ICES WGBEC REPORT 2017

SCICOM STEERING GROUP ON ECOSYSTEM PRESSURES AND IMPACTS

ICES CM 2017/SSGEPI:06

REF. SCICOM

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

13-17 March 2017

Reykjavik, Iceland

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

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

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

Recommended format for purposes of citation:

ICES. 2017. Interim Report of the Working Group on the Biological Effects of Contaminants (WGBEC), 13–17 March 2017, Reykjavik, Iceland. ICES CM 2017/SSGEPI:06. 26 pp. https://doi.org/10.17895/ices.pub.8566

Contents

Executive summary

The Working Group on the Biological Effects of Contaminants (WGBEC), chaired by Bjørn Einar Grøsvik (NO) and Ketil Hylland (NO), met in Reykjavik, Iceland, 13–17 March 2017. There were 13 attendees through the week, representing 8 countries, in addition two invited speakers from the University of Iceland.

WGBEC aims to address effects of chemical stressors on the marine environment and subsequent consequences for human health and resource use, taking into account other stressors and environmental factors. The group aims to develop, evaluate and quality assure methods and frameworks for environmental quality.

WGBEC raison d´être and communication with other expert groups

WGBEC is currently extending its activities to also encompass issues relevant to human health, in addition to progressing with seabirds and marine mammals, with an aim to address ecosystem-wide impacts of contaminants.

ToR a) Review effects of chronic oil exposure on marine organisms. WGBEC members are working on a review to be submitted by the end of 2017, led by Bjørn Einar Grøsvik (NO).

ToR b) Review available studies on marine seabird ecotoxicology. WGBEC members are involved in research projects and two papers on seabird genotoxicity are in preparation, to be submitted prior to WGBEC 2018.

ToR c) Review available studies on marine mammal ecotoxicology. With invited external experts, WGBEC discussed the serious situation for long-lived toothed whales in European waters, particularly killer whales. Populations in the UK, around Gibraltar and the Canary Islands have suffered dramatic declines in reproduction over the past decades. The same populations have very high contaminant concentrations. PCB was identified as the possibly most important group of contaminants and the group highlighted the need for European countries to reduce diffuse inputs of PCB.

ToR d) Review effects of contaminants on community composition. It is clearly important, but challenging, to link contaminant effects with impacts on biodiversity. The activity will be summarised at WGBEC 2018.

ToR e) Develop methods to evaluate effects of acute spills on marine organisms. The group considered national guidelines already available on the topic and group members have been approached to combine and review the information into a common guideline.

ToR f) Develop methods to evaluate effects of ocean acidification on marine organisms. The current state of science was reviewed during the meeting.

ToR g) Review interactions between essential nutrients or vitamins and contaminants in marine organisms. Although not clear at the moment, there is a need to further explore links between deficiencies in essential nutrients or vitamins and contaminant exposures. The planned workshop on thiamine methods could not be held and will be considered for WGBEC 2018.

ToR h) Review progress with marine plastic ecotoxicity to marine organisms. WGBEC members are involved in a range of national and international projects addressing issues

with macro- and microplastics. A promising technique for quantification of some plastics in sediments and tissues was presented.

ToR i) Review and update knowledge of environmental interactions and combined stressors in marine ecosystems. An outline for this review was discussed at the meeting. A scientific paper co-authored by WGBEC members will be submitted by the end of 2017, guided by an outline circulated by Ketil Hylland (NO) before the summer.

ToR j) Review effects of emerging contaminants on marine organisms. The group discussed the report from MCWG.

ToR k) Review the use of passive samplers and dosing in marine ecotoxicity studies. Recent papers and results reported at MSWG and MCWG were presented to the group and discussed.

Review the status of publications and consider requirements for new publications

Activities stemming from WGBEC has led to the publication of a special volume of Marine Environmental Research (Volume 124), edited by Ketil Hylland (current Chair) and Matthew Gubbins (former Chair). The volume described the ICON project and comprised 14 scientific papers. A TIMES document elicited by the group is currently under publication, Hanson *et al.* "Supporting variables for biological effects measurements in fish and blue mussel".

