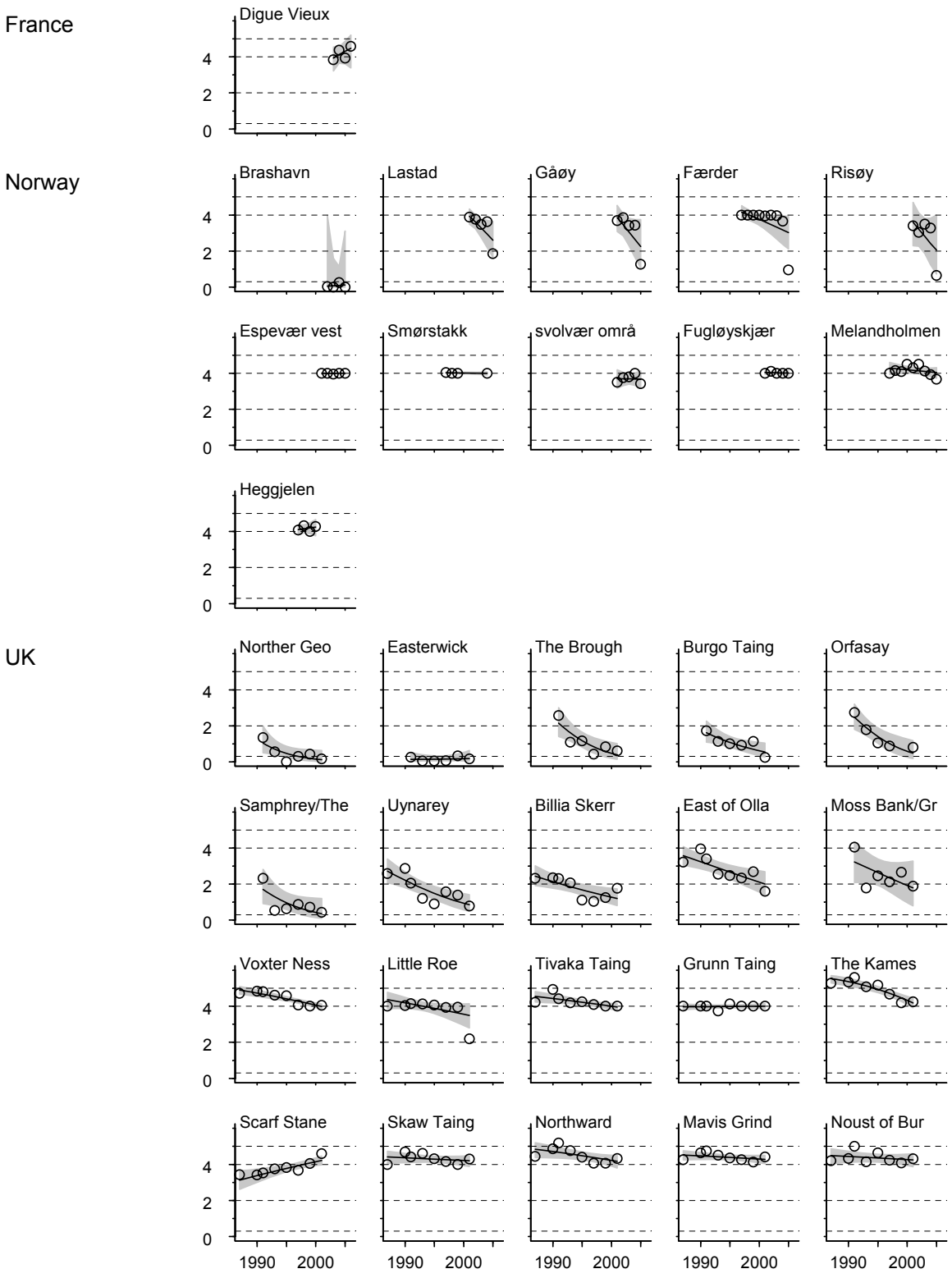

Figure A8.3. Assessment plots of VDSI in *Nucella lapillus*. Continued.

Annex 9: Biomonitoring programmes in Spain (BIOMEJIMED, MEDPOLIEO and CONOSPAR projects 2001–2006)

"Micropollutants bioavailability and biological effects along the Iberian mediterranean coast, using mussel (Mytilus galloprovincialis) as bioindicator". This structural project was fully funded by IEO (Spanish Institute of Oceanography). The leader project was Benedicto J. <benedicto@mu.ieo.es> from the Oceanographic Centre of Murcia (COMU, IEO).

Starting date: 01-01-2001. Completion date: 31-12-2005.

Mussel samples were collected annually for selected sites, from mid May to mid June (no spawning period)

Table A9.1. *Range size of the wild mussels were 3.0–4.5 cm.

MPIs	TECHNIQUE SELECTED	Nº STATIONS	FREQUENCY	YEARS	SPECIES
Lysosomal Membrane Stability	NRR Assay Moore and Lowe, 2004; UNEP/RAMOGE, 1999	10	annual	2002	*M. Gallo-provincialis**
		12	annual	2003	
		10	annual	2004	
		13	annual	2005	
Frequency of Micronuclei	Brunetti *et al*., 1992; UNEP/RAMOGE, 1999	17	annual	2003	
Metallothionein	Viarengo *et al*., 1997; UNEP/RAMOTE, 1999	15	annual	2001	
		16	annual	2002	
		19	annual	2003	
		10	annual	2004	
		12	annual	2005	

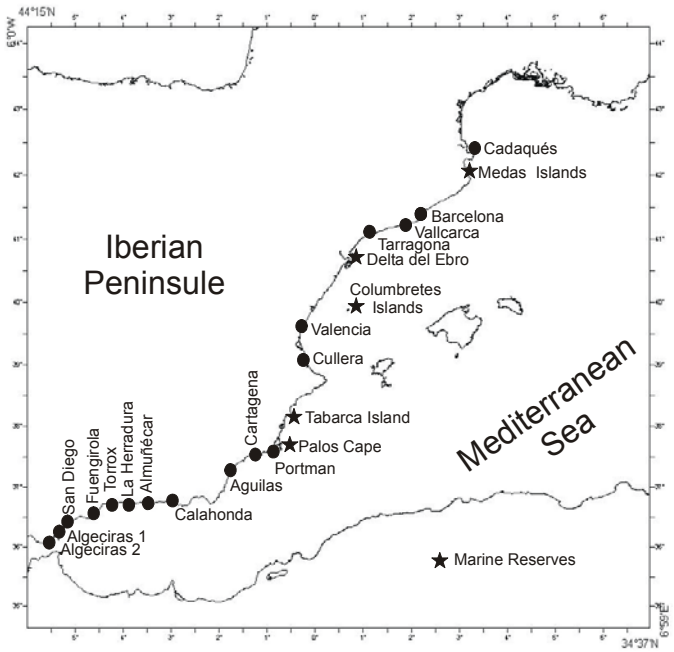

Figure A9.1 Sampling sites of *Mytilus galloprovincialis* populations by IEO (COMU) in May-June, from 2001to 2005.

