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Report of the ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-sea Areas (WKIMON III)

16–18 January 2007

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Contents

1 Opening of the meeting (Annex 1)

The meeting was opened at ICES Headquarters at 0900 hr on 16 January 2007 by Michala Ovens, who welcomed the delegates on behalf of the ICES Secretariat and provided information on the ICES computer network and various domestic matters. Twenty-one participants from 9 countries attended the meeting.

2 Adoption of the agenda (Annex 2)

Prior to the adoption of the agenda, Richard Emmerson of the OSPAR Secretariat gave a talk summarising the position and role of WKIMON in the OSPAR system, the importance of the Group to the OSPAR assessment processes, and the progress that had been made since WKIMON II.

The agenda was then accepted.

3 Review and note the Terms of Reference (WKIMON 7/1/2).

The terms of reference as provided by both OSPAR and ICES were reviewed and it was agreed that they were reflected in the agenda (Annex 2).

4 To note background documents, tabled presentations and suggested timetable and deliverables.

Draft Background documents for several determinands were presented at WKIMON II, and comments made regarding redrafting and other amendments. The expanded and revised suite of documents was made available to WKIMON III. New Background Documents for lysosomal stability and for DNA adducts of PAHs are presented in Annexes 3 and 4. Further new Background Documents on CYP1A (EROD) and bile metabolites of PAHs are included in Annexes 5 and 6.

5 Position paper: Review and comment on a position paper on the status of biological effects methods.

The authors of this document had been unable to progress its preparation before WKIMON III, primarily because the revisions and resubmissions of many of the Background documents had not been completed according to the timetable set out after WKIMON II.

6 Assessment criteria: Develop proposals for assessment criteria for biological effects methods under the CEMP on the basis of initial proposals in the available background documents and to develop additional proposals for assessment criteria where initial proposals are not available.

OSPAR (through SIME, MON and ASMO) have expressed a desire for several years to undertake an assessment of the biological effects data covering the Convention area. To date, this has not been possible for various reasons, including the rather small amount of information held in the ICES database, and the lack of assessment criteria. An inventory was prepared of the effects data held by ICES (Table 1 below). WKIMON was surprised and encouraged by the significant recent increases in the volume of effects data held by ICES, but noted that the data were probably concentrated in particular parts of the Convention area. In addition, several members of the WG indicated that they expected to be able to submit data to ICES soon, (which included VTG , DNA adducts, sediment bioassay, fish reproduction and

lysosomal latency data) and this served to emphasise the importance of developing assessment criteria.

Table 1 Progress of biological effects data submissions to ICES databases by Contracting Parties

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

As an introduction to the further development of assessment criteria for biological effects methods, WKIMON III received 3 presentations, which are summarised below:

a Background document on DNA adducts (Annex 7)

Brett Lyons, CEFAS, UK.

In the chemical carcinogenesis model, the initiating step is the covalent modification of DNA by a carcinogen (Miller and Miller, 1981). The measurement of covalent structures formed between environmental carcinogens and DNA, termed DNA adducts, can be utilised as a biological marker of exposure to genotoxic compounds. DNA adducts can be removed by cellular repair processes or by cell death, but during chronic exposures they often reach steady state concentrations in carcinogen target tissues such as the liver. As a consequence, DNA adducts have several important features which make them suitable as biomarkers of carcinogen exposure. For example, it is a quantifiable measurement of the biologically effective dose of a contaminant reaching a critical cellular target and therefore a useful

epidemiological biomarker for detecting exposure to environmental genotoxins. The document presented is offered to OSPAR as a Background Document on DNA adducts.

b) Predicting health of the environment: Lysosomal reactions in mussels and fish (Annex 8)

Michael Moore, Angela Köhler, Aldo Viarengo and David Lowe

The lysosomal-autophagic system appears to be a common target for many environmental pollutants as lysosomes accumulate many toxic metals and organic xenobiotics, which perturb normal function and damage the lysosomal membrane. In fact, lysosomal membrane integrity or stability appears to be an effective generic indicator of cellular well-being in eukaryotes. In bivalve molluscs and fish, lysosomal membrane stability is correlated with many toxicological responses and pathological reactions, and can also be mechanistically linked with many of these processes. Cellular changes, such as reduced lysosomal membrane stability, lipofuscin (age or stress pigment) accumulation and other lysosomally related assays, are good indicators of cell injury and animal health status. In a situation where exposure to environmental stressors is likely to be sustained, lysosomal biomarkers can be used to predict that further pathological changes will occur. Lysosomal and autophagic functional perturbations also appear to have potential as a measure of damage to ecological health, although caution is required in interpretation of data in respect of possible selection for tolerant phenotypes.

c) A model to predict background responses of EROD in female Dab (*Limanda limanda*) (Annex 9)

Ulrike Kammann

EROD is a well known biomarker and a lot of research work has been done during the last 15 years. It has been shown earlier that background variations of EROD in dab differ between seasons (Kammann *et al.*, 2005). Lange *et al.* (1995, 1998) have shown that water temperature exhibits a strong influence on the regional variability in activity of the 7-ethoxyresorufin-*O*-deethylase (EROD) in the liver of dab from the German Bight during the spawning and post spawning seasons. The correlation between EROD activity and temperature could not be explained by a direct temperature effect in terms of temperature compensation. Instead, the authors concluded that temperature influences EROD activity indirectly via its influence on the duration of the gonadal cycle and thus on the time of spawning, which may be coupled with the seasonal variation in EROD activity. The authors calculated the sum of month degrees from the bottom water temperature and adjusted the annual EROD cycle to this biological time scale. Thus a comparison of EROD data from different sites became possible. The sum of month degrees at the day the fish was caught is defined as the sum of mean month temperatures on a daily base starting at the 1st of September in the year before (this is the date of the assumed start of the spawning cycle).

Ulrike Kammann presented an application of this model to calculate background concentrations for EROD in the German Bight. The parameters of the model were fit to a data set from 1997/1998 concerning female dab 20–25 cm caught in different seasons. Monitoring data from other years which were inside the 95% confidence interval meets the prediction of the model. These data could therefore be regarded as lying in the range of background variation. Data outside this interval can be interpreted as effects. In Fig xx the model and some monitoring data are presented. 3 Data points could be identified as lying significantly outside the prediction.

Using this model it is possible to:

1) compare EROD values from different sites covered in one survey, and to

2) compare EROD values from different years or seasons.

The availability of suitable bottom water temperatures on a monthly base from the time before the fish were caught is a preposition. We used bottom water temperatures from the "Feuerschiff Deutsche Bucht", a fixed measuring device providing online bottom temperature

data for the German Bight, and temperature data from a model driven by surface temperature data observed by satellite. This model provides bottom temperature data for the whole North Sea in a good geographic resolution since 1999. Both temperature data sets were provided by the German Hydrographic Maritime Agency in Hamburg.

Model formula:

$$EROD = \frac{B}{\sqrt{2*\pi}*C*M} * e^{-\frac{(\ln M - \ln M_0)^2}{2*C^2}} + A*\left(1 - e^{-\frac{(M - M_1)^2}{2*D^2}}\right)$$

Parameters : M_0: 88,9; M_1: 58,8; A: 125; B: 14400; C: 0,134; D: 10,6. M: Month degrees

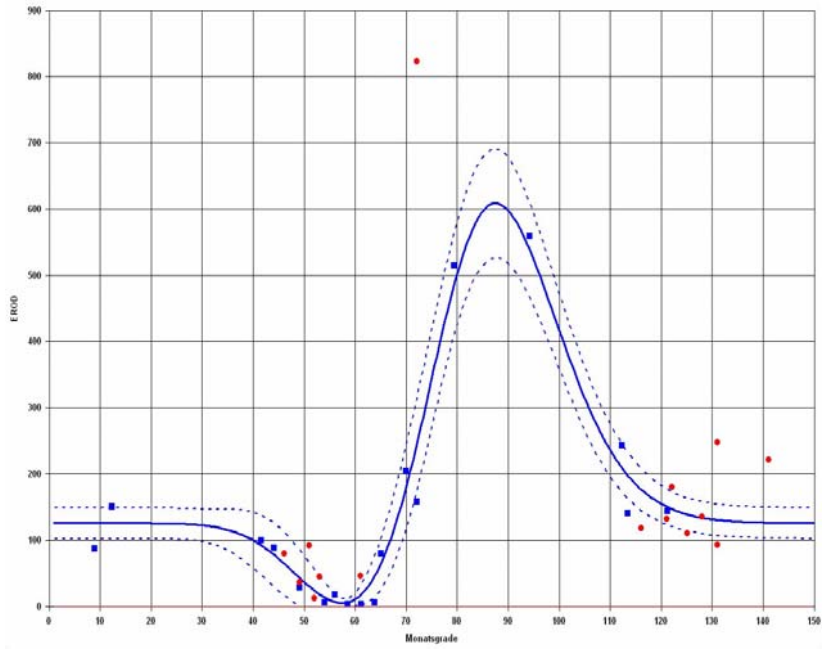

Fig. Xx: EROD Model (solid line) with 95% confidence intervals (dashed lines) for female dab from the North Sea. Model data from 1997/1998 (blue) and other monitoring data (red). Red dots above the curve were obtained in Mai 1999, August 2002 and September 2004. All dots a mean values from about 20 fish each.

Restrictions

The model was developed using a data set from 1997/1998 from the German Bight (383 female dab 20–25 cm). This selection was done due to the availability of data. Other regions or years may be a better choice for defining general background conditions. The quality of the model predictions depends on the availability of reliable bottom water temperatures for the year prior to the prediction. The model is at the moment the best tool we have to interpret monitoring data, however, it should be confirmed by a second data set. It is also possible that parameters additional to the temperature cycle may improve EROD prediction.

How to evaluate EROD data

Take the mean EROD value in pmol/(mg min) and calculate the corresponding month degrees by adding the mean bottom water temperatures from the months before sampling starting at the 1st of September. Put this data point into the curve presented in Fig. Xx and decide if it is inside or outside the 95% interval. A value outside the confidence interval indicates the presence of additional influence factors.

Application to other biomarkers

It is well known that other biomarkers also show annual cycles (Lacorn *et al.* 2001). Therefore the concept of a background model could be also applied to other parameters showing a significant annual cycle.

References:

Kammann U, Lang T, Vobach M, Wosniok W (2005) Ethoxyresorufin-O-deethylase (EROD) activity in dab (Limanda limanda) as biomarker for marine monitoring. Environ Sci Pollut Res, 12 (3) 140–145.

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

Lange U, Saborowski R, Siebers D, Buchholz F, Karbe L (1998) Temperature as a key factor determining the regional variability of the xenobiotic-inducible ethoxyresorufin-O-deethylase activity in the liver of dab (Limanda limanda). Canadian Journal of Fisheries and Aquatic Sciences 55 (2): 328–338.

Lange, U., Saborowski, R., Karbe, L., Siebers, D. (1995)A model for the Prediction of the Basal EROD-Activity Levels. ICES C.M. (E7).

Discussion

It was noted in discussion that assessment criteria were well established for TBT effects in gastropods, and more extensively for contaminant concentrations in sediment and in biota. The latter were of two types, firstly Background Concentrations (BCs) and associated Background Assessment Concentrations (BACs) which represented conditions where anthropogenic influence was absent or of low significance (e.g. pre-industrial concentrations in sediment cores, or concentrations in biota from remote areas). Secondly, there were Environmenta; Assessment Concentrations (EACs), concentrations below which adverse effects arising from the contaminants in the organisms concerned were unlikely to occur. Patrick Roose gave a description of the ways in which Groups such as WGMS, OSPAR MON etc had estimated BCs and BACs.

It was possible to conceive of analogous criteria for some biological effects. However, in most cases (e.g. EROD, Mt, bile metablites), it was likely to be easier to estimate the BC analogue and the equivalent of EACs. In some cases, the measures were more closely related to higher level effects and EAC-equivalents could be considered.

Patrick Roose described to the meeting how BCs and BACs had been derived for contaminant concentrations in sediment, fish and shellfish, by various ICES WGs and by OSPAR MON. BCs were estimated as the median of the medians of data sets from areas considered by data submitters to be reference/background areas. The BACs were derived from time series of data and took account a wide range of sources of variance in monitoring data and allowed simple "Green" tests to be applied to determine whether concentrations were at or close to background.

The meeting then separated into a series of Sub-Groups to review the background documents, and, in particular, to elaborate the assessment criteria proposed in the documents, or to propose criteria where none were suggested in the current texts. This process used data held in the ICES data base and additional data sets brought by contracting parties / scientists to the meeting. The composition of the sub-groups was as below:

Sub groups established at WKIMON III to develop assessment criteria

INTEGRATION DOCUMENT	COLIN MOFFAT	POSITION PAPER	JOHN THAIN KETIL HYLLUND
Sub-Group A EROD DNA adducts Bile metabolites	Ulrike Hammann Kris Cooreman Brett Lyons Jacqueline Jones Colin Moffat Thierry Burgeot Martin Larsen Ketil Hylland Patrick Roose	**Sub-Group B** Bioassays	Dick Vethaak Jose Fumega Ricardo Beiras Foppe Smedes John Thain
Sub-Group D Lysosomal stability/ neutral red retention time	Mike Moore Doris Scheidek	**Sub-Group C** Pathology Fish reproduction VTG	Grant Stentiford Werner Wosniok Steve Feist Jakob Strand

A series of proposals for assessment criteria were developed by the above Sub-Groups during the meeting, and are included as Annexes 14, 15 and 16 for Sub-Groups A, B and C respectively. Assessment criteria proposals from Sub-Group D are included in the Background Document for lysosomal stability (Annex 3).

Sub-Group A:

- Background concentrations for EROD are 80, 40 and 10 pmol/mg protein for cod, dab and flounder respectively, for fish collected out of the spawning season (months August - November). In order to use data from other times of the year, there is a need to extend the current model to take account of water temperature, spawning seasons and other factors. WKIMON recommends that an ad hoc OSPAR / ICES study group is formed to take this work forward intersessionally.
- For bile metabolites of PAHs, a provisional background response concentration of 220 and 0.95 1-OH pyrene (ug/ml; 341/383 nm fluorescence) for dab and cod respectively was derived. It is recommended that further work is conducted to validate these values and assessment criteria and Ketil Hylland (NO) and Dick Vethaak (NL) volunteered to take this forward intersessionally.
- Background response values for DNA adducts of PAHs are 7.86, 6.84 and 7.90 nmol adducts / mol DNA for dab, haddock and saithe respectively

From Sub-Group B:

- Three assessment classes were derived for sediment and water bioassays; a background response, a warning level and a level of serious concern. For the sediment bioassays (*Corophium* sp. and *Arenicola* sp.) the background responses were 0-30% and 0-10% mortality respectively, the level of serious concern was 100% mortality and the warning level between these values. For the water bioassays (*Tisbe* sp., *Acartia* sp., sea urchin and bivalve larvae) the background responses were 10%, 10%, 10% and 20% mortality (or deformity as appropriate) respectively; the level of serious concern was 100% mortality, and the warning level between these values.

For Sub-Group C:

- A fish disease index (FDI) has been developed by WGDPMO for the assessment of fish diseases (external and histopathological). WKIMON fully supported this

approach and recommended that WGDPMO complete the outstanding work to allow for its full implementation. Once completed, it should be forwarded to OSPAR for consideration.

For Sub-Group D:

- Background responses and assessment criteria for lysosomal stability was derived for the cytochemical and Neutral Red Retention (NRR) methods. For all species, the three levels of response were; i) background, ii) stressed but compensating and iii) severely stressed probably exhibiting pathology. The values recommended for adoption are: i) > 20 mins ii) <20 – >10 mins and iii) < 10 mins for the cytochemical method. For the NRR method, the recommended values are i) >120 mins ii) <120 – >50 mins and iii) <50 mins

WKIMON recommended that analogues of Background Assessment Concentrations for biological effects be developed intersessionally and collaboratively by members of WKIMON and ICES WGSAEM. WKIMON recommended that the assessment criteria developed at WKIMON III should be used on a trial basis in data assessments (e.e. by OSPAR MON 2007) to obtain experience of their use and indications of where further work is required.

Progression of development of guidance on integrated monitoring and assessment programmes.

A further series of three presentations was given, which had greater emphasis on the integration of data as background to the development of the guidance document on integrated monitoring and assessment. Summaries of the presentations are given below:

a) Fish disease – a top-level marker for marine health assessment. (Annex 10 and 11)

Grant Stentiford and Steve Feist, CEFAS Weymouth Laboratory, UK

Assessments of anthropogenic effects of contaminants on marine ecosystems require holistic and integrative approaches that allow for the combination of complex multivariate data. Specifically, chemical and biomarker data can be combined to assess the effect of exposure on the physiology of animals living within the marine ecosystem. Alternatively, expression of biomarkers can be monitored in order to inform on likely exposure to specific (unmeasured) contaminant types. Whilst several approaches to data analysis are available, multivariate approaches (such as those offered by software packages such as PRIMER^{TM}) are an accessible way to combine complex datasets and by identifying relationships between them, providing capacity for interpretation.

Fish diseases have been used for several decades to inform upon the general health status of commercial and non-commercial sentinel species. Since 2000, diseases of European flatfish (dab and flounder) have been monitored using international quality assurance standards developed under the BEQUALM program. Under the auspices of the Clean Safe Seas Environmental Monitoring Program (CSSEMP) (previously the National Marine Monitoring Program, NMMP) of the United Kingdom, external diseases and those specific to the liver (including cancer) are recorded alongside a suite of biomarkers, contaminants and other environmental variables. In this way, monitoring (and associated data collection) is carried out in a fully integrated manner.

Considering population disease status as a top-level indicator of historic exposure to stressors (including those of anthropogenic origin), we have developed a strategy based upon the use of multivariate analysis to investigate relationships between liver disease and chemical/biomarker measures collected simultaneously. Using this strategy it is possible to identify sites (potentially geographically isolated) with similar disease profiles and to use these profiles to assign a simple site classification (Type A, B and C) (see below). In particular, we have assigned Types based upon the degree of pre-neoplastic and neoplastic pathologies observed in the livers of fish captured therein. Once Types have been assigned, it

is then possible to cross-correlate specific effect biomarkers and contaminants to further classify the site profile (and potentially to investigate causality). Furthermore, comparison of site type profiles to apparently non-related data (e.g. oceanographic, population genetics etc.) can also be incorporated to offer further explanation to the observed pattern. Multi-year analysis using this approach allows for assessment of health trends over time.

TYPE A	TYPE B	TYPE C
• Generally low levels of ICES external diseases and almost complete absence of skin hyperpigmentation. • Approximately 30% of fish with no indication of BEQUALM liver pathology categories. • Low prevalence (<5%) of toxicopathic lesions and approximately 50% prevalence of inflammatory lesions (according to BEQUALM). • Low prevalence of FCA (<15%), benign tumour (<5%) and malignant tumour (0%) according to BEQUALM.	• Appearance of higher prevalence of ICES external diseases (incl. Lymphocystis and skin hyperpigmentation, the latter up to 20% at North Sea sites). • Between 10 and 20% of fish with no indication of BEQUALM liver pathology categories. • Low prevalence (generally <5%) of toxicopathic lesions but an elevated prevalence of inflammatory lesions (up to 90%) compared to Type A sites. • Prevalence of FCA can exceed 15% with mean benign tumour prevalence >10%. Appearance of malignant tumours at low prevalence.	• Elevated prevalence of several ICES external diseases (incl. Ulceration, parasites and skin hyperpigmentation, the latter up to 50% at some Dogger sites). • Low prevalence (<10%) of fish with no indication of BEQUALM liver pathology categories. • Prevalence of toxicopathic lesions generally >5% with prevalence of inflammatory lesions up to 100%. • High prevalence of FCA (>50%) of several types. Mean benign tumour prevalence of >15% (up to 25%) and malignant lesions more common but still relatively infrequent (up to 6%)

Successful integrated monitoring is based upon the use of ecologically familiar sentinels on which quality assured multivariate data is collected, stored and analysed. We consider disease as a top-level effect of exposure to the marine environment and to anthropogenic amendments made to it. Disease offers an assessment of health status at the population level and shifts in disease status can be used to monitor larger scale changes in the wider marine environment. Simultaneous measurement of contaminants and exposure biomarkers will allow us to assign which of these changes are due to natural processes and which are due to anthropogenic inputs.

The Fish Disease Index (FDI) currently being develop under ICES WG PDMO will provide an assessment tool for fish diseases. Data generated using the FDI will be directly applicable to multivariate analyses and will provide further clarification on the link between population health status and anthropogenic inputs across the OSPAR maritime area.

b) The Fish Disease Index as an assessment tool (Annex 12)

Werner Wosniok, Germany.

Fish disease prevalences are important markers of marine environmental health. They have been monitored since the early 1980's, following standard protocols, the resulting data is fed into the ICES database and summary reports are produced regularly by the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO). In order to provide a comprehensive summary of fish disease status, jointly for a large set of diseases and fish individuals of both sexes and all sizes, the WGPDMO developed a Fish Disease Index (FDI) (WGPDMO Report 2006, ICES 2006/MCC:01). This index and its use as an assessment tool were presented by W. Wosniok.

The present version of the FDI is constructed to summarize diseases of the common dab (*Limanda limanda*). The common dab is affected by a variety of externally visible diseases

and parasites as well as by a wide range of liver pathologies, including neoplastic changes, at varying degree of severity/intensity. Three categories of diseases are included in the construction of the FDI:

- externally visible diseases,
- macroscopically visible liver neoplasms > 2 mm in diameter (previously termed liver nodules > 2 mm), and
- histopathological liver lesions (early non-neoplastic toxicopathic lesions, pre-neoplastic lesions (foci of cellular alteration, FCA), benign tumours, malignant tumours).

These conditions enter the FDI with different weights, which reflect the severity of the conditions. Weights are given by experts' judgements. The FDI is then calculated for each individual fish by summing the weights of those diseases that were found on the fish and then normalized to a range between 0 and 100. A spreadsheet illustration of the calculation procedure is given in the WGPDMO 2006 report (p. 76–83). The FDI for a fish population is the mean of the individual FDI's. Four versions of the FDI have been formulated so far, one for each of the above disease categories and a summarizing version for all categories jointly.

As disease prevalence and parasite presence depend on sex and size (or age) of the fish, the FDI of several populations will in general differ simply as a consequence of different sex and length distributions in the populations. This can be compensated for by an adjusted FDI, which contains an additional length adjustment factor for each disease. This factor is small for fish lengths for which the disease is frequent, and high otherwise. The factor values are derived as reciprocals of the prevalence-length relation observed in a large reference data set. Also the adjusted FDI is normalized to a range of 0–100.

The construction principle of the FDI is universal in the sense that it can be carried over to other parameter (combinations) for which assessment tools are required. It is in fact frequently used in the field of (human) medicine in order to define action limits for medical interventions.

The FDI for externally visible diseases was calculated for diseases of dab (*Limanda limanda*) in the North Sea (data from the ICES Data Centre with additional information provided by the German Federal Fisheries Research Centre, for details see the section on the sue of the FDI as an assessment tool in this report). Inspection of the FDI time series revealed that (i) temporal trends and (ii) annual cycles exist, and that (iii) temporal trends and annual cycles are specific for area (ICES statistical rectangles). Based on these findings, a procedure to categorize FDI values into 3 assessment classes was proposed and applied to the current data (see the corresponding section in this report for sample results). Once the necessary weights and correction terms included in the FDI are finally established (expected to be finalized under the auspices of the WGPDMO in 2007), the FDI based assessment procedure can be implemented either on the side of the data recording, even onboard ship, or it can be implemented at ICES, where it could become a standard data product. Tasks to be accomplished by the WGPDMO in order to establish a functional version of the FDI are to review the current techniques applied for length, sex and season adjustment, and for the derivation of assessment class boundaries. Also, further fish disease experts are invited to contribute their judgements on the relative severity of diseases, which is an important component of the FDI definition.

c) Environmental prognostics: An integrated biomarker and modelling approach (Annex 13)

Michael Moore, Icarus Allen, Allan McVeigh, Nasir Jamal, Phil Dyke, Aldo Viarengo

The potential prognostic use of lysosomal reactions to environmental pollutants has been explored in relation to predicting animal health in marine mussels and flatfish (dab and

flounder), based on diagnostic biomarker data. Integration of multiple biomarker data is achieved using multivariate statistics and then mapped onto "health status space" by using lysosomal membrane stability as a measure of cellular well-being. This is viewed as a crucial step towards the derivation of explanatory frameworks for prediction of pollutant impact on animal health; and has facilitated the development of a conceptual mechanistic model linking lysosomal damage and autophagic dysfunction with injury to cells, tissues and the whole animal. This model has also complemented the creation and use of a cell-based bioenergetic computational models of molluscan hepatopancreatic cells and flatfish liver hepatocytes that simulate lysosomal and cellular reactions to pollutants. The use of coupled empirical measurements of biomarker reactions and modelling is proposed as a practical approach to the development of an operational toolbox for predicting the health of the environment.

Significant progress was made during the meeting with the drafting of **JAMP Guidelines for the Integrated Monitoring and Assessment of Contaminants and their effects,** and the current text is included as Annex 18. For the Guidelines to be fully utlilisable, it will be necessary to append Technical Annexes on integrated assessment and related topics. WKIMON suggested this this could be an appropriate task for WKIMON. .

7 To draft background documents and associated assessment criteria for those biological effects methods where documents have not been completed.

Background documents for all the biological effects measurements considered were available to WKIMON III. Some were perhaps not complete, but it was decided to concentrate on the development of assessment criteria for these and other measurements, in preference to elaborating the Background Documents. It is likely that all the existing Background Documents will be reviewed by SIME 2007, and those that do not pass for publication should be referred to WGBEC for completion.

8 Review whether recommendations at WKIMON II have been adequately addressed, including:

a) comment on draft guidelines for integrated monitoring and assessment of chemicals and their effects prepared following WKIMON II;

b) consider proposals for integrated assessment of data and how to take forward.

WKIMON III assessed the progress being made on the implementation of the recommendations made by WKIMON II, as below:

1) To OSPAR SIME: that a Technical Annex for integrated assessment should be developed to supplement the draft JAMP monitoring guidelines.

France had agreed at SIME to consider taking on the task, but reported to ASMO that they could not do it. Some work on a preamble to such a Technical Annex has been undertaken at WKIMON III.

2) To OSPAR SIME: that the JAMP guidelines for general and for contaminant-specific biological effects monitoring and, if necessary, their Technical Annexes, are rewritten in the light of comments made by WKIMON II, including inconsistencies in relation to liver histopathology.

This work is in progress through SIME (see SIME 2006 Annex 19).

3) To OSPAR SIME: to take account of developments in monitoring and establish a mechanism which develops Technical Annexes for emerging contaminants.

SIME has this work in hand (e.g. UK are drafting a TA on PBDEs).

4) To OSPAR SIME: that the Technical Annexes are published separately to the integrated and other guideline documents in order to facilitate their review and updating.

SIME gave this task to OSPAR Secretariat, who decided to wait until the full set of TAs was available before doing the job.

5) To OSPAR SIME: to review the comments of WKIMON II on the Technical Annexes and, where appropriate, to initiate a process for revising the annexes.

SIME has this in hand.

6) To OSPAR SIME: to consider the need for the preparation of a background document on the scope for growth technique. SIME should note that John Thain (UK) has indicated his willingness to assist in this process.

John Thain (UK) will do this in time for SIME 2007.

7) To OSPAR SIME: to consider the development of a position paper summarising the current status of biological effects techniques. SIME should note that Ketil Hylland (Norway) and John Thain (UK) have indicated their willingness to assist in this process.

Not yet done. However, it is hoped that some work on this will be done during WKIMON III.

8) To OSPAR SIME: to consider the development of a review of the experience within Norway with monitoring of offshore installations and the possible applicability of the specific techniques employed in a wider monitoring context. SIME should note that Ketil Hylland (Norway) has indicated his willingness to assist in this process.

Ketil Hylland (Norway) will do this for SIME 2007.

9) To OSPAR SIME: that oxidative stress should not be included as a recommended technique within the metal-specific suite of biological effects techniques as it is currently more suited to use as a research tool rather than in monitoring programmes.

10) To OSPAR SIME: to consider the need for the preparation of a background document on the monitoring of lysosomal stability. This could be based upon an existing TIMES document.

Document prepared for WKIMON III.

11) To OSPAR SIME: to make arrangements for the development of a Technical Annex outlining the methods for monitoring and assessing endocrine disruption. SIME should note that Dick Vethaak (The Netherlands) has indicated his willingness to assist in this process.

This task had been adopted by SIME, but the scope of the task requires clarification. A short paper will go to SIME 2007 to request clarification.

12) To OSPAR SIME: to consider the need for the completion of the methods table given as Annex 19 in the meeting report. SIME should note that John Thain (UK) has indicated his willingness to assist in this process.

John Thain will review this table, as it is linked to the position paper at 7) above.

13) To ICES: that WGBEC review all draft background documents not available at WKIMON II at its meeting in 2006.

SIME 2007 will review Background Documents for biological effects and any that are not signed off will be passed to WGBEC 2007.

14) To ICES: that a TIMES document be prepared on the determination of fish reproductive success.

This task has not been completed. Jakob Strand (Denmark) will discuss the way forward with the chairman of WGBEC.

15) To ICES: that WGBEC consider whether a new TIMES document should be prepared concerning the use of *Echinocardium* in whole sediment bioassays, as this test species is specified by OSPAR.

This proposal was discussed briefly at WGBEC 2006, and a TIMES document will be considered for production in about 2009.

16) To ICES: that WGBEC review the methodology for cellular energy allocation and its application.

WGBEC 2006 had been unable to address this task. It would be considered at WGBEC 2007, and contributions from Norway and Belgium are anticipated.

9 Prepare an analysis of the costs and benefits of integrated monitoring.

This subject was considered at WKIMON II, and the report is available in Annex 18 of that report. WKIMON III noted some of the comments made in relation to the value of biological effects monitoring made at SIME, and included in the introductory presentation from the OSPAR Secretariat made under agenda item 2. Criticisms included cost, inability to unambiguously interpret data, and weak links to the generally contaminant-oriented approach taken to OSPAR to hazardous substances.

In discussion, it was noted that some biological effects measurements are widely recognised as being of significant value. An example was the effects of TBT (imposex and intersex). In this case, the effects observations were at least as sensitive as routine chemical measurements, provided integration over time, could be linked to specific contaminants, and OSPAR had agreed assessment criteria that could be used to assess data over wide areas. These observations could give some insight into why the role of some other biological effects measurements in the context of OSPAR monitoring programmes has been questioned, as reported by Richard Emmerson in his introductory presentation.

WKIMON III felt that they were not able to further develop a cost benefit analysis at this time. While it might be possible to express the costs of an integrated biological effects programme, perhaps expressed as the marginal costs on top of an existing chemical programme, it was much more difficult to assign a monetary value to the results from an integrated biological effects programme. The participants in WKIMON III were not aware of any similar exercise having been undertaken for chemical monitoring programmes that might give them some guidance on how to do this for an integrated programme. WKIMON III agreed that it might be possible to address the task using qualitative expressions of the benefits of an integrated programme, and that this should best be done after assessment criteria for a wider range of biological effects measurements had been established and had been applied to CEMP data. Other work undertaken at WKIMON III on assessment criteria should allow them to be applied to monitoring data as part of the preparation for QSR 2010.

10 Develop proposals for the organization of an international pilot study applying the assessment criteria developed above

using data from several Contracting Parties, and apply it to both specific CEMP issues and to the general health assessment of a region

There has been a history in the OSPAR Convention area of successful international Practical Workshops testing established and new monitoring procedures. Examples include the exercise in Oslo Fjord Workshop, the Bremerhavn Workshop, and more recently the BECPELAG project. The new knowledge gained through these Workshops has influenced the subsequent development of the OSPAR monitoring programmes. The request for proposals for an international pilot study to apply the assessment criteria developed through WKIMON and by other mechanisms is therefore very appropriate.

Ketil Hylland, who has been involved in the Workshops mentioned above, informed the meeting that he had received initiation funding from the Norwegian oil and gas industry with a view to organising a further Workshop along the general lines of BECPELAG, but also including a benthoic component. The first meeting had not yet taken place, and therefore there was plenty of opportunity to influence the design and content of the project.

The WG agreed that this was opportune, and that the project offered great possibilities for the testing of assessment criteria, and for development and testing of integrated monitoring. WKIMON encouraged the organisers of the proposed Workshop to take account of OSPAR's interests in their planning, and specifically WKIMON noted the following outcomes that they would like to see from the new Project/Workshop:

- Demonstration that an integrated programme is cost effective and functional, delivering the required information that allows the questions on clean healthy, safe, biologically diverse seas and oceans to be answered
- An emphasis on coastal and inshore, recognising that the interests of possible funding agencies (e.g. offshore O&G) need to be given appropriate weight.
- Relevant policy customers to be involved – best achieved through OSPAR
- Inclusion of techniques that give an overview of effect e.g. lysosomal stability, genomics
- Inclusion of newer approaches to chemistry e.g. passive sampling to assess availability of pollutants in water and sediment
- Implementation of the WGBEC fish and mussel packages of effects measurements and chemistry
- It is important that OSPAR see a clear link from the Field Project/Workshop to the JAMP assessments
- Assessment criteria 'put into action' so as to give a picture of 'status' of the North Sea
- The biggest challenge will be to develop a process leading towards integrated assessment c.f. REGNs, Fullmonti, FDI
- Initial implementation of programme design and assessment methods, which will provide useful background and experience for proposed agenda items at WKIMON IV.

11 Any other business

11.1 Future of WKIMON

It was noted that both ICES and OSPAR have the expectation that Workshops (such as WKIMON) should have short lives (a few years at most) and they should be disbanded once they have completed their task (or their work has reached another appropriate concluding point). WKIMON III therefore recommended that OSPAR and ICES should review the progress they have made and the requirement for further activity in this area. Within that

context, WKIMON III noted that although very significant progress had been made, their work had not been completed. A proposal for a further meeting is therefore included as Annex 19 to this report.

11.2 QSR 2010

QSR 2010 is a major objective of OSPAR, both in relation to its own monitoring and assessment activities and broader objectives, but also as contribution to the expected initial environmental status assessment under the EU Marine Strategy Directive. The preparation of the QSR is to be coordinated by the Management Group for the QST 2010 (MAQ). A consultant, Dr Cornelius J.M. Kramer, had been appointed to act as convener for this Group. The Group was relatively new, but a draft structure for the QSR had been distributed to MAQ members. It was not clear what the role of integrated assessment might be in the QSR 2010 process and it was agreed that Ian Davies should attempt to clarify how MAQ envisaged integrated assessment might be used, and the process by which the work might be done. .

12 Final report for SIME by 2nd Feb and onward transmission to ASMO and ICES (ACME and MHC)

The meeting concluded at 1245 hr on 18 January. The report was compiled after the meeting and subsequently amended by a written procedure

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Annex 2: Agenda

WKIMON III Agenda

The meeting will start at 09:00 on Tuesday 16th January 2006

at: ICES, H. C. Andersens Boulevard 44-46, DK-1553, Copenhagen V, Denmark

1. Adoption of agenda.

2. Review and note the Terms of Reference (WKIMON 7/1/2).

3. To note background documents, tabled presentations and suggested timetable and deliverables.

4. Position paper: Review and comment on a position paper on the status of biological effects methods.

5. Assessment criteria: Develop proposals for assessment criteria for biological effects methods under the CEMP on the basis of initial proposals in the available background documents and to develop additional proposals for assessment criteria where initial proposals are not available.

6. To draft background documents and associated assessment criteria for those biological effects methods where documents have not been completed.

7. Review whether recommendations at WKIMON II have been adequately addressed, including:

    a. comment on draft guidelines for integrated monitoring and assessment of chemicals and their effects prepared following WKIMON II;

    b. consider proposals for integrated assessment of data and how to take forward.

8. Prepare an analysis of the costs and benefits of integrated monitoring.

9. Develop proposals for the organization of an international pilot study applying the assessment criteria developed above using data from several Contracting Parties, and apply it to both specific CEMP issues and to the general health assessment of a region.

10. Any other business.

11. Final report for SIME by 2nd Feb and onward transmission to ASMO and ICES (ACME and MHC).

Annex 3: Lysosomal stability as a global health status indicator in biomonitoring

Background

Lysosomal functional integrity is a generic common target for environmental stressors in all eukaryotic organisms from yeast and protozoans to humans (Cuervo, 2004), that is evolutionarily highly conserved, and lysosomal membrane stability is a good diagnostic biomarker of individual health status (Allen and Moore, 2004; Bayne and Moore, 1998; Burlando *et al*., 2002; Cajaraville *et al*., 1995, 2000; Dondero *et al*., 2006b; Galloway *et al*., 2002, 2004; Hankard *et al*., 2004; Klionsky and Emr, 2000; Köhler *et al*., 1992, 2002; Lekube *et al*., 2000; Lowe, 1988; Lowe *et al*., 1982, 1992, 1995, 2006; Marigomez and Baybay-Villacorta, 2003; Moore, 1976, 1985, 1988, 1990, 2002; Moore *et al*., 2004a; Moore *et al*., 2006a,b,c; Nicholson and Lam, 2005; Svendsen and Weeks, 1995; Svendsen *et al*., 2004; Winston *et al*., 2002). Dysfunction of lysosomal processes has been mechanistically linked with many aspects of pathology associated with toxicity and degenerative diseases (Cuervo, 2004; Köhler, 1991; Köhler *et al*., 2002, 2004; Moore *et al*., 2006a, b). Recent studies have shown that lysosomal autophagy provides a second line of defence against oxidative stress (Cuervo, 2004; Moore *et al*., 2006c), and the capability to effectively up-regulate this process is probably a significant factor contributing to the ability of some organisms to tolerate stressful and polluted environments.

Lysosomal membrane stability has recently been adopted by UNEP as part of the first tier of techniques for assessing harmful impact in the Mediterranean Pollution programme (MEDPOL Phase IV). Other lysosomal biomarkers including lipofuscin in molluscs (age/stress pignment), and lysosomal neutral lipid (chemically induced lipidosis) in molluscs and fish have been adopted as part of the second tier assessment methods (Krishnakumar *et al*., 1994; Moore, 1988; Moore *et al*., 2004b).

This biomarker can also be used prognostically to predict liver damage and tumour progression in the liver of various fish species (Broeg *et al*., 1999 a, b; Köhler *et al*., 2002; Köhler, 2004), and hepatopancreatic degeneration in molluscs (e.g., blue and green mussels, freshwater bivalves and snails, periwinkles, oysters), coelomocyte damage in earthworms, as well as enhanced protein turnover (i.e., lysosomal autophagy) as a result of radical attack on proteins; and energetic status (i.e., scope for growth) as a predictive indicator of fitness of individuals within a population (Allen and Moore, 2004; Kirchin *et al*., 1992; Köhler *et al*., 2002; Moore *et al*., 2004a, 2006a; Nicholson and Lam, 2005; Svendsen and Weeks, 1995; Svendsen *et al*., 2004).

Lysosomes are known to accumulate many metals and organic xenobiotics. Adverse lysosomal reactions to xenobiotic pollutants include swelling, lipidosis (pathological accumulation of lipid), lipofuscinosis (pathological accumulation of age/stress pigment) in molluscs but not fish, and loss of membrane integrity (Köhler *et al*. 2002; Moore, 1988; Moore *et al*., 2006a, b; Viarengo *et al*., 1985a). Metals such as copper, cadmium and mercury will also induce lysosomal destabilisation in mussels (Viarengo *et al*., 1981, 1985a, b), and if oxyradicals are generated then lipofuscinosis can also occur (1985b).

Lysosomal membrane integrity or stability in blue mussels is correlated with oxygen and nitrogen radical scavenging capacity (TOSC), protein synthesis, scope for growth and larval viability (oysters – *Crassostrea gigas*); and inversely correlated with DNA damage (incidence of micronuclei), lysosomal swelling, lipidosis and lipofuscinosis, which are characteristic of failed or incomplete autophagy (Dailianis *et al*., 2003; Kalpaxis *et al*., 2004; Krishnakumar *et al*., 1994; Moore *et al*., 2004a, b, 2006a; Regoli, 2000; Ringwood *et al*., 2004). In fish liver, lysosomal membrane stability is strongly correlated with a suppression of the activity of

macrophage aggregates (Broeg, 2003; Broeg *et al*., 2005), lipidosis and lipofuscinosis (Broeg *et al*., 1999 a,b; Broeg *et al*., in preparation; Köhler , 2004).

Lysosomal stability and other lysosomal biomarkers such as lipofuscin are strongly correlated with mussel tissue concentration of PAHs, which are ubiquitous contaminants (Cajaraville *et al*., 2000; Krishnakumar *et al*., 1994; Moore, 1990; Moore *et al*., 2006a, b, c; Viarengo *et al*., 1992), as well as organochlorines and PCB congeners in liver of fish (Köhler *et al*., 2002).

Lysosomal stability of various species of mussel and fish from different climate zones clearly reflects gradients of complex mixtures of chemicals in water and sediments (Da Ros *et al*., 2002; Pisoni *et al*, 2004; Schiedek *et al*., 2006, Barsiene *et al*., 2006; Sturve *et al*., 2005), single pollution events and accidents (Einsporn *et al*. 2005; Broeg *et al*., 2002, Nicholson and Lam, 2005) and also serves for the discovery of new "Hot Spots" of pollution (Bressling, 2006; Moore *et al*., 1997, 1998a,b; 2004).

A conceptual mechanistic model has been developed linking lysosomal damage and autophagic dysfunction with injury to cells, tissues and the whole animal; and the complementary use of cell-based bioenergetic computational model of molluscan hepatopancreatic cells that simulates lysosomal and cellular reactions to pollutants has also been demonstrated (Allen and McVeigh, 2004; McVeigh *et al*., 2006; Lowe, 1988; Moore *et al*., 2006a, b, c). The integration of biomarker data can be achieved using multivariate statistics and then mapped onto a two dimensional representation of "health status space" (see below) by using lysosomal membrane stability as a measure of cellular well-being (Allen and Moore, 2004; Clarke, 1999; Dagnino *et al*., 2007; Dondero *et al*., 2006a; Lowe, 1988; Moore, 1988; Moore *et al*., 2006a). This is viewed as a crucial step towards the derivation of explanatory frameworks for prediction of pollutant impact on animal health.

Health status space is analagous to phase space in physics. For a system of n first-order ordinary differential equations, the 2n-dimensional space consisting of the possible values of x is known as its phase space. In its simplest form it is a two dimensional graph where any point can be described in terms of two numbers the x and y coordinates. The dimensions of multi-dimensional health status space are multiple contaminant and biomarker data, environmental variabilty, space and time. Principal component analysis (PCA) has been used to reduce the dimensionality of the problem to a simple two-dimensional representation (Allen and Moore, 2004; Lowe *et al*., 2006; Moore *et al*., 2006a).

Confounding Factors

Lysosomal stability is an indicator of health status and will be affected by non-contaminant factors such as severe nutritional deprivation, severe hyperthermia and prolonged hypoxia (Moore *et al*., 1980; Moore *et al*., 2007). Processing for neutral red retention (NRR) in samples of molluscs adapted to low salinity environments should use either physiological saline adjusted to the equivalent ionic strength or else use ambient filtered seawater. The major confounding factor in respect of biomonitoring is the adverse effect of the final stage of gametogenesis and spawning in mussel, which is a naturally stressful process (Bayne *et al*., 1978). In general, this period should be avoided anyway for sampling purposes, as most physiological processes and related biomarkers are adversely affected (Moore *et al*., 2004b). However, for fish, spawning has only a minimal effect on lysosomal stability and does not mask harmful chemical induced damage to lysosomal membrane stability (Köhler, 1991).

Ecological Relevance

Lysosomal integrity is directly correlated with physiological scope for growth (SFG) and is also mechanistically linked in terms of the processes of protein turnover (Allen and Moore, 2004; Moore *et al*., 2006a), and Ringwood *et al*. (2004) have also shown that lysosomal stability in parent oysters is directly correlated with larval viability. Finally, lysosomal

stability is also directly correlated with diversity of macrobenthic organisms in an investigation in Langesund Fjord in Norway (Moore *et al*., 2006b).

Quality Assurance

Intercalibration exercises for lysosomal stability techniques have been carried out in the ICES/UNESCO-IOC-GEEP Bremerhaven Research Workshop and UNEP-MEDPOL programme, and for the neutral red retention method in the GEF Black Sea Environment Programme (Köhler *et al*., 1992; Lowe *et al*., 1992; Moore *et al*., 1997, 1998a, b; Viarengo *et al*., 2000). The results from these operations indicated that both techniques could be used in the participating laboratories in an effective manner with insignificant inter-laboratory variability.

The standards used in this intercalibration involved digestive glands from marine mussels prepared at the University of Genova / University of Eastern Piedmont, Alessandria (Italy). Comparisons of the cytochemical and the neutral red retention techniques have been performed in fish liver (ICES-IOC Bremerhaven Workshop, 1990) and in mussels experimentally exposed to PAHs (Lowe *et al*., 1995).

Background Responses and Assessment Criteria

Health status thresholds for NRR and cytochemical methods for lysosomal stability have been determined from data based on numerous studies (Cajaraville *et al*., 2000; Moore *et al*., 2006a).

Lysosomal stability is a biophysical property of the bounding membrane of secondary lysosomes and appears to be largely independent of taxa. In all organisms tested to date, which includes protozoans, annelids (terrestrial and marine), molluscs (freshwater and marine), crustaceans (terrestrial and aquatic), echinoderms and fish, the absolute values for measurement of lysosomal stability (NRR and cytochemical method) are directly comparable. Furthermore, measurements of this biomarker in animals from climatically and physically diverse terrestrial and aquatic ecosystems also indicate that it is potentially a universal indicator of health status. For the cytochemical method animals are considered to be healthy if the lysosomal stability is $\geq$20 minutes; stressed but compensating if <20 but $\geq$10 minutes and severely stressed and probably exhibiting pathology if <10 minutes (Moore *et al*., 2006a). Similarly for the NRR method, animals are considered to be healthy if NRR is $\geq$120 minutes; stressed but compensating if <120 but $\geq$50 minutes and severely stressed and probably exhibiting pathology if <50 minutes (Moore *et al*., 2006a).

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Annex 4: Draft Background document on DNA adducts

OSPAR CEMP Review – DNA adduct detection using the 32P-postlabelling method

Presented by the United Kingdom

Background

1) As part of the review of the CEMP and as input to WKIMON II, SIME 2005 agreed on, and identified lead countries for, a number of background documents on biological effects techniques under the CEMP. ASMO agreed that these should form part of the Review of the CEMP (see WKIMON 06/1/Info.2) and they are included under product 23 in the 2005/2006 ASMO work programme. The background documents were intended to provide:

c) an assessment of the applicability of the biological effects techniques across the OSPAR maritime area;

d) a review of the environmental variables that influence the biological effect;

e) an assessment of the thresholds when the response of a biological effects technique can be considered to be of concern and/or require a response;

f) proposals for assessment tools;

g) status of quality assurance techniques

2) The UK offered to prepare the attached background document on application of DNA adduct techniques in marine monitoring programmes.

Action requested

3) WKIMON is invited to examine and comment on the attached background document on DNA adduct detection methods and their application to marine monitoring programmes.

Background document on DNA adducts of PAHs

1. Background

In the chemical carcinogenesis model the initiating step is the covalent modification of DNA by a carcinogen (Miller and Miller, 1981). The measurement of covalent structures formed between environmental carcinogens and DNA, termed DNA adducts, can be utilised as a biological marker of exposure to genotoxic compounds. DNA adducts can be removed by cellular repair processes or by cell death, but during chronic exposures they often reach steady state concentrations in carcinogen target tissues such as the liver. As a consequence, DNA adducts have several important features which make them suitable as biomarkers of carcinogen exposure:

1) It is a quantifiable measurement of the biologically effective dose of a contaminant reaching a critical cellular target and therefore a useful epidemiological biomarker for detecting exposure to environmental genotoxins.

2) DNA adduct levels integrate multiple toxicokinetic factors such as uptake, metabolism, detoxification, excretion and DNA repair in target tissues.

3) DNA adducts are relatively persistent once formed (may last several months) and therefore they provide an assessment of chronic exposure accumulated over many weeks rather than a few days, as afforded by other PAH biomarkers such as EROD induction or the presence of bile metabolites.

4) Studies from North America have shown that risk factors for certain lesions can be generated by correlating the level of DNA damage with lesion occurrence, thus allowing the use of a relatively simple biomarker in predicting risk.

Polycyclic aromatic hydrocarbons (PAHs) are a ubiquitous and large group of environmental contaminants, some of which are known to cause genetic toxicity through the formation of DNA adducts. Over the past 25 years a growing body of research has investigated the uptake, bioaccumulation and metabolism of PAHs and there is now extensive experimental and field based evidence supporting their role in the initiation and progression of chemical carcinogenesis. Numerous field studies in both North America and Europe have established a correlation between PAH sediment concentrations and the prevalence of hepatic tumours in fish (Malins *et al*., 1985; Myers *et al*., 1991; Baumann, 1998). For example, liver and skin neoplasia in brown bullheads (*Ictaluvus nebulosus*) from the Black River, Ohio (USA) have been shown to be strongly correlated with PAH sediment contamination (Baumann, 1998). Further work carried out in Puget Sound (USA) has also found positive correlations between hepatic lesions including neoplasia (hepatocellular carcinomas and cholangiocellular carcinomas) and foci of cellular alteration (pre-neoplastic lesions) in English sole (*Parophrys vetulus*) and sediment PAH contamination (Malins *et al*., 1985). Therefore, the measurement of DNA adduct levels in marine organisms is an important step in assessing risk from exposure to environmental carcinogens and mutagens.

