Marine Environmental Research
March 2017, Volume 124, Pages 2-10
http://dx.doi.org/10.1016/j.marenvres.2015.11.006
http://archimer.ifremer.fr/doc/00297/40782/

How can we quantify impacts of contaminants in marine ecosystems? The ICON project

Hylland Ketil [1,*], Burgeot Thierry [2], Martínez-Gómez Concepción [3], Lang Thomas [4], Robinson Craig D. [5], Svavarsson Jörundur [6], Thain John E. [7], Vethaak A. Dick [8,9], Gubbins Matthew J. [2]

[1] Department of Biosciences, University of Oslo, PO Box 1066, Blindern, 0316 Oslo, Norway
[2] IFREMER, Laboratory of Ecotoxicology, Rue de l'Ile d'Yeu, B.P. 21105, 44311 Nantes Cédex 03, France
[3] nstituto Español de Oceanografía (IEO), Oceanographic Centre of Murcia, Varadero 1, PO Box 22, 30740 San Pedro del Pinatar, Murcia, Spain
[4] Thünen Institute of Fisheries Ecology, Deichstr. 12, 27472 Cuxhaven, Germany
[5] Marine Scotland Science, Marine Laboratory, 375 Victoria Road, Aberdeen AB11 9DB, UK
[6] University of Iceland, Askja – Natural Science Building, Sturlugata 7, 101 Reykjavík, Iceland
[7] Cefas Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, UK
[8] Deltares, Marine and Coastal Systems, P.O. Box 177, 2600 MH, Delft, The Netherlands
[9] VU University Amsterdam, Amsterdam Global Change Institute, Institute for Environmental Studies, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

* Corresponding author : Ketil Hylland, email address : ketilhy@ibv.uio.no

Abstract :

An international workshop on marine integrated contaminant monitoring (ICON) was organised to test a framework on integrated environmental assessment and simultaneously assess the status of selected European marine areas. Biota and sediment were sampled in selected estuarine, inshore and offshore locations encompassing marine habitats from Iceland to the Spanish Mediterranean. The outcome of the ICON project is reported in this special issue as method-oriented papers addressing chemical analyses, PAH metabolites, oxidative stress, biotransformation, lysosomal membrane stability, genotoxicity, disease in fish, and sediment assessment, as well as papers assessing specific areas. This paper provides a background and introduction to the ICON project, by reviewing how effects of contaminants on marine organisms can be monitored and by describing strategies that have been employed to monitor and assess such effects. Through the ICON project we have demonstrated the use of an integrating framework and gleaned more knowledge than ever before in any single field campaign about the impacts contaminants may have in European marine areas.

Highlights

► The historical and theoretical background for integrated contaminant monitoring is reviewed. ► The motivation, components and geographical coverage of the ICON project are presented. ► The ICON project has demonstrated the use of an integrating framework and provided more knowledge than in any single previous field campaign about the potential impacts of contaminants in European marine areas.

Keywords : ICON ; Contaminants ; North sea ; Mediterranean sea ; Biological effects ; Biomarkers ; Monitoring

1. Introduction

Marine ecosystems and organisms are influenced by many internal and external factors, including ecological processes and their interactions, fisheries, a changing climate, habitat modification, eutrophication and inputs of toxic chemicals. Exposure to contaminants[1] has the potential to affect cellular and physiological processes in marine organisms, as well as fundamental processes in marine ecosystems (Fleeger et al., 2003, Hylland et al., 2006b). The health of individuals or integrity of ecological processes will depend on many environmental factors, not only the presence of contaminants (see e.g. Hylland et al., 2009; Vestheim et al., 2012). Moreover, the consequences of contaminant exposure for the health of individual marine organisms will depend on the species, whether it is being exposed as adult, larvae, or embryo, and the life history of that species. Marine ecosystems are by nature dynamic and, particularly in temperate and polar regions of the globe, there is a pronounced annual seasonality in both abiotic and biological processes that modulate both partitioning of contaminants and effects caused by exposure to contaminants (Gagné et al., 2008; Jørgensen & Wolkers, 1999; Vijayan et al., 2006). Although it is close to impossible to single out how they influence marine organisms in any particular moment, it is important for regulatory reasons to be able to assess the extent to which contaminants actually cause effects and, whenever possible, to pinpoint the responsible contaminant(s). To this end it is crucial to be able to separate contaminant-related effects from changes caused by other environmental influences (see e.g. Hylland et al., 2009, Laane et al., 2012). In addition, we would ideally be able to compare effects across species and preferably identify and focus on the most sensitive species and endpoints for any particular contaminant. This is clearly a long-term endeavour, but significant progress has been made over the past couple of decades, and some ways to

[1] as Paracelsus published in 1538: "dosis facit venenum" - it is the dose that makes the poison; any chemical will be toxic at some dose and although that the term "contaminant" does not imply effects, it is widely used in ecotoxicology and will be used here to describe chemicals that may cause toxicity in marine ecosystems

handle this challenge are reported in this special volume (e.g. Vethaak et al.; Hylland et al.).

In the past, European countries chose different strategies by which to monitor concentrations and effects of contaminants in marine habitats. As a result of both national interests and international agreements, countries with a coastline initially implemented monitoring programmes that targeted concentrations of chemicals in marine organisms. The objectives of the early monitoring programmes were typically twofold: to ascertain that humans do not consume contaminated food and to quantify the presence and spatial extent of elevated concentrations of selected contaminants for regulatory purposes. Effects of contaminants on marine organisms were not at the forefront of concerns in most countries, but initial effect-oriented monitoring programmes were pioneered in the early 1980s in some European countries. Somewhat different strategies were chosen, depending on national priorities and both national and international scientific advice. A range of science-based activities was put in place from the 1980s onwards to investigate the applicability of biological effects techniques to quantify the impacts of contaminants on marine organisms, the GEEP workshop in Frierfjord, Norway (Bayne et al., 1988), the Bremerhaven workshop in the southern North sea (Stebbing et al., 1992), the Bermuda workshop (Addison & Clarke, 1990) and the workshop on contaminant effects in pelagic habitats, BECPELAG (Hylland et al., 2006b). Selected biological effects techniques were tried out, validated and subsequently made available for monitoring activities through the preparation of standardised protocols and setting of assessment criteria. Guidelines were subsequently established for international organisation with a monitoring role, i.e. OSPAR[2], HELCOM[3] and MEDPOL[4]. This activity has over the past two decades resulted in a harmonisation of the effect component of European contaminant monitoring programmes. At the moment, there is a process by which existing procedures and strategies are being carried over into the implementation of the Marine Strategy Framework Directive (MSFD), see e.g.

---

[2] OSPAR: Oslo and Paris Commissions, http://www.ospar.org
[3] HELCOM: Helsinki Commission, http://www.helcom.fi
[4] MEDPOL: the marine pollution assessment and control component of the Mediterranean Action Plan (UNEP), http://www.unepmap.org

Burgeot et al. (this issue), Law et al., 2010, Lyons et al. (2010; this issue), Thain et al. (2008), Vethaak et al. (this issue).

Although the process described above has had a particular focus on effects, it has been clear throughout that measurements of concentrations of selected contaminants in appropriate matrices would need to be included (Hylland, 2006, Thain et al., 2008). A framework for integrated chemical and biological monitoring of contaminants has recently been developed and is described in Vethaak et al. (this issue). The framework describes a comprehensive programme, aimed at identifying and quantifying both the presence and the effects of known and unknown contaminants. The framework comprises the main groups of chemical contaminants and a wide range of effect responses in selected marine organisms. The selection of effect methods for the framework was the result of comprehensive reviews by international working groups over the last two decades (summarised in Davies & Vethaak, 2012).