AQC activities for biological effect methods

An intercalibration for selected biological effects methods, i.e. EROD, AChE and micronucleus, is underway and will be performed early autumn 2017. Co-ordinators are Bjørn Einar Grøsvik (NO) and Steve Brooks (NO).

Communication with the ICES Data Centre

WGBEC support the development of a simplified data entry format, to increase the inflow of biological effect data into ICES databases.

Contact with OSPAR and other ICES WGs

Although there has been progress in developing integrated monitoring programmes to be accepted by OSPAR HASEC, there is a need for more data.

The collaboration with WGEEL in 2016 was discussed. This topic has left some loose threads that will be addressed at WGBEC 2018.

1 Administrative details

Working Group name

Working Group on the Biological Effects of Contaminants (WGBEC)

Year of Appointment

2016

Reporting year within current cycle (1, 2 or 3)

2

Chair(s)

Bjørn Einar Grøsvik, Norway

Ketil Hylland, Norway

Meeting venue

Reykjavik, Iceland

Meeting dates

13–17 March 2017

2 Terms of Reference a) – z)

a) Review effects of chronic oil exposure on marine organisms;

b) Review available studies on marine seabird ecotoxicology;

c) Review available studies on marine mammal ecotoxicology;

d) Review effects of contaminants on community composition;

e) Develop methods to evaluate effects of acute spills on marine organisms;

f) Develop methods to evaluate effects of ocean acidification on marine organisms;

g) Review interactions between essential nutrients or vitamins and contaminants in marine organisms;

h) Review progress with marine plastic ecotoxicity to marine organisms;

i) Review and update knowledge of environmental interactions and combined stressors in marine ecosystems;

j) Review effects of emerging contaminants on marine organisms;

k) Review the use of passive samplers and dosing in marine ecotoxicity studies.

3 Summary of Work plan

Year 1

The development of methods to assess effects of acidification is an ongoing issue, to be reported each year. Effects of emerging contaminants will be finalised (there has been activity on this issue over the last 3-year period). The group will finalise recently initialised work on interaction between contaminants and vitamins. Work will also focus on items to be reported in year 2 with status updates this year (a, b, c).

Year 2

This is an important reporting year during this 3-year cycle with a final reporting, i.e. review papers, on items a, b (chronic oil exposure, seabird toxicity), in addition to status updates for items f and h (effects of acidification and plastics).

Year 3

Final reporting (i.e. review papers) on items c, d and i (marine mammal ecotoxicology, effects on communities and interactions/combined effects) as well as status reports for ocean acidification.

4 List of Outcomes and Achievements of the WG in this delivery period

- The group reserved a full day to discuss the plight of toothed whales in European waters, specificially killer whale. Killer whales from the UK southwards have so low reproduction, most likely contaminant-related, that they will most likely go extinct within one or two decades.
- An entire issue of a periodical – Marine Environmental Research 124. Pages 1-138 (March 2017) – was dedicated to papers from a WGBEC-initiated project, ICON, with WGBEC members as main contributors.
- The group will continue to develop an ecosystem perspective that includes seabirds, marine mammals and humans alongside fish and invertebrates.
- Effects of plastics and interactions with contaminants in how they affect marine organisms will be in focus for the last year of the 3-year cycle.

5 Progress report on ToRs and workplan

WGBEC raison d´être

WGBEC is currently extending its activities to also encompass issues relevant to human health, in addition to progressing with seabirds and marine mammals. Work within the next year will include evaluating the relevance of animal models for assessing human health risks and the extent to which impacts in nature can be used to predict future consequences for man. A position paper is being produced to discuss the above aspects, led by Dick Vethaak (NL).

References to the text in each ToR can be found in Annex 3.

ToR a – review effecs of chronic oil exposure on marine organisms

WGBEC members are involved in different activities addressing how oil or oil-related components affect marine organisms. A study performed at the Sandgerdi field station (Iceland) highlighted major species differences (Atlantic cod, turbot) in their response to exposure to the water soluble components of oil (Holth *et al.*, in press). A recent study have shown how transcriptomics can be used to identify critical pathways for oil toxicity in fish (haddock) larvae (Sørhus *et al.*, 2017). Formation of DNA adducts in fish liver due to exposure of oil and PAHs was recently reviewed by Pampanin *et al.* (2017).