Of the techniques currently available for the detection of DNA adducts the most sensitive method for the detection of a wide range of compounds chemically bound to DNA is the ^{32}P-postlabelling assay (Gupta *et al*., 1982). The method possesses a number of advantages that make it suitable for the assessment of DNA adduct induced by environmental genotoxins (for a review see Beach and Gupta, 1992; Phillips, 1997, 2005). The technique is applicable to any tissue sample from which DNA can be isolated and is also extremely sensitive, capable of detecting one adducted nucleotide in 10^9–10^{10} undamaged nucleotides from 5–10 μg DNA. In addition, providing the adduct is amenable to the labelling reaction and subsequent thin layer chromatography, its prior characterisation is not required. It is this last feature that makes the assay particularly appropriate for aquatic biomonitoring, because it is suitable for the analysis of the diverse array of adducts induced by complex mixtures of environmental chemicals. It is important to note that ^{32}P-postlabelling is only semi-quantitative as not all DNA adducts are

labelled with the same efficiency and the various enrichment and chromatograph steps involved will preferentially select certain adducts. However, the assays sensitivity, coupled with the assays ability to detect a wide range of carcinogens (e.g. PAHs), has led to its wide spread use in environmental biomonitoring programmes using both vertebrate and invertebrate sentinel organisms (Van der Oost *et al*., 1994; Ericson *et al*., 1998; Lyons *et al*., 1999; Akcha *et al*., 2004; Lyons *et al*., 2004b; Balk *et al*., 2006), following exposure to specific environmental genotoxins (Ericson *et al*., 1999; Lyons *et al*., 1999) and to compounds present in organic extracts from PAH contaminated sediments (Stein *et al*., 1990; French *et al*., 1996).

2. Ecological relevance and validation for use in the field

The field validation of a biomarker of exposure, such as DNA adducts is essential in establishing their credentials when used in routine monitoring programmes. In North America the technique has been widely used (>30 marine and freshwater species) and guidelines for implementation are published in an ICES Times technical document (Reichert *et al*., 1999). Across the OSPAR maritime area the assay has been used in several biological effects monitoring programmes using a range of indicator species including blue mussels, *Mytilus* sp, perch (*Perca fluviatilis*), dab (*Limanda limanda*), European flounder (*Platichthys flesus*), eelpout (*Zoarces viviparous*) and cod (*Gadus morhua*) (Ericson *et al*., 1998; Lyons *et al*., 1999; Lyons *et al*., 2000; Ericson *et al*., 2002; Aas *et al*., 2003; Akcha *et al*., 2004; Lyons *et al*., 2004a,b; Balk *et al*., 2006). Studies from both North America and Europe have clearly demonstrated that when using non-migratory fish the levels of DNA adducts strongly correlate with the concentration of PAH sediment contamination (Van der Oost *et al*., 1994; Ericson *et al*., 1999; Lyons *et al*., 1999). For example, studies using the eel (*Anguilla anguilla*) demonstrated a significant relationship between the level of DNA adducts and PAH contamination of the sediment (Van der Oost *et al*., 1994). Laboratory studies have demonstrated that fish exposed to PAHs accumulate hepatic DNA adducts in both a time- and a dose-dependent manner (French *et al*., 1996). It is known from experimental studies using both fish and shellfish that such DNA adducts may persist for many months once formed and are therefore particularly suited to monitoring chronic exposure to genotoxic contaminants (Stein *et al*., 1990; French *et al*., 1996; Harvey and Parry, 1998). Significantly, field based studies have investigated the relationship between DNA adduct formation and neoplastic liver disease and it has been shown that at certain contaminated sites the prevalence of DNA adducts are associated with the prevalence of toxicopathetic lesions including foci of cellular alteration and neoplasia (for review see Reichert *et al*., 1998).

Studies from North America and Europe suggest that DNA adduct levels are not markedly influenced by factors such as age, sex, season or dietary status, which are known to confound the interpretation of other biomarkers (e.g. EROD). However, validation of any biomarker, including DNA adducts in a species of interest is essential to insure against any unforeseen species-specific responses (Reichert *et al*., 1999). While there is no evidence to suggest that environmental factors such as salinity and temperature significantly effect the formation of DNA adducts these factors should always be considered, as it is known that cellular detoxification systems (e.g. Cyp1A) are influenced by changes in environmental variables (Sleiderink *et al*., 1995).

3. Species selection and target tissue

The majority of hydrophobic genotoxins, such as PAHs, released into the marine environment quickly adhere or organic particular matter and settle into the sediment. Therefore, the majority of fish species used in PAH contaminant-monitoring programmes are benthic feeders, such as the marine flatfish. A particular advantage of the ^{32}P-postblabelling assay is that it is not species specific and therefore can be utilised on any organism deemed fit for purpose. As such it has been used widely in a range of species (both vertebrate and invertebrate), ranging from filter feeders to high-order predators. It should be noted that DNA

adducts are known to accumulate and persist over time (Stein *et al*., 1990; French *et al*., 1996) and consequently should be considered a cumulative index integrating both past and present genotoxic exposure. Therefore, care needs to be taken when undertaking studies in migratory fish species as the detectable levels of DNA adducts may not be a true representation of the genotoxic contaminants at the site of capture. It has been suggested by Reichert *et al*., (1999) that in such situations biomarkers, such as bile metabolite analysis, should be employed in parallel as this would provide a relatively accurate index of recent PAH exposure and would therefore indicate whether the levels of DNA adducts were due to exposure at the site of capture.

Of the affected organs, liver is the most commonly studied when fish are used as sentinel organisms. Field data infers a chemical aetiology for many of the commonly observed hepatic lesions seen in wild fish collected from contaminated areas. Laboratory data supporting this association stems from biochemical and molecular studies which have shown the liver to be the major site for contaminant detoxification pathways (e.g. cytochrome P-450-mediated biotransformation enzyme systems). Furthermore, contaminant metabolism studies have shown fish liver microsomes are capable of producing the ultimate carcinogenic forms of common environmentally relevant PAHs, including benzo[a]pyrene, which bind to DNA to form adducts (Sikka *et al*., 1991). As mentioned previously, a major strength of the ^{32}P-postabelling assay is that it is not tissue specific and assuming sufficient DNA can be extracted it can be applied in a fit-for-purpose manner in any tissue of choice. To this end it has been used successfully in a range of tissues (both invertebrate and vertebrate), including liver, intestine, gill, brain, gonad and digestive glad (Ericson *et al*., 1999; French *et al*., 1996; Lyons *et al*., 1997; Harvey and Parry, 1998).

4. Methodology and technical considerations

4.1 ^{32}P-postlabelling

In the ^{32}P-postlabeling method, DNA isolated from tissue is first hydrolysed enzymatically to 3'-monophosphates. The proportion of adducts in the enzyme hydrolysate are enriched by selective removal of unmodified nucleotides by enzymatic methods (Reddy and Randerath 1986) or by extracting the adducts into n-butanol (Gupta, 1985) before labelling the mononucleotides with ^{32}P-ATP. For hydrophobic aromatic DNA adducts, such as PAH-DNA adducts, the enrichment steps can enhance the sensitivity of the assay to detect 1 adduct in 10^9-10^{10} bases (Reichert *et al*., 1999). Following the adduct enrichment step, the 3'-monophosphates are radio-labelled at the 5'-hydroxyl using ^{32}P-ATP and T4-polynucleotide kinase to form 3', (^{32}P)5'-bisphosphates. Separation of the ^{32}P-labeled adducts is accomplished by multidimensional high-resolution anion exchange thin-layer chromatography. Autoradiography is then used to locate the radiolabelled adducts on the chromatogram and the radioactivity is measured by either liquid scintillation spectroscopy or storage phosphor imaging (IARC, 1993, Phillips and Castegnaro, 1999). Detailed methodologies have which have been through appropriate Quality Assurance (QA) programmes are now published by ICES and IARC (Phillips and Castegnaro, 1999; Reichert *et al*., 1999).

4.2 Radiation safety

The ^{32}P-postlabelling assay uses large amounts of ^{32}P, which is an energetic beta emitter (1.7 MeV) with a half-life of 14.3 days. Researchers using this isotope must receive detailed instruction before handling ^{32}P and must be frequently monitored for exposure to ^{32}P. In the UK the use of ^{32}P in scientific procedures is governed by Environment Agency. Institutes need to have an appointed Radiation Protection Supervisor (RPS) and follow designated licence consent criteria. Institutes wishing to conduct ^{32}P-postlabelling outside the UK must contact their own national licensing organisation to clarify the legislative procedures required.

Main considerations to help minimise and monitor ^{32}P exposure:

- All researchers who handle ^{32}P must wear a whole body film badge and a finger dosimeter on the inside of each hand where there is the highest potential for radiation exposure. These badges should be monitored regularly.
- All laboratory operations are planned to minimize the time spent handling radioactivity, the use of tongs and forceps to minimise handling of tubes and vials is recommended.
- Double latex gloves are worn while handling ^{32}P and they should be regularly checked for radioactivity by passing them under a radiation monitor. Gloves should immediately be changed and discarded if found to be contaminated.
- Laboratory working surfaces are checked frequently with the radiation monitor when handling ^{32}P. The monitor probe should be covered with a thin vinyl wrap to prevent contamination of the detector.
- After completion of work with radioactivity, the workers are to check themselves and their equipment with the radiation monitor. If any radioactivity is detected then they are to wash themselves and/or the equipment until free of radioactivity.

4.3 Equipment for handling and storage of ^{32}P

All ^{32}P is handled behind 1 cm Perspex/Plexiglas shielding. In addition, samples are kept in Perspex/Plexiglas containers that are at least 1 cm thick. Where possible all manipulations of eppendorfs and vials should be conducted using long armed tongs. It is recommended that radioactive waste is temporarily stored in a 1cm thick Perspex/Plexiglas boxes. Such radiation specific safety equipment is available from most large scientific suppliers. Researchers should insure that all safety procedures comply implicitly with their local radiation protection regulations. Detailed laboratory safety procedures are discussed in further in Castegnaro *et al*., (1993)

5. Status of quality control procedures and standardised assays

There are presently no active QA programmes running for the detection of DNA adducts using the ^{32}P-postlabeling method. Previous QA programmes have been conducted under the auspices of the EU funded Biological Effects Quality Assurance In Monitoring Programme (BEQUALM) and the International Agency for Research on Cancer (IARC). The IARC QA trial of the ^{32}P-postlabelling assay was conducted between 1994–1997 and involved 25 participants in Europe and the USA. The primary objectives of this project were to standardise the ^{32}P-postlabelling assay and improve inter-laboratory reproducibility. The IARC QA programme for ^{32}P-postlabelling led to a series of publications, which detailed a standardised protocol for the detection of bulky aromatic DNA adducts by the ^{32}P-postlabelling assay (IARC, 1993; Phillips and Castegnaro, 1999). The standardised protocol has now been adopted by the International Programme on Chemical Safety (IPCS)[1] and recommended for use in their guidelines for monitoring genotoxic carcinogens in humans (Richard *et al*., 2000). Essentially the same protocol is also published in an ICES Times technical document (Reichert *et al*., 1999)

6. 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

1 International Programme on Chemical Safety (IPCS) was established in 1980 under the WHO, for more information visit: http://www.who.int/ipcs/en/

unintended or unacceptable level of biological effect. Therefore, in order to understand and apply DNA adducts as a biomarker of genotoxic exposure it is of fundamental importance to gain information on the natural background levels in non-contaminated organisms. A number of studies have now examined fish collected from pristine areas (as supported by chemical and biomarker analyses) and the typical ^{32}P-postlaballing generated DNA adduct profiles either exhibited no detectable adducts or very faint diagonal radioactive zones (DRZs) (Fig 1A), suggesting minimal PAH exposure (Ericson *et al*., 1998; Reichert *et al*., 1998; Lyons *et al*., 2000; Aas, *et al.,* 2003; Balk *et al*., 2006). In contrast, DNA adduct profiles in fish exposed to a complex mixture of PAHs will form DRZs on the chromatogram (Figure 1B), which is a composite of multiple overlapping PAH-DNA adducts.

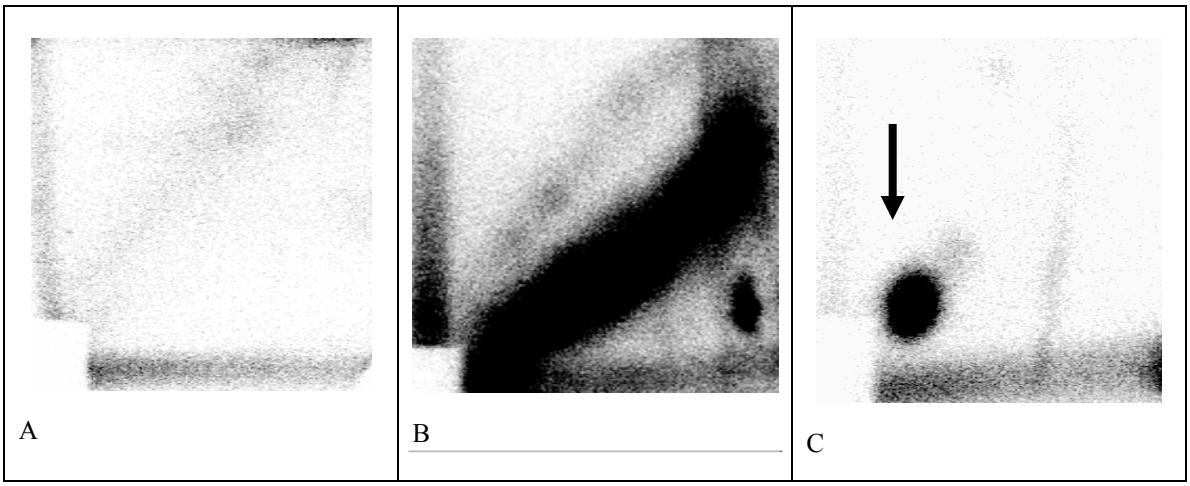

Figure 1: Representative hepatic DNA adducts profiles produced following ^{32}P-postlabelling. (A) DNA adduct profile obtained from a site with a low level of PAH contamination. A faint DRZ is visible, indicating a low level of DNA adducts representative of a clean reference location. (B) DNA adduct profile displaying a clear DRZ of ^{32}P-labelled DNA adducts indicating the fish has been exposed to a complex mixture of genotoxins. (C) Positive control consisting of BaP labelled DNA (115 nucleotides per 10^8 undamaged nucleotides) run with each batch (kindly provided by Prof. David Phillips and Dr Alan Hewer, Cancer Research Institute, Sutton UK). Figure adapted from Lyons *et al.,* 2004b).

Using such studies to define reference locations it should be possible to gain an international consensus (via ICES Working Group for the Biological Effect of Contaminants, WGBEC) on what level of DNA adducts should be considered background for a particular location and species. However, the following issues will require consideration:

- ^{32}P-postlabelling studies should be conducted using internationally agreed protocols incorporating appropriate positive and negative control samples (Phillips and Castegnaro, 1999; Reichert *et al*., 1999).
- All studies need to include supporting environmental data to confirm the contaminant load at the reference location and where possible supporting biomarker and histopathological data to confirm health status of the individual
- While the assay ^{32}P-postlabelling can be applied to any species deemed fit for purpose, it should only be applied to those species where there is sufficient background information available on life history traits and behaviour (e.g. migration).

7. Concluding remarks

- *DNA adducts as biomarkers of genotoxic exposure*. DNA adducts provide a measure of biologically active contaminant to have reached a critical cellular target (DNA). They are persistent and therefore considered a 'cumulative index'

of exposure to genotoxins and a significant body of research demonstrates their importance in the initiation and progression of carcinogenesis induced by important environmental contaminants (e.g. PAHs).*Safety considerations when conducting the 32p-postlabelling assay.* The ^{32}P-postlabeling assay uses large amounts of ^{32}P, which is an energetic beta emitter. This requires specialist laboratories may limit the use of the assay to a few appropriately equipped research groups. *Applicability across OSPAR maritime area*. DNA adducts have been applied in a wide range of species across the whole OSPAR maritime area including blue mussels, *Mytilus* sp, perch (*Perca fluviatilis*), dab (*Limanda limanda*), European flounder (*Platichthys flesus*), eelpout (*Zoarces viviparous*) and cod (*Gadus morhua*). A particular advantage of the ^{32}P-postblabelling assay is that it is not species specific and therefore can be utilised on any organism deemed fit for purpose.

- *Status of quality assurance*. There are presently no active QA programmes running for the detection of DNA adducts using the ^{32}P-postlabeling method. However, inter laboratory QA programmes have previously been conducted under the auspices of BEQUALM and IARC and standardised protocols are available in the form of an ICES Times technical document and IARC publications.
- *Assessment thresholds*. These have not been agreed, but are likely to be determined from comparisons of DNA adduct levels detected at pristine reference locations. It is recommended that further work to define baseline or background levels of DNA adducts using the 32-postlabelling assay is taken forward through the activities of ICES WGBEC.

8 References

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Annex 5: Cytochrome P4501A activity (EROD)

Compiled by Anders Ruus and Ketil Hylland (NIVA)

Introduction

EROD (7-Ethoxyresorufin-*O*-deethylase) is a cytochrome P450 catalysed reaction with ethoxyresorufin as the substrate (Burke and Mayer 1974; Stagg and McIntosh, 1998). Cytochrome P450 1A catalyse the deethylation of 7-ethoxyresorufin to resorufin.

The cytochrome P450 system is a superfamily of enzymes with several hundred forms comprising more than 250 different families, further divided into subfamilies. The CYP system is highly diversified and is found in bacteria, plants, lower eukaryotes and in animals. Members of the P450 subfamily CYP1A are particularly important in the metabolism of many pollutants. In the case of planar molecules, such as polycyclic aromatic hydrocarbons (PAHs) isoenzymes of CYP1A are responsible for the insertion of oxygen into the molecule, which is the first oxidative step in the biotransformation process (termed 'phase I'; Williams, 1974).

In addition to being substrates for biotransformation, planar compounds, such as PAHs, can also interact with cytochrome P450 1A as inducers, by binding to the cytosolic Ah (aryl hydrocarbon)-receptor. EROD is a tool used to quantify this induction. The induction of cytochrome P450 enzymes in fish liver was first suggested as an indicator of environmental contamination in the 1970s by Payne (1976). It has later gained widespread use (see e.g. Förlin *et al*., 1990; George *et al.*, 1995; Goksøyr *et al.*, 1991; Whyte *et al.*, 2000).

Dose-response

Whyte *et al.* (2000) rank chemicals according to the level of EROD activity they induce in treated or exposed fish when compared with untreated or control fish. Contaminants that induce EROD less than 10-fold above control levels are considered "weak" inducers, 10- to 100-fold are "moderate" inducers, and chemicals that elicit > 100-fold induction are considered "strong" inducers. Dioxins, planar PCBs and PAHs (benzo[a]pyrene) are categorised as "strong" inducers. Over 25 studies have observed induction of hepatic EROD by benzo[a]pyrene in 15 species of fish (Whyte *et al.* 2000).

Relevance of other factors

Several factors have been shown to affect hepatic EROD, both endogenous and exogenous. The most important endogenous factors for most fish species are developmental stage (juvenile-mature), gender, reproductive status and age, all of which can be controlled through sampling design. In addition, environmental temperature has been shown to affect EROD (Sleiderink *et al*., 1995; Lange *et al*, 1999). Seasonal cycles in EROD induction have been observed for e.g. rainbow trout (Förlin and Haux 1990), flounder (Hylland *et al*., 1998), salmon (Larsen *et al*., 1992), most likely due to both to changes in water temperature and reproductive cycles (which it is not really possible to separate in the field).

Several species have baseline EROD activities within the same order of magnitude among different studies/measurements and also show greater than 10-fold EROD induction after contaminant exposure (Whyte *et al.* 2000). These are, however, mostly freshwater species.

Developmental stage of the fish is very important. The main age-related factors are time of exposure/accumulation, food selection and reproductive stage.

The mechanism for CYP1A suppression in spawning females is related to 17β-estradiol (E_2) (or xenoestrogen) levels. The hormone controls the induction of vitellogenin (VTG; egg yolk protein) production during gonadal recrudescence. Some of the inter-gender differences during spawning can be attributed to increased levels of CYP isoenzymes in males rather than suppression of levels in females.

Dietary factors can be important for the induction of CYP1A. Firstly, of course, AhR ligands can be presented to the organism through the food. Secondly, proper nutrition is a prerequisite for enzyme systems to function properly. Hylland *et al*. (1996) reported an elimination of EROD response (i.e. to control levels) in BaP-treated flounder deprived of food for one month.

Background responses

Baseline levels of EROD in four marine species have been estimated from results derived from the Norwegian monitoring programme (Ruus *et al*., 2003). The baseline value for Atlantic cod has been suggested to be 9-95 pmol/min/mg protein including fish from the Norwegian west coast and if only fish from the Barents sea had been included, the values would have been 9–25 pmol/min/mg protein. For flounder, baseline values are in the range 10–43 pmol/min/mg protein, for dab 123–529 pmo/min/mg protein and for plaice 33–146 pmol/min/mg protein. The fish were sampled from reference locations (i.e. no known local sources of contamination) in the autumn, the data includes males and females and the water temperature at the sampling locations was 9–11°C.

Assessment criteria

As many factors are known to influence EROD and it is not feasible to correct for all in the design, it is advisable always to include an appropriate reference group in studies that include EROD as an endpoint. Experience suggests that an EROD value in most marine species above twice the upper limit of baseline values indicate an ecosystem influenced by planar organic contaminants.

Quality assurance

Cytochrome P4501A is possibly the most widely used biomarker. There have been two international intercalibrations for the method, both within BEQUALM. The intercalibrations have pinpointed variability relating to most steps in the analytical process, excepting possibly the enzyme kinetic analysis itself. It is imperative that laboratories have internal quality assurance procedures, e.g. use internal references samples with all batches of analyses.

Acknowledgement

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Table 1. Dose-response, background response and sensitivity in experimental studies with gadoid fish.

SPECIES	SUBSTANCE(S)	LOWEST-HIGHEST CONCS	EXPOSURE TIME	BASELINE/CONTROL (LEVEL/ACTIVITY)	INDUCTION (FOLD)	REFERENCE
Polar cod Boreogadus saida juvenile	Crude oil (Oseberg C)	~200 mg/kg (i.p. inj.)	10 and 21 d post inj.	~30 pmol/min/mg	~8 and ~2.5 (245 and 80 pmol/min/mg)	(George *et al.*, 1995)
Polar cod Boreogadus saida male	Crude oil (Oseberg C)	~200 mg/kg (oral)	21 d post exposure	28 pmol/min/mg ± 6 (n=12)	~5 (132 ± 14 pmol/min/mg)	(George *et al.*, 1995)
Polar cod Boreogadus saida female	Crude oil (Oseberg C)	~200 mg/kg (oral)	21 d post exposure	8 pmol/min/mg ± 2 (n=14)	~5 (42 ± 6 pmol/min/mg)	(George *et al.*, 1995)
Polar cod Boreogadus saida juvenile	β-naphthoflavone	50 mg/kg (i.p. inj.)	21 d post inj.	~30 pmol/min/mg	~12.5 (380 pmol/min/mg)	(George *et al.*, 1995)
Cod, Gadus morhua juvenile	2,3,7,8-TCDD	0.008 mg/kg oral dose twice, d 0 and d 4	9 and 17 d post exposure	55.4 (d 9) and 91.4 (d 17) pmol/min/mg	~4 and ~3 (230 and 277 pmol/min/mg)	(Hektoen *et al.*, 1994)
Cod, Gadus morhua juvenile	PCB-105	10 mg/kg oral dose twice, d 0 and d 4	measure at d 9 and d 17	55.4 (d 9) and 91.4 (d 17) pmol/min/mg	1.5 and 1.2	(Bernhoft *et al.*, 1994)
Cod, Gadus morhua juvenile	β-naphthoflavone	100 mg/kg (i.p. inj. at d 0 and d 4)	measure at d 7	84 pmol/min/mg ± 8 (n=5)	~13 (1074 ± 340 pmol/min/mg)	(Goksoyr *et al.*, 1987)
Cod, Gadus morhua	β-naphthoflavone	100 mg/kg (2 i.p. inj.)	measure 3-4 d after last injection	40 pmol/min/mg	~72 (2870 pmol/min/mg)	(Goksoyr *et al.*, 1991)
Cod, Gadus morhua juvenile	Crude oil (North Sea)	0.06 – 1 ppm	30 days	~2 pmol/min/mg	~ 2- 5.5 (~ 4 – 11 pmol/min/mg)	(Aas *et al.*, 2000)

Table 2. Dose-response, background response and sensitivity in field studies with gadoid fish.

SPECIES	SUBSTANCE(S)	LOWEST-HIGHEST CONCS	EXPOSURE TIME	BASELINE/CONTROL (LEVEL/ACTIVITY)	INDUCTION (FOLD)	REFERENCE
Rockling, Ciliata mustella	Crude oil (Gullfaks; M.V. Braer spill, Shetland)	85000 tons spill 129 ± 38 ng /g dry wt. of PAHs (selected 2- and 3-ring) detected in muscle.	3 months after spill	~160 pmol/min/mg ± 50	~9 (1480 pmol/min/mg)	(George *et al.*, 1995)
Roundnose grenadier, Coryphaenoides rupestris	i.a. PAHs and PCBs			260 ± 20 (Male) ~170 (Female) pmol/min/mg	~2 (530 ± 70 (male) and ~350 (female) pmol/min/mg)	(Lindesjoo *et al.*, 1996)
Hake, Urophycis spp.	Pollution (PAH) from oil platforms (Gulf of Mexico) <100m from platforms			10.9 ± 6.4 and 11.7 ± 10.5 pmol/min/mg (>3000 m from platforms)	<1 (10.6 ± 3.8 and 10.5 ± 7.1 pmol/min/mg)	(McDonald *et al.*, 1996)

Annex 6: PAH metabolites in bile

Compiled by Lars-Petter Myhre (IRIS-Akvamiljø) and Ketil Hylland (NIVA)

Background

Analyses of PAH metabolites in fish bile have been used as a biomarker of exposure to PAH contamination since the early 1980s. The presence of metabolites in bile (and in urine) is the final stage of the biotransformation process whereby lipophilic compounds are transformed to a more soluble form and then passed from the organism in bile or urine.

As a biomarker of exposure, measuring PAH metabolites in bile has many advantages over other techniques that require sophisticated tissue preparation protocols. The pretreatment of bile samples requires relatively simple dilution steps prior to analysis by direct fluorescence measurement. The bile is diluted in methanol : distilled water (1:1) and fluorescence is measured with a fluorometer. Fixed wavelength fluorescence is a suitable screening method for samples while HPLC/F or GC-MS SIM is utilized for qualitative and quantitative measures (Ariese *et al*., 2005; Jonsson *et al*., 2003; Lin *et al*., 2006; Aas *et al*., 2000a, 2000b).

Bile is generally stored in the gall bladder prior to episodic release into the esophagus where bile salts have a function to perform as part of the digestive process. This period of storage permits a degree of accumulation of metabolites and hence an increase in their concentration. The periodic release of bile does however introduce a variable into the technique, which must be accounted for. The feeding status of fish has been shown to influence both the volume and the density of the bile (Collier and Varanasi, 1991).

The ability of fish to biotransform PAHs into less lipophilic derivatives means that reliance on the detection of parent PAHs alone may lead to an underestimation of the *in vivo* exposure level of PAH in the fish. PAH metabolite detection, on the other hand, represents a quantification of the flux of PAHs streaming through the fish's body. From a toxicological point of view, flux information is more relevant for estimating the actual biotic stress due to PAH exposure, than the body burden data of the unmetabolised parent PAH compounds in tissues (most often liver). Despite this, body burden measurements are still more commonly used within monitoring studies than metabolite determination.

Dose-response (species specific)

The PAH compounds are metabolised rapidly in the organisms and it is the endpoint of this metabolisms that is measured in the bile. The compounds are measured using chemical analysis. A consistent dose-response relationship has been demonstrated in laboratory studies between PAH exposure and the subsequent presence of metabolites in bile (Beyer *et al.*,1997; Aas *et al.*, 2000). To establish a good dose-response relationship in field studies it is necessary to focus on aspects that influence the excretion of bile.

The method requires that bile is available in the gall bladder. Since the fish renew bile as part of normal metabolism and excrete it during digestion, it is important to know about the dietary status of the organism to establish a dose- response relationship. If the fish feed just before sampling, the gall bladder may become more or less empty. After the gall bladder has been emptied it will fill up and metabolites will be concentrated up to a plateau level corresponding to the exposure regime. Consequently the time since last digestion is important for the dose-response relationship. Fish generally have a very efficient metabolic excretion of most PAHs and it has been shown that most of the PAH will excreted after 2–8 days following exposure. This means that the PAH metabolites determined in bile will represent exposures on the scale of days and, at most, two weeks.

It has been shown in several field and laboratory studies that there is a good correlation between PAH exposure and bile metabolites. Because of the rapid metabolism and the correlation between bile content and digestive status it is difficult to make a dose-response relationship that can be used to quantify the exposure. Work has been done to try to correlate bile metabolite concentration to digestive status, by correlating it to the amount of protein or biliverdin in the bile. Absorbance at 380 nm is also used (similar to biliverdin) (Hylland, unpublished). This normalisation is not standardised because it has been shown to only explain parts of the variability, but it is recommended to be part of the explaining factors in the interpretation of results. In laboratory studies it is normal to stop the feeding some days before sampling to ensure the bile quality. In field sampling this can be taken into account by letting the fish go some days in tanks before sampling, but this has some logistical challenges.

Species sensitivity

The background level differs between species so it is important to establish good baseline before using new species. It may be expected that species with fatty livers, i.e. most gadiids, may metabolise PAHs more slowly as more will partition into fat, but this has not been documented experimentally.

Relevance of other factors

As mentioned above, food availability will affect the concentration of PAH metabolites in bile. In an assessment of data for more than 500 individual cod sampled through five years of national monitoring, variables such as size/age and sex explained some variability in multiple regression models (Ruus *et al.*, 2003). This could be due to different feeding preferences, but also endogenous processes. In addition, the fat-content of the liver (measured as liver-somatic index, LSI) came out as significant, presumably because fat decreases the availability of PAH to the cellular compartments of liver cells.

Background responses

Baseline levels of PAH metabolites have been established for many of the species relevant for monitoring in Norwegian coastal and offshore waters. From Ruus *et al.* (2003) values for the relevant species are: (all values standardised to absorbance at 380 nm) Atlantic cod: 0.6–4 µg/kg bile, flounder 27–89 µg/kg bile, dab 3.1–34 µg/kg bile, plaice 0.4–3 µg/kg bile (all quantified using HPLC separation and fluorescence detection and quantification). Standardisation at 380 nm is used to remove variability due to bile salts.

Assessment criteria

It is possible to establish global criteria for individual PAH metabolites. Baseline data for individual species may be used to test against to determine whether fish have been exposed to PAHs. As mentioned above, some variation in PAH metabolites in bile appear to be related to sex and size/age (Ruus *et al.*, 2003), knowledge of which should be included in the sampling design.

Quality assurance

A general protocol outlining analytical strategies and their strengths as well as weaknesses has recently become available (Ariese *et al.*, 2005). There have been international intercalibration exercises for the determination of PAH-metabolites in fish bile, arranged in collaboration between an EU-project and QUASIMEME[2]. Reference bile samples were generated as part of

2 QUASIMEME – organisation that offers quality assurance for chemical endpoints; http://www.quasimeme.org

the aforementioned EU project and are now available through IRMM, JRC, Geel, Belgium (http://www.irmm.jrc.be/html/homepage.html).

Acknowledgement

The current review has been derived from an overview prepared for the Norwegian offshore companies through OLF (Hylland *et al.*, 2006).

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Table 1. Overview of field and laboratory studies – PAH metabolites measured by fixed fluorescence.

SPECIES	SUBSTANCE (LAB/FIELD)	TEST CONCENTRATIONS/AREA	EXPOSURE TIME	METABOLITE	BASELINE	CONTROL OR REFERENCE	EXPOSED /CONTROL	REFERENCE/COMMENTS
Cod (Gadus morhua)	Feral fish	Barents Sea	Baseline					Aas *et al.*, 2003
Cod (Gadus morhua)	Feral fish	Egersund	Baseline non polluted area	Naph type	5.3 ug/ml			Klungsøyr *et al*. 2003
				Pyren type	0.8 ug/ml			
				BaP type	0.4 ug/ml			
Cod (Gadus morhua)	Feral fish	Sleipner	Baseline polluted area?	Naph type	6.1 ug/ml			Klungsøyr *et al*. 2003
				Pyren type	1.0 ug/ml			
				BaP type	0.5 ug/ml			
Cod (Gadus morhua)	Feral fish	Statfjord	Baseline polluted area?	Naph type	5.9 ug/ml			Klungsøyr *et al*. 2003
				Pyren type	0.9 ug/ml			
				BaP type	0.3 ug/ml			
Cod (Gadus morhua)	Feral fish	Frøy, ceased installation 10 000 m (ref) 2000 m - 200 m	Baseline polluted area?	Naph type		3.9 ug/ml	1.1 - 1.1	Beyer *et al.* 2003
				Pyren type		0.6 ug/ml	1.1 - 0.9	
				BaP type		0.3 ug/ml	0.9 - 0.9	
Cod (Gadus morhua)	Feral fish	Barents sea	Baseline	Naph type	2,15 ug/g			Sundt, 2002
				Pyren type	1,63 ug/g			
				BaP type	0,69 ug/g			
Cod (Gadus morhua)	Feral fish	Barents sea	Baseline	Naph type	5,8 ug/g			Aas and Børseth, 2002
				Pyren type	1,7 ug/g			
				BaP type	0,8 ug/g			

SPECIES	SUBSTANCE (LAB/FIELD)	TEST CONCENTRATIONS/AREA	EXPOSURE TIME	METABOLITE	BASELINE	CONTROL OR REFERENCE	EXPOSED /CONTROL	REFERENCE/COMMENTS
Cod (Gadus morhua)	Laboratory	1 ppm crude oil Statfjord B	14 days					Aas *et al*., 2002
Cod (Gadus morhua)	Laboratory	0.06 - 0.25 - 1 ppm Oil	average 3, 7, 14, 24 days	Naph type		3,9 ug/g	7,5 - 23,7 - 31,4	Skadsheim *et al*., 2004
				Pyren type		2,6 ug/g	3,6 - 10,6 - 13	
				BaP type		1,0 ug/g	1,7 - 2,4 - 2,2	
Cod (Gadus morhua)	Laboratory	0.06 - 0.25 - 1 ppm Oil	average 3, 17, 31 day	Naph type		*53.1 ug/g*	0.7 - 2.3 - 2.9	Skadsheim *et al.*, 2004
				Pyren type		7.0 ug/g	1 - 2.9 - 3.3	
				BaP type		1.0 ug/g	1.1 - 1.5 - 1.5	
Cod (Gadus morhua)	Laboratory	Oil 0.06 - 0.25 - 1 ppm	30 days	Naph type		7.1 fi	5.1 - 9.5 - 227.5	Aas *et al.* 2000
				Pyren type		2 fi	6.4 - 12.7 - 43.3	
				BaP type		0.8 fi	2.3 - 3.6 - 9.6	
Cod (Gadus morhua)	Laboratory	PW Oseberg, 1:1000 - 1:200 - 0.2 ppm oil - 0.2 ppm oil + PAHmix	15 days	Naph type		12.6 ug/ml	1.3 - 2.5 - 3.6 - 5.4	Sundt, 2004
				Pyren type,		4 ug/ml	1.7 - 3.7 - 4.1 - 17.8	
				BaP type,		1.8 ug/ml	1.3 - 1.8 - 1.5 - 2.4	
Cod (Gadus morhua)	Field, Caged	North Sea - Statfjord, 10000 m - 2000m - 500 m German bight G	5.5 weeks	Naph type	7.5 ug/ml	0,7	1.7 - 1.9 - 2.1	Aas *et al.*, in press
				Pyren type	3.1 ug/ml	0,7	1.2 - 1.5 - 1.6	
				BaP type	1.2 ug/ml	0,8	1.2 - 1.1 - 1.2	
Cod (Gadus morhua)	Field, Caged	German bight G4 (Ref) G1 - G2 - G3	5.5 weeks	Naph type	7.5 ug/ml	0,4	0.9 - 0.9 - 1.6	Aas *et al.*, in press
				Pyren type	3.1 ug/ml	0,5	0.8 - 0.9 - 1.7	
				BaP type	1.2 ug/ml	0,7	0.8 - 1 - 1.3	
Cod (Gadus morhua)	Field, Caged	North Sea - Troll, 1000 m - 500m	6 weeks	Naph type	4.6 ug/ml	1,4	1.7 - 2.5	Børseth *et al.*, 2004
				Pyren type	2.4 ug/ml	0,9	1.1 - 1.3	
				BaP type	0.9 ug/ml	1,1	1.1 - 1.3	

SPECIES	SUBSTANCE (LAB/FIELD)	TEST CONCENTRATIONS/AREA	EXPOSURE TIME	METABOLITE	BASELINE	CONTROL OR REFERENCE	EXPOSED /CONTROL	REFERENCE/COMMENTS
Cod (Gadus morhua)	Field, Caged	North Sea - Tampen, 10000 - 2500 - 1000 – 500	6 weeks	Naph type		8.8 ug/ml	1.0 - 1.5 - 1.2 - 1.2	Hylland *et al.*, 2005
				Pyren type				
				BaP type		1.4 ug/ml	0.9 - 0.7 - 0.8 - 0.9	
Haddock (Melanogrammus aeglefinus)	Feral fish	Egersund	Baseline non polluted area	Naph type	5.1 ug/ml			Klungsøyr *et al.* 2003
				Pyren type	1.4 ug/ml			
				BaP type	0.7 ug/ml			
Haddock (Melanogrammus aeglefinus)	Feral fish	Sleipner	Baseline polluted area?	Naph type	6.8 ug/ml			Klungsøyr *et al.* 2003
				Pyren type	1.9 ug/ml			
				BaP type	0.8 ug/ml			
Haddock (Melanogrammus aeglefinus)	Feral fish	Statfjord	Baseline polluted area?	Naph type	11.2 ug/ml			Klungsøyr *et al.* 2003
				Pyren type	2.5 ug/ml			
				BaP type	0.7 ug/ml			
Haddock (Melanogrammus aeglefinus)	Feral fish	Barents sea		Naph type	2.52 ug/g			Sundt, 2004
				Pyren type	1.69 ug/g			
				BaP type	0.77 ug/g			
Haddock (Melanogrammus aeglefinus)	Feral fish	Barents sea		Naph type	2.0 ug/g			Aas and Børseth, 2004
				Pyren type	1.3 ug/g			
				BaP type	0.6 ug/g			
Haddock (Melanogrammus aeglefinus)	Feral fish	Frøy, ceased installation 10 000 m (ref) 2000 m - 200 m	Baseline polluted area?	Naph type		5.6 ug/ml	1.3 - 2.2	Beyer *et al.*, 2003
				Pyren type		1.4 ug/ml	1.4 - 0.7	
				BaP type		0.75 ug/ml	1.8 - 0.6	
Sheepshead minnow	Laboratory	North sea oil A 0.1 - 0.4 - 0.7 ppm	5 weeks	Naph type		6916	2.3 - 6.2 - 9.3	Bechmann *et al.* 2004
				Pyren type		569	2.5 - 5 - 6.3	
				BaP type		107	4 - 13.1 - 19.2	
Sheepshead minnow	Laboratory	North sea oil B 0.1 - 0.9 - 5.6 ppm	6 weeks	Naph type		18164	1.8 - 4.3 - 12.5	Bechmann *et al.* 2004
				Pyren type		438	5.6 - 12.6 - 30.8	
				BaP type		110	12.6 - 42.7 - 123.9	
Sheepshead minnow	Laboratory	2 - 14 - 214 ppb	5 weeks	Naph type		267280	0.9 - 2.2 - 18.6	Bechmann *et al.* 2004

SPECIES	SUBSTANCE (LAB/FIELD)	TEST CONCENTRATIONS/AREA	EXPOSURE TIME	METABOLITE	BASELINE	CONTROL OR REFERENCE	EXPOSED /CONTROL	REFERENCE/COMMENTS
				Pyren type		9926	0.9 - 1.5 - 9.6	
				BaP type		5152.7	3 - 17.4 - 207	
Polar cod (Boreogadus saida)	Laboratory , feral fish 2001, 2002	1.5 ppm StatfjA oil , baseline, control	14 days	Naph type	16.0 ug/g	2	16,9	Sundt and Bechmann, 2004
				Pyren type	0.9 ug/g	5,5	74,4	
				BaP type	0 ug/g	0	1,8	

Table 1. PAH-metabolites in marine fish – measured by GC-MS.

SPECIES	SUBSTANCE (LAB/FIELD)	TEST CONCENTRATIONS	EXPOSURE TIME	METABOLITE	BASELINE	CONTROL OR REFERENCE	EXPOSED/CONTROL	REFERENCE
Cod (Gadus morhua)	Feral fish	Barents sea	baseline	Naph sum	150,6 ng/g			Aas and Børseth, 2002
				Phen sum	61,2 ng/g			
				Pyren	4,6 ng/g			
Cod (Gadus morhua)	Feral fish	Barents sea	baseline	Naph sum	1285 ng/g			Sundt, 2004
				Phen sum	220 ng/g			
				Pyren	3.5 ng/g			
Cod (Gadus morhua)	Feral fish	Egersund	Baseline non polluted area	Naph sum	2005.1 ng/g			Klungsøyr *et al*. 2003
				Phen sum	230.2 ng/g			
				Pyren	3.9 ng/g			
Cod (Gadus morhua)	Feral fish	Sleipner	Baseline polluted area?	Naph sum	1296.1 ng/g			Klungsøyr *et al*. 2003
				Phen sum	197.8 ng/g			
				Pyren	0 ng/g			
Cod (Gadus morhua)	Feral fish	Statfjord	Baseline polluted area?	Naph sum	1361.7 ng/g			Klungsøyr *et al*. 2003
				Phen sum	351.1 ng/g			
				Pyren	4.0 ng/g			
Cod (Gadus morhua)	Laboratory	0.06 - 0.25 - 1 ppm Oil	average 3, 7, 14, 24 days	Naph sum		2549 ng/g	4.6 - 13.4 - 23.6	Skadsheim *et al*., 2004
				Phen sum		691 ng/g	7.7 - 22.9 - 34.9	
				Pyren		27 ng/g	7.3 - 16.2 - 25.1	
Cod (Gadus morhua)	Laboratory	0.06 - 0.25 - 1 ppm Oil	average 3, 17, 31 day	Naph sum		5702 ng/g	4 - 13.3 - 12,7	Skadsheim *et al*., 2004
				Phen sum		377 ng/g	10,5 - 40,3 - 48,7	
				Pyren		5 ng/g	8,6 - 63 - 88,4	
Cod (Gadus morhua)	Field, Caged	North Sea - Statfjord, 500 - 2000 - 10000 m		Naph sum		1150 ng/g	3.0 - 2.0 - 1.3	Aas *et al*., in press
				Phen sum		340 ng/g	3.5 - 2.7 - 2.5	
				Pyren		-	-	
Cod (Gadus morhua)	Field, Caged	North Sea - Troll, 1000 m - 500m	6 weeks	Naph sum	1515.1 ng/g	1,1	1.1 - 1.2	Børseth *et al*., 2004
				Phen sum	327.2 ng/g	1,6	2.1 - 2.0	
				Pyren	173.2 ng/g	1,2	0.9 - 1.2	

Cod (Gadus morhua)	Field, Caged	North Sea - Tampen, 10000 - 2500 - 1000 - 500	6 weeks	Naph sum		965.3 ng/g	0.9 - 1.7 - 0.9 - 1	Hylland *et al*., 2005
				Phen sum		934.5 ng/g	1.4 - 3 - 1.8 - 1.5	
				Pyren		3.7 ng/g	0 - 0 - 0.5 - 0.0	
Cod (Gadus morhua)	Field, Caged	North Sea - Statfjord, 10000 m – 2000m - 500 m	5.5 weeks	Naph sum	228 ng/g	0,2	0.9 - 1.1 - 0.9	Aas *et al*., in press
				Phen sum	482 ng/g	2,0	3 - 4.5 - 6.7	
				Pyren	28 ng/g	10,2	29.5 - 31.1 - 41.5	
Cod (Gadus morhua)	Field, Caged	German bight G4 (Ref) G1 - G2 - G3	5.5 weeks	Naph sum	228 ng/g	0,8	1 - 1 - 1.9	Aas *et al*., in press
				Phen sum	482 ng/g	1,0	0.7 - 0.8 - 0.8	
				Pyren	28 ng/g	0,0	0 - 0 - 0	
Haddock (Melanogrammus aeglefinus)	Feral fish	Egersund	Baseline non polluted area	Naph sum	1346.9 ng/g			Klungsøyr *et al*. 2003
				Phen sum	526.8 ng/g			
				Pyren	5.7 ng/g			
Haddock (Melanogrammus aeglefinus)	Feral fish	Sleipner	Baseline polluted area?	Naph sum	1111.5 ng/g			Klungsøyr *et al*. 2003
				Phen sum	331.5 ng/g			
				Pyren	10.4 ng/g			
Haddock (Melanogrammus aeglefinus)	Feral fish	Statfjord	Baseline polluted area?	Naph sum	1279.7 ng/g			Klungsøyr *et al*. 2003
				Phen sum	331.9 ng/g			
				Pyren	3.1 ng/g			
Haddock (Melanogrammus aeglefinus)	Feral fish	Barents sea		Naph sum	1474 ng/g			Sundt, 2004
				Phen sum	165 ng/g			
				Pyren	0			
Polar cod (Boreogadus saida)	Laboratory, feral fish 2001, 2002	1.5 ppm StatfjA oil , baseline, control	14 days	Naph sum	1330 ng/g	1,3	114	Sundt and Bechmann, 2004
				Phen sum	538 ng/g	0,9	90	
				Pyren	52 ng/g	14,6	60	

ANNEX 7

DNA adduct detection using the ^{32}P-postlabelling method

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Cefas

Background information

Cefas

What are DNA adducts?

- DNA adducts are formed when genotoxic chemicals covalently bind to DNA bases (A,C,T,G).
- If left un-repaired (or miss-repaired) DNA adducts may go on to cause permanent alterations to the genome (gene mutation).
- A number of important environmentally relevant genotoxins, such as PAHs are known to induce their carcinogenic effects via the formation of DNA adducts.

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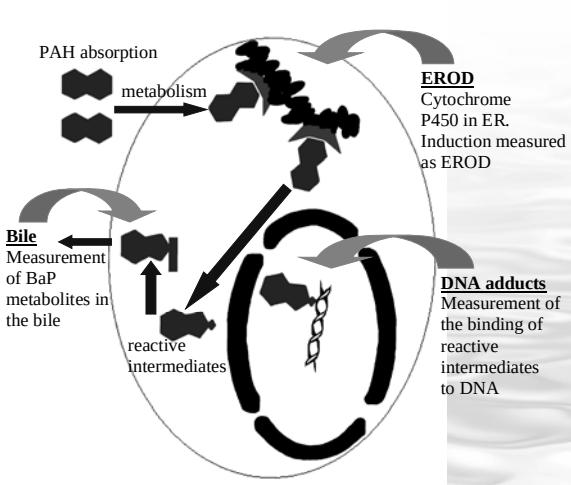
DNA adduct formation



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DNA adducts as biomarkers (1)

- They are semi- quantifiable measurement of the biologically effective dose of contaminant reaching a critical cellular target.
- Thereby integrating multiple toxciokinetic factors including: bioavailability, metabolism, detoxification and DNA repair mechanisms.

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DNA adducts as biomarkers (2)

- DNA adducts are relatively persistent (known to last several months) and therefore provide an assessment of chronic exposure.
- Studies have demonstrated that the levels of DNA adducts positively correlate with the prevalence of certain neoplastic and pre-neoplastic lesions in several fish species.
- Integration of analytical and biological effect data is required for meaningful environmental assessment.

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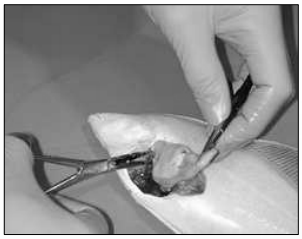
Methodology, sampling and safety considerations

How are DNA adducts measured?

- ^{32}P-postlabelling assay has proved the most popular method of detecting DNA adducts in both laboratory and environmentally exposed organisms.
- Method possesses a number of advantages that make it suitable for the assessment of DNA adducts induced by environmental genotoxins:
  - ^{32}P-postlabelling is sensitive (1 adducted DNA base per 10 $^{9/10}$ undamaged DNA bases).
  - Prior knowledge of contaminant not required.
  - DNA adducts can be detected in any organism/tissue from which DNA can be extracted.

Sampling requirements



- Target organ: Any tissue can be used but usually liver is selected. 100-200mg dissected out and immediately snap frozen in liquid nitrogen before subsequent storage at –80°C.
- Depending on laboratory up up to 20-30 samples can analysed per experiment 1-2 times per week.
- Requirement for integration with other biomarkers and pathology!

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- Standard methods used to extract DNA (5–10 μg).
- DNA digestion to 3'monophosphate nucleotides (contains both adducted and normal nucleotides.
- Adducts enriched (nuclease P1 or butanol enrichment).
- Adducted 3'monophosphate radioactively labelled using T4 polynucleotide kinase catalysed transfer of the terminal phosphate from ^{32}P-ATP to the 5'position of the 3'monophosphate leading to the formation of a [5'^{32}P]-3',5'biphosphate nucleotide.
- Adducts resolved using 2D thin layer chromatography (TLC).
- Visualised using autoradiography and/or commercial ^{32}P-scanners

DNA Isolation

↓

DNA Digestion

Np (Ap+Cp+Gp+Tp) + Xp e.g.

