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

11-15 January 2010

Dublin, Ireland

ICES International Council for the Exploration of the Sea

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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

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Contents

Executive Summary

The Working Group on the Biological Effects of Contaminants [WGBEC], chaired by John Thain, UK, met at Trinity College, Dublin, from 11–15 January 2010. There were 21 attendees representing 13 countries.

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

WGBEC includes in its membership scientists from national government institutes, academia, industry and management. The group also has a diverse membership of expertise, ranging from chemists, biologists, biochemists and environmental scientists. This is beneficial as requests in the past, particularly from OSPAR have been wide-ranging. This year there were thirteen items on the agenda, including three items from ICES and one from OSPAR. Priority was given to the latter items. Presentations and discussions took place in plenary, with the exception of a sub-group working on specific requests from the ICES data centre. All items on the agenda (covering all ToR) were completed and are reported.

Progress with national and international monitoring activities. Presentations were received from Spain, Ireland, Sweden, Italy (MEDPOL), and the Baltic Sea. These included new monitoring activities such as in Ireland to well established programmes in other countries. Of particular importance for WGBEC to note were the developments on integrated assessment being pursued in the Baltic (BEAST), North Sea (ICON) and MEDPOL and the QC intercalibrations involving WGBEC members being facilitated by Italian and Spanish group members. The WGBEC considered the progress made by the Marine Strategy Directive (MDS) descriptor 8 Task Group. The WG fully supported the recommendations made by the Task Group which placed a strong emphasis on biological effect monitoring as developed by the WGBEC in the past decades and currently used by OSPAR.

Review the methodology used by the OSPAR workshop on the development of Chapter 11 of the QSR 2010 (Utrecht workshop). The WG considered the OSPAR report and concluded that there were serious shortcomings to the methodology of the workshop and its conclusions. There were a number of concerns in relation to the poor scientific basis under pining several of the conclusions reached. It was noted that the workshop considered that this type of broader ecosystem assessment could be a useful contribution to an Initial Assessment for the Marine Strategy Framework Directive (MSFD) in 2012. WGBEC would be concerned if this were the case without further underpinning science and engagement with experts in the specific scientific fields.

Report to SSGHIE on potential and current contributions of your EG to the Strategic Initiative on Coastal and Marine Spatial Planning (SICMSP). The WG briefly reviewed the SICMSP and suggest three specific examples in respect to contaminants that should be considered that are directly relevant to area-based management: these are point sources of contaminants (i.e. industry, offshore platforms, and rivers), diffuse sources (e.g. harbours, urban areas) and contaminants in sediments.

Report to SSGHIE on your plans to promote cooperation between EGs covering similar scientific issues. After intensive discussions on the scope and future activities of the WG, and taken consideration of the various ICES vision and strategy documents, WGBEC identified 9 areas of its core business and 8 areas for future directions. While some of these activities are considered as "true" core business, others clearly

attend to broaden the scope of WGBEC, towards more ecology and wider ecosystem effects. WGBEC also identified how and where it fitted in to the ICES Science Plan and its potential to collaborate with other expert groups.

Review ICES WGBEC list of recommended biological effects methods for monitoring purposes and define how this fits in for both OSPAR and EU MSFD purposes. The WGBEC discussed and amended the 'promising' and 'recommended' monitoring techniques that had been last updated in 2007.

Cooperation with ICES / OSPAR SGIMC conduct intersessional work for review at 2010 meeting based on the outcome of the SGIMC Aberdeen Workshop, October 2009. The outcome of the SGIMC report was reviewed and noted and tasks deferred to WGBEC were considered. These included a review of draft background document on the Comet assay and amendments to background document on DNA adducts.

Review progress with publication and electronic dissemination of biological effects techniques in the ICES TIMES series. The group reviewed the status of publications that were in preparation or had been commissioned. Considering the number of manuscripts commissioned by the group, still in preparation (9) all with draft resolutions, it was decided to focus WGBEC efforts on delivery of these, rather than commission any new manuscripts.

Answer queries / requests from the ICES Data Centre. WGBEC responded to several requests from the ICES data centre relating to; legacy data, data quality issues, parameter codes and also to queries from WGBEC data submitters. In order to assist in data submission to ICES, WGBEC recommended that WGBEC / ICES data centre develop a live 'working document' to be added to at future WGBEC meetings to explain how biological effect data should be entered into the database and keep track of WGBEC advice on database issues.

Review of emerging and novel contaminants. Reviews were received and discussed on nanoparticles, marine litter and plastics, and contaminants in eels and associated biological effects.

1 Opening of the meeting

The Chair, John Thain (UK), opened the meeting at 09.30 on Monday, 11 January 2010, and thanked Michelle Giltrap (IE), for hosting the meeting at the Zoology Department, Trinity College, Dublin and for organising the meeting arrangements and hotel accommodation, etc. The Chair then invited the participants to introduce themselves and their affiliations and describe their area of interest and field of expertise. 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 in particular those requests from ICES and OSPAR. 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 6 was a request from OSPAR and Agenda Item 7 and 8 was a recent request from ICES.

One item was agreed under any other business, the election of a new Chair person(s).

3 Appointment of rapporteurs

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

4 Review progress with national /international monitoring activities; to include / integrated assessment / and application of biological effect techniques within OSPAR / MEDPOL / WFD / HELCOM / EU MSD + any other; (ToR c).

4.1 Spain

Concepción Martinez-Gomes (ES) gave a presentation on progress with the national programme for monitoring marine pollution in Spain and details can be found in Annex 5. In summary, two major biomonitoring programmes, along the Northern Iberian coast and along the Iberian Mediterranean coast have been conducted through several research projects over the past decade. However, between 2010–2012 a new biomonitoring programme will be instigated to meet the obligations of the both the OSPAR and Barcelona conventions and if possible to contribute to the GES assessment for the Marine Strategy Framework Directive.

The new programme along the Northern Iberian coast includes a chemical and biological effect integrated approach in order to establish clear relationships between results of chemical monitoring of pollution and the pollutant concentrations that may cause ecological damage. This will include (i)biological effect studies on sediment elutriates, using the sea urchin embryo-larval bioassay; (ii) conducting sediment toxicity assessments using the amphipod survival bioassay; and (iii) biological effects studies using molecular responses in mussels (GST and AChE).

In addition, to fulfil obligations for biological effect monitoring in MEDPOL Phase IV (2006–2013), the Contracting Parties to the Barcelona Convention adopted the strategy for the development of Mediterranean Marine Pollution Indicators (MPIs). This strategy will be adopted on the Spanish Mediterranean coast as well as extending this to include Spanish monitoring research activities with more biomarker measurements

in mussels and fish as well as contaminant concentrations in surface sediments and fish.

In both programmes, measurements are performed yearly (excepting temporal trends in sediments that are conducted biannually, in the case of the Mediterranean program) and the application of both chemical and biological effect techniques (biomarkers/bioassays) is included. A summary of the sampling strategy and timescale is given in Table 4.1.1.

Table 4.1.1. Sampling strategy, parameters, timescales and matrices included in the Spanish monitoring programme.

BIOMONITORING IEO	SPANISH ATLANTIC MONITORING	SPANISH MEDITERRANEAN MONITORING
Sediment (S)	Yearly	Autumn (Sept-Oct)
Fish (MB/MM)	Autumn	Autumn (Sept-Oct) Post-spawning
Mussels (MG)	Autumn (Oct-Nov) Pre-spawning	Spring (May-June) Pre-spawning
Sampling NR/NL	Yearly	not
Parameter	Matrix	Matrix
Trace metals	MG/MM/S	MG/MB/S
PAHs	MG/S	MG/MB/S
Organochlorinated Compounds	MG/MM/S	MG/MB/S
BFRs	MG/MM/S	not
TBTs	NR/NL/S	not
Imposex	NR/NL	not
SFG	MG	not
SoS	not	MG
LMS	not	MG
MT	not	MG/MB/S
AChE	MG	MG/MB/S
Antioxidant enzymes	MG*	MG*
GST	MG	MG*
MN	not	MG/MB/S
EROD	not	MB
Genotoxicity	not	MB
Sea urchin Embryotoxicity assay	S	S*
Amphipod bioassay	S	not
CI/CF	MG/MM	MG/MB
GSI	not	MB

MG: ***Mytilus galloprovincialis***

MB: ***Mullus barbatus***

MM: ***Merluccious merluccious***

NL: ***Nucella lapillus***

NR: ***Nassarius reticulatus***

S: Surficial sediments

***pilot study**

More details of the sampling procedures, geographical spread of the sampling sites, testing methodology and proposed method for conducting the integrated assessment are given in Annex 5

WGBEC were in full support of the activities being conducted in the Spanish monitoring programme, both in the approach and the MEDPOL, OSPAR and MSFD harmonization aspects. WGBEC look forward to seeing data from this programme in 2011 and 2012.

4.2 Marine monitoring in Ireland

Michelle Giltrap (IR) presented an update on the project entitled "Biological Effects and Chemical Measurements for the Assessment of Pollution in Irish Marine Waters". The structure of the project is outlined in WGBEC report 2009. Tier I site sampling was completed at 8 sites around the coast of Ireland in 2009. For Tier I analysis samples were taken for clearance rate, stress on stress, condition index, sediment toxicity testing and chemical analysis. Sediment toxicity testing included whole sediment tests with *Corophium volutator* and *Arenicola marina*, porewater and elutriate testing with *Tisbe battagliai* and *Skeletonema costatum* and the microtox test with *Vibrio fischeri*. Results from Tier I analysis will direct analysis to 3 sites for full biological, ecotoxicological and chemical assessment. Tier II site analysis will take place in 2010 and involves analysis with a battery of biomarkers in mussels and fish, chemical analysis, fish and mussel histopathology, benthic monitoring, sediment bioassays and imposex/intersex analysis. The battery of bioassays for mussels include scope for growth, stress on stress, condition index (CI), lysosomal membrane stability (LMS), metallothionein (MT), acetylcholinesterase (AChe), alkali labile phosphate (ALP) and comet assay. For fish, the battery of biomarkers include CI, EROD, bile metabolites, vitellogenin induction, AChe, MT and comet assay. Natural reproductive cycles will be investigated for mussels from a control location on the west coast of Ireland with the use of the adipogranular scoring index as reported by Bignell et al. 2008 (REF). Flounder will be sampled quarterly in 2010 from 2-3 estuaries for histology/VTG in blood plasma and total protein to investigate reproductive cycles before commencement of more in depth fish studies. Sampling for benthic monitoring and imposex analysis in snails will commence in February 2010. Development of chemical methodology for natural and synthetic steroid estrogens in water and biota is underway at the Marine Institute, Galway. As well as Tier I/II site analysis, various caging study case studies are being conducted for the investigation of sewage related effects/chemical analysis in mussels. These sites include Kinvarra Bay and Mutton Island (Co. Galway), the North Bank Lighthouse in Dublin Bay. Potential sites for future caging studies include Haulbowline Island (Cork Harbour) and others yet to be confirmed. Passive sampling and stable isotope analysis is being conducted at MI for various case study sites also. An in-vivo exposure system is now set up in the Shannon Aquatic Toxicity Laboratory. A pilot study has been performed with mussels and the exposure of ethinylestradiol. This study was performed to demonstrate a positive control for alkali labile phosphate biomarker and also intertidal and submerged uptake of this contaminant will be investigated with chemical analysis. Further studies with flounder/dab exposures and alkylphenols will be investigated in 2010/2011. Collaborations with the Galway and Mayo Institute of Technology (EPA funded), Athlone Institute of Technology and MI (STRIVE EPA funded) shall allow for testing of pharmaceuticals (gemfibrozil and diclofenac) using proteomics, yeast estrogen screening assay and norovirus respectively.

References

Bignell, J. P., Dodge, M. J., Feist, S. W., Lyons, B., Martin, P.D., Taylor, N. G. H., Stone, D., Travalent, L., and Strentiford, G.D. 2008 Mussel histopathology: effects of season, disease and species, Aquatic Biology, Vol. 2: 1–15.

4.3 Sweden

Halldóra Skarphéðinsdóttir (SE) from Stockholm University gave a presentation on recent studies on DNA adducts in blue mussel, fish and herring gulls and included field and experimental data.

DNA adducts are formed when a compound or its metabolite binds covalently to DNA and is commonly used as a biomarker of PAH exposure and effects. Adduct formation may occur as an integrative response to multiple factors such as uptake, metabolism, detoxification, and DNA repair. It may persist for weeks or months and can ultimately lead to physiological consequences if the DNA is not functioning right, can cause cancer and mutations if in germ cells resulting in effects on genetic diversity.

Mussels

In a study conducted in Iceland DNA adduct levels were measured in mussels at different sites with suspected contamination. Mussels were transplanted in the sub tidal and tidal zone for a period of six weeks in summer and winter. The results showed: that DNA adduct levels were elevated in mussels at sites with suspected contamination; highest DNA adduct levels were found in gills of mussels in Reykjavik harbour; DNA adduct levels in mussels transplanted in Reykjavik harbour for six weeks were not similar to those of native mussels in the same area; there is a possibility that seasonal variation in adduct level occurs. This work is fully reported in Ericson *et al* 2002.

Following on from this some laboratory experiments were conducted to better understand the formation of DNA adducts in blue mussels, and to improve the interpretation of field results. Blue mussels were exposed to the genotoxic compound, benzoapyrene, and DNA adduct formation studied. The results showed that: BaP uptake at the end of the 4 day exposure was linear with dose in all the studied tissues; highest tissue concentration was found in the digestive gland; the uptake was linear with dose in all tissues; adducts were only significantly formed in the gills, no increase in DNA adduct levels in the digestive gland; there was only dose response up to 50ug BaP/l. with no difference in adduct levels between mussels exposed to 50 and 100 ug BaP/l; *BaP uptake during the 6 days exposure was rapid and linear over time (*Maximum levels were 107ug BaP/g dw 1 day after the end of exposure); DNA adducts were persistent in gills for at least 2 weeks, but BaP tissue levels decreased fast. This work is fully reported in Skarphéðinsdóttir *et al* 2003.

In a further study, seasonal variation in DNA adduct levels in blue mussels (*Mytilus edulis*), was investigated along with the impact of intertidal exposure on the DNA adduct levels, i.e. to explore if DNA adduct levels in mussels in the intertidal zone differ from those in the sub tidal zone. Blue mussels were deployed separately in the intertidal and sub tidal zone at a contaminated and a reference site in Iceland, and sampled regularly during one year. Gill DNA adduct levels were found to be higher in mussels in the intertidal zone compared to the sub tidal zone at the contaminated site, the difference being largest in winter. Total PAH tissue levels were also higher in mussels in the intertidal zone. Seasonal variation was observed in both DNA adduct and PAH tissue levels in mussels at the contaminated site, with lower levels from the

time of transplantation in summer to autumn, maximum levels in winter, which decreased to lower levels again in spring and summer the following year. DNA adducts and PAH levels were low or below the detection limits in mussels at the reference site at all times, both in the intertidal and sub tidal zone. Concluding that intertidal differences and seasonal differences need to be taken account when using DNA adducts measurements in biomonitoring programmes. This work is fully reported Skarphedinsdottir *et al.* 2005.

In a field monitoring programme DNA adducts in gills and digestive gland, as well as polycyclic aromatic hydrocarbon (PAH) tissue levels were analysed in blue mussels (*Mytilus* spp.) from Nordic coastal areas (Iceland, Norway and Sweden) with diffuse or point sources of PAHs of various origins. Both DNA adduct and PAH tissue levels were generally low, indicating low PAH exposure to the mussels in the areas studied. DNA adducts were found to be higher in gills than in digestive gland of the mussels at all sites studied. Elevated DNA adduct levels in gills were found at 6 sites out of 18 compared to reference sites in respective coastal zones. Adduct levels ranged from 0.5 to 10 nmol adducts/mol normal nucleotides, being highest in mussels from Reykjavík harbour, Iceland (intertidal mussels), and from Fiskaatangen, Norway (sub tidal mussels). Total PAH tissue levels in the mussels ranged between 40 and 11,670 ng/g dry wt., and were significantly correlated with DNA adduct levels ($r^2 = 0.73$, $p < 0.001$). PAH ratio values indicated that the PAHs were in most cases of pyrolytic origin. Thos work is fully reported in Skarphedinsdottir et al 2007.

Fish

DNA adducts have been analysed in several fish species at ITM, Stockholm University, Sweden and these include; perch, pike, cod, haddock, saithe, halibut, greenland halibut, long rough dab, and more. In many studies these have been related to Norwegian oil platforms or produced water: lab experiments, field experiments, monitoring. In one study (Aas *et al* 2003), 11 species from pristine areas were analysed for liver DNA adducts in order to study "natural background" levels. Adduct values above 1 nmol add/mol normal nucleotides can be considered an effect, but values below that are too low to be considered an effect. In a laboratory study Atlantic cod were exposed for up to 44 weeks to environmentally relevant concentrations (resembled North Sea produced water) of low-molecular weight PAHs (2-4 ring), and short chained APs. Three treatments were used: - low (0.54ug PAH/L +1.14ug APs); high (5.4ug PAH/L +11.4ug APs); Pulsed (high dose and control exposures alt. at 2 weeks interval). DNA adducts were analysed after 0, 16 and 44 weeks. Few adducts were formed after 16 weeks so the period was extended and 44 weeks exposure was needed for formation of DNA adducts in female cod. No adducts were measured in the pulsed treatment: this might indicate that tissue contaminant loads were reduced during control exposure periods, even though bile PAH metabolite levels were maintained. A possible explanation may be that during such periods of control exposure, a continued metabolism and excretion of tissue contaminants could allow the rate of DNA repair to exceed adduct formation. This work is reported fully in Holth *et al* 2009.

Birds

A survey in both Sweden and Iceland has shown that adult herring gulls (*Larus argentatus*) are exposed to genotoxic chemicals as seen in elevated DNA adducts, analysed with the ^{32}P postlabelling method. DNA adducts were highest in the liver, with levels ranging up to 72.6 nmol adducts/mol normal nucleotides, thereafter in kidneys, intestinal mucosa, and lowest in blood. Gulls from the urban site Skåne (Malmö harbour)

had significantly higher liver DNA adduct levels than Iceland, the control (P = 0.01), while the rural sites Blekinge and Södermanland did not (P > 0.05). Liver DNA adducts were detected in Swedish pulli, but not in pulli from Iceland. Frequency of micronucleated erythrocytes in adult birds was similar in all the regions studied, ranging from 0.18–0.28 ‰. Neither liver DNA adducts nor erythrocyte micronuclei levels were associated with observed hepatomegaly, poor condition or paralytic symptoms observed in the Swedish birds (P > 0.05), the DNA adduct levels are however suspected to reflect different pollution load of the respective regions. This work is full reported in Skarphedinsdottir et al (submitted 2010).

References

Aas *et al*. 2003. DNA adduct levels in fish from pristine areas are not detectable or low when analysed using the nuclease P1 version of the 32P postlabelling technique. Biomarkers, 8(6), 445–460.

Ericson, G., Skarphéðinsdóttir, H., Dalla Zuanna, L., Svavarsson, J. 2002.DNA adducts as indicators of genotoxic exposure in indigenous and transplanted mussels, *Mytilus edulis* L. from Icelandic coastal sites. 2002. Mutation Research, 516: 91–99.

Holth, T.F., Beylich, B.A., Skarphéðinsdóttir, H. Liewenborg, B. Grung, M., and Hylland, K. 2009. Environ. Sci. Technol., 43: 3329–3334:

Skarphéðinsdóttir, H., Ericson, G., Dalla Zuanna, L. and Gilek, M. 2003. Tissue differences, dose-response relationship and persistence of DNA adducts in blue mussels, (*Mytilus edulis* L.), exposed to benzo[a]pyrene. 2003. Aquatic Toxicology, 62: 165–177.

Skarphedinsdottir, H., Ericson, G., Halldorsson, H.P., and Svavarsson, J. 2005. Seasonal and intertidal impact on DNA adduct levels in gills of blue mussels (*Mytilus edulis* L.) Environmental Pollution, 136: 1–9.

Skarphedinsdottir, H., Ericson, G., Svavarsson, J., and Næs, K. 2007. DNA adducts and polycyclic aromatic hydrocarbon (PAH) tissue levels in blue mussels (Mytilus spp.) from Nordic coastal sites 2007. Marine Environmental Research 64 (4): 479–491.

Skarphedinsdottir H., Gunnar T. Hallgrimsson , Tomas Hansson, Per-Åke Hägerroth, Birgitta Liewenborg , Ulla Tjärnlund, Gun Åkerman, Janina Baršienė, and Lennart Balk. Genotoxic effects in herring gulls (Larus argentatus) in Sweden and Iceland. Submitted to Mutation Research 2010.

4.4 Italy

MEDPOL – PHASE IV MONITORING ACTIVITIES

Prof. Aldo Viarengo (IT) presented progress with the MEDPOL – Phase IV monitoring activities.

The main purpose of the programme is to evaluate the biological effects of pollutants on marine organisms along the Mediterranean coasts. The programme highlights three important aspects. Firstly the choice of test species which is *Mytilus* spp. because of it is widely distributed and easily collected. Secondly, the use of biomarkers to evaluate the level of the stress syndrome induced by pollutants in the selected organisms; these are schematically categorized into two classes, biomarkers of stress and biomarker of exposure. A biomarker of stress reveals the stress syndrome by integrating the effects of a wide range of environmental pollutants and include the techniques; lysosomal membrane stability, micronuclei frequency, neutral lipid accumulation, lipofuscin accumulation. Biomarkers of exposure reflect the response of the organisms to a specific class of chemicals and include the techniques; metal-

lothionein content, exposure to heavy metals (Cd, Hg, Cu, Zn, etc.), stress on stress. The third aspect is the provision of a QA (Quality Assurance) Program, and this was achieved by a) the distribution of a "UNEP/MAP manual" for biomarker utilization, b) distribution of produced by RAMOGE in collaboration with UNEP/ MAP showing biomarker methodologies, c) organization of Training Courses to prepare the researchers to participate in the biomonitoring programs, and d) organization of an "Intercalibration Program": the first one that has ever been realised to achieve a standardization of biomonitoring data.

Prof. Aldo Viarengo then presented the results from the UNEP – MAP MEDPOL programme from the last three years. Seventeen laboratories from different Mediterranean countries being involved to the UNEP MAP activity and they have contributed to the Mediterranean coast biomonitoring: these were Italy, Greece, Slovenia, Croatia, Tunisia, Monaco, France, Spain, Algerie, Morocco, Syria, Israel, Turkey, Albania, Malta, Egypt, Libanon, Palestine. Standardised protocols were agreed and used for, animal collection, transport of animals, storage of biological samples, biomarker choice and application and this included a training course at the MedPol Reference Centre in Alessandria and the equipping of laboratories in Egypt, Syria and Moracco. The biomarkers data was collected by following standard procedures and all results were sent to UNEP MAP by using a standard data transmission protocol. The results for lysosomal membrane stability, lysosomal lipofuscin content, and lysosomal neutral lipid accumulation showed that all the laboratories involved were able to identify the blind samples obtained from control and exposed mussels. The results for the metallothionein content intercalibration exercise shoed that two labs were not able to correctly determine the metallothionein content. This indicates the need of yearly organized intercalibration activity and the importance of the training courses and of periods of training for the researcher involved in the program.

The current intercalibration exercise (2009–2010) is the first intercalibration exercise putting togheter Med Pol and ICES laboratories. It commenced in October 2009 and results from the sixteen laboratories are expected to be available by mid 2010. It is hoped that this programme will help to develop AQC harmonisation, including organising regular training courses, guarantee the quality of data, validate data collected throughout the year by laboratories and improve knowledge exchange between laboratories.

At previous meetings of WGBEC the "2-tier appraoch" used in MEDPOL to assess levels of pollution-induced distress syndrome in sentinel organisms had been described. At this meeting Prof Aldo Viarengo gave an update on these strategies and how they may be used in MEDPOL IV. These strategies have been developed and improved during the last decade, supported by funds from national and international programmes BEEP (EC), RAMOGE, NOMIRACLE (EC) and MED-POL (UNEP). The important use of this strategy is for biomonitoring/ecological risk assessment.

The 2-tier approach strategy is described as

Tier 1 – one biomarker: lysosomal stability + Stress on Stress + mortality

Tier 2 – full biomarker battery: 6 biomarkers – lipofuscin, neutral lipids, micronuclei test, metalothionein, acetylcholineesterase, lysosome/cytoplasm ratio and stress on stress

As result of the TIER I analyses: a) no effects on lysosomal membrane stability → clean sites → no other analysis necessary (biological or chemical); b) increased mortality → direct chemical analysis to identify pollutants that induce biological effects.

Apply TIER 2 analysis in sites where there are alterations in lysosomal membrane stability and use battery of biomarkers to quantify the stress syndrome.

This approach has been found to be cost effective. In order to assist the interpretation of results mussels are caged and sampled after 30 days exposure at the site of interest. This is important for the following reasons:

- using the same stock means the animals have similar genetic and physiological characteristics; and similar minimal content of toxic chemicals.
- variations occurring in the polluted sites allow comparison with chemical data obtained for organisms sampled in control/clean sites. This involves an accumulation of chemicals directly related to the month of mussel exposure in the polluted sites.
- it is not easy to correlate organism health status data to pollutant content in wild mussels that accumulate chemicals for years and detoxify them in order to survive in polluted areas.
- in wild mussels, it is possible to observe variations in biological parameters (such as gonad maturation) which can lead to problems with data analysis and interpretation. Caged mussels (3–4 weeks) mantain similar gonad maturation level.
- the use of caged mussels permits geographical referencing of the sampling sites.