An international workshop on marine integrated contaminant monitoring (ICON) was initiated to test the above framework in practice on a Europe-wide scale. ICON was initially planned to evaluate effects of contaminants in the North Sea with Iceland as a reference area, but was later extended to the Baltic, France (Seine Bay) and Spanish Mediterranean waters.

This paper provides a background and introduction to the ICON project, by reviewing how effects of contaminants on marine organisms can be monitored and by describing strategies that have been employed to monitor and assess such effects. In addition to testing an implementation of the suggested monitoring framework, the ICON project aimed at providing an integrated assessment of selected estuarine, inshore and offshore marine areas encompassing European coastal waters from Iceland in the north to the Mediterranean in the south.

2. Monitoring effects of contaminants on marine organisms

A large volume of scientific literature produced over the past decades addresses how and whether chemicals affect marine organisms and how such effects may be detected and monitored. The overarching concepts for including biological effects in marine monitoring activities has been discussed in e.g. Depledge et al. (1993), Hylland (2006), Hylland et al. (2006a), Laane et al. (2012), Vethaak & ap Rheinallt (1992) and in international working groups, particularly ICES WGBEC[5]. Over the past three decades, there have been a vast number of studies that show a relationship between exposure to some stressor or contaminant and biological responses under controlled conditions in the laboratory, but this does not necessarily mean that the same method would be useful to monitor effects of contaminants in nature. The implementation of methods in environmental monitoring programmes is a sequential process from scientific discovery, through validation and verification to actual use. As for any other assessment tool, some degree of formalisation is required, as monitoring results will feed into a regulatory process, which could imply substantial costs for national authorities or commercial interests. As a rule of thumb, the following criteria should be met for any effect-based method prior to implementation on a national or international level (developed from ICES WGBEC, 2010): (i) the method should be able to separate contaminant-related effects from natural processes or the influence of other stressors, including knowledge of confounding factors, (ii) there should be some knowledge of dose-dependency, (iii) the mechanism of toxicity should at least partly be understood, (iv) quality assurance should be established, and finally (v) assessment criteria must be established for responses in relevant species.

Any method that is to be used to quantify effects of contaminants in nature must enable a separation of contaminant-related responses from changes caused by other exogenous or endogenous factors. There has therefore been a focus on identifying effect responses that are highly responsive to contaminant stress while not being strongly affected by other endogenous or exogenous factors. It is however important to remember that contaminant-related responses in an organism do not take place in a vacuum, but in biological systems with internal

[5] ICES Working Group on Biological Effects of Contaminants; http://www.ices.dk/community/groups/Pages/WGBEC.aspx

feedback and regulation. It is therefore to be expected that other physiological processes affect such responses, and it is clearly important to be able to adjust for them (Hylland et al., 2009).

Methods that are highly contaminant-specific, such as CYP1A induction (Whyte, 2000) or ALA-D inhibition (Hylland et al., 2009), are generally not predictive of impacts on individual health or populations. On the other hand, methods that reflect properties relevant to populations or communities, such as increased disease prevalence (Vethaak et al., 2009, Lang et al., this issue), reduced individual condition or growth (Hansen et al., 2004) or impoverished community composition (Næs et al., 1997) are strongly affected by factors other than contaminants. Observed changes in populations or communities can in most cases not be directly associated with elevated concentrations of contaminants. A contaminant-directed monitoring programme should therefore include a range of effect methods, some with high contaminant-specificity, others with relevance to the health of populations or communities. In this paradigm, there is a sequential development of increasingly more serious consequences of exposure to contaminants, from molecular interactions, through cellular compensatory mechanisms to physiological responses in individuals (Peakall & Shugart, 1993). The latter may or may not have knock-on effects on populations or communities, but it has to be admitted that there is limited knowledge on how to bridge the gap from individual health to "higher order" effects on populations.

Responses to contaminants in biological systems are generally referred to as "biological effects", or "biomarkers" for methods that quantify sublethal effects in individuals. In human toxicology and ecotoxicology, a "biomarker" is widely acknowledged to be a measurement that indicates exposure, susceptibility or effect of a toxic substance (see e.g. Peakall & Shugart, 1993). To avoid confusion, the reader should be aware that there are other uses of the term "biomarker" in environmental science, e.g. in analysing or tracking sewage components (Adnan et al., 2012), to characterise phytoplankton assemblages (Véron et al., 1998), to geochemically fingerprint different crude oils (Peters & Moldowan, 1993), and to describe the origin of lipids in sediments (Pearson et al., 2011). It is furthermore important to keep in mind that a biomarker measurement in ecotoxicology is a proxy for environmental degradation, and as such probably not the most sensitive or ecologically relevant expression of such degradation. The quantification of vitellogenin in male fish in a coastal area is an example: high concentrations of vitellogenin indicate the presence of oestrogens in that environment (Allen et al., 1999; Scott et al., 2006, Vethaak et al., 2002). Concentrations of vitellogenin up to mg/mL plasma in male fish are however probably not the most sensitive or ecologically most relevant measurement. We would probably not be that concerned about the male fish producing an unnecessary protein, even in large amounts, but more about whether larval stages of that or other species became feminised. The observed environmental oestrogen concentrations may even have some other consequence that we are not yet aware of. In this context, increased concentration of plasma vitellogenin in male fish is a biomarker for the presence of oestrogens in that coastal marine ecosystem.

A prerequisite for using any biological effect response, biomarker, to quantify responses in a field study is an *a priori* understanding of whether the biomarker response can be expected to increase or decrease with increasing exposure up to a realistic exposure level, whether there is a threshold above which a response will be expected, and whether the response will peak and then decrease at higher exposures (Depledge et al., 1993). The information required can only be generated through an iterative process between laboratory, mesocosm and field studies. Biomarkers most widely used for environmental effect assessment reflects important mechanisms of toxicity, such as biotransformation (Eggens et al., 1996, Grinwis et al., 2001, Wessel et al., 2010), genotoxicity (Devier et al., 2012, Vethaak et al., 1996), neurotoxicity (Bocquené et al. 1993, Burgeot et al. 2006), endocrine disruption (Kuiper et al., 2008), metal homeostasis (Hylland et al., 2009) and membrane stability (Broeg et al., 2012, Holth et al., 2012), but there are clearly other important mechanisms of toxicity that have not yet been sufficiently developed in ecotoxicology, e.g. immunotoxicity, developmental toxicity and reproductive toxicity. A ubiquitous mechanism of cellular toxicity, oxidative stress, is a general response in cells and tissues which may be associated with contaminants, but will also be affected by other factors (Regoli et al., 2011). Oxidative stress clearly reflects vital processes of relevance in ecotoxicology, but the complexity of the responses in relation to species, tissue, temporal changes and nutritional status has until now limited the use of oxidative stress in contaminant monitoring programmes. There is a clear need to develop methods to evaluate oxidative stress in marine organisms, not least due to knock-on effects on overt toxicity, e.g. through genotoxicity and cell pathology. Results for oxidative stress in the context of ICON are reported in Carney Almroth et al. (this issue).

It could be argued that it is not really important to know the mechanism of toxicity as long as a response has been shown to be specific to contaminant stress. In accordance with Hill's criteria for causation (Hill, 1965), however, it strengthens the confidence in the response if the mechanism is known. In addition, any links back to specific contaminants or groups of contaminants, allowing regulatory measures to be put in place to reduce environmental degradation, requires knowledge of which contaminants may cause the observed response. Implementation of any method for monitoring purposes, be it chemical analyses, biomarker analyses or disease diagnosis, requires quality assurance, which means that all laboratories performing any given analysis for national or international monitoring programmes need to establish internal protocols and procedures and participate in international intercalibration exercises. Such intercalibrations have been performed for biomarkers over the past couple of decades through different organisations, primarily BEQUALM[6] and for chemical analyses through QUASIMEME[7].