MEDPOLIEO Project, "Spatial distribution, temporal trends and biological effects related to chemical contamination on coastal and reference stations along the Iberian Mediterranean coast". IEO main researcher: Benedicto J. <benedicto@mu.ieo.es> from the Murcia Oceanographic Centre (COMU, IEO).

Starting date: 01-01-2005. Completion date: 31-12-2006.

This project is the result of the collaboration agreement between the Main Directorate of Coasts of the Ministry of Environment and the Spanish Institute of Oceanography (IEO). Their main objectives are:

- To evaluate the spatial distribution the temporal trends of the levels of chemical polluting agents on coastal and reference stations along the Mediterranean littoral of the Iberian Peninsula
- To evaluate the temporal trends of the biological effects of the pollution on coastal and reference stations along the Mediterranean littoral of the Iberian Peninsula

Target matrices: (*Mytilus galloprovincialis* and *Mullus barbatus*) and superficial sediments.

Biota samples are collected annually for selected sites. The samples of *Mytilus galloprovincialis* will be collected from mid May to mid June (no-spawning period). The samples of *Mullus barbatus* and sediment *will* be collected in mid April.

Table A9.2. Biomarkers measurements at sampling stations in 2006. MEDPOLIEO project (COMU, IEO); KEYS: MG (*Mytilus galloprovincialis*), MB (*Mullus barbatus*). DNA= Genotoxic damages; EROD activity; MT= Metallothionein content; LMS= Lysosomal membrane stability Range size of the wild mussels were 3.0–4.5 cm; Range size of the feral red mullet 12–18 cm.

		CONTAMINANT CONCENTRATIONS OCPS, PAHS, TRACE METALS,			BIOMARKERS			
CODE	STATION	In sediments	Organisms	Species	DNA	EROD	MT	LMS
1	Medas I.		□	MG			□	□
2	Barcelona		□	MG			□	□
3	Tarragona		□	MG			□	□
4	Ebro river		□	MG			□	□
		□	□	MB	□	□	□	
5	Valencia		□	MG			□	□
		□	□	MB	□	□	□	
6	Cullera		□	MG			□	□
7	Santa Pola		□	MG			□	□
		□	□	MB	□	□	□	
8	Cartagena		□	MG			□	□
		□	□	MB	□	□	□	
9	La Herradura		□	MG			□	□
10	Fuengirola		□	MG			□	□
11	San Diego		□	MG			□	□
12	Algeciras		□	MG			□	□

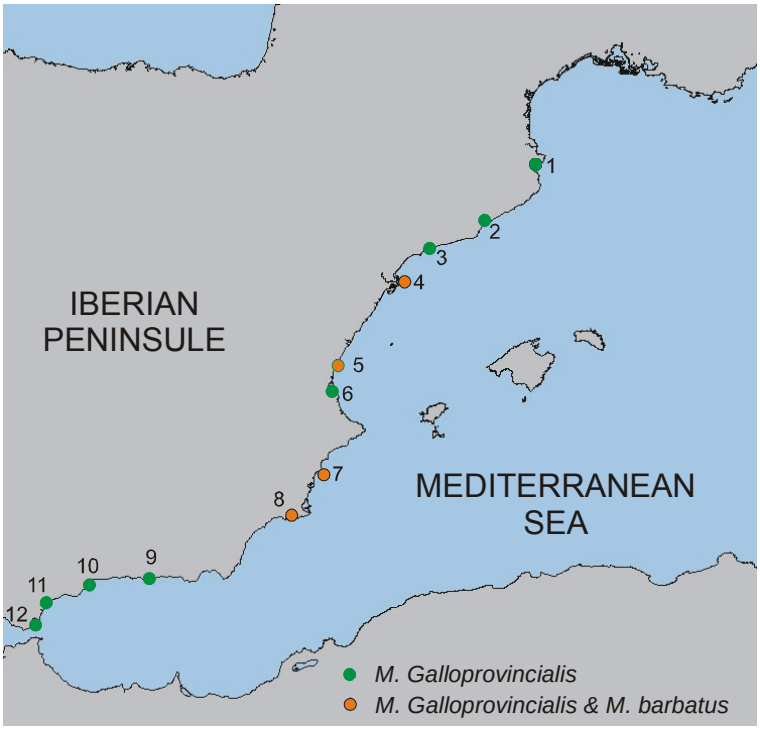

Figure A9.2. Map showing sampling sites of *Mytilus galloprovincialis* and *Mullus barbatus* for the biomarker monitoring at 2006. Project MEDPOLIEO (COMU, IEO).

Table A9.3. Range (minimum - maximum mean values) found in *Mullus barbatus* in MEDPOLIEO Project for each biomarker. Biomarkers were tested in wild specimens sampled in 4 stations at 2006.

SAMPLING PERIOD	BIOMARKER	TECHNIQUE	MINIMUM AND MAXIMUM MEAN VALUES
April 2006	EROD activity	Eggens and Galgani, 1992; UNEP/RAMOGE, 1999	Min: 86.81 pmol/min*mg prot cit. Max : 138.20 pmol/min*mg prot cit
April 2006	Metallothionein	Benedicto *et al*, 2005; UNEP/RAMOGE, 1999	Min: 405.65 µg/g hepatic tissue Max: 642.81 µg/g hepatic tissue
April 2006	Genotoxic damage	Kohn *et al*., 1981; UNEP/RAMOGE, 1999	Min: 152.55 (k x 1000) Max: 102.80 (k x 1000)

Table A9.4. Range (minimum - maximum mean values) found in *Mytilus galloprovincialis* in BIOMEJIMED and MEDPOLIEO projects for each biomarker. Biomarkers were tested in wild specimens sampled in 10–18 stations in the period 2001–2006. Data for Micronuclei frequency were obtained at 2003.

SPECIES	BIOMARKER	TECHNIQUE	MINIMUM AND MAXIMUM MEAN VALUES
M. galloprovincialis	Lysosomal stability	Moore and Lowe, 2004; UNEP/RAMOGE, 1999	Min. 4.69 minutes Max. 129.38 minutes
M. galloprovincialis	Metallothionein	Viarengo *et al.*, 1997; UNEP/RAMOGE, 1999	Min. 97.9 micro/g tissue Max. 271.0 micro/g tissue
M. galloprovincialis	Micronuclei	Brunetti *et al.*, 1992; UNEP/RAMOGE, 1999	Min. 1.53 micron. / 1000 cells Max. 12.3 micron. / 1000 cells

CONOSPAR Project. IEO main researcher: Fumega J. <jose.fumega@vi.ieo.es> from the Vigo Oceanographic Centre (COVI, IEO).