It is well know that there are many difficulties in integrating biological effect and chemical data and to present the data objectively and meaningfully to decision makers. With this in mind Prof Aldo Viarengo then described an approach using an expert system using data from laboratory and field studies. A five-fold classification scheme has been derived (Stress Syndrome Level) from A (no stress) to E (pathologic stress) considering the alteration each biomarker on the basis of its stress response profile and the level of biological organisation (cell, tissue or organism). Further details on this appraoch can be found in Dondero *et al*., 2006.

Prof Aldo Viarengo also gave a short presentation on ecological risk assessment using modifications to the "Triad approach" using a case study on the Bormida river with fresh water and soil data.

References

Dondero, F., Dagnino, A., Jonsson, H., Caprì, F., Gastaldi, L., and Viarengo, A. 2006. Assessing the occurrence of a stress syndrome in mussels (*Mytilus edulis*) using a combined biomarker/gene expression approach. Aquatic toxicology (Amsterdam, Netherlands); 78(1): S13–24.

Recommendation

WGBEC fully supported the proposed intercalibration exercise (Sept 2010) on lysosomal stability(NRR method) to be held in Alexandria in Italy; the first intercalibration exercise putting together MEDPOL and ICES laboratories. This is an important step forward for harmonisation between OSPAR, MEDPOL and HELCOM biomonitoring activities. WGBEC would recommend that ICES supports this initiative and recommends further support and uptake from organisations and laboratories within these communities.

4.5 Baltic Sea Issues: Report on ICES SGEH activities (Kari Lehtonen, by correspondence)

The ICES Study Group for the Development of Integrated Monitoring and Assessment of Ecosystem Health in the Baltic Sea (SGEH) will meet in Gdynia (PL), 1–5 March 2010. The group is chaired by Kari Lehtonen (FI), a WGBEC member. In short, the SGEH focuses its main activities on matters related to biological effects of contaminants in marine organisms in the Baltic Sea, a field with a significantly lesser research emphasis in this geographical area compared e.g. to eutrophication, biodiversity and fisheries, and high and urgent needs for development. Information on the effects of contaminants on biodiversity is also closely followed.

To achieve the target of developing assessments of Ecosystem Health in the Baltic Sea links with groups dealing with fisheries and eutrophication impacts will be established with expected participation of experts having data and information relevant to SGEH. Important aspects are identification of links between SGEH work related to HELCOM, OSPAR, EU (with a special reference to the Marine Strategy Framework Directive [MSFD]) and other ICES EGs, especially WGBEC, SGIMC and WGIAB. In regard to the MSFD, suggested criteria and methodological standards for the descriptors will be discussed in this group in the 2010 meeting. Since OSPAR is working on the Quality Status Report for 2010, SGEH will follow the outcome of this report, and it will also be discussed in the 2010 meeting.

Progress made through the BONUS+ programme BEAST project (Biological Effects of Anthropogenic Chemical Stress: Tools for the Assessment of Ecosystem Health [2009–2011]) and other similar activities in and outside the Baltic Sea will be reviewed at the 2010 SGEH meeting, with discussions on development of especially the parts of the project related to development of integrated monitoring (WP 2) and assessment of ecosystem health (WP 3) to serve the goals of the SGEH and Baltic Sea Action Plan (BSAP).

4.6 MSD

The WGBEC considered the progress made by the Marine Strategy Directive (MDS) descriptor 8 Task Group. No report was yet available but Dick Vethaak (NL) presented the executive summary (Annex 6). The WG fully supported the recommendations made by the Task Group which placed a strong emphasis on biological effect monitoring as developed by the WGBEC in the past decades and currently used by OSPAR. Given the recommendations made by the Task Group, the WGBEC anticipates that further work on biological effects monitoring and harmonisation of methods may increase in the next years. This is generally well in line with the WG's activities in this area.

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

5.1 Review progress within the BONUS+ Programme BEAST project (Kari Lehtonen, by correspondence)

The BEAST project (Biological Effects of Anthropogenic Chemical Stress: Tools for the Assessment of Ecosystem Health) was launched under the Baltic Sea BONUS+ Programme (2009–2011). The BEAST consists of 16 partners from all nine Baltic Sea countries. Detailed information on the BEAST project is available in the WGBEC Report 2009, at the BONUS+ website (http://www.bonusportal.org/research_projects), and at the BEAST project website (www.bonusportal.org/research_projects/...projects/beast/).

In short, the BEAST project consists of three thematic Work Packages (WP), see Figure 5.1.1:

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

- basic research: testing and validation of biomarkers in Baltic Sea species and environmental conditions

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

- recommendations and practical guidelines for the integration of chemical-biological monitoring of hazardous substances in Baltic Sea monitoring programmes (mainly HELCOM)

WP3: Developing tools for ecosystem health assessment in the Baltic Sea

- testing and developing approaches (e.g. indices) for the assessment of Ecosystem Health in different sub-regions of the Baltic Sea

Research activities in the three WPs are organised under five sub-regional Tasks, i.e. field and experimental studies in the Gulf of Bothnia, G. of Finland, G. of Riga, G. of Gdansk and the Belt Sea (Fig. 5.1.2). In addition to WP leaders also each sub-regional Task has a responsible leader.

BEAST sampling campaigns started in April 2009 in the Gulf of Riga and they were continued at all target areas (except for the Gulf of Bothnia). The largest research activity in 2009 was the GOF-IA (Integrated Multidisciplinary Assessment of the Ecosystem Health of the Gulf of Finland) joint 2-week research cruise of r/v Aranda (FI) and r/v Walther Herwig III (DE) in August-September. Unfortunately, no permission to sample in Russian waters could be obtained and the original sampling plan had to be adjusted. Sampling was carried out at 20 point stations (Aranda) and 9 fishing areas (WHIII) in different parts of the Gulf of Finland within the Finnish and Estonian EEZ. The research performed consists of measurements of several biological and chemical parameters with emphasis on selected biomarkers (Table 5.1.1). The main aim is to use the data (plus additional existing data sets) for an integrated assessment of ecosystem health in the different sub-regions of the Gulf of Finland by using methods tested and developed under WP3. The BEAST sampling campaigns will continue in 2010 but no sampling is planned for the last year of the project, 2011.

In regard to WP2, a draft of a handbook with guidelines and standard operating procedures (SOPs) has been produced based on a document produced during the EU funded BEEP project (2001–2004). Harmonisation of the guidelines and SOPs with those under preparation for OSPAR is a further development plan.

An Excel-based project database has been developed in WP3 and is ready to receive data. The intention is also feed in data from the BEEP project and other available and relevant data from the Baltic Sea. These will be used for the testing and development of integrated indices and sub-regional assessments.

Collaboration with another BONUS+ project dealing with biological effects, BALCOFISH, has been established and aimed to be strengthened during 2011. In addition to practical collaboration activities (e.g. sampling, workshops) the aim is to start preparations for the coming call for projects for the Joint Baltic Sea Research Programme (BONUS-169). The objective of BONUS-169 is to enhance the Baltic Sea region research capacity to ensure a more sustainable development of the region. The Commission proposes to contribute € 50 million to a joint research investment with

eight EU Baltic Sea Member States. The € 100 million programme will provide a framework for the coordination of their environmental research.

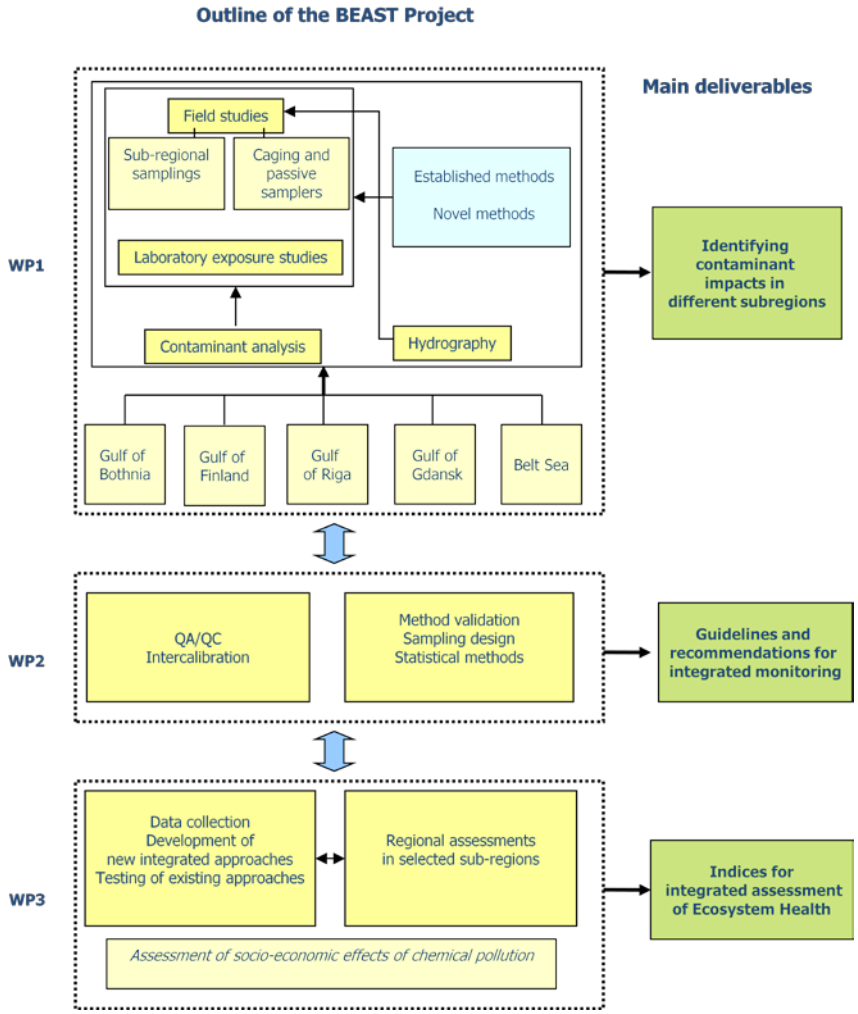


Figure 5.1.1. Outline of the BEAST project.

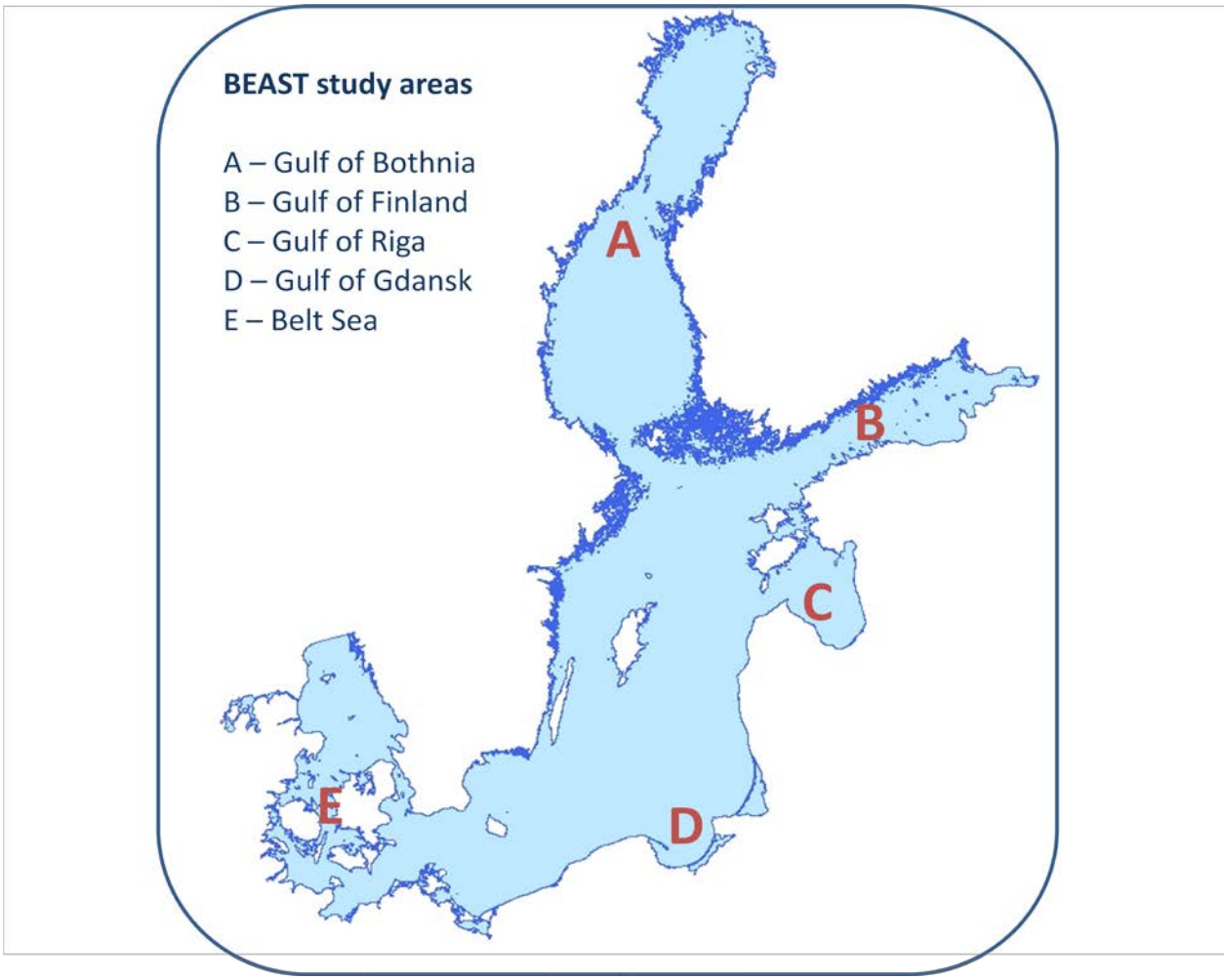


Figure 5.1.2. Study areas of the BEAST project.

Table 5.1.1. Sampling scheme in the Gulf of Finland during the GOF-IA cruises with r/v Aranda and r/v Walther Herwig III in August-September 2009. The sampling was carried out at 20 point stations and 9 fishing areas around the G. of Finland in Finnish and Estonian EEZ.

SAMPLING TYPE	SAMPLING DEVICE	PARAMETER	SPECIFIC PARAMETER	SPECIES	FUNCTION OR PROCESS
Hydrography	Near-bottom O2	Oxygen concentration	O2 content	-	Eutrophication
Hydrography	CTD	Salinity	Salinity	-	Background data
Hydrography	CTD	Temperature	C-degrees	-	Background data
Hydrography	CTD rosette	Nutrients	NO3, NO2, NH4, PO4, SiO2	-	Eutrophication
Phytoplankton comm.	Water sampler	Community structure	Indicators & indices	-	Disturbed structure
Zooplankton comm.	Zooplankton net 100 um	Community structure	Indicators & indices	-	Community description
Macrozooplankton comm.	Zooplankton net 500 um	Community structure	Indicators & indices		Community description
Benthic comm.	Bottom grab/corer	Community structure	Indicators & indices	-	Disturbed structure
Benthic comm.	Bottom grab/corer	Abundance and biomass	Abundance and biomass	-	Anomalous growth
Near-bottom & surface water	Water sampler	Algal toxins/water	Nodularin/hepatotoxins	*Nodularia spumigena*	Increase in natural toxins
Near-bottom & surface water	Water sampler	Algal toxins/particulates	Nodularin/hepatotoxins	*Nodularia spumigena*	Increase in natural toxins
Sediment surface layer	Bottom grab/corer	Algal toxins	Nodularin/hepatotoxins	*Nodularia spumigena*	Increase in natural toxins
Sediment surface layer	Bottom grab/corer	Heavy metals	Cu, Zn, Cd, Hg, Pb	-	Anthropog. contamination
Sediment surface layer	Bottom grab/corer	Bioassays	Amphipod tests (2)	*Corophium, Gmelinoides*	Anthropog. contamination
Benthic organisms	Bottom grab/corer	Heavy metals	Cu, Zn, Cd, Hg, Pb	*Macoma balthica*	Anthropog. contamination
Benthic organisms	Bottom grab/corer	PAH compounds	16 priority list	*Macoma balthica*	Anthropog. contamination
Benthic organisms	Bottom grab/corer	Organochlorine compounds	PCBs, DDTs	*Macoma balthica*	Anthropog. contamination
Benthic organisms	Bottom grab/corer	Butyltins	TBT, DBT, MBT	*Macoma balthica*	Anthropog. contamination
Macrozooplankton	Zooplankton net 500 μm	Biomarkers	Oxidative stress (6 param.)	*Limnocalanus*	Anthropog. contamination
Benthic organisms	Bottom grab/corer	Biomarkers	Oxidative stress (5 param.)	*Macoma balthica*	Anthropog. contamination
Benthic organisms	Bottom grab/corer	Biomarkers	Neurotoxicity: AChE	*Macoma balthica*	Anthropog. contamination
Benthic organisms	Bottom grab/corer	Biomarkers	Genotoxicity: MN	*Macoma balthica*	Anthropog. contamination
Fish	Trawling	Population data	Abundance	Pelagic: herring	Reduction in stock
Fish	Trawling	Population data	Abundance	Benthic: flounder, eelpout	Reduction in stock
Fish	Trawling	Community data	Abundance	All species	Disturbed structure

Sampling type	Sampling device	Parameter	Specific parameter	Species	Function or process
Fish	Trawling	Algal toxins/liver & muscle	Nodularin/hepatotoxins	Pelagic: herring	Increase in natural toxins
Fish	Trawling	Algal toxins/liver & muscle	Nodularin/hepatotoxins	Benthic: flounder, eelpout	Increase in natural toxins
Fish	Trawling	Diseases, parasites, histopath.	Various parameters	Pelagic: herring	Anthropog. contamination
Fish	Trawling	Diseases, parasites, histopath.	Various parameters	Benthic: flounder, eelpout	Anthropog. contamination
Fish	Trawling	Biomarkers	Oxidative stress (5 param.)	Pelagic: herring	Anthropog. contamination
Fish	Trawling	Biomarkers	Oxidative stress (5 param.)	Benthic: flounder, eelpout	Anthropog. contamination
Fish	Trawling	Biomarkers	Neurotoxicity: AChE	Pelagic: herring	Anthropog. contamination
Fish	Trawling	Biomarkers	Neurotoxicity: AChE	Benthic: flounder, eelpout	Anthropog. contamination
Fish	Trawling	Biomarkers	Genotoxicity: MN	Pelagic: herring	Anthropog. contamination
Fish	Trawling	Biomarkers	Genotoxicity: MN	Benthic: flounder, eelpout	Anthropog. contamination
Fish	Trawling	Biomarkers	General stress: LMS	Pelagic: herring	Anthropog. contamination
Fish	Trawling	Biomarkers	General stress: LMS	Benthic: flounder, eelpout	Anthropog. contamination
Fish	Trawling	Biomarkers	PAH exposure: PAH metabol.	Pelagic: herring	Anthropog. contamination
Fish	Trawling	Biomarkers	PAH exposure: PAH metabol.	Benthic: flounder, eelpout	Anthropog. contamination
Fish	Trawling	Biomarkers	Immunocompetence	Pelagic: herring	Anthropog. contamination
Fish	Trawling	Biomarkers	Immunocompetence	Benthic: flounder, eelpout	Anthropog. contamination

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

Ketil Hylland (NO) provided an overview and brief update on progress with the ICON (Integrated Assessment of Contaminant Impacts on the North Sea) project. The steering group for the project is Ketil Hylland (Chair)[Norway], Thomas Lang [Germany], Alistair McIntosh and Matt Gubbins [Scotland], Dick Vethaak [Netherlands], John Thain [England], Jörundur Svavarsson [Iceland].

The main objective of ICON, a practical workshop, is to provide a demonstration programme for the framework developed through the OSPAR/ICES WKIMON process (integrated chemical and biological monitoring). In addition the programme will allow the assessment of effects of contaminants over a range of North Sea, Icelandic and Mediterranean habitats and provide the opportunity to develop research topics and improve the underpinning science. The project was initiated by a kick-off meeting spring 2007: subsequently, samples have been collected during cruises and sampling campaigns in 2008 (all offshore locations, some inshore) and 2009 (additional inshore locations, including Iceland and UK). Locations to be included cover both coastal and offshore areas in the North Sea, Iceland and Mediterranean (figure 5.2.1).

Figure 5.2.1. Overview of locations to be included in ICON (stars).

Samples collected in 2008 have been distributed to participating laboratories across Europe for processing and analysis. These include dab samples from 10 locations, haddock from 4 locations and flounder from six locations. Further samples of flounder and mussels were taken from Iceland and the UK in 2009. The samples taken in Iceland were taken to determine "background responses"; Iceland is regarded as a pristine environment.

Coordination of the data and its assessment is crucial to the programme and in order to facilitate this process a central database has been established by the steering group at the University of Oslo (contact Ketil Hylland email: k.d.e.hylland@bio.uio.no).

Analysis of samples is ongoing and is due to be completed by August 2010. Each expert laboratory will assess its own data. Once all data has been submitted to the University of Oslo database the integrated assessment of the data will commence along the lines suggested by the OSPAR integrated approach or as deemed appropriate by the steering group.

It is anticipated that the integrated assessment will be completed in early autumn to enable a wrap-up conference to be held in November 2010 and subsequently to publish the outcomes in the open literature in 2011. ICON will also communicate the results of the programme to OSPAR and ICES in early 2011 in order to fulfil its obligation of running a demonstration programme on integrated chemical-biological effects as requested by OSPAR SIME in 2007.

In 2009 a poster was presented on the ICON programme at SETAC and a one page contribution was included in the OSPAR QSR 2010. In addition, a presentation will be made at the ICES ASC, Nantes, in the theme session (F) on monitoring of biological effects and contaminants.

6 Extending marine assessment and monitoring framework used in Chapter 10 of the QSR 2010 (OSPAR request 2010/1) - To review the methodology used by the OSPAR workshop on the development of Chapter 11 of the QSR 2010 (Utrecht workshop); (ToR j).

The working group considered the OSPAR report on the "biodiversity assessment workshop for the QSR 2010 ("Chapter 11" regional assessments)". The workshop, held in Utrecht in February 2009, comprised 66 experts in different disciplines of marine science. The work during the workshop was divided into 8 groups, each with a Chair and Rapporteur, i.e. seabirds, cetaceans, seals, fish, rock and biogenic reef habitats (0–200 m depth), shallow sediment habitats (0–50 m depth), shelf sediment habitats (50–200 m depth), deep-sea habitats (>200 m depth). The aims for the workshop were very ambitious, i.e. (i) assess the quality status of the marine environment in each OSPAR Region, as represented by selected ecosystem components, (ii) assess trends since the QSR 2000 and provide an outlook on likely future trends (next 20 years), (iii) rank the pressures from human activities, based on their impact on the marine environment, (iv) identify priorities for future assessment, monitoring and management measures, recognizing the need for indicator development under the MSFD for the GES descriptors and any limitations in the data available. Although one would accept a large degree of uncertainty in any such assessment, this was clearly a process in which it would be equally important to indicate lack of knowledge as definite conclusions.

The outcome of the workshop will necessarily reflect the areas of expertise represented by its participants. This is would particularly be the case when the main

methodology is expert judgement, as in the present case. WGBEC was surprised that the workshop organisers had not seen it useful to include experts that are active in ICES and OSPAR working groups on effects of contaminants in marine ecosystems. The output from the workshop appeared to reflect a lack of sufficient scientific basis in marine ecotoxicology. Potential risks associated with the presence of contaminants in the selected compartments was generally evaluated as being low by the workshop, with worst case scenarios for hazardous substances limited to the effect of TBT on gastropods, PCB contamination in seals and effects on seabirds following the Prestige oil spill.

There were serious shortcomings to the methodology of the workshop and its conclusions. In addition to issues relating to a lack of ecotoxicological competence, the workshop decided not to include pelagic ecosystems and processes, thereby excluding the main marine primary producers of the oceans and the organisms that form the basis for most marine food webs (phyto- and zooplankton).

The workshop concluded that there is low risk from contaminants in cetaceans, seabirds and seals. The working group cannot see that this conclusion can be supported by current knowledge in any way. It is well known that populations of seabirds, toothed whales and seals in many of the regions included in the report have sufficiently high concentrations of a range of contaminants for there to be health impacts and such effects have indeed been shown (see e.g. Bustnes, 2006; De Swart *et al*., 1994; Hall *et al*., 2006; Reijnders *et al*., 1986; Ross *et al*., 1996).

The workshop identified harbour sediments as a worst case situation for shallow water sediments due to effects from TBT on gastropods. This is probably the case although the issue with TBT could probably be extended to include larger parts of coastal areas and some estuaries and fjords. In addition, there are a range of other contaminants in many harbours that could be expected to cause effects on sediment-dwelling organisms, e.g. PAHs, chlorinated POPs and metals.

It appears that the participants themselves were not entirely comfortable with the outcome and that there was a pressure to "produce" data even though the required information may have been lacking.

It was noted that the workshop considered that this type of broader ecosystem assessment could be a useful contribution to an Initial Assessment for the Marine Strategy Framework Directive (MSFD) in 2012. WGBEC would be concerned if this were the case without further underpinning science and engagement with experts in the specific scientific fields.

To conclude, the working group found the report to reflect an insufficient insight into levels and effects of contaminants in marine compartments and its conclusions should not in any way be used in future processes.

References

Bustnes, J. 2006. Journal of Toxicology and Environmental Health, Part A: 69(1–2).

De Swart *et al*., 1994; Environ Health Perspect, 104: 823–828.