3. Confounding factors

One of the largest challenges in evaluating effects of contaminant-related stress on marine organisms is the confounding influence of endogenous and exogenous factors. Above all, even closely related species cannot be expected to respond in a

---

[6] http://www.bequalm.org
[7] http://www.quasimeme.org

similar way to what would appear to be the same exposure, for example there are inter-species differences in sensitivity and response magnitudes (Balk et al., 2011). Furthermore, differences in habitat and species availability between geographical locations will also require environmental monitoring programmes to include more than one species. In the framework of international monitoring, responses can be compared between species using species-specific assessment criteria, as described in Vethaak et al. (this issue). Another possible strategy is to evaluate the sensitivity of representative species at different trophic levels in marine food chains. Ellesat et al. (2011) investigated an *in vitro* strategy by which the contaminant sensitivity of different species sampled in the same location could be quantified, simply by extracting cells (in that case hepatocytes) and performing an immediate, on-site quantification of their relative sensitivity to different contaminants as determined using cytotoxicity. Although a promising technique, *in vitro* exposure of cells from an individual does of course not provide the same information as an *in vivo* exposure study. Within a species, life stage, gender (Vethaak et al., 2009), stage in reproductive period (Hylland et al., 1998), food availability (Hylland et al., 1996), nutritional status, general health status and life history traits (Vethaak & ap Rheinallt, 1992) may modulate responses to contaminant exposure. Exogenous factors that may affect responses to any given contaminant are other contaminants (mixture toxicity) (Sandvik et al., 1997), dissolved and particulate organic material in water or sediment (Vestheim et al., 2012), turbidity (water), grain size distribution (sediment), temperature, salinity, sudden changes in temperature and salinity (Vethaak et al., 2011) as well as UV radiation (Chiang et al., 2003). There is some knowledge about how many of the above factors modulate the responses of different biomarkers in the most widely studied monitoring species (Davies & Vethaak, 2012). The biomarkers that are currently recommended by ICES WGBEC for use in environmental monitoring (Table 1) have been evaluated to be specific and as robust in relation to modulation by other factors. In a monitoring context, confounding factors are addressed through a careful and standardised sampling design, e.g. sampling only females of a certain size at a time well outside the period of reproductive activity, and through quantifying relevant endogenous factors such as disease and environmental factors such as temperature, salinity and organic content.

Contaminants in the tissues of an organism are not necessarily biologically active. It is therefore not surprising if tissue residues do not correlate well with biological responses. This also means it is not possible to convert directly from concentrations to effects or *vice-versa*. For lipophilic contaminants, there will clearly be an equilibrium between concentrations in tissues and concentrations in plasma, potentially causing responses, and the nature of the association of contaminants with cells may make it possible to generalise over effects, as observed with high concentrations of lipophilic contaminants causing narcotisation. In general, however, one would expect major influences of contaminants during external exposure or in periods when tissue-bound contaminants are mobilised due to physiological processes such as reproduction (Jørgensen et al., 2006), moulting or starvation (Jørgensen et al., 1999) or when an individual has recently migrated from an unpolluted to a polluted area. The common denominator for the three situations is increased internal exposure to contaminants. There is a need for more knowledge about the dynamics and consequences of such mobilisation and interactions with speciation and accumulation of contaminants in tissues. In a monitoring context, this issue can be tackled through sampling design, i.e. sampling the selected organism at times of the year when mobilisation is at a maximum (for a worst case scenario).

4. Monitoring strategies

Different strategies have been chosen by European countries to assess effects of contaminants in marine ecosystems. As mentioned above, the main focus was initially on monitoring concentrations of selected contaminants in marine biotic or abiotic matrices. Starting in the 1980s, there was however an increasing awareness in many European countries of the need to for biological effects measurements to understand contaminant impacts in marine ecosystems. Some examples of different strategies are highlighted here, but similar processes were also taking place in other countries. In Germany, an approach was developed to link contaminant effect monitoring to fisheries, focusing on assessment of fish embryonal aberrations and fish disease (Dethlefsen et al., 1984; Lang 2002; von Westernhagen et al., 1987, 1989; Wosniok et al., 2000). Embryos from a number of fish species were sampled on an annual basis from both coastal and offshore areas, beginning in the early 1980s. Results from the first decade showed large spatial variation and very high frequency of aberrations in the embryos from some species, e.g. dab (*Limanda limanda*) and whiting (*Merlangius merlangus*) (von Westernhagen et al., 1989). The frequency of aberrations decreased in the 1990s, coinciding with decreased inputs of persistent pollutants from the Rhine and Elbe/Weser. A similar decrease was seen for prevalence of liver tumours in fish from the same area (reviewed by Hylland et al., 2006a). Disease conditions in fish have recently been integrated into an integrating index, fish disease index - FDI, facilitating comparison between years and areas (see Lang et al., this issue). In summary, the German monitoring programme focused on a few, ecologically important endpoints over a large spatial scale, but no direct relation to contaminant inputs.

The strategy of the Swedish monitoring programme initiated in the early 1980s was very different to the approach in Germany: in Sweden a few locations were selected for comprehensive annual surveys, including biomarkers, health assessment, assessment of growth and reproduction, population assessment, measurement of environmental factors and chemical analyses (Hanson et al., 2006, 2009). The programme includes two main locations in reference areas, one in the Baltic, the second on the Skagerrak coast. Two fish species have been used in the Swedish programme: perch (*Perca fluviatilis*) in the Baltic and eelpout (*Zoarces viviparus*) in the Skagerrak. There have been subtle changes in contaminant related responses over the period since the monitoring started (1980s) that would not have been detectable with a shorter period of monitoring, e.g. changes in gonad size and biotransformation activity in perch and large multifactorial changes in biotransformation activity in eelpout (Hanson et al., 2006, Hedman et al., 2012). In summary, the Swedish programme comprises a comprehensive annual assessment at few, relatively unpolluted locations with a main focus on selected fish species.

A similar strategy was chosen in France with a pilot site in the Seine Bay. The main objective was to apply an integrated program comprising biological effects and chemical contaminants in sediment, flounder (*Platichthys flesus*), dab and mussels (*Mytilus edulis*). A limited set of biomarkers and bioassays were applied in sediment and the chosen sentinel species. This programme has focused on different mechanisms of action, each including different biomarkers, e.g. quantifying genotoxicity through measuring micronucleus aberrations, DNA strand breaks and DNA adducts. Such an approach provides a more robust estimation of any mechanism of action that if only one of the biomarkers would be included.

In the Dutch national programme, fish-disease monitoring with dab and flounder has been integrated with chemical analyses, including exposure biomarkers such as bile PAH metabolites and contaminants in sediment, as well as supporting biological and hydrographical data (Bovenlander & Langenberg, 2006). The integrated approach allowed evaluation of one facet of coastal and estuarine ecosystem health, but at the same time demonstrates that migration patterns play a critical role in explaining the distribution of chronic diseases such as liver neoplasms in flatfish (Vethaak et al., 2009).

The contaminant programme implemented by OSPAR signatory countries in the late 1990s was expanded to include contaminant-related effects (OSPAR 1998a, b). This programme aimed to include a limited set of biomarker analyses in the same individuals as used for chemical analyses. Both polluted and less polluted locations were sampled in most countries and this strategy made it possible to investigate links between contaminants and biomarker responses in selected species (see e.g. Hylland et al., 2009, Schipper et al., 2009, Vethaak et al., 2009).