↓ Gp

Adduct Enhancement

(Nuclease P1 or Butanol)

↓

^{32}P-Labelling

^{32}pNp + ^{32}pGp

↓

TLC and Quantification

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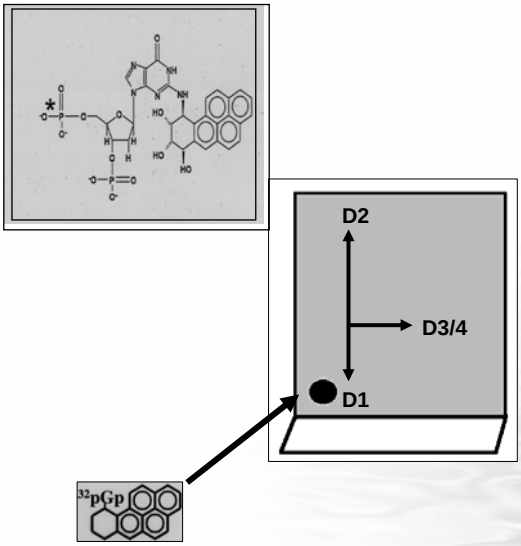
^{32}P-Postlabelling of a B[*a*]P-DNA adduct



- DNA extraction, digestion to 3'monophosphate, labelling to 3'5' biphosphate using ^{32}P-ATP.
- Labelled adducts spotted and resolved (2-D) on TLC plates to separate adducts.
- ^{32}P-tag allows quantification of number of DNA adducts.

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^{32}P-TLC plate reader

- Storage phosphor imaging systems are currently used for qualitative and semi-quantitative analysis of samples.
- Liquid scintillation spectrometry (LSS) can se used where phosphor imaging systems are not available.

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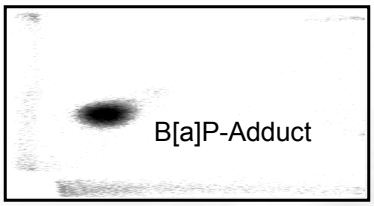
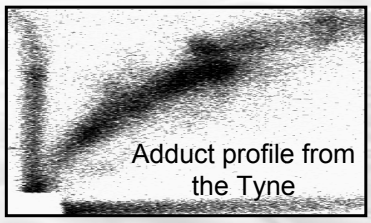
^{32}P-postlabelling and DNA adduct detection

- ^{32}P-assay detects bulky hydrophobic DNA adducts, including those formed by the known 4-5 ringed carcinogenic PAHs (e.g. B[a]P).
- Complex mixtures of genotoxins form over lapping areas of DNA adducts termed DRZ's.
- Generates qualitative and semi quantitative data (expressed as number damaged adducts per 10 normal nucleotides; nmol adducts/mol DNA, amol adducts/ug DNA).





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Safety considerations

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Safety considerations

- ^{32}P-postlabelling assay uses relatively large amounts of high specific activity ^{32}P-ATP.
- ^{32}P- is an energetic beta emitter (1.7 MeV) with ½ life of 14.3 days.
- Designated radiation laboratory and licensed disposal and storage facilities.
- In the UK use of radioactive isotopes is governed via Environment Agency, through a consent licence covering purchase, storage and disposal.

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Safety equipment to minimise 32P exposure

- Appropriate shielding (1cm Perspex).
- Experiments planned to minimise risk, use of long arm tongs to reduce exposure, long arm gloves etc.
- Frequent monitoring of work station (Geiger counter).
- Personal monitoring via radiation medicals and body dosimeter badges.

Castegnaro *et al*., . (1993). Some safety procedures for handling ^{32}P during postlabelling assays, In Postlabelling Methods for Decetion of DNA Adducts. Ed. D.H. Phillips, M. Castegnaro & H. Bartsch. Lyon, International Agency for Research on Cancer. IRAC Scientific Publications 124, 87-92.

Cefas

Ecological relevance and validation for use in the field

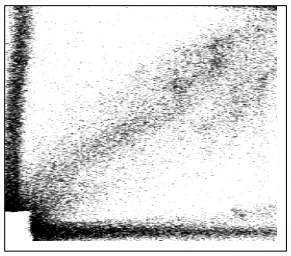
Cefas

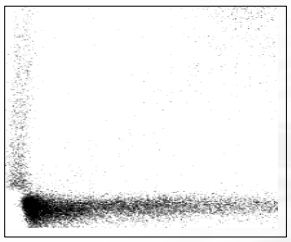
Ecological relevance

- In North America the technique has been used widely (>30 marine and freshwater species).
- Across OSPAR maritime area the assay has been applied in numerous biological effects monitoring programmes using a range of species including, blue mussels, *Mytilus* sp, perch (*Perca fluviatilis*), dab (*Limanda limanda*), European flounder (*Platichthys flesus*), eelpout (*Zoarces viviparous*) and cod (*Gadus morhua*).
- Actually can be used in any species and tissue in a “fit for purpose” manner.

Cefas

Offshore hepatic DNA adduct profiles





- Offshore species dab UK NNMP (*Limanda limanda*) but amenable to a wide range of species across OSPAR.
- Qualitative profiles indicate that in some locations you can detect faint DRZs indicative of PAH exposure.
- Majority of sites quantitative data indicates that levels of DNA adducts fall within levels that are considered background (in agreement with most data from Europe).

Cefas

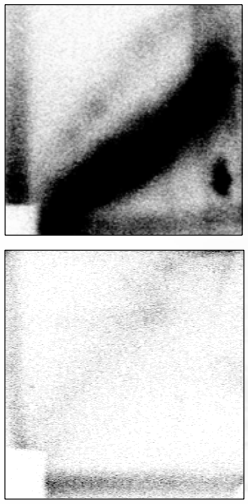
Quantitative DNA adduct levels offshore

Station	CSEMP	Adducts per 10^8 nucleotides
Firth of Forth	165	3.2 ± 0.8 [a] (7) [b]
Amble	244	7.6 ± 2.0 (10)
Flamborough	344	2.0 ± 0.5 (10)
West Dogger	285	5.4 ± 1.0 (10)
Off Humber	345	4.3 ± 1.1 (10)
Celtic deep	605	10.6 ± 2.1 (10)
Outer Cardigan Bay	665	6.0 ± 1.3 (10)
Burbo Bight	705	2.3 ± 1.3 (10)
Liverpool Bay	715	7.5 ± 1.2 (10)
Red Warfe Bay	776	8.2 ± 1.9 (10)

Levels of hepatic DNA adducts (DNA adducts per 10^8 undamaged nucleotides) in dab from UK coastal waters.
[a] *Mean adduct levels ± SE*
[b] *Numbers in parentheses represent number of individual liver samples analysed*

Cefas

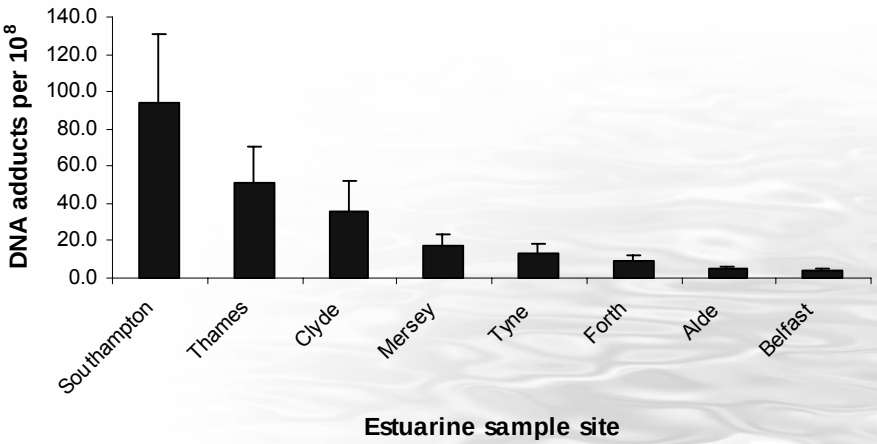
DNA adduct profiles from estuarine sites



- DNA adduct profile from contaminated estuaries indicative of exposure to complex mixtures of genotoxins.
- In contrast low or background levels of DNA adducts (similar to offshore sites) are seen at reference sites in the majority of published studies.

Cefas

DNA adduct levels European flounder



Cefas

Status of quality control procedures and standardised assays

Status of AQC and standard methods (1)

- Presently no active AQC programmes.
- Previous AQC programmes conducted under the auspices of BEQUALM and the International Agency for Research on Cancer (IARC).
- IARC standardisation and validation trial ran between 1994-1997. 25 participating laboratories in Europe and USA.

Status of AQC and standard methods (2)

- IARC trial led to series of publications which detailed a standardised assay protocol (Phillips & Castegnaro, 1999)[1].
- An ICES times document[2] available using protocol essentially as outlined in Phillips & Castegnaro (1999).
- The standardised protocol has now been adopted by the International Programme on Chemical Safety (IPCS) and recommended for use in their guidelines for monitoring genotoxic carcinogens in humans (Richard *et al*., 2000)[3].

[1] Phillips, D.H., Castegnaro, M. (1999). Standardization and validation of DNA adduct postlabelling methods:report of interlaboratory trails and production of recommended protocols. Mutagenesis 14 (3), 301-315.

[2] Reichert, W.L., French, B.L,. Stein, J.E. (1999). Biological effects of contaminants: Measurements of DNA adducts in fish by ^{32}P-postlabelling. ICES Techniques in Marine Environmental Sciences. No. 25.

[3] Richard J. Albertini, R.J., Diana Anderson, D Dougla, D.R., Hagmar, L., Hemminki, K, Merlo, F., Natarajan, A.T., Norppa, H., Shuker, D.E.G., Tice, R., Waters, M.D., Aitio, A (2000). IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. Mutation Research-Reviews in Mutation Research. 463 (2), 111-172.

Cefas

Assessment criteria and requirements

Cefas

Assessment

- Setting background/baseline responses essential in order to set limits for unintended or unacceptable levels of biological effect.
- Studies which have examined fish/shellfish from pristine areas (supported by biomarkers and chemistry) demonstrate clearly that adducts are at or near the limits of detection.
- Using such defined reference locations it should be possible to gain an international consensus (via ICES WGBEC) on the levels of DNA adducts, which can be considered background for a particular species and location. Will require:-
  - All data to be generated using standardised protocols (e.g ICES Times protocol and IARC).
  - Species used to have sufficient background data available on life history traits, behaviour etc.

Cefas

Conclusions

Cefas

Summary (1)

- DNA adducts provide a measure of biologically active contaminant to have reached a critical target (DNA).
- Background information demonstrating importance in the initiation and progression of carcinogenesis induced by important environmental contaminants (e.g. PAHs).
- Applicable to species across the whole OSPAR maritime area, so suitable for use in any species deemed 'fit for purpose'.

Cefas

Summary (2)

- Standardised protocols available via IACR and ICES.
- **Not high through put**. Should used to focus in on environments where other biomarkers (EROD, bile) have highlighted a potential problem due to contaminant exposure (e.g. PAHs).
- Though to obtain really meaningful environmental assessment data their determination needs to be integrated into a comprehensive analytical and biological effects programme.

Cefas

Annex 8: PREDICT 2 - Mussels and fish

PREDICT 2: Predicting Health of the Environment – Lysosomal Reactions in Mussels & Fish

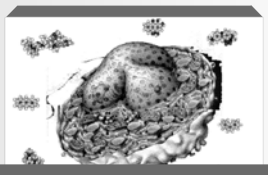
Mike Moore (PML), David Lowe (PML), Jenny Shaw (PML), Jim Readman (PML), Icarus Allen (PML), Allen McVeigh (PML/BMT-CORDAH), Nasir Jamal (PML), Bob Clarke (PRIMER-€), Paul Somerfield (PML), Phil Dyke (Univ. of Plymouth), Angela Köhler (AWI, Germany), Aldo Viarengo (Univ. Eastern Piedmont, Italy), John Thain (CEFAS), Steve Brooks (CEFAS), Brett Lyons (CEFAS), Grant Stentiford (CEFAS), John Bignall (CEFAS) & Steve Feist (CEFAS)

Supported by Defra/NERC

Plymouth Marine Laboratory

Environmental Prognostics

Harmful environmental agents



"Distress Signals"

Cell Injury

- Description & mechanisms
- Integration of disparate data sets
- Explanation & prediction

Harmful environmental agents

Physiomics: Whole Animal Function

Disease & Death

Ecological Consequences

Plymouth Marine Laboratory

Why biomarkers?

- Many biomarkers do not tell us anything about the health status of the animal
- Without this information, much of the available exposure biomarker data cannot be effectively interpreted or used in prognosis
- Biomarkers of cell injury (e.g., lysosomal reactions) can be used as an index of cellular function or dysfunction and also to provide a measure of animal health status
- Can *lysosomal* biomarkers be used to predict higher level effects?

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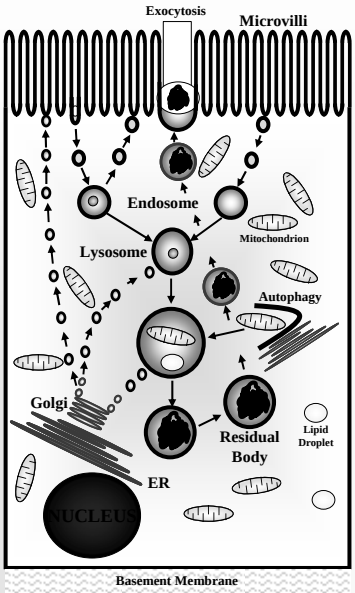
Cellular Vesicular Traffic: Endocytosis, Golgi, Lysosomal-Vacuolar System & Autophagy



Endocytosis of food & receptor-bound ligands

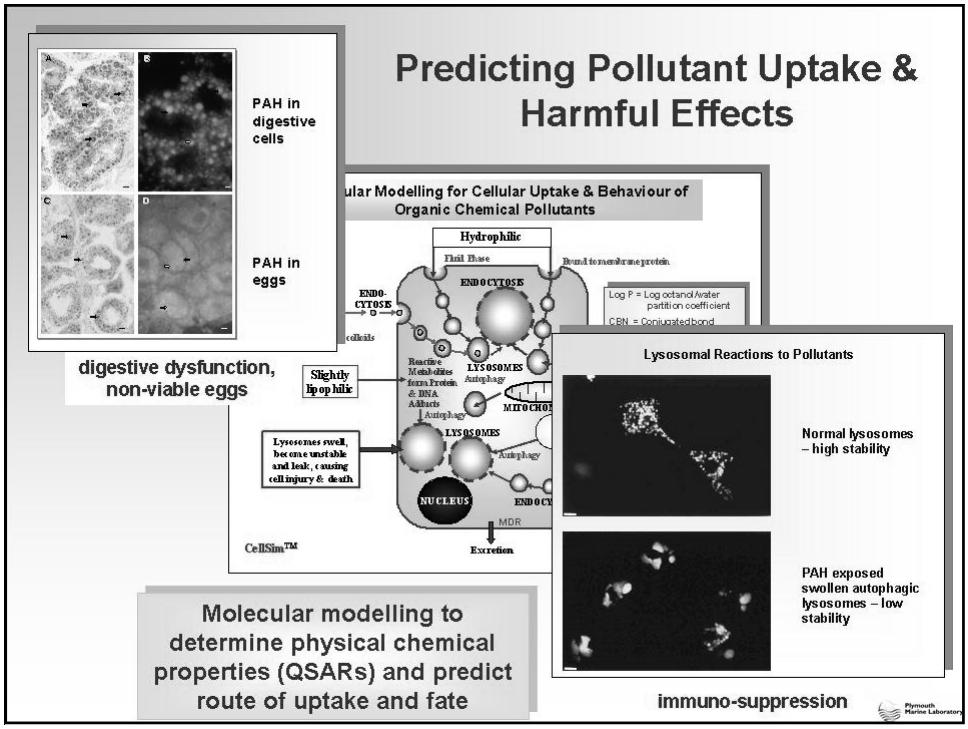
Transport to Endosome & membrane/receptor recycling

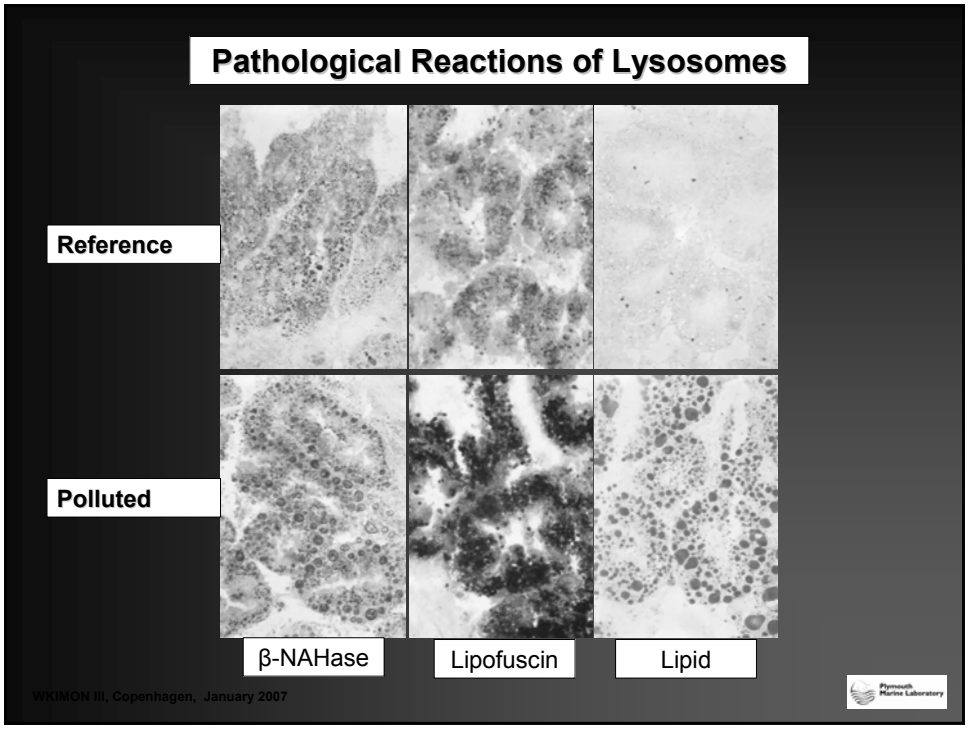
Formation of Secondary Lysosome & digestion of endocytosed materials

Autophagy of damaged or redundant proteins & organelles (Apg factors)

Formation of Residual Body containing Lipofuscin, which may then be exocytosed

CellSim™

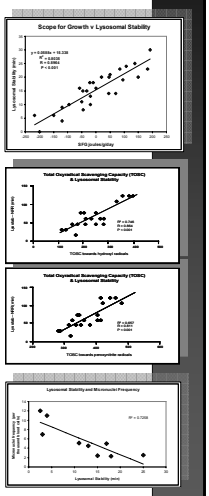

Predicting Pollutant Uptake & Harmful Effects
PAH in digestive cells
PAH in eggs
digestive dysfunction, non-viable eggs
ular Modelling for Cellular Uptake & Behaviour of Organic Chemical Pollutants
Hydrophilic
Fluid Phase
ENDOCYTOSIS
Log P = Log octanol:Water partition coefficient
Slightly lipophilic
LYSOSOMES
Autophagy
Lysosomes swell, become unstable and leak, causing cell injury & death
NUCLEUS
MDR
Excretion
CellSim™
Lysosomal Reactions to Pollutants
Normal lysosomes – high stability
PAH exposed swollen autophagic lysosomes – low stability
immuno-suppression
Molecular modelling to determine physical chemical properties (QSARs) and predict route of uptake and fate


Pathological Reactions of Lysosomes
Reference
Polluted
β-NAHase
Lipofuscin
Lipid
WKIMON III, Copenhagen, January 2007

Lysosomal Reactions, Cell Injury & Pathophysiology

Lysosomal stability is correlated with:

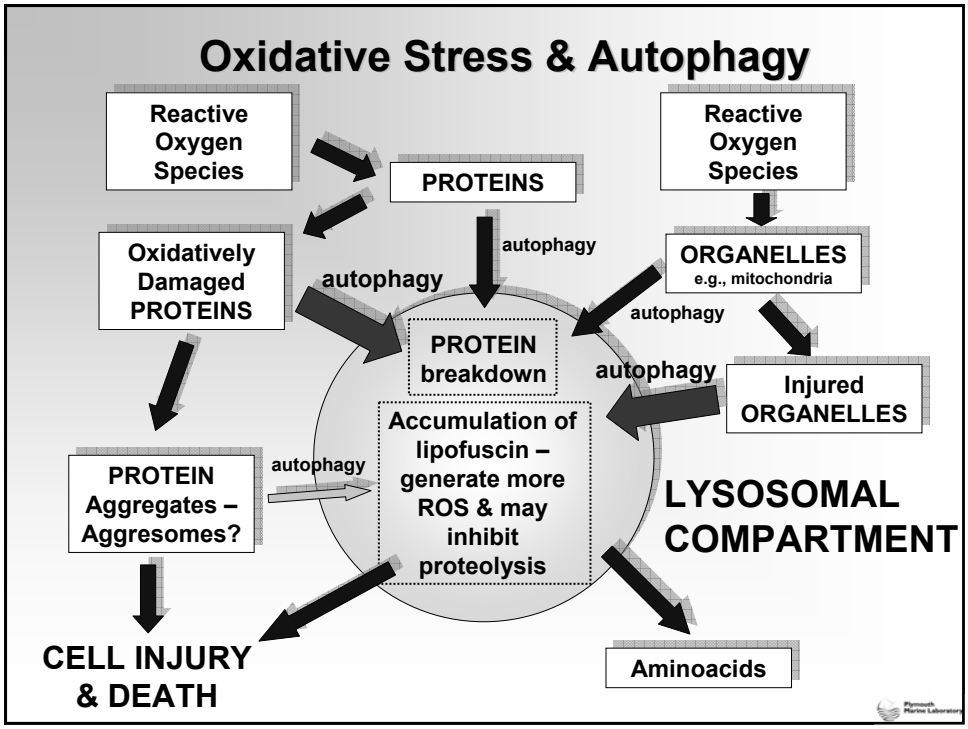
- Larval viability
- Scope for growth
- Protein synthesis
- Total oxyradical scavenging capacity (TOSC)



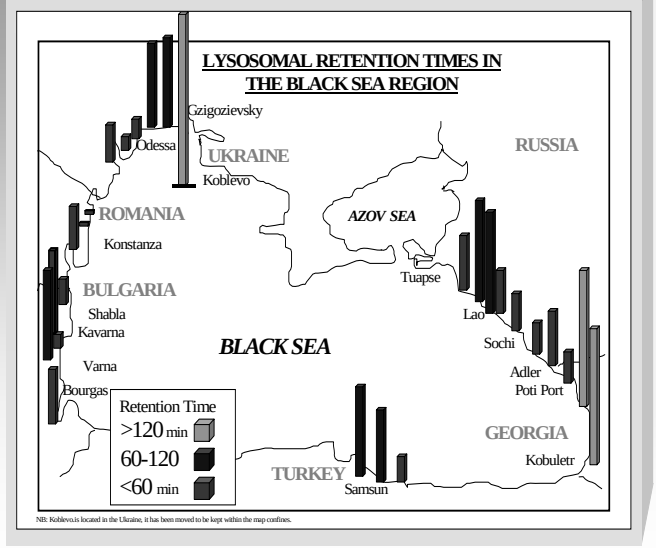
Lysosomal stability is inversely correlated with:

- Protein catabolism
- Lipofuscin (stress or age pigment)
- Lysosomal lipidosis
- Lysosomal swelling
- DNA damage
- Liver cancer in flatfish

Oxidative Stress & Autophagy

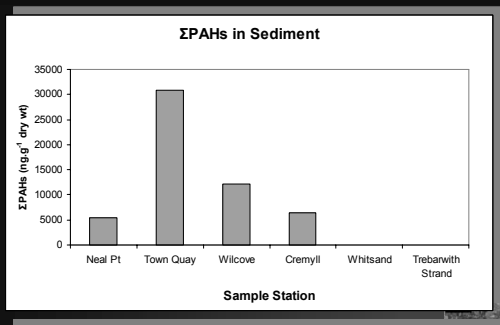


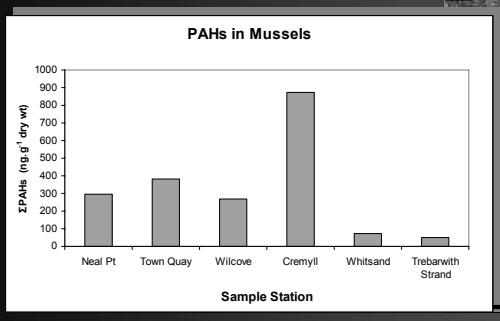
UNESCO - IOC Black Sea Mussel Watch



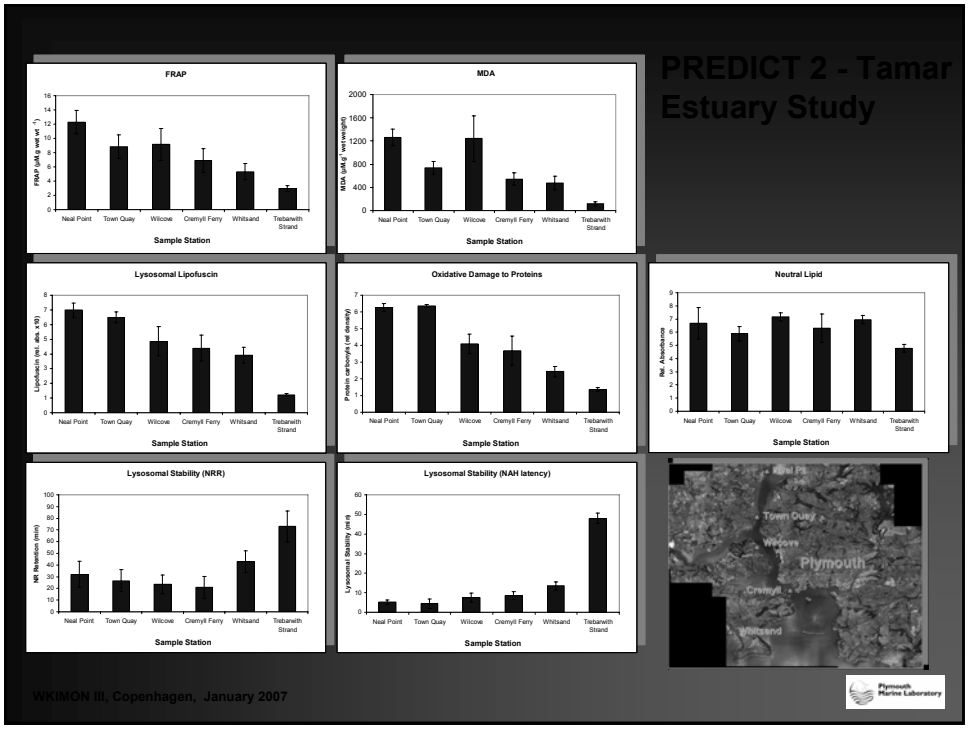
11

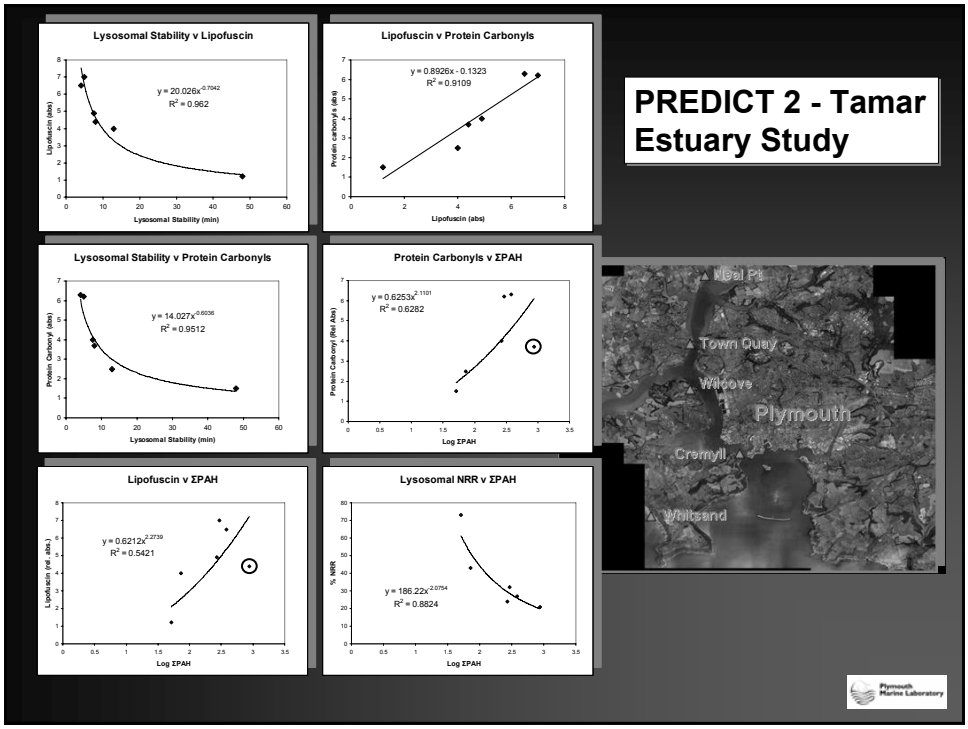
PREDICT 2 - Tamar Estuary Study



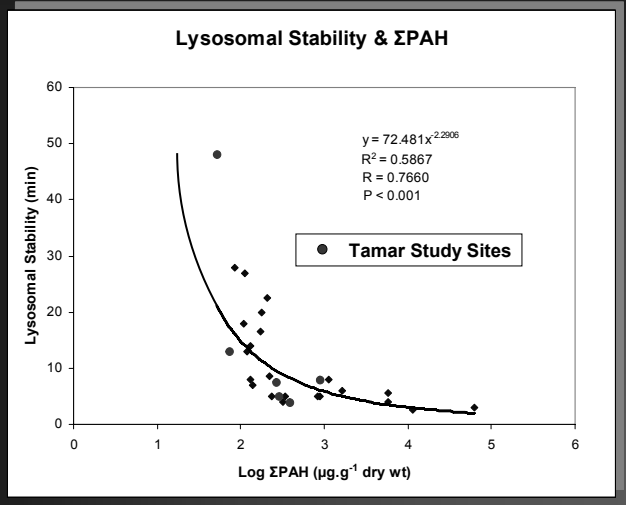


WKIMON III, Copenhagen, January 2007


PREDICT 2 - Tamar Estuary Study
FRAP
MDA
Lysosomal Lipofuscin
Oxidative Damage to Proteins
Neutral Lipid
Lysosomal Stability (NRR)
Lysosomal Stability (NAH latency)
Neal Point
Town Quay
Wilcove
Cremyll Ferry
Whitsand
Trebarwith Strand
Sample Station
Plymouth
Cremyll
Whitsand
WKIMON III, Copenhagen, January 2007
Plymouth Marine Laboratory


Lysosomal Stability v Lipofuscin
y = 20.026x-0.7042
R² = 0.962
Lipofuscin v Protein Carbonyls
y = 0.8926x - 0.1323
R² = 0.9109
PREDICT 2 - Tamar Estuary Study
Lysosomal Stability v Protein Carbonyls
y = 14.027x-0.6936
R² = 0.9512
Protein Carbonyls v ΣPAH
y = 0.6253x2.1101
R² = 0.6282
Lipofuscin v ΣPAH
y = 0.6212x2.2739
R² = 0.5421
Lysosomal NRR v ΣPAH
y = 186.22x-2.0754
R² = 0.8824
Neal Pt
Town Quay
Wilcove
Plymouth
Cremyll
Whitsand
Plymouth Marine Laboratory

PREDICT 2 - Tamar Estuary Study



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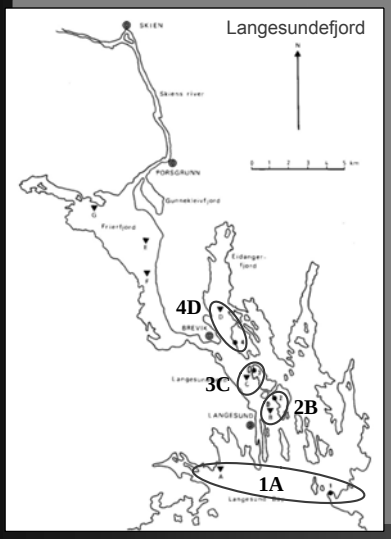
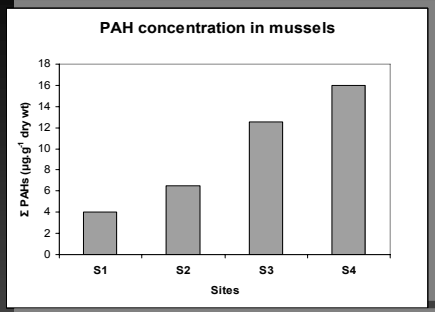
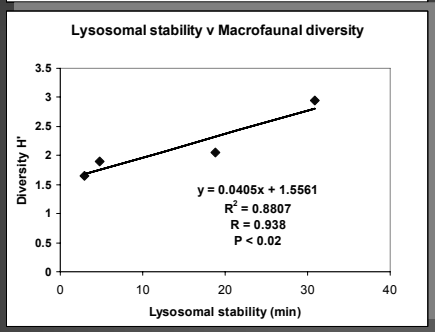
UNESCO-IOC GEEP / ICES 1986







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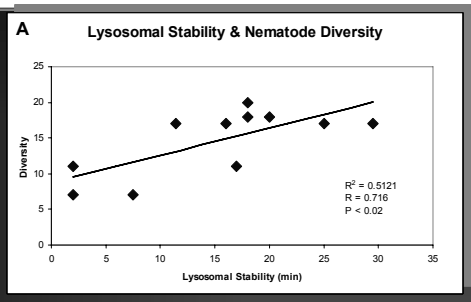
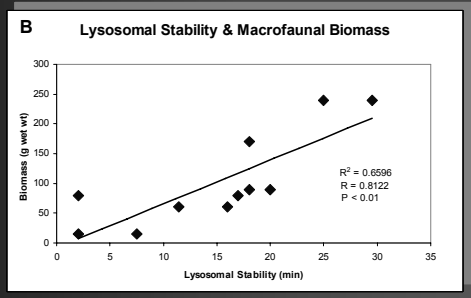
Dab Liver Health and Ecological Status from a Transect in the North Sea





Spearman Rank Correlation Analysis (PRIMER-BIOENV) of Flatfish (Dab – *Limanda limanda*) liver and larval biomarkers and benthic ecological community data for the Bremerhaven – North Sea Transect.

Benthic Community	Biomarkers*	Correlation r_s
Macrobenthos	2, 9, 14	0.927
Macrobenthos	2, 5, 10, 14	0.915
Copepods	2, 6, 12, 14	0.952
Copepods	2, 6, 10, 14, 15	0.927
Nematodes (top 2 cm)	2, 6, 10, 13, 15	0.758
Nematodes (2-10 cm)	2, 6, 10, 13, 15	0.818

*Biomarkers:

1. EROD (hepatocytes)
2. Lysosomal Stability (acridine orange uptake)
3. Lysosomal Stability (neutral red retention)
4. Pinocytosis
5. Generation of Reactive Oxygen Species (ROS)
6. Proliferation of Endoplasmic Reticulum (ER)
7. N-*ras* Oncoprotein
8. Lipid Vacuolation
9. Foci of Cellular Alteration
10. Larval Morphological Abnormalities
11. EROD (liver)
12. Cytochrome P-450 (CYP 1A1)
13. Superoxide Dismutase (SOD)
14. Catalase (CAT)
15. Glutathione Peroxidase (GPX)
16. Acetylcholine Esterase (gill tissue)
17. Metallothionein (MT)
18. Liver Nodules (>2 mm)
19. Unsaturated Neutral Lipid
20. Lysosomal Stability (cytochemical method)

Summary

1. Clear gradient of oxidative damage and harmful cellular effects in Tamar system
2. Evolutionarily conserved lysosomal function, autophagy and anti-oxidant protection is essential for normal cell function and provides an index of health status
3. Perturbation of lysosomal and autophagic function results in “failed autophagy”, cell injury and cell death
4. Biomarkers of lysosomal dysfunction are predictors for tissue, organ and whole animal pathology and have been used to derive an expert system
5. Can lysosomal biomarkers be predictive for ecosystem status?

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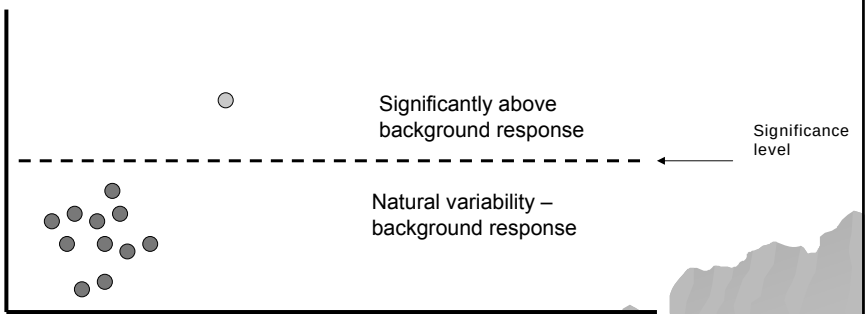
Annex 9 Background values for EROD

A way to regional background values of EROD activity in dab

U. Kammann & H.-J. Kellermann
Federal Fisheries Research Centre
Germany

Background response

- Elevated levels compared to background response indicate that a biological effect caused by hazardous substances can occur.



Natural variability

- Natural variability is included in background responses.
- A possible contaminant effect can only be detected by a biomarker when it is significantly different from natural variability.

Two types of background responses

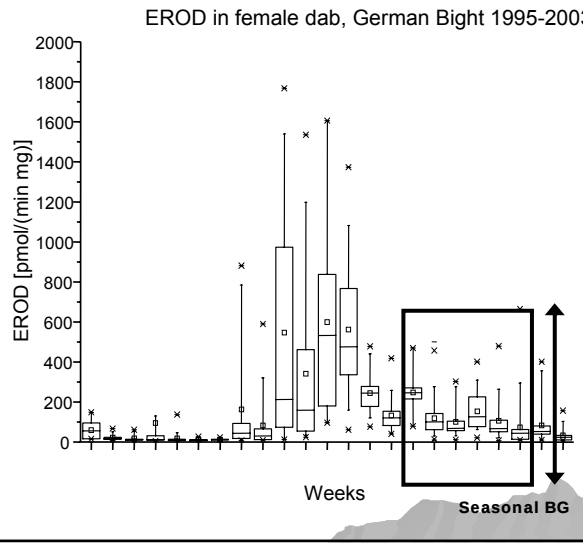
- Background doesn't vary from site to site

  Calculate "global values" for background response. The choice of global/regional reference sites defines the background response. One Number for the whole North Sea
  …. Imposex …

- Background varies from site to site
  Biomarkers, where "regional values" are needed to compare results from different sites. Reference conditions have to be defined for each single region (season).

  … EROD …

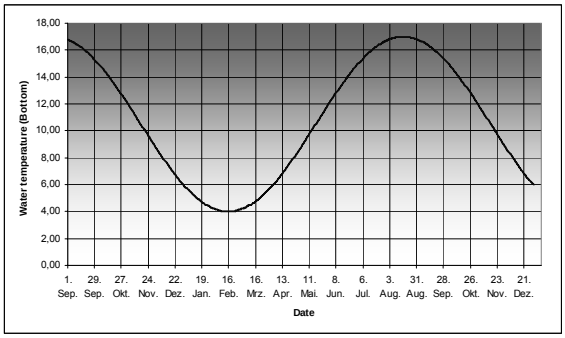
EROD annual cycle



Temperature is the key to EROD

- EROD is closely linked to spawning
- The driving force for the duration of gonadal development is the temperature
- Spawning time varies from year to year according to the temperature in the winter before.
- → Sum up month degrees to predict EROD

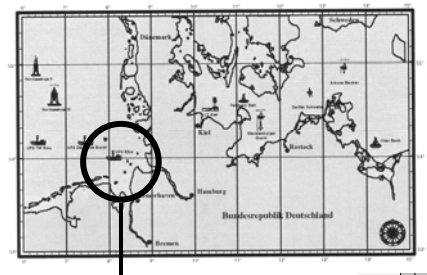
Month degrees

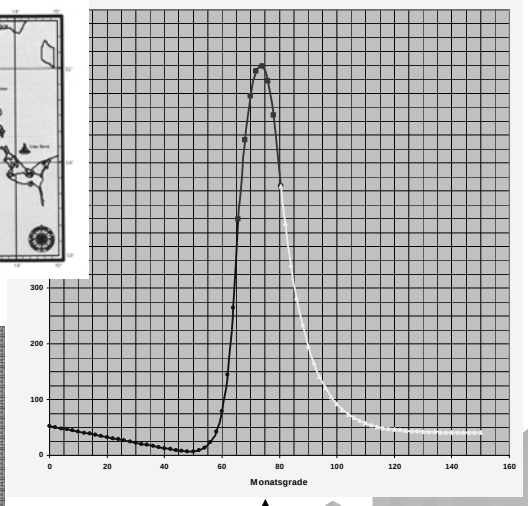


Sum of the mean bottom water temperature at the location in every month starting at 01. September until the date when the fish is caught.

$$Month\deg rees(day) = \int_{1.Sep}^{day} Temp(t) \cdot dt$$

Constructing the model





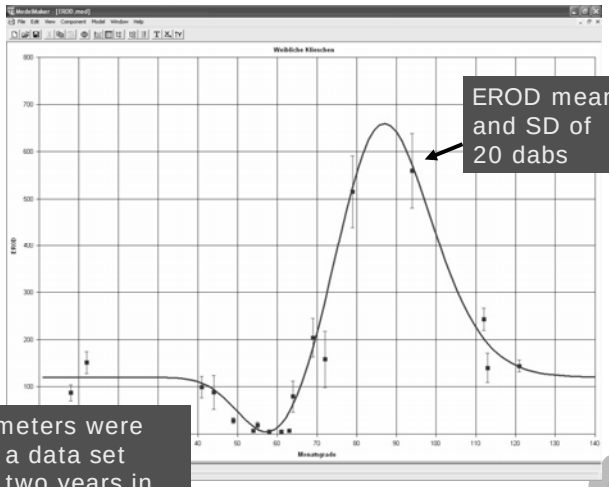
Blue: $2.4*10^{-5} * e^{0.29*x} + 52.4 - x$

Red: $-4.42 * (x-73.5)^2 + 700$

Yellow: $3.4 * 10^{6} * e^{-0.11*x} + 40.6$

Lange, U., Saborowski, R., Karbe, L., Siebers, D.: A Model for the Prediction of the Basal EROD-Activity Levels. ICES C.M. (E7), 1995.

Dataset 1997/1998



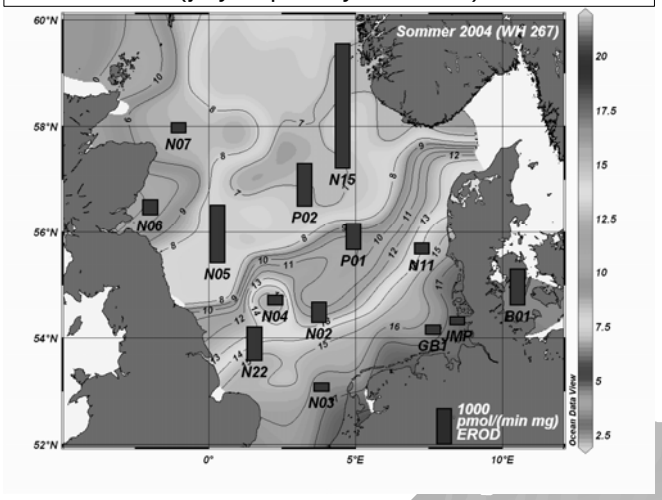
Model Parameters were adjusted to a data set collected in two years in the German Bight

Next Step

- Knowing the background variability we can have a look on the monitoring data.

Monitoring data

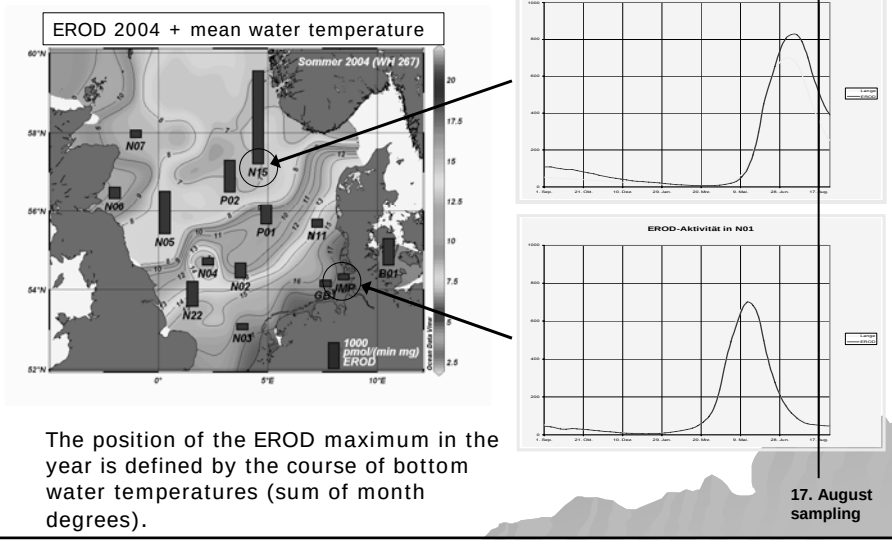
EROD 08/09 2004 + mean water temperature (july-sept 25 year mean)



Now ..

- With the model we have the possibility to compare EROD values from different regions.
- Results could be given in relation to the expected background value.
- The model provides a „global“ EROD background

Spatial EROD monitoring?



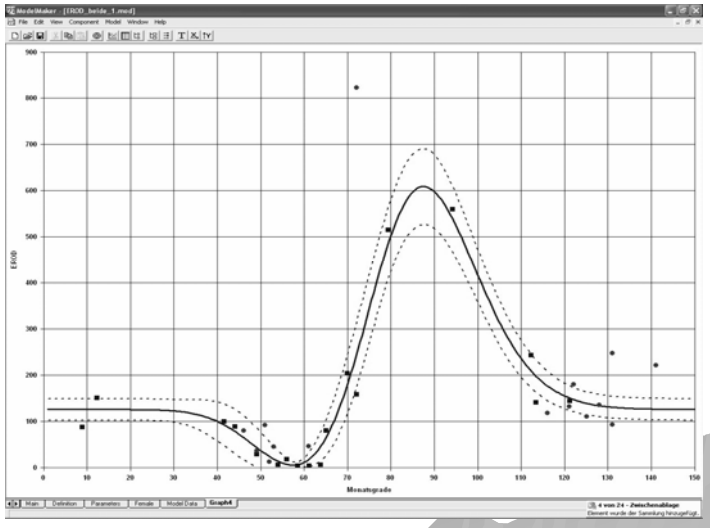
The position of the EROD maximum in the year is defined by the course of bottom water temperatures (sum of month degrees).

17. August sampling

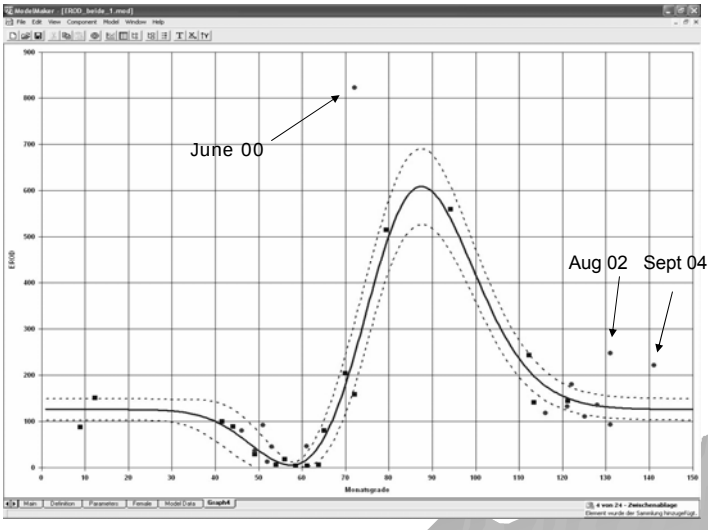
Sampling date

- We have our background response model for EROD in the German Bight.
- Is there a difference between the years?

Our data and the model



Our data with the model



Model benefits

- The model provides a background variation which is independent from sampling date (region)
- If data are outside the 95% confidence interval they are outside the expected natural background response.
- Evaluation criteria are possible

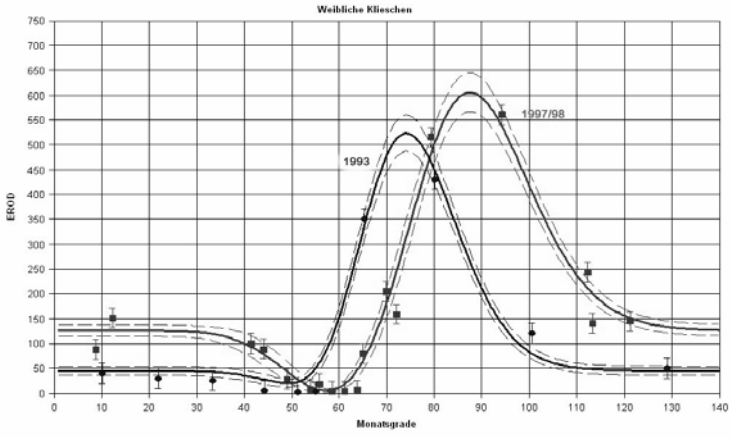
Model problems

- All we know is restricted to one area and to two years.
- We believe that we can transfer this knowledge to other locations or years but we have no proof.
- Maybe not all relevant influence factors have been considered.
- The model needs reliable bottom water temperatures for the sampling location (from year before).
- → We need to test the model

Testing a model

- To test a model constructed from one data set you need a second set of data and see if they fit to the model.