A review of chronic oil effects will comprise the following:

i) description of chronic exposure, including degradation and environmental modification (BEG);

ii) tissue and environmental concentrations following chronic exposure (SB);

iii) effect methods – fish (BEG, KH, SB);

iv) effect methods – invertebrates/communities (JB, SB);

v) effect methods – seabirds, mammals (KH);

vi) comparison lab – field; thresholds/assessment values (BE);

vii) modelling exposure and effects.

Action: Work is in progress with a review paper on effects of chronic oil spills. The review will be prepared and submitted by the end of 2017. Bjørn Einar Grøsvik (NO) will circulate a draft outline before the summer to WGBEC.

ToR b – review available studies on marine seabird ecotoxicology

Ketil Hylland (NO) presented recent studies at the University of Oslo, focusing on involvement of environmental contaminants on DNA strand breaks in white blood cells from seabirds sampled at Ny Ålesund, Svalbard, i.e. black guillemot, kittiwake, eider, Arctic skua and large skua (Haarr *et al.*, in prep), as well as herring gull from Norway (Keilen, 2017).

Hrönn Jörundsdóttir (IS) presented a study with a large spatial coverage of persistent organic contaminants in black guillemot eggs, with a surprisingly different patterns for different contaminants. All contaminants were highest in eggs from the Baltic, PBDEs higher in Iceland eggs than eggs sampled along the Norwegian coast, whereas different fluorinated substances varied in their concentrations, with PFOSA highest at Sklinna (Trøndelag, Norway). A study on Iceland showed that skua eggs were most contaminated with tern and eider eggs having the lowest contaminant loads.

Action: Follow up specifically on the consequences of plastic ingestion by seabirds at WGBEC 2018.

ToR c – review available studies on marine mammal ecotoxicology

Paul Jepson (UK) and Rune Dietz (DK) presented their ongoing work on killer whales, toothed whales and other marine mammals. Their studies show that the bioaccumulation of persistent and toxic pollutants in both humans and wildlife lead to elevated tissue concentrations and associated detrimental effects on important immune, endocrine and reproductive functions. Marine mammals, especially killer whales (*Orcinus orca*), are particularly vulnerable to bioaccumulation as they have long life spans and are top predators feeding at high trophic levels.

A recent paper has linked long term population declines of European killer whale and other cetaceans populations to reproductive impairment caused by PCBs (Jepson *et al.*, 2016). Many of these highly PCB-exposed populations of killer whales and bottlenose dolphins in both the NE Atlantic and the Mediterranean Sea have small or declining populations associated with very low rates of reproduction in adult females. PCBs have all but stopped declining in marine mammals in Europe over the past 15–20 years. Since most adult female dolphins can only normally produce a single calf every 2–4 years – any further PCB-induced suppression of fecundity can and will have catastrophic consequences on population viability. The killer whale is now close to extinction within the industrialised regions of Europe. In a recent review of the PCB threat to marine apex predators (including seabirds), the bioaccumulation and biomagnification of PCBs was conserved a significant threat not just to killer whales but also to bottlenose dolphins (resident/coastal ecotypes); false killer whales (globally); polar bears (Arctic); river dolphins and porpoises (SE Asia) and numerous marine apex predator shark species (Jepson and Law 2016).

WGBEC discussed the need for evaluating the effects of realistic pollutant exposure on *in vitro* immune function in killer whales in order to generate data for a population model of contaminant effects in killer whales. Ongoing work at Aarhus University and collaborators are using peripheral blood to isolate immune cells from individual whales being cryopreserved until laboratory analysis. The *in vitro* immune assays to be tested in the present study include mitogen-induced lymphocyte proliferation, natural killer cell activity and cytokine production, and these represent important aspects of innate and acquired immunity (De Guise *et al.*, 1997; Mori *et al.*, 2006). Immune function are assessed dose-dependently using a contaminant cocktails extracted from killer whale blubber, thus representing the actual mixture of contaminants present in killer whales. This work will feed into a population-level model of contaminant effects in wild killer whales, allowing us to estimate how current and future contaminant levels in wild animals are influencing population growth and risk of viral epidemics. Such information is crucial for on-going conservation efforts for killer whales, which continue to have extremely elevated body burdens of persistent organic pollutants (POPs).