This project is the result of the collaboration agreement between the Main Directorate of Coasts of the Ministry of Environment and the Spanish Institute of Oceanography (IEO).

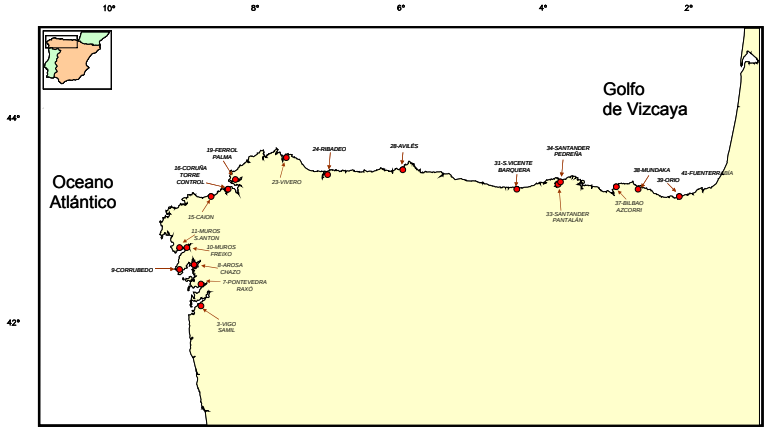

Figure A9.3. Sampling stations of TBT concentrations in whole tissue of mussels (*Mytilus galloprovincialis*). Survey 2005.

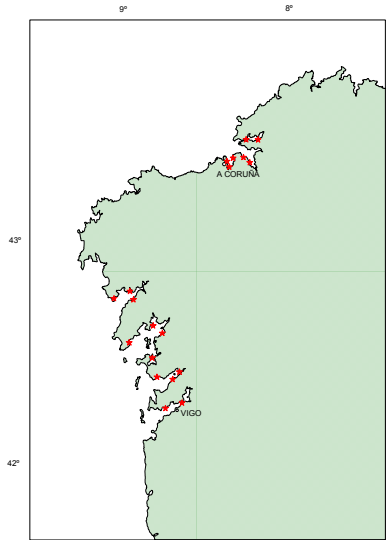

Figure A9.3. Sampling stations of TBT concentrations and incidence of imposex in *Nucella sp.* Surveys 2005 and 2006.

References

Benedicto, J., Martínez-Gómez, C., and Campillo, J. 2005. Induction of metallothionein in Mullus barbatus as specific biomarker of metal contamination: A field study in the western Mediterranean. Ciencias Marinas, 31(1B): 265–274.

Brunetti, R., Gabriele, M., Valerio, P., and Fumagalli, O. 1992. The micronucleus test: Temporal pattern of baseline frequency in *Mytilus galloprovincialis*. Mar.Ecol.Prog.Ser. 83 (1): 75–78.

Burke, M. D., and Mayer, R. T. 1974. Ethoxyresorufin: Direct fluorimetric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metabolism and Disposition 2, 583–588.

Eggens, M. L., and Galgani, F. 1992. Hepatic EROD-activity in flatfish: fast determination with fluorescence plate-reader method. Marine Environmental Research 33, 213–221.

Kohn, K. W., Ewig, R. A. G., Erickson, L. C., and Zwelling, L. A. 1981. Measurement of strand breaks and cross-links by alkaline elution in: DNA repair. In: Friedberg and Hanawalt (Eds.), A laboratory manual of research procedures, Vol. 1(B). Marcel Dekker Inc., New York, pp. 379–401.

Moore, M. N., and Lowe D. 2004. Biological effects in contaminants: measurement of lysosomal membrane stability. ICES Techniques in Marine Enviromental Sciences. Nº 36. 39 pp.

Porte, C., Escartín, E., García, L. M., Biosca, X., and Albaigés, J. 2002. Assessment of coastal pollution by the combined determination of chemical and biochemical markers in Mullus barbatus. Mar. Ecol. Prog. Ser., 235: 205–216.

UNEP/RAMOGE: Manual on the biomarkers recommended for the MED POL biomonitoring programme. UNEP Athens, 1999.

Viarengo, A., Ponzano, F., and Fabri, R. 1997. A simple spectrophotometric method for metallothionein evaluation in marine organisms. An application to the Mediterranean and Antarctic molluscs. Mar. Environ. Res. 44: 69–84.

Annex 10: Monitoring activities in research projects in Spain (BIOMARC and BEEP projects)

Determination of exposure degree to metallic and organic contamination in fish from Spanish Mediterranean areas of the Iberian Peninsula under low and high human activity by means of the use of specific biomarkers (Metallothionein and EROD)". Coordinated Project partially funded by the Spanish Institute of Oceanography (IEO ceanogrOceanografía)) and the General Secretariat Of Maritime Fishing (SGPM). The Project was leaded by Benedicto J. <benedicto@mu.ieo.es>, from the IEO (Oceanographic Centre of Murcia, COMU) and Dr. Porte C. <cpvqam@cid.csic.es>, from the IIQAB-CSIC (Department of Environmental Chemistry, Barcelona).

BIOMARKERS	Nº STATIONS	FREQUENCY	YEARS	SPECIES
EROD activity	10	2 at year	1999	*M. barbatus*
MT content	6	2 at year	1999	*M. barbatus*

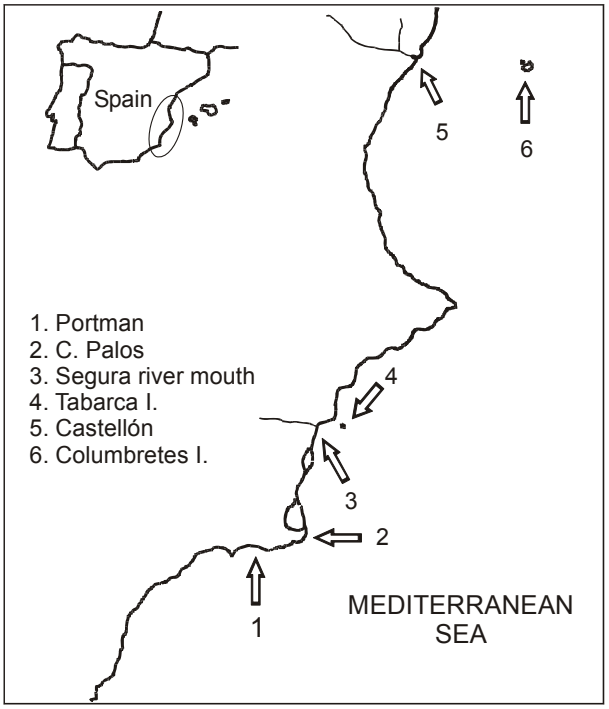

Figure A10.1. Sites of *Mullus barbatus* sampled by IEO (COMU) on April–October 1999.