Hall *et al*., 2006; Environ Health Persp., 114: 704-711.

Reijnders, 1986. Nature, 324: 456–7.

Ross *et al*. 1996. Aquat Tox., 34: 71–84.

7 Report to SSGHIE on potential and current contributions of your EG to the Strategic Initiative on Coastal and Marine Spatial Planning (SICMSP); (ToR k)

The WG considered the draft strategy document on "Area-based science and management of marine ecosystems: from the coast to the high seas". The activity links up to the ICES mission statement "*To advance the scientific capacity to give advice on human activities affecting, and affected by, marine ecosystems*". This is an ongoing process and a comprehensive review of the draft document would not be appropriate, but the WG would like to emphasise that contaminant inputs and their effects in marine ecosystems are highly relevant to the ICES mission statement and a necessary component of marine ecosystem management. There are specific challenges associated with contaminants in terms of area-based management since contaminants and their effects may be associated with large areas. Three specific examples directly relevant to area-based management are point sources of contaminants (i.e. industry, offshore platforms, and rivers), diffuse sources (e.g. harbours, urban areas) and contaminants in sediments.

8 Report to SSGHIE on your plans to promote cooperation between EGs covering similar scientific issues; n (ToR l)

As an introduction the Chair, John Thain gave a presentation on the new ICES structure, the ICES Vision, Science Plan, Strategic Initiative and the role of EGs within the new SCICOM Steering Groups. WGBEC sits within the Steering Group on Human Interactions on Ecosystems (SGHIE), Chair: Erik Olsen (NO)

8.1 WGBEC core activities and future directions

After intensive discussions on the scope and future activities of the WG, and taken consideration of the various ICES vision and strategy documents, WGBEC identified the core business and 8 areas for future directions. While some of these activities are considered as core business, others clearly attend to broaden the scope of WGBEC, towards more ecology and wider ecosystem effects.

8.1.1 WGBEC core business

1) Development of strategies for biological effects in integrated monitoring and assessment and provide advice on appropriate methods for monitoring;

2) The role of biological effects techniques in environmental risk assessment;

3) Increase fundamental understanding of ecotoxicological processes;

4) Provide advice on effects of novel / emerging compounds;

5) Facilitate harmonisation and AQC concerning biological effects methods;

6) Improve understanding on how and whether contaminants in the marine environment interacts with other environmental factors and processes;

7) Improve ecosystem-oriented understanding of how contaminants affect marine systems and processes;

8) Initiate transnational cooperative research and monitoring (e.g. BECPELAG, ICON, BEQUALM);

9) Provide guidance to international organisations / conventions as required and agreed by ICES (OSPAR, HELCOM, AMAP).

Research needs required for the implementation of MSFD are not yet available but may be relevant. International cooperative research / monitoring areas research proposals should be initiated. This has successfully been done in the past, e.g. BECPELAG, ICON, BONUS+, BEAST and MEDPOL.

8.1.2 New future directions identified for WGBEC

8.1.2.1 Impacts of contaminants on food webs and ecosystem function / processes:

Continued attention should be given to top predators such as marine mammals, but also sea birds. Special emphasis should be placed on lower levels of the trophic food web, such as the impact of contaminants on benthic, pelagic algae and microbial populations and communities and their potential impact on carrying capacity of marine and coastal waters. Over the long term, knowledge of lower food web population and community effects can also result in new indicators to be included as additional components for integrated monitoring and assessment. It was pointed out that this type of research is very challenging due to complexity / diversity of plankton and that it will require experimental work. There are also clear interactions with eutrophication. This type of research needs modelling and energy budgeting.

8.1.2.2 Development of bioassays and/or biomarkers for detecting and determining the effects of contaminants on the immunocompetence and fitness of organisms

This seems particularly relevant to clarify the contributing role of contaminants in the recently observed epizootics in marine mammals and fish.

8.1.2.3 Ecogenetics.

There is increasing knowledge on the effects of contaminants on population genetics and for example antibacterial resistance development. So far WGBEC only considered this research field rarely, but this will deserve more attention in the future.

8.1.2.4 Mixture of toxicity and interactions with natural factors should receive increasing attention

This is a very challenging field of research, but essential to clarify the role of contaminants in cumulative stress impact assessments.

8.1.2.5 More focus on modelling fate of contaminants and effects

Most models are lacking an effect module on top of fate modelling. WGBEC could play a contributing role here. This should also include increased effort on expert system modelling for biomarkers based on data collected all around Europe. Such an approach was done in the late 90's but failed due to shortage of suitable data. Hence it will be particularly worthwhile to revisit the expert system approach.

8.1.2.6 Genomics / proteomics / metabolomics

Already regularly on the WGBEC agenda, this area will require increasing attention and effort. In the future the technology will make this easier and there will be much work in applying this technology in monitoring and assessment approaches.

8.1.2.7 Climate change including ocean acidification

The WG already conducted some work on the effects of climate change on ecotoxicological processes and environmental quality issues. Future work should also include the changes of PH on the bioavailability, uptake and other ecotoxicological processes.

8.1.2.8 Plastic particles - (addressed here under agenda item 14)

The WG envisaged this increasing environmental problem as a particular urgent area for future direction, given its potential impact on food chain energetics, food web transfer of contaminants, and increased risk for contaminant exposure and effects. The influence of plastic particle presence in sediments and their confounding effects on chemical and bioassay analysis results should be assessed.

A possible mechanism to widen the WG science basis is to yearly invite non-member experts that can add value and broaden scope. There is also a clear need to collaborate with WGs in other areas (see Table 8.3.1 below).

8.2 WGBEC activities relevant to ICES Science plan

WGBEC viewed the Science Plan and noted that sixteen research topics have been identified as being of strategic importance to the advisory needs of ICES Member Countries and clients in the coming decade. These topics have been organized in three thematic areas.

1) Understanding Ecosystem Functioning

2) Understanding Interactions of Human Activities with Ecosystems

3) Development of options for sustainable use of ecosystems

The sixteen research topics were discussed and activities relating to WGBEC activities were noted as follows:

8.2.1 Understanding ecosystem functioning

The WG has provided advice on possible effects of climate change, an activity which led to the production of a paper (Schiedek *et al*. 2007). The group has planned activities on ocean acidification. Members of the group are involved in studies on impacts modelling based on contaminant loading and climate change models.

There are links between contaminant-related responses and marine biodiversity. This is an area WGBEC wishes to invest more time for in the years to come. Some contaminant-related methods will be relevant to predict local population declines or even extinction.

Contaminant effects are highly relevant for top predators of marine ecosystems and need to be included. There is evidence of contaminant effects on marine birds and mammals (see e.g. Bustnes, 2006; De Swart *et al*., 1994; Hall *et al*., 2006; Reijnders *et al*., 1986; Ross *et al*., 1996).

The WG has ongoing work with sensitive areas and ecosystems, e.g. contaminants in the Arctic.

8.2.2 Understanding interactions of human activities with ecosystems

This thematic area is at the basis of WGBECs activities. Trawling will increase resuspension resulting in increased contaminant availability in areas with elevated concentration of contaminants in sediments. WGBEC members are involved in projects addressing contaminant inputs from aquaculture, which is relevant to mariculture carrying capacity. WGBEC has a range of activities relevant to understanding contaminant impacts on populations and communities. This theme is within the core activity of the working group.

8.2.3 Development of options for sustainable use of ecosystems

There is no direct link between the work of the group and living resource management tools, but WGBEC is interested and has appropriate connections to modelling work in this area. Impacts of oil spills may benefit from modelling toxic effects.

There is a potential impact of development activities (as a result of MSP) on contaminant loading and there is a need to look at socioeconomic impacts of contaminant effects in future.

8.3 Collaboration with other expert groups

The WG has a history of collaboration with a range of other ICES EGs as well as MEDPOL and HELCOM (Table 8.3.1).

Table 8.3.1. Overview of EGs with which WGBEC has had collaboration of with which WGBEC would envisage possible future interactions.

Expert Groups	Worked before?	Interested in joint activity?	Joint meeting?
WGPDMO	Yes	Yes	Yes
MCWG	Yes	Yes	Potential
MSWG	Yes	Yes	Potential
ICZM	No	Potential	No
SGONS	No	No	No
WGMASC	No	No	No
WGEIM	No	Yes	Potential
WGHABD	No	Potential	No
WGEXT	No	No	No
WGFCCIFS	No	No	No
WGAGFM	Yes	Yes	Potential
WGEEL	No	Yes	Potential
WGMME	No	Yes	No
SGIMC	Yes	Yes	No
SGEH	No	Yes	Potential
MEDPOL	Yes	Yes	Yes

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

Many common activities, e.g. interaction between contaminants and disease. Joint efforts to develop integrated monitoring and assessment. Scope for future development of immunotoxicological end points.

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

Several areas of common interest, e.g. passive sampling and TIE.

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

Have worked together in the past on developing concepts for sediment bioavailability and there is a current need to develop common projects on passive sampling. There is a large overlap concerning the integrated monitoring strategy.

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

Contaminants are important in ICZM, but there has been little direct contact until now.

Working Group on Environmental Interactions of Mariculture (WGEIM)

Common ground with contaminant discharges from finfish farms and environmental interactions.

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

Some overlap on toxicological effects and interactions of HAB toxins on toxicological endpoints.

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

Interest from WGBEC on methods they use and potential applicability to field of toxicogenomics / population genetic effects from contaminants.

Joint EIFAC/ICES Working Group on Eels (WGEEL)

Interested in contaminant effects in eels and future joint activity.

Study Group in Integrated Monitoring of Contaminants and Biological Effects (SGIMC)

Already closely working in this area. WGBEC output feeding into SGIMC in Jan 2010.

Study Group for the Development of Integrated Monitoring and Assessment of Ecosystem Health in the Baltic Sea (SGEH)

Reports from SGEH members received by WGBEC at past meetings. Much overlap, need to coordinate work between SG and WG more.

MEDPOL monitoring group

Not ICES, but important to harmonise activities here. Common ground on AQC and integrated strategies. Joint workshops already planned.

Working Group on Marine Habitat Mapping (WGMME)

WGBEC believes contaminants in marine mammals play an important role and there is potential interaction in this field between the groups.

The Chair will report back to SSGHIE for comment and investigate how some of these activities may be taken forward.

References

Bustnes, J. 2006. Journal of Toxicology and Environmental Health, Part A, 69: 1–2.

De Swart *et al*., 1994. Environ Health Perspect., 104: 823–828.

Hall *et al*. 2006. Environ Health Persp., 114: 704–711.

Reijnders, 1986. Nature, 324: 456–7.

Ross *et al*. 1996. Aquat Tox., 34: 71–84.

Schiedek, D., Sundelin, B., Readman, J., and Macdonald, R. W. 2007; Mar. Pollut. Bull., 54: 1845–1856.

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

The WGBEC discussed the 'promising' and 'recommended' monitoring techniques that had been last updated in 2007.

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 and which national programmes are currently using them. Methods should be used as part of an integrated package c.f. SGIMC / WKIMON integrated framework. (See SGIMC 2010 report, (annex 16 and 17) www.ices.dk)

During the 2010 meeting the WGBEC confirmed that recommended methods for monitoring programmes should conform to the following criteria:

1) A recommended method needs to be an established technique that is available as a published method, preferably in the TIMES series.

2) A recommended method (or combination of methods) should have been shown to respond to contaminant exposure in the field.

3) 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. Tables 9.1 and 9.2 have been edited to include a direct reference to the ICES Techniques in Marine Environmental Sciences publication (where available) and also included information relating to those countries where specific techniques are currently in use and those international monitoring programmes where their use is proposed.

Changes to the tables

Induction/inhibition of multidrug/multixenobiotic resistance (MDR/MXR) in *Mytilus* species was removed from Table 9.1b (Recommended techniques for biological monitoring programmes at the national or international level - methods for invertebrates) and grouped with MDR/MXR detection methods in fish and invertebrates in Table 9.2. (Promising biological effects monitoring methods that require further research before they can be recommended for monitoring both fish and invertebrates). This was due to the fact that the method specific to *Mytilus* lacked an ICES TIMES Series document, was only used in a limited number of laboratories had no current AQC programme in place.

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 and micronucleus assay will be developed intersessionally and updates presented at the 2011 meeting to allow an assessment of their suitability for recommendation to be made.

Other points arising

Issues were raised concerning the current status of some of the ICES Times documents. For example, certain biomarkers including CYP1A analysis and Metal-

lothionein induction are now routinely measured using qPCR techniques. The working group discussed producing a general set of recommended guidelines for using molecular techniques in the determination of gene expression levels.

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

Method	Organism	AQC	ICES TIMES	Issues addressed	Biological significance	Current National active use	International programme	Ref.
Bulky DNA adduct formation	Fish	No current AQC programme.	No. 25	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.	N, SE, UK* *(ad hoc only)	WKIMON	1-6
AChE inhibition	Fish	No current AQC programme.	No. 22	Organophosphates and carbamates or similar molecules	Measures exposure.	F, N. UK, E, IRE	BEAST WKIMON	7-10
Metallothionein induction	Fish	No current AQC programme.	No. 26	Measures induction of metallothionein protein by certain metals (e.g., Zn, Cu, Cd, Hg)	Measures exposure and disturbance of copper and zinc metabolism.	UK, N, S, E, IRE	MEDPOL,	11-15
EROD or P4501A induction	Fish	B (last run 2009)	No. 23 and No. 14	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.	UK, B, N, S, F, E, IRE	MEDPOL, WKIMON	16-23
ALA-D inhibition	Fish	No current AQC programme.	No. 34	Lead	Index of exposure.	N, E* *(ad hoc only)		24-25
PAH bile metabolites	Fish	No current AQC programme.	No. 39	PAHs	Measures exposure to and metabolism of PAHs.	IRE, UK, N, NE, B, D	WKIMON, BEAST	26-27
Lysosomal stability using histochemical detection	Fish	IMARE workshop		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.	D, more expected to take it up after 2008 IMARE workshops	WKIMON, BEAST	28-31

Method	Organism	AQC	ICES TIMES	Issues addressed	Biological significance	Current National active use	International programme	Ref.
Early toxicopathic lesions, pre-neoplastic and neoplastic liver lesions by and histopathology	Fish	B (last run 2009)	No. 38	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.	UK, D, NE, IRE	WKIMON, BEAST	32 - 42
External visible lesions and parasites	Limanda limanda, Platichthys flesus, Gadus morhua	B (last run 2009)	No. 19	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.	UK, NE, GER, F	WKIMON, BEAST	43-44
Vitellogenin induction	Male and juvenile fish	No current AQC programme.	No. 31	Oestrogenic substances	Measures feminization of male fish and reproductive impairment.	IRE, UK, NO, D* *(Ad hoc)	WKIMON	45-48
Intersex	Male flounder,eelpout, dab	No current AQC programme.	In prep.	Oestrogenic substances	Measures feminization of male fish and reproductive impairment.	IRE , D, UK	BEAST, WKIMON	49-50
Reproductive success in Zoarces viviparus	Zoarces viviparous	No current AQC programme.			Measures reproductive output and survival of eggs and fry in relation to contaminants. Restricted to period when young are carried by female viviparous fish.	SE, D, DE	BEAST	51
Alkylphenol- bile metabolites	Fish (cod)	No current AQC programme	In press	Alkyl phenols	Measures exposure to and metabolism of Alkylated phenols	NO	WKIMON	Awaiting publications

B: BEQUALM; Q: QUASIMEME. ICES TIMES: http://www.ices.dk/products/techniques.asp.

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

METHOD	ORGANISM	QA	ICES TIMES	ISSUE ADDRESSED	BIOLOGICAL SIGNIFICANCE	NATIONAL	INTERNATIONAL	REF
AChE inhibition	Molluscs and crustaceans	No current AQC programme.	No. 22	Organophosphates and carbamates or similar molecules Possibly algal toxins	Measures exposure to a wide range of compounds and a marker of stress.	E, F, IRE, UK* *(ad hoc)	WKIMON, MEDPOL, BEAST	52-53
Metallothionein induction	Mytilus	Programme run under MEDPOL in 2009.		Measures induction of metallothionein protein by certain metals (e.g., Zn, Cu, Cd, Hg)	Measures exposure and disturbance of copper and zinc metabolism.	E, UK, IRE	MEDPOL	54-55
Lysosomal stability (including NRR)	Mytilus. Oyster	MEDPOL training workshop 2010 ring trial 2011.	No. 36	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.	IT, IRE, UK, N, NE, IS	WKIMON, MEDPOL, BEAST	56- 70
Scope for growth	Bivalve molluscs, e.g.,Mytilus spp. and oysters	No current AQC programme.	No. 40	Responds to a wide variety of contaminants	Integrative response, a sensitive sub-lethal measure of energy available for growth.	IRE *, E*, UK*, IS* (*Ad hoc only)		71-72, 148
Imposex	Neogastropod molluscs (Nucella lapillus, Buccinum undatum, Hinia reticulata, Neptunea antiqua)	Q	No. 24 (N. lapillus)	Specific to organotins	Reproductive interference Estuarine and coastal littoral waters (Nucella) and offshore waters (Buccinum).	IRE, E, FR, UK, IRE, HE, DK, N		73-82
Intersex	Littorina littorea	Q	No. 37	Specific to reproductive effects of organotins	Reproductive interference in coastal (littoral) waters.	NE, (Ad hoc as replacement for Nucella)		83

Histopathology	Blue mussels	Cefas run histopathology workshop (2010)	In prep.	Not contaminant-specific	General responses	UK, IRE, NO, D, E, FR, I	MEDPOL, WKIMON	87 - 89
Embryo aberrations in field-collected amphipod crustaceans	Amphipods	No current AQC programme.	No. 41	Contaminant-specific	Measures frequency of different types of lethal embryo aberrations; allows for separating effects of contaminants and environmental climate variables	SE	BEAST	90 - 94

B: BEQUALM; Q: QUASIMEME. ICES TIMES: http://www.ices.dk/products/techniques.asp

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

METHOD	ORGANISM	ICES TIMES	QA	ISSUE ADDRESSED	BIOLOGICAL SIGNIFICANCE	NATIONAL	PROGRAMMES	REFERENCES
Benthic community analysis	Macro-, meio-, and epibenthos		B (low uptake outside UK)	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.	B, UK, N, NE, E, F, IRE	Coastal waters driven under WFD	95 – 100
Whole sediment bioassays	Corophium (problems with stocks i.e. look to get standards) Arenicola, Ampelisca brevicornis Other species may be used.	No. 29 (Arenicola) No. 28 (Corophium)	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	UK, E, NE, N, IRE, I	WKIMON MEDPOL, BEAST	101 – 102

Bioassays of sediment pore waters, sea water elutriates, sea water samples, extracts	Bivalve embryo Acartia, Sea urchin embryos, tisbe	No. 11 (Oyster embryo). Sea urchin in prep.	No current AQC programme.	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.	UK, NE, N, DE, D, F, E, I, IRE	WKIMON, MEDPOL	103 – 104
CALUX	Reporter gene assay		MODELKEY	Ah receptor-active compounds	Predictor of dioxin like toxicity	NE, UK, N, F* *(ad hoc)		105
YES	Reporter gene assay (yeast)		MODELKEY	Oestrogen receptor-active compounds	Potential endocrine disruption	UK, N, NE, IRE		106 – 107
YAS	Reporter gene assay (yeast)		MODELKEY	Androgen receptor-active compounds	Potential endocrine disruption	UK, N, NE		108 – 109

B: BEQUALM; Q: QUASIMEME. ICES TIMES: http://www.ices.dk/products/techniques.asp

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
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.	110 –112
BaP Hydroxylase -like enzymes	Invertebrates	Induced enzyme response to PAHs, planar PCBs, dioxins and/or furans	Measures exposure to organic contaminants.	113 – 114
Induction/inhibition of Multidrug/multixenobiotic resistance (MDR/MXR)	Fish and invertebrates including Mytilus	Multiple contaminants (organics and metals)	Adaptation/inhibition in response to xenobiotic stress.	84 – 86, 115 – 119.
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	120 – 122
Oxidative stress	Fish, invertebrates	Not contaminant-specific, will respond to a wide range of environmental contaminants	Measures the presence of free radicals.	123 – 126

Immunocompetence	Fish, invertebrates	Not contaminant-specific, will respond to a wide range of environmental contaminants	Measures factors that influence susceptibility to disease.	127
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.	128
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.	129 – 130
Bulky DNA adduct formation	Mussels, invertebrates	PAHs, other synthetic organics	Measures genotoxic effects	131 – 134
Gene arrays	Fish, mussels	Various	Combined responses from various biomarkers	135 – 137
Histopathology	Invertebrates (other than Mytilus)	Not contaminant-specific	General responses	Awaiting publications
Spiggin	Three-spined stickleback	Androgens	Measures environmental androgens	138
Micronuclei	Fish, bivalve molluscs	Not contaminant-specific	Exposure to aneugenic and clastogenic	139 - 141
Peroxisomal proliferation (enzyme assays)	Fish and invertebrates	Contaminant-specific	Potential alterations in lipid metabolism, non-genotoxic carcinogenesis	142 -144
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	145

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.	146
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.	147-148

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

References Agenda Item 9

1) Reichert, W. L., French, B. L., andStein, J. E. 1999. Biological effects of contaminants: Measurements of DNA adducts in fish by 32P-postlabelling. ICES Techniques in Marine Environmental Sciences. No. 25.

2) Varanasi, U., Reichert, W. L., and Stein, J. E. 1989. 32P-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. 32P-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. 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) Hylland, K. 2004. Biological effects of contaminants: Quantification of d-aminolevulinic acid dehydratase (ALA-D) activity in fish blood. ICES Techniques in Marine Environmental Sciences. 9 pp.

26) Ariese, F., Beyer, J., Jonsson, G., Visa, C.P., Krahn, and M.M. 2005. Review of analytical methods for determining metabolites of polycyclic aromatic compounds (PACs) in fish bile. 41 pp.

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) Stentiford G.D., Bignell J.P., Lyons B.P., Feist S.W. (2009) Site-specific disease profiles in fish and their use in environmental monitoring. Mar Ecol Prog Ser., 381: 1–15.

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. 42pp

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. & 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) Smith, A.J., Thain, J.E., and Barry, J. 2006. Exploring the use of caged *Nucella lapillus* to monitor changes to TBT hotspot areas: A trial in the River Tyne estuary (UK). Marine Environmental Research 62 (2), pp. 149–163.

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

85) 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 system in the Pacific oyster *Crassostrea gigas*. Marine Environmental Research, 54: 455–459.

86) 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.

87) Bignell, J.P., Dodge, M.J., Feist, S.W., Lyons, B.P., Martin, P.D., Taylor, N.G.H., Stone, D., Travalent, L., and Stentiford, G.D. 2008. Mussel histopathology: effects of season, disease and species. Aquatic Biology Vol.2: 1–15, 2008.

88) 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.

89) 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.

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

91) 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.

92) 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.

93) 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.

94) 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.

95) 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.

96) 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.

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

98) 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.

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

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

101)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.

102)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.

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

104)Beiras, R., Fernández, N., Bellas, J., Besada, V., González-Quijano, adnA.Y nunes, T. 2003 Integrative assessment of marine pollution in Galician estuaries using sediment chemistry, mussel bioaccumulation, and embryolarval toxicity bioassays. Chemosphere, 52: 1209–1224.

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)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.

107)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.

108)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.

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

110)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.

111)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.

112)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. An-

nual 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.

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

114)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.

115)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.

116)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.

117)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.

118)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.

119)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.

120)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.

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

122)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.

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

124)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.

125)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.

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

127)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.

128)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.

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

130)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.

131)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.

132)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.

133)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.

134)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.

135)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.

136)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.

137)Geoghegan F., Katsiadaki I., Williams T.D., Chipman J.K (2008). A cDNA microarray for the three-spined stickleback, Gasterosteus aculeatus L., and analysis of the interactive effects of oestradiol and dibenzanthracene exposures (2008) Journal of Fish Biology, 72 (9), pp. 2133-2153.

138)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.

139)Grisolia CK and Cordeiro CMT (2000) Variability in micronucleus induction with different mutagens applied to several species of fish. Genetics and Molecular Biology 23(1):235-239.

140)Izquierdo, J.I., Machado, G. Ayllon, F., d'Amico, V.L. Bala, L.O. Vallarino, E. Elias, R., Garcia-Vazquez, E. (2003). Assessing pollution in coastal ecosystems: a preliminary survey using the micronucleus test in the mussel Mytilus edulis, Ecotoxicology and Environmental Safety, 55, pp. 24–29.

141)Rybakovas, A., Baršiene, J., Lang, T. (2009). Environmental genotoxicity and cytotoxicity in the offshore zones of the Baltic and the North Seas. Marine Environmental Research 68 (5), pp. 246-256.

142)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.

143)Cancio I, Ibabe A, Cajaraville MP (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.

144)Orbea A, Cajaraville MP (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.

145)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.

146)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.

147)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.

148) 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.

149) J. Widdows, P. Donkin, F. J. Staff, P. Matthiessen, R. J. Law, Y. T. Allen, J. E. Thain, C. R. Allchin and B. R. Jones (2002). Measurement of stress effects (scope for growth) and contaminant levels in mussels (*Mytilus edulis*) collected from the Irish Sea. Marine Environmental Research 53(4): 327–356.

10 In close cooperation with ICES / OSPAR SGIMC conduct intersessional work for review at 2010 meeting based on the outcome of the SGIMC Aberdeen Workshop, October 2009.; (ToR e).