Among a cocktail of industrial pollutants, PCBs are possibly the most important drivers for reproductive, immunotoxic and carcinogenic effects. While environmental PCB concentrations were indeed observed declining after legal mitigation, large body burdens remain in many top predators, especially in the North Atlantic. Moreover, both intentional and unintentional production of PCBs, as well the use and recycling of PCB-containing equipment, are contemporary primary and secondary sources (Dietz *et al.* 2016).

The Stockholm Convention therefore urges its ratifying parties to cease using PCB-containing equipment by 2025 and perform environmentally sound waste management by 2028. This means nonetheless that PCBs will continue to leach into the environment over the next decade. Given present-day observed reproductive failure in several killer whale populations we must urgently reduce the ultimate industrial PCB phase-out deadline before conservation of this species surpasses a tipping point.

The IUCN Red List of Threatened Species does not state concern for killer whales as data are deficient, mainly because there is scientific uncertainty around whether killer whales are one or more species. Nonetheless, there is a need for an international risk assessment, using both *in vivo* and *in vitro* approaches to determine physiological effect thresholds of realistic PCB exposures that will allow us to identify meaningful population impacts using state-of-the–art modelling. Worldwide collaborative efforts are crucial to identify populations at risk of extinction and those that could maintain this iconic species. Killer whales are excellent marine sentinel species, indicating that not one nation can address the persistent threat that is environmental PCB pollution. The choice for international PCB mitigation is both timely and urgent - in order not to lose this "canary in the coalmine".

Action: WGBEC will follow up on contaminant effects in sensitive marine mammal populations in 2018.

Recommendation: WGBEC are deeply concerned about the PCBs loads on killer whales and other toothed whale populations in the North Atlantic since they appear to have devastating consequences. WGBEC invites the regional seas conventions to take note of these serious findings and encourage member countries to dramatically increase the rate of clean-up of contaminated landfills, rivers, estuaries and coastal areas. Marine mammals and seabirds appear to be sensitive components of marine ecosystems and the conventions are invited to include relevant components in ongoing monitoring programmes.

ToR d – review effects of contaminants on community composition

The input to the discussion of community was contributed by Juan Bellas (ES), with inputs from other members of WGBEC. 'Community ecotoxicology' has been defined as the study of the effects of chemicals on species abundance, diversity and interactions (Newman and Clements 2008). The concept of biological classification of aquatic systems according to the degree of pollution was introduced by Richard Kolkwitz and Maximilian Marsson in their classical freshwater studies at the beginning of the twentieth century, developing the *Saprobic System* (Kolkwitz and Marsson, 1908, 1909). The application of the "bioindicator approach" to assess the spatial impact of pollution was applied to marine ecosystems by Reish (1955), who was the first to publish on the proliferation of a polychaete species (e.g. *Capitella capitata*) in marine sediments with elevated concentrations of organic matter. Because of its relative stability and due to the relatively long-life cycle of macrobenthic species, benthic communities are less sensitive to short variations of the physico-chemical water characteristics, compared for instance to the high sensitivity of planktonic communities (Reish 1986). Therefore, benthic communities are thought to be particularly suited to study the effects of marine pollution at medium-to-long term.

In a comprehensive review, Pearson and Rosenberg (1978) proposed the paradigm according to which organic matter enrichment can trigger the abundance of a few opportunistic species (typically polychaetes), which causes alterations in benthic community structure, by decreasing the diversity, abundance and biomass of macrobenthic species. In order to detect slight changes in the community structure caused by pollution in a given area a complete sample of indicator species is required. It is now generally agreed that this paradigm has universal application for environmental stress in the broad sense, and Gray (1989) shows that marked changes in communities do not only occur at high levels of impact and, for instance, both specific richness and diversity can increase with moderate levels of disturbance. Current approaches includes the calculation of several biotic and diversity indices, but are still based on the same principle. Increasing levels of stress will eliminate more sensitive species, and tolerant/opportunistic species will expand their ranges of distribution in absence of competition, dominating impacted communities. Although initially used to describe eutrophication processes, benthic community analyses have also been extensively used to monitor other stressors, e.g. the release of drilling muds around oil platforms (Gray *et al.*, 1999) and hypersedimentation due to mining activities (Olsgard & Hasle, 1993). There has however been extensive discussions, also within WGBEC, as to whether benthic community analysis are useful in the monitoring of pollution by environmental contaminants (WGBEC, 2006).