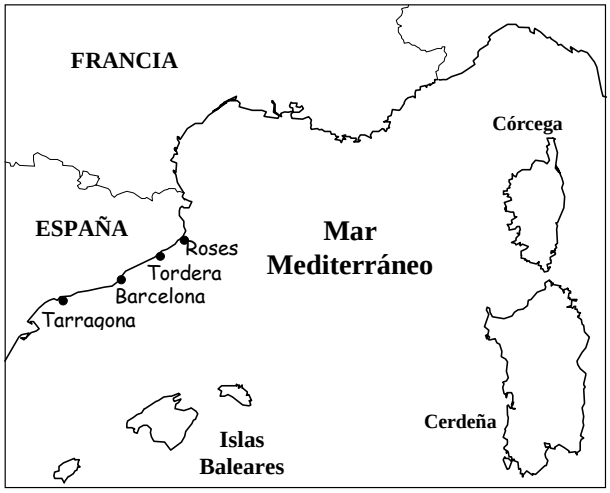

Figure A10.2. Sites of *Mullus barbatus* sampled by IIQAB-CSIC on April–August 1999.

Table A10.1. Range (minimum – maximum mean values) found in *Mullus barbatus* in BIOMARC Project for each biomarker. Biomarkers were tested in wild specimens sampled in 10 stations during 1999.

SAMPLING PERIOD	BIOMARKER	TECHNIQUE	MINIMUM AND MAXIMUM MEAN VALUES
April 1999	EROD activity	Burke and Mayer, 1974	Min: 39.6 pmol/min*mg prot cit. Max : 258.4 pmol/min*mg prot cit.
May 1999	EROD activity	Burke and Mayer, 1974	Min: 26.84 pmol/min*mg prot cit. Max : 36.88 pmol/min*mg prot cit
August 1999	EROD activity	Burke and Mayer, 1974	Min: 160.9 pmol/min*mg prot cit. Max : 760.1 pmol/min*mg prot cit
October 1999	EROD activity	Burke and Mayer, 1974	Min: 25.5 pmol/min*mg prot cit. Max : 55.48 pmol/min*mg prot cit
April 2006	EROD activity	Burke and Mayer, 1974	Min: 86.81 pmol/min*mg prot cit. Max : 138.20 pmol/min*mg prot cit
May 1999	Metallothionein	Spectrophotometry MEDPOL Guidelines, 1999	Min: 284.39 µg/g hepatic tissue Max:573.95 µg/g hepatic tissue
October 1999	Metallothionein	Spectrophotometry MEDPOL Guidelines, 1999	Min: 103.78 µg/g hepatic tissue Max:186.89 µg/g hepatic tissue

BEEP project. 2001–2004. *"Biological Effects of Environmental Pollution in Marine Coastal Ecosystems"*. Contract EVK3-CT2000-00025. BEEP was a three-year EU research program. Data obtained from BEEP project are limited to 4 samplings surveys along 4 stations for mussels and 3 stations for red mullets in areas under Spanish jurisdiction. As Spanish partners, Dr. Prof. Miren P. Cajaraville was the head of the group of the University of the Basque Country (http://www.ehu.es/GrupoBCTA) and Dr. Cinta Porte (cpvqam@cid.csic.es) was the head of the Environmental Toxicology group of the Environmental Chemistry department (IIQAB-CSIC).

Table A10.2. Mussel sampling locations. BEEP project.

COUNTRY	NUMBER	STATION	COORDINATES	MUSSEL SIZE	DESCRIPTION
Spain	4	Montjoy cove	42º15'00 N 03º 14'50 E	4.120 ± 0.026	Clean seawater, tourist area in summer
	2	Fangar bay	40º46'40 N 00º45'60 E	4.987 ± 0.584	Rice agriculture, marine farms, fishing
	1	Alfacs bay	40º 36'70 N 00º 36'30 E	5.088 ± 0.450	Rice agriculture, marine farms, fishing
	3	Barcelona harbour	41º 22'55 N 02º 11'80 E	5.106 ± 0.187	Intense maritime traffic, industrial effluents

Table A10.3. Red mullets sampling locations. BEEP project.

SITE	STATION	COORDINATES	DEPTH (M)	LENGTH (CM)
Catalonian coast	Altafulla	41º06'48 N / 1º20'61 E	---	16.96 ± 1.92
	Delta of Ebro	40º31'18 N / 0º36'50 E	---	13.50 ± 1.30
	Roses	42º13'47 N / 3º09'94 E	30	18.81 ± 1.12

Sampling Stations in Spain

BEEP Project Surveys:

- BEEP 01: 2nd May – 23rd May 2001 (mussels and red mullet);
- BEEP 02: 10th September – 30th September 2001 (mussels and red mullet);
- BEEP 04: 4th September – 24th September 2002 (mussels and red mullet);
- BEEP 05: 25th April – 12th May 2003 (only mussels samples).

Table A10.6. Range (minimum - maximum values) found during the BEEP monitoring programme for each biomarker for both mussels and red mullets. Biomarkers were tested in wild animals sampled in 12 stations for mussels and in 4-7 stations in red mullet during September 2001, May 2002, September 2002 and May 2003.

SPECIES	BIOMARKER	TECHNIQUE	MINIMUM AND MAXIMUM VALUES
M. galloprovincialis	Lysosomal stability	Histochemistry	May: 2.5–23.3; Sep: 2.15–36.93 min
M. galloprovincialis	Lysosomal volume density	Histochemistry	May: 0.93–10.81x103; Sep: 0.97–11.45x 103µm3/µm3
M. galloprovincialis	MT by DPP	Biochemistry	May: 12.18–179.59; Sep: 12.59–50.05 MT µg/mg prot
M. galloprovincialis	AOX activity	Biochemistry	May: 0.30–4.11; Sep: 0.35–2.33 mU/mg prot
M. galloprovincialis	Gonad development	Histology	May: Spawning gonads; September: Resting gonads
M. barbatus	Lysosomal stability	Histochemistry	May: 3.49–29.1; Sep: 6.62–32.11 min
M. barbatus	MT by DPP	Biochemistry	May: 8.1–204.68; Sep: 1.52–9.1 MT µg/mg prot
M. barbatus	AOX activity	Biochemistry	May: 2.9–8.3; Sep: 1.9–6.8 mU/mg prot
M. barbatus	Peroxisomal volume density	Histochemistry	May: 3.94–77.24x10^4; Sep: 13.11–130.4x10^4 $\mu m^3/\mu m^3$
M. barbatus	Gonad development	Histology	May: Spawning gonads; September: Resting gonads

Annex 11: Monitoring research activities on the Basque coast using cell and tissue level biomarkers in mussels.