The different approaches described above were developed into an integrated chemical and biological contaminant monitoring framework, as described in Vethaak et al. (this issue). The framework comprises both biotic and abiotic components. The biotic components included are mussel, gastropod and fish, each with species-specific effect endpoints covering contaminant-specific biomarkers up to indicators of individual health status, chosen from the list of ICES WGBEC recommended methods (Table 1), as well as chemical analyses of mussels and fish. The abiotic components comprise water and sediment, mainly for hydrography and chemical analyses, but with bioassays as options (Vethaak et al., this issue). The basis of the assessment in this framework is criteria developed for each and every one of the chemical determinants and species-specific biological responses. The output from a scoring of each method determined at each location is an assessment that can be combined across methods for a given location or across locations for a regional assessment. An overall assessment for the studies included in this issue can be found in Hylland et al. (this issue).

5. An international workshop on marine integrated contaminant monitoring (ICON)

The objective of the ICON project was to evaluate the status of selected estuarine, inshore and offshore marine areas in Europe with regard to contaminant impacts, using the monitoring framework described above. The project comprised a series of sampling campaigns covering the North Sea, Iceland coastal waters, Seine bay, the Baltic, the western Wadden Sea and the Spanish Mediterranean coast (Figure 1). A comprehensive sampling and analytical effort was performed as part of the project (Table 2). The selected fish species were not all found at all sites, and mussels were, for obvious reasons, only available at coastal sites. At two sites, the Seine estuary and in the Baltic, two of the target species, dab (*Limanda limanda*) and flounder (*Platichthys flesus*) could be sampled at the same location, and in Iceland and offshore Firth of Forth dab and another target species, haddock (*Melanogrammus aeglefinus*), could be sampled at the same location and responses compared. Red mullet (*Mullus barbatus*) and the Mediterranean mussel (*Mytilus galloprovincialis*) were target species in the Mediterranean. Samples from field campaigns were analysed at different laboratories throughout Europe and the results reported in the papers of this issue. The results are reported in this special volume in the form of method-oriented papers addressing chemical analyses (Lang et al., this issue; Robertson et al. this issue), PAH metabolites (Kammann et al., this issue), oxidative stress and biotransformation (Carney Almroth et al., this issue), lysosomal membrane stability (Broeg et al., this issue; Martinez-Gomez et al., this issue), genotoxicity (Hylland et al., this issue), disease in fish (Lang et al., this issue), sediment toxicity (Vethaak et al., this issue), as well as papers addressing specific areas, i.e. the Humber-Wash estuary (UK; Lyons et al., this issue), Cartagena marine area (Spain; Martinez-Gomez et al., this issue) and Seine bay (France; Burgeot et al., this issue).

6. Study areas

The North Sea is an invaluable resource to the surrounding countries. There are substantial commercial fisheries in this semi-enclosed basin, but at the same time, due to urbanisation and anthropogenic activities, it is a repository for chemical waste from land-based and offshore sources (OSPAR, 2010). North Sea ecosystems have been and are subject to many pressures, including intensive fishing pressure, eutrophication, habitat modification and contaminant inputs. The available data suggest that North Sea ecosystems and organisms are under pressure from a wide range of contaminants, but the magnitude of the impact is largely unknown (Hylland et al., 2006). It may well be that the entire North Sea is polluted, making it difficult to find a reference location. For this reason it was important to include an area where the main target species could be found, but with lower pollution load. Iceland was chosen as an appropriate reference area.

Iceland was considered as an optimal reference area as background pollution is much lower than in the North Sea and the main (northern) species of interest could be sampled, i.e. dab, haddock, flounder and blue mussel (Table 2). The Firth of Forth is a contaminated estuary where flounder could be sampled in the inner parts and dab and haddock at the offshore part. The Baltic and Seine bay are coastal areas that are known to be contaminated and are important in the monitoring programmes of Germany (Lehtonen et al., 2014) and France (Burgeot et al., 1992, Cachot et al., 2012, Devier et al., 2012, Minier et al., 2000). Two of the fish species, dab and flounder, were sampled in both areas. The Wadden Sea is a moderately polluted coastal area. In addition to coastal sites, four offshore locations in the North Sea were included in ICON: Ekofisk, Egersund Bank, the German Bight and Dogger Bank. The Ekofisk area is affected by offshore activity (Brooks et al., 2011), the German Bight is a heavily contaminated area from different sources. Dogger Bank and Egersund Bank are not directly affected by contaminant inputs. The studies in the Mediterranean focused on the Cartagena area (NW Mediterranean), with Palos Cape as a reference area. Organisms in the Cartagena area have been and are being exposed to inputs of chemicals from a range of anthropogenic activities, including intense commercial and recreational boating, naval military activity, urban development and past mining activity. The Cartagena bay receives inputs from urban, harbour and industrial activities of the city and the nearby industrial zone, Escombreras Valley, identified as a priority pollution hot spot in the Mediterranean Sea (Martínez-Gómez et al., 2012). In addition to inputs of organic pollutants, marine sediments from Cartagena are contaminated by trace metals as a result of a continuous marine dispersal of mining waste from the nearby Portmán bay area (Benedicto et al., 2008). The reference area, Palos Cape, is a marine reserve with minor local point sources of contaminant inputs.

7. Conclusions

Through the ICON programme we have gleaned more knowledge than ever before in any single field campaign about any impacts hazardous substances may have along our coasts and in the open waters of the North Sea and other European marine areas.

The programme successfully demonstrated the application of assessment criteria (BAC/EAC) developed by SGIMC (2011), a framework for integration (Vethaak et al., this issue) and an integrated chemical and biological assessment as described in Hylland (this issue).

Acknowledgements

The authors wish to acknowledge the work by numerous colleagues in ICES and OSPAR working group, i.e. WGBEC, WKIMON, SGIMC, as well as the cruise leaders, cruise participants and crews of R/V Walther Herwig III (Germany), R/V Scotia, R/V Alba na Mara (Scotland), R/V Gwen Drez (France) and R/V Endeavour (UK). The French participation was funded by IFREMER and ONEMA. B. A. Svendsen is thanked for constructive criticism of an earlier version of this manuscript and N Bølling for preparing the map in Figure 1.

References

Addison, R.F., Clarke, K.R., 1990. The IOC/GEEP Bermuda Workshop. Journal of Experimental Marine Biology and Ecology, 138, 1−8.

Adnan, N.H., Zakaria, M.P., Juahir, H., Ali, M.M., 2012. Faecal sterols as sewage markers in the Langat River, Malaysia: Integration of biomarker and multivariate statistical approaches. Journal of Environmental Sciences, 24, 1600−1608.

Allen, Y., Scott, A. P., Matthiessen, P., Haworth, S., Thain, J. E., Feist, S. 1999. Survey of estrogenic activity in United Kingdom estuarine and coastal waters and its effects on gonadal development of the flounder *Platichthys flesus*. Environmental Toxicology and Chemistry, 18, 1791−1800.

Balk, L., Hylland, K., Hansson, T., Berntssen, M.H.G., Beyer, J., Klungsoeyr, J. 2011. Biomarkers in Natural Fish Populations Indicate Adverse Biological Effects of Offshore Oil Production. PLoS ONE 6(5): e19735. doi:10.1371/journal.pone.0019735

Barranger A., Akcha, F., Rouxel, J., Brizard, R., Maurouard, E., Pallud, M., Menard, D.,Tapie, N., Budzinski, H., Burgeot, T., Benabdelmouna, A. 2014. Study of genetic damage in the Japanese oyster induced by an environmentally-relevant exposure to diuron: Evidence of vertical transmission of DNA damage. Aquatic Toxicology 146: 93-104.