Two data sets

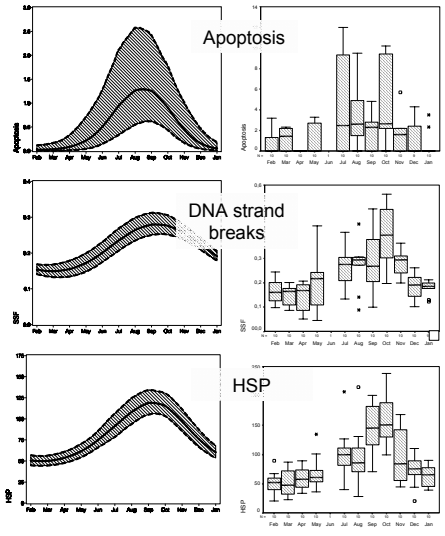


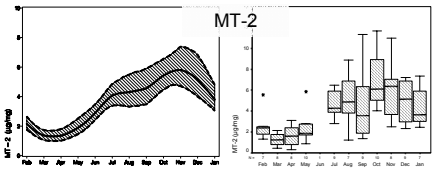
Same curve shape but shifted in month degrees

Lessons learned

- A model is only as good as the data and assumptions it is based on.
- The model explains a lot of the regional EROD variation and is at the moment the best tool we have to interpret monitoring data.
- Looking at a second data set can be surprising.
- → OUR MODEL IS NOT READY YET.
- The model needs improvement. Maybe an important influence parameter is still missing.

A look at other biomarkers in female dab in the German Bight





etc...

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

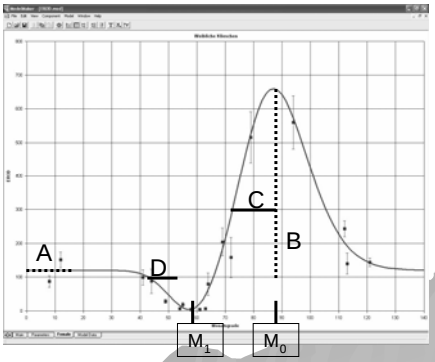
Outlook

- Improve the model to calculate regional EROD background responses
- Think of annual cycles as general base for background responses of biomarkers.

Model Parameters

$$EROD = \frac{B}{\sqrt{2*\pi}*C*M} * e^{-\frac{(\ln M - \ln M_0)^2}{2*C^2}} + A*\left(1 - e^{-\frac{(M-M_1)^2}{2*D^2}}\right)$$

	BFAFi 1997/1998	BAH 1993
M_0	88,9	75,1
M_1	58,8	52,2
A	125	44,5
B	14400	12400
C	0,134	0,134
D	10,6	10,6



Annex 10 Fish Disease monitoring

ICES/OSPAR WKIMON III, 16-18 January, Copenhagen

Fish disease and liver pathology for general and PAH-specific biological effects monitoring

- Current status and outlook-

Stephen Feist

The Centre for Environment, Fisheries and Aquaculture Science

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

Cefas

Background

- First studies in Europe during the late 1970s
- Role of ICES – seagoing workshops (intercalibration) 1984,88 & 94; publication of guidelines
- ICES Sub-group on statistical analysis of fish disease data (1992-1996)
- Monitoring programmes conducted by Germany, The Netherlands and the UK (other data from Denmark & Belgium)
- Fish disease data used in environmental assessments – NSTF 1993; OSPAR QSR 2000; HELCOM assessments 1996, 2002
- Increasingly integrated with monitoring for chemical contaminants and biological effects of contaminants

Cefas

Overview*

- Applicability across the OSPAR Maritime Area
- Status of Quality Assurance
- Influence of environmental variables
- Assessment of thresholds
- Proposals for assessment tools

*OSPAR CEMP Review – Externally visible diseases, liver nodules and liver pathology including liver neoplasia/hyperplasia. Final version submitted July 2006.

Cefas

Applicability across the OSPAR Maritime Area

- Used for many years as an integrative response measuring health status in general biological effects monitoring
- Applicable for a variety of species, eg. Dab, flounder, cod
- Contaminant specific liver pathology – liver cancer and liver histopathology
- Application to many other species, eg. Eelpout, dragonet, plaice and pelagic species
- Appropriate for use across the OSPAR maritime area

Cefas

Status of Quality Assurance

- QA procedures are in place and operational through ICES activities and BEQUALM.
- Covering survey methodology, sampling, diagnosis, reporting and statistical analysis
- Workshops, ring-tests and intercalibration exercises.
- ICES TIMES publications on external fish diseases and liver histopathology (nos 19 & 38)
- Increasing BEQUALM participation

Cefas

Influence of environmental variables

- Multifactorial aetiology of disease is accepted
- Environmental pollution is one factor
- Contaminants cause specific and non-specific changes at various levels of organisation
- Liver cancer and pre-neoplastic lesions are associated with exposure to carcinogenic contaminants eg. PAHs
- Integrated monitoring effort ensure collection of various biological and environmental parameters
- Allows statistical analysis eg. Wosniok et al. 2000

Cefas

Assessment of thresholds

Currently under development via ICES WGPDMO

- Determined from comparisons of disease prevalence between reference site(s) and quantitative change over time (trends)
- Further work needed to define and implement reference and threshold values

Presentation by Werner Wosniok – this meeting

Cefas

Proposals for assessment tools

Currently under development via ICES WGPDMO

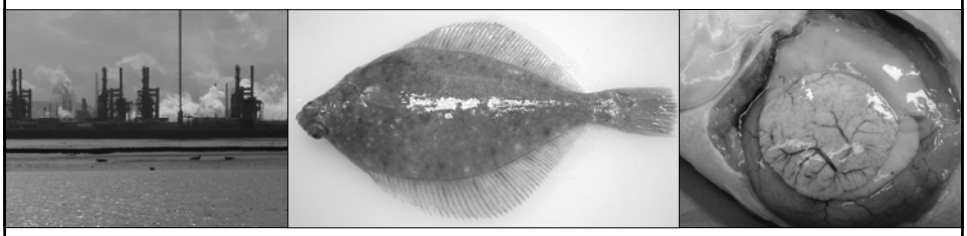
- Development of the Fish Disease Index (FDI)
- Quantitative information summarising data on prevalence and intensity of various disease conditions into one figure
- Multivariate statistical approaches offer good potential

Presentations by Grant Stentiford & Werner Wosniok – this meeting

Cefas

Annex 11 Fish disease integration

Fish disease - a marker for marine environment health



Integration of disease, biomarker and chemistry data

Dr Grant Stentiford and Dr Stephen Feist

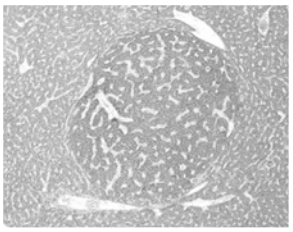
Cefas Weymouth Laboratory, UK

Fish diseases

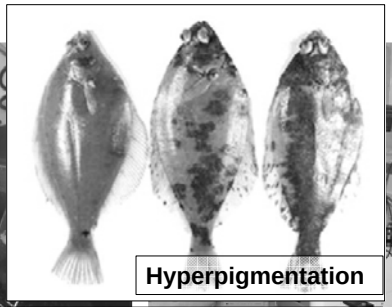
- Top-level indicator of fish population health status
- Used in environmental monitoring programs for two decades
- Utility of approach recognised internationally (e.g. OSPAR CEMP) and nationally (e.g. UK CSEMP)
- External fish disease and liver pathology afforded the highest CEMP ranking and are conducted on a voluntary basis by member states
- Activities surrounding this field have been undertaken by the ICES Working Group on Pathology and Diseases of Marine Organisms (WG PDMO).

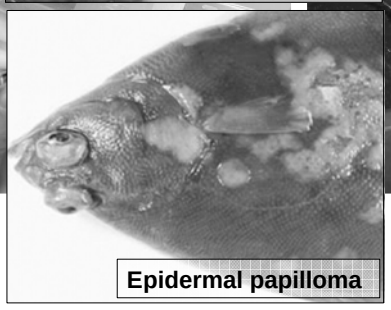
BEQUALM

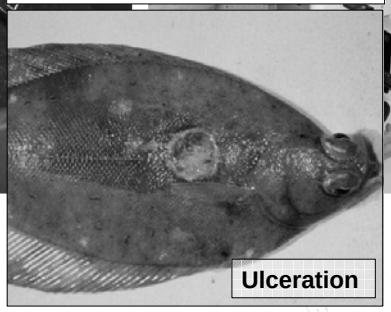
- External fish disease and liver pathology data collected using protocols devised under BEQUALM
- Common approach across Europe
- Ring tests, inter-calibration exercises and workshops
- QA disease data since 2000
- Ongoing



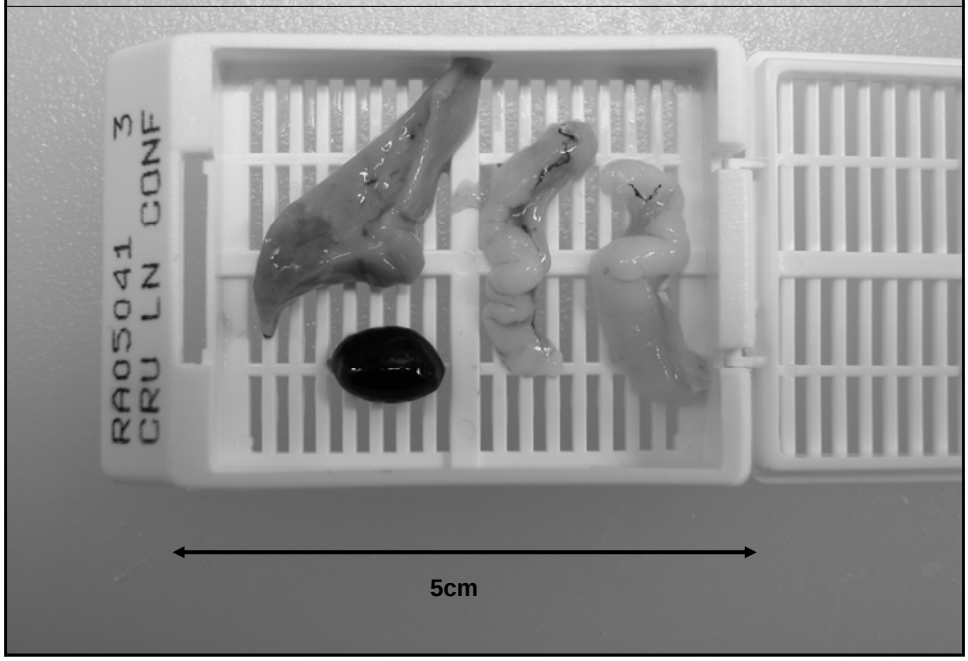
Investigating disease





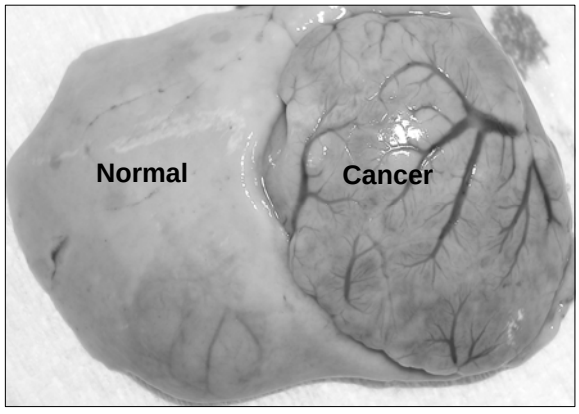


Investigating disease

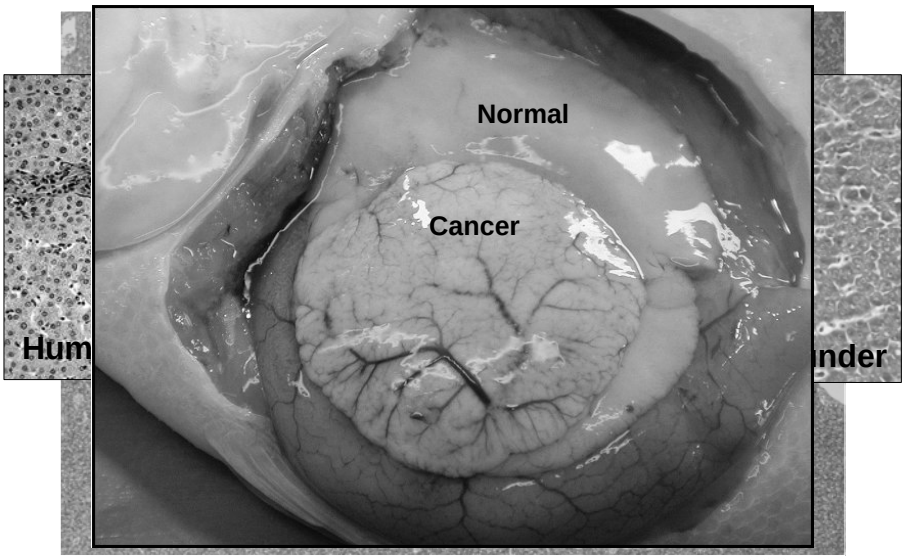


Liver disease

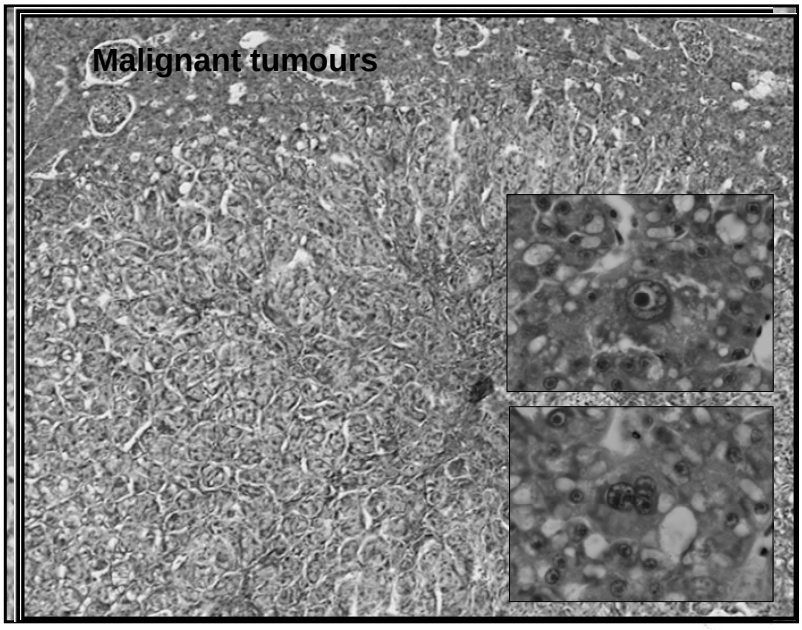
- BEQUALM provides criteria for the diagnosis of liver cancer in sentinel European flatfish

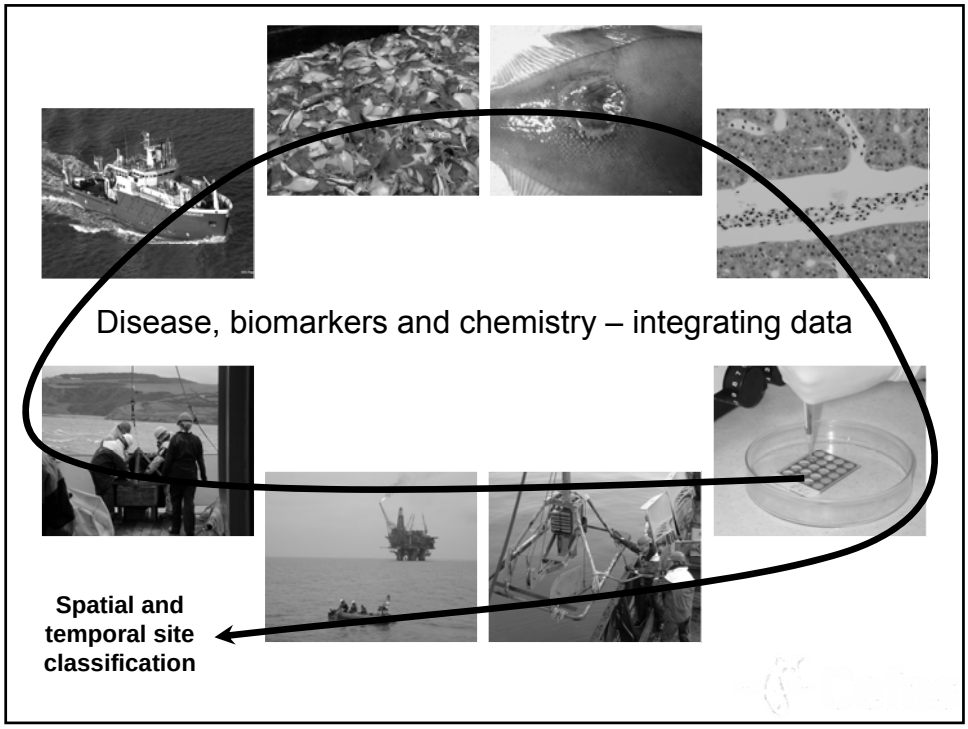


Liver disease

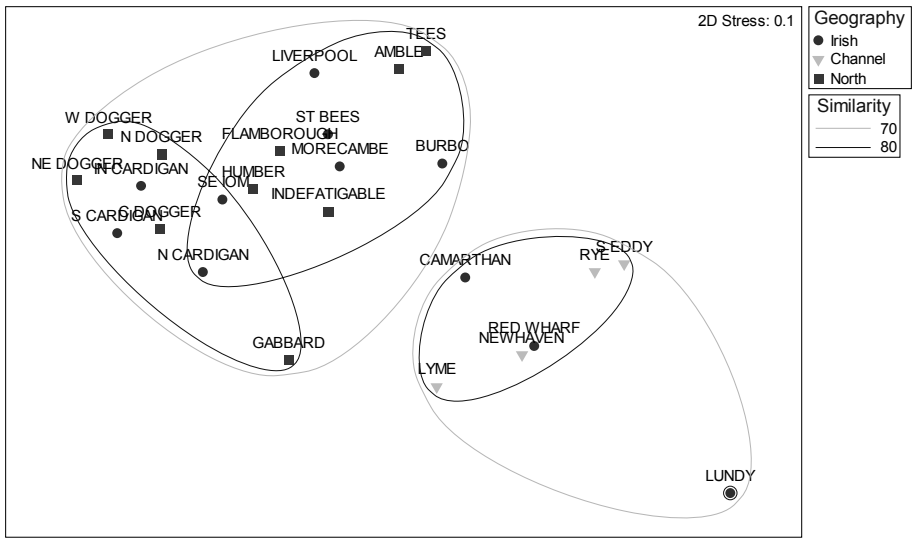


Development of liver cancer



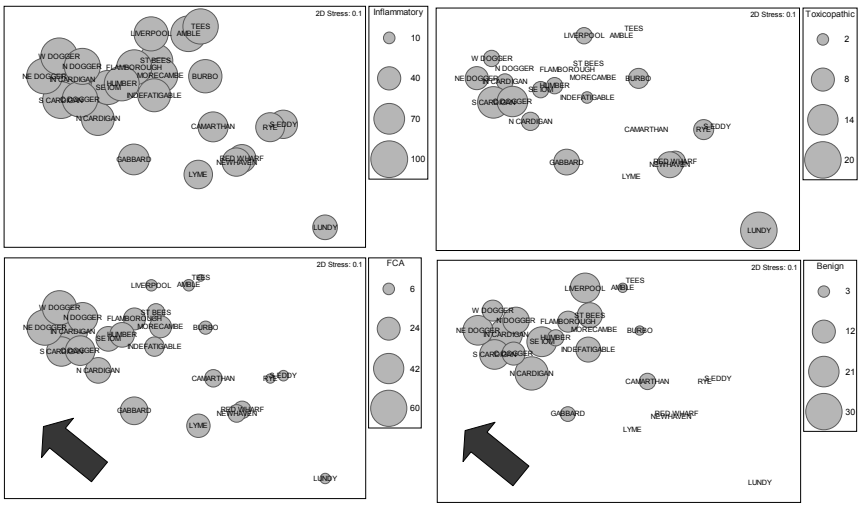


Site discrimination based upon liver pathology (PRIMER)



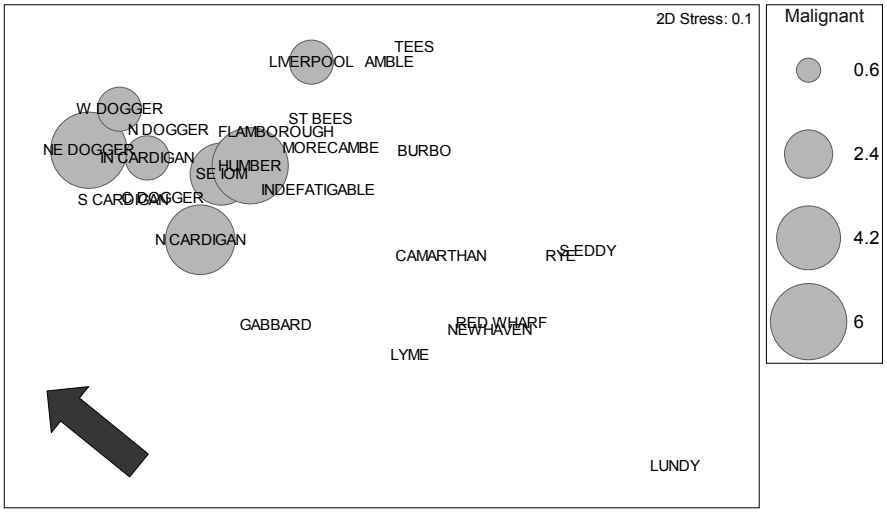
Grant Stentiford

Drivers for site discrimination - liver pathology (PRIMER)



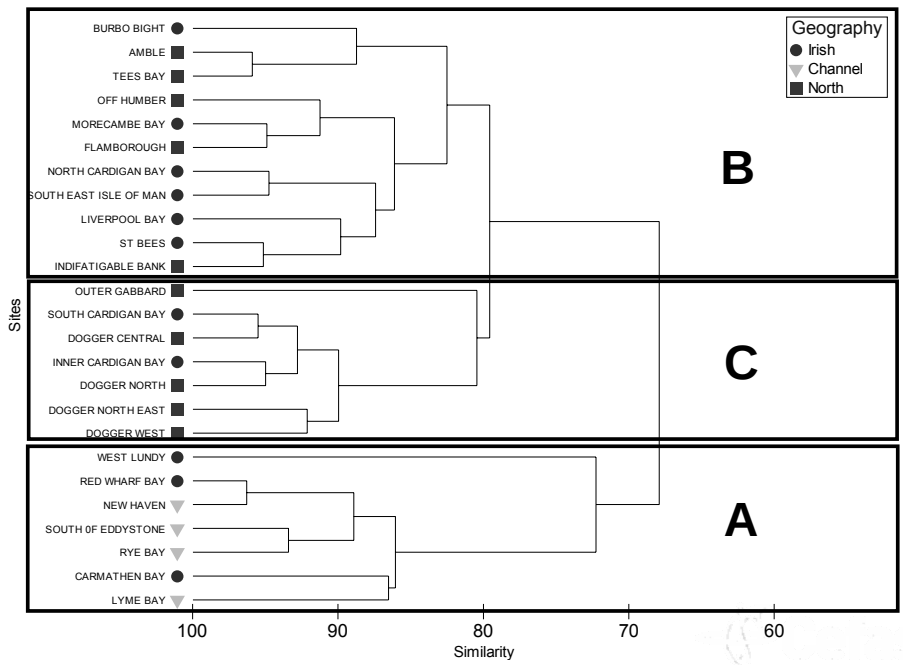
Grant Stentiford

Drivers for site discrimination - liver pathology (PRIMER)



Grant Stentiford

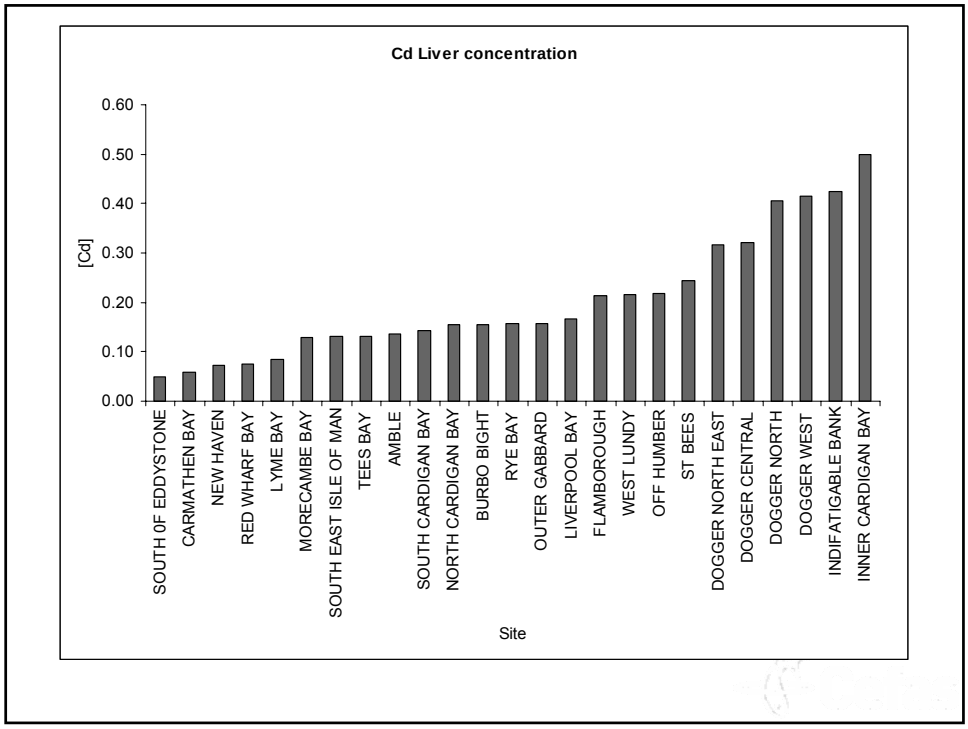
Site classification

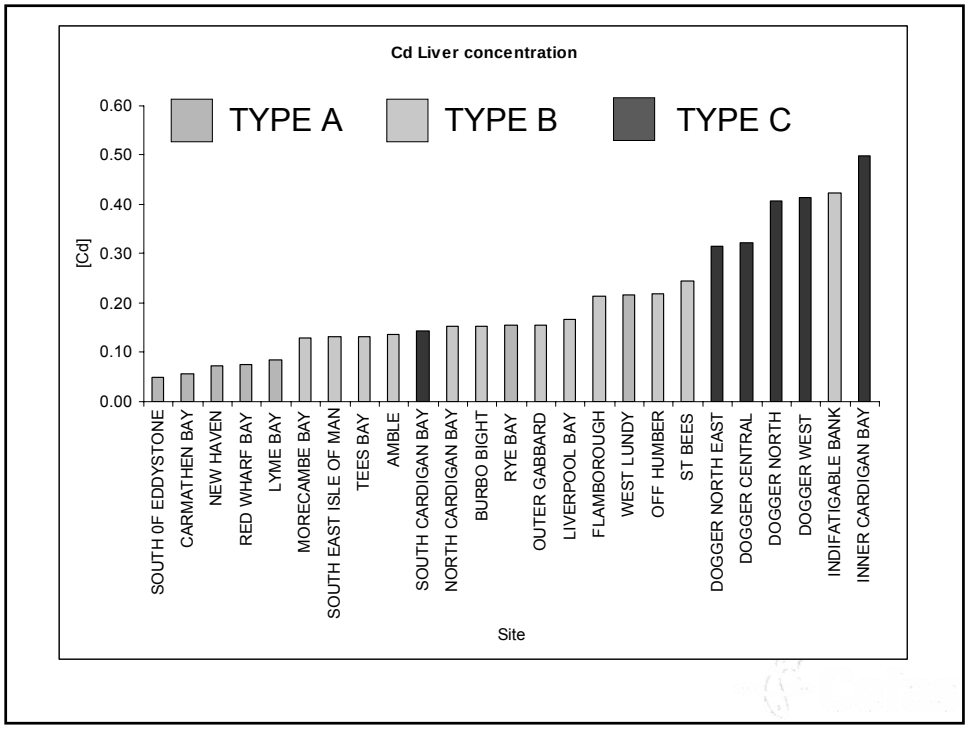


Site classification

TYPE A	TYPE B	TYPE C
• Generally low levels of ICES external diseases and almost complete absence of skin hyperpigmentation. FDI? • Approximately 30% of fish with no indication of BEQUALM liver pathology categories. • Low prevalence (<5%) of toxicopathic lesions and approximately 50% prevalence of inflammatory lesions (according to BEQUALM). • Low prevalence of FCA (<15%), benign tumour (<5%) and malignant tumour (0%) according to BEQUALM.	• Appearance of higher prevalence of ICES external diseases (incl. Lymphocystis and skin hyperpigmentation, the latter up to 20% at North Sea sites). FDI? • Between 10 and 20% of fish with no indication of BEQUALM liver pathology categories. • Low prevalence (generally <5%) of toxicopathic lesions but an elevated prevalence of inflammatory lesions (up to 90%) compared to Type A sites. • Prevalence of FCA can exceed 15% with mean benign tumour prevalence >10%. Appearance of malignant tumours at low prevalence.	• Elevated prevalence of several ICES external diseases (incl. Ulceration, parasites and skin hyperpigmentation, the latter up to 50% at some Dogger sites). FDI? • Low prevalence (<10%) of fish with no indication of BEQUALM liver pathology categories. • Prevalence of toxicopathic lesions generally >5% with prevalence of inflammatory lesions up to 100%. • High prevalence of FCA (>50%) of several types. Mean benign tumour prevalence of >15% (up to 25%) and malignant lesions more common but still relatively infrequent (up to 6%)

Grant Stentiford


Cd Liver concentration
[Cd]
0.60
0.50
0.40
0.30
0.20
0.10
0.00
SOUTH 0F EDDYSTONE
CARMATHEN BAY
NEW HAVEN
RED WHARF BAY
LYME BAY
MORECAMBE BAY
SOUTH EAST ISLE OF MAN
TEES BAY
AMBLE
SOUTH CARDIGAN BAY
NORTH CARDIGAN BAY
BURBO BIGHT
RYE BAY
OUTER GABBARD
LIVERPOOL BAY
FLAMBOROUGH
WEST LUNDY
OFF HUMBER
ST BEES
DOGGER NORTH EAST
DOGGER CENTRAL
DOGGER NORTH
DOGGER WEST
INDIFATIGABLE BANK
INNER CARDIGAN BAY
Site


Cd Liver concentration
TYPE A
TYPE B
TYPE C
[Cd]
0.60
0.50
0.40
0.30
0.20
0.10
0.00
SOUTH 0F EDDYSTONE
CARMATHEN BAY
NEW HAVEN
RED WHARF BAY
LYME BAY
MORECAMBE BAY
SOUTH EAST ISLE OF MAN
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AMBLE
SOUTH CARDIGAN BAY
NORTH CARDIGAN BAY
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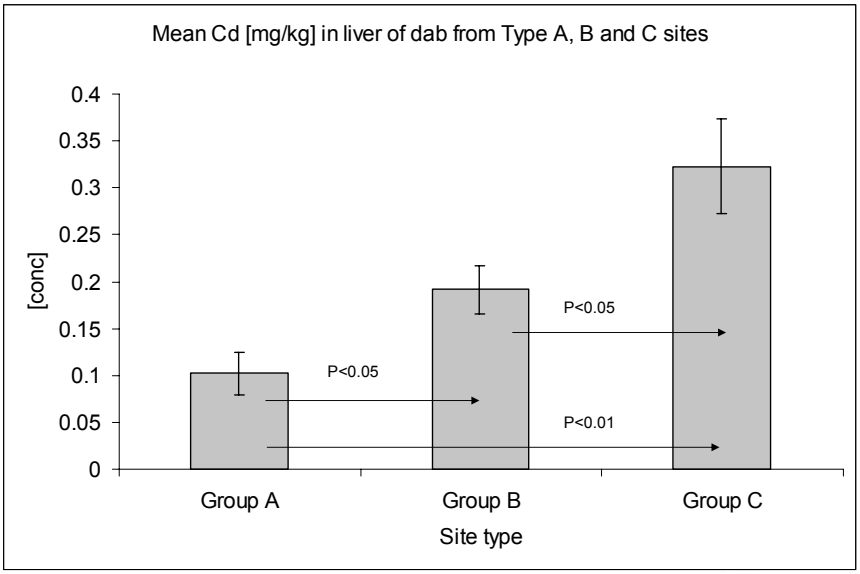
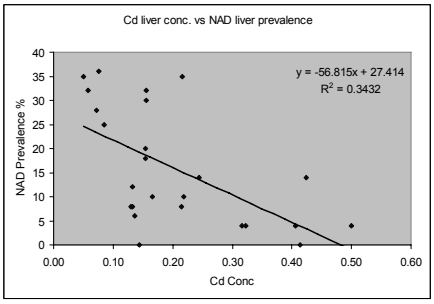
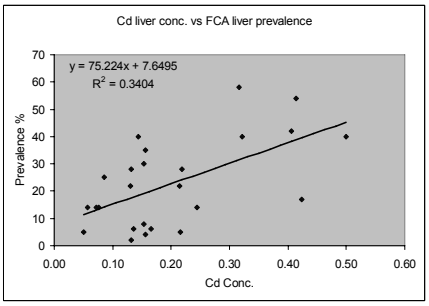
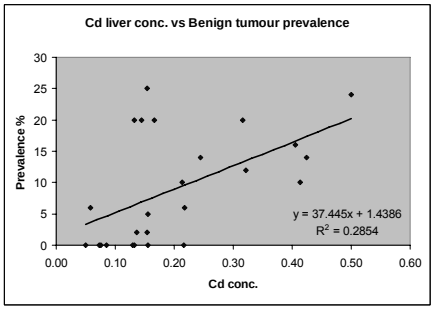
LIVERPOOL BAY
FLAMBOROUGH
WEST LUNDY
OFF HUMBER
ST BEES
DOGGER NORTH EAST
DOGGER CENTRAL
DOGGER NORTH
DOGGER WEST
INDIFATIGABLE BANK
INNER CARDIGAN BAY
Site

Site classification correlates



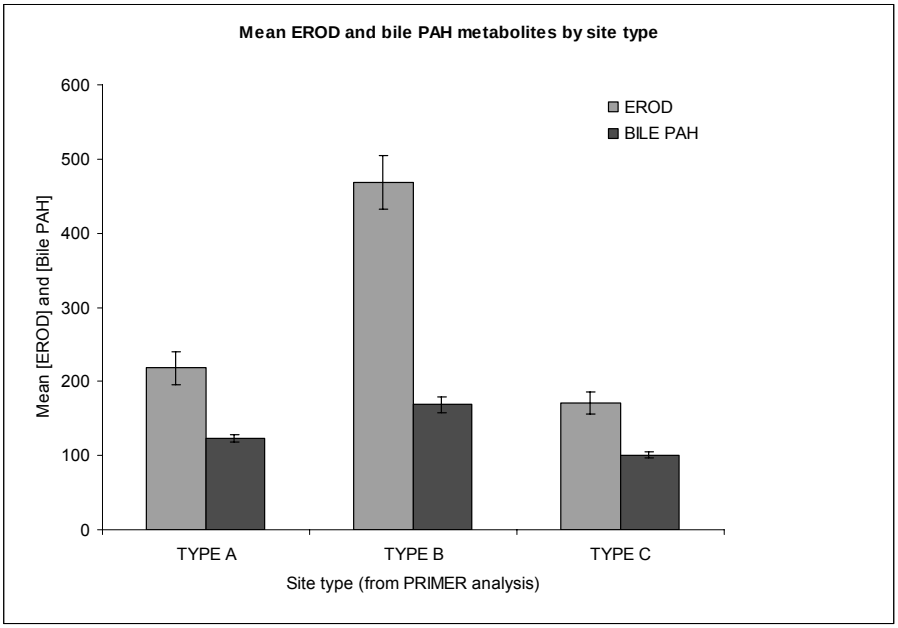






Negative correlation between liver Cd conc. and prevalence of 'normal' liver

Positive correlation between liver Cd conc. and preneoplastic (FCA) and benign neoplasms



Conclusions from preliminary data analysis – liver disease

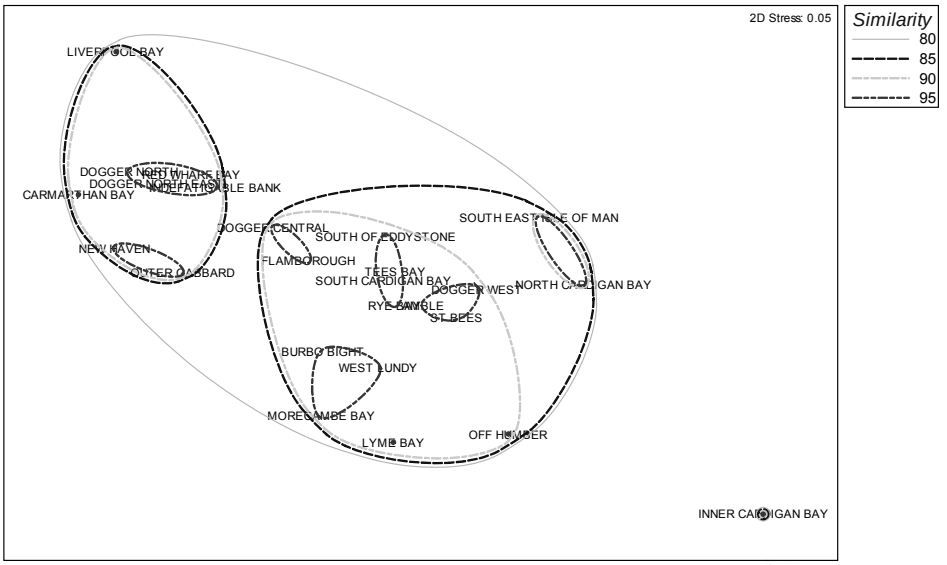
1. PRIMER analysis of liver disease as top-level effect consistently discriminates sites from each another
2. Allows for spatial and temporal analysis of disease/marker/chem data
3. Possible to devise site grading based upon disease
4. Site typing moves us towards evidence-based comparisons of site-site similarity (e.g. fish from Type C sites in North Sea are similar to Type C sites in Irish Sea).
5. Site typing can be used to find cross-correlates from biomarker and chemical data collected from the same fish.

   e.g. Differences in [liver Cd] between site types

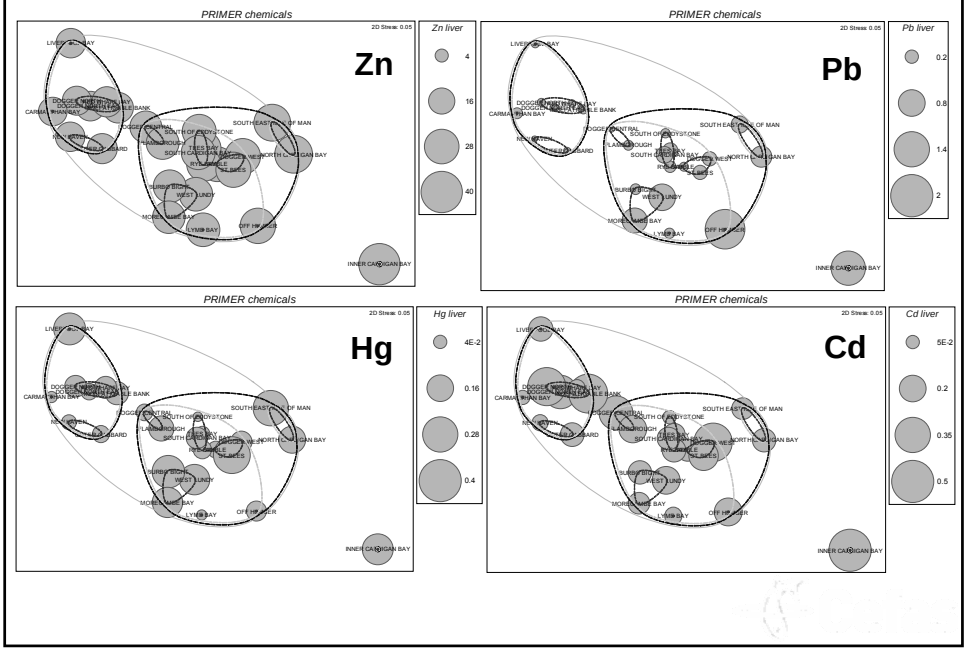
What about using chemistry as the site type selector?

Contaminants

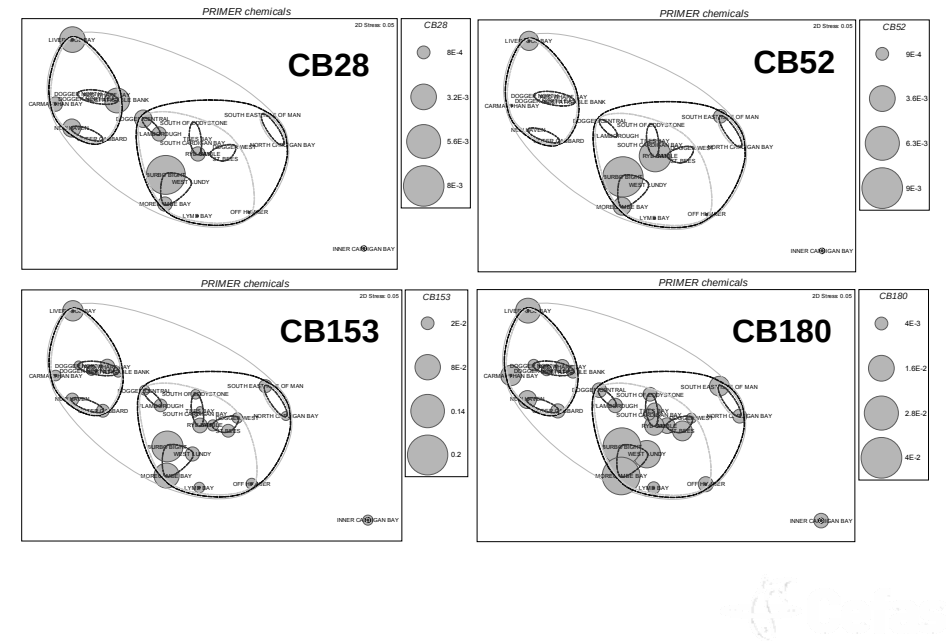
PRIMER chemicals



Drivers - metals



Drivers - organics

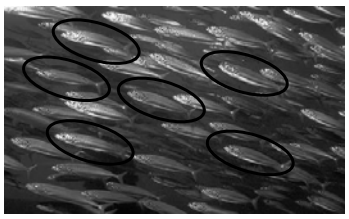


Conclusions from preliminary data analysis – chemistry

- Feasible to assign site type based upon chemical profile in tissues
- Cross relate disease and biomarker profiles to tissue chemistry
- Sites may discriminate as predominantly 'metal' or 'organic' impacted
- Technique allows for chemical classes to be cross related to specific biomarker or disease profiles
- Approach depends on philosophy of study – i.e. relation of chemical profile to disease profile or vice versa

Where now?

- Disease, marker and chemistry data should be collected in an integrated manner using QA principles such as BEQUALM etc
- All data needs to be easily accessible (with metadata) for multivariate assessment
- Continued effort to better understanding life history parameters of our sentinels is critical (e.g. via population genetics)
- Differential health status of sentinels suggest that they do contain the answers to our questions – we just need to think what we are asking and whether our tools are appropriate to find the answers



Not all fish are the same

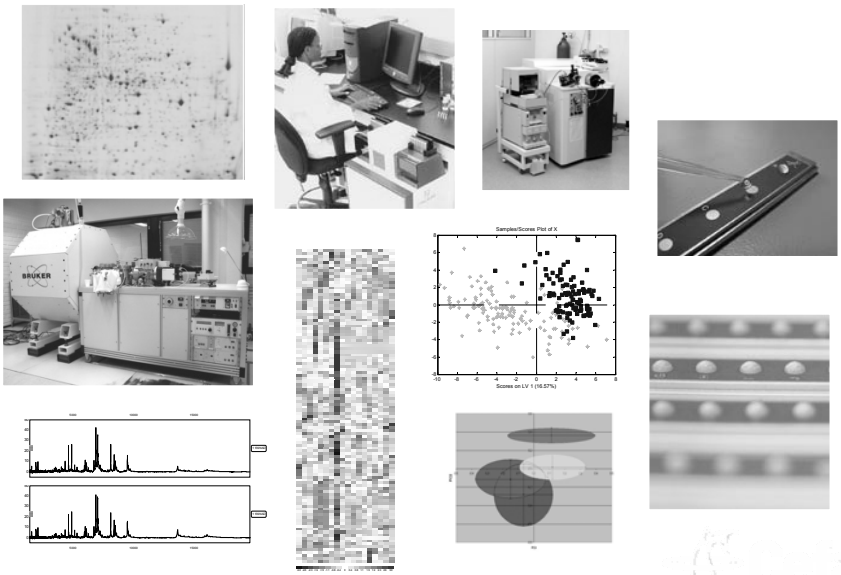
Status in 2005

Higher cancer prevalence in some parts of North Sea and Irish Sea

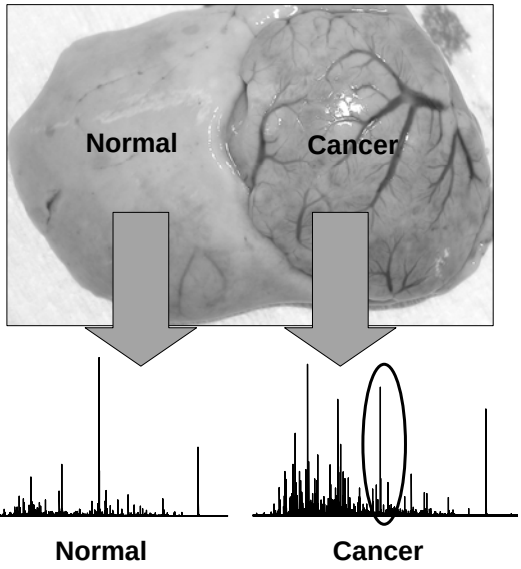
Visible liver cancer prevalence up to 15%

Microscopic liver cancer prevalence up to 25%

New technologies and fish liver cancer



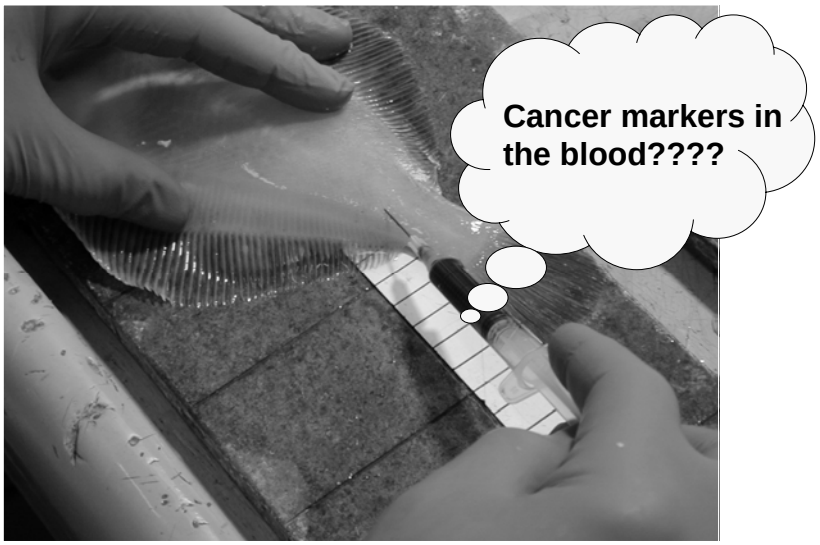
Can we compare fish and human cancer?