From 1991 to 1993 the BCTA group (url: www.ehu.es/GrupoBCTA) from the University of the Basque Country/UPV (Spain) have developed a first monitoring programme along the coast of Biscay around Bilbao, funded by the Spanish Ministry of Education and Science. A similar pollution monitoring programme was carried out in the Urdaibai´s Reserve of the Biosphere from 1991 to 1994 funded by the Basque Government. Since 2000 we monitor the biological impact of effluents from oil refineries in Bilbao under contract with Petronor (Table A11.1).

After the *Prestige* oil spill in November 2002, they started a survey to assess the biological impact of the oil spill along the North Iberian coast, from Galicia to the Basque coast (www.ehu.es/ImpactoBiologicoPrestige). During the first year 2003 (April, July, September), six localities were studied in the Basque coast (Muskiz, Arrigunaga, Gorliz, Mundaka, Orio and Hondarribia) (Marigómez *et al.*, 2006; Orbea *et al.*, 2006). During the following 8 campaigns (February, April, July and October in 2004; April, July and October in 2005 and April in 2006) two localities more were studied (Bakio and Mutriku). The biological parameters measured were lysosomal responses as changes in lysosomal structure (Vv_L, Sv_L, S/V_L, Nv_L) and in lysosomal membrane stability (labilization period, LP), accumulation of intracellular neutral lipids, peroxisome proliferation as induction of acyl-CoA oxidase (AOX) activity, changes in the morphology of digestive alveoli as mean luminal radius to mean epithelial thickness (MLR/MET), changes in cell type composition in digestive gland epithelium (volume density of basophilic cells, Vv_{BAS}), histopathology of digestive gland and gonad, micronuclei frequency, flesh condition index (FCI), gonad index (GI) and vitellogenin-like protein levels.

According to biomarker data, impacted mussel populations started to recover in mid 2004 (Cajaraville *et al.*, 2006). However, once the Prestige monitoring ended in April 2006, the assessment of biological effects of environmental pollution has been continued in the Basque coast within the IMPRES project with two objectives. First, to obtain basal values of the parameters studied in order to use them as reference values for future possible accidental oil spills, and, second, to evaluate the effects of chronic pollution, which is of relevance in some areas of the Basque coast. For this, mussels have been collected in October 2006 and will be sampled in April and October 2007 in ten localities of the Basque coast (Muskiz, Arriluze, Arrigunaga, Plentzia, Gorliz, Mundaka harbour and beach, Mutriku, Pasaia and Hondarribia) (Figure A11.1, Table A11.2). Most of the localities have been sampled during the Prestige monitoring since 2003 (Muskiz, Arrigunaga, Gorliz, Mundaka beach, Mutriku and Hondarribia) plus two sampling localities were added in polluted harbours of interest (Arriluze, Pasaia) and two more in harbours sampled in the frame of the Water Quality Assessment of the Basque coast by AZTI (Plentzia, Mundaka). Up to now all the studies have been performed on native mussels. Additionally, as the use of caged mussels can reduce variability and is widely applied in biomonitoring programmes in the last years, we plan to use caging strategies in 2 to 4 selected locations in the next sampling (April 2007).

The biological parameters measured in this monitoring of the Basque coast are: 1) intralysosomal metal levels as marker of exposure to metals, 2) peroxisome proliferation as marker of exposure to organic contaminants, 3) lysosomal membrane stability, structure of digestive alveoli, histopathology and stress on stress as indicative of general health status of organisms, 4) lipid peroxidation as marker of oxidative damage, 5) micronuclei frequency as marker of genotoxic damage, and 6) gonad index and vitellogenin-like protein levels as indicators of reproductive status (Table A11.3). These biological responses from molecular and cellular level to organism level have been selected based on our previous experience and

other monitoring experiences and are expected to provide an integrated view of pollutant effects on studied mussel populations. Chemical analysis of main pollutant classes will be also performed with confirmative purposes.

Samples from the first sampling campaign in October 2006 are being processed. Preliminary results on stress on stress generally show that polluted sites like harbours of Pasaia and Plentzia can be readily discriminated from cleaner sites such as Gorliz and Mundaka.

Table A11.1: Selected data of biological responses measured in mussels collected at different localities along the Basque coast.

AUTHORS	SEASON	STATION	BIOLOGICAL PARAMETERS
Cajaraville *et al.* 2006	Apr, Jul, Sep 2003 Feb, Apr, Jul, Sep 2004	Muskiz, Arrigunaga, Gorliz, Bakio, Mundaka, Mutriku, Orio, Hondarribia	AOX, LP, VvL, SvL, S/VL, NvL, MLR/MET, FCI, GI, MN frequency
Orbea *et al.*, 2006	Apr, Jul, Sep 2003	Muskiz, Arrigunaga, Gorliz, Mundaka, Orio, Hondarribia	AOX, LP, VvL, SvL, S/VL, NvL, VvNL, FCI, GI
Marigómez *et al.*, 2006	Apr, Jul, Sep 2003	Muskiz, Gorliz, Mundaka	AOX, LP, VvL, SvL, S/VL, NvL, VvBAS, MLR/MET, FCI, GI
Orbea and Cajaraville, 2006	Nov 1998	Muskiz, Arriluze, Plentzia, Mundaka	AOX, Vvp, catalase, SOD, GPX
Orbea *et al.*, 2002	Jul 1996 Feb 1997	Laida, Txatxarramendi, Arteaga, Plentzia	Catalase, SOD, GPX, Vvp
Cancio *et al.*, 1999	Monthly from Aug 1995 to Jul 1996	Plentzia	AOX, catalase, DAOX, Vvp, VvNL
Marigómez *et al.*, 1999	Sep 1987 Mar, Aug 1988	Galea, Zierbena, Plentzia, Lekeitio	MET and digestive tubule necrosis
Orbea *et al.*, 1999	Bimonthly from Sep 1992 to Oct 1992	Plentzia, Galea	Catalase activity, peroxisomal Vvp
Soto and Marigómez, 1997	Jan-Feb 1992 Jul-Aug 1992 Sep-Oct 1992	Zierbena, Arrigunaga, Plentzia, Meñakoz, Mundaka, Laga	VvBSD
Cajaraville *et al.*, 1996	Sep 1991 Jan, Jun, Sep 1992	Zierbena, Santurtzi, Arrigunaga, Galea, Meñakoz, Plentzia	Extent of hemocyte infiltration in connective tissue of the digestive gland
Marigómez *et al.*, 1996	Monthly from Sep 1991 to Sep 1992	Zierbena, Santurtzi, Arrigunaga, Galea, Meñakoz, Plentzia	VvL, SvL, S/VL, NvL
Etxeberria *et al.*, 1995	Bimonthly from Sep 1991 to Sep 1992	Txatxarramendi, Laidatxu, Mundaka, Laida, Laga	VvL, SvL, S/VL, NvL
Etxeberria *et al.*, 1994	Mar 1988 Mar 1989	Meñakoz	VvL, SvL, S/VL, NvL
Agirregoikoa *et al.*, 1991	Aug 1988	Galea, Zierbena, Plentzia, Lekeitio	FCI, MET