10.1 SGIMC work update

Dick Vethaak (NL) presented the outcome of the ICES/OSPAR Workshop on Assessment Criteria for Biological Effects Measurements (SKIMC) held in UK from 14 – 16 October 2009. The workshop focused on assessment criteria for PAH-related effect measurements and how they can be used in an integrated way. The following tasks were completed:

a) Review and updating of OSPAR Background Documents on a range of biological effects measurements.

b) Review and confirmation of assessment criteria for biological effects measurements, and development of new assessment criteria for a range of effects.

c) Elaboration of an integrated scheme for the assessment of biological effects and environmental chemistry data for use in environmental quality assessment.

d) Updating of the forward work programme for SGIMC, and for cooperation with WGBEC.

The output of the workshop will be reviewed by SGIMC 2010 and intermediate comments from WGBEC would be very welcomed. The work should result in proposals for adoption of assessment criteria formulated for adoption by OSPAR through ASMO. At this stage a complete draft report was not available, but 3 tables representing the progress made by the Workshop were presented and discussed, viz: Table A on progress on assessment criteria; Table B update of OSPAR Background Documents; and Table C proposed work programme for SGIMC from January 2009 to January 2011.

WGBEC appreciated the progress made in the workshop and emphasised the importance of this work in relation to the integrated monitoring approach by OSPAR and the implementation process of monitoring for the MSFD.

In relation to Table A the following remarks were made. For DNA adducts, there was limited amount of assessed data and some uncertainty about the chosen reference site. Halldóra Skarphéðinsdóttir(SE) will provide new data to SGIMC which can then be used for adjustment of the assessment criteria. Further it was noted that the range of biological effects measurements presented in Table A were not all included in the integrated approach. This particularly seems to be the case for the types of bioassays that have to be used for the sediment component in the integrated scheme. WGBEC suggested to ask SGIMC to recap the integrated approach to see if the appropriate biomarkers and bioassays have been taken into account. DV will take this action on board of WGIMC2010.

The OSPAR background document on biological effects techniques with proposed changes was made available to the WGBEC participants and Ricardo Beiras (ES) agreed to provide additional changes for bioassay chapters 8, 9, 10 for consideration by SGIMC later this month.

In relation to Table C; the status of actions to be delivered by WGBEC and to be fed back to SGIMC were:

- Extraction protocol for bioassays: Times series manuscript not yet finalized but a completed draft will be send to SGIMC2010 (action John Thain)
- VTG: no progress made, but state of the art will be send to SGIMC2010 (task John Thain/Matt Gubbins)
- VTG mRNA: this method will be discussed at WGBEC2011
- Acetylcholinesterase: Background document will be provided for SGIMC 2010,, assessment criteria will follow in March 2010 (action Thierry Burgeot)
- Micronucleus assay and comet assay: Background document has been provided and reviewed by WGBEC and will be made available to SGIMC, assessment criteria are not yet available (action Brett Lyons)
- Bioassays: update of Background documents, see above.

In addition the background document on DNA adducts was reviewed by Halldóra Skarphéðinsdóttir(SE), an expert in this field and revised as appropriate. Halldóra Skarphéðinsdóttir is in the process of revising the assessment criteria for haddock based on background data from Iceland.

The working group further considered the strategy outlined for the integrated assessment of biological effects and concentrations of chemicals in the preliminary report from SKIMC in Aberdeen in October 2009. The suggested strategy would divide biological responses into those indicative of contaminant exposure and those indicative of effects. Each biological effect response and chemistry endpoint would be categorised into "background" (green), "exposed" (yellow) and "possibly deleteriously affected" (red). The division of responses between the three categories would then be summed up for each group of methods/determinands, resulting in a % score for green, yellow and red, respectively. Such scores were then averaged to provide a grand score for each location, thereby including biological effects and chemical determinands in one index.

The WG appreciated the concepts underlying the suggested assessment framework, but had a number of issues with the proposed framework and suggestions for how such an assessment framework may be designed.

Three main questions need to be resolved: (1) the level of aggregation for the range of components in the integrated programme, (2) the choice of quantification for the resulting components (each of which would comprise multiple responses/determinands) – e.g. averages, "one out – all out", (3) how to resolve lacking responses/determinands.

In addition, the group discussed the possibility of selecting a subset of responses to address specific questions, e.g. PAH or oestrogen effects.

(1) Level of Aggregation

As was suggested by WKIMC, the group agreed that biological effects should be divided into early effect, sensitive methods (for which there would be only two categories – background and exposed) and methods that could indicate deleterious effects (for which there would be three categories (background, exposed and affected). With this in mind, the components available in the integrated framework would be the following: mussel – early effects, mussel – deleterious effects, mussel – chemical, fish

– early effects, fish – deleterious effects, fish – chemical, sediment – bioassay, sediment – chemical, gastropod – deleterious effects (only imposex/intersex). There was some discussion in the group as to which methods should be included as representing "deleterious effects" for each of the organism groups. Some members of the group were of the opinion that e.g. lysosomal stability should not be given the same weight as e.g. liver tumours.

The group discussed to which extent the components could be aggregated without losing essential information and ended up with the following suggestion for SGIMC:

A. mussel – early effects

B. mussel – deleterious effects

C. fish – early effects

D. fish – deleterious effects

E. gastropods – effects

F. sediment bioassays

G. levels of chemical determinands in mussel, fish or sediment

Except for E, all the above components would contain more than one measurement. There is therefore a requirement for a mechanism of quantification.

(2) Quantification

The procedure suggested by WGIMC involved a mechanism for averaging out responses within each of the components. WGBEC disagreed with this approach. Alternatives put forward were multivariate techniques, expert systems and "one out – all out" type quantification. The input to this analysis would be categorical, i.e. "green", "yellow" or "red" (the latter only for components B, D, E, F, G) for each of the measurements within each component.

Component E will only have one component (whichever measure of imposex/intersex is used) and there is no need for further aggregation.

The group thought that the approach suggested by WGIMC, i.e. averaging out "greens" and "yellows", could be appropriate for components A and C. If there would be more "yellows" than "greens" this would result in the component being "yellow". Those two components will never become "red", but will provide added information as to the type of chemical stress present.

WGBEC was of the opinion that "one out – all out" would be the most appropriate for components B, D, F & G, i.e. if one of the chemical determinands produced an EAC value producing a "red", a sediment bioassay resulted in "red" or one of the deleterious effects measurements for either fish or mussel produced a "red", this would cause the output to be "red" for this component. Similarly, one "yellow" would cause the index to become "yellow".

There was some discussion of whether an EAC for a chemical or a group of chemicals (G; indicative of a level that may cause effect) should be given the same weight as measured effect (B, D, F), but as long as the components are kept separate this knowledge can be used in the subsequent assessment.

(3) Lacking responses or determinands

Although efforts should clearly be made to design monitoring programmes according to the proposed framework, it may happen that there are problems involved in sampling specific species or samples may be lost in analyses. There will need to be a requirement for a minimum number of methods to be included for B, D, F and G if the assessment is to be valid. Components A and C are more robust towards decreased number of methods included, but lacking methods will weaken the subsequent assessment.

Future development of the assessment framework should address the possibility of using a subset of methods for specific assessments (e.g. PAH, TBT, oestrogens).

In addition to a range of published strategies (MEDPOL: Viarengo, France: Narbonne, Spain: Bilbao, Germany: Broeg, UK: Galloway), HELCOM are currently developing an assessment strategy called CHASE.

Recommendation

WGBEC members have been involved with the ICES/OSPAR WKIMON / SGIMC process and there has been important consultation and support between the two groups over the past few years. WGBEC would recommend continued involvement with the work and strive to complete the revision of the integrated strategy as appropriate and where required by SGIMC for completion in 2011.

10.2 To receive Background Documents and draft assessment criteria from ICES WGBEC on: Acetyl cholinesterase, Mussel histopathology, Micronucleus and Comet assay, MT and ALA-D, and Intersex in fish

Background documents for acetyl cholinesterase, mussel histopathology and intersex in fish and micronucleus were in various stages of draft. Acetyl cholinesterase was almost complete and may be available for the SGIMC 2010 meeting. The MT and ALA-D background documents had been submitted to SGIMC but there was still some work to be done in agreeing assessment criteria. The background document on the COMET assay was presented at the meeting by Brett Lyons (UK).

10.2.1 Background document: Comet assay as a method for assessing DNA damage in aquatic organisms; Author: Brett Lyons (UK)

Background

The analysis of modified or damaged DNA has been shown to be a highly suitable method for assessing exposure to genotoxic contaminants in aquatic environments. In general, the methods developed are sensitive to a range of contaminant concentrations, applicable to a wide range of species and have the advantage of detecting and quantifying exposure to genotoxins without a detailed knowledge of the contaminants present. The Single Cell Gel Electrophoresis (SCGE) or comet assay was first applied to ecotoxicology over 15 years ago, and has since become one of the most widely used tests for detecting DNA strand breaks in aquatic animals[1-5]. The comet assay has many advantages over other methods commonly used to assess genotoxic exposure, including (1) genotoxic damage can be detected in most eukaryotic cell types at the single cell level; (2) only a small number of cells are required; (3) it is a rapid and sensitive technique; (3) Due to the nature of DNA strand break formation it provides an early warning response of genotoxic exposure.

As a consequence of the advantages listed above the comet assay has been used widely in both laboratory and field based studies to assess genotoxic exposure in many freshwater and marine organisms. However, unlike mammalian genotoxicology, where the focus is limited to a small number of model species, efforts in the aquatic field have generally lacked coordination and have used an extensive range of sentinel species[1,3,5]. While guidelines relating to the use of the comet assay have been published for mammalian genotoxicology[6,7], no standard protocols currently exist for environmental studies. Consequently, the variations in protocols can lead to major differences in results and an inability to directly compare studies. Despite these obvious limitations the comet assay provides a well-researched tool for studying genotoxicity in aquatic species.

10.3 Confounding factors: Protocols, cell types and target organs

The majority of aquatic studies published to date have used circulating blood cells (either haemocytes or erythrocytes), as target cells for comet assay analysis. This is likely to be due to the practical advantage of processing tissues from a ready-made supply of nucleated cells in suspension. Solid tissues such as gill or fish hepatocytes require dissociation prior to analysis, with the potential of introducing damage through enzymatic or mechanical processes. Studies have also demonstrated that different cell types responded with different sensitivities to contaminant exposure. When comparing cells types it is usually reported that circulating cells are less sensitive than hepatocytes or gill cells[8-13]. Blood and to a lesser extent the haemolymph of bivalve molluscs (e.g. mussels) are ''buffered'' tissues, in which contaminants arrive having crossed numerous biological barriers. Gill cells appeared to be the most sensitive following MNNG exposure, while liver and digestive gland were more sensitive to B(a)P, suggesting that uptake routes and bioaccumulation mechanisms need to be taken into account when designing experiment systems[12].

Mammalian studies have demonstrated that certain tissue types may have higher background levels of DNA damage due to presence of alkali sensitive sites in cells with highly condensed chromatin[14]. Similar studies comparing basal levels of DNA migration in mussel gill cells, haemocytes and fish erythrocytes under both mild alkaline (pH 12.1) and alkaline versions (pH > 13) of comet assay have supported this assumption[15, 16]. Indicating that the mild alkaline version of the assay should be employed when dealing with certain cell types (e.g. fish erythrocytes), in order to prevent higher background levels of DNA strand breaks inhibiting data interpretation. Indeed, this problem has been highlighted in other studies using fish species where excessive DNA tail migration has inhibited the interpretation of results[17].

In addition to the variation in response depending on cell type, it is also apparent a range of comet assay protocols (differing in terms of agarose concentrations, lysing and electrophoresis parameters) have been used in studies with aquatic organisms[1-5]. Therefore, effort is required to establish standardized protocols for the main species and cell type commonly used in environmental studies. The production of standard protocols, or the initiation of inter laboratory ring testing workshops focused on aquatic species are essential if the comet assay is to develop further as an environmental monitoring tool.

10.4 Ecological relevance:

Marine invertebrates

Marine invertebrates have been widely used as sentinel species in environmental monitoring programs. This is mainly due to their sessile nature, ability to bioaccumulate contaminants and general ease of capture[18-20]. The majority of work has focused on coastal and estuarine environments. For example, Hartl *et al*., used the clam (*Tapes semidecussatus*) as an indicator species for the presence of potentially genotoxic substances in estuarine environments, demonstrating an increase in DNA damage in haemocytes, gill and digestive gland cells of animals exposed to contaminated sediments[8]. The study also highlighted the differences in sensitivity between cell types, with gill and digestive gland cells appearing to be the most sensitive target tissues for detecting genotoxic exposure. The Mediterranean mussel (*Mytilus galloprovincialis*) has also been extensively deployed as a sentinel organism to assess the genotoxic effects of crude oil spills[21-23]. Studies have demonstrated the sensitivity of mussels to oil exposure and laboratory studies have clearly linked the total polycyclic aromatic hydrocarbon (TPAHs) content of oils with the level of DNA damage observed[21]. In Northern European studies the Blue mussels (*M. edulis*) has also been used to differentiate sites receiving waste treatment effluent, with positive correlations detected between the presence of selected contaminants and the level of DNA damage[24].

Mussels have also been used extensively in the field as part of transplantation studies[25-27]. The use of indigenous organisms is often hampered by the absence of a suitable sentinel species, or if present, the genotoxic responses obtained may be influenced by local physiological adaptations. Furthermore the use of transplanted organisms also offers advantages over indigenous species, such as ensuring genetic homogeneity, developmental/reproductive status and controlling the precise exposure window. Validation studies have been under taken with the comet assay to assess the time course variations in DNA damage following field transplantation experiments[25, 26]. It was observed that within the first 7 days following transplantation the level of DNA damage can fluctuate, which is likely to be caused by manipulation disturbance, then after 2 weeks the level reaches a plateau. Such data suggests that transplantation experiments lasting less that 2 weeks may give spurious results, with the levels of DNA damage detected attributable to artefacts associated with the sampling procedure rather than genotoxic exposure. Studies conducted in a coastal area of Denmark, impacted by a disused chemical site, have also highlighted that the levels of DNA damage in mussels can be affected by seasonal variations in baseline levels[25]. Such results are likely to be influenced by the seasonal variations, which are known to exist for a range of physiological and reproductive processes in mussels[28, 29].

The sampling location has also been shown to influence the results of field-based surveys. For example, mussels (*M. edulis*) sampled from the intertidal zone in Reykjavik harbour had higher levels of DNA damage when compared with mussels collected from the sub tidal zone at the same site[30]. While the study supports the use of DNA strand breaks as a measure of environmental pollution it also highlights the high levels of intra site variability in DNA damage that can occur. As such the study further serves to underline the importance of validating experimental protocols and sampling procedures to ensure that non-contaminant related factors (e.g. physiological and biochemical responses to variations in oxygen availability and temperature stress) do not adversely affect biomarkers data.

Marine vertebrates

There are a limited number of comet assay studies utilizing marine fish species in comparison to those using freshwater species (for detailed review see[1, 4, 5]). This is mainly due to the logistical problems associated with collecting fish at sea (e.g. need for a research vessels) and technical problems inherent within the assay, such as the difficulty of performing electrophoresis reproducibly at sea (e.g. dealing with adverse weather conditions). To date those studies undertaken have mainly focused on flatfish and bottom-feeding species, which due to their close association with sediment bound contaminants are widely used in marine monitoring programmes[31, 32]. In vivo studies have been undertaken to investigate oxidative stress in the European eel (*Anguilla anguilla*)[33]. The comet assay has also proven to be a useful tool for studying the genotoxic effects of non bio-accumulating contaminants in the marine environment. For example, the environmental effects of the known mutagen and potential carcinogen styrene has been studied in the mussel (*M. edulis*) and fish (*Symphodus mellops*)[34]. Styrene hasn't previously been considered to be harmful to marine fauna due to its high volatility and low capacity to bio-accumulate. However, it was shown to cause a statistically significant increase in DNA damage in blood cells, probably due to the formation of a radical styrene metabolite, which is thought to have potent oxidative capacity. Hatchery-reared turbot (*Scophthalmus maximus* L.) have been used successfully to investigate the genotoxic potential of PAH and heavy metal contaminated sediment from sites in Cork Harbour (Ireland)[35]. Eelpout (*Zoarces viviparus*) have been used in site-specific investigative monitoring following a bunker oil spill in Goteborg harbour, Sweden. The comet assay was deployed along site a battery of other bioassays and elevated levels of DNA damage were correlated to the presence of PAH metabolites in the bile of fish[36]. The marine flatfish dab (*Limanda limanda*) is a commonly used flatfish species in offshore monitoring programmes and it has been used in a number of studies investigating the impacts of genotoxic contaminants in coastal and estuarine waters[37-39]. Studies have shown that both sex and age of the fish have a significant effect on the presence of DNA strand breaks, which again highlights the influence other factors (i.e. reproductive status) may have on the extent of DNA damage.[37, 38].

10.5 Quality assurance

No formal quality assurance programmes are currently run within the marine monitoring community. However, a series of comet assay workshops have taken place with the aim of drafting a common regulatory strategy for industrial genotoxicology screening[6,7]. Final guidelines drafted after the 4th International Workgroup on Genotoxicity testing: Results of the in vivo Comet assay workgroup[7] provide a useful starting point for developing quality assurance programmes specifically focused on protocols employed in marine species. These include consideration of 1) cell isolation processes[if required]; 2) cryopreservation processes; 3) concurrent measures of cytotoxicity; 4) Image analysis and scoring method.

Currently data can be reported in a number of formats. % DNA in tail has been reported to be the most linearly related to exposure dose[7]. However there is no clear consensus of which measure of DNA migration should be used (% DNA in tail, Tail moment, Tail length). This difference in scoring criteria hinders our ability to develop a consensus background response and assessment criteria.

10.6 Background responses and assessment criteria

It is recognised that setting baseline/background response levels have an important role in integrating biological effect parameters into environmental impact assessments of the marine environment. The general philosophy is that an elevated level of a particular biomarker, when compared with a background response, indicates that a hazardous substance has caused an unintended or unacceptable level of biological effect. Therefore, in order to understand and apply the Comet Assay as a biomarker of genotoxic exposure it is of fundamental importance to gain information on the natural background levels in non-contaminated organisms. Table 1 summaries a number of studies that have utilised commonly deployed bioindicator species collected from reference locations (as supported by chemical and biomarker analyses) or kept under control conditions in the laboratory. While these studies provide a starting point for determining "background" levels of DNA damage they also serve to highlight the number of different tissues, protocols and endpoints currently reported.

Table 10.2.1. Assessment of "control DNA damage" by Comet assays after in vivo exposure to commonly used biomonitoring organisms.

Organism	Cell type	Agent	Exposure time	Parameter	Control response	Ref.
Invertebrates						
M. edulis	Haemocytes	MMS	0-4 days	Tail Moment	2.08 ± 3.43 2.96 ± 4.60	25
M. edulis	Haemocytes	Tritiated water	96 hrs	% DNA Tail	<10	40
M. edulis	Haemocytes	TBT	7 days	% DNA Tail	5-10	41
M. edulis	Haemocytes	MMS	3-7 d	% DNA Tail	<10	44
M. edulis	Gill cells	Cd Cr Cr VI	10 days 7 days injection	% DNA Tail	<15	42
M. edulis	Gill cells	MMS		Tail Moment	1.87 ±2.23 0.60 ± 1.05 3.84 ± 3.61 1.22 ± 1.47	25
M. edulis	Gill cells	Field site	In situ	Tail Moment	<1.5	45
M. edulis	Gill cells	Field site	In situ	Tail Moment	<5	46
M. edulis	Digestive gland	H202, BaP	1hr	% DNA Tail	< 10	43
Vertebrates						
L.limanda	Erythrocytes	Field	In situ	Tail Moment	<5	39
L.limanda	Erythrocytes	Field	In situ	% DNA Tail*	4-6	37
P. olivaceus	Erythrocytes	Field	In situ	Tail length (μm)	<10	47
Zoarces viviparus	Erythrocytes	Field	In situ	% DNA Tail	<15	36

10.7 *Mean square root of percent tail DNA measured

The requirement now is to establish a common set of protocols for those tissues/species rountine used in biomonitoring programmes. Once established it will be

possible to define internationally accepted background levels of DNA damage and from their establish assessment criteria

Required steps:

- Consensus on standardized protocol from main species currently used in marine biomonitoring programmes (OSPAR, HELCOM, MEDPOL and MSFD).
- Establish minimum acceptable reporting criteria (cellular toxicity, +/- control etc)
- Agree data reporting format to allow cross study comparisons of data (Tail moment, % DNA in Tail, Tail moment).

References

1) C.L. Mitchelmore and J.K. Chipman, DNA strand breakage in aquatic organisms and the potential value of the comet assay in environmental monitoring, Mutat. Res., 1998, 399: 135–147

2) S. Cotelle and J.F. Fèrard. Comet assay in genetic ecotoxicology: a review, Environ. Mol. Mutagen., 1999, 34: 246–255.

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

4) A.N. Jha. Ecotoxicological applications and significance of the comet assay, Mutagenesis, 2008, 23(3): 207-221

5) G. Fenzilli, M. Nigro, B.P. Lyons. The Comet assay for evaluation of genotoxic impact in aquatic environments. Mutat Res., 2009: 681, 80–92.

6) R.R. Tice, E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.C. Ryu and Y.F. Sasaki, 2000. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing, *Environ. Mol. Mutagen,* 35: 206–221.

7) B. Burlinson, R.R. Tice, G. Speit, E. Agurell, S.Y. Brendler-Schwaab, A.R. Collins, P. Escobar, M. Honma, T.S. Kumaravel, M. Nakajima, Y.F. Sasaki, V. Thybaud, Y. Uno, M. Vasquez and Andreas Hartmann, Fourth International Workgroup on Genotoxicity testing: results of the in vivo Comet assay workgroup, *Mutat. Res*., 2007, 627: 31–35.

8) M.G. Hartl, B.M. Coughlan, D. Sheehan, C. Mothersill, F.N. van Pelt, S.J. O'Reilly, J.J. Heffron, J. O'Halloran and N.M. O'Brien. 2004. Implications of seasonal priming and reproductive activity on the interpretation of Comet assay data derived from the clam, Tapes semidecussatus Reeves 1864, exposed to contaminated sediments, Mar. Environ. Res., 2004, 57: 295–310.

9) W.H. Siu, J. Cao, R.W. Jack, R.S. Wu, B.J. Richardson, L. Xu, and P.K. Lam. 2004. Application of the comet and micronucleus assays to the detection of B[*a*]P genotoxicity in haemocytes of the green-lipped mussel (*Perna viridis*), Aquat. Toxicol., 66: 381–392.

10) S. Lemiere, C. Cossu-Leguille, A. Bispo, M.J. Jourdain, M.C. Lanhers, D. Burnel and P. Vasseur, 2005. DNA damage measured by the single-cell gel electrophoresis (Comet) assay in mammals fed with mussels contaminated by the 'Erika' oil-spill, Mutat. Res., 581: 11–21.

11) S. Pandey, N.S. Nagpure, R. Kumar, S. Sharma, S.K. Srivastava and M.S. Verma. 2006. Genotoxicity evaluation of acute doses of endosulfan to freshwater teleost *Channa punctatus* (Bloch) by alkaline single-cell gel electrophoresis, *Ecotoxicol. Environ. Saf*., 65, 56–61.

12) I.Y. Kim and C.K. Hyun. 2006. Comparative evaluation of the alkaline comet assay with the micronucleus test for genotoxicity monitoring using aquatic organisms, *Ecotoxicol. Environ. Saf.*, 64, 288–297.

13) D. Huang, Y. Zhang, Y. Wang, Z. Xie and W. Ji, Assessment of the genotoxicity in toad *Bufo raddei* exposed to petrochemical contaminants in Lanzhou Region, China, *Mutat. Res*., 2007, 629, 81–88.

14) N.P. Singh, D.B. Danner, R.R. Tice, M.T. McCoy, G.D. Collins and E.L. Schneider, Abundant alkali-sensitive sites in DNA of human and mouse sperm, *Exp. Cell. Res*., 1989, 184, 461–470.

15) G. Frenzilli, V. Scarcelli, F. Taddei and M. Nigro, Adaption of SCGE as a candidate for monitoring marine ecosystems, *Neoplasma*, 1999, 46, 6–7.

16) M. Moretti, M. Villarini, G. Scassellati-Sforzolini, A.M. Santroni, D. Fedeli and G. Falcioni, Extent of DNA damage in density-separated trout erythrocytes assessed by the 'comet' assay, *Mutat. Res.* 1998, 397, 353–360.

17) G. Wirzinger, L. Weltje, J. Gercken and H. Sordyl, Genotoxic damage in field-collected three-spined sticklebacks (*Gasterosteus aculeatus* L.): a suitable biomonitoring tool? *Mutat. Res.*, 2007, 628, 19–30.

18) B.L. Bayne. Watch on mussels. *Mar. Pollut. Bull*., 1976 7(12), 217–128.

19) R. Seed. Ecology. In: B.L. Bayne, Marine Mussels, their ecology and physiology, International Biological Programme (13-65), Cambridge University Press, 1976, 13–65

20) M.H. Salazar and S.M. Salazar. In situ bioassays using transplanted mussels: I. Estimating chemical exposure and bioeffects with bioaccumulation and growth. In Hughes JS, Biddinger GR, Mones E, Eds. Environmental Toxicology and Risk Assessment – Third Volume. Philadelphia: American Society for Testing and Materials STP, 1995 2118, 216–241.