CANCER RESEARCH UK

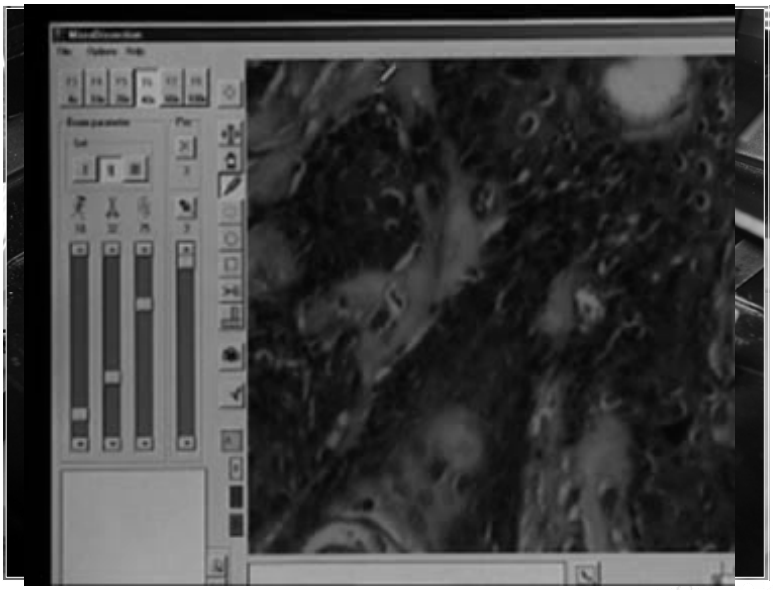
Stentiford, G.D., Johnson, P.J., Martin, A., Wenbin, W., Ward, D.G., Viant, M., Lyons, B.P., Feist, S.W. (2005). Liver tumours in wild flatfish: a histopathological, proteomic and metabolomic study. *OMICS: Journal of Integrative Biology 9*, 281-299

Taking things further

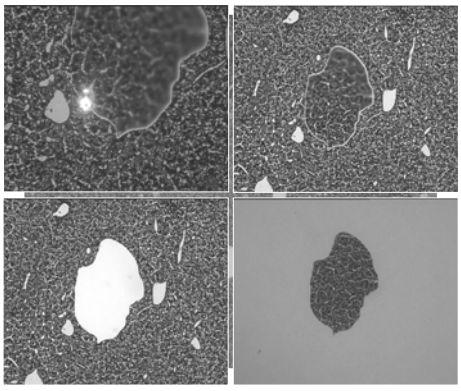


Ward, D.G., Martin, A., Johnson, P.J., Wenbin Wei, Chen, Y., Lyons, B.P., Feist, S.W., Stentiford, G.D. (2006). Plasma proteome analysis reveals the geographical origin and liver disease status of dab (*Limanda limanda*) from UK marine waters. *Environmental Science and Technology* 40 (12), 4031 -4036

Laser Capture Microscopy (LCM)



Laser Capture Microscopy (LCM)



- Small lesions (too small to see)
- Pre-cancerous lesions
- Gene/protein/metabolite profile changes
- Chain of events (normal cell to cancer)

Du Corbier, F.A., Stentiford, G.D., Lyons, B.P., Rotchell, J.M. (2005). Isolation of the *Retinoblastoma* cDNA from the marine flatfish dab (*Limanda limanda*) and evidence of mutational alterations in liver tumors. *Environmental Science and Technology* 39, 9785-9790

Annex 12 The Fish Disease Index

Title

The Fish Disease Index: an Assessment Tool

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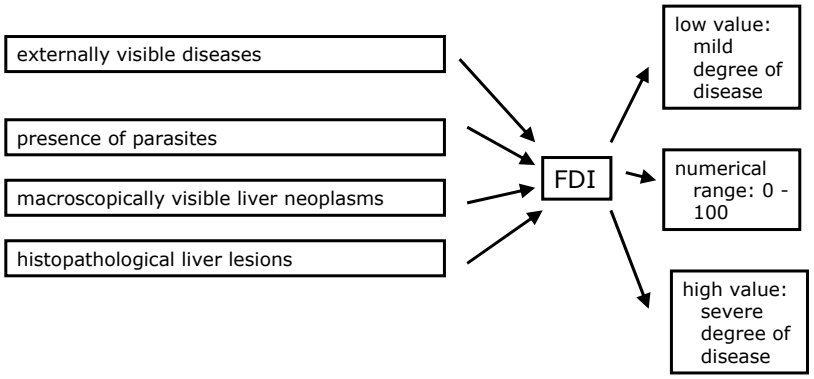
WKIMON III, ICES HQ Copenhagen, 16-18 January 2007

The purpose

The purpose of the Fish Disease Index (FDI)

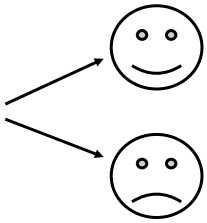
Summarize the severity of diseases



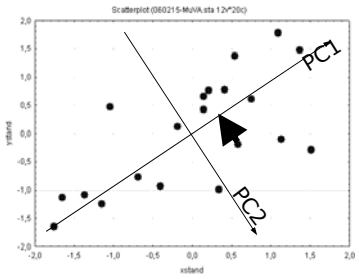
The FDI compared to other measures

The Fish Disease Index (FDI)…

… provides an **ASSESSMENT** of the observed set of diseases



… does **NOT** summarize variation (as do methods like PCA, MDS, …)



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Construction of the FDI (no adjustment)

disease (grade)	disease score		disease status (one fish)		FDI contribution
Lymphocystis grade 1	3	*	0	=	0
Lymphocystis grade 2	6	*	1	=	6
Lymphocystis grade 3	9	*	0	=	0
Epidermal Papilloma grade 1	3	*	1	=	3
Epidermal Papilloma grade 2	6	*	0	=	0
Epidermal Papilloma grade 3	9	*	0	=	0
● ● ●					
Lepeoptheirus grade 3	2		0		0

Reference: WGPDMO report 2006, p. 68-83

determined according to guideline | expert's judgment | observed | FDI = c *9 | calculated (sum)

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Disease scores in the FDI

the disease score …

… expresses the severity of the disease and its grade

… the higher, the more severe

… assigned by expert's judgment

… judgments of several experts can be melted into one score (per disease grade): e.g. Bradley-Terry approach

Bradley-Terry approach overcomes problem of ranking many items by evaluation of pairwise comparisons

Bradley-Terry approach for disease scores (1)

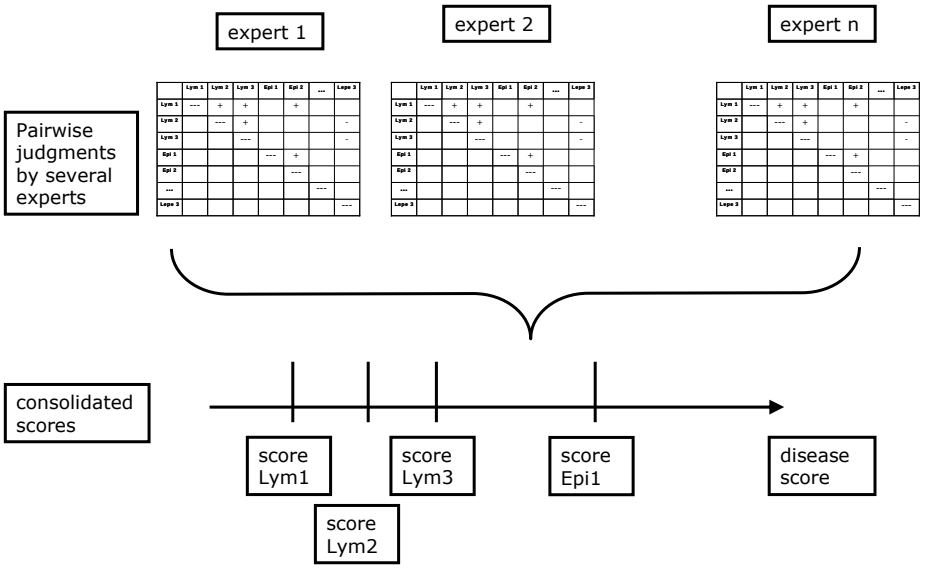
Matrix of pairwise comparisons. Need not be complete.

+ : column disease **more** severe than row

- : column disease **less** severe than row

	Lym 1	Lym 2	Lym 3	Epi 1	Epi 2	…	Lepe 3
Lym 1	---	+	+		+		
Lym 2		---	+				-
Lym 3			---				-
Epi 1				---	+		
Epi 2					---		
…						---	
Lepe 3							---

Bradley-Terry approach for disease scores (2)



Numerical example from WGPDMO report 2006

WGPDMO 2006: Non-adjusted (length, gender) Health Indices for externally visible diseases/parasites (EDV Index), macroscopically visible liver neoplasms (MVLN Index) and liver histopathology (LH Index) in common dab (*Limanda limanda*) (n.e.: not examined)

				Fish 1		Fish 2		Fish 3		Fish 4	
			Size (cm total length)	15		22		25		32	
			Gender	Male		Female		Male		Female	
A: Externally visible diseases (EDV)	**Grade**	**Disease code**	**Disease-specific weight**	**presence of disease**	**score**	**presence of disease**	**score**	**presence of disease**	**score**	**presence of disease**	**score**
Lymphocystis	1	1	2	1	2,00	1	2,00	0	0,00	1	2,00
	2	2	2	0	0,00	0	0,00	0	0,00	0	0,00
	3	3	2	0	0,00	0	0,00	1	6,00	0	0,00
Epidermal Hyperplasia/Papilloma	1	1	2	0	0,00	0	0,00	0	0,00	0	0,00
	2	2	2	0	0,00	0	0,00	0	0,00	0	0,00
	3	3	2	0	0,00	0	0,00	1	6,00	0	0,00
Acute/Healing Skin Ullcerations	1	1	3	0	0,00	0	0,00	0	0,00	0	0,00
	2	2	3	1	6,00	1	6,00	0	0,00	0	0,00
	3	3	3	0	0,00	0	0,00	1	9,00	1	9,00
X-cell gill disease	1	1	9	0	0,00	0	0,00	1	9,00	0	0,00
Hyperpigmentation	1	1	3	0	0,00	0	0,00	0	0,00	1	3,00
	2	2	3	1	6,00	1	6,00	0	0,00	0	0,00
	3	3	3	0	0,00	0	0,00	1	9,00	0	0,00
Acute/Healing Fin Rot/Erosion	1	1	6	0	0,00	0	0,00	0	0,00	0	0,00
Stephanostomum sp.	1 (1-10 cysts)	1	1	1	1,00	1	1,00	0	0,00	0	0,00
	2 (11-50 cysts)	2	1	0	0,00	0	0,00	0	0,00	0	0,00
	3 (> 50 cysts)	3	1	0	0,00	0	0,00	1	3,00	1	3,00
Acanthochondria sp.	1 (1 specimen)	1	1	0	0,00	0	0,00	0	0,00	1	1,00
	2 (2 specimens)	2	1	0	0,00	0	0,00	0	0,00	0	0,00
	3 (> 2 specimens)	3	1	0	0,00	0	0,00	1	3,00	0	0,00
Lepeophtheirus sp.	1 (1 specimen)	1	1	1	1,00	1	1,00	0	0,00	0	0,00
	2 (2 specimens)	2	1	0	0,00	0	0,00	0	0,00	0	0,00
	3 (> 2 specimens)	3	1	0	0,00	0	0,00	1	3,00	0	0,00
			EDV raw score		16,00		16,00		48,00		18,00
			EDV Index		**70,37**		**70,37**		**11,11**		**66,67**

Using the FDI in populations

Remember:
the FDI is to be used for the assessment of **populations**

But:

different populations (different time, different location) have different sex / length distributions

fish samples are not representative for the population they come from (arbitrary bias possible)

disease prevalence tends to be specific for sex and length

adjustment for variation in sex proportion and length distribution is needed

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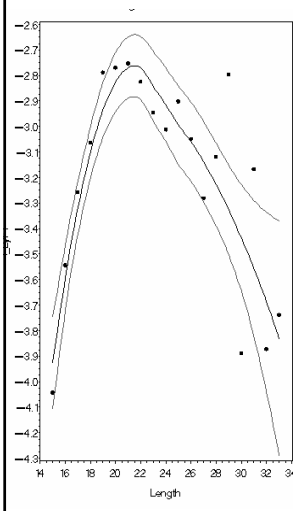
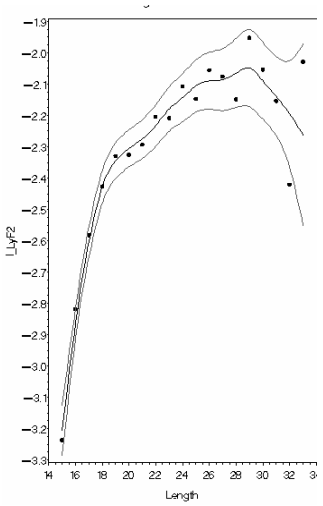
Disease prevalence and length

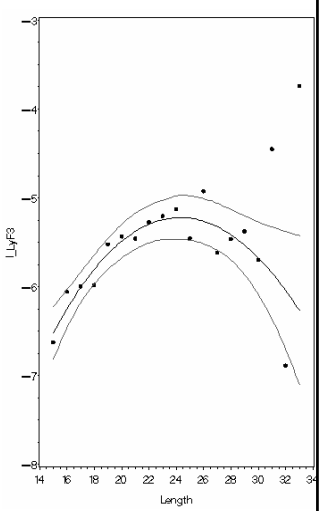
Ly grade 1



Ly grade 2



Ly grade 3



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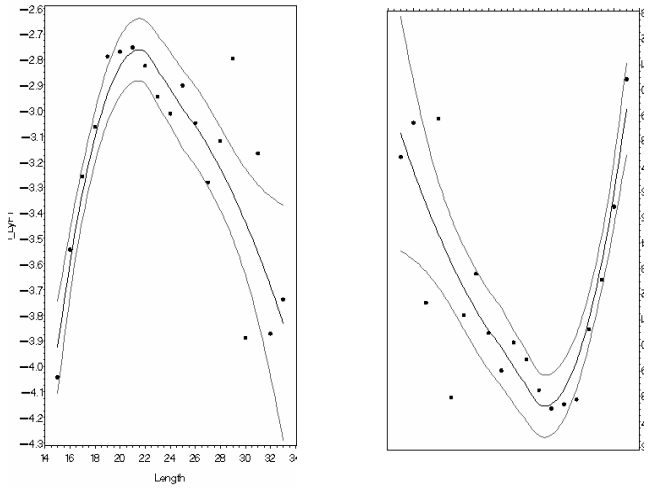
The strategy of length adjustment

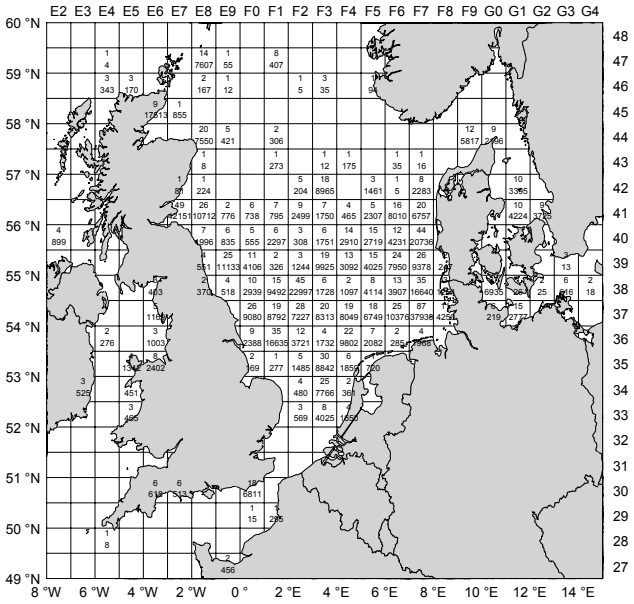
Ly grade 1 prevalence (transformed)

correspon-ding weight



FDI for North Sea 1990 – 2005

- „North Sea": ICES rectangles 33E7 – 46F8
- male and female dab (*Limanda limanda*) 15cm ≤ length ≤ 33 cm
- 150 000 individuals
- data source: German Fisheries Research Centre (data also in ICES data base, but not uniformly with grades)



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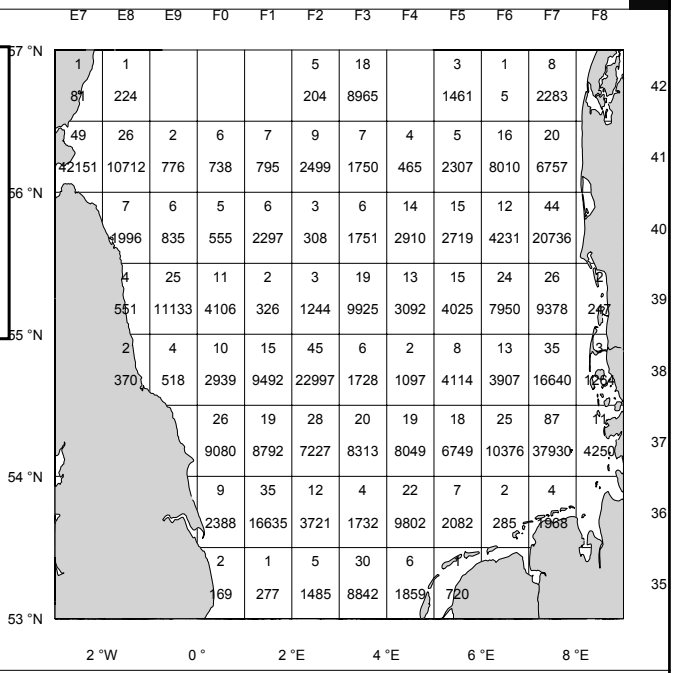
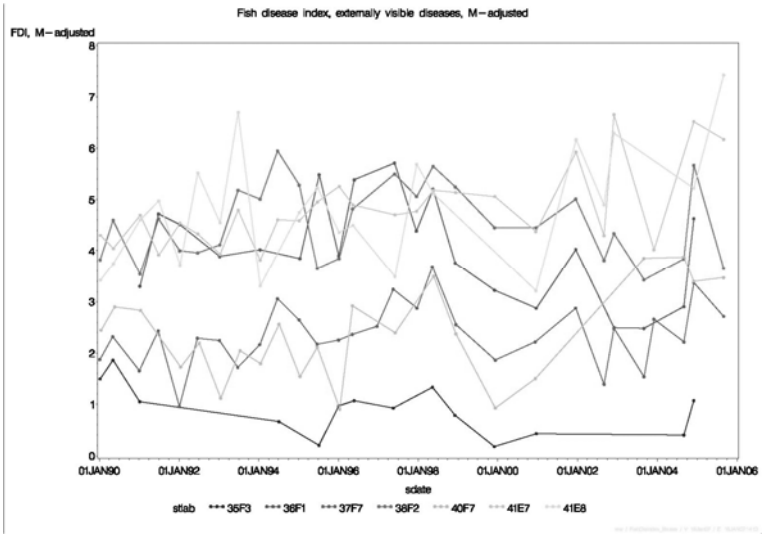


FDI for North Sea 1990 – 2005, WITHOUT sex/length adjustment

FDI, not length adjusted, means per date, per ICES rectangle



FDI for North Sea 1990 – 2005: sources of variation

Observation from previous FDI time series:

- seasonal effects seem to exist
- fitting a mathematical model

FDI = temporal trend + seasonal variation + random variation

confirms

- significant size of trend and season
- trends and seasonal variation are specific for area (ICES rectangle)

Consequence:

Using the FDI for assessment as intended (BC, EAC …) needs

- adjustment for season (or restriction to a selected season)
- consideration per geographical area (no uniform BC, EAC over large geographical area)

FDI: Background level? EAC?

Background level:

Is there an area which we can assume to deliver a background level?

No.

Can we infer a background level from the FDI (or the underlying disease prevalence) time series?

No.

- Each observed prevalence (or FDI) can contain anthropogenic effects.
- "Zero" is not a reasonable background, as diseases are known to exist since long time (exceptions exist).

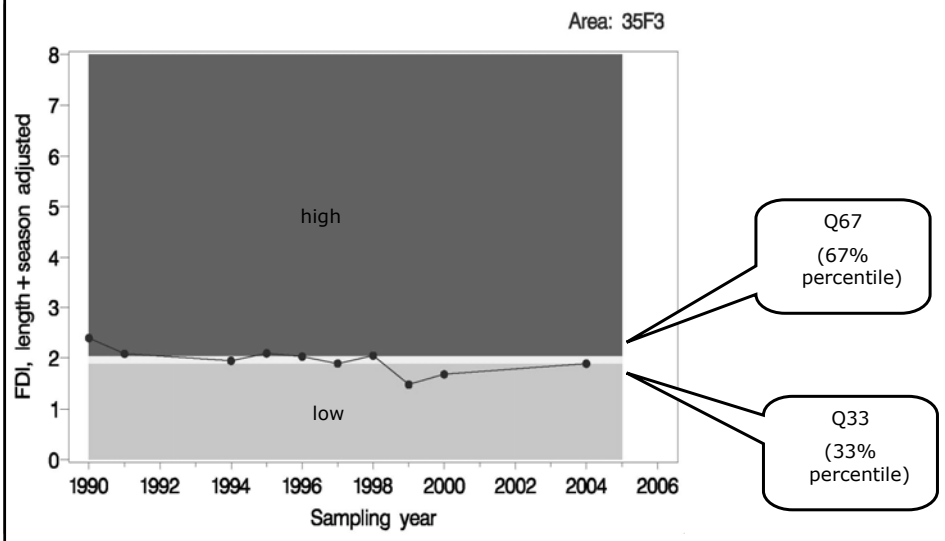
FDI: Background level? EAC?

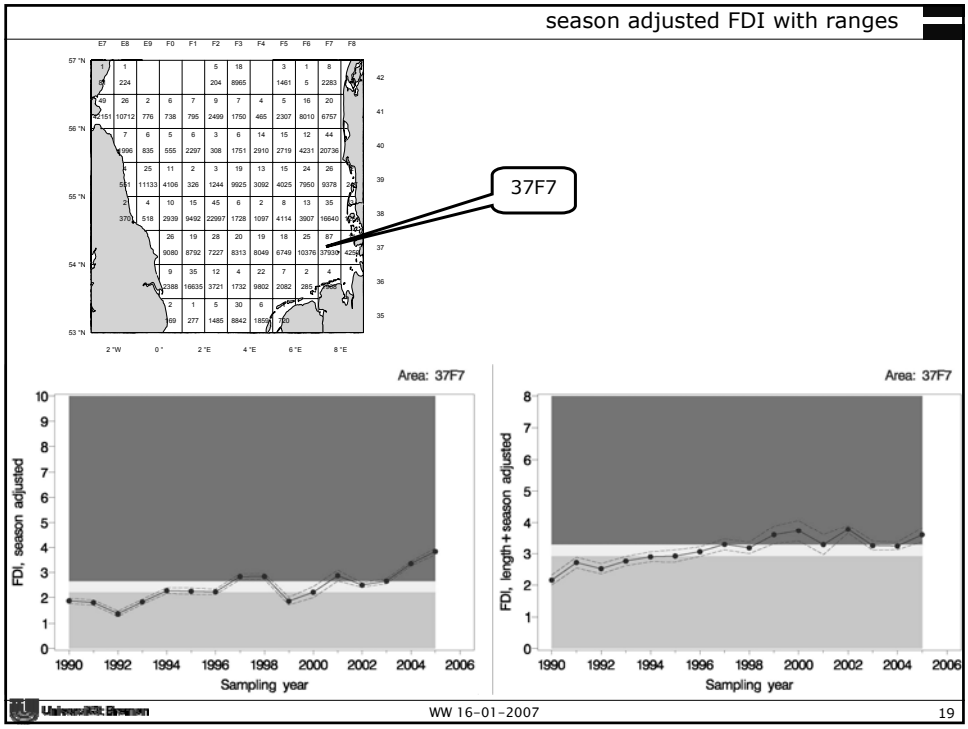
What else can we do to use the FDI for an assessment with regard to temporal change, spatial differences?

For each area, use past FDI means to identify ranges of "low", "moderate" and "high" levels.

- Method: empirical quantiles for probabilities 33% ("Q33") and 67% ("Q67")
- Interpretation: obvious. A change
  - low → (moderate or high) or
  - moderate → high
- is undesirable, a change
  - high → (moderate or low) or
  - moderate → low
- is desirable.
- The "low" range of observed values may take over the function of a "background level". Values in this range have occurred, i.e. are possible, and exceeding them should be avoided.

FDI ranges




season adjusted FDI with ranges
37F7
Area: 37F7
Area: 37F7
FDI, season adjusted
FDI, length+season adjusted
Sampling year
Sampling year
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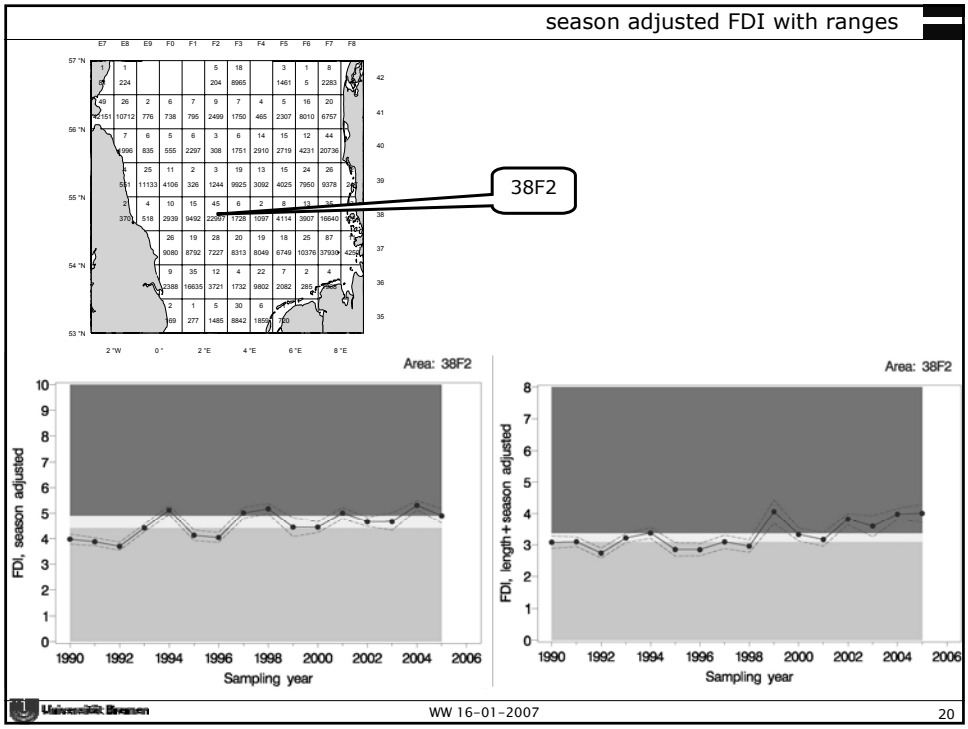
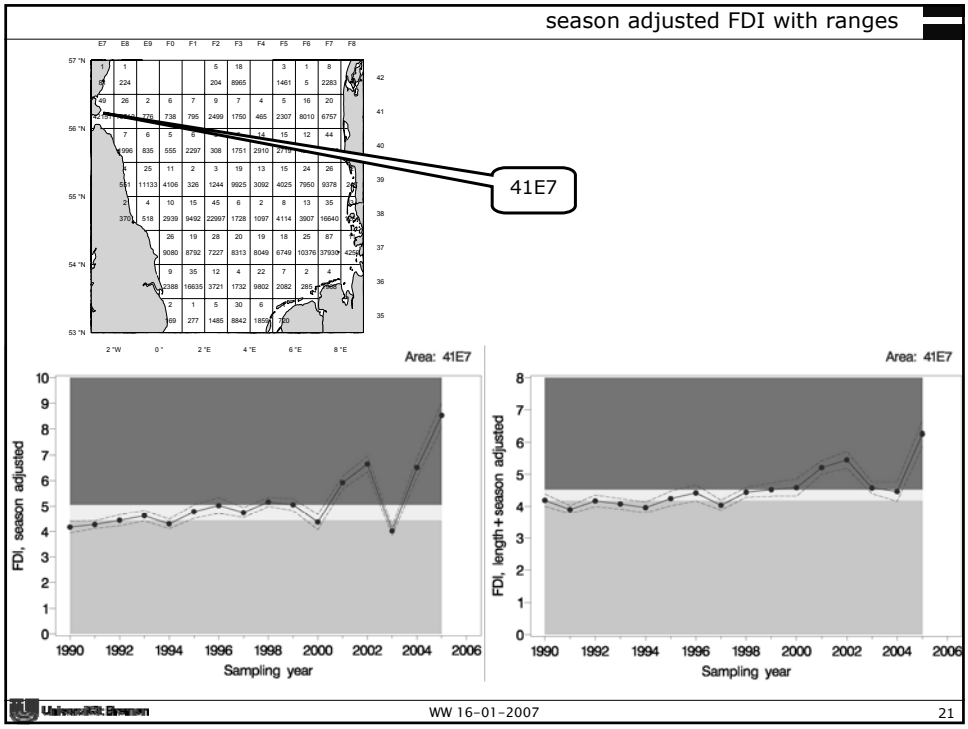
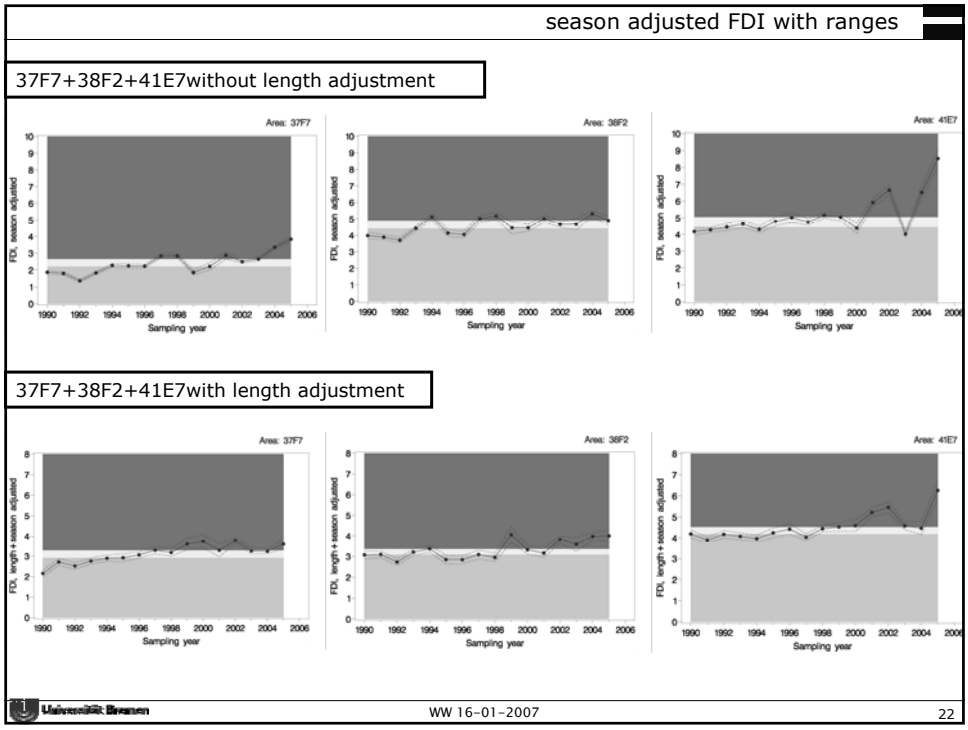
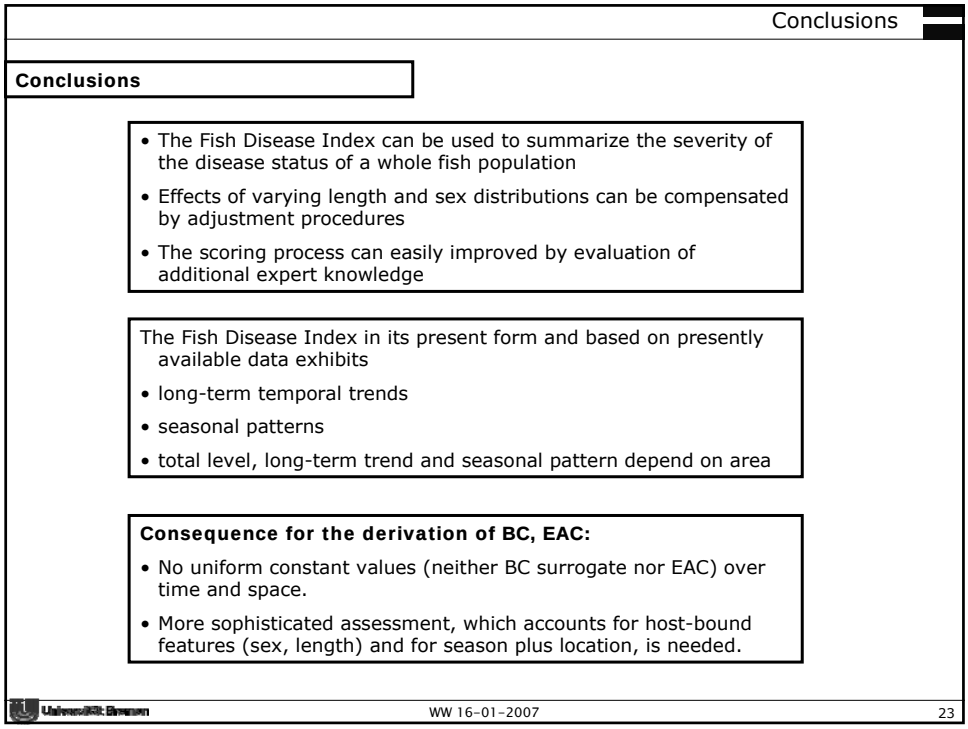

season adjusted FDI with ranges
38F2
Area: 38F2
Area: 38F2
FDI, season adjusted
FDI, length+season adjusted
Sampling year
Sampling year
WW 16-01-2007
20


season adjusted FDI with ranges
41E7
Area: 41E7
Area: 41E7
FDI, season adjusted
FDI, length+season adjusted
Sampling year
Sampling year
WW 16-01-2007
21


season adjusted FDI with ranges
37F7+38F2+41E7without length adjustment
Area: 37F7
Area: 38F2
Area: 41E7
37F7+38F2+41E7with length adjustment
Area: 37F7
Area: 38F2
Area: 41E7
Sampling year
WW 16-01-2007
22


Conclusions
Conclusions
• The Fish Disease Index can be used to summarize the severity of the disease status of a whole fish population
• Effects of varying length and sex distributions can be compensated by adjustment procedures
• The scoring process can easily improved by evaluation of additional expert knowledge
The Fish Disease Index in its present form and based on presently available data exhibits
• long-term temporal trends
• seasonal patterns
• total level, long-term trend and seasonal pattern depend on area
Consequence for the derivation of BC, EAC:
• No uniform constant values (neither BC surrogate nor EAC) over time and space.
• More sophisticated assessment, which accounts for host-bound features (sex, length) and for season plus location, is needed.
WW 16-01-2007
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Annex 13 PREDICT 2 - Biomarker approach

PREDICT 2: Predicting Health of the Environment – An Integrated Biomarker Approach

Mike Moore (PML), **Icarus Allen (PML), Allen McVeigh (PML/BMT-CORDAH), Nasir Jamal (Univ. of Plymouth/PML), Phil Dyke (Univ. of Plymouth), Christophe Minier (Univ. of Le Havre, France), Angela Köhler (AWI, Germany) & Aldo Viarengo (Univ. of Eastern Piedmont, Italy)**

Supported by Defra/NERC

Plymouth Marine Laboratory

Deep Simplicity – Order from Complexity!

- Whole system "middle-out approach"
- Cross-cutting – connectivity and integration
- Simplification – at the root of complex behaviours are simple rules (Kauffman, Gribben)
- Interpretation - development of a health status index for use with biomarker data

Environmental prognostics – a "systems approach" for predicting to higher levels (*decision support and simulation*)

- Ecosystem status consequences
- Prediction of the consequences of future events – "scenario simulation"

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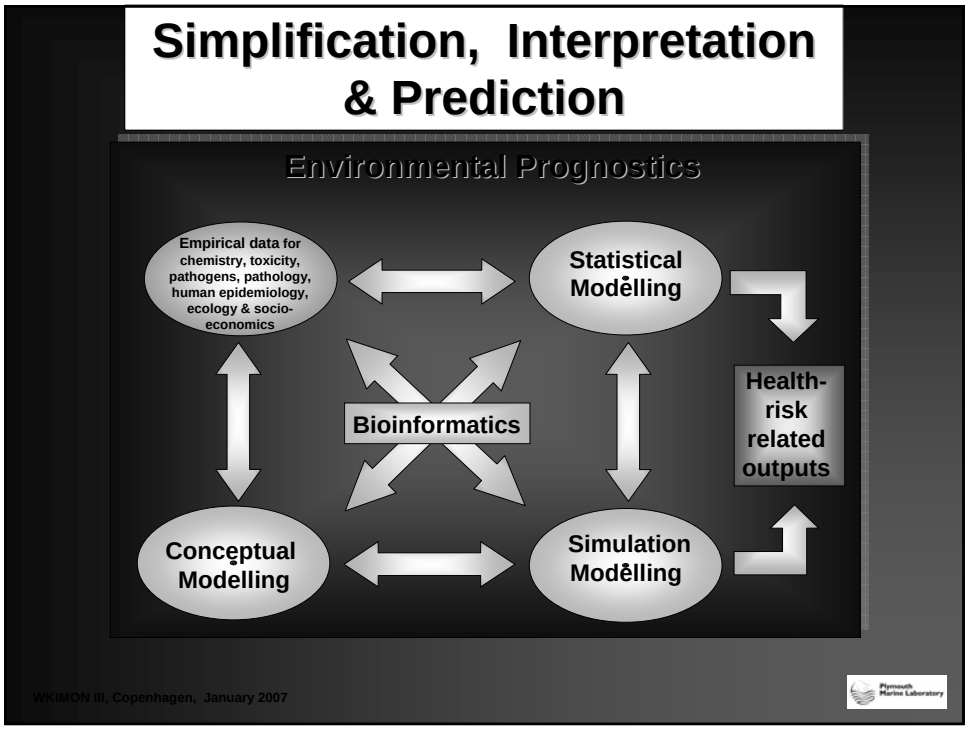
Simplification, Interpretation & Prediction
Environmental Prognostics
Empirical data for chemistry, toxicity, pathogens, pathology, human epidemiology, ecology & socio-economics
Statistical Modelling
Health-risk related outputs
Bioinformatics
Conceptual Modelling
Simulation Modelling
WKIMON III, Copenhagen, January 2007
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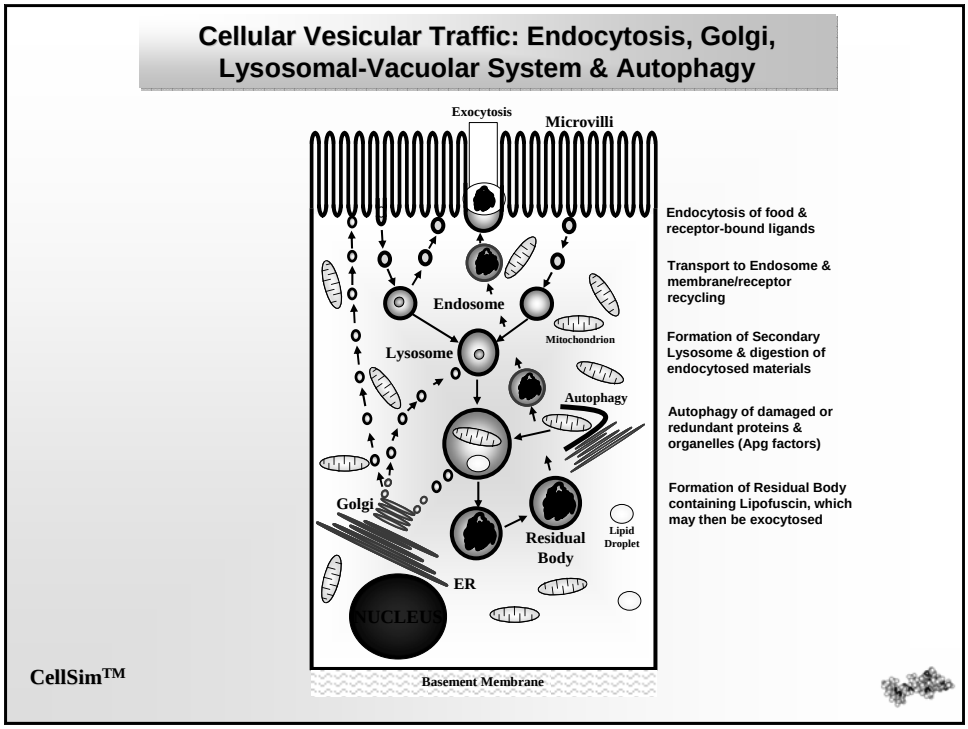
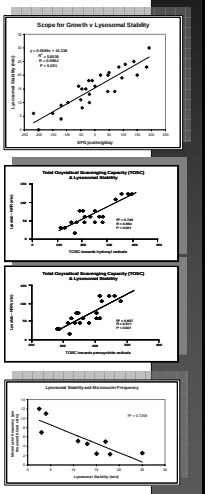

Cellular Vesicular Traffic: Endocytosis, Golgi, Lysosomal-Vacuolar System & Autophagy
Exocytosis
Microvilli
Endosome
Mitochondrion
Lysosome
Autophagy
Golgi
Residual Body
Lipid Droplet
ER
NUCLEUS
Basement Membrane
Endocytosis of food & receptor-bound ligands
Transport to Endosome & membrane/receptor recycling
Formation of Secondary Lysosome & digestion of endocytosed materials
Autophagy of damaged or redundant proteins & organelles (Apg factors)
Formation of Residual Body containing Lipofuscin, which may then be exocytosed
CellSim™

Lysosomal Reactions, Cell Injury & Pathophysiology

Lysosomal stability is correlated with:

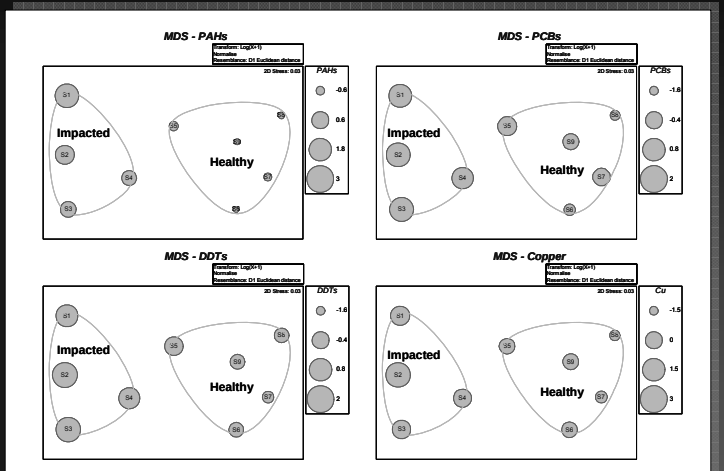
- Larval viability
- Scope for growth
- Protein synthesis
- Total oxyradical scavenging capacity (TOSC)



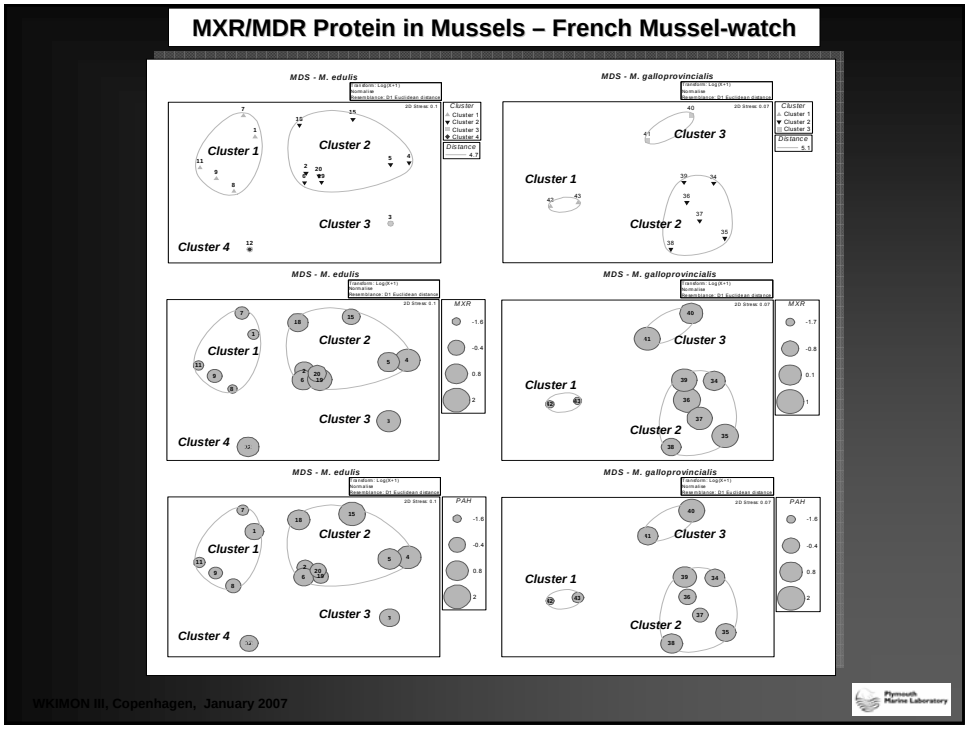
Lysosomal stability is inversely correlated with:

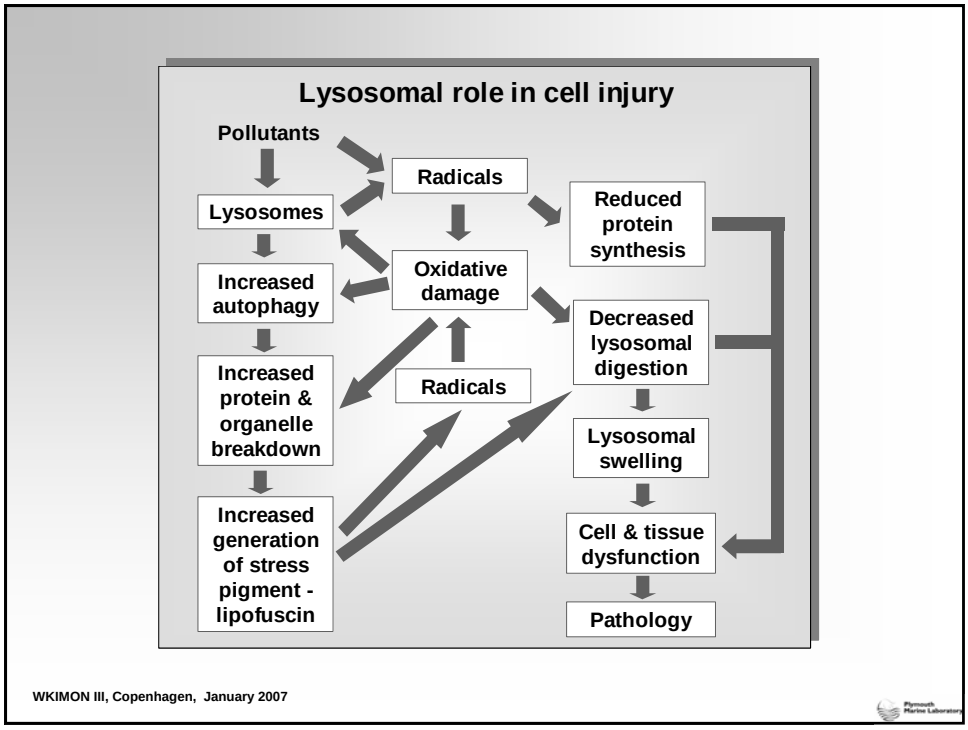
- Protein catabolism
- Lipofuscin (stress or age pigment)
- Lysosomal lipidosis
- Lysosomal swelling
- DNA damage
- Liver cancer in flatfish

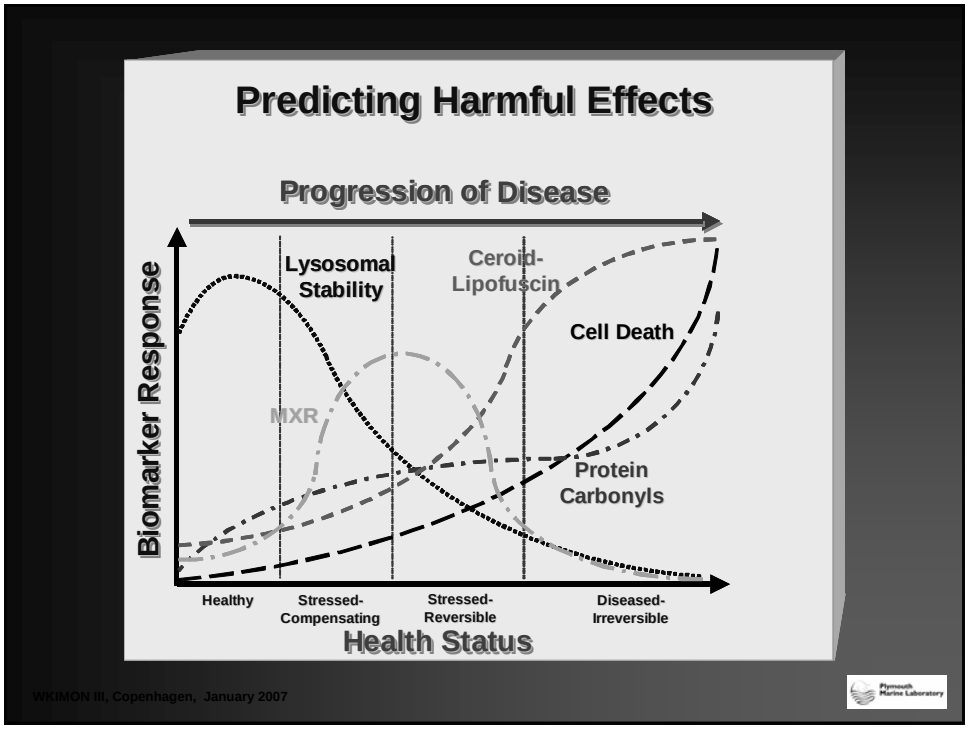
Multiple Biomarkers in Mussels: NOAA - Puget Sound

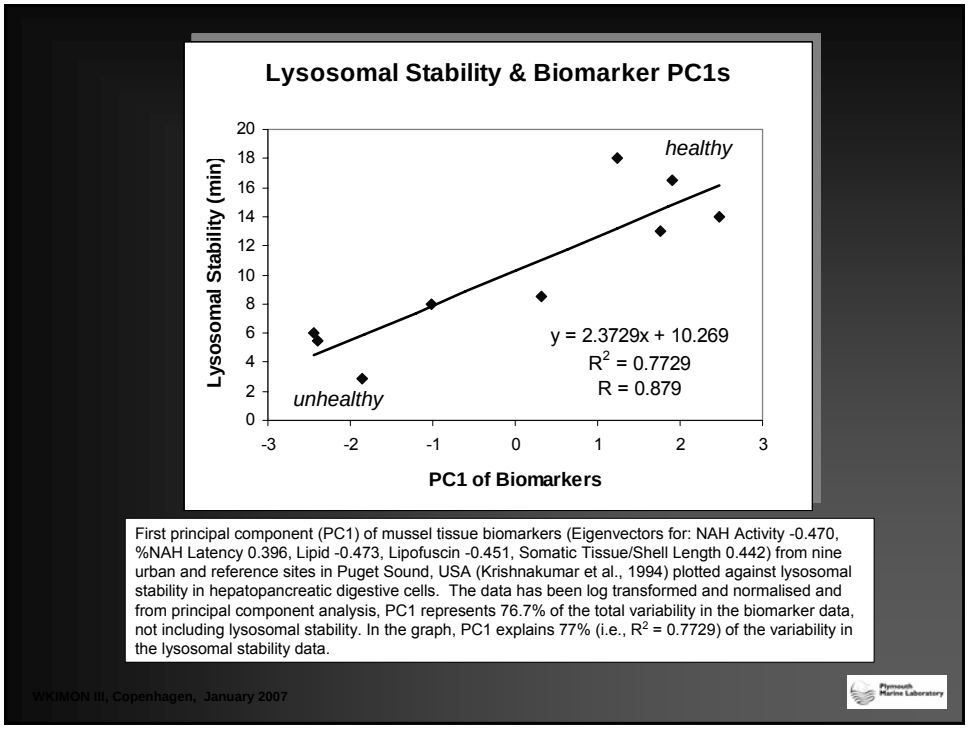


Multi-dimensional scaling (MDS) analysis of the biological responses (lysosomal stability, NAH activity, NAH latency, lipid, lipofuscin and somatic tissue index - measured as somatic tissue weight/shell length) for blue mussels from Puget Sound. Bubble plots for selected individual chemical contaminants (PAHs, PCBS, DDTs & Copper) determined have been serially superimposed. Samples are grouped as urban or reference sites and cluster analysis has been overlaid. Original data derived from Krishnakumar et al. (1994).