It should also be noted that although changes in populations or communities are relevant in terms of ecological significance, it is usually very difficult to distinguish the variation of community structure caused by environmental differences across localities or by anthropogenic (pollutants) factors, and natural factors vary and may co-vary with the contamination in different ways depending on the area.

More effort is therefore needed for the validation and application of these techniques in biomonitoring (efficiency, reliability, and cost-effectiveness). As we ascend in the organization level the ecological relevance increases, but specificity, rapidity of response and easiness of standardization as a routine technique for environmental monitoring decrease, and vice versa. The way forward for their application may arise in part from their combined use with biomarkers, which would help to establish the link between cellular and molecular specific changes and the effect at the individual/population level, and provide a mechanistic explanation of pollution effects. Integrated approaches such as the Sediment Quality Triad may help to establish the link between the presence of pollutants in the environment and their ecological effects, allowing to discern between the impact of pollution on biological communities from natural factors.

Another issue to consider is that changes in community structure due to pollution not only correspond to differences in sensitivity among individual species, that are manifested as direct responses of the community (e.g. loss of sensitive species, reduced species richness), but also to changes in the interactions between populations (competition, predation) that may cause indirect community responses. At low levels of environmental stress predation regulates community structure, at moderate levels of environmental stress competition is the main factor regulating community structure and at high levels of stress, pollution would be the factor driving the variation in community structure. More experimental work should then be devoted to the study of the effect of pollutants on species interactions. The relevance of community resistance and resilience to pollution as well as the role of 'keystone species' that present a great impact on the community, may also be considered.

ToR e – develop methods to evaluate effects of acute spills on marine organisms

All countries with a coastline have more or less detailed guidelines on how to evaluate effects of acute spills. Craig Robinson (UK) described strategies in England and Scotland. Information on the occurrence of spills is collated annually by the Advisory Committee on the Protection of the Seas (ACOPS) on behalf of the Maritime and Coastguard Agency (MCA) using data collected from the shipping, ports & harbours and offshore oil and gas industries. Monitoring the impacts of spills is implemented in a case-specific manner, depending upon the nature of the spill (including its size, the substance(s) involved and their chemical properties, toxicity, etc.).

In the event of spills occurring, the UK follows the National Contingency Plan (https://www.gov.uk/government/publications/national-contingency-planncp). UK competent agencies regularly test the Contingency Plan and their ability to respond to incidents via mock spill scenario exercises. Post-spill monitoring guidelines and procedures for initiating and coordinating monitoring and impact assessment surveys have been developed (www.cefas.co.uk/premiam) in order to respond to, and understand the environmental significance of, major spills.

Following on from the Macondo spill in the Gulf of Mexico, the Scottish Government has been working to develop hydrographic models to investigate how a spill in the deep water to the west of Scotland would behave, and has been putting in place procedures to allow the monitoring of any such incident, these are based on the Premiam guidelines (Law *et al.*, 2011); following a conference in June 2016, amended guidelines will be published in 2017.

Ecotoxicological monitoring is not an automatic requirement following a spill; the guidelines recommend a decision on whether to employ ecotoxicological monitoring be taken based upon the nature of the compound spilled and its mode of action, amongst other things. Bioassays may then be undertaken to examine effects/toxicity of the spill in the water, sediment and biota compartments. Recommended bioassays are those with guidelines and protocols, such as ICES TIMES papers and OSPAR background documents/technical annexes. Integrated approaches to assessing impacts are recommended.

Action: Approach WGBEC member James Readman to lead in the development of a guideline manuscript, combining European approaches, to be submitted by WGBEC 2018.

ToR f – develop methods to evaluate effects of ocean acidification on marine organisms

An update on ocean acidification was provided by Hrönn Egilsdóttir (IS).