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

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

23) I.C. Taban, R.K. Bechmann, S. Torgrimsen, T. Baussant and S. Sanni. 2004. Detection of DNA damage in mussels and sea urchins exposed to crude oil using comet assay, *Mar. Environ. Res.*, 58: 701–705.

24) J. Rank, K. Jensen and P.H. Jespersen. 2005. Monitoring DNA damage in indigenous blue mussels (*Mytilus edulis*) sampled from coastal sites in Denmark, Mutat. Res./Genet. Toxicol. Environ. Mutagen., 585, 33–42.

25) J. Rank, K.K. Lehtonen, J. Strand and M. Laursen. 2007. Aquatic toxicology, DNA damage, acetylcholinesterase activity and lysosomal stability in native and transplanted mussels (*Mytilus edulis*) in areas close to coastal chemical dumping sites in Denmark, *Aquat. Toxicol.*, 84, 50–61.

26) F. Regoli, G. Frenzilli, R. Bocchetti, F. Annarumma, V. Scarcelli, D. Fattorini and M. Nigro, Time-course variations of oxyradical metabolism, DNA integrity and lysosomal stability in mussels, *Mytilus galloprovincialis*, during a field translocation experiment, *Aquat. Toxicol.*, 2004, 68, 167–178.

27) M. Nigro, A. Falleni, I. Del Barga, V. Scarcelli, P. Lucchesi, F. Regoli and G. Frenzilli. 2006. Cellular biomarkers for monitoring estuarine environments: transplanted versus native mussels, *Aquat. Toxicol.*, 77, 339–347.

28) A. Hines, G.S. Oladirana, J.P. Bignell, G.S. Stentiford, M.R. Viant. 2007. Direct sampling of organisms from the field and knowledge of their phenotype: key recommendations for environmental metabolomics, *Environ. Sci. Tech.*, 41, 3375-3381.

29) J.P. Bignell, M.J. Dodge, S.W. Feist, B.P. Lyons, P.D. Martin, N.G.H. Taylor, D. Stone, L. Travalent, G.D. Stentiford, G.D. 2008. Mussel histopathology: effects of season, disease and species. *Aquatic Biology*, 2: 1–15.

30) H.P. Halldorsson, G. Ericson and J. Svavarsson. 2004. DNA strand breakage in mussels (*Mytilus edulis* L.) deployed in intertidal and subtidal zone in Reykjavik harbour, *Mar. Environ. Res.*, 58, 763–767.

31) S.W. Feist, T. Lang, G.D. Stentiford, A. 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.

32) JAMP guidelines for general biological effects monitoring. *Joint Assessment and Monitoring Programme*. Oslo and Paris Commissions, 1998, 38 pp.

33) F. Regoli, G.W. Winston, S. Gorbi, G. Frenzilli, M. Nigro, I. Corsi and S. Focardi, Integrating enzymatic responses to organic chemical exposure with total oxyradical absorbing capacity and DNA damage in the European eel *Anguilla anguilla*: toward development of a more holistic biomarker assessment, *Environ. Toxicol. Chem.*, 2003, 22, 2120–2129.

34) E. Mamaca, R.K. Bechmann, S. Torgrimsen, E. Aas, A. Bjornstad, T. Baussant and S.L. Floch, The neutral red lysosomal retention assay and Comet assay on haemolymph cells from mussels (*Mytilus edulis*) and fish (*Symphodus melops*) exposed to styrene, *Aquat. Toxicol.*, 2005, 75, 191–201.

35) M.G.J. Hartl, M. Kilemade, D. Sheehan, C. Mothersill, J. O'Halloran, N.M. O'Brien and F.N.A.M. van Pelt, Hepatic biomarkers of sediment-associated pollution in juvenile turbot, *Scophthalmus maximus* L, *Mar. Environ. Res.*, 2007, 64, 191–208.

36) G. Frenzilli, V. Scarcelli, I. Del Barga, M. Nigro, L. Forlin, C. Bolognesi and J. Sturve, DNA damage in eelpout (*Zoarces viviparus*) from Goteborg harbour, *Mutat. Res.*, 2004, 552, 187–195.

37) F. Akcha, G. Leday and A. Pfohl-Leszkowicz, Potential value of the comet assay and DNA adduct measurement in dab (*Limanda limanda*) for assessment of in situ exposure to genotoxic compounds, *Mutat. Res.*,2003, 534, 21–32.

38) F. Akcha, F. Vincent Hubert and A. Pfohl-Leszkowicz, Measurement of DNA adducts and strand breaks in dab (*Limanda limanda*) collected in the field: effects of biotic (age, sex) and abiotic (sampling site and period) factors on the extent of DNA damage, *Mutat. Res.*, 2004, 552, 197–207.

39) B.P. Lyons, G.D. Stentiford, J. Bignell, F. Goodsir, D.B. Sivyer, M.J. Devlin, D. Lowe, A. Beesley, C.K. Pascoe, M.N. Moore and E. Garnacho, A biological effects monitoring survey of Cardigan Bay using flatfish histopathology, cellular biomarkers and sediment bioassays: findings of the Prince Madog Prize 2003, *Mar. Environ. Res.*, 2006 62, S342–S346.

40) Jha, A.N., Dogra, Y., Turner, A., Millward, G.E. 2005. Impact of low doses of tritium on the marine mussel, *Mytilus edulis*: Genotoxic effects and tissue-specific bioconcentration. Mut. Res.Genet. Toxicol. Environ. Mutagen 586 (1), pp. 47–57

41) Hagger, J.A., Depledge, M.H., Galloway, T.S. (2005). Toxicity of tributyltin in the marine mollusc *Mytilus edulis*. Mar. Poll. Bull. 51 (8-12), pp. 811–816.

42) Emmanouil, C., Smart, D.J., Hodges, N.J., Chipman, J.K. (2006) Oxidative damage produced by Cr(VI) and repair in mussel (*Mytilus edulis* L.) gill. Mar. Environ. Res., 62(1): 292–296.

43) Mitchelmore, C.L., Birmelin, C., Livingstone, D.R., Chipman, J.K. 1998. Detection of DNA Strand Breaks in Isolated Mussel (*Mytilus edulis* L.) Digestive Gland Cells Using the ''Comet'' Assay. Ecotox and Environ. Saf. 41, 51Ð58 (1998).

44) Canty, M.N., Hutchinson, T.H., Brown, R.J., Jones, M.B., Jha, A.N. (2009). Linking genotoxic responses with cytotoxic and behavioural or physiological consequences:

Differential sensitivity of echinoderms (Asterias rubens) and marine molluscs (Mytilus edulis). Aquat. Toxicol., 94 (1), pp. 68-76.

45) Rank J. (2009) Intersex in Littorina littorea and DNA damage in Mytilus edulis as indicators of harbour pollution Ecotox. Environ. Saf., 72 (4), pp. 1271-1277.

46) Rank J. Lehtonen, K.K., Strand, J., Laursen, M. (2007). DNA damage, acetylcholinesterase activity and lysosomal stability in native and transplanted mussels (Mytilus edulis) in areas close to coastal chemical dumping sites in Denmark Aquat.Toxicol., 84 (1), pp. 50-61.

47) Woo, S., Kim, S., Yum, S., Yim, U.H., Lee, T.K. (2006). Comet assay for the detection of genotoxicity in blood cells of flounder (Paralichthys olivaceus) exposed to sediments and polycyclic aromatic hydrocarbons Mar. Poll. Bull. 52 (12), pp. 1768–1775.

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

The group reviewed the status of publications that were in preparation or were commissioned at last year's meeting. Two draft manuscripts were received at or just prior to the meeting (Alkylphenol bile metabolites & sea urchin embryo bioassays). These were still pending external peer review, but were placed on the SharePoint for consideration by the group. WGBEC members are to respond to Matt Gubbins (WGBEC TIMES coordinator by the end of February with any comments).

Considering the number of manuscripts commissioned by the group, still in preparation (9) all with draft resolutions. It was decided to focus WGBEC efforts on delivery of these, rather than commission any new manuscripts.

During review of the table of WGBEC TIMES manuscripts it was noted that several documents were nearing completion and could be expected imminently (EROD, OEB, Extraction methods).

The current status of manuscripts is given in table 11.1 below:

C. Res	Method	ICES Deadline	Status
2002/1E03	The report on Biological Effects of Contaminants: Oyster (Crassostrea gigas) Embryo Bioassay by J.E. Thain (UK)	31/12/09 (now expected end of Jan 2010)	Reviewed at WGBEC 2009. Minor edits required. Final draft available from author within 2 weeks of meeting. Peer review not required.
2006/1/MHC06	The Protocol for Extraction Methods for Bioassays. Hans Klamer and John Thain (UK)	31/12/09	Draft available from authors for SGIMC meeting at end of January 2010.
2006/1/MHC07	The protocol for conducting EROD determinations in flatfish By M. Gubbins	31/12/09	Reviewed at WGBEC 2009. Minor edits required. Peer review not required.
2007/1/MHC02	Blue Mussel Histopathology, John Bignell, Steve Feist & Miren Cajaraville	01/03/10	David Lowe is no longer an author of this MS. Main author is awaiting input from co-authors on specific pathologies. In preparation.

C. Res	Method	ICES Deadline	Status
2008/1/MHC13	Protocol for measuring dioxin-like activity in environmental samples using CALUX assays. Dick Vethaak (Netherlands)	31/03/10 (1st draft expected)	Estrogenic receptor method has been removed from manuscript. In preparation by author. Deadline revised with ICES.
2008/1/MHC14	Protocols for measuring micronucleus formation in cells as an indicator of toxicant induced genetic damage. Brett Lyons & Awadesh Jha (UK).	30/04/10	Manuscript will be based on recent background document. New co-authors identified. Revised deadline reported to ICES.
2008/1/MHC15	Protocol for measuring estrogen/androgen activity in environmental samples using YES/YAS yeast screen assays. J Thain (UK), Kevin Thomas (Norway)	01/05/10	No update on progress from the author. WGBEC to chase up and provide progress report.
2008/1/MHC12	The protocol for gonadal histology in flounder. S Feist et al.	31/03/10	Progress by author. 1st draft expected by the end of the month.
	Reproductive success in eelpout. Jakob Strand	31/10/10	In preparation.
	Alkylphenol bile metabolites. Jonny Beyer	31/03/10	Manuscript produced and awaiting review
	Sea urchin embryo bioassay. Ricardo Beiras	31/03/10	Manuscript produced and awaiting review

In 2009, WGBEC noted that there were some restrictions on the availability of the CALUX method (2008/MHC13) and that this may limit the usefulness of pursuing a TIMES manuscript on this method. Dick Vethaak reported that an alternative source of cell lines was readily available for research purposes and that the manuscript should progress.

The role of TIMES coordinator for WGBEC was discussed. Matt Gubbins indicated that he would stand down in this role as he was taking on chairmanship duties. Ricardo Beiras was appointed to this role by the group.

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

A spreadsheet of biological effects data submissions to the ICES database was provided to the WG by an ICES data manager. WGBEC reviewed this and noted that there were some substantial additions to the database in 2009. These included the following parameter types:

- TBT effects / imposex / intersex: France, UK, Norway, Sweden, Netherlands
- PAH bile metabolites: Germany, Norway, and UK

- EROD: UK, France, Norway, Spain
- Bioassay: Spain

New submissions of biological effects data and legacy data conversion activity over the last year has brought data quality issues to the attention of the data centre that now requires WGBEC advice. These were directed to the group by Marilynn Sorensen and addressed as indicated below:

The DATSU checks proposed intersessionally for imposex were approved by WGBEC:

- Values for VDS should be an integer from 0–6
- VDSI should be a variable from 0–6
- IS = INTS should be an integer from 0–4
- ISI = INTSI should be a variable from 0–4
- PCI should be a variable from 0–3.5
- IMP = IMPSI should be a variable from 0–6

Additional checks were suggested:

For all population level parameters for TBT effects (i.e., when MUNIT = 'index') a warning should be triggered if the condition "NOINP >39" is not met for parameters VDSI, INTSI, IMPSI and PCI.

For data on individuals, if the condition "NOINP = 1" is not met for parameters VDS, INTS, IMPS, a non-critical error should be triggered. Critical errors are reserved for database requirements.

For parameters EROD and CYP1A, if the condition "NOINP > 19" is not met, a warning should be triggered.

The lists of DATSU checks and FINFL (factors influencing results) codes were also reviewed by a WGBEC sub-group. It was noted that there were very few data quality checks currently included for biological effects data and that it was up to WGBEC to provide these to the data centre. WGBEC members were asked to suggest appropriate data checks for parameters that they were particularly involved with. These will be developed intersessionally before the next meeting, but will focus initially on EROD.

For conversion of legacy data, standardisation of EROD units and matrices is required. WGBEC advises that the recommended unit for EROD is picomole/minute/milligram protein and all the varying units that appear in the submitted legacy data are either equivalent to this or can be converted to this (conversion factors were provided to the data centre for converting data submitted as nanomole/minute/milligram liver to picomole/minute/milligram protein where data are available on protein content of liver. Legacy data in the database as codes ERODS, ERODM, and ERODL were assumed by the WG to be S9, microsomal fraction and normalised to liver weight values respectively. All were converted to parameter EROD with matrices liver S9 (LIS9), liver microsomes (LIMIC) and liver (LI) respectively. For ERODL it is unclear whether measurements were done on S9s or microsomes so data should be assessed with caution (e.g. were not included in the derivation of BACs). Legacy data with codes CODLM, ECODL, RODLM, and PRODP should not be converted.

Norway has data on CYP1A measured by ELISA. The unit of measurement requested by a data submitter for use in the database (absorbance 450/milligram protein) was

not appropriate for this technique. WGBEC advised the data centre that UNITS/milligram protein was the relevant unit for this technique as it is semi-quantitative and the ELISA results are unit less.

WGBEC was asked to clarify WGBEC2009's request for condition factor / index and somatic indices data into the ICES database. The response to the data centre was for fish to rely on existing parameter codes: length (LNMEA/LNMIN/LNMAX), weight (WTMEA/WTMIN/WTMAX) and for some countries gutted weight (GUTWTMEA etc.), and somatic weight. The data centre pointed out that data are being submitted without length/weight etc. but the new OSPAR contaminant cofactor checks in DATSU will check for some of these parameters. Parameter codes LISOI and GOSOI are already available for hepato-somatic and gonado-somatic indices respectively. In mussels a new parameter code for mussel condition index needs to be developed and defined in the OSPAR background document on condition indices and supporting parameters for mussel integrated monitoring.

A scope for growth parameter code (SFG) and MUNIT code were previously recommended by WGBEC. Since there are various combinations of methods for scope for growth, the data centre recommended that METOA codes be developed specifically for SFG method variations to enable a quality check by DATSU at the time of submission. It was decided that clearance rate measurements alone would not be acceptable but that clearance rate / oxygen consumption and clearance rate / oxygen consumption / excretion calculations should be accepted but recorded separately. Failure to submit the method information will trigger a non-critical error.

Gametogenesis in mussels. WGBEC2009 recommended that a new parameter code "Gonadal stage" was used for this information by reporting an alphanumeric value in the range of 1–5 for pre-spawning and 1s-5s for post-spawning. The ICES database requires that the field "VALUE" is a number and therefore suggested the use of the existing "condition of specimen" field "CONES" in the database for recording this information. Multiple options could be added for pre- and post-spawning. WGBEC prefers that specific parameter codes are used to avoid confusion and it was therefore agreed that two parameters would be added: pre-spawning gonadal stage and post-spawning gonadal stage should be created and acceptable values for these should be integers from 1-5 where MUNIT = stage. These parameters should be allowed for individuals only, i.e. if the condition "NOINP=1" is not met, an error will be triggered.

The Data Centre requested an update on specifications for the new Quality Assurance database for storing biological effects intercalibration results. WGBEC advised that the existing QUASIMEME z-score system already established within ICES data centre was appropriate for TBT effects, but that for other methods a more flexible approach would be required. This will require further development as QA schemes are developed by WGBEC in the future, but it was envisaged that WGBEC might pass data files to ICES data centre containing lists of participating laboratories and pass/fails for specific parameters by monitoring year.

WGBEC members also had questions for the ICES data centre. These were:

1) What procedures should be followed to extract data from the database?

The ICES data centre will supply WGs with data extractions as required for meetings. This can be as a standing request for data in ToR or simply by emailing accessions@ices.dk. Individual labs can check their own submitted data files by accessing DOME.ices.dk, choosing "submitted files" and filtering on their lab code. Alterna-

tively, anyone can download data (up to 50 000 lines) from the EcoSystemData website ecosystemdata.ices.dk by going to the 'inventory' on the website, selecting a region on the interactive map and filtering by parameter. For > 50 000 lines of data you can email accessions@ices.dk. Downloads are in csv format and are simpler in structure than submitted files in V3.2 format but lack method and QA information.

2) What possibilities are there for training in data submissions?

One can always contact a data manager for help. In addition, the ICES data centre offered to host a training workshop in Copenhagen. A call for interest from the group did not identify much demand for this. It is suggested that expertise is enhanced by direct communication with ICES data centre and through the generation of guidance documents by WGBEC (see recommendation).

3) How should data submitters record the information that fish in a single sample may have come from multiple trawls of an area?

To indicate that the fish came from multiple trawls, fill in the field NOAGG in the sample record with the number of hauls taken to comprise the sample. A single sample record is required when linking of individual fish measurements with pooled measurements from bulks is needed.

4) How best to record bottom water temperature as a supporting parameter for EROD?

Use a 92 record (site description) associated with each 91 (station) record. A new parameter for 'bottom water temperature' will need to be created. This type of parameter is usually kept separate from the hydrographic database.

5) Which member states use a central reporting system and what issues are associated with this?

UK, Denmark, Germany submit all their data via a centralised reporting institute. Advantages are that fewer people require training in ICES reporting formats. Disadvantages are that data submitters are then more removed from the data being submitted, which can make issues harder to resolve. Some WGBEC members expressed a desire to move towards such a system. Some submitters noted that some aspects of data submission were handled in different organisations. ICES pointed out that if samples are being analysed by multiple laboratories (biological effects at one laboratory, organics at another, and metals at a third), a single data file by reporting laboratory and monitoring year was required so all sample analyses can be linked and assessed together.

WGBEC would like information on how biological effects data submission is handled by HELCOM and MEDPOL to see if there are any possibilities for standardisation across systems.

Recommendations

That WGBEC members (and ICES member states) actively submit biological effects monitoring data onto the ICES database using the relevant (v3.2) reporting formats. To assist in this process WGBEC recommends that WGBEC / ICES data centre develop a live 'working document' to be added to at future WGBEC meetings to explain how biological effect data should be entered into the database and keep track of WGBEC advice on database issues.

Actions: Develop guidance document on biological effects data submissions to include example data files that comprise multiple effects parameters and BULKID

codes to show how to handle data for pooled samples (UK), Supporting parameters for each method, DATSU and FINFL checks for BE data (All WGBEC members)

13 Review progress with AQC procedures for biological effect methods and include harmonisation activities within OSPAR, Baltic and MED-POL maritime areas; (ToR d)

13.1 Harmonisation activities within OSPAR, Baltic and MEDPOL

SGIMC in 2009 proposed a ICES/OSPAR practical workshop on lysosomal membrane stability (LMS) by using the neutral red retention (NRR) assay to be organized by Spain (lead IEO/C. Martinez). This workshop was aimed at scientists who have certain experience and are familiar with the NRR assay, but need further training to progress with the harmonised interpretation criteria for this semiquantitive technique. C. Martínez-Gómez (IEO, Spain) informed WGBEC that a funding proposal will be send in January 2010 to the Coordinator for Training Programme in ICES, to support the organization of this practical training workshop. Apart from LMS, it was proposed to include also practical sessions concerning the biomarker Stress on Stress, also recommended as a biological effect method in the OSPAR integrated mussel component.

Prof. Aldo Viarengo(IT) informed WGBEC that a training course on the use of certain biomarkers including LMS and genotoxicity biomarkers in mussels) will be held in 13-17 September 2010 at the University of Piemonte Orientale, Alessandria (Italy). The course will be organized by Prof. Aldo Viarengo and supported by high-profile scientists and instructors (Dr. M.N. Moore and C. Bolognesi), as part of the QA work required in the framework of the MED POL Programme. Additional aims of this training course is to help build capacity in the MED POL biomonitoring programme and to provide special support to new scientists/Institutions involved in the monitoring activities of the riparian Mediterranean countries.

Since the aims and activities of both workshops largely overlap, WGBEC proposed to combine both workshops. Prof. Aldo Viarengo (IT) happily agreed to do this. It is expected that a combined training workshop will attract a higher number of participants and also facilitates harmonisation of biological effects methods within and between OSPAR and MEDPOL maritime areas. A draft proposal of the MEDPOL-ICES/OSPAR Training Workshop will be made available for SGIMC in January 2010 for further elaboration (Task CM and AV). See also 4.4 above.

13.2 Review of progress with AQC procedures.

Quality assurance is a necessity for any method to be used for national or international monitoring and it is important to be aware that this is a continuous process, not a one-off intercalibration or other exercise. Through the past decade there have been various rounds of training workshops and intercalibrations for biological effects methods within BEQUALM, QUASIMEME, MEDPOL and HELCOM, as well as through EU research projects such as BEEP and COMPREHEND. BEQUALM (http://www.bequalm.org) has been the only organisation to offer QA for a broad range of methods, including benthic community studies, fish histopathology, bioassays and biomarkers. The current linking of the UK NMMP with BEQUALM in offering QA for benthic community studies appear to be satisfactory and should be retained as it is. Likewise, BEQUALM QA for fish histopathology is an ongoing activity that appears to be providing the required services for the scientific and monitoring community.

There is, however, a need for a renewed strategy for bioassay and biomarker QA. Critical components of such a programme are regularity (annual or biannual) and cost. A requirement for submitting data to the ICES database is that there is a certified AQC scheme. In order to take this forward WGBEC felt it should review this process and assess whether it was feasible to coordinate from within the group.

Following discussions in the working group it was agreed to launch a low-cost programme for methods included in the integrated monitoring framework. A basic web site will be launched (Cefas) to provide information to prospective users and WGBEC members will be the scientific basis and main users of the services provided by this activity. The results from intercalibration exercises will be evaluated by WGBEC members during a half-day meeting prior to the main meeting on an annual basis and a brief report produced. The activity will need a WGBEC member to co-ordinate sampling, shipment, communication with participants and registering of results. A steering committee (Ketil Hyland, Matt Gubbins, and John Thain) (in communication with MEDPOL and HELCOM) was formed to communicate bimonthly and coordinate necessary activity to deliver this programme.

The methods would be divided into three categories: (1) material could be prepared and distributed (most methods, e.g. AChE, EROD); (2) a workshop or similar would be required (LMS, Comet); (3) prepared toxic mixtures would be distributed (bioassays).

Liver, plasma, bile, blood cell and muscle samples would be collected at one polluted, one intermediate and one clean site during routine monitoring cruises organised by WGBEC members. Samples from 15-20 individual fish would be pooled, homogenised and aliquotted into cryovials before storage at -80. Protocols for sample preparation are available from BEQUALM and would involve homogenisation of tissues under liquid nitrogen (for non-liquid matrices). The only direct cost to participating laboratories would be shipment of samples. An agreement would have to be signed by participating laboratories to ensure timely reporting of results.

The methods required for the integrated approach (fish) would comprise DNA adducts, AChE inhibition, EROD/CYP1A activity, PAH metabolites, lysosomal membrane stability and vitellogenin concentration (Table 13.2.1). Optional methods for fish are Comet (workshop planned), metallothionein (no activity planned), ALA-D (no activity planned) and reproductive success in fish (activity will be clarified with HELCOM).

Table 13.2.1. Overview of methods for the integrated approach (fish) which require WGBEC QA.

METHOD	STATUS	LIKELY UPTAKE
Bulky DNA adduct formation	None currently available; was done through BEQUALM; could be done by sending fish liver samples	limited, maybe 3 labs (France, Sweden, Italy)
AChE inhibition	BEEP; none currently active; could be done by sending fish muscle tissue	probably >10 labs (Norway, UK, Spain, Italy, Netherlands, Portugal etc)
EROD or P4501A induction	BEQUALM; last intercalibration 2008; could be done by sending fish liver samples	> 10 labs
PAH bile metabolites	Quasimeme; last intercalibration 2002; either as part of proposed AP intercalibration or from research cruises; methodology needs to be reported for each participant (FF, SS, HPLC, GC/MS)	probably >10 labs

Lysosomal membrane stability	MEDPOL and ad hoc; need to be done as workshop; planned activity AWI (cytochemical methods)	>5 labs
Vitellogenin induction	COMPREHEND; none currently active; different species need specific antibodies and standards; antisera commercially available, but standards for most species not available	>5 labs

The methods required for the integrated approach (mussel) would comprise scope for growth, AChE inhibition, lysosomal membrane stability and micronucleus formation (Table 13.2.2). Histopathology should be handled by BEQUALM (will need to be confirmed). Optional methods for mussel are metallothionein induction (intercalibration by MEDPOL), Comet (workshop planned) and stress on stress (training and intercalibration can be done in parallel with LMS workshop). The group proposes to remove MXR from the framework.

Table 13.2.2. Overview of methods for the integrated approach (mussel) which require WGBEC QA.