Bayne, B.L., Addison, R.F., Capuzzo, J.M., Clarke, K.R., Gray, J.S., Moore, M.N., Warwick, R.M. 1988. An overview of the GEEP workshop. Marine Ecological Progress Series, 46, 235-243.

Benedicto, J., Martínez-Gómez, C., Guerrero, J., Jornet, J., Rodríguez, C. 2008. Metal contamination in Portmán Bay (Murcia, SE Spain) 15 years after the cessation of mining activities. Ciencias Marine, 34, 389-398.

Bocquené, G., Galgani, F., Burgeot, T., Le Déan, L., Truquet, P. 1993. Acetylcholinesterase levels in marine organism along French coasts. Marine Pollution Bulletin, 26, 101-106.

Brooks, S. J., Harman, C. , Grung, M. , Farmen, E., Ruus, A. , Vingen, S., Godal, B. F., Baršiene, J., Andreikenaite, L., Skarphéðinsdóttir, H., Liewenborg, B., Sundt, R. C. 2011. Water Column Monitoring of the Biological Effects of Produced Water from the Ekofisk Offshore Oil Installation from 2006 to 2009. Journal of Toxicology and Environmental Health, Part A, 74, 7, 582 — 604

Burgeot, T., Bocquené, G., Truquet, P., Le Déan, L., Poulard, J.C, Dorel, D., Souplet, A., Galgani, F. 1992. The Dragonet (*Callionymus lyra*), a target species used for evaluation of the biological effects of chemical contaminants on French coasts. Marine Ecological Progress Series, 97, 309-316

Burgeot, T., Faucet, J , Ménard, D, Grosjean, P., Bocquené, G. 2006. Variations of benzo(a)pyrene hydroxylase and cholinesterase activities in mussels caged in the North Sea (German Bight and Statfjord). Special Issue. Environmental Toxicology and Chemistry. 25, 171-185.

Burgeot, T., Akcha, F., Ménard, D., Robinson C.R., Loizeau, V., Brach-papa, C., Martìnez-Gómez , C., Le Goff, J., Budzinski, H., Le Menach, K., Cachot, J., Minier, C., Broeg, K., Hylland, K. Integrated monitoring of chemicals and their effects on four sentinel species, *Limanda limanda*, *Platichthys flesus*, *Nucella lapillus* and *Mytilus* sp, in Seine Bay: a key step towards applying biological effects to monitoring (this issue)

Broeg, K., Kammann, U., Hoeher, N., Lang, T. Lysosomal membrane stability in the liver of dab (*Limanda limanda*) – Applicability and reliability of assessment criteria under concrete contaminant-related monitoring conditions of coastal-, estuarine-, and offshore locations. (this issue)

Bovenlander, R.W., Langenberg, V.T. 2006. National evaluation report of the joint assessment and monitoring programme of the Netherlands 2004. RIKZ Report, 2006.002. RWS/National Institute for Coastal and Marine Management, The Hague, 45 pp.

Carney Almroth, B, Hultman, M, Wassmur, B, Sturve, J. Is oxidative stress evident in dab (*Limanda limanda*) in the North Sea? (this issue)

Chiang, W.L., Au, D.W.T., Yu, P.K.N., Wu, R.S.S. 2003. UV-B Damages Eyes of Barnacle Larvae and Impairs Their Photoresponses and Settlement Success. Environmental Science & Technology. 37, 1089–1092.

Davies, I.M., Vethaak, A.D. (Eds.), 2012. Integrated monitoring of chemicals and their effects. ICES Cooperative Research Report 315, 227 pp.

Depledge, M. H., Amaral-Mendes, J. J., Daniel, B., Halbrook, R. S., Kloepper-Sams, P., Moore, M. N., Peakall, D. B. 1993. The conceptual basis of the biomarker approach. In *Biomarkers* (pp. 15–29). Springer.

Dethlefsen, V., Cameron, P., von Westernhagen, H. 1984. On the emergence of abnormal fish embryos in the southern North Sea. *Inf. Fischwirtsch.*, 32: 22-27

Devier M.H., Ledû-Lacoste, M., Akcha, F., Morin, B., Pelhuet, L., Le Menach, K., Burgeot, T., Budzinski, H. 2012. Biliary PAH metabolites, EROD activity and DNA damage in dab (*Limanda limanda*) from Seine Estuary (France). Environmental Sciences and Pollution Research, 20, 708-722.

Eggens, M. L., Vethaak, A. D., Leaverz, M. J., Horbach, G. J., Boon, J. P., Seinen, W. 1996. Differences in CYP1A response between flounder (*Platichthys flesus*) and plaice (*Pleuronectes platessa*) after long-term exposure to harbour dredged spoil in a mesocosm study. Chemosphere, 32, 1357-1380.

Ellesat, K., Yazdani, M., Holth, T.F., Hylland, K. 2011. Comparative cytotoxicity of statins and copper to primary hepatocytes of three marine fish species plaice (*Pleuronectes platessa*), long rough dab (*Hippoglossoides platessoides*) and Atlantic cod (*Gadus morhua*). Marine Environmental Research, 72: 216-224.

Fleeger, J.W., Carman, K.R., Nisbet, R.M., 2003. Indirect effects of contaminants in aquatic ecosystems. Science of the Total Environment. 317, 207-233.

Gagné, F., Burgeot, T., Hellou, J., St-Jean, S., Farcy, E., Blaise, C., 2008. Spatial variations in biomarkers of *Mytilus edulis* mussels at four polluted regions spanning the Northern Hemisphere. Environmental Research. 107*,* 274-289.

Grinwis, G. C. M., van den Brandhof, E.J., Engelsma, M.Y., Kuiper, R.V., Vaal, M.A., Vethaak, A.D., Wester, P.W., Vos, J.G., 2001. Toxicity of PCB-126 in European flounder (*Platichthys flesus*) with emphasis on histopathology and cytochrome P4501A induction in several organ systems. Archives of Toxicology, 75, 80-87.

Grinwis, G. C. M., Vethaak, A. D., Wester, P. W., Vos, J. G., 2000. Toxicology of environmental chemicals in the flounder (*Platichthys flesus*) with emphasis on the immune system: field, semi-field (mesocosm) and laboratory studies. Toxicology Letters. 112, 289-301.

Hansen, J. A., Lipton, J., Welsh, P. G., Cacela, D., MacConnell, B. 2004. Reduced growth of rainbow trout (*Oncorhynchus mykiss*) fed a live invertebrate diet pre-exposed to metal-contaminated sediments. Environmental Toxicology and Chemistry, 23, 1902–1911.

Hanson, T., Lindesjöö, E., Förlin, L., Balk, L., Bignert, A., Larsson, Å., 2006. Long-term monitoring of the health status of female perch (*Perca fluviatilis*) in the Baltic Sea shows decreased gonad weight and increased hepatic EROD activity. Aquatic Toxicology, 79, 341-355.

Hanson, N., 2009. Population level effects of reduced fecundity in the fish species perch (*Perca fluviatilis*) and the implications for environmental monitoring. Ecological Modelling, 220, 2051-2059.

Hedman, J.E., Rüdel, H., Gercken, J., Bergek, S., Strand, J., Quack, M., Appelberg, M., Förlin, L., Tuvikene, A., Bignert, A., 2011. Eelpout (*Zoarces viviparus*) in marine environmental monitoring. Marine Pollution Bulletin. 62, 2015–2029.

Hill, A.B., 1965. The Environment and Disease: Association or Causation? Proceedings of the Royal Society of Medicine. 58, 295–300.

Holth, T.F., Beckius, J., Zorita, I., Cajaraville, M.P., Hylland, K.. 2011. Assessment of lysosomal membrane stability and peroxisome proliferation in the head kidney of Atlantic cod (*Gadus morhua*) following long-term exposure to produced water components. Marine Environmental Research. 72, 127–134.