ToR g – review interactions between essential nutrients or vitamins and contaminants in marine organisms

The main focus in the preceding two years have been on thiamine deficiency and retinol (vitamin A) metabolism. While there is still scope to investigate both aspects further, there are other aspects of nutrition toxicology that deserve attention. Two issues that will be addressed at the 2018 WGBEC meeting are nutrition toxicology in aquaculture and nutrition changes due to modified diet caused by e.g. climate change.

Action: WGBEC will follow up on nutrition toxicology in 2018, particularly issues relevant to aquaculture.

ToR h – review progress with marine plastic ecotoxicity to marine organisms

Members of WGBEC are involved with a range of different projects on macro- and microplastics, many of which will have relevant results to be reported to the group in 2018. Thomas Maes (UK) informed on a newly published method for identification of microplastics based on selective fluorescent staining using Nile Red (Maes *et al.*, 2017). Michiel

Kotterman (NL) informed on studies of how microplastics could affect PCB uptake in salmon.

The IFREMER host in 2018 is a leading researcher in the field. Aspects that will be addressed include links between contaminants and plastics, whether plasticisers leach sufficiently from plastic to result in significant exposure for marine species and the quality of exposure experiments.

Action: WGBEC will arrange a one-day workshop on plastics in marine ecosystems during the 2018 meeting.

ToR i – review and update knowledge of environmental interactions and combined stressors in marine ecosystems

The main input for this discussion came from Juan Bellas (ES). The issues were further discussed during the meeting, leading to a framework for a scientific paper to be coordinated by Ketil Hylland (NO).

One of the main problems which are currently limiting the applied use of biomarkers in routine monitoring, and their further implementation within a legislative context, is the interpretation of the results in terms of whether the alteration of the biological response is due to the presence of pollutants or to natural factors, i.e. **confounding factors**. For this reason, international guidelines recommend taking samples during the same season, either on late autumn/early winter, when mussels and fish are as far away from the spawning season as possible (e.g. OSPAR, 2010).

Environmental variables that affect biochemical, metabolic and physiological activities act as confounding variables that may alter the organisms' responses to pollution (Moriarty, 1999). Ignoring these factors will affect the discriminative capacity of biomarkers between the effects of marine pollution and the genetic (inter-individual) and environmental (seasonal) sources of variability.

There are sometimes no clear link between pollutant exposure and biological effects in e.g. mussel populations. A lack of relationship can be attributed to the presence of unmeasured pollutants, but confounding factors such as differences in food availability, age differences or the period of the reproductive cycle are obviously important (Koehler, 1989; Regoli, 1998; Viarengo *et al.*, 2007; Albentosa *et al.*, 2012; Bellas *et al.* 2014). Strong influence by non-pollutant variables makes it challenging to define the basal levels of biomarkers, limiting their usefulness (Coulaud *et al.* 2011). A more holistic approach, in which pollutants are not considered as the only source of variability for the biological responses of organisms, but as one of many variables, will need to be implemented to better understand the variance of the data, and to discern between effects due to the presence of pollutants or to other factors. Some such factors are predominantly external, such as salinity, food availability and temperature (see below), whereas other have an endogenous basis, e.g. variation in endocrine processes and gonad development (which may be triggered by external factors, of course).

Temperature is one of the most important determinants of the general physiology of ectotherms and its relevance for biological responses associated with pollutant exposure is clear (Viarengo *et al.*, 1998; Zippay and Helmuth, 2012). In fact, seasonal variation of biomarkers in ectotherm marine organisms has been partially explained as a result of temperature oscillation, and the activities of several biomarkers have been reported to be affected by temperature variations, masking the effect of pollution (Sleiderink *et al.*, 1995, Viarengo *et al.*, 1998; Jarque *et al.*, 2014). Laboratory experiments with animals acclimated to constant temperature may help to discern the effect of ambient temperature on the activity of these enzymatic biomarkers (e.g. Sleiderink and Boon 1996, Vidal-Liñán and Bellas 2013). Temperature can however affect the bioavailability of the toxicant due to changes in the kinetic reactions of chemicals (Leon *et al.*, 2004; Sokolova and Lanning, 2008) and thereby affect the induced biological response (Watkins and Simkiss, 1988). The combined exposure of ectotherm organisms to temperature and pollution stress has been reported to increase the sensitivity to toxicants of thermal-stressed organisms and decrease the thermal tolerance of pollution-stressed organisms (Sokolova and Lanning, 2008). The consequences of such interactions in the context of current global climate change, including acidification and increased CO_2 levels, need to be evaluated at different levels of biological organization (Sokolova and Lanning, 2008, Zippay and Helmuth, 2012).