RECOMMENDED METHODS (INVERTEBRATES)	EXTERNAL QA COMMENTS	LIKELY UPTAKE
AChE inhibition	No current activity on intercalibration. Possible to do by simple ring test distribution of material.	At least 3 labs: Spain, Finland (clams not mussels), France
Lysosomal stability	MEDPOL and BEQUALM; two methods (cytochemical and NRR); need to be done as workshop; planned activity MEDPOL/SGIMC	>10 labs
Scope for growth	Originally run by PML (WGBEC initiated); needs workshop	Limited; 3-5 labs?
Micronucleus formation	None currently available; slides can be prepared and distributed	>5 labs

Bioassay intercalibration will have to be done either by workshops or by distribution of toxic mixtures to be tested. WGBEC involvement with such intercalibration should be investigated intersessionally.

To take the AQC process forward WGBEC intends to form a small steering group (KH, JT, MG) to coordinate QA activities to deliver the above work plan.

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

14.1 Continuing review of emerging and novel contaminants including nanoparticles; presented by Jim Readman (UK)

As described at previous WGBEC meetings, urban and industrial sewage effluents contain important quantities of emerging pollutants (including pharmaceuticals, personal care products and endocrine disrupters). Many of these substances are emitted in substantial quantities and our lack of knowledge concerning their environmental behaviour and long-term ecotoxicological impacts need to be addressed if we are to understand the environmental, economic and human health implications. JR provided a presentation on recent research into this topic. He described a selection of emerging contaminants, commencing with pharmaceuticals, personal care products and phenolic endocrine disrupters. All three are amenable to a single analytical protocol to investigate their behaviour. Recent research regarding toxicological investigations of pharmaceuticals including paracetamol, a beta-blocker (propanolol) and Tamiflu were also described. For endocrine disrupters, challenges relating to the quantification, particularly of biologically active concentrations of female steroids, and most recent approaches, were summarised. Next, the approach to measure contaminants in biological fluids so as to evaluate the biologically available fraction (frequently at concentrated levels) was described, using the analysis of a fungicide in crab urine with a bacterial bioreporter, as an example. Environmental implications associated with ubiquitous synthetic musks (in particular galaxolide and tonalide) was then addressed.

Integration of biological effects assessments with chemical fingerprinting in the context of shipping accidents was then discussed. The case of the MCS Napoli was used as an example and demonstrates biological effects in limpets associated with spilled fuel oil from the ship.

Finally, research relating to nanoparticles was described. This included the uptake and biological effects of fullerenes and carbon nano-tubes on the marine mussel, the impact and effects of silver nanoparticles on bacterial communities and the subsequent potential for antibiotic resistance. Iron nanoparticles are becoming increasingly used as a food supplement, and results from preliminary toxicological research on these was summarised.

Bradford, A., Handy, R., Readman, J.W., Atfield, A., and Mühling, M. 2009. Impact of silver nanoparticle contamination on the genetic diversity of natural bacterial assemblages in estuarine sediments. Environmental Science and Technology, 43 (12), 4530–4536. doi:10.1021/es9001949.

Grover, D.P., Zhang, Z.L., Readman, J.W., and Zhou, J.L. 2009. A comparison of three analytical techniques for the measurement of steroidal estrogens in environmental water samples. *Talanta*, 78, 1204-1210. doi:10.1016/j.talanta.2008.12.049

Guitart, C. and Readman, J.W. 2010. Critical evaluation of the determination of pharmaceuticals, personal care products, phenolic endocrine disrupters and faecal steroids by GC/MS and PTV-GC/MS in environmental waters. Analytica Chimica Acta, doi:10.1016/j.aca.2009.10.066.

Hutchinson, T.H., Beesley, A., Frickers, P.E., Readman, J.W., Shaw, J.P., and Straub, J.O. 2009. Extending the Environmental Risk Assessment for Oseltamivir (Tamiflu®) Under Pandemic Use Conditions to the Coastal Marine Compartment. Environment International, 35, 931–936.

Kadar, E., Lowe, D.M., Sole, M., Fisher, A., Jha, A.N., Readman, J.W. and Hutchinson, T.H. 2010. Uptake and biological responses to nano-Fe versus soluble $FeCl_3$ in excised mussel gills. Analytical and Bioanalytical Chemistry, doi:10.1007/s00216-009-3191-0.

Lewis, C., Beggah, S., Pook, C., Guitart, C., Redshaw, C., Roelof van der Meer, J., Readman, J.W. and Galloway, T.S. 2009. Novel use of a whole cell *E. coli* bio-reporter as a urinary exposure biomarker. Environmental Science and Technology, 43 (2), 423–428. doi:10.1021/es801325u.

Mühling, M., Bradford, A., Readman, J.W., Somerfield, P.J. and Handy, R. 2009. An investigation into the effects of silver nanoparticles on antibiotic resistance of naturally occurring bacteria in estuarine sediments. Marine Environmental Research, 68: 278-283. doi:10.1016/j.marenvres.2009.07.001.

Moore, M.N., Readman, J.A.J., Readman, J.W., Lowe, D.M., Frickers, P.E. and Beesley, A. 2009. Carbon nanoparticle-induced lysosomal membrane injury in blood cells of marine mussels (*Mytilus galloprovincialis*): an *in vitro* study. Nanotoxicology, 3 (1), 40-45. doi:10.1080/17435390802593057

Readman, J.W. 2009. Chemical Analysis of Hydrocarbons in Petroleum Oils and the Assessment of Environmental Contamination. In: Microbiology of Hydrocarbons, Oils, Lipids, and Derived Compounds. Kenneth N. Timmis (Ed.). Springer-Verlag, Heidelberg. Part 32, 3573-3582. doi:10.1007/978-3-540-77587-4_280.

Solé, M., Shaw, J.P., Frickers, P.E., Readman, J.W. and Hutchinson, T.H. 2010. Effects on feeding rate and biomarker responses of marine mussels experimentally exposed to propranolol and acetaminophen. Analytical and Bioanalytical Chemistry, doi:10.1007/s00216-009-3182-1.

Sumner, N.R., Guitart, C., Fuentes, G. and Readman, J.W. 2010. Inputs and distributions of synthetic musk fragrances in an estuarine and coastal environment; a case study. *Environmental Pollution*, 158, 215–222. doi:10.1016/j.envpol.2009.07.018.

14.2 Emerging and novel arising contaminants: marine litter and plastics; presented by Thomas Maes (UK)

There have been many new emerging contamination problems in the last half-century, but one of the most instantly observable is the ubiquity and abundance of marine debris. It is a growing problem which will persist for centuries. From what started as an aesthetic problem of littering, the number of potentially harmful implications of debris that have been identified has escalated and include the accumulation and transport of persistent organic pollutants and carcinogenic, mutagenic or toxic for reproduction (POPs and CMRs; Mato *et al*. 2001), the release of toxic compounds, including medicines, the assistance of alien invasions (Barnes 2002), the distribution of algae associated with red tides (Masó *et al*. 2003), the entanglement in and ingestion of plastic by marine organisms with associated mortality (Katsanevakis 2008), alteration of the structure of benthic communities (Katsanevakis *et al*. 2007), and socioeconomic impacts such as the threat of floating debris to navigation, reduction of the recreational value of beaches and lost tourism, and damages to fishing gear.

Future policy drivers in relation with this emerging problem are the Marine Strategy Framework Directive (MSFD). The definition of marine litter and good environmental status (GES) for this descriptor are stated below:

Marine litter is any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment. Marine litter consists of items that have been made or used by people and deliberately discarded or unintentionally lost into the sea or coastline including such materials transported into

the marine environment from land by rivers, drainage or sewage systems or wind. This definition does not include semi-solid remains of for example mineral and vegetable oils, paraffine and chemicals that sometime litter sea and shores.

Good environmental status is defined by the commission as "Properties and quantities of marine litter do not cause harm to the coastal and marine environment".

"Harm" is subdivided in different matrices:

- Social (e.g. reduction in aesthetic value and public safety)
- Economic (e.g. cost to tourism, damage to vessels, fishing gear and facilities, losses to fishery operations, cleaning costs)
- Ecological (e.g. mortality or sublethal impacts to plants and animals through entanglements, physical damage and ingestion including uptake of microplastics including chemical pollutants, assist the invasion of alien species, alter the benthic biocommunity structure).

Definitions of the acceptable levels of harm in these categories and good environmental status must consider impacts as assessed by

- the amount of litter in different compartments of the marine environment (seabed, sea surface, water column, coastline)
- ecological effects of the litter (e.g. plastics ingested by marine organisms; entanglement rates)
- problems associated with degradation of litter (microplastics) as well as social and economic aspects.

An overriding objective for marine litter pollution will be a **measurable decrease** in the total load of litter in the environment by 2020.

Debris are progressively fragmenting in the environment (Colton *et al*. 1974; Thompson et al. 2004). In addition, the use of plastic granules as abrasives in skin cleaning products has increased considerably in recent years. The prevalence of small pieces and granules (<5mm in diameter) varies considerably among habitats. Quantities of plastic microparticles in excess of 100,000 items m^{-2} (Gregory 1978) or 1250 items $250g^{-1}$ of natural material (Zubris & Richards 2005) have been reported, while in intertidal habitats near Plymouth more than 10% small (<5mm) plastic pieces by weight have been reported (Browne et al. in review). As well as these small pieces, in 2004, Thompson et al. reported on the accumulation of microscopic plastic fragments (≥ 20μm diameter) on shorelines and in the water column around the UK (Thompson *et al., 2004)*. Similar debris has been reported in India (Reddy *et al*., 2006) and Singapore (Ng & Obbard 2006) and a recently completed global survey confirmed that polyethylene, polyvinyl chloride and polypropylene fragments are now present on shorelines worldwide (Barnes *et al*., 2009). Production of plastic is increasing rapidly and since conventional plastics will not biodegrade it is inevitable that the abundance of small fragments like these will increase over the next few decades. Such fragments have a considerably larger surface area to volume ratio and hence a greater potential to transport and release contaminants than larger items. In addition, because of their size they are available to a wide range of organisms including deposit feeders, filter feeders and detritivores (Thompson *et al*., 2004). Ingestion of microplastic material therefore presents a likely route by which chemicals could pass from plastics to the food chain. Ryan *et al*. (1988) found a positive correlation between the mass of ingested plastics and PCB concentrations in the fat tissue of Great Shearwaters *Puffinus*

gravis, and presented the first indication that marine organisms can assimilate toxic chemicals from ingested plastics.

A range of potentially toxic chemicals, including flame retardants, plasticizers and antimicrobials are frequently added during the production of plastics. Because of the nature of the plastic surface, hydrophobic pollutants such as PCBs are accumulated on the pellets from the surrounding seawater with concentration factors of up to 10^6. The high pollutant concentrations in the plastic pellets may also be due to the marine microlayer where hydrophobic contaminants are known to be enriched (e.g., Teuten *et al*., 2007; Teuten *et al*., 2009; Endo *et al*., 2005; Ogata *et al*., 2009). Maybe one of the only positive aspects of this is the utility of these particles as monitoring media for contaminants in coastal waters with low-cost of sampling and shipping as compared with conventional monitoring using water, sediment and biological samples.

Recommendation

WGBEC recognises that the field of microparticles is an important research area and would recommend that work in this field (nanoparticles, microplastics) is reviewed at its 2011 meeting. This review should include; studies that are undertaken to understand dose response relationship for microparticles; studies of biological effects from contaminants attached to particles and bioavailability / biomagnification of contaminants in microparticles; and strategies for monitoring micoparticles in the marine environment.

References

Barnes, D. K. A. 2002 Invasions by marine life on plastic debris. Nature, 416: 808–809. (doi:10.1038/416808a).

Barnes, D.K.A. and Milner, P. 2005. Drifting plastic and its consequences for sessile organism dispersal in the Atlantic Ocean. Marine Biology, 146: 815–825.

Barnes , D.K.A., Galgani, F., Thompson, R. C., and Barlaz, M. 2009 Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society B, 1985–1998.

Browne, M. A., Galloway, T., and Thompson, R. 2007 Microplastic—an emerging contaminant of potential concern. Integr. Environ. Assess. Manag. 3: 559–566.

Browne, M. A., Dissanayake, A., Galloway, T. S., Lowe, D. M. & Thompson, R. C. 2008 Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). Environ. Sci. Technol. 42: 5026–5031. (doi:10.1021/es800249a)

Browne, M. A., Galloway, T. S., and Thompson, R. C. in review Influence of size and density on the transportation of plastic debris by wind and tide. Environmental Science and Technology.

Colton,J. B., Knapp, F. D., and Burns, B. R. 1974 Plastic particles in surface waters of the Northwestern Atlantic. Science, 185: 491–497.

Derraik, J.G.B. 2002. The pollution of the marine environment by plastic debris: a review. Marine Pollution Bulletin, 44, 842–852.

Endo, S., Takizawa, R., Okuda, K., Takada, H., Chiba, K., Kanehiro, H., Ogi, H., Yamashita, R. and Date, T. 2005 Concentration of polychlorinated biphenyls (PCBs) in beached resin pellets: Variability among individual particles and regional differences. Marine Pollution Bulletin, 50: 1103–1114.

Katsanevakis, S. 2008. Marine debris, a growing problem: Sources, distribution, composition, and impacts. In: Hofer TN (ed) *Marine Pollution: New Research*. Nova Science Publishers, New York. pp. 53–100.

Katsanevakis, S. 2009. Estimating abundance of endangered marine benthic species using Distance Sampling through SCUBA diving: the *Pinna nobilis* (Mollusca: Bivalvia) example. In: Columbus AM, Kuznetsov L (eds) Endangered Species: New Research. Nova Science Publishers, New York. pp. 81–115.

Masó, M., Garcés, J., Pagès, F., and Camp, J. 2003 Drifting plastic debris as a potential vector for dispersing Harmful Algal Blooms (HAB) species. Sci. Mar., 67: 107–111.

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., and Kaminuma, T. 2001 Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. Environ. Sci. Technol. 35, 318–324. (doi:10.1021/es0010498)

Ng, K. L. & Obbard, J. P. 2006 Prevalence of microplastics in Singapore's coastal marine environment. Marine Pollution Bulletin 52, 761–767.

Ogata Y., Takada H., Mizukawa K., Hirai H., Iwasa S., Endo S., Mato Y., Saha M., Okuda K., Nakashima A., Murakami M., Zurcher N., Booyatumanondo R., Pauzi Zakaria M., Quang Dung L., Gordon M., Miguez C., Suzuki S., Moore C., Karapanagioti H. K., Weerts S., McClurg T., Burres E., Smith W., Van Velkenburg M., Selby Lang J., Lang R. C., Laursen D., Danner B., Stewardson N., Thompson R. C. 2009 International Pellet Watch: Global monitoring of persistent organic pollutants

(POPs) in coastal waters. 1. Initial phase data on PCBs, DDTs, and HCHs. Marine Pollution Bulletin 58 (2009) 1437–1446.

Ryan, P.G. (1988). Effects of ingested plastic on seabird feeding: evidence from chickens. Marine Pollution Bulletin, 19: 125–128.

Ryan, P.G. 2008. Seabirds indicate changes in the composition of plastic litter in the Atlantic and south-western Indian Oceans. Marine Pollution Bulletin 56: 1406–1409.

Ryan, P.G., Connell, A.D. and Gardner, B.D. (1988). Plastic ingestion and PCBs in seabirds: is there a relationship? *Marine Pollution Bulletin*, *19*, 174–176.

Ryan, P.G., Moore, C.J., Van Franeker, J.A., and & Moloney, C.L. 2009 Monitoring the abundance of plastic debris in the marine environment. Phil. Trans. R. Soc. B. 364. (doi: 10.1098/rstb.2008.0207).

Teuten, E. L., Rowland, S. J., Galloway, T. S. & Thompson, R. C. 2007 Potential for plastics to transport hydrophobic contaminants. Environmental Science and Technology, 41: 7759–7764.

Teuten, E. L., Saquing, J. M., Knappe, D. R. U., Barlaz, M. A., Jonsson, S., Björn, A., Rowland, S. J., Thompson, R. C., Galloway, T. S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P., Tana, T. S., Prudente, M., Boonyatumanond, R., Zakaria, M. P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino , Y., Imamura, A., Saha, M., and Takada, S. 2009 Transport and release of chemicals from plastics to the environment and to wildlife. Philosophical Transactions of the Royal Society B, 364: 2027–2045.

Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W. G., McGonigle, D., and Russell, A. E. 2004 Lost at sea: where is all the plastic? Science 304, 838. (doi:10.1126/science.1094559)

Thompson, R., Moore, C., Andrady, A., Gregory, M.,Takada, H., and Weisberg, S. 2005 New directions in plastic debris. Science 310, 1117.

Thompson, R. C., Moore, C., vom Saal, F. S. and Swan, S. H.2009 Plastics, the environment and human health: current consensus and future trends. Phil. Trans. R. Soc. B 364.(doi:10.1098/rstb.2009.0053)

15 Review current knowledge and research on contaminants in eel and associated biological effects; (ToR i).

Comments by WGBEC on recent publications and reports relating to the decline in eel populations during recent years.

Due to the complex life cycle of eels, geographic range and habitat requirements, this species has been difficult to manage and faces a broad range of threats. Threats and potential causes of decline in eels include: overharvesting, habitat loss/degradation, oceanographic conditions, parasites and contaminants. During the 2009 WGBEC meeting, the Report of the 2007 session of the Joint EIFAC/ICES Working Group on Eels was reviewed. It was concluded that a trend is clearly appearing indicating a reduction in the eel populations. Eels are unusual in that their fat content is an order of magnitude higher than that of other fish and that levels of lipophilic contaminants generally reflect the elevated fat content, being 5 to 10-fold higher than in other fish and invertebrates, depending on the contaminant and the species. The elevated lipid content is important as an energy reserve and is regulated through steroidal/endocrine systems, although these fat reserves appear to be declining overall. The relationships between lipid contents and environmental variables have been studied by analysing extensive contaminant datasets, and statistical modelling demonstrates that especially highly chlorinated PCBs, DDT (and related compounds) and Cd have a negative impact on the lipid content of eels (Belpaire and Goemans, 2006; Geeraerts *et al*., 2007). Other characteristics that render environmental evaluation of eels difficult include identification of gender and that eels only reproduce once in each generation. The latter impairs contaminant losses through gonadal releases. PCB-loaded females negatively influence the survival of larvae (Palstra *et al*., 2006). A negative correlation exists between embryo survival time and TEQ levels in the gonads implying TEQ-induced teratogenic effects. The disrupting effects were caused at levels below 4 pg TEQ/g wet weight gonad which are below the EU maximum consumption limit for dioxin in food (Palstra *et al*., 2006). Van Ginneken *et al.* (2009) also indicated that transoceanic spawning migration is altered by PCBs.

The European eel (*Anguilla anguilla*) is now included in the OSPAR list of threatened and/or declining species and habitats and an OSPAR background document on eel is currently being developed (led by France) and publication may be expected in the first half of 2010.

Whilst it was considered that the Report of the 2007 session of the Joint EIFAC/ICES Working Group on Eels thoroughly reviewed much of the available literature, WGBEC in 2009 believed that some areas would benefit from further scrutiny:

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

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

WGBEC 2009 recommended:

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

Considering the very recent reports reviewing available data (Geeraerts and Belpaire, 2009; EIFAC/ICES WGEEL report 2009) WGBEC 2010 supports the recommendations, given by Geeraerts and Belpaire (2009), and made the following comments

- lipid content reductions occurring on large geographical scales should be examined.
- contaminant loads on the large geographical scales require mapping.
- older substances such as PCBs, heavy metals and some pesticides are relatively well studied. On the other hand more extensive research is needed to evaluate how 'newer' substances (e.g. BFRs, bisphenol A, VOCs, PFOS, alkylphenols, phthalates, TBT etc.) are detrimental to eel populations.
- as controlled reproduction of *A. japonica* is now possible on an experimental scale, new opportunities for experimental work on effects of contaminants on the different life stages of eel have become feasible.
- the development of adequate biomarkers with great sensitivity for both the concentration and length of exposure will be another challenge and would allow detection of the genetic variation underlying environmentally dependent fitness traits in eels.
- for the EU-eel recovery plan (European Commission 2007), whose efforts are concentrated on increasing the quantity of silver eels leaving continental waters, it is recommended to include quality aspect in the stock wide recovery plan. Quality targets should include contamination levels, biomarker responses, lipid content and condition. Generating a comprehensive overview of the quality of the silver eel population all over Europe seems to be an essential and urgent objective for the global eel management.

Recommendation

To take forward any future work on contaminants and their effects on eels it is recommended that WGBEC liaise with WGEEL and work intersessionally to progress, (review the most recent information on effects in eels) and report back on developments and research in this area.

References

Belpaire, C., and Goemans, G. 2007. The European eel Anguilla Anguilla, a rapporteur of the chemical status for the Water Framework Directive? Life and Environment, 57: 235–252.

ICES. 2009. Report of the 2009 Session of the Joint EIFAC/ICES Working Group on Eels (WGEEL). ICES Advisory Committee; ICES CM 2009/ACOM:15; REF. ACOM, DFC.; 7–12 September 2009. Göteborg, Sweden. DRAFT.

Geeraerts C., Belpaire. 2009. The effects of contaminants in European eel: a review. Ecotoxicology. DOI 10.1007/s10646-009-0424-0

Geeraerts C, Goemans G, Belpaire C. 2007. (Dutch) Ecologische en cotoxicologische betekenis van verontreinigende stoffen gemeten in paling. MIRA/2007/05; INBO/R/2007/40. INBO, Groenendaal-Hoeilaart, pp 241.

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

Palstra, A.P., Antonissen, E., Clavero, M.E., Nieveen, M., Niemantsverdriet, P., van Ginneken, V.J.T., and van den Thillart, G.E.E.J.M.. 2006. The fate of fat in silver eels: lipid requirements for spawning migration. In: Palstra, A.P. Energetic requirements and environmental constraints of reproductive migration and maturation of European silver eel (Anguilla anguilla L.). PhD dissertation. Leiden, The Netherlands: University of Leiden. 184 pp.

Van Ginneken, V., Dufour, S., Sbaihi, M., Balm, P., Noorlander, K., de Bakker, M., Doornbos, J., Palstra, A., Antonissen, E., Mayer, I. & van den Thillart, G. (2007). Does a 5,500-km swim trial stimulate early sexual maturation in the European eel (*Anguilla anguilla* L.)? Comparative Biochemistry and Physiology, in press, doi: 10.1016/j.cbpa.2007.03.021.

16 Any other business

16.1 Election of Chairperson

John Thain (current Chair), had indicated at previous meetings the need to elect a new Chairperson but no volunteers had been found. After some discussion it was agreed that WGBEC should opt for two co-chairs for the 2011 meeting. Matt Gubbins was unanimously elected as one chairperson and in the absence of another candidate John Thain agreed to act as a supporting co-chair for the next meeting.

16.2 ICES ASC 2010 in Nantes

The Chairperson reminded WGBEC members of the ICES Annual Science Conference in Nantes, France in September 2010. In particular the theme session relating to biological effects and asked for the group to support this if possible.

Details of the session were outlined as described in the ICES call for submissions:-

Session F: Monitoring biological effects and contaminants in the marine environment: where do we go from here? Conveners: John Thain (UK), Catherine Couillard (Canada), and Dick Vethaak (The Netherlands).

Many countries within the ICES community have monitoring programmes to measure chemical contaminants and biological effects in coastal and offshore waters. The programmes are carried out to measure the "health status" of the marine environment and to meet national and international obligations e.g. OSPAR, HELCOM, EU WFD, LEM, U.S. Clean Water Act, Canadian Oil Pollution Act, etc. With the OSPAR QSR 2010 looming and the introduction of the EU Marine Strategy Directive (MSD) it is time to take stock and ask important questions.

Do our measurements tell us anything useful?

- Are they fit for purpose and cost effective?
- Can the data be assessed in an integrated manner?

- Do we have good indicators of environmental "health"?
- Are the indicators being applied for management purposes?
- Are past and current measurements useful for the future and are they broadly applicable?

The emphasis of the theme session will be to address these issues and to this end contributions are invited on:

- Marine chemical contaminant data and their assessment
- Biological effect data and their assessment
- Development and implementation of chemical contaminant and biological effect indicators
- Chemical contaminant and biological effect integrated assessment
- Integrated assessment of chemical contaminant and other environmental stressors
- Use of chemical contaminant and biological effect data for risk assessment purposes
- Management applications.

Contributions as presentations / posters can include case studies, assessment of long-term data sets or just be scientifically stimulating, but must aim to address the issues stated above. In particular, presentations will be highly ranked if they provide good evidence and a strategy for monitoring chemical contaminants and their effects as part of ecosystem health assessment into the future.

John Thain, CEFAS, UK [e-mail: john.thain@cefas.co.uk]

Catherine Couillard, Institut Maurice-Lamontagne, Fisheries and Oceans Canada [e-mail: couillardc@dfo-mpo.gc.ca]

Dick Vethaak, The Netherlands [e-mail: dick.vethaak@deltares.nl]

17 Recommendations and action list

17.1 Recommendations

1 Recommendation (From agenda item 4.4):

WGBEC fully supported the proposed intercalibration exercise (Sept 2010) on lysosomal stability(NRR method) to be held in Alexandria in Italy; the first intercalibration exercise putting together MEDPOL and ICES laboratories. This is an important step forward for harmonisation between OSPAR, MEDPOL and HELCOM biomonitoring activities. WGBEC would recommend that ICES supports this initiative and recommends further support and uptake from organisations and laboratories within these communities.

2 (Recommendation From agenda item 7 & 8):

Recommendations for further WGBEC work areas

During discussion of the future role and scope of WGBEC under agenda items 7 & 8, several new work areas were raised as potential priority areas and of emerging interest to ICES and the group. These are considered in turn below together with consideration of how WGBEC might action these issues at future meetings. WGBEC would recommend these to SGHIE for comment and advice.