MXR/MDR Protein in Mussels – French Mussel-watch
MDS - M. edulis
MDS - M. galloprovincialis
Cluster 1
Cluster 2
Cluster 3
Cluster 4
MXR
PAH
WKIMON III, Copenhagen, January 2007

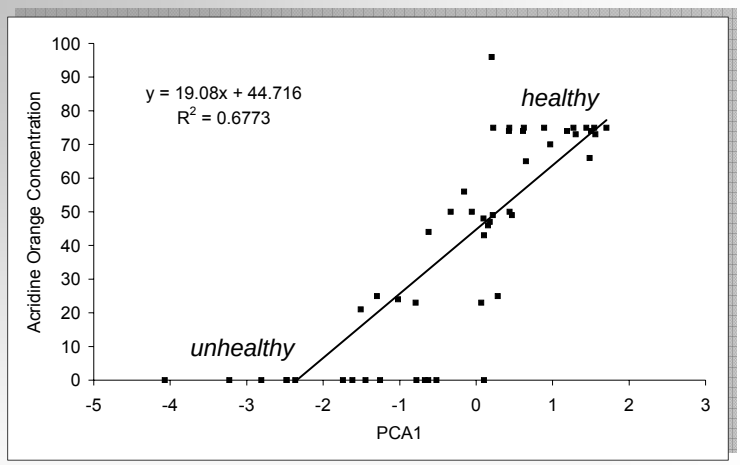

Lysosomal role in cell injury
Pollutants
Lysosomes
Increased autophagy
Increased protein & organelle breakdown
Increased generation of stress pigment - lipofuscin
Radicals
Oxidative damage
Radicals
Reduced protein synthesis
Decreased lysosomal digestion
Lysosomal swelling
Cell & tissue dysfunction
Pathology
WKIMON III, Copenhagen, January 2007





First principal component (PC1) of mussel tissue biomarkers (Eigenvectors for: NAH Activity -0.470, %NAH Latency 0.396, Lipid -0.473, Lipofuscin -0.451, Somatic Tissue/Shell Length 0.442) from nine urban and reference sites in Puget Sound, USA (Krishnakumar et al., 1994) plotted against lysosomal stability in hepatopancreatic digestive cells. The data has been log transformed and normalised and from principal component analysis, PC1 represents 76.7% of the total variability in the biomarker data, not including lysosomal stability. In the graph, PC1 explains 77% (i.e., $R^2 = 0.7729$) of the variability in the lysosomal stability data.

Relationship between Lysosomal Stability and Other Biomarkers



First principal component of Dab liver biomarkers (EROD –0.258, Pinocytosis 0.488, Oxyradicals –0.571, Endoplasmic reticulum –0.334, and Lipid vaculation –0.507) data from the Bremerhaven workshop (MEPS 1992) plotted against the concentration of acridine orange in the lysosomes (lysosomal stability) of liver cells. The data used in the PCA has been normalised to a mean of zero and a standard deviation of 1 and represents 46% of the total variability in the data (Allen & Moore, 2004). PC1 explains 68% of the variability in the lysosomal stability data.

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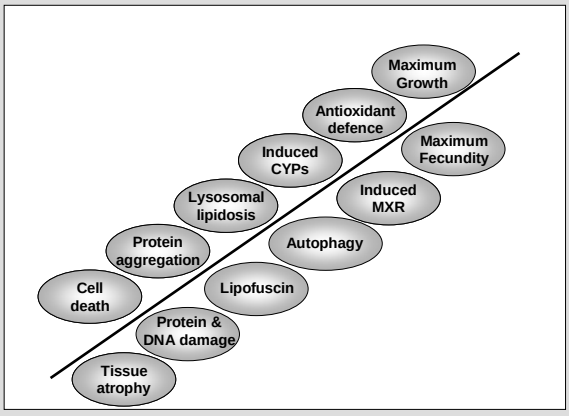
Interpreting Biomarker Reactions



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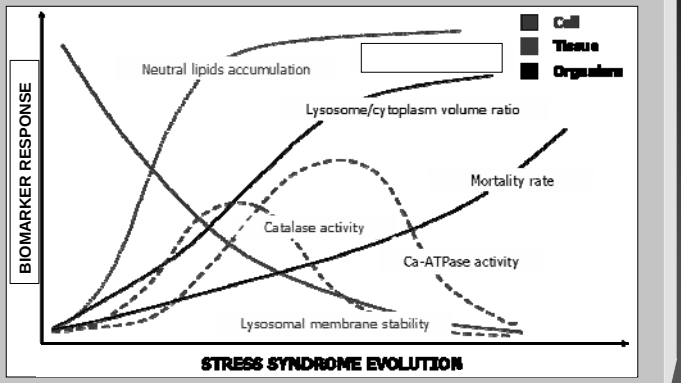
Response trends of biomarkers in a pollution gradient, related to the development of the stress syndrome: (a) decreasing, (b) increasing and (c) bell-shaped profile.



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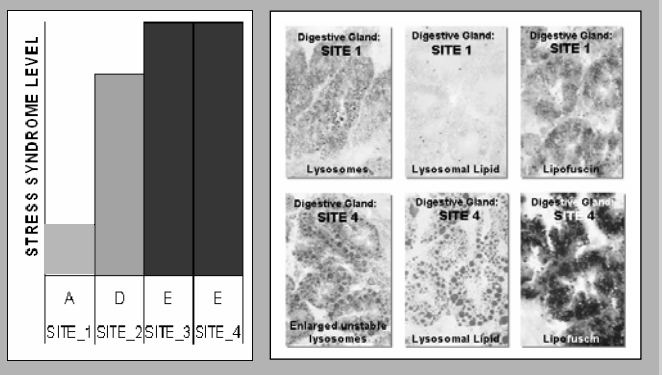
Decision Support System



Stress syndrome level in mussel sampled along Langesund in Norway. Data from Sites 2, 3 and 4 are compared to those from the reference site (Site 1, Langesund Bay, Oslo Fjord) located outside the fjord (data from scientific literature, Moore, M.N., Marine Ecology Progress Series, vol.46: 81-89,1988).

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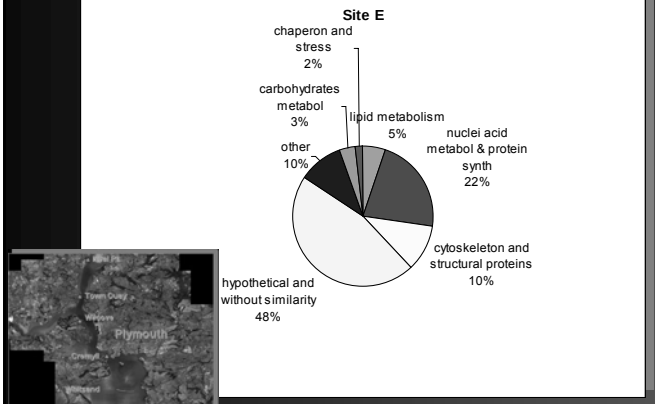
Molecular Functions

Site E Whitsand (58 DE genes)



Nucleic acid metabolism and *protein synthesis*; *cytoskeleton and structural* proteins are present as main molecular classes

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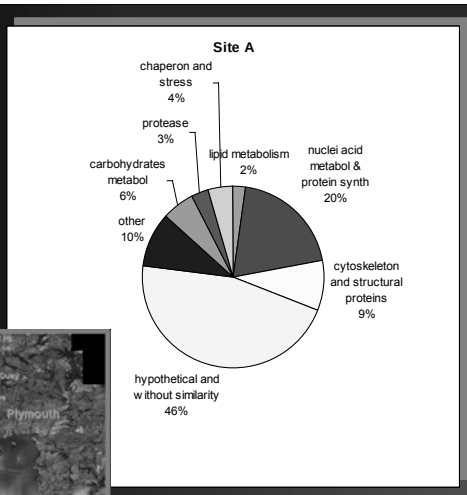
Molecular Functions

Site A Neal Point (135 DE genes)



***Chaperonin and stress proteins* (DNAJ, HSP90, Cyclophilin, Interferon inducible protein, etc) is more represented and *Proteases* are present as a new class**

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Changes in ecological assemblages:

- responses are often manifest at taxonomic levels higher than species, commonly as high as phyla
- organisms in different phyla have different sensitivities to stress
- autophagic and antioxidant protection mechanisms are highly conserved among eukaryotes

We hypothesise that:

- ability to up-regulate these processes vary among phyla
- underlies differences in their ability to colonise parts of the coastal and estuarine environment
- contributes to their differential sensitivities to anthopogenic stress

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Systems Biology:
Predictive Computational Ecotoxicological Modelling

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Mussel Model

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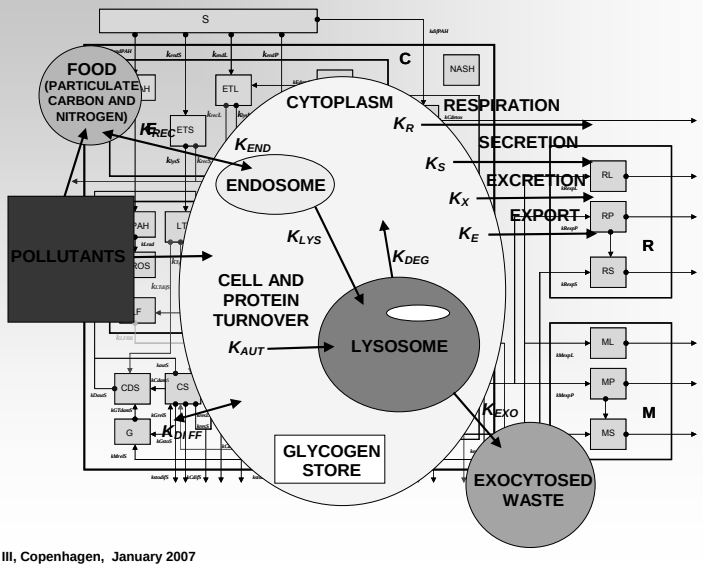
Cell Model Schematic



CellSim™

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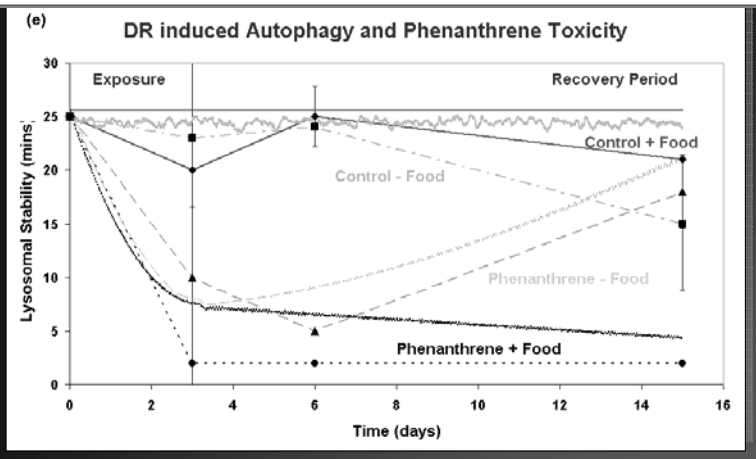
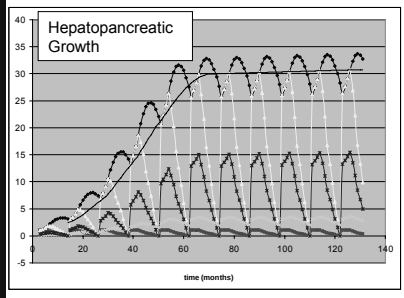
Toxicity Simulation



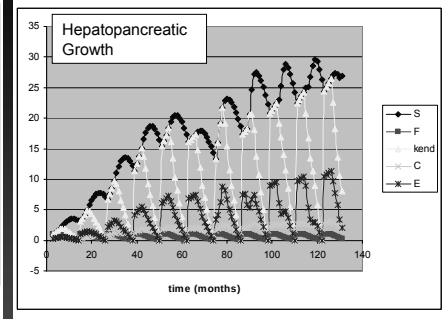
Exposure Concentration 50µg phenanthrene/mussel/day for 3 days. Measured values from Moore (2004), *Marine Environmental Research*, 54, 603-607

Clean Site – Digestive Gland

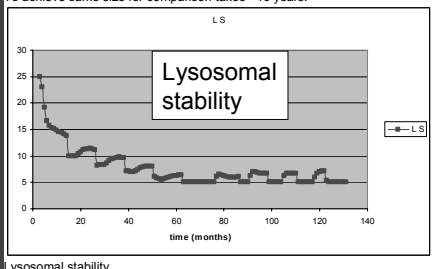


Black line is annual average size. Approximate 6 fold increase from year 1 to year 5. Thereafter levelling off. Required export to be made an increasing function over time up to a maximum after year 4.

Dirty Site – digestive gland



To achieve same size for comparison takes ~10 years.



Lysosomal stability

Fish Liver Model

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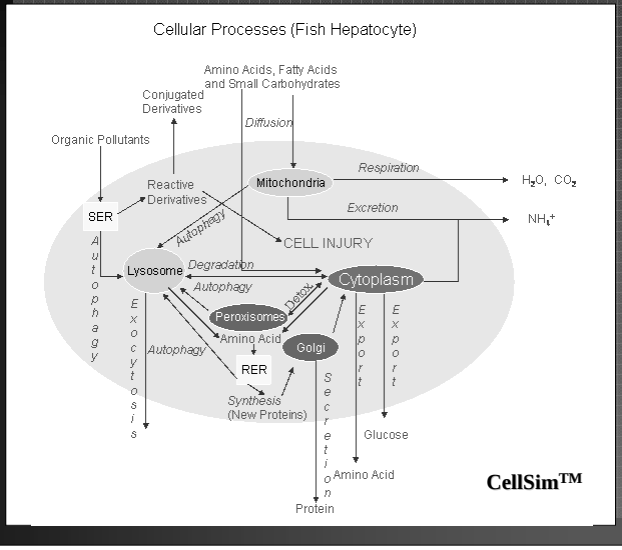

Conceptual model of fish liver hepatocyte. The hepatocyte has been divided into seven different compartments. Nutrient molecules and environmental contaminants enter into the hepatocyte by diffusion. Various cellular processes like autophagy, respiration, excretion, etc., are shown with the help of arrows and products of these processes such as water, ammonia, glucose, amino acids, etc. are released from the cell.

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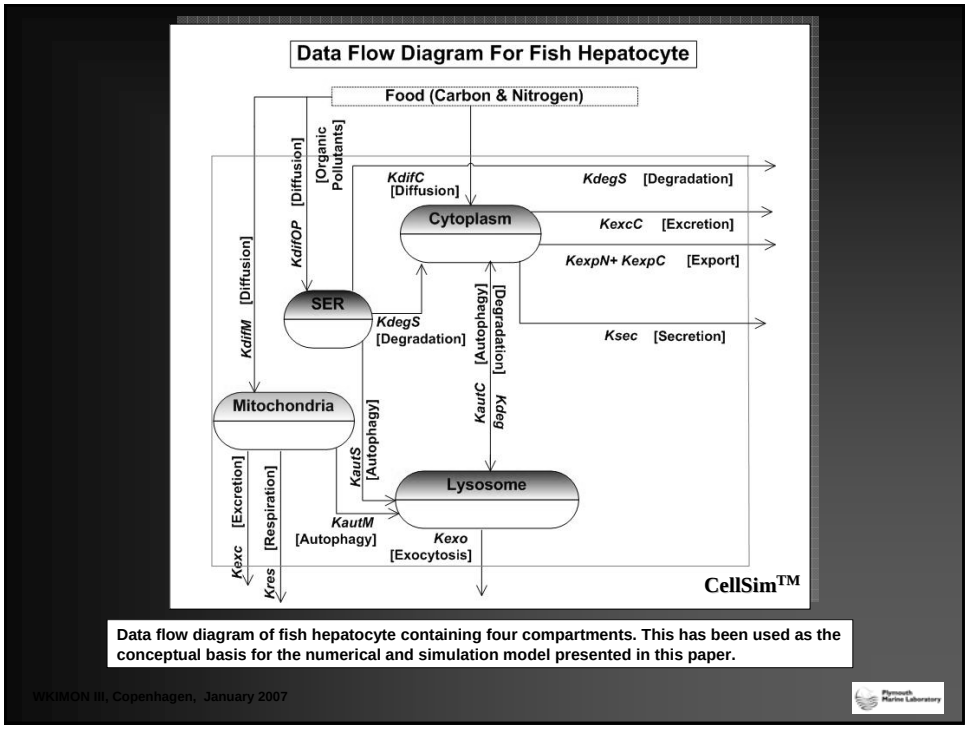

Data flow diagram of fish hepatocyte containing four compartments. This has been used as the conceptual basis for the numerical and simulation model presented in this paper.

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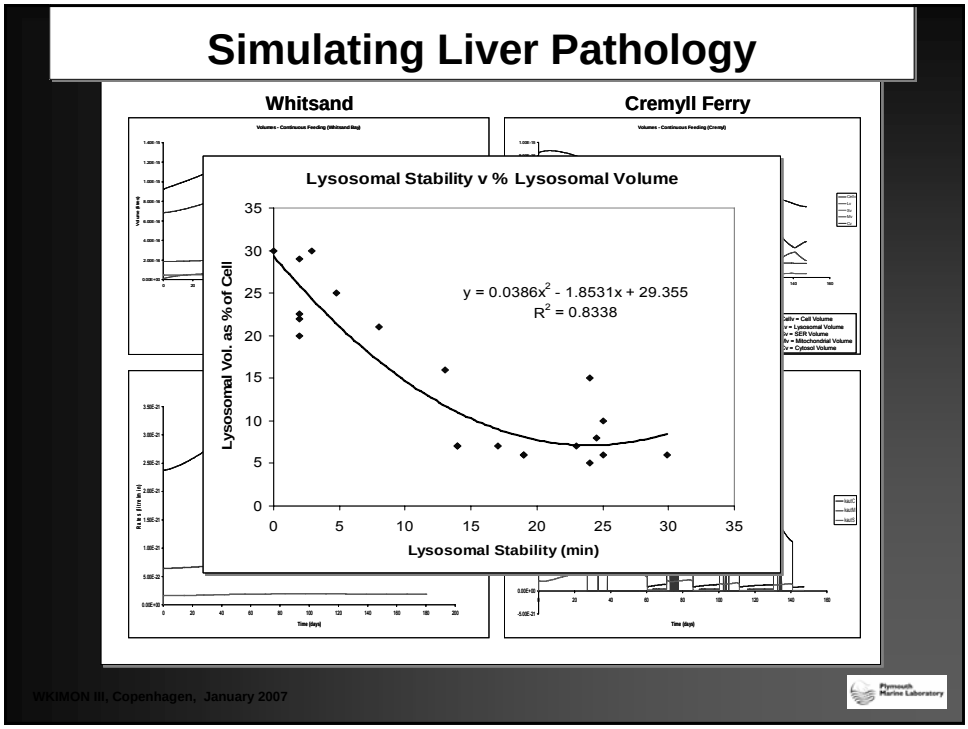

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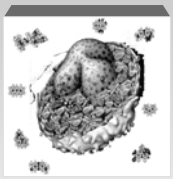
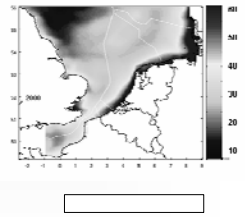
Process modelling in PML – the bigger picture



Modelling from the cell to the ecosystem



Wider environmental impacts

- Cell models as proxies for communities nested into large scale ecosystem and pollutant transport models
- Predicting effects of climate change on ecotoxicological processes
- Ecotoxicological correlates as predictors for deleterious human health and socio-economic effects

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Summary

1. A whole system “middle-out” approach based on cells and tissues as the starting point has enabled us to derive simple mathematical tools for interpreting multiple biomarker responses
2. Lysosomal stability can be used as an index of animal health status which facilitates mapping and interpretation of multiple biomarker responses
3. Biomarkers of lysosomal dysfunction are predictors for tissue, organ and whole animal pathology and have been used to derive an expert system
4. Computational modelling of cellular and tissue function in molluscan and fish livers is increasing our predictive capability

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The Way Forward

1. Targeted use of diagnostic indicators in samples of mussels from sites already tested for contaminants
2. Use chemical data to input into models
3. Compare predicted and real results
4. Develop rapid throughput methods for diagnostic biomarkers related to pathology
5. Refine models and expert systems for use in an ecosystem-based approach for environmental health status

Plymouth Marine Laboratory

Annex 14: Assessment criteria for EROD, DNA adducts and bile metabolites of PAHs

1 Assessment criteria for EROD activity in fish liver

During the meeting data was collated on EROD, 1-OH-pyrene and DNA adducts originating from Belgium, Denmark, France, Germany, the Netherlands, Norway and the United Kingdom, resulting in a database containing 11645 records. The majority of the data, more than 8000 records, concerned EROD measurements.

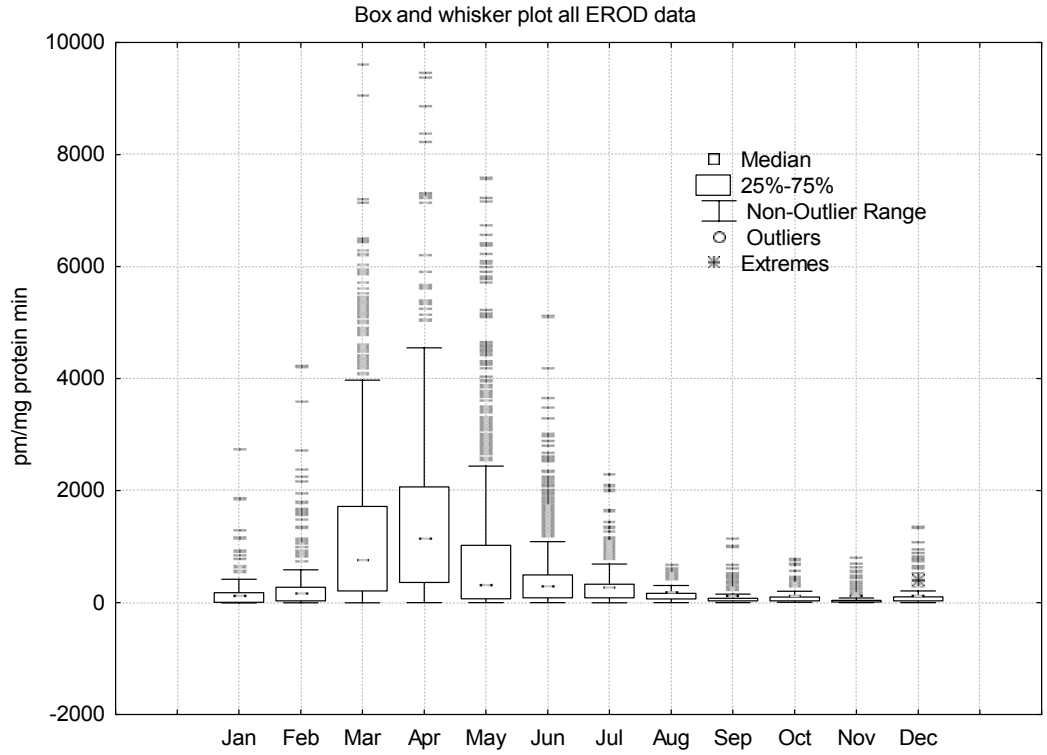

Figure 1: Overall EROD seasonal induction pattern in the OSPAR area.

A first glance at all the data for EROD revealed a very distinct seasonal pattern in the data with EROD induction reaching its peak during spring (figure 1). The dataset was then further separated out according to species and sex. Three main species where considered: dab, flounder and cod. Most of the EROD data was for dab with major contributions from Belgium, Germany and the UK but also from France, Denmark and the Netherlands.

For dab, being the largest dataset, no real differences could be observed between EROD induction for males and females (figure 2). The seasonal pattern discussed above was clearly present. For the other species there was not enough data to reach a similar firm conclusion.

With assessment criteria in mind, male and females were not considered separately. Also, calculation of assessment criteria was limited to the period August-November, where the lowest induction is observed. This is also the period indicated in the ICES paper as optimal for EROD monitoring? Basically, median values were calculated for those months and for stations which contracting parties consider being reference stations. For those medians, an overall median was calculated as the background EROD induction level. The resulting values are given in Table 1.

TABLE 1: PROPOSED BASELINE EROD INDUCTION VALUES IN PMOL/MG PROTEIN MIN FOR THE PERIOD AUGUST TO NOVEMBER	
Species	**Value**
Atlantic cod	80
Dab	40
Flounder	15

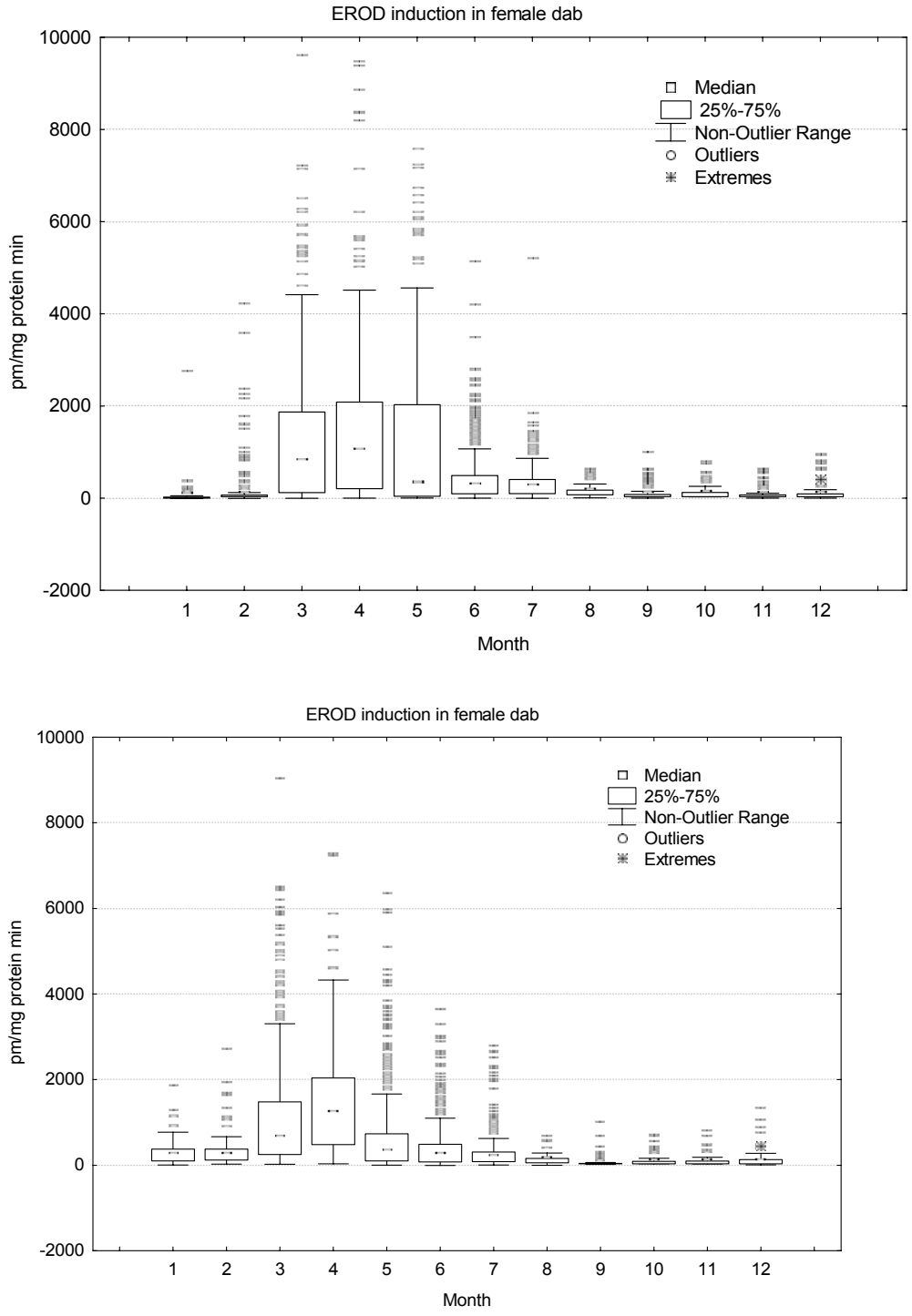

Figure 2: EROD induction in male and female dab.

2 Assessment criteria for DNA adducts of PAHs

Data were available for dab, haddock, saithe and Atlantic cod from UK and NO reference areas. The data was compiled and medians computed for each group of fish taking account of station, sex and date. The median of all entries was then used to derive a median and 80 percentile for males and females for each species (Table #1).

Data from UK was reported in adducts/108 nucleotides, whereas data from NO was reported as nmol adducts/mol DNA. All data was converted to nmol adducts/mol DNA using the conversion factor #.

Table #1. Proposed background values for DNA adducts in the indicated species using data from reference areas; N indicates the number of groups for which medians have been used to calculate median and 90 percentile as indicated.

SPECIES	N	MEDIAN	90 PERCENTILE	COMMENT
Dab (*Limanda limanda*)	23	3.75	7.86	Data from UK
Haddock (*?*)	15	3.86	6.84	Data from NO
Saithe (?)	53	2.06	7.90	Data from NO

3 Pyrene metabolites in fish bile

Data were available for dab, flounder, Atlantic cod, haddock and saithe from UK, NL and NO reference areas. The data was compiled and medians computed for each group of fish taking account of station and date. No differences were observed between sexes. The median of all entries was then used to derive a median and 80 percentile for each species (Table #2).

Data were not standardised, but used as µg/g bile. Only data derived using the fixed fluorescence method (Ariese et al., TIMES) for 1-OH-pyrene was used. See Ariese et al. (TIMES) for a discussion of alternative methods.

The concentration of PAH metabolites in the bile will be affected by feeding history, which is why biliverdin or the absorbance at e.g. 380 nm has been used as standardisation. There is some discussion as to whether this simply increases variability in measurements and the derived background have therefore not included such data.

Table #2. Proposed background values for 1-OH pyrene (µg/mL; 341/383 nm fluorescence) in the indicated species using data from reference areas; N indicates the number of groups for which medians have been used to calculate median and 90 percentile as indicated.

SPECIES	N	MEDIAN	90 PERCENTILE	COMMENT
Dab (*Limanda limanda*)	14	134	220	data from UK
Atlantic cod (*Gadus morhua*)	4	0.56	0.95	data from NO

Annex 15: Assessment criteria for bioassays

Two background documents have been produced, one for (water) bioassays which include bulk water and concentration techniques and a second for sediment bioassays which include whole sediment, pore water and elutriate techniques. The document on (water) bioassays also provides an overview of bioassays in a more general fashion (including in vitro bioassays) in respect to their use and assessment tools.

Water, sediment bioassays, in vivo and in vitro bioassays include techniques that use specific testing regimes and species. Therefore for the purposes of developing background responses and assessment values each technique will require a separate review. Data available to undertake this task was sourced from contracting parties at the meeting, and included information from the Netherlands, Spain and United Kingdom.

1 Whole sediments

Whole sediment assays that are currently in JAMP and the ICES list are: *Corophium* sp. acute and *Arenicola* sp acute. Methods for these 2 bioassays are developed and described in the ICES TIMES SERIES and Quality Assurance via BEQUALM is in place.

Sediment bioassays are used for three different purposes:

- For toxicity assessment of known or spiked contaminants (hazard assessment for known compounds or verification following field assessment)
- Field assessment for environmental sediment quality (field assessment)
- Site-specific investigations or for dredge material testing (site- specific purposes)

The method to derive the background response level is basically the same for all types of whole sediment bioassays. Within the methodology, the experimental design requires a control sediment, a positive control sediment (reference toxic compound) and/or reference sediment. A *control sediment* is defined as "pristine and contaminant-free" with known characteristics and defined ranges, with respect to; contaminants, particle size composition, organic carbon content, ammonia, sulphide and known response of the organism in this sediment. This control sediment is used each time the test is performed and all test sample responses are compared to the control response. A *positive control sediment* is used in every test to assess the performance of the testing procedures, including the sensitivity of the test organism. The positive control consists of the control sediment spiked with a reference compound (for example Zn). A *reference sediment* is usually used for site specific programmes and may be considered as the control sediment for the sampling area or region under investigation and is different from the control sediment in that it has the same or similar physical / chemical characteristics as the sediments under investigation. Ideally, this should give the same response as the control sediment (but in practice this is difficult to achieve due to different sediment characteristics).

Assessing the data

In all assays the validity criteria for each assay should be met, e.g. temperature, ammonia, etc. Confounding factors (e.g. ammonia, sulfide, etc) must also be measured to be sure that the response is related to contaminants.

The background response is defined as the upper level of natural variation and can be determined as a percentile (for instance 90%) of the individual responses (mortality) of the control sediment or reference sediment as appropriate. Experience suggests that for *Corophium* sp this would be 10% (Spain), 20% (UK) or between 3 and 30% (NL). Similarly,

for *Arenicola marina* it is 10% (UK). These figures however need to be defined and further established. (see also Table 1 below). Above the background response level is the warning level warranting further investigation (mortality between the background response and 100% mortality, indicating possible individual and population effects). The next level is the serious concern level (100% mortality) with immediate impact on individual and population levels. In addition, when multiple bioassays are applied, the Predicted Affected Fraction (PAF) approach (which has been used successfully in the Netherlands) can be used to define a more accurate environmental assessment level (see Background document on water bioassays).

Pore water and elutriate bioassay can be considered with water bioassays insofar that these techniques involve a sediment manipulation procedure followed by the use of small volume water *in vivo* bioassays.

2 Water bioassays

The species recommended for water bioassays are:

- Copepod (*Tisbe battaglii* and *Acartia* sp); 48hr exposure using mortality as the end point.
- Bivalves (*Crassostrea gigas, Mytilus* sp) embryos: 24 hr exposure using Percent Net Response as the end point.
- Sea urchin (*Paracentrotus lividus*): 24 hr embryo exposure using percent normal development and larval length as the end points.

The methodology for water bioassays is well developed and available through ICES TIMES and/or OECD. Quality Assurance is provided via BEQUALM for the bivalve tests and *Tisbe* assay.

In all water bioassays a control and positive control is used. The control is a "pristine water" of known water quality and characteristic ie no contamination, full salinity, appropriate pH and dissolved oxygen eg natural seawater from the Atlantic from ICES reference station or Cape Wrath. The *control water* is used in all tests and test animal response in all field and test samples are compared to the test animal response in the control water. A positive control is always used in each experimental design to assess the performance of the testing procedures, including the sensitivity of the test organism. The *positive control* consists of the control water spiked with a reference compound (usually Zn). A *reference water* may also be included for site-specific programmes and may be considered as the control water for the sampling area or region under investigation and ideally should give the same response as the control water.

Assessing the data

The data for water bioassays can be considered in much the same way as for sediment bioassays ie the background response is defined as the upper level of natural variation and can be determined as a percentile (for instance 90%) of the individual responses (mortality or malformation) of the control water.

From experience in the UK, Netherlands and Spain the maximum background level response is of the order of 10% for *Tisbe* sp and *Acartia* sp bioassays, 10% for sea urchin and 20% for the bivalve embryo bioassay (see also Table 1). Above these values would be the warning level and at 100% this would be categorised as a level of serious concern. Responses at the warning level would prompt further sampling and assay in terms of spatial/geographical spread and frequency of sampling (possibly time-integrated water sampling). Responses at the serious concern level would initiate further assay of the water test samples using a dilution series in order to quantify the toxicity using an ECx (percent dilution causing a x% reduction in the endpoint) or toxic units (TU=100/ECx) approach. A phased Toxicity Identification

Evaluation (TIE) can be conducted to further describe the nature of the toxicity or potential toxicants present.

Sediment pore water and elutriates.

Any of the four water bioassays listed above can be used to assess the toxicity of pore water and elutriates. The procedures used to prepare the pore water and elutriates are well documented and described elsewhere. For the assessment of elutriate data, confounding factors that must be considered are; volume of sediment and the sediment -water ratio, ammonia, sulphide, sediment quality – (i.e. sand or mud). For the assessment pore water data confounding factors that must be considered are; salinity, pH, DO, ammonia and sulphide. Data is produced and assessed in the same manner as for the water bioassays above and the results may be expressed in term of EC50 values and/or toxic units, depending on the purpose and the objectives of the study. Toxic elutriates and pore water can be diluted for testing if appropriate.

3 In vitro assays

In vitro bioassays are being developed for use with water, and sediment bioassays. In general this requires sample manipulation and/or concentration techniques, and clean-up using extraction procedures in analog to chemical compounds. These procedures and QA are currently being developed and documents for ICES and OSPAR are being prepared by the UK and NL. When they are fully in place it will be appropriate to develop the background responses and assessment criteria for these techniques. This needs to be progressed within the current ICES OSPAR framework.

Preliminary assessment of background response level of available data

A preliminary derivation of background response levels was attempted at the meeting for the whole sediment bioassays *Arenicola marina* and *Corophium* sp and the water bioassays using *Tisbe bataglii*, bivalve embryo and echinoderm embryo. However, it should be noted that the raw data available at the meeting was limited and only tentative background responses could be calculated. The data were entered into a template (see table 2) and the following calculations made. Data from controls were collected for several tests from different sources. When individual datasets were obtained these were averaged per sample and listed in a databases with standard deviation. From resulting samples the averaged per lab/country was calculated together with the 10, 50 (median) and 90 percentile. In case more datasets (*Corophium*) were available the same was done with lab/countries datasets.

Table 1 Preliminary results of background response levels for water and whole sediment bioassays

Test	lab		Average	0.1 perc	0.5 perc	0.9 perc	
			10.9	3.3	8.6	19.7	
			Average	0.1 perc	0.5 perc	0.9 perc	n
Corophium	RIKZ	Control	12.3	6.6	10.5	19.3	4
Corophium	Cefas	Control	9.5	0.0	6.7	20.0	21
Test	lab		Average	0.1 perc	0.5 perc	0.9 perc	n
Arenicola	Cefas	Control	4.7	0.0	0.0	13.3	20
Test	lab		Average	0.1 perc	0.5 perc	0.9 perc	n
Mussel embryo	IEOV	Control	14.1	12.0	13.5	16.5	3

Test	lab		Average	0.1 perc	0.5 perc	0.9 perc	n
Copepods	Tisbe	Control	1.3	0.0	0.0	5.0	28

Test	lab	Average	0.1 perc	0.5 perc	0.9 perc
		10.9	3.3	8.6	19.7

			Average	0.1 perc	0.5 perc	0.9 perc	n
Corophium	RIKZ	Control	12.3	6.6	10.5	19.3	4
Corophium	Cefas	Control	9.5	0.0	6.7	20.0	21

Test	lab		Average	0.1 perc	0.5 perc	0.9 perc	n
Arenicola	Cefas	Control	4.7	0.0	0.0	13.3	20

Test	lab		Average	0.1 perc	0.5 perc	0.9 perc	n
Mussel embryo	IEOV	Control	14.1	12.0	13.5	16.5	3

Test	lab		Average	0.1 perc	0.5 perc	0.9 perc	n
Copepods	Tisbe	Control	1.3	0.0	0.0	5.0	28

Table 2: Template of data available during the meeting used for calculations of background responses for water and whole sediment bioassays (Median, Min and max are optional)

Test	**Name of the test**
reference	Reference to the origin of the data
year	Year of production
Country	
lab	Laboratory that performed the analyses
type	Is it a control or other type of sample
Endpoint	Type of measurement
unit	
idnr	Sample number within a data set
Replicates	Number of replicates
Result	Average value of the control
Median	Median of the individual data
Min	*Minimum of the individual data*
Max	*Maximum of the individual data*
Stdv	Standard deviation f the individual exposures
Sed-ino	Information about sediment properties

Recommendation

Considerably more raw data needs to be sourced to derive the background response values for each bioassay. Raw data should be sent to Dick Vethaak (NL) for completion at WGBEC 2007.

Annex 16: Assessment criteria for fish diseases, vitellogenin, and reproductive success in eelpout etc

Drafted by

W. Wosniok, T. Lang, S.W. Feist, G. Stentiford, Jacob Strand

1 Assessment Criteria for Fish Diseases

Introduction

Fish disease prevalences are important markers of marine environmental health. They have been monitored since the early 1980's, following standard protocols, the resulting data is fed into the ICES data base and summary reports are produced regularly by the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO). However, the various diseases exhibit specific temporal trends and differences between sites, thus generating a complex picture. In order to provide a comprehensive summary of fish disease status, jointly for a large set of diseases and fish individuals of both sexes and all sizes, the WGPDMO developed a Fish Disease Index (FDI) (WGPDMO Report 2006, ICES 2006/MCC:01). The index and its proposed use as an assessment tool is described in this document.

Construction of the Fish Disease Index

The index in its present form is constructed to summarize diseases of the common dab (*Limanda limanda*) which is the most common flatfish species in the North Sea and adjacent areas and has been the major target species for monitoring fish diseases and biomarkers in these areas for more than two decades. The common dab is affected by a variety of externally visible diseases and parasites as well as by a wide range of liver pathologies, including neoplastic changes, at varying degree of severity/intensity. Three categories of diseases are included in the construction of the FDI:

- externally visible diseases,
- macroscopically visible liver neoplasms > 2 mm in diameter (previously termed liver nodules > 2 mm), and
- histopathological liver lesions (early non-neoplastic toxicopathic lesions, pre-neoplastic lesions (foci of cellular alteration, FCA), benign tumours, malignant tumours).

These conditions enter the FDI with different weights, which reflect the severity of the conditions. Weights are given by experts's judgements. The FDI is then calculated for each individual fish by summing the weights of those diseases that were found on the fish. This sum is normalized to a range between 0 and 100 by multiplication with 100/(maximally achievable sum of weights). A spreadsheet illustration of the calculation procedure is given in the WGPDMO 2006 report, p. 76–83. The FDI for a fish population is the mean of the individual FDI's. Four versions of the FDI have been formulated so far, one for each of the above disease categories and a summarizing version for all categories jointly.

As disease prevalence and parasite presence depend on sex and size (or age) of the fish, the FDI of several populations will in general differ simply as a consequence of different sex and length distributions in the populations. This can be compensated for by defining an adjusted FDI, which contains an additional length adjustment factor for each disease. This factor is small for fish lengths for which the disease is frequent, and is high otherwise. The numerical values are derived as reciprocals of the prevalence-length relation observed in a large reference data set. The adjusted FDI is normalized to a range of 0–100 as the unadjusted version.

The construction principle of the FDI is a universal one in the sense that it can be carried over to other parameter (combinations) for which assessment tools are required. It is in fact frequently used in the field of (human) medicine in order to define action limits for medical interventions.

Use of the Fish Disease Index as an assessment tool

The FDI for externally visible diseases ((lymphocystis, epidermal hyperplasia/ papilloma, acute/ healing skin ulcerations, X-cell gill disease, hyperpigmentation, acute/ healing fin rot/ erosion, Parasites: *Stephanostomum sp*., *Acanthochondria sp*., *Lepeophtheirus* sp.) was calculated for diseases of dab (*Limanda limanda*) in the North Sea (data from the ICES Data Centre with additional information provided by the German Federal Fisheries Research Centre, North Sea between ICES statistical rectangles 33E7 and 46F8, dab with lengths between 15 and 33 cm, both sexes, time range 1990–2005, 155 500 individual fish). Non-adjusted and length/sex-adjusted FDIs were calculated. Analysis of the FDI with respect to temporal and spatial changes showed that

- temporal trends exist
- annual cycles exist
- temporal trends and annual cycles are specific for area (ICES statistical rectangles).

Consequently, any assessment based on the FDI has to be area-specific and has to use a correction term for the season of observation.

Some fish diseases are a natural phenomenon. Due to the absence of data from the pre-industrial era there is no definitive reference area for fish diseases in the North Sea. For that reason, and in reaction to the findings above, the following procedure is proposed as an assessment procedure.

1) Calculate the FDI from appropriate fish disease data. For the length/ sex adjusted FDI version use the factors given in Table ###.

2) Adjust for the season of observation by adding the adjustment terms given in Table (##+1) to the FDI.

3) Determine the assessment class for each of the two FDIs from Table (##+2)..

The boundaries between assessment classes in Table (##+2) separate observed values in the presently used data set into proportions of equal size. No assumptions about the statistical distribution of the FDI were necessary to find the boundaries.

Values in assessment class I are considered as desirable in that area, values in class II are considered as acceptable. Values in class III are uncommonly high. The assessment should further focus on the temporal development in the area: any change from a higher assessment class to a lower one is desirable, while a change into a higher class is not.

For a spatial comparison the FDI values can be used directly as input to standard statistical tests.

Figures ##1a–1c show three examples of unadjusted FDI time series and their associated assessment classes.

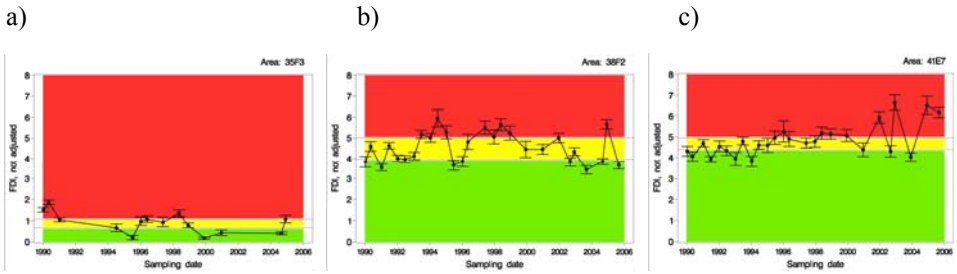

Fig. 1. Time series of the unadjusted FDI for three areas in the North Sea (a: ICES Statistical rectangle 35F3, b: 38F2, c: 41E7). Green, yellow and red areas correspond to assessment classes I, II, III. The FDI series exhibit area-specific levels, temporal trends and seasonal variation.

The assessment consists of two sub-assessments, one based on the unadjusted FDI, the other on the FDI. If they coincide, this is the final assessment. In the case of non-coincidence the final assessment is given by the adjusted FDI, but the non-coincidence signals additionally a change in the length-prevalence relationships.

Table ##: Length adjustment factors for lymphocystis in female dab (excerpt, preliminary – see "Remaining tasks")

SEX	DISEASE	LENGTH (CM)	LENGTH ADJUSTMENT FACTOR
female	lymphocystis, grade 1	15	2.32
		20	0.80
		25	0.90
		30	1.44

Table ##+1. Season adjustment terms (excerpt, preliminary – see "Remaining tasks")

AREA	NOVEMBER, DECEMBER, JANUARY	MAY, JUNE	JULY, AUGUST, SEPTEMBER
35F3	0	1.42	-0.29
38F2	0	0.53	-1.25
41E7	0	-0.02	-2.36

Table ##+2. Assessment classes for the Fish Disease Index (preliminary – see "Remaining tasks")

AREA	I: LOW RANGE	II: MEDIUM RANGE	III: HIGH RANGE
35F3	< 0.81	0.81 – 1.05	>1.05
38F2	< 4.42	4.42 – 4.90	> 4.90
41E7	< 4.44	4.44 – 5.06	> 5.06

Implementation of the FDI assessment procedure

The intention is to develop an automated system for the calculation of the FDI. The FDI per individual can then be calculated either in real-time onboard ship (where it can be used for immediate interpretation) and then reported to ICES alongside raw data, alternatively, it can be calculated within ICES on actual and archive data. The establishment of the necessary tables will be finalized under the auspices of the WGPDMO in 2007.

Remaining tasks

The numbers given in Tables ##–##+2 are derived from a preliminary calculation and therefore only an excerpt of the complete tables is provided here. They will be reviewed by the WGPDMO during its forthcoming meeting (March 2007) and completed intersessionally. This review will focus on the techniques applied for length, sex and season adjustment, and for the

derivation of assessment class boundaries. Also, further expert opinions with regard to the weighting of diseases will be obtained, using a Bradley-Terry to join these.

2 Assessment criteria for fish vitellogenin as a biomarker of exposure to xenoestrogens

The draft document entitled 'Fish vitellogenin as a biomarker of exposure to xenoestrogens (presented by the UK)' was reviewed, specifically the section on setting of background concentrations. The aim of the subgroup was to define background concentrations for vitellogenin (VTG) across fish species used in biological effects monitoring surveys. For this we used information contained within the background document and raw data provided for the WKIMON III workshop.

Two major factors were discussed by the subgroup. The first related to the limit of detection of the VTG assay (ELISA) and the second regarded new information on potential seasonality of VTG expression in non-exposed controls (cod data from Norway).

1) The background concentrations for VTG suggested in the background document were 0.13 μg/ml for flounder (River Alde, UK, 1996-2001 data) and 0.22 μg/ml for cod (caged males, North Sea reference site). However, observation of the raw data for flounder showed a lower limit of 0.2 μg/ml (i.e. above the suggested background concentration suggested for this species). Further consultation with colleagues responsible for the collection of this data confirmed that 0.2 μg/ml is regarded as the lower limit of detection for the assay in this species. Since data for flounder from the River Alde falls below this limit, the background concentration is effectively set at the limit of detection. For this reason, it is proposed that setting a background concentration of 0.13 μg/ml for flounder is not appropriate. Since the provisional background concentration for VTG in cod (0.22 μg/ml) is presumably close to the limit of detection for the flounder VTG assay (to be confirmed by authors), it is suggested that the background concentration has also effectively been set at the limit of detection.