Alongside temperature, **food availability** is probably the single environmental factor with the strongest influence on the condition of organisms. The intimate relationships among these factors in the natural environment make it difficult to understand how they separately affect biological responses to pollution in field studies. For instance, recent field studies conducted with mussels in large-scale monitoring programs confirmed that different trophic conditions in different areas caused a strong effect on molecular and physiological biomarkers, masking their responses to pollution (Albentosa *et al.*, 2012; Bellas *et al.*, 2014).

The effect of food availability in the bioaccumulation of pollutants has been evidenced in different studies. In general, bioaccumulation depends on the levels of the pollutant in the environment and on the incorporation of the pollutant into the tissues, which depends, among other factors, on the trophic characteristics of the area, reflected in the condition of the organisms, which is also affected by the reproductive cycles. Thus, the relationship between condition and bioaccumulation of contaminants has been established (Jørgensen *et al.*, 1999). Usually, this relationship is explained by the dilution effect associated with the high growth rates of well-fed organisms and/or by the reduction in tissular lipid concentration of food deprivated organisms (Jørgensen *et al.*, 1999). In laboratory studies with mussels, however, González-Fernández *et al.* (2015) reported that lower food absorption rates in well-fed mussels and autophagy in nutritionally-stressed mussels, may explain respectively lower and higher bioaccumulation than expected due to the level of pollutants in the environment. Autophagy may however allow for a more efficient removal of damaged proteins and facilitate detoxification, increasing pollution resistance in nutritionally-stressed organisms (Moore 2004). This challenges one of the key premises in environmental monitoring, that indicator organisms (*biomonitors*) accumulate pollutants in direct proportion to environmental concentrations. Biochemical and physiological biomarkers have also been found to be more affected by nutrition than by toxicant exposure, resulting in higher values in organisms under nutrition stress, whereas the effect of toxicant was not always evident, masked by nutrition (González-Fernández *et al.* 2015).

Variations in biological responses are not only affected by the amount of food available to organisms, but also to the food quality. The effects of such differences in biological responses to pollution have been relatively understudied. In a recent study, higher bioaccumulation was reported for mussels fed with diatoms, which was also related to an increase in biomarker responses, in comparison with mussels fed with dinoflagellates (González-Fernández *et al.* 2016a), pointing to food quality as a confounding factor of pollution effects.

The annual variation in the **reproductive and physiological cycle** of marine organisms is controlled by light (depending on the latitude), food abundance and temperature, all subject to seasonal cycling. Gonadal development is an energy-demanding process which consists of several steps from the accumulation of nutrients and the proliferation of the gonad to the spawning and a subsequent resting period (Giese and Pearse 1974). The availability of suitable food resources provides the necessary energy for maintenance and somatic growth of individuals, but also for the reproductive process. This process causes relevant changes on the biochemical composition of the organisms, mainly on the levels of lipids and carbohydrates, due to the accumulation of nutrient reserves needed to fuel gametogenesis. This natural cycle is also accompanied by physiological and metabolic variations that occur in different ways between males and females, affecting the levels the biological responses used to measure pollution effects. As a result, it has been recommended to conduct sampling for pollution monitoring purposes during the reproductive resting stage or when gametogenesis has a limited effect on the biological responses measured (Eggens *et al.*, 1995; Thain *et al.*, 2008).