AOX: peroxisomal acyl-CoA oxidase activity, LP: lysosomal labilization period, Vv_L: lysosomal volume density, Sv_L: lysosomal surface density, S/V_L: lysosomal surface to volume ratio, Nv_L: lysosomal numerical density, Vv_p: peroxisomal volume density, MLR/MET: morphology of digestive alveoli as mean luminal radius to mean epithelial thickness, Vv_{BAS}: changes in cell type composition in digestive gland epithelium as volume density of basophilic cells, MN: micronuclei frequency, MET: mean epithelial thickness, SOD: superoxide dismutase, GPX: glutathione peroxidase, DAOX: peroxisomal D-amino acid oxidase, Vv_{NL}: accumulation of intracellular neutral lipids as volume density of neutral lipids, Vv_{BSD}: intralysosomal metal levels as volume density of black silver deposits, FCI: flesh condition index, GI: gonad index.

Table A11.2: Description of the sampling locations for the monitoring of the Basque coast.

LOCATION	DESCRIPTION
Muskiz	Near oil refineries
Arrigunaga	Moderately polluted beach near the industrialized Bilbao metropolitan area
Arriluze	Sport and leisure-harbour, receives industrial inputs from the Bilbao metropolitan area
Plentzia	Small harbour of a tourist village in summer
Gorliz	Near small tourist village in summer
Mundaka harbour	Small fishery harbour
Mundaka beach	Small tourist village in summer
Mutriku	Receives river effluents from Deba river, moderately polluted beach
Pasaia	Industrial harbour
Hondarribia	Near a sport and leisure harbour, receives domestic effluents

Table A11.3: Summary of the chemical and biological parameters measured in each locality of the Basque coast.

MEASUREMENT	Nº MUSSELS	TECHNIQUE	TISSUE	ISSUE
-Lysosomal responses	10	Histochemistry	Digestive gland	General stress
-Peroxisome proliferation	20	Biochemistry	Digestive gland	Organic exposure
-Vitellogenin-like protein levels			Mantle	Endocrine disruption
-Lipid peroxidation	10	Biochemistry	Digestive gland	Oxidative stress
-Intralysosomal metal levels	13	Autometallography	Digestive gland	Metal exposure General stress
-Histopathology		Histology		
-Gonad index		Histology	Mantle	Alteration in reproduction
-Micronuclei frequency	10	Cytology	Hemolymph	Genotoxicity
-Stress on stress	50		Whole mussel	General stress
-Chemistry-PAHs	20	GC-MS	Whole mussel	
-Chemistry-metals	20	Atomic absorption	Whole mussel	

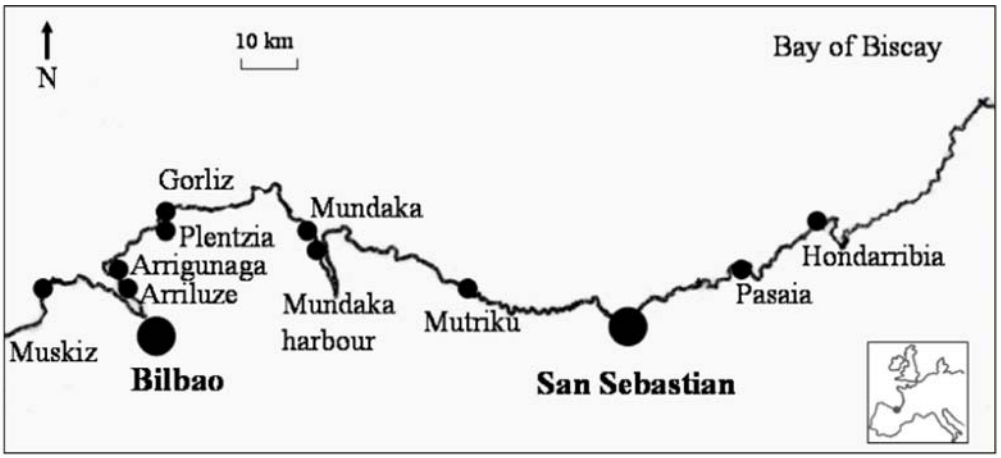

Figure A11.1: Map of the sampling stations in the Basque coast.

References

Agirregoikoa, M.G., Pérez, M.A., Marigómez, J.A/, Angulo, E. 1991. Relationship between quantitative indices of individual and digestive cell conditions in the common mussel, Mytilus edulis L., from the Biscay Coast. Acta. Hydrochim. Hydrobiol. 19, 29–37.

Cajaraville, M.P., Olabarrieta, I., and Marigómez I. 1996. In vitro activities in mussel hemocytes as biomarkers of environmental quality: a case study in the Abra estuary (Biscay Bay). Ecotoxicol. Environ. Saf. 35: 253–260.

Cajaraville, M. P., Garmendia, L., Orbea, A., Werding, R., Gómez-Mendikute, A., Izagirre, U., Soto, M., and Marigómez, I. 2006. Signs of recovery of mussels health two years after the Prestige oil spill. Mar. Environ. Res., 62: 337–341.

Cancio I., Ibabe, A., and Cajaraville, M P. 1999. Seasonal variation of peroxisomal enzyme activities and peroxisomal structure in mussels Mytilus galloprovincialis and its relationship with the lipid content. Comp. Biochem. Physiol. 123, 135-144.

Etxeberria, M., Cajaraville, M.P., and Marigómez, I. 1995. Changes in digestive cell lysosomal structure in mussels as biomarkers of environmental stress in the Urdaibai estuary (Biscay coast, Iberian Peninsula). Mar. Pollut. Bull., 30: 599–603.

Etxeberria, M., Sastre, I., Cajaraville, M.P., and Marigómez, I. 1994. Digestive lysosome enlargement induced by experimental exposure to metals (Cu, Cd and Zn) in mussels collected from a Zn-polluted site. Arch. Environ. Contam. Toxicol., 27: 338–345.