Hylland, K. 2006. Biological effects in the management of chemicals in the marine environment. Marine Pollution Bulletin. 53, 614-619.

Hylland, K., Beyer, J., Berntssen, M., Klungsøyr, J., Lang, T., Balk, L. 2006a. May persistent organic pollutants affect fish populations in the North Sea? Journal of Toxicology and Environmental Health. Part A, 69, 125-138.

Hylland, K., Gubbins, M.J., Robinson, C., Burgeot, T., Martínez-Gómez, C., Lang, T., Svavarsson, J., Thain, J.E., Vethaak, AD. Integrated chemical and biological assessment of contaminant impacts in selected European coastal and offshore marine areas (this issue)

Hylland, K., Lang, T., McIntosh, M., Thain, J.E., Utvik, T.I.R., Vethaak, A.D., Wosniok, W., 2006b. Biological effects of contaminants in pelagic ecosystems: the BECPELAG workshop. In: Biological effects of contaminants in pelagic ecosystems. Hylland, K, Vethaak, AD, Lang, T (Eds), SETAC special volume, p 3-8.

Hylland, K., Nissen-Lie, T., Christensen, P.G., Sandvik, M. 1998. Natural modulation of cytochrome P4501A and metallothionein in flounder, *Platichthys flesus*. Marine Environmental Research. 46, 51-55.

Hylland, K, Ruus, A, Grung, M, Green, N. 2009. Relationships between physiology, tissue contaminants and biomarker responses in Atlantic cod (*Gadus morhua* L.). Journal of Toxicology and Environmental Health. Part A, 72, 226-233.

Hylland, K., Sandvik, M:, Skåre, J.U., Beyer, J., Egaas, E., Goksøyr, A. 1996. Biomarkers in flounder (*Platichthys flesus*): an evaluation of their use in pollution monitoring. Marine Environmental Research. 42, 223-227.

Hylland, K., Skei, B.B., Gubbins, M.J., Lang, T., Brunborg, G., Le Goff, J., Burgeot, T., Genotoxicity in dab (*Limanda limanda*) and haddock (*Melanogrammus aeglefinus*) from European seas (this issue)

Hylland, K., Sköld, M., Gunnarsson, J., Skei, J. 1997. Interactions between eutrophication and contaminants. IV. Effects on sediment-dwelling invertebrates. Marine Pollution Bulletin. 33, 90-99.

Jørgensen, E.H., Vijayan, M.M., Killie, J.-E.A., Aluru, N., Aas-Hansen, Ø., Maule, A. 2006. Toxicokinetics and Effects of PCBs in Arctic Fish: A Review of Studies on Arctic Char. Journal of Toxicology and Environmental Health. Part A, *69*, 37–52.

Jørgensen, E. H., Wolkers, J. 1999. Effect of temperature on the P4501A response in winter-and summer-acclimated Arctic char (*Salvelinus alpinus*) after oral benzo [a] pyrene exposure. Canadian Journal of Fisheries and Aquatic Sciences, 56, 1370–1375.

Kammann, U., Akcha, F., Budzinski, H., Burgeot, T., Gubbins, M.J., Lang, T., Le Menach, K., Vethaak, A.D., Hylland, K. PAH metabolites in fish bile: from the Seine Estuary to Iceland (this issue)

Kuiper, R. V., Vethaak, A. D., Anselmo, H., Dubbeldam, M., van den Brandhof, E. J., Leonards, P. E., van den Berg, M. 2008. Toxicity of analytically cleaned pentabromodiphenylether after prolonged exposure in estuarine European flounder (*Platichthys flesus*), and partial life-cycle exposure in fresh water zebrafish (*Danio rerio*). Chemosphere. 73, 195-202.

Laane, R.W.P.M., Slijkerman, D., Vethaak, A.D., Schobben, J.H.M., 2012. Assessment of the environmental status of the coastal and marine aquatic environment in Europe: A plea for adaptive management. Estuarine, Coastal and Shelf Science, 96, 31-38.

Laane, R.W.P.M., Slijkerman, D., Vethaak, A.D., Schobben, J.H.M., 2012. Assessment of the environmental status of the coastal and marine aquatic environment in Europe: A plea for adaptive management. Estuarine, Coastal and Shelf Science, 96, 31-38.

Lang, T., 2002. Fish disease surveys in environmental monitoring: the role of ICES. ICES Marine Sciences Symposium, 215, 202-212.

Lang, T., Feist, S.W., Stentiford, G.D., Bignell, J., Vethaak, A.D., Wosniok, W. Diseases of dab (*Limanda limanda*): analysis and assessment of data on externally visible diseases, macroscopic liver neoplasms and liver histopathology at offshore sites in the North Sea, Baltic Sea and off Iceland (this issue)

Lang, T., Kruse, R., Haarich, M., Wosniok, W. Methylmercury in dab (*Limanda limanda*) from the North Sea, Baltic Sea and Icelandic waters: relationship to host-specific variables (this issue)

Law, R., Hanke, G., Angelidis, M., Batty, J., Bignert, A., Dachs, J., Davies, I., Denga, Y., Duffek, A., Herut, B., Hylland, K., Lepom, P., P. Leonards, J. Mehtonen, H. Piha, P. Roose, J. Tronczynski, V., Velikova, V. and Vethaak, D.

2010. Marine Strategy Framework Directive Task Group 8 Report Contaminants and pollution effects. Joint Report Prepared under the Administrative Arrangement between JRC and DG ENV (no 31210 – 2009/2010), the Memorandum of Understanding between the European Commission and ICES managed by DG MARE, and JRC's own institutional funding Editor: H. Piha EUR 24335 EN – 2010.

Lehtonen, K.K., Sundelin, B., Lang, T., Strand, J. 2014. Development of Tools for Integrated Monitoring and Assessment of Hazardous Substances and Their Biological Effects in the Baltic Sea. AMBIO. 43, 69-81.

Lyons, B., Thain, JE, Stentiford, GD, Hylland, K, Davies, I, Vethaak, AD. 2010. Using biological effects tools to define Good Environmental Status under the European Union Marine Strategy Framework Directive. Marine Pollution Bulletin, 60. 1647-1651.

Lyons B.P., Bignell, J.P., Stentiford, G.D., Bolam, T., Rumney, H.S., Bersuder, P., Barber, J., Askem, C.W., Maes T., Thain, J.E. Determining Good Environmental Status under the Marine Strategy Framework Directive: case study for descriptor 8 (chemical contaminants) (this issue)

Martínez-Gómez C., Burgeot T., Robinson, C.D., Gubbins, M.J., Halldorsson, H.P.' Albentosa, M., Bignell J.P., Hylland, K., Vethaak A.D. Lysosomal membrane stability and Stress on Stress in mussels as common Pan-European contaminant-related biomarkers (this issue)

Martínez-Gómez C., Fernández B., Robinson, C.D., Campillo J.A., León V.M., Benedicto J., Hylland, K., Vethaak A.D. Assessing the good environmental status (GES) of the Cartagena coastal zone (W Mediterranean) using an integrated framework of chemical and biological effect data: a practical case study (this issue)

Martínez-Gómez, C., Fernández, B., Benedicto, J.M., Valdés, J., Campillo, J.A., León, V.M., Vethaak, A.D. 2012. Health status of red mullets from polluted areas of the Spanish Mediterranean coast, with special reference to Portmán (SE Spain). Marine Environmental Research. 77, 50-59.

Minier C., Levy F., Rabel D., Boquené G., Godefroy D., Burgeot T., Leboulenger F., 2000. Flounder health status in the Seine Bay. A multibiomarker study. Marine Environmental Research. 50, 69-73.