2) Information provided by a WKIMON III participant highlighted the potential for seasonal variation in VTG concentrations in control cod (from laboratory trials). Some evidence suggests that background levels can elevate by a factor of ten throughout the year. The subgroup discussed the implication of this for VTG analysis in cod and in other species with regard to the setting of appropriate background concentrations for VTG.

Evidence from seasonal studies of control cod has shown that VTG expression may alter by a factor of 10 over a year. Bearing in mind a limit of detection in the assay of 0.2 μg/ml, the background concentration for control specimens would be up to 2 μg/ml. Consequently, the background concentration for cod VTG is appropriately set at 2 μg/ml.

Although seasonal data is currently not available for control populations of flounder, we cannot assume that seasonal patterns do not also exist in this (and other) species. In the absence of such information, it was proposed that a similar correction factor be applied, setting the background concentration for flounder VTG at 2 μg/ml.

We recommend that for the development of assessment tools, background concentrations for all species be set at 2 μg/ml. However, to provide additional confidence in this value, further information is required, particularly with regard to seasonality and size/age effects on VTG levels in control fish species (see requirements below).

Requirements:

a) Confirm detection limit of cod and flounder VTG assay.

b) Obtain seasonality and size/age data for cod (to confirm correction factor for background).

c) Investigate potential for seasonality effects in other species.

3 Assessment criteria for reproductive success in eelpout (*Zoarces viviparus*)

The WKIMON-III subgroup propose the following assessment criteria based on the Background document on reproductive success in eelpout (*Zoarces viviparus*) (document WKIMON 07/3/8-E, presented by Denmark), where the assessment also is described in more details.

Assessment criteria related to mean frequencies of abnormal larvae in broods

A three class scheme of assessment criteria is proposed and it includes the following parameters for abnormal development of embryo and larvae in eelpout broods (Neuman *et al.*, 1999, Strand *et al*., 2004);

- Malformed larvae: larvae with morphological and/or skeletal gross anomalies. This includes yolk sac or intestinal defects, bent spine or spiral shapes of the spinal axis, eye defects including rudimentary or missing eye(s), cranio-facial defects and conjoined/Siamese twins more or less separated.
- Late dead larvae: dead larvae without malformations.
- Growth retarded larvae: normal developed larvae which are smaller than the three highest length classes in the broods.

The derivation of assessment criteria was based on 52 datasets from 14 sampling stations regarded as reference sites in the Baltic Sea, the Kattegat and the Skagerrak from the period 1994–2004 are available for the analyses.

However, an important assumption in the derivation was that the sites in the Baltic Sea, the Kattegat and the Skagerrak selected as reference sites are adequate reference sites, although these waters are generally regarded to be more polluted compared to the North Sea and the North Atlantic. However, with regard to data availability, the selected sites are considered the best choice currently possible.

In areas, which were considered as reference sites, generally small mean frequencies of abnormal larvae have been found, if any. Values of 90 percentiles have been found to be 1% malformed larvae, 2% late dead larvae and 4% growth retarded larvae, respectively.

Statistical analysis shows that frequencies of >2%, >3% and >6% of malformed, late dead and growth retarded larvae, respectively, are significantly different from the 90 percentiles, when the sample size consists of 40 pregnant female eelpouts with minimum 40 larvae in each brood ($G > 3.84$, $p < 0.05$, 2×2 contingency table).

The range between 90% percentiles and the level significant different from this can be regarded as a zone of uncertainty where effects induced by environmental factors like contaminants cannot be excluded (Table 1).

Table 1. Proposal for assessment criteria for the mean frequencies of malformed larvae, late dead larvae and growth

ASSESSMENT CLASS	I	II	III
	"Background response". The upper limit is the 90% percentile of response at reference sites.	Effects cannot be excluded.	Significant effect level compared to background response.
Mean frequency of malformed larvae	0 – 1%	>1% - 2%	>2%
Mean frequency of late dead larvae	0 – 2%	>2% - 3%	>3%
Mean frequency of growth retarded larvae	0 – 4%	>4% - 6%	>6%

Annex 17

Fish disease and liver pathology for general and PAH-specific biological effects monitoring

- Current status and outlook-

hen Feist

The Centre for Environment, Fisheries and Aquaculture Science

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

Cefas

Background

- First studies in Europe during the late 1970s
- Role of ICES – seagoing workshops (intercalibration) 1984,88 & 94; publication of guidelines
- ICES Sub-group on statistical analysis of fish disease data (1992-1996)
- Monitoring programmes conducted by Germany, The Netherlands and the UK (other data from Denmark & Belgium)
- Fish disease data used in environmental assessments – NSTF 1993; OSPAR QSR 2000; HELCOM assessments 1996, 2002
- Increasingly integrated with monitoring for chemical contaminants and biological effects of contaminants

Cefas

Overview*

- Applicability across the OSPAR Maritime Area
- Status of Quality Assurance
- Influence of environmental variables
- Assessment of thresholds
- Proposals for assessment tools

*OSPAR CEMP Review – Externally visible diseases, liver nodules and liver pathology including liver neoplasia/hyperplasia. Final version submitted July 2006.

Cefas

Applicability across the OSPAR Maritime Area

- Used for many years as an integrative response measuring health status in general biological effects monitoring
- Applicable for a variety of species, eg. Dab, flounder, cod
- Contaminant specific liver pathology – liver cancer and liver histopathology
- Application to many other species, eg. Eelpout, dragonet, plaice and pelagic species
- Appropriate for use across the OSPAR maritime area

Cefas

Status of Quality Assurance

- QA procedures are in place and operational through ICES activities and BEQUALM.
- Covering survey methodology, sampling, diagnosis, reporting and statistical analysis
- Workshops, ring-tests and intercalibration exercises.
- ICES TIMES publications on external fish diseases and liver histopathology (nos 19 & 38)
- Increasing BEQUALM participation

Cefas

Influence of environmental variables

- Multifactorial aetiology of disease is accepted
- Environmental pollution is one factor
- Contaminants cause specific and non-specific changes at various levels of organisation
- Liver cancer and pre-neoplastic lesions are associated with exposure to carcinogenic contaminants eg. PAHs
- Integrated monitoring effort ensure collection of various biological and environmental parameters
- Allows statistical analysis eg. Wosniok et al. 2000

Cefas

Assessment of thresholds

Currently under development via ICES WGPDMO

- Determined from comparisons of disease prevalence between reference site(s) and quantitative change over time (trends)
- Further work needed to define and implement reference and threshold values

Presentation by Werner Wosniok – this meeting

Cefas

Proposals for assessment tools

Currently under development via ICES WGPDMO

- Development of the Fish Disease Index (FDI)
- Quantitative information summarising data on prevalence and intensity of various disease conditions into one figure
- Multivariate statistical approaches offer good potential

Presentations by Grant Stentiford & Werner Wosniok – this meeting

Cefas

Annex 18: Draft JAMP Guidelines for the Integrated Monitoring and Assessment of Contaminants and their effects

Contents

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1. General Introduction

Our seas and oceans are both dynamic and variable. They represent a fundamental component of our global ecosystem and as such we need to be able to assess the health status of our marine environment. Furthermore, we need to be able to detect anthropogenically-induced changes in our seas and oceans and to be able to identify the reasons for these changes. It is only through such understanding that we can advise on necessary and appropriate remedial responses, such as regulatory action, or else report on the improvements resulting from OSPAR measures. There is, however, a basic problem in that we need to express clearly what is meant by the 'health' of the marine environment and for that purpose we require indicators of ecosystem health. Such tools are under development in a variety of fora covering a range of topics including biodiversity and hazardous substances.

The marine environment receives inputs of hazardous substances through riverine inputs and direct discharges as well as by atmospheric deposition and is the ultimate repository for complex mixtures of persistent chemicals. This means that organisms are exposed to a range of substances, many of which have the potential to cause metabolic disorders, an increase in disease prevalence and, potentially, effects on populations such as changes in growth, reproduction and survival. There is general agreement that the best way to assess the environmental quality of the marine environment, with respect to hazardous substances, is by using a suite of chemical and biological measurements in an integrated fashion. In the past, monitoring to assess the 'impact' of hazardous substances has been based primarily on measurements of concentration. This was because the questions being asked concerned concentrations of such substances in water, sediment and biota and such measurements were possible. However, in order to more fully assess the health of our maritime area, questions about the bioavailability and impact of hazardous substances on the biota are now being posed. As such, biological effects techniques have become increasingly important in recent years. The OSPAR Joint Assessment and Monitoring Programme (JAMP) (Agreement No 2003/22) now specifies requirements for information on the biological effects of the substances on the OSPAR List of Chemicals for Priority Action and on any emerging problems related to the presence of hazardous substances in the marine environment. The specific focus is on determining whether there are any unintended/unacceptable biological responses, or unintended/unacceptable levels of such responses, as a result of exposure to hazardous substances. Sometimes a biological response can be observed when the causative substance is below current chemical analytical detection limits; the development of imposex in gastropod molluscs due to tributyltin (TBT) is a point in case. As a consequence, studies on TBT-specific biological effects are now mandatory under the OSPAR Coordinated Environmental Monitoring Programme (CEMP). More general biological effects measurements, and those associated with PAHs and metals, are now part of the CEMP, but some are awaiting development of quality assurance procedures and assessment criteria.

The original JAMP Guidelines for monitoring contaminants in biota and sediment or biological effects do not provide guidance for the optimum approach to monitoring to support the integrated assessment of concentrations and effects of contaminants across the OSPAR Maritime Area, although some of them contain references to supporting measurements (chemical data, physical data, biological data) which aid the interpretation of monitoring data. Consequently, chemical analytical and biological effects data have usually been collected, reported and assessed separately. Also, in some cases, the original Guidelines do not provide guidance on the specific compounds (e.g CB congeners or specific PAH compounds) which should be determined in order to be able to explicitly link concentrations and effects. An integrated approach to monitoring is based on the simultaneous measurement of contaminant concentrations (in biota, sediments and possibly water), biological effects parameters and a range of physical and other chemical measurements so as to permit normalization and assessment. This supports improved assessment of contaminant effects by providing assessors

with information on measurements of related concentrations and effects and on the environmental variables which influence the effects measurement.

Integrated monitoring of contaminants and their effects requires better co-ordination of the field sampling and sample handling techniques which are already being used in monitoring programmes, utilising the same species/population/individual for both types of measurement, from the same area and sampled within the same time frame. Furthermore, the set of supporting parameters should be measured at the same time and these data have to be available for utilisation in the final assessment, since many biological effects can be influenced by temperature, stage of maturation, age of fish, and other factors. Integration of effort in this way will yield additional information in a cost-effective manner, whilst also reducing the inter-annual variance of the data.

OSPAR has obligations to measure and monitor the quality of the marine environment and its compartments (water, sediments, and biota), the activities and inputs that can affect that quality and the effects of those activities and inputs, and to assess what is happening in the marine environment as a basis for identifying priorities for action. OSPAR, together with HELCOM, have agreed on an ecosystem approach to managing the marine environment under which OSPAR has committed to monitoring the ecosystems of the marine environment, in order to understand and assess the interactions between, and impact of, human activities on biota. Integrated monitoring and assessment of contaminants in the marine environment and their effects will contribute more effectively to the integrated assessment of the full range of human impacts on the quality status of the marine environment as part of the ecosystem approach.

2. The OSPAR Hazardous Substances Strategy

The objective of the OSPAR Hazardous Substances Strategy (Annex 3) is to prevent pollution of the maritime area by continuously reducing discharges, emissions and losses of hazardous substances, with the ultimate aim of achieving concentrations in the marine environment near background values for naturally occurring substances and close to zero for man-made synthetic substances. The Hazardous Substances Strategy further declares that the Commission will implement this Strategy progressively by making every endeavour to move towards the target of the cessation of discharges, emissions and losses of hazardous substances by the year 2020. In association with this, and the other five OSPAR strategies, OSPAR has developed a Joint Assessment and Monitoring Programme (JAMP). This provides the basis for the monitoring activities undertaken by Contracting Parties to assess progress towards achieving OSPAR objectives (Figure 1). In relation to hazardous substances, the JAMP seeks to addresses the following questions:

- What are the concentrations in the marine environment, and the effects, of the substances on the OSPAR List of Chemicals for Priority Action ("priority chemicals")? Are they at, or approaching, background levels for naturally occurring substances and close to zero for man made substances?
- Are there any problems emerging related to the presence of hazardous substances in the marine environment? In particular, are any unintended/unacceptable biological responses, or unintended/unacceptable levels of such responses, being caused by exposure to hazardous substances?

The primary means of addressing these questions on an OSPAR wide basis is the Coordinated Environmental Monitoring Programme (CEMP; OSPAR Agreement 2005–5). The CEMP requires temporal and spatial monitoring of a range of contaminants, and effects measurements, as detailed in the CEMP Appendices. Realisation of the CEMP requires monitoring guidelines (including technical details of monitoring procedures), quality assurance procedures and assessment tools for each component. OSPAR has been pursuing this approach through the development of Background Concentrations (BCs), Background

Assessment Concentrations (BACs) and Environmental Assessment Criteria (EACs) for contaminants in sediments, biota and sea water. Assessment criteria have also been developed for TBT-specific biological effects. Further assessment criteria are being developed which will encompass a range of parameters covering specific and more general effects and will incorporate the concept of unintended/unacceptable levels of biological responses.

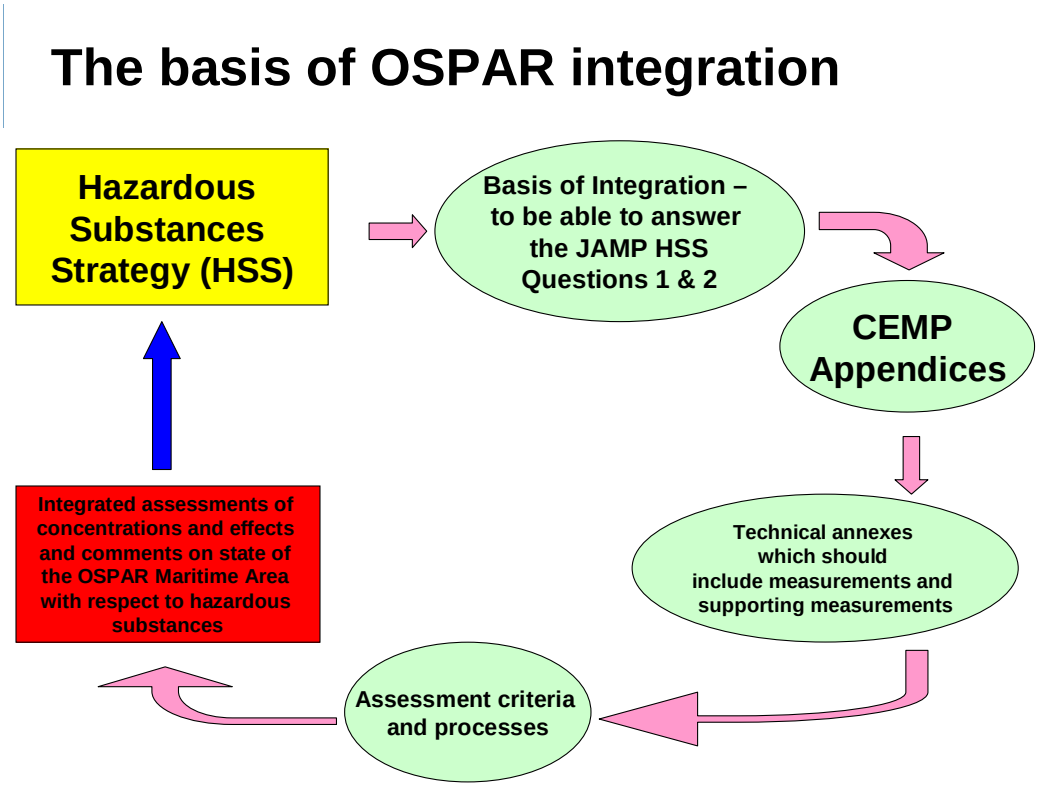

Figure 1: The integrated monitoring OSPAR cycle.

In order to address the JAMP hazardous substances questions, there is a need for the integrated assessment of concentrations and effects. To support this there is a need to adopt an integrated approach to monitoring contaminants in the marine environment and the biological responses to the presence of hazardous substances. Such an approach would provide greater interpretative power in assessments of the state of the OSPAR Maritime Area with respect to hazardous substances and an improved assessment of progress towards achieving the objectives of the OSPAR Hazardous Substances Strategy.

3. Wider Initiatives

The marine environment is a precious heritage that must be protected, restored and treated as such with the ultimate aim of providing biologically diverse and dynamic oceans and seas that are safe, clean, healthy and productive. It is in this context that the European Union has over the last decade developed its water policies such that significant Europan Legislation incorporating marine waters and the lakes and rivers which ultimately flow into our coastal ecosystems. The Water framework Directive (Directive 2000/06/EC) establishes a framwork for Community action in the field of water policy, central to which is good ecological status for water bodies. This is described on the basis of biological quality elements, hydromorphological quality elements and physico-chemical quality elements. More recently, the European Union had initiated a process liley to lead to a Europane Marine Startegy Directive. This legislation is currently under development, but at its heart is the concept of

'good environmental status' for all Euopean waters and the provision of a framework for the protection and preservation of the marine environment, the prevention of its deterioration and where practicable the restoration of that environment in areas where it has been adversely affected. Good environmental status will be described on a regional basis and as such the programmes of the various Regional Sea Conventions, including OSPAR, will provide the data for the assessments that will be required under a Directive.

4. Purpose of the Guidelines

The purpose of this document is to provide guidance on integrated chemical and biological effects monitoring within the OSPAR area, with special reference to the Coordinated Environmental Monitoring Programme (CEMP) issues and the list of OSPAR priority chemicals. In addition, it provides a link to the Technical Annexes which provide guidance on the supporting parameters which have to be measured either when collecting the samples from the marine environment e.g. temperature and salinity, or else when processing the samples e.g. sex, fish length.

5. Quantitative Objectives – Temporal Trend and Spatial Programmes

The ultimate objectives of OSPAR monitoring activities relating to hazardous substances are:

- to assess status (existing level of marine contamination and its effect) and trends across the OSPAR maritime area;
- to assess the effectiveness of measures taken for the reduction of marine contamination;
- to assess harm (unintended/unacceptable biological responses) to living resources and marine life;
- to identify areas of serious concern/hotspots and elucidate their underlying causes
- to identify unforeseen impacts and new areas of concern;
- to create the background to develop prediction of expected effects and the verification thereof (hindcasting); and
- to direct future monitoring programmes.

By being clear about the objective of the monitoring, the parameters for inclusion in the programme of work, the sampling strategy, methods of statistical analysis and assessment method can all be specified. In the context of integrated monitoring, the planning aspect is crucial as it will ensure that operating procedures can be put in place which clearly detail all the chemical, physical and biological samples and data to be collected.

Ultimately, through exploratory sampling, the intention would be to estimate the concentration of a particular contaminant and/or the level of biological effect at a particular time or place, to describe the normal range of values of the measurement and its spatial variability. Furthermore, locating an area of concern and determining the extent of such an area has value. Finally, there is a need to perform monitoring which will identify differences over time and across geographical space. This will divide monitoring into two generic types:

Spatial monitoring - monitoring to identify geographical variation within the OSPAR maritime area

Temporal Monitoring – monitoring aimed at identifying changes over time.

Although these two types of monitoring have been specifically detailed, there is no reason why the two activities cannot be carried out simultaneously, as long as this is incorporated into the design of the programme. The processes of integration for both these types of monitoring are closely related and hence should be developed simultaneously.

When designing a monitoring programme, some *a priori* knowledge of the system to be monitored is required. This will inform the frequency of monitoring and the required spatial distribution of sites that will allow a regional-specific temporal trend monitoring programme to have a power (e.g. 90%) to detect a change in contaminant concentration (e.g. 50%) or level of a biological effect measurement over a specified period of time (e.g. 10 years). By ensuring that the formal objectives are clearly specified, the monitoring programmes become operational.

There will, of course, be occasions where purely exploratory monitoring is being conducted and in this context it may be necessary to define the sample sizes and determine the power of the approach *a posteriori*. In such cases, the formalized objectives must at least describe the intended comparisons. However, it should be the intention to always gather as much information as is possible in developing and designing the monitoring programme with an objective clearly defined. Within OSPAR, there is now a recognized process for undertaking assessments at regular intervals. Such assessments will provide, increasingly, the basis for future monitoring as the data will be of a defined quality and collected over a sufficiently long time period.

6. An Integrated Approach

The contribution made by an integrated programme, involving both chemical and biological effects measurements, is primarily that the combination of the different measurements increases the interpretive value of the individual measurements. For example, biological effects measurements will assist in the assessment of the significance of measured concentrations of contaminants in biota or sediments. Conversely, chemical measurements (or additional effects measurements) will aid in the identification of the causative substances giving rise to the observed effects. An integrated assessment can further lead to an improved ability to explain the causes for hotspots detected during monitoring programmes. Ultimately, an integrated approach also has the advantage of pulling the various disciplines together, of acheieving a greater understanding amongst those performing marine assessments of the contributions from the different components of a monitoring programme and has the clear technical advantage that sampling of all parameters will be assured. An integrated porogramme will bring together the various parallel programmes which have operated historically over many years. However, in developing this integrated approach it may be the case that new (and perhaps therefore additional) ‘integtared’ sites will have to be developed. How such sites will relate to exisiting ‘chemical’ or ‘effects’ monitoring sites is unclear, but what is clear is that an integrated approach requires sites to be assigned, and thus sampled, accordingly. The economic benefit of an integated approach comes from the fact that the samples and data are gathered during a one cruise and that the data can be directly compared/used with holistic assessment tools to provide truly integrated assessments.

Fundamental aspects of the design of an integrated programme include the selection of appropriate combinations of biological effects and chemical measurements and the design of sampling programmes to enable the chemical concentrations, the biological effects data and other supporting parameters to be combined for assessment (see Figure 2).

As part of an integatred approach, a range of desirable properties for relevant biological effects methods have been identified (Reference – 2006 WGBEC Report) and include:

It is essential that methods have an ability to separate contaminant-related effects from influence by other factors (e.g. natural variability, starvation)

The methods used should ideally have an ability to predict effects on “ecosystem health”

They should be sensitive to contaminants, i.e. provide “early warning”

"general health" of the organism should be reflected in at least one of the methods selected

The range of methods used should include a range of mechanisms of toxic action, e.g. estrogenicity/androgenicity, carcinogenicity, genotoxicity and mutagenicity.

The ICES Working Group on Biological Effects has recommended a number of biological effects measurements for inclusion in an integrated programme. These core methods are:

the concentration of PAH-metabolites,
CYP1A/EROD induction,
lysosomal stability,
liver histopathology,
external fish disease,
liver (microscopic) neoplasms and
plasma vitellogenin concentration

In addition, some physiological characteristics of individual fish are required including GSI, LSI and condition factor. On the basis of the above, a general design for fish and mussels is presented in Figures 3 and 4.

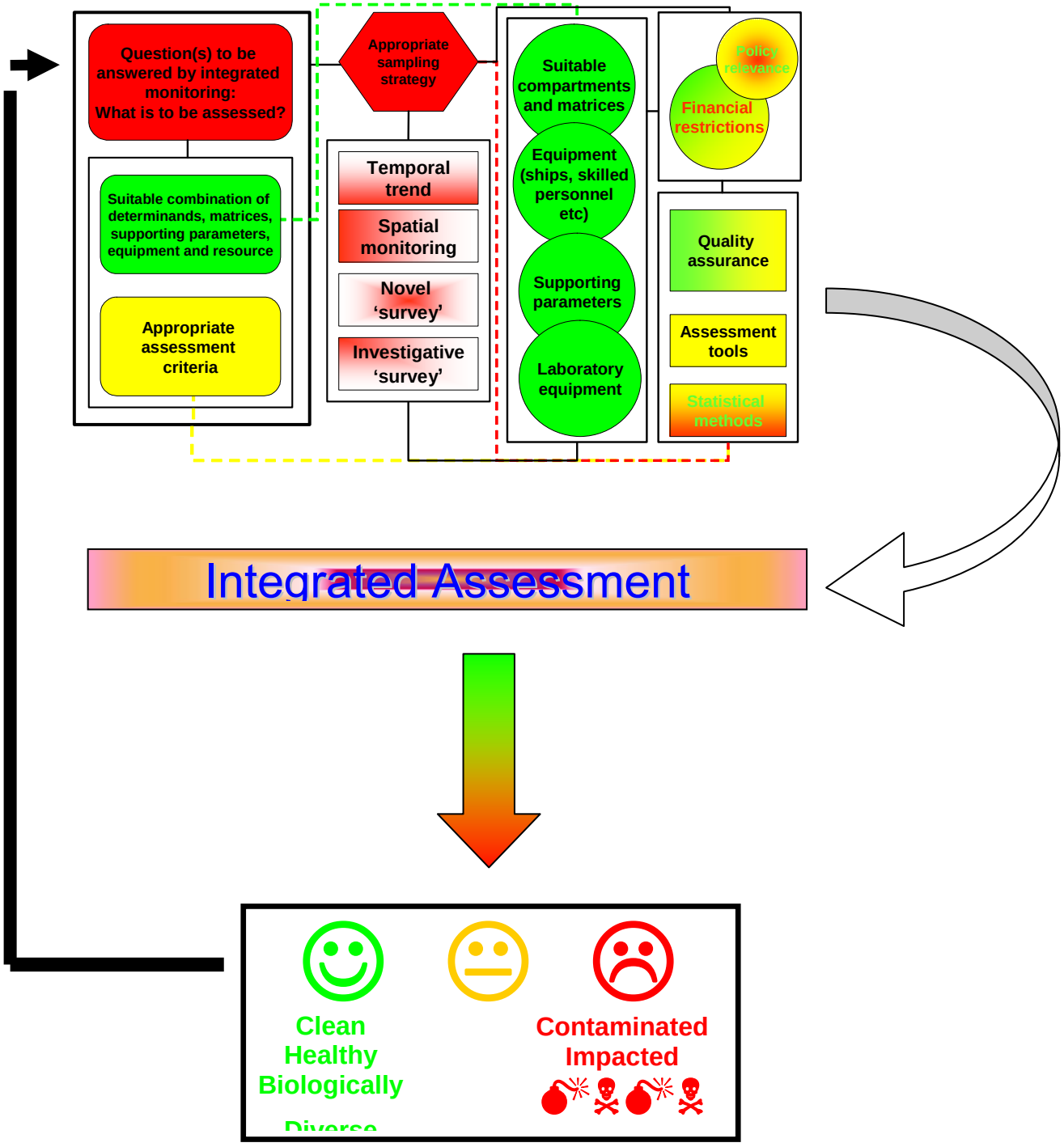

Figure 2. Outline of procedures relating to integrated monitoring and assessment leading to conclusions of status and associated feedback loop.

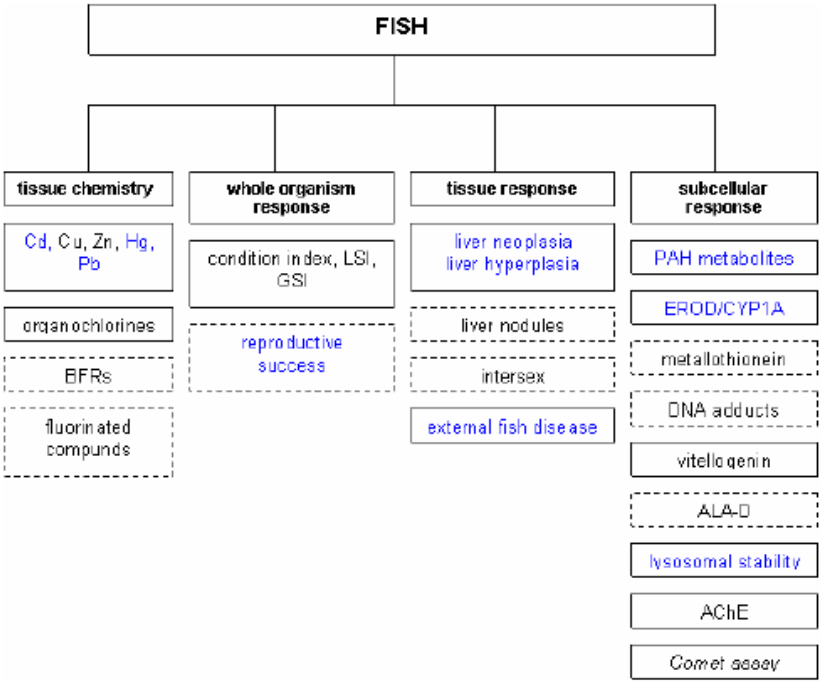

Figure 3: Overveiw of methods to be included in an integrated programme for selected fish species. (Blue: included in CEMP; solid-line boxes: prioritised components (only applies to tissues and subcellular responses); italics: ICES WGBEC promising method

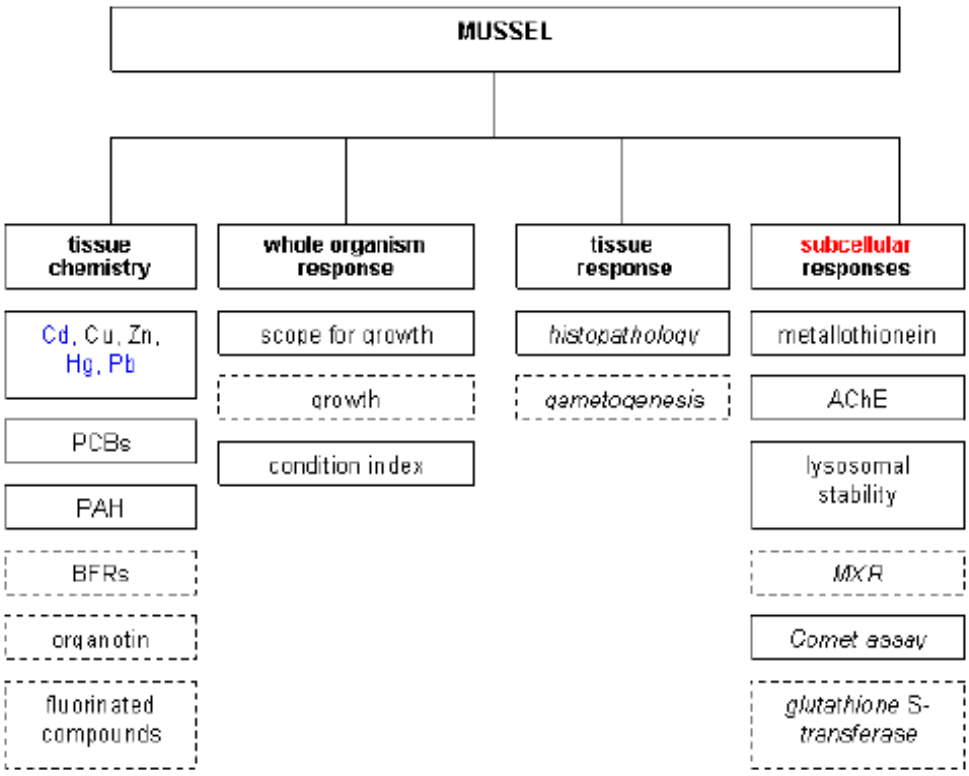

Figure 4: Overveiw of methods to be included in an integrated programme for selected blue mussel. (Blue: included in CEMP; solid-line boxes: prioritised components (only applies to tissues and subcellular responses); italics: ICES WGBEC promising method

Technical annexes describing biological effects techniques should also include a list of the supporting parameters which should be determined in an integrated programme, as well as the chemical determinands relevant to the effects being studied. Assessment criteria and procedures should define how supporting measurements and chemical parameters should be used in the interpretation of the effects data.

The technical annexes will also provide guidance in relation to sampling. In the context of integrated guidelines, the integration of sampling has four distinct connotations:

sampling and analyses of same tissues and individuals;

sampling of individuals for effects and chemical analyses from the same population as that used for disease and/or population structure determination at a common time;

sampling of water, the water column and sediments at the same time and location as collecting biota;

more or less simultaneous sampling for and determination of primary and support parameters (e.g. hydrographic parameters) at any given location.

It is also essential to consider the rate of change of concentrations or effects in the different matrices being sampled (see Figure 3).

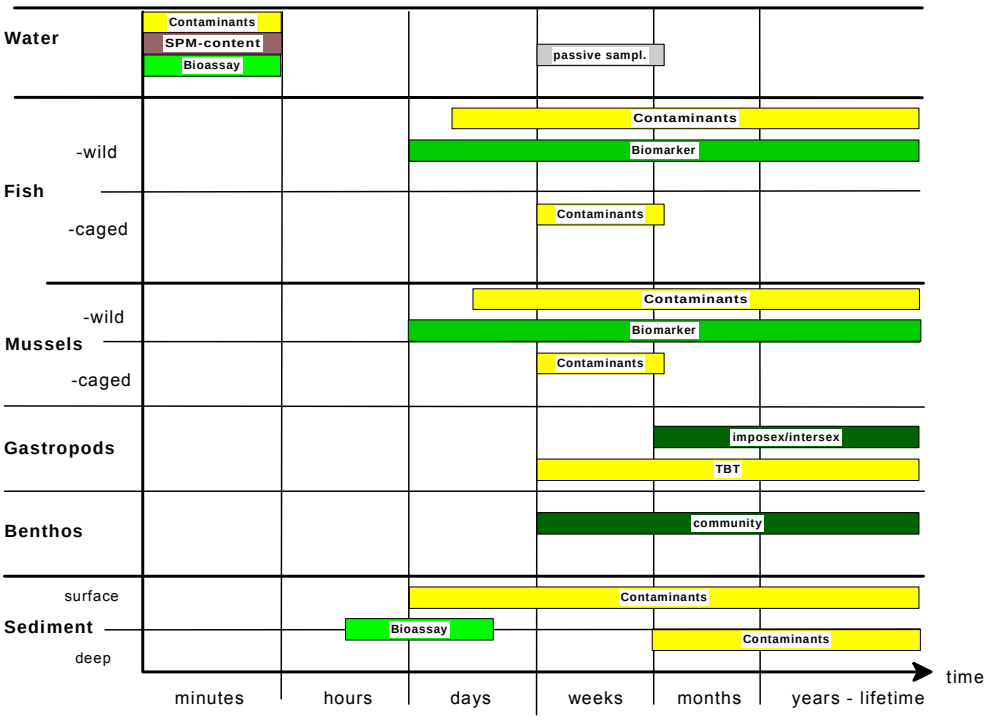

Figure 3. Differing timescales for processes operating in different matrices. Interpretation of data. – add comments

Interpretation of data will be dependent on having a clear understanding of the differing timescales covering the observation of effects and the associated concentrations of contaminants. Such considerations are discussed in more detail in Technical Annex New.1 (Table 2).

7. Technical Annexes

OSPAR has prepared a series of five 'Guidelines for Monitoring' (Annex 1) covering topics such as general biological effects monitoring, monitoring contaminants in biota, monitoring contminants in sediment, contaminant-specific biological effects monitoring and monitoring the environmental impact of offshore oil and gas activities. These Guidelines follow a common format comprising a preamble and then a series of Technical Annexes (Table 1). During WKIMON II these 25 Technical Annexes were reviewed in the context of developing integreated monitoring and recommendations were made for amendments to these Technical Annexes (see Annex 20 of the Report of WKIMON II and Annex 10 of the Summary record for SIME 06). These modified Technical Annexes, together with new Annexes, provide the specific methodologies with respect to sampling and analysis and have been altered to take account of the need for integrated monitoring and assessment. It is clear that there is a dynamic process in operation and OSPAR, in association with ICES, have in place a process for ensuring that relevant documentation is updated as required (Annex 6 – to be written once process is agreed). However, in addition, guidance on the selection of suitable sites for integrated monitoring together with the preferred methodology for the integatred assessment is presented in Technical Annex New.1 – to be prepared by intersessional work and WKIMON IV.

8. Assessment Criteria

It is not sufficient simply to co-ordinate sampling; integration must also involve a combined assessment of the monitored parameters (the 'Integarted Assessment in Figure 2), which must themselves be selected with the assessment aim in mind. Such a combined assessment may

involve using environmental parameters as covariates in statistical analyses or they may be used to standardise effect-variables, e.g. temperature or seasonal effects on biomarker responses. The use of all available methods to generate indices of environmental perturbation is a second example of an integrated assessment Ultimately, the purpose of the integared JAMP monitoring programme is to provide the necessary data to facilitate integrated assessments so that the status of the OSPAR Martime area can be described and Quality Status Reports prepared which clearly describe progress towards delivering the OSPAR Startegies and assessing the effectiveness of regulation. Assessment criteria have been developed within the OSPAR process. Examples include Background Concentrations (BCs), Background Assessmernt Concentrations (BACs) and Enviroenmental Assessment Criteria (EACs) (Reference). Further assessment criteria have been developed to aid interpretation of biological effects data and again there is a dynamic process on-going which will have to take account of scientific progress. The current situation with regard to assessment criteria is summarized in Table 3.

9. Archiving Data

In order to undertrake assessments, the data must be readily accessible. This requires the data to be submitted to the relevant database timesously. In this context, data should be submitted to the ICES database by Contracting Parties. The Working Group on Concentrations Trends and Effects of Substance in the Marine Environment (SIME) undertakes an assessment of the monitoring undertaken by Contracting Parties in response to what Contracting Parties have indicated they will perform in a given year. A traffic light system is used[3] with a 'green light' being assigned only when the data has been submitted to, and is recorded on, the ICES database. Having data readily available in a recognized format is a fundamental requirement of assessments.

10. References

To be finalised

11. Glossary of Definitions and Abbreviations

6PPD	4-(dimethylbutylamino)diphenylamin
AChE	Acetylcholine Esterase
ACS	American Chemical Society
AgNO3	Silver Nitrate
Al	Aluminum
Al2O3	Aluminium Oxide
ALA-D	δ-amino levulinic acid dehydratase
ANOVA	Analyses of variance
As	Arsenic

3 The basis of the traffic light system is that in year X (e.g. 2007), the situation will be reviewed for year X-2 (e.g. 2005) and categorised as follows:

a. had planned for X-2 but had not performed (red stations);

b. had performed in X-2, but for which data has not, so far, been included in the ICES data base (yellow stations); and

c. had performed in X-2, and for which data is included in the ICES data base (green stations).

ASE	Accelerated Solvent Extraction
Ba	Barium
BACI –	Before-After-Control-Impact
BACs	Background Assessment Concentrations ()
BaPH	Benzo[*a*]pyrene hydroxylase
BCR	Bureau Communautaire de Référence
BCs	Background Concentrations
BE	Belgium
BSA	**Bovine serum albumin**
CAT	catalase
CB	chlorinated biphenyl
Cd	Cadmium
CEMP	OSPAR Coordinated Environmental Monitoring Programme
Cr	Chromium
CRM	Certified Reference Material
Cu	Copper
CYP 1A	Cytochrome P4501A
DBT	dibutyltin
DDT	dichlorodiphenyltrichloroethane
DE	Germany
DK	Denmark
DPhT	diphenyltin
DTNB	5,5-dithio-*bis*(2-nitrobenzoic acid)
EACs	Environmental Assessment Criteria.
EDTA	ethylenediaminetetraacetic acid
EI	Electron impact ionization (in mass spectrometry)
ELISA	Enzyme-Linked Immunosorbent Assay
EPN	ethyl O-(p-nitrophenyl) phenylphosphonothionate
EROD	7-ethoxyresorufin *O*-deethylase
ES	Spain
ESE	Enhanced Solvent Extraction
EU	European Union
FCA	foci of cellular alterations
FPrL	Average Prostate Length of Females
FR	France
GC	gas chromatography
GC-FID	Gas chromatography with flame ionisation detection
GC-AAS,	Gas chromatography with detection by atomic absorption spectrophotomeeyr
GC-AED	atomic emission detector
GC-FPD,	flame photometric detector
GC-MS	gas chromatography / mass spectrometry
GC-PFPD	pulsed- flame photometric detector
GC-ECD	Gas Chromatography electron capture detector
GLP	Good laboratory practice
GPC	gel permeation chromatography
GPX	glutathione peroxidase
GSI	gonad somatic index
GST	Glutathione S-Transferase
H3BO3	boric acid
HCB	Hexachlorobenzene
α-HCH	α-hexachlorocyclohexane

γ-HCH	γ- hexachlorocyclohexane Lindane
HCH	hexachlorocyclohexane isomers
HF	hydrofluoric acid
Hg	Mercury
HMDS	hexamethyldisiloxane
HNO_3	Nitric acid
HPLC	high performance liquid chromatography)
HPLC-UV	High Performance Liquid Chromatography Ultra Violet Detection
ICES	International Council for the Exploration of the Sea
ICP-MS	inductively-coupled plasma mass spectrometer
IgG	***Immunoglobulin G***
IMS	industrial methylated spirit
INAA	instrumental neutron activation analysis
IOC	International Oceanographic Commission
IR	Ireland
IRM	internal reference material
IS	Iceland
ISI	Intersex Index
ITD	ion trap detection
JAMP	Joint Assessment and Monitoring Programme
Li	Lithium
LRM	laboratory reference material
LSI	liver somatic index
MBT	monobutyltin
MDGC	multidimensional gas Chromatography
MFO	mixed-function oxygenase
Mg	magnesium
MMCs	Melanomacrophage centres
MPhT	monophenyltin
MT	metallothionein
MW	molecular weight
NADPH	Nicotinadenine Dinucleotide Phosphate
NCI	Negative chemical ionization (in mass spectrometry)
NGOs	Non-Governmental Organisation
Ni	Nickel
NIST	National Institute of Standards and Technology
NL	Netherlands
NO	Norway
NP/NPEs	nonylphenol/ethoxylates and related substances
NPDs	Σ naphthalene, phenanthrene/anthracene, dibenzothiophene and their C1-C3 alkyl-homologues.
OCP	organochlorine compound
OSPAR	Convention for the Protection of the Marine Environment of the North-east Atlantic
OSPAR CPs,	Contracting Parties
p,p´-DDD (TDE),	Metabolite of DDT
p,p´-DDE,	dichlorodiphenyldichloroethylene (principle metabolite of DDT)
p,p´-DDT,	dichlorodiphenyltrichloroethane
PACS	Polycyclic Aromatic Hydrocarbons Reference Material
PAH	Polyaromatic hydrocarbon
Pb	Lead
PBG	porphobilinogen
PCBs	polychlorinated biphenyls
PCDDs	polychlorinated dibenzodioxins

PCDFs	polychlorinated dibenzofurans
PCI	Penis Classification Index
PCP	pentachlorophenol
PFOS	perfluorooctanyl sulphonic acid and salts
POPs	Persistent organic pollutants
PTFE	polytetrafluorethene
QC – QA	Quality Control and Quality Assurance
QIS	quantification internal standards
QUASIMEME	Quality Assurance of Information for Marine Environment Monitoring in Europe
RIA	Radio Immuno Assay
RIS	recovery internal standards ()
RNA	Ribonuecleic acid
RPLI	Relative Penis Length Index
RPSI	Relative Penis Size Index
SCCP	short chained chlorinated paraffins
SCX	silica-based ion-exchange
SE	Sweden
SFE	Supercritical fluid extraction
Sn	Tin
SOD	superoxide dismutase
SOP	standard operating procedures
TBA	tetrabutylammonium salts
TBBP-A	tetrabromobisphenol A
TBT	tributyltin
THC	Total hydrocarbon content
TIMES	ICES TIMES series publication
TOC	Total Organic Carbon
TPhT	triphenyltin
UK	United Kingdom
UNESCO	UN Educational Scientific and Cultural Organisation
VDSI	Vas Deferens Sequence Index
WGMS	Marine Sediments in Relation to Pollution
WKIMON	Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open Sea areas
XRF	X-ray fluorescence analysis
Zn	Zinc

Table 1. Summary of OSPAR Monitoring Guidelines and associated Technical Annexes together with the revised numbering system adopted following publication of the JAMP Technical Annexes as separate documents from the Guideline documents so as to facilitate updating of the technical annexes.

GUIDELINES	NO OF TECHNICAL ANNEXES	DETAILS	REVISED NUMBER
JAMP Guidelines for General Biological Effects Monitoring Ref No: 1997-7	10	Technical Annex 1: Whole sediment bioassays	
		Technical Annex 2: Sediment pore-water bioassays	
		Technical Annex 3: Sediment sea water elutriates	
		Technical Annex 4: Water bioassays	
		Technical Annex 5: CYP1A9	
		Technical Annex 6: Lysosomal stability	
		Technical Annex 7: Liver neoplasia/hyperplasia	
		Technical Annex 8: Liver nodules	
		Technical Annex 9: Externally visible fish diseases	Updating (DE & UK)
		Technical Annex 10: Reproductive success in fish	Updating (DK)
JAMP Guidelines for Monitoring Contaminants in Biota Ref. No: 1999-2	3	Technical Annex 1: Organic contaminants	
		Technical Annex 2: Metals	
		Technical Annex 3: PAHs in biological materials	
JAMP Guidelines for Monitoring Contaminants in Sediments Ref. No: 2002-16	6	Technical Annex 1: Statistical aspects of sediment monitoring	
		Technical Annex 2: Determination of chlorobiphenyls in sediments - analytical method	
		Technical Annex 3: Determination of PAHs in sediments	
		Technical Annex 4: Determination of mono-, di- and tributyltin in sediments - Analytical methods	
		Technical Annex 5: Normalisation of contaminant concentrations in sediments	
		Technical Annex 6: Determination of metals in sediments	
JAMP Guidelines for Contaminant-specific Biological Effects Monitoring Ref. No: 2003-10	3	Technical Annex 1: Metal-specific biological effects monitoring	Updating (N)
		Technical Annex 2: PAH-specific biological effects monitoring	
		Technical Annex 3: TBT-specific biological effects monitoring	
OSPAR Guidelines for Monitoring the Environmental Impact of Offshore Oil and Gas Activities Ref. No: 2004-11E	3	Technical Annex 1: OSPAR guidelines and international standards of relevance to sediment and water column monitoring related to offshore activities	
		Technical Annex 2: Recommended procedures for sediment monitoring related to offshore activities	
		Technical Annex 3: Recommended procedures for water column monitoring related to offshore activitie	

Table 2. Jamp Technical Annexes produced separately from the production of Guideline Documents (post 2006) – Requires Updating in relation to information from SIME 07, WGBEC 07, MCWG 07 and WGMS 07

TECHNICAL ANNEX[1]	TITLE/SUBJECT	COMMENT	STATUS
	Integrated Assessment	To supplement the draft JAMP monitoring guidelines	On hold
	Assessing endocrine disruption in the marine environment		In prepn (Netherlands)
	PBDEs in biota	Under auspices MCWG	Draft available (UK)
	HBCD in biota	Under auspices MCWG	Draft Available (UK)
New 1	Combining data relating to different timescales	Concentrations of contaminants and biological effects data are indicative of exposures over various timescales. Similarly, the presence of effects cover different time scales. Guidance on this in relation to interpretaing data is required.	Proposed new Technical Annex
New 2			
New 3			
New 4			

[1]**Numbering system to follow-on from that adopted by OSPAR following separation of the Technical Annexes from the specific Guideline Dcouments and used in Table 1.**

Table 3. Current and Developing Assessment Criteria (To be reviewed and data added at SIME 07)

TITLE	DEFINITION/USE	COMMENT	REFERENCE
Background/Reference Concentrations (B/RCs)			
Background Concentrations (BCs)			
Background Assessment Concentrations (BACs)			
Ecotoxicological Assessment Criertia			
Environmental Assessment Criteria			
TBT-specific effects			

Note: Relevant figure to be inserted following SIME 07

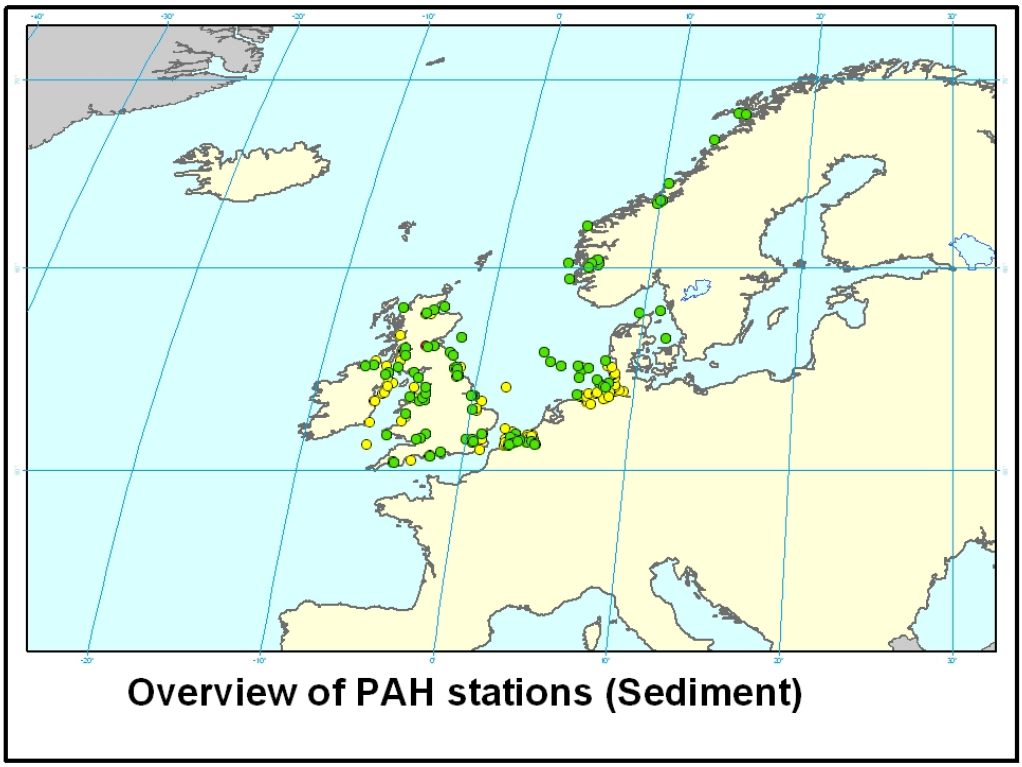

Figure 1. Example of output from the OSPAR assessment of monitoring undertaken by Contracting Parties under the CEMP

Annex 1 List of documents used to prepare the 'Guidelines on Integrated Monitoring'

JAMP Guidelines for Monitoring Contaminants in Sediments Ref. No: 2002-16

JAMP Guidelines for Monitoring Contaminants in Biota Ref. No: 1999-2

JAMP Guidelines for General Biological Effects Monitoring Ref No: 1997-7

JAMP Guidelines for Contaminant-specific Biological Effects Monitoring Ref. No: 2003-10

OSPAR Guidelines for Monitoring the Environmental Impact of Offshore Oil and Gas Activities Ref. No: 2004-11E

Draft ICES/OSPAR Guidelines for Integrated Chemical and Biological Effects Monitoring in Coastal and Offshore Areas (Annex 17 to the Report of the ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas (WKIMON) 10 – 13 January 2005)

Report of the Second ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-sea Areas (WKIMON II), Copenhagen, Denmark, 17 – 19 January 2006.