Marigómez, I., Agirregoikoa, M., Pérez, M.A., and Angulo, E. 1999. Seasonal, geographical and zonational variations in the quantitative structure of the digestive tubules of mussels, Mytilus galloprovincialis Lamark. Iberus, 17: 115–125.

Marigómez, I., Soto, M., Cancio, I., Orbea, A., Garmendia, L., Cajaraville, M.P. 2006. Cell and tissue biomarkers in mussel, and histopathology in hake and anchovy from Bay of Biscay after the Prestige oil spill (Monitoring campaign 2003). Mar. Pollut. Bull., 53: 287–304.

Marigómez, I., Orbea, A., Olabarrieta, I., Etxeberria, M., Cajaraville, M.P. 1996. Structural changes in the digestive lysosomal system of sentinel mussels as biomarkers of environmental stress in mussel-watch programmes. Comp. Biochem. Physiol., 113: 291–297.

Orbea, A., and Cajaraville, M.P. 2006. Peroxisome proliferation and antioxidant enzymes in transplanted mussels of four Basque estuaries with different levels of polycyclic aromatic hydrocarbon and polychlorinated biphenyl pollution. Environ. Toxicol. Chem., 25: 1616–1626.

Orbea, A., Garmendia, L., Marigómez, I., and Cajaraville, M.P. 2006. Effects of the "Prestige" oil spill on cellular biomarkers in intertidal mussels: results of the first year of studies. Mar. Ecol. Prog. Ser., 306: 177–189.

Orbea, A., Marigómez, I., Fernández, C., Tarazona, J.V., Cancio, I., Cajaraville, M.P. 1999. Structure of peroxisomes and activity of the marker enzyme catalase in digestive epithelial cells in relation to PAH content of mussels from two Basque Estuaries (Bay of Biscay): seasonal and site-specific variations. Arch. Environ. Contam. Toxicol., 36: 258–166.

Orbea, A., Ortiz-Zarragoitia, M., Solé, M., Porte, C., and Cajaraville, M.P. 2002. Antioxidant enzymes and peroxisome proliferation in relation to contaminant body burdens of PAHs and PCBs in bivalve molluscs, crabs and fish from the Urdaibai and Plentzia estuaries (Bay of Biscay). Aquat. Toxicol., 58: 75–98.

Soto, M., and Marigómez, I. 1997. Metal bioavailability assessment in "mussel-watch" programmes by automated image analysis of autometallographical black silver deposits (BSD) in digestive cell lysosomes. Mar. Ecol. Prog. Ser. 156, 141–150.

Annex 12: Publications concerning studies about biological responses and the Prestige oil spill (Spain).

Barata, C., Calbet, A., Saiz, E., Ortiz, L., and Bayota, J.M. 2005. Predicting single and mixture toxicity of petrogenic polycyclic aromatic hydrocarbons to the copepod *Oithona davisae*. Environmental Toxicology and Chemistry. 24(11): 2992–2999.

Beiras, R., and Saco Alvarez, L. 2006. Toxicity of seawater and sand affected by the Prestige fuel-oil using bivalve and sea urchin embryogenesis bioassays. Water, Air, and Soil Pollution. 177(1–4): 457–466.

Bilbao, E., Diaz de Cerio, O., Cajaraville, M.P., Cancio, I. 2006. Cloning and expression pattern of peroxisomal enzymes in the mussel *Mytilus galloprovincialis* and in the thicklip grey mullet *Chelon labrosus*: generation of new tools to study peroxisome proliferation. Marine Environmental Research, 62: 109–112

Cajaraville, M.P., Garmendia, L., Orbea, A., Werding, R., Gómez-Mendiuka, A., Izaguirre, U., Soto, M., and Marigómez, I. 2006. Signs of recovery of mussels health two years after the *Prestige* oil spill. Mar. Environ. Res. 62 Suppl:S337–341.

Casado, S., Babín, M., Tarazona, J.V. and Navas, J.M. 2006. Activation of the Aryl hydrocarbon receptor by the water-soluble fraction of the Prestige fuel oil. Marine Environmental Research. 62(Suppl.1): S75–S76.

Fernández, N., César, A., González, M., and delValls, T. A. 2006. Level of contamination in sediments affected by the Prestige oil spill and impact on the embryo development of the sea urchin. Ciencias Marinas. 32(2B):421–427.

Franco, M.A., Viñas, L., Soriano, J.A., de Armas, D., González, J.J., Beiras, R., Salas, N., Bayota, JM., and Albaigés, J. 2006. Spatial distribution and ecotoxicity of petroleum hydrocarbons in sediments from the Galicia continental shelf (NW Spain) after the *Prestige* oil spill. Mar. pollut. Bull. 53 (5–7): 260–271.

Labarta U., Fernández-Reiriz M.J., Garrido J.L., Babarro J.M.F., Bayona J.M. and Albaigés J. 2005. Response of mussel recruits to pollution from the "Prestige" oil spill along the Galicia coast. A biochemical approach. Marine Ecology Progress Series. 302(4): 135-145.

Laffon, B., Rabade, T., Pasaro, E., and Mendez, J. 2006. Monitoring of the impact of the *Prestige* oil spill on *Mytilus galloprovincialis* from Galician coast. Environ. Int. 32(3): 342–348.

Marigómez, I., Soto, M., Cancio, I., Orbea, A., Garmendia, L., and Cajaraville, M.P. 2006. Cell and tissue biomarkers in mussel, and histopathology in hake and anchovy from Bay of Biscay after the *Prestige* oil spill (monitoring Campaign 2003). Mar. Pollut. Bull. 53(5–7): 287–304.

Mariño-Balsa, J.C., Pérez, P., Estévez-Blanco, P., Saco-Alvarez, L., Fernández, E. and Beiras, R. 2003. Assessment of the toxicity of sediment and seawater polluted by the *Prestige* fuel spill using bioassays with clams (*Venerupis pollastra*, *Tappes decussatus* and *Venerupis rhomboideus*) and the microalga *Skeletonema costatum*. Ciencias Marinas. 29(1): 115–122.

Martínez-Gómez, C., Campillo, J.A., Benedicto, J., Fernández, B., Valdés, J., García, I and Sánchez, F. 2006. Monitoring biomarkers in fish (*Lepidorhombus boscii* and *Callionymus lyra*) from the Northern Iberian shelf after the *Prestige* oil spill. Mar. Pollut. Bull. 53(5–7): 305–314.

Morales-Caselles, C., Jiménez-Tenorio, N., de Canales, M.L., Sarasquete, C., and del Valls, T.A. 2006. Ecotoxicity of sediments contaminated by the oil spill associated with the tanker "Prestige" using juveniles of the fish *Sparus aurata*. Arch. Environ. Contam. Toxicol. 51(4): 652–660.