Næs, K., Hylland, K., Oug, E., Förlin, L., Ericson, G. 1999. Accumulation and effects of aluminium-smelter generated PAHs in soft bottom invertebrates and fish. Environmental Chemistry & Toxicolology. 18, 2205-2216.

OSPAR, 1998a. JAMP guidelines for general biological effects monitoring. Oslo and Paris Commissions, 15 p.

OSPAR, 1998b. JAMP guidelines for contaminant-specific biological effects monitoring. Oslo and Paris Commissions, 38 p.

OSPAR, 2010. Quality Status Report 2010. OSPAR Commission, London, 176 pp.

Peakall, D. B., Shugart, L. R. 1993. *Biomarkers: research and application in the assessment of environmental health* (Vol. 68). Springer.

Pearson, A., McNichol, A. P., Benitez-Nelson, B. C., Hayes, J. M., Eglinton, T. I. 2001. Origins of lipid biomarkers in Santa Monica Basin surface sediment: a case study using compound-specific Δ14C analysis. Geochimica et Cosmochimica Acta. 65, 3123–3137.

Peters, K.E., Moldowan, J.M. 1993. *The Biomarker Guide, Interpreting Molecular Fossils in Petroleum and ancient Sediments*. Englewood Cliffs, New Jersey: Prentice Hall.

Regoli, F., Giuliani, M. E., Benedetti, M., Arukwe, A. 2011. Molecular and biochemical biomarkers in environmental monitoring: A comparison of biotransformation and antioxidant defense systems in multiple tissues. Aquatic Toxicology. 105, 56–66.

Robinson, C.D., Webster, L., Martínez-Gómez, C., Burgeot, T., Gubbins, M.J., Thain, J.E., Vethaak, A.D., McIntosh, A.D., Hylland, K. Assessment of contaminant concentrations in sediments, fish and mussels sampled from the North Atlantic and European regional seas within the ICON project (this issue)

Sandvik, M., Beyer, J., Goksøyr, A., Hylland, K., Egaas, E., Skåre, J.U., 1997. Interaction of benzo[a]pyrene, 2,3,3',4,4',5-hexachlorobiphenyl (PCB-156) and cadmium on biomarker responses in flounder (*Platichthys flesus* L.). Biomarkers. 2, 153-160.

Schipper, C.A., Lahr, J., van den Brink, P.J., George, S.G., Hansen, P.-D., da Silva de Assis, H.C., van Murk, A.J., Grinwis, G.C.M., Klamer, H., Kater, B.J., Postma, J.F., van der Werf, B., and Vethaak, A.D., 2009. A retrospective analysis to explore the applicability of fish biomarkers and sediment bioassays along contaminated salinity transects. ICES Journal of Marine Science. 66, 2089–2105.

Scott, A.P., Katsiadakis, I., Whittames, P., Hylland, K., Davies, I.M., McIntosh, A.D., Thain, J. 2006. Vitellogenin in the blood plasma of male cod (*Gadus morhua*): a sign of oestrogenic endocrine disruption in the open sea? Marine Environmental Research. 61, 149-160.

Stebbing, A.R.D., Dethlefsen, V., Addison, R.F., Carr, M., Chapman, P.M., Cofino, W., Heip, C.H.R., Karbe, L., Moore, M.N., Vethaak., A.D., 1992. Overall summary and some conclusions from the Bremerhaven workshop. Marine Ecology Progress Series. 91, 1-8.

Thain, JE, Vethaak, AD, Hylland, K. 2008. Contaminants in marine ecosystems: developing an integrated indicator framework using biological effects techniques. ICES Journal of Marine Scienc., 65, 1508-1514.

Véron, B., Dauguet, J.-C., Billard, C., 1998. Sterolic biomarkers in marine phytoplankton. II. Free and conjugated sterols of seven species used in mariculture. Journal of Phycology, 34, 273–279.

Vethaak, A. D., Ap Rheinallt, T. 1992. Fish disease as a monitor for marine pollution: the case of the North Sea. Reviews in Fish Biology and Fisheries. 2, 1-32.

Vethaak, A. D., Jol, J. G., & Martínez-Gómez, C. 2011. Effects of cumulative stress on fish health near freshwater outlet sluices into the sea: a case study (1988–2005) with evidence for a contributing role of chemical contaminants. Integrated environmental assessment and management. 7, 445-458.

Vethaak, A. D., Jol, J.G., Meijboom, A., Eggens, M.L., ap Rheinallt, T., Wester, P.W., van de Zande, T. 1996. Skin and liver diseases induced in flounder (*Platichthys flesus*) after long-term exposure to contaminated sediments in large-scale mesocosms. Environmental Health Perspectives. 104, 1218.

Vethaak, A.D., Davies, I.M., Thain, J.E., Gubbins, M.J., Martínez-Gómez, C., Robinson, C.D., Moffat, C.F., Burgeot, Maes, Wosniok, Giltrap , M., Lang, T., Strand, J., Hylland, K. Integrated indicator framework and methodology for monitoring and assessment of hazardous substances and their effects in the marine environment (this issue)

Vethaak, A.D., Hamers, T., Martínez-Gómez, C., Kamstra, J.H., de Weert , J., Leonards, P., Smedes, F. Toxicity profiling of marine surface sediments: a case study using rapid screening bioassays of exhaustive total extracts, elutriates and passive sampler extracts (this issue)

Vijayan, M. M., Aluru, N., Maule, A. G., Jørgensen, E. H. 2006. Fasting augments PCB impact on liver metabolism in anadromous arctic char. Toxicological Sciences. 91, 431−439.

Wessel, N., Ménard, D., Pichavant-Rafini, K., Ollivier, H., Le Goff, J., Burgeot, T., Akcha, F., 2012. Genotoxic and enzymatic effects of fluoranthene in microsomes and freshly isolated hepatocytes from sole (*Solea solea*). Aquatic Toxicology. 18, 33-41.

Wessel, N., Santos, R., Ménard, D., Le Ménach, K., Buchet V., Lebayon, N., Loizeau, V., Burgeot, T., Budzinski, H., Akcha, F. 2010. Relationship between PAH biotransformation as measured by biliary metabolites and EROD activity, and genotoxicity in juveniles of sole (*Solea solea*). Marine Environmental Research. 69, S71-S73.

von Westernhagen, H., Cameron, P., Dethlefsen, V. and Janssen, D. 1989. Chlorinated hydrocarbons in North Sea whiting (*Merlangius merlangus* L.), and effects on reproduction. I. Tissue burden and hatching success. Helgoländer Meeresuntersuchungen. 43, 45-60.