Effects on algae / primary production / eutrophication

Primary production by micro algae embodies the carrying capacity of marine and coastal ecosystems and has primarily been linked to nutrient availability in policy studies. However recently it is indicated that certain industrial chemicals (e.g. TBT, PAHs, irgarol, atrazine, azaarenes) may have a direct impact on coastal phytoplankton communities by (photo)toxicity and hence on the carrying capacity on estuarine and marine ecosystems. Recent results already show that Irgarol, a substitute for TBT compounds on ships to prevent anti fouling, already reduces the primary production at very low concentrations in a few days. The impact of toxicants on aquatic ecosystems has been recognized to interact with eutrophication. Also the frequency and intensity of algae blooms are increasing globally, resulting in increased levels of toxin prospected to affect coastal ecosystems. These different chemical stressors (toxicants and natural toxins) are hypothesised to disturb regulatory mechanisms with algae communities, modifying the competitive abilities of individual species and resulting in shifts from highly nutritious to unfavorable algal species that destabilize the food chain. The research on this issue will be of relevance for reaching a good ecological or environmental status. In the Netherlands, several research groups are working on this topic. Dick Vethaak agreed to report on the Dutch progress and provide a short review paper on this subject for WGBEC 2011. Other participants will be asked to provide relevant information on this topic.

Immunotoxicology

A gap in the current battery of recommended techniques for biological effects monitoring is a method for determining the level of immune system suppression potentially caused by toxicant exposure (immunotoxicity). This is a potential missing link in integrated monitoring frameworks between contaminant exposure and fish disease prevalence. A number of techniques are available such as measures of macrophage activity and for the non-specific immune system, but it is not clear how these could be employed in a monitoring context on wild fish rather than by examining effects over a controlled time course following pathogen challenge. WGBEC should review the range of available techniques at its next meeting and consider their applicability in an integrated monitoring context. Some experts in this field could be invited to present this issue to the group. One group member also has experience of using some of these techniques in the field (Andrea Johnson) and may be able to present some of the methodology at the 2011 meeting.

Benthic community structure and its relationship to contaminants

WGBEC would like to review the links between contaminant exposure and effects on benthic community structure. Although not a new area of interest to the group, WGBEC would like to resolve some outstanding areas of uncertainty. Benthic community analysis is a recommended technique for biological effects, however there has been little formal consideration by the group of the evidence for the causal links. WGBEC would like to ask BEWG to review this issue at their 2010 meeting so that it can be considered by the group. Some of the key issues are:

1) To what extent is benthic community structure affected by sediment contaminant levels compared to other environmental factors (sediment structure, organic material etc)?

2) What monitoring data is available from across contamination gradients in the field to support the use of this method as an indicator for contaminant

effects (taking confounding factors such as change in sediment composition across the same gradient into account).

3) To what extent are changes in benthic community structure in offshore environments influenced by lower levels of contaminant exposure?

4) What consideration of contaminant levels has been taken into account in assessment of national benthos monitoring data across the ICES area?

Recommendation: "WGBEC requests that BEWG review the evidence for benthic community structure as a suitable method for monitoring effects of contaminants, taking into account the considerations above and provide WGBEC with a report (or presentation) to consider at their meeting in March 2011"

Comparison of qPCR measurements of mRNA responses to traditional biomarkers

Current technology allows easy measurement of gene expression, also in cases where it may be challenging to determine the concentration of a biomarker protein (e.g. vitellogenin). Gene expression and protein measurements do however reflect different components of a temporal response to contaminant stress. There is a need for a review of relationships between "traditional" biomarker measurements and gene expression as well as suggestions for how to assess the latter in current frameworks and how the two may be combined in environmental assessment.

Species specific differences (including bioassay test animals)

It has been taken for granted that biomarker responses can be more or less directly transferred between different fish or mussel species. This is clearly not the case and there is a need for a review outlining such differences for the relevant biological effects methods.

Most toxicity tests use clonal cultures which may commonly be lab-specific. In contrast, sediment bioassays rely to a large extent on field-collected individuals which may have different nutritional status, reproductive status or contamination history. The advantage of using field-collected individuals is of course that results may be more directly applied to a natural situation, whereas it is less obvious that this will be the case for a more or less homozygous lab-population.

Ocean acidification

One of the likely marine problems associated with climate change is decreasing pH. Consequences for marine organisms could be dramatic. There is a need to consider the existing knowledge concerning ocean acidification, which organisms would be most likely to be most sensitive and possible biomarkers for such effects.

3 Recommendation (From agenda item 10.1):

WGBEC members have been involved with the ICES/OSPAR WKIMON / SGIMC process and there has been important consultation and support between the two groups over the past few years. WGBEC would recommend continued involvement with the work and strive to complete the revision of the integrated strategy as appropriate and where required by SGIMC for completion in 2011.

4 Recommendation (From agenda item 12):

That WGBEC members (and ICES member states) actively submit biological effects monitoring data onto the ICES database using the relevant (v3.2) reporting formats. To assist in this process WGBEC recommends that WGBEC / ICES data centre de-

velop a live 'working document' to be added to at future WGBEC meetings to explain how biological effect data should be entered into the database and keep track of WGBEC advice on database issues.

5 Recommendation (From agenda item 14.2):

WGBEC recognises that the field of microparticles is an important research area and would recommend that work in this field (nanoparticles, microplastics) is reviewed at its 2011 meeting. This review should include; studies that are undertaken to understand dose response relationship for microparticles; studies of biological effects from contaminants attached to particles and bioavailability / biomagnification of contaminants in microparticles; and strategies for monitoring micoparticles in the marine environment.

6 Recommendation (From agenda item 15):

To take forward any future work on contaminants and their effects on eels it is recommended that WGBEC liaise with WGEEL and work intersessionally to progress, (review the most recent information on effects in eels) and report back on developments and research in this area.

17.2 Actions

1 (From agenda item 12)

Develop guidance document on biological effects data submissions to include example data files that comprise multiple effects parameters and BULKID codes to show how to handle data for pooled samples (UK), Supporting parameters for each method, DATSU and FINFL checks for BE data (All WGBEC members)

2 From agenda item 13.2)

WGBEC intends to form a small steering group (KH, JT, and MG) to coordinate QA activities to both develop and deliver a work plan.

18 Adoption of the report and closure of the meeting

Text for the report where available was edited and agreed at the meeting, in particular those items relating to ICES and OSPAR requests. Other editing to be conducted by correspondence.

The Chairperson thanked Michelle Giltrap again for hosting the meeting and the hospitality provided by Trinity College Dublin and finally thanked the group members for their contribution and closed the meeting at 15:00 hrs

Annex 1: List of participants

NAME	ADDRESS	PHONE/FAX	EMAIL
Ricardo Beiras	Universidad de Vigo Campus Universitario C.P. Illa de Toralla, s/n ES-36330 Vigo (Pontevedra) Spain	TEL: +34 647 343 060 FAX: +34 986 498 626	rbeiras@uvigo.es
Johnny Beyer	IRIS Biomiljø Mekjarvik 12 N-4070 Randaberg Norway	TEL: +47 5187 5504 FAX: +47 5187 5540	Jonny.Beyer@iris.no
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
Lisa Devriese	ILVO – Fisheries Ankerstraat 1 8400 Oostende Belgium	TEL. +3259569820 FAX: +3259330629	Lisa.devriese@ilvo.vlaanderen.be
Michelle Giltrap	Zoology Department Trinity College Dublin College Green Dublin 2 Ireland	TEL: +353 1 8962571	giltrapm@tcd.ie
Matt J. Gubbins	Fisheries Research Services Marine Laboratory P.O. Box 101 375 Victoria Road Aberdeen AB11 9DB UK	TEL: +44(0)122 429 5681	m.gubbins@marlab.ac.uk
Halldór Pálmar Halldórsson	Suðurnes University Research Centre University of Iceland Garðvegur 1 245 Sandgerði Iceland	Tel +354 525 5226 GSM +354 848 8811	halldor@hi.is
Andrea Johnson	Department of Natural Sciences LMRCSC, Carver Hall University of Maryland Eastern Shore Princess Anne, MD 21853 USA	TEL:(410) 651-8447 FAX: (410) 651-7739	akjohnson@umes.edu

NAME	ADDRESS	PHONE/FAX	EMAIL
Ketil Hylland	Norwegian Institute for Water Research (NIVA) Gaustadalléen 21 N-00349 Oslo Norway	TEL: +47 22185170 FAX: +47 22185200	ketil.hylland@niva.no ketilhy@bio.uio.no
Kari Lehtonen *By correspondence*	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 UK	TEL: +44 1305 206600 FAX: +44 1305 206601	brett.lyons@cefas.co.uk
Thomas Maes	CEFAS Pakefield Road NR330HT Lowestoft UK	TEL. +44 (0)1502 524433	thomas.maes@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	Concepcion.martinez@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
Johan Robbens	ILVO – Fisheries Ankerstraat 1 8400 Oostende Belgium	TEL. +3259569820 FAX: +3259330629	Johan.robbens@ilvo.vlaanderen.be
Rolf Schneider	Institut für Ostseeforschung Seestrasse 15 D-18119 Rostock Germany	TEL: +49 381 5197 213	rolf.schneider@io-warnemuende.de
Halldóra Skarphéðinsdóttir	Department of Applied Environmental Science ITM Stockholms Universitet 106 91 Stockholm Sweden	TEL: +46 867	halldora.sk@gmail.com

NAME	ADDRESS	PHONE/FAX	EMAIL
John Thain ***(Chair)***	CEFAS Burham-on-Crouch Laboratory Remembrance Avenue Burnham-on-Crouch UK-Burnham-on-Crouch CM0 8AH UK	TEL: +44 (0)1621787239 FAX: +44 (0)1621784989	j.e.thain@cefas.co.uk
Dick Vethaak	Deltares, Unit Marine and Coastal Systems, Section Ecosystem Analysis and Assessment (ESA) Rotterdamseweg 185 2629 HD Delft The Netherlands	TEL: +31 15-2858659 / +31 651232412	dick.vethaak@deltares.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: Terms of Reference for 2010

2009/2/SSGHIE01 The **Working Group on Biological Effects of Contaminants** (WGBEC), chaired by John Thain, CEFAS, UK, will meet in Dublin, Ireland, 10–15 January 2010:

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

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

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

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

e) In close cooperation with ICES / OSPAR SGIMC conduct intersessional work for review at 2010 meeting based on the outcome of the SGIMC Aberdeen Workshop, October 2009.

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

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

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

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

j) Extending marine assessment and monitoring framework used in Chapter 10 of the QSR 2010 (OSPAR request 2010/1)

To review the methodology used by the OSPAR workshop on the development of Chapter 11 of the QSR 2010 (Utrecht workshop)[1] and taking into account, inter alia, ICES work on integrated assessment, provide advice on the following aspects:

1 Although the workshop title referred to Chapter 11. the output has subsequently been reflected in Chapter 10 of the QSR.

i) improvements that could be made to the thresholds between different assessment classes, including any scientific basis for proposed thresholds;

ii) extending the methodology to support the assessment of plankton communities;

iii) improving the method for working at different scales, such as the level of an OSPAR Region, the level of sub-Regions such as the Irish Sea or the Channel or the level of an estuary or an MPA;

k) Report to SSGHIE on potential and current contributions of your EG to the Strategic Initiative on Coastal and Marine Spatial Planning (SICMSP).

l) Report to SSGHIE on your plans to promote cooperation between EGs covering similar scientific issues.

WGBEC will report by 15 February 2010 (via SSGHIE) for the attention of SCICOM and ACOM.

Supporting Information

Priority	The activities of this group will enable ICES to advise on issues relating to the design, implementation and execution of regional research and monitoring programmes pertaining to hazardous substances in the marine environment. To develop procedure for quality assurance of biological effects data and to improve assessments of data relating to the biological effects of contaminants in the marine environment.
Scientific justification	a) It is important for WGBEC to keep track of publication progress with biological effects methods it has sponsored. Protocols are needed for national and international programmes as well as the OSPAR programmes. b) Biological effects data is increasingly being entered into the ICES database and WGBEC is encouraging this and monitors this activity. In addition as more data is being submitted technical queries arise and WGBEC can assist with answering queries from the ICES Data Centre. c) WGBEC has found it of value to discuss, feedback and support national monitoring programmes across the maritime areas and this is a valuable opportunity to improve and harmonise programme designs and assessment of data (e.g. OSPAR / MEDPOL / WFD / HELCOM / EU FWM); d) AQC is vital to support, report and assess data, particularly for cross maritime areas and developments and harmonisation in this area need to be taken forward in a coordinated manner. e) ICES / OSPAR SGIMC have a heavy work programme and WGBEC have noted that this Study Group have already identified tasks for ICES WGBEC, both intersessionally and at the WGBEC meeting. These tasks are not insignificant and WGBEC are willing to provide the support and expertise for taking this important work forward; f) WGBEC last reviewed the list of biological effect recommended and promising monitoring techniques in 2007. There has been considerable developments over three years and WGBEC feels it is necessary to conduct a major review, including the rationale for recommending techniques and how they fit in with SGIMC and EUMSFD activities; g) As information on emerging contaminants becomes available it is important to be in a position to advise and assess their impact on biological systems and the environment and to advise on suitable monitoring techniques, and nanoparticles have been identified as a fast moving research area. h) The ICON demonstration programme and the Baltic Beast programme underpins the integrated chemical – biological effects approach advocated by OSPAR and in the Baltic. WGBEC needs to monitor and evaluate these activities. i) It has been identified (see ICES WGEEL reports) that contaminants and

	associated biological effects may be contributing to the demise in eel populations across Europe and WGBEC will review what research there is available to support this suggestion. j) This is an OSPAR request (2010/1) k) This strategic initiative is currently being planned and suggestions from EGs on their engagement in the SICMSP are sought. l) Collaboration across EGs is encouraged and may be facilitated by e.g. inviting EG chairs and/or key members to attend meetings of your EG, and to use teleconferencing and videoconferencing as means to engage participants remotely .
Resource requirements	The main input to this group is from National experts. Each attendee is self-funded from their own / organisation / institute resources.
Participants	The Group is normally attended by ca. 16 members and guests.
Secretariat facilities	None required.
Financial:	No financial implications.
Linkages to advisory committees	ACOM
Linkages to other committees or groups	There are linkages with WGSAEM, MCWG, WGMS and WGPDMO.
Linkages to other organizations	None identified.

Annex 3: Agenda

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

Dublin, from 11–15 January 2010

1) Opening of the meeting;

2) Adoption of the agenda;

3) Appointment of rapporteurs;

4) Review progress with national /international monitoring activities; to include / integrated assessment / and application of biological effect techniques within OSPAR / MEDPOL / WFD / HELCOM / EU MSD + any other; (ToR c).

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

6) Extending marine assessment and monitoring framework used in Chapter 10 of the QSR 2010 (OSPAR request 2010/1) - To review the methodology used by the OSPAR workshop on the development of Chapter 11 of the QSR 2010 (Utrecht workshop); (ToR j).

7) Report to SSGHIE on potential and current contributions of your EG to the Strategic Initiative on Coastal and Marine Spatial Planning (SICMSP); (ToR k).

8) Report to SSGHIE on your plans to promote cooperation between EGs covering similar scientific issues; (ToR l).

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

10) In close cooperation with ICES / OSPAR SGIMC conduct intersessional work for review at 2010 meeting based on the outcome of the SGIMC Aberdeen Workshop, October 2009.; (ToR e) "to receive Background Documents and draft assessment criteria from ICES WGBEC on:

- Acetyl cholinesterase
- Mussel histopathology
- Micronucleus and Comet assay
- MT and ALA-D
- Intersex in fish"

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

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

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

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

15) Review current knowledge and research on contaminants in eel and associated biological effects; (ToR i).

16) Any other business;

17) Recommendations and action list;

18) Adoption of the report and closure of the meeting

19) WGBEC will report by 15 February 2010 (via SSGHIE) for the attention of SCICOM and ACOM

Annex 4: Tentative timetable

Date	Approx. Time	Agenda Item	Rapporteurs Contributors	Issue
Monday 11 January	09:30	1	JT	Introduction by Chairperson and Michelle Giltrap, housekeeping issues, tour de table.
	10:00	2	JT	Adoption of agenda, tabling of documents
	10:15	3	JT	Appointment of rapporteurs.
		7	DV + KH + JT	Report to SSGHIE on potential and current contributions of your EG to the (SICMSP).
	12:45			Lunch
	13:30	8	DV + KH + JT	Report to SSGHIE on your plans to promote cooperation between EGs covering similar scientific issues.
	17/18:00			Close of business.
Tuesday 12 January	09:00	11	MG	. Review progress with publication and electronic dissemination of biological effects techniques in the ICES TIMES series.
		16	JT	Proposal of new chairperson
		9		Review ICES WGBEC list of recommended biological effects methods for monitoring purposes and define how this fits in for both OSPAR and EU MSFD purposes.
	12:45			Lunch
	14:30	12	TM + MG + RB + TB + liaise ICES	Assess the amount of biological effects data submitted to the ICES database and answer queries / requests from the ICES Data Centre.
	15:30	6	JT + KH + DV	. Extending marine assessment and monitoring framework used in Chapter 10 of the QSR 2010 (OSPAR request 2010/1).
	16:15			Close of business.
Wednesday 13 January	09:00	10	DV	Review SGIMC meeting report and provide support as requested.
		ditto	BL	Micronuclei + Comet
		ditto	HS	DNA adducts
		4a	RB	Review progress with national /international monitoring
	12:45			Lunch
	13:30	5	KH + KL	Review progress with the ICON (NSHEALTH) and Baltic BEAST programme.
		13	AV + CM + JB + KH	Review progress with AQC procedures for biological effect methods and include harmonisation activities within OSPAR, Baltic and MEDPOL maritime areas.
	16:00			Close of business

Date	Approx. Time	Agenda Item	Rapporteurs Contributors	Issue
Thursday 14 January	09:15	4b		Review progress with national /international monitoring activities; within OSPAR / MEDPOL / WFD / HELCOM / EU MSD + any other
		ditto	HS	Sweeden DNA
		ditto	MG	Ireland
		ditto	CM	Spain
		ditto	AV	MEDPOL
		ditto	DV already had	MSFD
	12:45			Lunch
	13:45	15	JR + …???	Review current knowledge and research on contaminants in eel and associated biological effects.
		14	TM	Continue to review of emerging and novel contaminants as they arise….micriplastics
		ditto	JR	…….nanoparticles and emerging contaminats
	16:00	16		Any other business.
		ditto	JT	ICES ASC Nante
	17/18:00			Close of business.
Friday 15 January	09:00	17	JT	Recommendations and action list.
	10:30	18		Adoption of the report.
	12:30			Lunch
	15:00			Closure of the meeting.

Annex 5: Progress in the national programme for monitoring marine pollution in Spain

From agenda item 4.

1. BACKGROUND

Two major biomonitoring programmes, along the Northern Iberian coast and along the Iberian Mediterranean coast have been conducted through several research projects since past decades until 2009, by the Instituto Español de Oceanografía (IEO). In January 2010, an agreement has been finally signed by the *Instituto Español de Oceanografía* (IEO) and the *Ministerio de Medio Ambiente Rural y Marino* (MARM) for 2010-2012 in order to conduct a biomonitoring program to meet the obligations of the both conventions (OSPAR and Barcelona), but also to potentially contribute to the GES assessment in the Marine Strategy Framework Directive.

Recent progress in the Atlantic Spanish marine pollution Monitoring Program, conducted by *Instituto Español de Oceanografía* (IEO) includes the adoption of an integrative approach that includes CEMP chemical methods and pre-CEMP biological methods. In order to establish clear relationships between results of chemical monitoring of pollution and the pollutant concentrations that may cause ecological damage, we are intending to carry out the following actions taking into account the general biological effects considered by the CEMP and PreCEMP: (i) To obtain data for conducting a study on the biological effects of sediment elutriates by using the sea.urchin embryo-larval bioassay; (ii) To obtain data for conducting a study on the toxicity of sediments by using the amphipod survival bioassay; (iii) To conduct a study on the biological effects of chemical pollutants on molecular responses in mussels (GST and AChE).

For the new organisation of the biological effects monitoring in MEDPOL Phase IV (2006-2013), the Contracting Parties to the Barcelona Convention adopted the strategy for the development of Mediterranean Marine Pollution Indicators (MPIs). This strategy will be considered as the basis for the preparation of marine ecosystem health assessments in a manner which could facilitate the development and implementation of a policy for the protection and conservation of the Mediterranean Sea and coastal areas (UNEP, 2003). Therefore, Spanish monitoring research activities in Mediterranean waters were recently also extended with more biomarker measurements in mussels and fish as well as contaminant concentrations in surficial sediments and fish. In order to make an integrated assessment of the quality/health status of the marine ecosystem, chemical contaminant concentrations (mussels, fish and sediments) and biomarker responses (mussels and fish) are analysed in selected areas.

In both programmes, measurements are performed yearly (excepting temporal trends in sediments that are conducted biannually, in the case of the Mediterranean program) and the application of both chemical and biological effect techniques (biomarkers/bioassays) is included (Table 1).

BIOMONITORING IEO	SPANISH ATLANTIC MONITORING	SPANISH MEDITERRANEAN MONITORING
Sediment (S)	Yearly	Autumn (Sept-Oct)
Fish (MB/MM)	Autumn	Autumn (Sept-Oct) Post-spawning
Mussels (MG)	Autumn (Oct-Nov) Pre-spawning	Spring (May-June) Pre-spawning
Sampling NR/NL	Yearly	not
Parameter	Matrix	Matrix
Trace metals	MG/MM/S	MG/MB/S
PAHs	MG/S	MG/MB/S
Organochlorinated Compounds	MG/MM/S	MG/MB/S
BFRs	MG/MM/S	not
TBTs	NR/NL/S	not
Imposex	NR/NL	not
SFG	MG	not
SoS	not	MG
LMS	not	MG
MT	not	MG/MB/S
AChE	MG	MG/MB/S
Antioxidant enzymes	MG*	MG*
GST	MG	MG*
MN	not	MG/MB/S
EROD	not	MB
Genotoxicity	not	MB
Sea urchin Embryotoxicity assay	S	S*
Amphipod bioassay	S	not
CI/CF	MG/MM	MG/MB
GSI	not	MB

MG: ***Mytilus galloprovincialis***
MB: ***Mullus barbatus***
MM: ***Merluccious merluccious***
NL: ***Nucella lapillus***
NR: ***Nassarius reticulatus***
S: Surficial sediments
***pilot study**

2. METHODOLOGY AND WORKING PLAN FOR THE ATLANTIC MONITORING

2.1. Sampling

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

Sediment elutriates intended for embryo-larval bioassays will be obtained following Beiras (2002) by rotatory mixing of 100 g of sediment and 500 ml of control FSW at 60 rpm for 30 min in airtight polypropylene flasks with no head space. After overnight decantation at 20ºC in the dark, the liquid phase (elutriate) is siphoned into a separate beaker and then aerated for 10 min to discard any potential toxicity caused by H_2S.

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

2.2. Study of temporal trends

Data corresponding to temporal trend studies of the monitoring program in 2007 and 2008 will be analyzed in order to fill the data gaps that may exist.

The study of the temporal trends of the sediment toxicity will be carried out through annual sampling cruises in four areas (Vigo, Pontevedra, Gijón-Avilés and Gulf of Cádiz) from 2010 to 2012 (Figure 1).

The study of the temporal trends of the biomarker levels in mussels will be carried out through annual sampling cruises in seven areas of interest, including reference sites (Vigo, Pontevedra, Arousa, A Coruña, Avilés, Santander y Bilbao) from 2010 to 2012 (Figure 2).

2.3. Study of the spatial distribution

Data corresponding to the spatial distribution studies of the monitoring program in 2007 and 2008 will be analyzed in order to fill the data gaps that may exist.

The spatial distribution of the sediment toxicity will be carried out in 2010 on sampling sites along the coast including inner areas in Rías and estuaries (Figure 3).

During 2010 mussels will be collected at the sampling sites indicated in Figure 2 in order to study the spatial distribution of the biomarker levels.

2.4. Sea-urchin embryo-larval bioassay

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

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

MATRIX: Sediments

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

The toxicity study of approximately 50 sediment samples concurrently with chemical data from the sediments will be part of the integrative monitoring program.

2.3. Amphipod survival bioassay

PreCEMP COMPONENT: Amphipod survival study in sediment samples.

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

MATRIX: Sediments

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

The toxicity study of approximately 25 sediment samples concurrently with the study of the associated biota and the chemical data from the sediments will be part of the integrative monitoring program.

2.4. Biomarkers in mussels

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

MATRIX: Biota

The GST enzymatic activity in mussel gills (*Mytilus galloprovincialis*) will be determined following the method of Habig et al. (1974) adapted to microplate. The enzymatic activity will be determined by measuring the increase in absorbance at 340 nm every 20 seconds for 5 minutes. AChE will be measured according to Bocquené and Galgani (1998) and adapted to microplate.

The toxicity study of approximately 50 mussel samples concurrently with the study of the chemical data in mussel tissues will be part of the integrative monitoring program.

3. METHODOLOGY AND WORKING PLAN FOR THE MEDITERRANEAN MONITORING

Current biomonitoring programme conducted in Spanish Mediterranean Waters comprises different partial monitoring programmes and the main objectives to achieve are:

1) The determination of spatial distribution and temporal trends of selected contaminants in mollusc, fish and sediments in coastal, hot spots and reference areas;

2) To seek evidence and assess over time the detrimental biological effects in mollusc and fish.