Annex 2 The OSPAR Hazardous Substances Startegy

1. Objective

1.1 In accordance with the general objective, the objective of the Commission with regard to hazardous substances is to prevent pollution of the maritime area by continuously reducing discharges, emissions and losses of hazardous substances with the ultimate aim of achieving concentrations in the marine environment near background values for naturally occurring substances and close to zero for man-made synthetic substances.

2. Guiding Principles

2.1 The strategy will use the following principles as a guide:

a) assessments made, and programmes and measures adopted, to achieve the objective and implement the strategy will be in accordance with the general obligations as set out in Article 2 of the OSPAR Convention and consequently will involve the application of:

i) the precautionary principle;

ii) the polluter pays principle;

iii) best available techniques and best environmental practice, including, where appropriate, clean technology;

b) in addition, the principle of substitution, i.e. the substitution of hazardous substances by less hazardous substances or preferably non-hazardous substances where such alternatives are available[4], is a means to reach this objective;

c) emissions, discharges and losses of new hazardous substances shall be avoided, except where the use of these substances is justified by the application of the principle of substitution;

d) in the work to achieve the objective, the scientific assessment of risks (in connection with the criteria stipulated at Appendix 2 of the 1992 OSPAR Convention and in connection with Annex IV of the 1992 OSPAR Convention) is a tool for setting priorities and developing action programmes.

3. Strategy

3.1 The Commission will develop programmes and measures to identify, prioritise, monitor and control (i.e., to prevent and/or reduce and/or eliminate) the emissions, discharges and losses of hazardous substances which reach, or could reach, the marine environment. To this end the Commission will:

a) complete and maintain a dynamic selection and prioritisation mechanism to select the hazardous substances to be given priority in its work;

Criteria to be used in this selection and prioritisation mechanism include that the substances or groups of substances:

i) due to their highly hazardous properties, are a general threat to the aquatic environment;

ii) show strong indications of risks for the marine environment;

iii) have been found widespread in one or more compartments of the maritime area, or may endanger human health via consumption of food from the marine environment;

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4 "Available" in the context of substitution must be understood in the same sense as in the definition of Best Available Techniques in the OSPAR Convention 1992 and should take into account the principles contained in the definition of Best Environmental Practice in the OSPAR Convention 1992 related to substitution of products.

iv) reach, or are likely to reach, the marine environment from a diversity of sources through various pathways;

The Commission will stimulate the further development of the criteria for hazardous substances namely toxicity, persistency and liability to bioaccumulate with respect to the marine environment and improve their operation as part of the work to implement this strategy. As working definitions, the Commission will use the criteria which it adopted in 20015, or any subsequent modification. The application of these criteria should both reflect the hazardous characteristics of substances or groups of substances and give priority to their actual or potential occurrence and effects in the maritime area;

b) carry forward the drawing up of programmes and measures in relation to the OSPAR List of Chemicals for Priority Action, as it is up-dated from time to time;

c) apply the selection mechanism to substances and groups of substances of concern including those substances and groups of substances set out in the OSPAR List of Substances of Possible Concern, as it stands from time to time, in order to review the OSPAR List of Chemicals for Priority Action and to apply the prioritisation mechanism to rank these substances in order of priority;

d) support the work of other relevant international bodies (e.g. UNEP, UN-ECE, OECD and IMO) and countries in taking the necessary measures to control persistent organic pollutants (POPs), heavy metals and other hazardous substances, on the grounds that these substances may enter the Convention Area and have otherwise been phased-out or are under action by OSPAR;

e) as soon as possible, develop or adopt, as part of the selection mechanism, a means of identifying substances which give reasonable grounds for concern that they are endocrine disruptors, and on this basis identify the substances on the OSPAR List of Substances of Possible Concern which give rise to such concerns. To this end, the Commission will:

i) develop and apply appropriate evaluation criteria (involving the use of internationally recognised testing procedures where these are available) to establish whether substances on these lists of potential endocrine disruptors list have the potential to cause adverse effects to organisms in the marine environment;

ii) collaborate with various international forums with a view to optimising international research effort on endocrine disruptors leading to the development of testing and assessment tools for identifying substances of concern and their occurrence and distribution and effect in the marine environment;

f) address, in developing programmes or measures in relation to any substance, all relevant aspects of that substance, including its toxicity and its ability to disrupt endocrine processes;

g) keep the selection mechanism, including the means of identifying endocrine disruptors, under review to ensure that it remains effective to identify all aspects of hazard and risk which should give rise to reasonable grounds of concern about substances taking account of developments in the International Forum on Chemical Safety and the UN-ECE Convention on Long-range Transboundary Air Pollution.

4. Timeframe

4.1 The Commission will implement this strategy progressively by making every endeavour to move towards the target of the cessation of discharges, emissions and losses of hazardous substances by the year 2020.

5 OSPAR Agreement 2001-1.

5. Implementation

5.1 This strategy will be implemented and the details developed in line with the Commission's commitment to an ecosystem approach and according to the periodic work programmes which will establish priorities, assign tasks, and set deadlines and targets. These commitments will concentrate on substances of the highest concern to the marine environment and make best use of resources. This is likely to involve developing stronger links with other international bodies.

5.2 Effective action is to be taken when there are reasonable grounds for concern that hazardous substances introduced into the marine environment, or which reach or could reach the marine environment, may bring about hazards to human health, harm living and marine ecosystems, damage amenities or interfere with other legitimate uses of the sea, even when there is no conclusive evidence of a causal relationship between the inputs and the effects.

5.3 With regard to hazardous substances identified by the Commission for action, such action should include:

a) identifying the sources of hazardous substances and their pathways to the marine environment, using, *inter alia*, information derived from monitoring, research, specific surveys and assessment activities;

b) establishing with the help of an appropriate combination of monitoring, modelling, risk characterisation and risk assessment techniques, whether these sources represent either a widespread problem or a problem restricted to regional or local environments within the maritime area;

and, as a result,

c) the identification of relevant measures to deal with the problem, including the adoption of measures to reduce discharges, emissions and losses of hazardous substances and taking into account the sources and pathways of hazardous substances and the substitution of hazardous substances with less hazardous (or, preferably, non-hazardous) substances, taking into account the sources and pathways of the hazardous substances.

5.4 There is limited experience with the scientific assessment of the risk of potential hazardous substances in the marine environment, particularly as regards the consequences of extremely large dilution, low degradation rates and long term exposure on marine organisms. The Commission therefore will address the following issues as a matter of urgency:

a) the development of the relevant scientific tools for assessing risks of potential hazardous substances in the marine environment. The Commission will cooperate with the EU in accelerating progress in improving such tools, drawing upon the relevant elements in the existing EU Technical Guidance in Support of Directive 93/67/EEC on Risk Assessment for New Notified Substances and Regulation EC 1488/94 on Risk Assessment for Existing Substances, and future expansions of that guidance;

b) the extent to which methodologies and results of a freshwater risk-assessment, or of any other relevant risk assessment, can be translated to and used for the assessment of the risk that a substance poses to the marine environment.

5.5 Measures should be selected taking into account:

a) the sustainability of the marine ecosystem;

b) the guiding principles;

c) an assessment of the advantages, disadvantages and effectiveness of proposed measures.

In order to support sustainable development and consumption, measures should also, to the greatest extent possible, encourage the principles of "green chemistry" as described in paragraph 5.8 below. When deciding upon the implementation of such measures the most cost-effective measures should have the highest priority. Risk reduction measures should be developed and/or applied in the light of the requirements laid down in the definitions of BAT and BEP in the OSPAR Convention. If in this process hazardous substances are to be substituted by other available6 substances, it has to be ensured that less hazardous, or preferably non-hazardous, substances are to be selected.

5.6 The Commission and Contracting Parties, individually or jointly, will endeavour to maintain and develop further a constructive dialogue with regard to hazardous substances with all parties concerned, including producers, manufacturers, user groups, authorities and environmental NGOs. This should ensure that all relevant information, such as reliable data on production volumes, use patterns, emission scenarios, exposure concentrations and on properties of substances, is available for the work of the Commission in connection with this strategy.

5.7 The Commission will invite industry to cooperate in fulfilling the objective of OSPAR with regard to hazardous substances.

5.8 Taking into account the increased environmental awareness, industry could help in achieving this OSPAR objective through:

a) the incorporation, as a strategy, of the objective in their development of clean production and clean products, and in this context the promotion of "green chemistry", including:

iii) the encouragement of the use and development of environmentally sound products and the development of less hazardous, or preferably non-hazardous, substances;

iv) the employment of usages and practices during the manufacture, use and ultimate disposal of chemicals (whether as intermediates, products or residues), including waste handling and waste management, that reduce, or preferably avoid, the use of hazardous substances and that avoid losses of hazardous substances to the environment;

v) the provision of alternatives to the use of hazardous substances in processes other than the manufacture of hazardous substances;

b) the provision of reliable data on production volumes, use patterns, emission scenarios, exposure concentrations and properties of substances.

The attitude of regulatory authorities can influence these approaches.

5.9 Pollution from diffuse sources becomes in comparison with point sources more and more important. Various (groups of) substances, products and pollutants from many different diffuse sources continue to pose a serious threat to the environment. Such sources are large in number, highly diverse and extend over a wide geographical area and the pollutants often follow a complex path through different environmental media / compartments before entering or reaching the marine environment. In some cases the sources are mobile, and even create transboundary effects and may cause varying loadings over time. These problems will be taken into account in analysing the options for action with regard to hazardous substances.

6 "Available" in the context of substitution must be understood in the same sense as in the definition of Best Available Techniques in the OSPAR Convention 1992 and should take into account the principles contained in the definition of Best Environmental Practice in the OSPAR Convention 1992 related to substitution of products.

5.10 The management of dredged materials containing hazardous substances requires special consideration because of the existing occurrence of such substances in sediments and the problem of their removal. Such management is regulated by the OSPAR Guidelines on the Management of Dredged Materials (as revised from time to time), and any programmes or measures adopted under Annex II of the OSPAR Convention.

5.11 In order to achieve internationally harmonised approaches and to avoid duplication of work, on hazardous substances, the Commission will ensure that measures and information (e.g. principles and methodologies, specific targets and BAT/BEP work) which have already been agreed (inter alia by means of legally binding instruments, recommendations or by way of political commitments) or which are being negotiated by Contracting Parties in other forums7 are considered by the Commission, as appropriate, in the development of measures and initiatives to control hazardous substances within OSPAR. Contracting Parties shall bring these measures and this information to the attention of the Commission. When significant common ground has been identified in measures and initiatives proposed by OSPAR and those of other forums, the Commission will initiate appropriate discussions to determine what level of co-operation and liaison is necessary.

5.12 Contracting Parties which participate in other forums will, if appropriate, endeavour to ensure that programmes and measures on hazardous substances developed within these other forums are compatible with any relevant programmes and measures adopted by the Commission.

5.13 The implementation of this strategy should take due account of Article 24 on regionalisation and Annex IV on assessment of the quality of the marine environment of the OSPAR Convention 1992.

6. Overall Evaluation and Review of Progress

6.1 The Commission will review progress achieved through this strategy within the framework of the Joint Assessment and Monitoring Programme. In the light of such reviews, the periodic Ministerial Meetings of the Commission will consider whether any changes to the strategy are needed.

7 Other forums include the EU (e.g. through relevant EC Directives and Regulations, in particular, the IPPC Directive (96/61/EC) and the future the European Community Directive of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy (2000/60/EC), OECD, UN-ECE, UNEP (the Global Programme of Action for the Protection of the Marine Environment against Pollution from Land-Based Sources), the Helsinki and Barcelona Conventions, the international river organisations, the Arctic Council and the North Sea Conference

Annex 3 OSPAR List of Chemicals for Priority Action

Type	Group of substances / substances	CAS No	EINECS No	Identified at †: Lead country: Background document
				A: CHEMICALS WHERE A BACKGROUND DOCUMENT HAS BEEN OR IS BEING PREPARED8
Aromatic hydrocarbon				
Metallic compound	**cadmium**			OSPAR/MMC 1998: Spain: Published 2002 (ISBN: 0 946956 93 6)
Metal/organometallic compounds	**lead and organic lead compounds**			OSPAR/MMC 1998: Norway: Published 2002 (ISBN 1-904426-00-X)
	mercury and organic mercury compounds			OSPAR/MMC 1998: United Kingdom: Published 2000 (ISBN: 0 946956 54 5)
Organometallic compounds	organic tin compounds ♣			OSPAR/MMC 1998: The Netherlands: Published 2000 (ISBN: 0 946956 56 1) addressing TBT and TPT
Organic ester	**neodecanoic acid, ethenyl ester**	51000-52-3	256-905-8	**OSPAR 2001: United Kingdom**
Organohalogens	**perfluorooctanyl sulphonic acid and its salts (PFOS**) ♦	1763-23-1	217-179-8	OSPAR 2003: United Kingdom
	tetrabromobisphenol A (TBBP-A)	79-94-7	201-236-9	OSPAR 2000: United Kingdom: Published 2004 (ISBN: 1-904426-39-5)
	1,2,3-trichlorobenzene	87-61-6	201-757-1	**OSPAR 2000: Belgium & Luxembourg: Published 2003 (ISBN 1-904426-10-7)**
	1,2,4-trichlorobenzene	120-82-1	204-428-0	**OSPAR 2000: Belgium & Luxembourg: Published 2003 (ISBN 1-904426-10-7)**
	1,3,5-trichlorobenzene	108-70-3	203-608-6	**OSPAR 2000: Belgium & Luxembourg: Published 2003 (ISBN 1-904426-10-7)**

8 OSPAR 2005 agreed to remove 4-*tert*-butyltoluene (CAS no 98-51-1), hexachlorocyclopentadiene (HCCP) (CAS No 77-47-4) and triphenylphosphine (CAS No 603-35-0) from the list since they are not PBT substances for the reasons set out in the updated Agreement 2004-13 available on the OSPAR website (see OSPAR 2005 Summary Record, OSPAR 05/21/1 paragraph 7.5).

Type	Group of substances / substances	CAS No	EINECS No	Identified at †: Lead country: Background document
	brominated flame retardants			OSPAR/MMC 1998: Sweden: Published in 2001 (ISBN: 0 946956 70 7) addressing: polybrominated diphenylethers; polybrominated biphenyls; hexabromocyclododecane
	polychlorinated biphenyls (PCBs) ♠			OSPAR/MMC 1998: Germany & Belgium: Published 2001 (ISBN: 0 946956 78 2)
	polychlorinated dibenzodioxins (PCDDs) **polychlorinated dibenzofurans (PCDFs)**			OSPAR/MMC 1998: Denmark & Belgium: Published 2002 (ISBN: 0 946956 92 8)
	short chained chlorinated paraffins (SCCP)			OSPAR/MMC 1998: Sweden: Published 2001 (ISBN: 0 946956 77 4)
Organic nitrogen compound	**4-(dimethylbutylamino)diphenylamin (6PPD)**	793-24-8	212-344-0	OSPAR 2002: Germany
Organophosphate				
Organosilicane	**hexamethyldisiloxane (HMDS)**	107-46-0	203-492-7	OSPAR 2000: France: Published 2004 (ISBN: 1-904426-41-7)
Pesticides/Biocides/ Organohalogens	**dicofol**	115-32-2	204-082-0	OSPAR 2000: Finland: Published 2002 (ISBN: 0 946956 97 9)
	endosulphan	115-29-7	204-079-4	OSPAR 2000: Germany: Published 2002 (ISBN: 0 946956 98 7)
	hexachlorocyclohexane isomers (HCH)			OSPAR/MMC 1998: Germany: Published 2002 (ISBN: 0 946956 94 4)
	methoxychlor	72-43-5	200-779-9	OSPAR 2000: Finland: Published 2002 (ISBN: 0 946956 99 5)
	pentachlorophenol (PCP)			OSPAR/MMC 1998: Finland: Published 2001 (ISBN: 0 946956 74 X)
	trifluralin	1582-09-8	216-428-8	OSPAR 2002: Germany: Published 2004 (ISBN: 1-904426-37-9)
Pharmaceutical	**clotrimazole**	23593-75-1	245-764-8	OSPAR 2002: France: Published 2004 (ISBN: 1-904426-38-7)

Type	Group of substances / substances	CAS No	EINECS No	Identified at †: Lead country: Background document
Phenols	**2,4,6-tri-tert-butylphenol**	732-26-3	211-989-5	OSPAR 2000: United Kingdom: Published 2003 (ISBN 1-904426-14-X)
	nonylphenol/ethoxylates (NP/NPEs) and related substances			OSPAR/MMC 1998: Sweden: Published 2001 (ISBN: 0 946956 79 0)
	octylphenol	140-66-9	205-426-2	OSPAR 2000: United Kingdom: Published 2003 (ISBN 1-904426-15-8)
Phthalate esters	**certain phthalates: dibutylphthalate, diethylhexylphthalate**			**OSPAR/MMC 1998: Denmark & France**
Polycyclic aromatic compounds	**polyaromatic hydrocarbons (PAHs)** §			OSPAR/MMC 1998: Norway: Published 2001 (ISBN: 0 946956 73 X)
Synthetic musk	**musk xylene**			OSPAR/MMC 1998: Switzerland: Published 2000 (ISBN: 0 946956 55 3) addressing musk xylene, musk ketone, moskene and musk tibetene. Revised Background Document: Published 2004 (ISBN: 1-904426-36-0)

Type	Group of substances / substances	CAS No	EINECS No	Identified at †: Lead country: Background document
				A: CHEMICALS WHERE A BACKGROUND DOCUMENT HAS BEEN OR IS BEING PREPARED9
Aromatic hydrocarbon				
Metallic compound	**cadmium**			OSPAR/MMC 1998: Spain: Published 2002 (ISBN: 0 946956 93 6)
Metal/organometallic compounds	**lead and organic lead compounds**			OSPAR/MMC 1998: Norway: Published 2002 (ISBN 1-904426-00-X)
	mercury and organic mercury compounds			OSPAR/MMC 1998: United Kingdom: Published 2000 (ISBN: 0 946956 54 5)

9 OSPAR 2005 agreed to remove 4-*tert*-butyltoluene (CAS no 98-51-1), hexachlorocyclopentadiene (HCCP) (CAS No 77-47-4) and triphenylphosphine (CAS No 603-35-0) from the list since they are not PBT substances for the reasons set out in the updated Agreement 2004-13 available on the OSPAR website (see OSPAR 2005 Summary Record, OSPAR 05/21/1 paragraph 7.5).

Type	Group of substances / substances	CAS No	EINECS No	Identified at †: Lead country: Background document
Organometallic compounds	organic tin compounds ♣			OSPAR/MMC 1998: The Netherlands: Published 2000 (ISBN: 0 946956 56 1) addressing TBT and TPT
Organic ester	**neodecanoic acid, ethenyl ester**	51000-52-3	256-905-8	**OSPAR 2001: United Kingdom**
Organohalogens	**perfluorooctanyl sulphonic acid and its salts (PFOS) ♦**	1763-23-1	217-179-8	OSPAR 2003: United Kingdom
	tetrabromobisphenol A (TBBP-A)	79-94-7	201-236-9	OSPAR 2000: United Kingdom: Published 2004 (ISBN: 1-904426-39-5)
	1,2,3-trichlorobenzene	87-61-6	201-757-1	**OSPAR 2000: Belgium & Luxembourg: Published 2003 (ISBN 1-904426-10-7)**
	1,2,4-trichlorobenzene	120-82-1	204-428-0	**OSPAR 2000: Belgium & Luxembourg: Published 2003 (ISBN 1-904426-10-7)**
	1,3,5-trichlorobenzene	108-70-3	203-608-6	**OSPAR 2000: Belgium & Luxembourg: Published 2003 (ISBN 1-904426-10-7)**
	brominated flame retardants			OSPAR/MMC 1998: Sweden: Published in 2001 (ISBN: 0 946956 70 7) addressing: polybrominated diphenylethers; polybrominated biphenyls; hexabromocyclododecane
	polychlorinated biphenyls (PCBs) ♠			OSPAR/MMC 1998: Germany & Belgium: Published 2001 (ISBN: 0 946956 78 2)
	polychlorinated dibenzodioxins (PCDDs) **polychlorinated dibenzofurans (PCDFs)**			OSPAR/MMC 1998: Denmark & Belgium: Published 2002 (ISBN: 0 946956 92 8)
	short chained chlorinated paraffins (SCCP)			OSPAR/MMC 1998: Sweden: Published 2001 (ISBN: 0 946956 77 4)
Organic nitrogen compound	**4-(dimethylbutylamino)diphenylamin (6PPD)**	793-24-8	212-344-0	OSPAR 2002: Germany
Organophosphate				
Organosilicane	**hexamethyldisiloxane (HMDS)**	107-46-0	203-492-7	OSPAR 2000: France: Published 2004 (ISBN: 1-904426-41-7)

Type	Group of substances / substances	CAS No	EINECS No	Identified at †: Lead country: Background document
Pesticides/Biocides/ Organohalogens	**dicofol**	115-32-2	204-082-0	OSPAR 2000: Finland: Published 2002 (ISBN: 0 946956 97 9)
	endosulphan	115-29-7	204-079-4	OSPAR 2000: Germany: Published 2002 (ISBN: 0 946956 98 7)
	hexachlorocyclohexane isomers (HCH)			OSPAR/MMC 1998: Germany: Published 2002 (ISBN: 0 946956 94 4)
	methoxychlor	72-43-5	200-779-9	OSPAR 2000: Finland: Published 2002 (ISBN: 0 946956 99 5)
	pentachlorophenol (PCP)			OSPAR/MMC 1998: Finland: Published 2001 (ISBN: 0 946956 74 X)
	trifluralin	1582-09-8	216-428-8	OSPAR 2002: Germany: Published 2004 (ISBN: 1-904426-37-9)
Pharmaceutical	**clotrimazole**	23593-75-1	245-764-8	OSPAR 2002: France: Published 2004 (ISBN: 1-904426-38-7)
Phenols	**2,4,6-tri-tert-butylphenol**	732-26-3	211-989-5	OSPAR 2000: United Kingdom: Published 2003 (ISBN 1-904426-14-X)
	nonylphenol/ethoxylates (NP/NPEs) and related substances			OSPAR/MMC 1998: Sweden: Published 2001 (ISBN: 0 946956 79 0)
	octylphenol	140-66-9	205-426-2	OSPAR 2000: United Kingdom: Published 2003 (ISBN 1-904426-15-8)
Phthalate esters	**certain phthalates: dibutylphthalate, diethylhexylphthalate**			**OSPAR/MMC 1998: Denmark & France**
Polycyclic aromatic compounds	**polyaromatic hydrocarbons (PAHs)** §			OSPAR/MMC 1998: Norway: Published 2001 (ISBN: 0 946956 73 X)
Synthetic musk	**musk xylene**			OSPAR/MMC 1998: Switzerland: Published 2000 (ISBN: 0 946956 55 3) addressing musk xylene, musk ketone, moskene and musk tibetene. Revised Background Document: Published 2004 (ISBN: 1-904426-36-0)

Type	Group of substances / substances	CAS No	EINECS No	Identified at †: Lead country: Background document

Type	Group of substances / substances	CAS No	EINECS No	Identified at †: Lead country: Background document
	B:CHEMICALS WHERE NO BACKGROUND DOCUMENT IS BEING PREPARED BECAUSE THEY ARE INTERMEDIATES IN CLOSED SYSTEMS ‡			
Aliphatic hydrocarbons	**1,5,9 cyclododecatriene**‡	4904-61-4	**225-533-8**	**OSPAR 2002: not applicable**
	cyclododecane‡	294-62-2	**206-033-9**	**OSPAR 2002: not applicable**
	*C: CHEMICALS WHERE NO BACKGROUND DOCUMENT IS BEING PREPARED BECAUSE THERE IS NO CURRENT PRODUCTION OR USE INTEREST**			
Organohalogens	**2-propenoic acid, (pentabromo)methyl ester**	59447-55-1	261-767-7	**OSPAR 2003: not applicable**
	2,4,6-bromophenyl 1-2(2,3-dibromo-2-methylpropyl) *	36065-30-2	252-859-8	**OSPAR 2001: not applicable**
	pentabromoethylbenzene*	85-22-3	**201-593-0**	**OSPAR 2001: not applicable**
	heptachloronorbornene*	28680-45-7 2440-02-0	249-153-7	**OSPAR 2001: not applicable**
	pentachloroanisole*	**1825-21-4**	-	**OSPAR 2001: not applicable**

Type	Group of substances / substances	CAS No	EINECS No	Identified at †: Lead country: Background document
Organohalogens (cont.)	**polychlorinated naphthalenes*, ††**			
	trichloronaphthalene*	1321-65-9	**215-321-3**	**OSPAR 2001: not applicable**
	tetrachloronaphthalene*	1335-88-2	**215-642-9**	**OSPAR 2001: not applicable**
	pentachloronaphthalene*	1321-64-8	**215-320-8**	**OSPAR 2002: not applicable**
	hexachloronaphthalene*	1335-87-1	**215-641-3**	**OSPAR 2001: not applicable**
	heptachloronaphthalene*	32241-08-0	250-969-0	**OSPAR 2001: not applicable**
	octachloronaphthalene*	2234-13-1	**218-778-7**	**OSPAR 2001: not applicable**
	naphthalene, chloro derivs. *	70776-03-3	**274-864-4**	**OSPAR 2002: not applicable**
Organic nitrogen compound	**3,3'-(ureylenedimethylene)bis(3,5,5-trimethylcyclohexyl) diisocyanate***	55525-54-7	259-695-6	**OSPAR 2001: not applicable**
Pesticides/Biocides	**ethyl O-(p-nitrophenyl) phenyl phosphonothionate (EPN)***	2104-64-5	218-276-8	**OSPAR 2001: not applicable**
	flucythrinate*	70124-77-5	274-322-7	**OSPAR 2001: not applicable**
	isodrin*	465-73-6	207-366-2	**OSPAR 2001: not applicable**
	tetrasul*	2227-13-6	218-761-4	**OSPAR 2001: not applicable**
Pharmaceutical	**diosgenin***	512-04-9	208-134-3	**OSPAR 2002: not applicable**

Endnotes

† The substances in this list were identified at the following OSPAR Commission meetings:

OSPAR/MMC 1998: Agreement reference number 1998-16 (Annex 2 to the OSPAR Strategy with regard to Hazardous Substances);

(Note: When identifying the substances or groups of substances, OSPAR/MMC 1998 has not allocated CAS and EINECS registration numbers. Background documents adopted by the OSPAR Commission for these substances or groups of substances may indicate which substances have been addressed so far by OSPAR)

OSPAR 2000: Agreement reference number 2000-10;

OSPAR 2001: Agreement reference number 2001-2;

OSPAR 2002: Agreement reference number 2002-18;

OSPAR 2003: Agreement reference number 2003-19.

‡ **The identification of these substances and the consequent action required is explained in § 7.6 of the OSPAR 2002 Summary Record. In brief, these substances have rankings in terms of persistency, liability to bioaccumulate and toxicity which are of equal concern as the other substances on this list. However, to the best of OSPAR's knowledge, on the basis of information from industry, OSPAR accepts that this substance is produced and used exclusively as an intermediate in closed systems in the production of other substances, under conditions where the safeguards applying are sufficient to avoid reasonable concerns that discharges, emissions or losses of the substance could reach the marine environment. Therefore, every five years, commencing in 2003, Contracting Parties and, where appropriate, observers representing the chemicals industries should report to OSPAR:**

a. whether they have found any evidence that these chemicals are being produced, used or discharged without being subjected to safeguards to avoid reasonable concerns that discharges, emissions or losses of the substances could reach the marine environment, and, if so, what that evidence is, and what action (if any) has been taken;

b. whether there have been any cases where applications have been made for approvals involving these chemicals, and, if so, what decision was taken.

* **The identification of these substances and the consequent action required is explained in § 4.13 of the OSPAR 2001 Summary Record. In brief, these substances have rankings in terms of persistency, liability to bioaccumulate and toxicity which are of equal concern as the other substances on this list. However, to the best of OSPAR's knowledge, there is no current production or use in the OSPAR states. Therefore, commencing in 2003 and every five years thereafter, or earlier, if information becomes available, Contracting Parties and, where appropriate, observers representing the chemicals industries should report to OSPAR:**

a. whether they have found any evidence that these chemicals are being produced, used or discharged, and, if so, what that evidence is, and what action (if any) has been taken;

b. whether there have been any cases where applications have been made for approvals involving these chemicals, and, if so, what decision was taken.

†† Polychlorinated naphthalenes should be treated as a group of substances (OSPAR 02/21/1, § 7.7).

♦ PFOS is the highly persistent and toxic breakdown product of a number of perfluorooctanyl sulphonyl compounds. Several PFOS precursors have been selected on the OSPAR List of Substances of Possible Concern. The background document will identify these precursors and, if necessary, appropriate control measures will be proposed. CAS and EINECS numbers refer only to the acid form of PFOS.

§ The following substances belonging to the group of polyaromatic hydrocarbons have been deselected from the OSPAR List of Substances of Possible Concern on the grounds that they do not meet the cut-off values for persistence in the Selection Criteria used in the Initial Selection Procedure adopted by OSPAR 2001 (*Reference Number: 2001-1*) and are therefore not considered to be a priority for action by OSPAR: naphthalene, 2-methyl- (CAS No. 91576); 1-phenanthrenecarboxylic acid, 1,2,3,4,4a,4b,5,6,10,10a-decahydro-1,4a-dimethyl-7-(1-methylethyl)-, methyl ester, [1R-(1.alpha.,4a.beta.,4b.alpha.,10a.alpha.)]- (CAS No. 127253); 1-phenanthrenemethanol, 1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,4a-dimethyl-7-(1-methylethyl)- (CAS No. 127366); 7H-dibenzo[c,g]carbazole (CAS No. 194592); 13H-dibenzo[a,i]carbazole (CAS No. 239645); 1H-3a,7-methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3alpha,3abeta,7beta,8aalpha)]- (CAS No. 469614); 1-phenanthrenemethanol, 1,2,3,4,4a,4b,5,6,10,10a-decahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1R-(1.alpha.,4a.beta.,4b.alpha.,10a.alpha.)]- (CAS No. 666842); cedrene- (CAS No. 11028425); 1-phenanthrenemethanol, tetradecahydro-1,4a-dimethyl-7-(1-methylethyl)- (CAS No. 13393936); 1-phenanthrenecarboxylic acid, tetradecahydro-1,4a-dimethyl-7-(1-methylethyl)-, methyl ester, [1R-(1alpha,4abeta,4balpha (CAS No. 19941287).

♣ The following substance belonging to the group of organic tin compounds has been deselected from the OSPAR List of Substances of Possible Concern on the grounds that it does not meet the cut-off value for persistence in the Selection Criteria used in the Initial Selection Procedure adopted by OSPAR 2001 (*Reference Number: 2001-1*) and is therefore not considered to be a priority for action by OSPAR: stannane, tributyl(1-oxododecyl)oxy- (CAS No. 3090366).

♠ The following substance belonging to the group of polychlorinated biphenyls has been deselected from the OSPAR List of Substances of Possible Concern on the grounds that it does not meet the cut-off value for persistence in the Selection Criteria used in the Initial Selection Procedure adopted by OSPAR 2001 (*Reference Number: 2001-1*) and is therefore not considered to be a priority for action by OSPAR: 1,1'-biphenyl, 4,4'-dichloro- (CAS No. 2050682).

Annex 4 Background Documents Currently under development for Biological Effects Techniques (as at WKIMON III, January 2007)

Biological effect technique	Type	Lead country	ICES TIMES Series report10	Status
Currently included in the CEMP				
Metallothionein	Metal-specific	Mr Norman Green (Norway)	26	Received 5/12 Presented to WKIMON III
ALA-D	Metal-specific	Mr Norman Green (Norway)	34	Received 5/12 Presented to WKIMON III
Oxidative stress11	Metal-specific			
Cytochrome P4501A	PAH-specific	Dr Kris Cooreman (Belgium)12	23	Paper presented at WKIMON III to be developed

10 ICES Techniques in Marine Environmental Sciences Reports (www.ices.dk/products/techniques.asp)

11 WKIMON II (January 2006) recommended that this technique should not be developed and as such no Background Document should be prepsrec

12.5 These techniques to be reviewed as a group

DNA adducts	PAH-specific	Dr Kris Cooreman (Belgium)[4]	25	Paper presented at WKIMON III to be developed
PAH metabolites	PAH-specific	Dr Kris Cooreman (Belgium)[4]	39	Paper presented at WKIMON III to be developed
Whole sediment bioassays	General	Mr Jose Fumega (Spain) assisted by UK		Paper presented to WKIMON III
Sediment pore-water bioassays	General	Mr Jose Fumega (Spain) assisted by UK		Paper presented to WKIMON III
Sediment sea water elutriates	General	Mr Jose Fumega (Spain) assisted by UK		Paper presented to WKIMON III
Water bioassays	General	Mr Victor Langenberg (The Netherlands)		Paper presented at WKIMON III
Lysosomal stability	General	?	36	Paper presented by Mike Moore at WKIMON III
Liver pathology including neoplasia/hyperplasia	General	Mr Andrew Franklin (UK)[5]	38	Received 6/12 Presented to WKIMON III
Liver nodules	General	Mr Andrew Franklin (UK)[5]		Received 6/12 Presented to WKIMON III

Externally visible fish diseases	General	Mr Andrew Franklin (UK)[5]		Received 6/12 Presented to WKIMON III
Reproductive success in fish	General	Jakob Strand (Denmark)		Document on viviparous blenny received from Denmark 5/12
To be considered for inclusion in the CEMP				
Vitellogenin	Endocrine disruption	Mr Andrew Franklin (UK)		Paper from UK prented at WKIMON III
Scope for growth[13]	General	?		
Cellular energy allocation[14]	General	?		

13 WKIMON II (January 2006) recommended that this method should be further developed and as such a Background Document should be developed

14 WKIMON II (January 2006) recommended that this method should be further developed and as such a Background Document should be devloped

Annex 5 Priority chemicals where a Background Document has been, or is being, prepared

Substance / group of substances (identified for priority action / lead country identified)	**Lead country**	**Background Document completed**	**Examined by SPDS/HSC or future meetings**	**Reference**
brominated flame retardants (OSPAR/MMC 1998 / idem)	Sweden	OSPAR 2001	review in 2008/2009 †	publication on OSPAR web site
cadmium **(OSPAR/MMC 1998 / HSC 2001)**	Spain	OSPAR 2002	review in 2008/2009 †	publication on OSPAR web site
clotrimazole (23593-75-1) (OSPAR 2002 / idem)	France	OSPAR 2004	SPDS 2003, HSC 2004	publication on OSPAR web site
dicofol (115-32-2) (OSPAR 2000 / HHSC 2000)	Finland	OSPAR 2002	review in 2006/2007 †	publication on OSPAR web site
endosulphan (115-29-7) (OSPAR 2000 / idem)	Germany	OSPAR 2002	review in 2007/2008 †	publication on OSPAR web site
HCCP (77-47-4; 1,2,3,4,5,5-hexachloro-1,3-cyclopentadiene) (OSPAR 2000 / idem)	Netherlands	OSPAR 2004		publication on OSPAR web site
HCH (hexachlorocyclohexane isomers) (OSPAR/MMC 1998 / OSPAR 2000)	Germany	OSPAR 2002	review in 2007/2008 †	publication on the OSPAR web site
HMDS (107-46-0; hexamethyldisiloxane) (OSPAR 2000 / idem)	France	OSPAR 2004		publication on OSPAR web site
lead and organic lead compounds (OSPAR/MMC 1998 / OSPAR 1999)	Norway	OSPAR 2002	review in 2007/2008 †	publication on the OSPAR web site

Substance / group of substances (identified for priority action / lead country identified)	**Lead country**	**Background Document completed**	**Examined by SPDS/HSC or future meetings**	**Reference**
mercury and organic mercury compounds (OSPAR/MMC 1998 / idem)	United Kingdom	OSPAR 2000	review in 2008/2009 †	publication on the OSPAR web site
methoxychlor (72-43-5) (OSPAR 2000 / HSC 2001)	Finland	OSPAR 2002	review in 2007/2008 †	publication on the OSPAR web site
musk xylene (OSPAR/MMC 1998 / idem)	Switzerland	OSPAR 2000, revised OSPAR 2004	†	Current publication on the OSPAR web site
neodecanoic acid, ethenyl ester (51000-52-3) (OSPAR 2001 / ---)	UK		Progress sheet expected for SPDS 2005	
NP/NPEs (nonylphenol/ethoxylates) and related substances (OSPAR/MMC 1998 / idem)	Sweden	OSPAR 2001	review in 2008/2009 †	publication on the OSPAR web site
octylphenol (140-66-9) (OSPAR 2000 / idem)	United Kingdom	OSPAR 2003	†	**12.1.1.1.1** publicati on on the OSPAR web site
organic tin compounds (OSPAR/MMC 1998 / idem)	The Netherlands	OSPAR 2000	review in 2008/2009 †	publication on the OSPAR web site
PAHs (polyaromatic hydrocarbons) (OSPAR/MMC 1998 / idem)	Norway	OSPAR 2001	review in 2007/2008 †	publication on the OSPAR web site
PCBs (polychlorinated biphenyls) (OSPAR/MMC 1998 / idem)	Germany & Belgium	**OSPAR 2001**	review in 2007/2008 †	publication on the OSPAR web site

Substance / group of substances (identified for priority action / lead country identified)	**Lead country**	**Background Document completed**	**Examined by SPDS/HSC or future meetings**	**Reference**
PCDDs & PCDFs (polychlorinated dibenzodioxins and polychlorinated dibenzofurans (OSPAR/MMC 1998 / idem)	Denmark & Belgium	**OSPAR 2002**	review in 2006/2007 †	publication on the OSPAR web site
PCP (pentachlorophenol) (OSPAR/MMC 1998 / PRAM 1999)	Finland	**OSPAR 2001**	review in 2009/2010 †	publication on the OSPAR web site
PFOS (perfluorooctanyl sulphonic acids and its salts) (1763-23-1)	**UK**	target OSPAR 2005	**SPDS 2004, HSC 2005**	
certain phthalates – dibutylphthalate and diethylhexylphthalate (OSPAR/MMC 1998 / DIFF 1998)	**Denmark & France**	**target OSPAR 2005**	**written procedure, HSC 2005**	
6PPD (793-24-8; 4-(dimethylbutylamino)diphenylamin) (OSPAR 2002 / ---)	**Germany**	**target OSPAR 2005**	**SDPS 2004, HSC 2005**	
SCCPs (short chained chlorinated paraffins) (OSPAR/MMC 1998 / idem)	Sweden	OSPAR 2001	review in 2008/2009 †	publication on the OSPAR web site
TBBP-A (79-94-7; tetrabromobisphenol A) (OSPAR 2000 / idem)	United Kingdom	OSPAR 2004	†	publication on the OSPAR web site
4-tert-butyltoluene (98-51-1) (OSPAR 2000 / OSPAR 2001)	Germany	OSPAR 2003	†	publication on the OSPAR web site
trichlorobenzene (87-61-6; 1,2,3-trichloro benzene) (OSPAR 2000 / idem)	Belgium (Flemish Region of Belgium) &	OSPAR 2003	† Belgium/Luxembourg to advise HSC 2005 of review date	publication on the OSPAR web site
1,2,4-trichlorobenzene (120-82-1) (OSPAR 2000 / idem)				

Substance / group of substances (identified for priority action / lead country identified)	**Lead country**	**Background Document completed**	**Examined by SPDS/HSC or future meetings**	**Reference**
1,3,5-trichlorobenzene (108-70-3) (OSPAR 2000 / idem)	Luxembourg			
trifluralin (1582-09-8) (OSPAR 2002 / idem)	Germany	OSPAR 2004	†	publication on the OSPAR web site
triphenyl phosphine (603-35-0) (OSPAR 2001 / idem)	Germany	OSPAR 2003	review in 2008/2009 †	publication on the OSPAR web site
2,4,6-tri-tert-butylphenol (732-26-3) (OSPAR 2000 / HSC 2000)	UK	OSPAR 2003	†	publication on the OSPAR web site

† Implementation of actions recommended in Background Documents published by OSPAR are being kept under review in the relevant groups, this may require activity by HSC.

Annex 6 OSPAR Procedure for Updating documentation covering JAMP Guidelines, Technical Annexes and Assessment Criteria

To be written once process agreed

Annex 19: WKIMON IV terms of reference for the next meeting

The Fourth ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-sea Areas [WKIMON III] (Co-chairs: one designated by OSPAR and one by ICES) will meet in Copenhagen in January 2008 to:

a) Assess whether the recommendations made at WKIMON III have been adequately addressed. If not, to make proposals to achieve this;

b) To undertake further development of assessment criteria both for specific biological effects methods and of the integrated approach to monitoring

c) To review the organisation and scope of an international pilot study of integrated biological effects and chemical monitoring and its application to both specific CEMP issues and to the general health assessment of a region.

d) To review the merged Guidelines for the Integrated Monitoring and Assessment of Contaminants and their effects, and to develop the necessary Technical Annexes.

WKIMON IV will report to SIME by 1st Feb 2008 for onward transmission to OSPAR (ASMO) and ICES (ACME & MHC).

Supporting Information

PRIORITY:	The current activities of this group are directed towards developing an integrated approach to monitoring and assessment of both chemical and effects for application within OSPAR monitoring programmes. Consequently these activities are considered to have a very high priority.
SCIENTIFIC JUSTIFICATION AND RELATION TO ACTION PLAN:	Action Plan No: 1. Term of Reference a) In order to maintain the momentum generated by the WKIMON workshop approach. No other forum within the ICES/OSPAR system has the necessary expertise. Term of Reference b) Required to underpin the application of the integrated approach to monitoring. Term of Reference c) Plans were initiated at WKIMON III to take advantage of a Norwegian initiative in the area of North Sea Health. Various planning meetings will have occurred during 2007 (ie since WKIMON III) and a wider review of the plans and progress is required to ensure that they adequately take account of the interests of OSPAR in developing integrated monitoring for Regional assessments. Term of Reference d) It is anticipated that the merged Guidelines will be developped further by SIME 2007, and that the requirements for Technical Annexes will then be clarified. It will probably be necessary to undertake intersessional drafting work to provide texts of Technical Annexes for discussion and elaboration at WKIMON IV.
RESOURCE REQUIREMENTS:	The research programmes which provide the main input to this group are already underway, and resources are already committed. The additional resource required to undertake additional activities in the framework of this group is negligible.
PARTICIPANTS:	The Group is normally attended by some 17 – 22 members.
SECRETARIAT FACILITIES:	None.
FINANCIAL:	No financial implications.
LINKAGES TO ADVISORY COMMITTEES:	The reports of the WKIMON group go to ACME.
LINKAGES TO OTHER COMMITTEES OR GROUPS:	The reports of the WKIMON group go to MHC.
LINKAGES TO OTHER ORGANIZATIONS:	The work of this group is undertaken directly for OSPAR, reporting to SIME .
SECRETARIAT MARGINAL COST SHARE:	

Annex 20: WKIMON 3 Recommendations

To OSPAR: That the "Position Paper" on the status of biological effects methods should be drafted intersessionally. JohnThain (UK) and Ketil Hylland (NO) have indicated their willingness to undertake this task.

To OSPAR: That CPs be encouraged to submit relevant biological effects field data to the ICES Data Centre to allow OSPAR MON to assess these data in preparation for OSPAR QSR2010.

To OSPAR: At WKIMON III, data were made available for a range of biological effects methods. The data were assessed to establish provisional background responses and assessment criteria. WKIMON recommends that OSPAR adopt the assessment criteria as described in Annexes 14, 15 and 16, and as included in Annex 3, and as summarised below:

- For VTG, a background assessment concentration of 2ug / l was derived for flounder and cod. It is recommended that this is accepted as a provisional value for "all species" until more data are available.
- For reproduction in eelpout, three assessment classes are proposed that consider the frequencies of malformed larvae, late dead larvae and larval growth. The three classes cover background responses, a class where effects of contaminants cannot be excluded, and a third class where significant effect levels compared to the background responses are evident.
- Background concentrations for EROD are 80, 40 and 10 pmol/mg protein for cod, dab and flounder respectively, for fish collected out of the spawning season (months 8-11). In order to use data from other times of the year, there is a need to extend the current model to take account of water temperature, spawning seasons and other factors. WKIMON recommends that an ad hoc OSPAR / ICES study group is formed to take this work forward intersessionally.
- For bile metabolites of PAHs, a provisional background response concentration of 220 and 0.95 1-OH pyrene (ug/ml; 341/383 nm fluorescence) for dab and cod respectively was derived. It is recommended that further work is conducted to validate these values and assessment criteria and Ketil Hylland (NO) and Dick Vethaak (NL) volunteered to take this forward intersessionally.
- Background response values for DNA adducts of PAHs are 7.86, 6.84 and 7.90 nmol adducts / mol DNA for dab, haddock and saithe respectively.
- Three assessment classes were derived for sediment and water bioassays; a background response, a warning level and a level of serious concern. For the sediment bioassays (*Corophium* sp. and *Arenicola* sp.) the background responses were 0-30% and 0-10% mortality respectively, the level of serious concern was 100% mortality and the warning level between these values. For the water bioassays (*Tisbe* sp., *Acartia* sp., sea urchin and bivalve larvae) the background responses were 10%, 10%, 10% and 20% mortality (or deformity as appropriate) respectively; the level of serious concern was 100% mortality, and the warning level between these values.
- Background responses and assessment criteria for lysosomal stability were derived for the cytochemical and Neutral Red Retention (NRR) methods. For all species, the three levels of response were; i) background, ii) stressed but compensating and iii) severely stressed probably exhibiting pathology. The values recommended for adoption are: i) > 20 mins ii) <20 - >10 mins and iii) < 10 mins for the cytochemical method. For the NRR method, the recommended values are i) >120 mins ii) <120 - >50 mins and iii) <50 mins.

To ICES: A fish disease index (FDI) has been developed by WGDPMO for the assessment of fish diseases (external and histopathological). WKIMON fully supported this approach and recommended that WGDPMO complete the outstanding work to allow for its full implementation. Once completed, it should be forwarded to OSPAR for consideration.

To OSPAR and ICES: That analogues of Background Assessment Concentrations for biological effects be developed intersessionally and collaboratively by members of WKIMON and ICES WGSAEM.

To OSPAR: WKIMON recommends that the assessment criteria developed at WKIMON III should be used on the trial basis in data assessments to obtain experience of their use and indications of where further work is required.

To OSPAR: That the set of updated and new Background Documents available at WKIMON III (and additional documents such as that in preparation on Scope for Growth) be forwarded to SIME for adoption. Any that are considered not yet ready for adoption should be forwarded to ICES WGBEC for further elaboration.

To OSPAR: That the draft merged Guidelines for the Integrated Monitoring and Assessment of Contaminants and their effects included as Annex 18 to this report should be further developed and presented to SIME. Colin Moffat (UK) indicated his willingness to undertake this work.

To OSPAR: That SIME should put in place a process for the drafting of such Technical Annexes as will be required for the merged Guidelines for the Integrated Monitoring and Assessment of Contaminants and their effects, and that this would be an appropriate task for WKIMON.

To OSPAR: WKIMON III recommends that OSPAR endorse a demonstration programme and international pilot study applying assessment criteria for biological effects measurements (NSHEALTH), provided that the interests of OSPAR listed in Section 10 of the WKIMON II report are satisfactorily accommodated in the project design. Ketil Hylland (NO) agreed to report back on progress through SIME and WKIMON on a regular basis.

To OSPAR and ICES: WKIMON III recommends that OSPAR (SIME and ASMO) and ICES review the progress being made through WKIMON and assess the need for a further workshop (WKIMON IV) be held in January 2008 with terms of reference to include the items in Annex 18 of the WKIMON II report.

Action List

NSHEALTH steering committee (Chair Ketil Hylland) prepare a proposal for a pilot study and demonstration programme and forward this for information and approval to SIME 2007.

Action: Ketil Hylland.

Information on progress with the demonstration programme to be circulated at regular intervals to WKIMON members.

Action: NSHEALTH steering group.

In order to make more complete use in data assessments of the EROD data held in the ICES databases, there is a need to extend the model used by WKIMON III to cover other times of the year, and to take account of water temperature, spawning cycles and other factors. It was recommended to take this forward intersessionally through an ad hoc OSPAR / ICES study group.

Action: Kris Coorman (BL) and Werner Wosniock (G) agreed contribute to this work

Validation of bile metabolite background values and assessment criteria is required and further data need to be collated for this purpose.

Action: Ketil Hylland (NO) and Dick Vethaak (NL) volunteered to take this forward intersessionally.