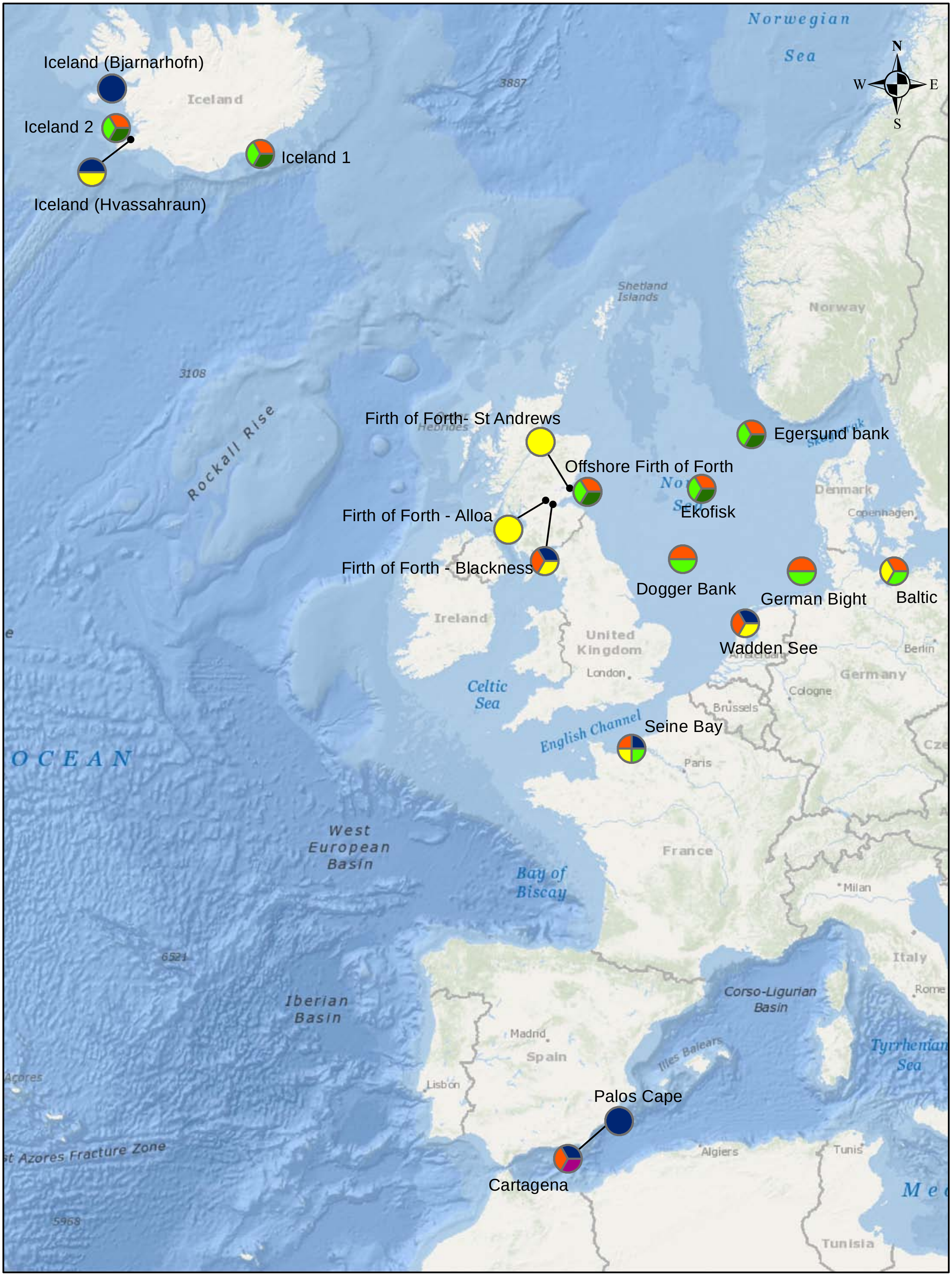
von Westernhagen, H., Dethlefsen, V. Cameron, P., Janssen, D. 1987. Chlorinated hydrocarbon residues in gonads of fish and effects on reproduction. Sarsia. 72, 419-422.

Whyte, J.J., Jung, R.E., Schmitt, C.J., Tillitt, D.E., 2000. Ethoxyresorufin *O*-deethylase (EROD) Activity in Fish as a Biomarker of Chemical Exposure. Critial Reviews in Toxicology. 30, 347-570.

Wosniok, W., Lang, T., Dethlefsen, V., Feist, S.W., McVicar, A.H., Mellergaard, S., Vethaak, A.D. 2000. Analysis of ICES long-term data on diseases of North Sea dab (*Limanda limanda*) in relation to contaminants and other environmental factors. ICES CM 2000/S:12, 15 pp.

Figure legend

Figure 1. Sampling locations; colours denote samples taken at any location; sediment: red; mussels: dark blue; dab: green; flounder: yellow; haddock: dark green; red mullet: violet.



Iceland (Bjarnarhofn)
Iceland 2
Iceland 1
Iceland (Hvassahraun)
Firth of Forth- St Andrews
Offshore Firth of Forth
Egersund bank
Ekofisk
Firth of Forth - Alloa
Firth of Forth - Blackness
Dogger Bank
German Bight
Baltic
Wadden See
Seine Bay
Palos Cape
Cartagena
N
W
E
S

Table 1. Biological effects methods recommended by ICES WGBEC; excerpted and updated from ICES WGBEC (2010); Countries: are using or have been using method in monitoring programme(s) within the last 10 years; TIMES: accepted protocol, Techniques in Marine Environmental Science, volume number.

Method	**Species**	**Countries**	**TIMES**	**Comment**
Bulky DNA adduct formation	fish	NO, SE, UK	25	
AChE inhibition	fish, molluscs, crustaceans	FR, ES, IE, UK	22	
Metallothionein induction	fish, mussels	ES, IE, IT, NO, SE	26	
EROD or P4501A induction	fish	BE, ES, FR, IE, NO, SE, UK	14, 23	
ALA-D inhibition	fish	NO		
PAH bile metabolite concentration	fish	BE, IE, GE, NL, NO, UK	39	
Alkylphenol bile metabolite concentration	fish	NO		
Lysosomal stability (including NRR)	mussels, oyster	IE, IS, IT, NL, NO, UK	36	
Lysosomal stability using histochemical quantification	fish, mussels	ES, GE	34	
Early toxicopathic lesions, preneoplastic and neoplastic liver lesions by histopathology	fish	GE, UK	38	
External visible lesions and parasites	dab, flounder, cod	GE, UK	19	
Vitellogenin induction	male, juvenile fish	GE, IE, NO, UK	31	species-specific antibody
Intersex	male dab, flounder, eelpout	GE, IE, UK		
Reproductive success	eelpout	DK, GE, SE		one species only
Scope for growth	mussel	ES, IE, IS, UK	40	
Imposex	neogastropods	DK, ES, FR, IE, NO, UK	24	
Intersex	periwinkles	NL	37	
Histopathology	mussels	DK, ES, FR, IE, IT, NO, UK		
Embryo aberrations	amphipods	SE	41	field only

Table 2. Locations and matrices sampled.

Location	Code	Type	Country	Matrices sampled
Hvassahraun	HV	Inshore	Iceland	Mussel, flounder
Bjarnarhöfn	BH	Inshore	Iceland	Mussel
SE Iceland	IS1	Offshore	Iceland	Dab, haddock, sediment
SW Iceland	IS2	Offshore	Iceland	Dab, haddock, sediment
Egersund bank	EB	Offshore	Norway	Dab, haddock, sediment
Ekofisk	EF	Offshore	Norway	Dab, haddock, sediment
Firth of Forth - Alloa	AL	Estuary	Scotland	Flounder
Firth of Forth - Blackness	BL	Estuary	Scotland	Mussel, flounder, sediment
Firth of Forth – St Andrews Bay	SAB	Inshore	Scotland	Flounder
Firth of Forth	FF	Offshore	Scotland	Dab, haddock, sediment
Dogger Bank	DB	Offshore	Germany	Dab, sediment
German Bight	GB	Offshore	Germany	Dab, sediment
Baltic Sea	BA	Inshore	Germany	Flounder, dab, sediment
Wadden Sea	WS	Inshore	Netherlands	Flounder, mussel, sediment
Seine estuary	SE	Estuary	France	Dab, flounder, mussel, sediment
Seine bay	PAR	Inshore	France	Dab, flounder, mussel, sediment
Cartagena	CAR	Inshore	Spain	Red mullet, mussel, sediment
Cape Palos	CP	Inshore	Spain	Mussel

*From coastal locations adjacent to the sampling point.