Morales-Caselles, C., Kalman, J., Riba, I., and del Valls, T.A. 2006. Comparing sediment quality in Spanish littoral areas affected by acute (*Prestige* 2002) and chronic (Bay of Algeciras) oil spills. Environ. Pollut., 146(1): 233–240.

Navas, J.M., Babin, M., Casado, S., Fernández, C., and Tarazona, J.V. 2006. The Prestige oil spill: a laboratory study about the toxicity of water-soluble fraction of the fuel oil. Mar. Environ. Res. 62 Suppl: S352–355.

Orbea, A., Garmendia, L., Marigómez, I., and Cajaraville, M.P. 2006. Effects of the "Prestige" oil spill on cellular biomarkers in intertidal mussels: Results of the first year studies. Marine Ecology Progress Series. 306(11): 177–189.

Ordas, MC., Albaigés, J., Bayota, J.M., Ordás, A., and Figuera, A. 2007. Assessment of in vivo effects of the *Prestige* fuel oil spill on the Mediterranean mussel immune system. Arch. Environ. Contam. Toxicol. 52(2): 200–206.

Pérez-Cadahia, B., Laffon, B., Pasaro, E., and Méndez, J. 2004. Evaluation of PAH bioaccumulation and DNA damage in mussels (*Mytilus galloprovincialis*) exposed to spilled Prestige crude oil. Comp. Biochem. C. Toxicol. Pharmacol. 138(4): 453–460.

Raingeard, D., Cancio, I., Cajaraville, M.P. 2006. Cloning and expression pattern of peroxisome proliferator-activated receptor α in the thicklip grey mullet *Chelon labrosus*. Marine Environmental Research, 62: 113–117.

Annex 13: WGBEC draft resolutions

Category 1

Permission is requested to publish the method 'Blue Mussel Histopathology' in the ICES Techniques in Marine Environmental Science (TIMES) series. Authors have been identified (Steve Feist, David Lowe & Miren Cajaraville) and will be formally requested to prepare the manuscript following acceptance of the draft resolution.

The Working Group on Biological Effects of Contaminants agrees to submit the final draft of the proposed publication by September 2008.

Supporting Information

PRIORITY:	Histopathology in mussels is an ICES WGBEC recommended technique for monitoring biological effects in invertebrates. This publication has high priority since common protocols are urgently needed for biological effects methods to ensure consistency in application of the technique and comparability of data to be submitted to the ICES database. The authors have been identified and are ready to commence preparation of the manuscript.
SCIENTIFIC JUSTIFICATION AND RELATION TO ACTION PLAN:	Biological effects in mussels is an important component of the proposed integrated monitoring guidelines prepared as a result of the ICES OSPAR WKIMON process. Identification of pathology in mussels is an important link between contaminants and effects on organism health and potential population effects that needs to be considered in integrated assessments. A standardised protocol is required to ensure consistency of application between laboratories and member states and comparability of reported data for assessment purposes.
RESOURCE REQUIREMENTS:	This manuscript is expected to be 20 – 30 pages long and may require colour figures. Publication is not expected to incurr any unusual expense. Online publication of this TIMES method will reduce demand for hard copies.
PARTICIPANTS:	Identified UK authors, ICES and ICES TIMES biological effects editor
SECRETARIAT FACILITIES:	For Secretariat
FINANCIAL:	For Secretariat
LINKAGES TO ADVISORY COMMITTEES:	Publication of this manuscript has been recommended by ICES WGBEC in their reports. These are approved by ACME
LINKAGES TO OTHER COMMITTEES OR GROUPS:	None.
LINKAGES TO OTHER ORGANIZATIONS:	Publication of this common method will also be of benefit to OSPAR and WKIMON (ICES/OSPAR Workshop on Integrated Monitoring)

In addition, WGBEC seeks permission to identify and contact authors with a view to submitting draft resolutions in 2008 for the following ICES TIMES paper topics:

1) YES / YAS assays
2) CALUX assays
3) Reproductive success in fish

Justification

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

Category 2

The **Working Group on Biological Effects of Contaminants** [WGBEC] (Chair: John Thain, CEFAS, UK) will meet in Sete in France from 31 March to 4 April 2008 to:

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

b) Consider progress with BEQUALM and national and international biological effect monitoring activities e.g. HELCOM, MEDPOL and WFD, and ICES ASC Indicator Workshop;

c) Assess the amount of biological effects data submitted to the new ICES database and answer queries / requests from the ICES Data Centre;

d) Evaluate the use of biological effects techniques for risk assessment purposes;

e) Evaluate documents prepared intersessionally on intersex in fish, micronuclei, comet assay, oxidative stress, in vitro – in vivo screening techniques and assessing toxicity of contaminants algae (phytoplankton and macrophytes);

f) Review confounding factors of salinity, temperature, season and genetic differences and how they affect biological effect responses;

g) Review the progress on the development of assessment criteria and integrated chemical-biological effect assessment tools

h) Review progress with the ICON demonstration programme

i) Review progress with OPSAR / MEDPOL / WFD / HELCOM / EU FM initiatives discussed at the 2007 meeting and develop a way forward for the use and application of biological effects techniques.

WGBEC will report by [DATE] 2008 for the attention of the Marine Habitat Committee and ACME.

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) AQC for biological effects methods has been identified as critical by OSPAR and international organisations; BEQUALM provides AQC for biological effect measurements and it is important that WGBEC keeps track of developments with this programme. In addition it is important that WGBEC keeps track and evaluates other national and international programmes that can influence the uptake of biological effect techniques, e.g., within MEDPOL, HELCOM etc. c) 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. d) Biological effect measures are increasingly being used for risk assessment purposes, such as for dredge material disposal and WFD and WGBEC would like to evaluate current strategies being advocated and developed. e) 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" f) As biological effects data is being used for developing assessment criteria and ultimately for regional assessments it is important to fully understand and evaluate the importance of confounding factors that can

	influence the biological effect measurements g) Provisional background responses and assessment criteria have been developed through WKIMON and WGBEC members. Further developmental work needs to be conducted and WGBEC needs to contribute to and evaluate this work as an ongoing process. 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 needs to continue to explore and develop the links (OSPAR / MEDPOL / WFD / HELCOM / EU FWM) already made during its 2007 meeting in respect harmonisation of AQC, application of techniques and interpretation of data.
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:	ACME
LINKAGES TO OTHER COMMITTEES OR GROUPS:	There are linkages with WGSAEM, MCWG, WGMS and WGPDMO.
LINKAGES TO OTHER ORGANIZATIONS:	None identified.