With such aims, IEO is conducting two field samplings yearly along the Iberian Mediterranean coast. The first field sampling is conducted between 15 May and 15 June (outside spawning period for *Mytilus galloprovincialis*) in order to collect native mussel samples for chemical and biological effects along the Iberian Mediterranean coastline (Figure 4). The second survey is conducted between 1 and 15 October (post-

spawning period for *Mullus barbatus*) in order to collect in coordinated way sediments and also fish samples for chemical and biological effects in selected areas of concern along the Iberian Mediterranean coast.

For chemical analysis and biomarkers in biota, the temporal monitoring programme comprises a number of locations that are sampled yearly, while the spatial monitoring programme comprises a larger number of locations that are sampled once every 5 years (Figure 4).

For chemical concentration in sediments, the temporal monitoring programme will be conducted at least once every 2 years, once the appropriated areas have been identified. At present, a pilot study (2006-2010) is being conducted to identify suitable sediment sampling areas along the Spanish Mediterranean coast with the best characteristics (undisturbed bottoms by anthropogenic activities with high sedimentation rate, percentage of fine fraction and content of organic matter, etc.).

3.1. Integrated assessment

The approach of an integrated assessment of the health status of the marine environment is being stressed also in the MEDPOL Programme and in order to progress on it and optimize the funding resources available, fish sampling is being carried out in a coordinated way with sediment sampling and catching fish from main fish grounds in the regional vicinity of sediment sampling areas. MCBE (IEO) started at 2006 the integrated assessment of the chemical contamination in some selected sites/areas chemically well characterized as hot-spots. In such areas, biomarkers and supporting parameters are measured in fish and/or mussels, and selected contaminants (PAHs, OCPs and trace metals) are measured both in biota and surficial sediments. Surficial sediments samples from the same box corer are being also sampled to perform sea urchin embryo toxicity bioassays. The final objective to achieve in 2012 is to identify the main areas of concern along the inner continental shelf (<70 m) to perform the integrated monitoring approach in fish underpinned with the data obtained from chemical trend monitoring in sediments.

3.2. Caged mussels and two-tier approach:

During last Workshop on the MED POL Biological Effects programme, the use of caged specimens was highly recommended (Alexandria, 2006). Developments in the biomonitoring programme conducted by IEO in the Mediterranean Spanish waters will continue using native mussels for study temporal trends on chemical concentration and the long-term effects on biological effects but also will use transplanting mussels to solve the problem of scarce natural mussel stocks in certain areas. To initiate and validate the use of caged mussels and two-tier approach recommended in MEDPOL Phase IV, the IEO initiated in 2008-2009 pilot field studies using caged mussels at selected locations along the SE Spanish coast. Results of these studies should help to make a final decision if the spatial biomonitoring with native mussels can be simplified using the two-tier approach and if in particular cases, the use of caged mussels is appropriate.

4. References

ASTM, 1995. Standard guide for conducting static acute toxicity tests with echinoid embryos. American Society for Testing and Materials. E 1563-95, pp. 962-980.

Beiras, R., Fernández, N., Bellas, J., Besada, V., González-Quijano, A., Nunes, T., 2003. Integrative assessment of marine pollution in Galician estuaries using sediment chemistry, mussel bioaccumulation, and embryo–larval toxicity bioassays. Chemosphere 52, 1209-1224.

Beiras, R., 2002. Comparison of methods to obtain a liquid phase in marine sediment toxicity bioassays with *Paracentrotus lividus* sea urchin embryos. Arch. Environ. Contam. Toxicol. 42, 23-28.

Bellas, J., Fernández, N., Lorenzo, J.I., Beiras, R. (2008). Integrative assessment of coastal pollution in a Ría coastal system (Galicia, NW Spain): Correspondence between sediment chemistry and toxicity. Chemosphere, 72: 826-835.

Bocquené, G. and Galgani, F., 1998. ICES Techniques in Marine Environmental Sciences. 22, 1-3.

Carr RS (1998) Marine and estuarine porewater toxicity testing. In: Wells PG, Lee K, Blaise CB (eds) Microscale testing in aquatic toxicology. Advances, techniques, and practice. CRC Press, Boca Raton, pp. 523-538.

Habig, W.H., Pabst, M.J., and Jakoby, W.B., 1974. Glutathione S-tranferase. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130-7139.

Stenersen, J., Kobro, S., Bjerke, M., Arend, U. (1987). Comp. Biochem. Physiol C, 86: 73-82.

Weiss C.M. (1958). The determination of cholinesterase in the brain tissue of three species of fresh water fish and its inactivation *in vivo*. Ecology, 39: 194-199.

Kobayashi, N., 1995. Bioassay data for marine pollution using echinoderms. In: P.N. Cheremisinoff (ed.) Encyclopedia of Environmental Control Technology Vol. 9, Chapter 16, pp. 539-609.

His, E., Beiras, R., Seaman, M.N.L., 1999. The assessment of marine pollution-bioassays with bivalve embryos and larvae. In: Southeward, A.I., Tyler, P.A., Young, C.M. (Eds.). Advances in Marine Biology, vol. 37. Academic Press, London, pp. 1-178.

Vidal-Liñán, L., Bellas, J., Campillo, J.A., Beiras, R. (2010). Integrated use of antioxidant enzymes in mussels, *Mytilus galloprovincialis*, for monitoring pollution in highly productive coastal areas of Galicia (NW Spain). Chemosphere, 78: 265-272.

Thain, J., and Roddie, B. 2001. Biological effects of contaminants: *Corophium* sp. sediment bioassay and toxicity test. ICES Techniques in Marine Environmental Sciences No. 28, 21 pp.

Figure 1. Sampling sites for the study of temporal trends of sediment toxicity in the Atlantic coast of Spain using sea-urchin and amphipod bioassays.

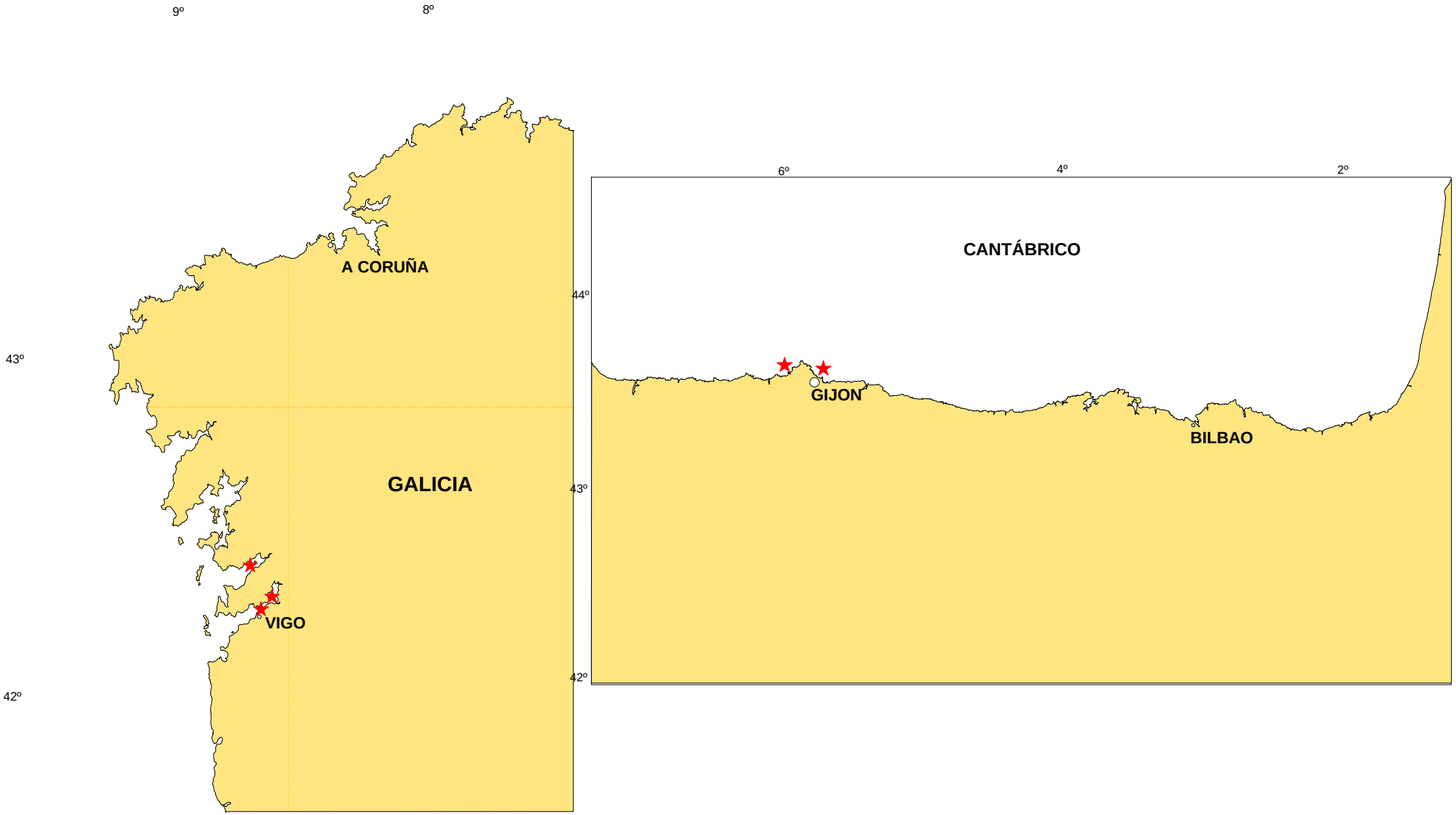


Figure 2. Sampling sites for the study of temporal trends of mussel (*M. galloprovincialis*) biomarkers (GST and AChE) in the Atlantic coast of Spain.

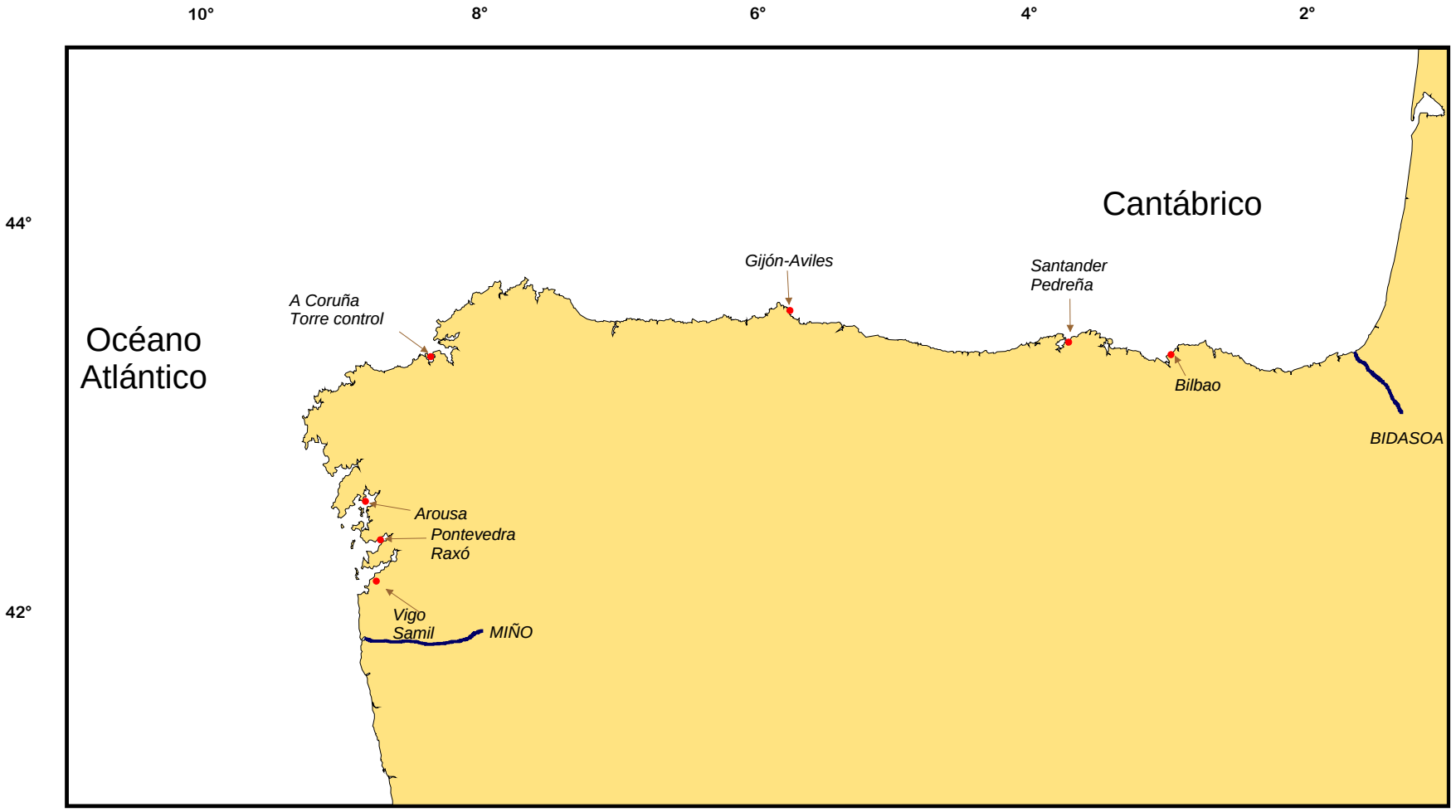
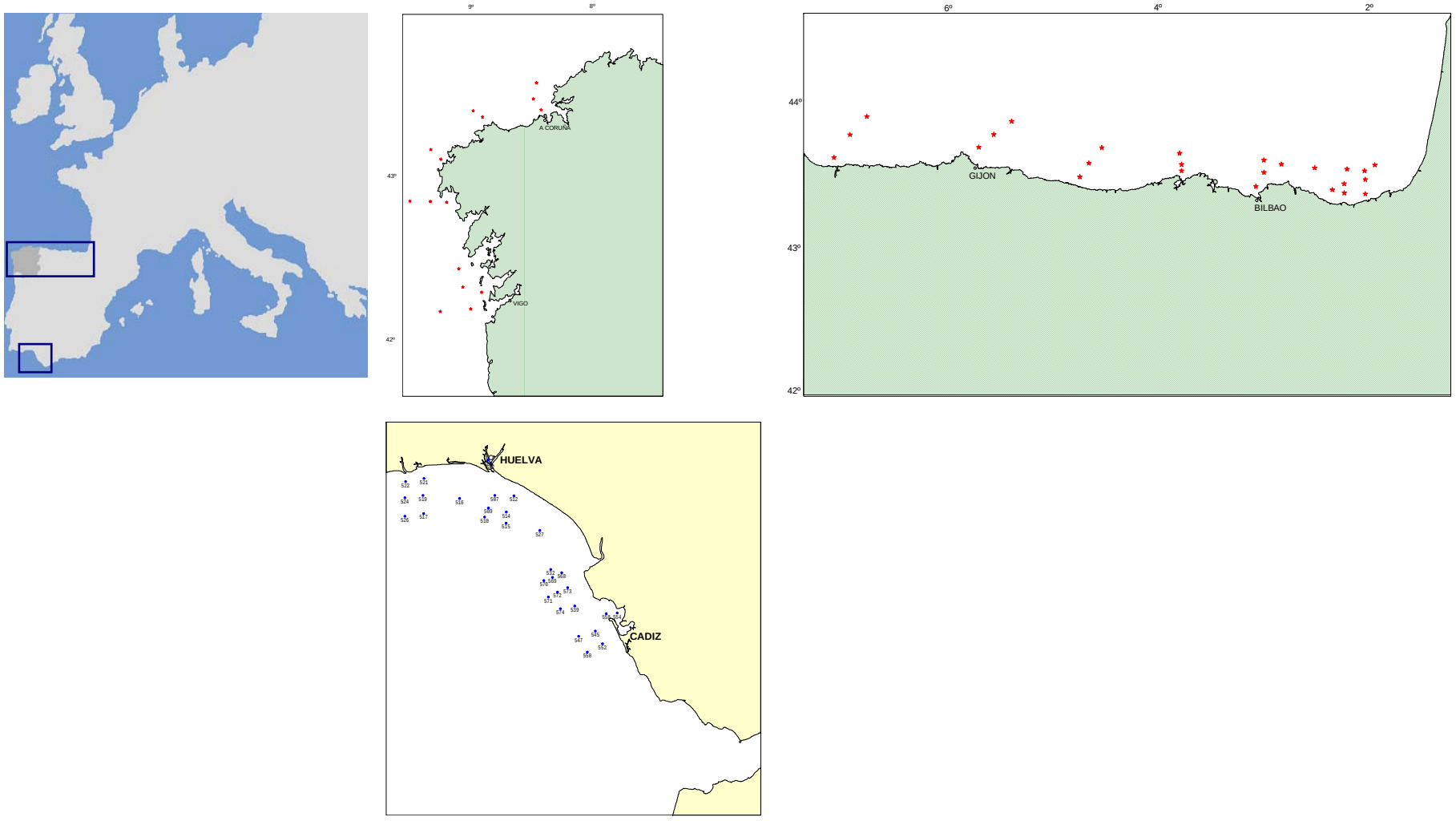


Figure 3. Sampling sites for the study of the spatial distribution of sediment toxicity in the Atlantic coast of Spain using sea-urchin and amphipod bioasays.



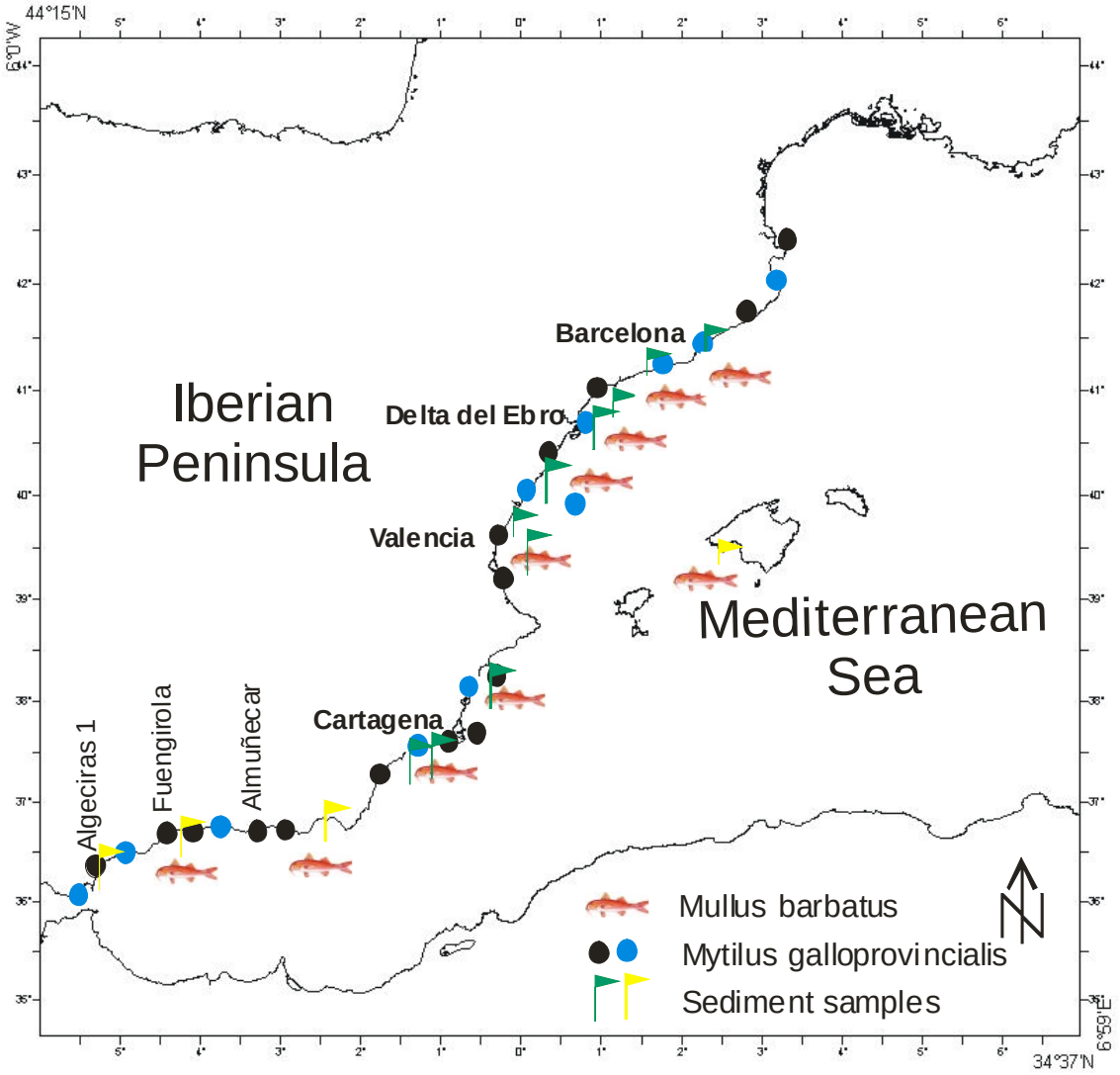


Fig. 4 Sampling sites corresponding to field samplings conducted since 2006 along the Spanish Mediterranean coast by the Instituto Español de Oceanografía (IEO): Mussel sampling stations (only contaminant concentrations in black circles; contaminants and biomarker responses in mussels in blue circles), contaminant content and biomarkers in fish and contaminant concentrations in sediments (yellow flags stations proposed to be sampled in the period 2010-2012).

Annex 6: From agenda item 4, MSD executive summary

Executive summary

We recommend that the assessment of achievement of GES under MSFD Descriptor 8 "Concentrations of contaminants are at levels not giving rise to pollution effects" should be based upon monitoring programmes covering the concentrations of chemical contaminants and also biological measurements relating to the effects of pollutants on marine organisms in each of the assessment Regions. The combination of conventional and newer, effect based, methodologies , with the assessment of environmental concentrations of contaminants provides a powerful and comprehensive approach. As the occurrence of adverse effects at various levels of organisation (organism, population, community, ecosystem) needs to be avoided, monitoring schemes should also indicate the approaching of critical values as early warning.

Therefore, for the purpose of implementing Descriptor 8 under the MSFD, three core elements of data assessment are recommended:

- Concentrations of contaminants in water, sediment and biota are below assessment thresholds identified on the basis of toxicological data.
- Levels of pollution effects are below assessment thresholds representing harm at organism, population, community and ecosystem levels.
- Concentrations of contaminants in water, sediment and biota, and the occurrence and severity of pollution effects, should not be increasing.

Monitoring programmes should include the assessment of concentrations of priority contaminants in environmental matrices, i.e. water, sediment, and the tissues of biota. Monitoring programmes should also include the quantification of biological effects of contaminants at different levels of biological organisation. The selection of priority contaminants, monitoring species and biological effects measurements should be made for each assessment Region by the Member States with responsibility for implementation of MSFD in each Region. Therefore, the priority monitoring matrices, and chemical and biological measurements made may vary between assessment Regions in response to Regional concerns and environmental conditions. However, monitoring and assessment should be harmonised to the greatest possible degree between assessment Regions.

Monitoring data should be interpreted against the objective described by Descriptor 8 through a series of assessment thresholds, expressed as concentrations of chemical contaminants, or levels of biological response. In particular, monitoring data should be interpreted against assessment thresholds that are designed to protect against the occurrence of pollution effects. Examples of suitable assessment thresholds include Environmental Quality Standards (EQS) derived under the WFD, Environmental Assessment Criteria (EACs) as defined within OSPAR for water, sediment and biota, and parallel assessment thresholds used by other Regional Conventions or Member States for the interpretation of monitoring data. Biological effects will be assessed against threshold levels of response that are indicative of significant harm to the organisms concerned. The aim is to prevent pollution effects occurring at the organism, population, community and ecosystem level.

In addition, monitoring data should be assessed against background concentrations of contaminants or levels of biological response to enable added-risk approaches to be used in the derivation of assessment thresholds, to enable greater use to be made

of monitoring data in interpreting the causative agents of pollution effects, and to give early warnings of potential developing problems.

Increasing contaminant concentrations increase the likelihood of pollution effects. In order to minimize the risk of deleterious effects, concentrations of contaminants in water, sediment and biota, and the occurrence and severity of pollution effects, should not be increasing. Regional Conventions have developed robust statistical approaches to the analysis of time series of monitoring data to detect significant trends over time. These should be applied to chemical and biological effects monitoring data.

The integration of the results of chemical monitoring programmes, and combination of data from chemical and biological effects monitoring, is an active area of science within the Regional Conventions (i.e. OSPAR, HELCOM, MEDPOL). Current experience indicates that integration is greatly facilitated by coherent and consistent sets of assessment thresholds (EQSs, EACS, etc). Further development work is necessary, through the EU, Regional Conventions or MS, to expand the range of assessment thresholds to include a greater number of contaminants and biological effects. Integrated monitoring programmes, data collation, interpretation and presentation schemes are being developed and applied by the Regional Conventions, and we recommend that this work continues and that Member States apply the best international advice applicable to MSFD Regions for which they have responsibility.

A core of both chemical analytical methods and biological effects methods exists which can be applied now. There are considerable benefits to be gained from the international experience in programme design, measurement methodology and data management and interpretation available from the Regional Convention programmes, and the EU (e.g. WFD). Detailed implementation of programmes for MSFD Descriptor 8 should build upon these, and upon existing data, to ensure that assessments against GES as robust as possible. However, marine monitoring science continues to develop, and the implementation strategy for MSFD should allow for programmes and procedures to evolve with time so as to maintain and improve the level of protection for marine ecosystems.