Reproductive condition is therefore considered as a relevant confounding factor of biological responses to pollution, but has been usually investigated in terms of the seasonal variability of the biological responses in the field (e.g. Jiménez *et al.* 1990, Eggens *et al.* 1995, Sheehan and Power, 1999, Vidal-Liñán *et al.*, 2010, Nahrgang *et al.* 2013), being difficult to distinguish the effect from related factors such as temperature or food availability (quantity and quality). Laboratory studies with mussels have demonstrated that the effect of the reproductive status was greater than the effect of the toxicant in biochemical and physiological biomarkers, being their values higher at the reproductive stage than at the resting stage (González-Fernández *et al.* 2016b). Not only biological responses, but also the bioaccumulation of pollutants was found to be affected by the annual variation of the reproductive cycle, as a result of variations in the biochemical composition of the organisms, which favors or hinders the accumulation or purification of organic pollutants (Rantamäki 1997), with higher bioaccumulation of animals with the same nutritive condition observed during the gonadal resting period (González-Fernández *et al.*, 2016b).

The process of **ageing** has been linked to an increase in oxidative stress at a cellular level (Lesser, 2006). In mussels, this has been explained as a progressive decrease of the glutathione content (due to an increased rate of oxidation, increased degradation or decreased synthesis), which affects the activity of several antioxidant enzymes, with the consequent increase in the level of endogenous ROS and higher susceptibility of organisms to oxidative stress (Viarengo *et al.* 1991, Canesi and Viarengo, 1997). At the physiological level, a negative effect of age on feeding has also been reported for mussels, which ultimately caused decreased growth rates (Sukhotin *et al.* 2002, Sukhotin *et al.* 2003, Bellas *et al.* 2014). However, unlike other organisms, bivalves, as well as many other benthic invertebrates, do not present a limiting size in their growth, but they continue to increase their body size throughout their life (Sukhotin *et al.*, 2002). Since their physiological rates are size-dependent, a pattern of covariation with both factors (size and age) is expected.

Sampling individuals of the same size in monitoring programs is highly recommended. However, since mussels' growth rates are determined by the environmental characteristics of the sampling area, individuals of the same size but of different ages are being used for monitoring purposes in large-scale programs, and the age-factor is being disregarded when interpreting bioaccumulation and biological responses data.

Sex-related differences in pollution biomarkers have been relatively well documented in fish. For instance, EROD activities and cytochrome P4501A contents have been reported to be much higher in male fish, which has been associated with biochemical changes during maturation and spawning, attributable to hormonal factors (Stegeman *et al.* 1984, Lindström-Seppä *et al.* 1995). This biochemical and hormonal changes have been pointed out as a causal factor of sex differences in oxidative stress responses observed in fish (Winzer *et al.* 2001). Differences in metallothionein content and metal bioaccumulation have also been recorded, with females having significantly higher MT content than males (Hylland *et al.* 1992, Hylland *et al.* 1998).

Sex differences in bivalves have been reported as a major source of metabolic variability. It has been argued that biochemical differences are caused, at least in part, by sperm- and egg-associated structures within the mantle (Hines *et al.* 2007). As a result, Hines and co-workers expect considerable temporal changes in mantle metabolome as part of the annual reproductive cycle of mussels, particularly the storage and utilization of glycogen reserves. In this line, it may be assumed that other biological indicators such as oxidative stress also would present a great variability, and the sex of the analyzed specimens would need to be considered in order to interpret such variation. Sex-related differences in bivalves have been also described in situations of nutrient stress, in relation to the mobilization of energy reserves. In females, lipids remain constant during the starvation period until the final stage, whereas males consume their lipid reserves from the outset of the nutritive stress period (Albentosa *et al.* 2007). It would be therefore reasonable to assume important differences in biological responses to pollution stress if the catabolic processes that trigger nutritional stress are different in males than in females.

Action: Prepare a review manuscript to be submitted by the end of 2017, coordinated by Ketil Hylland (NO).

ToR j – review effects of emerging contaminants on marine organisms

WGBEC took particular note of the results reported under ToR c, i.e. how an "old" and non-emerging contaminant such as PCBs may cause "new" effects. It is clearly important not to forget possible effects of contaminants that have been present in marine ecosystems for decades.

ToR k – review the use of passive samplers and dosing in marine ecotoxicity studies

Craig Robinson (UK) presented a review of recent literature on the use of passive dosing in ecotoxicity studies for the group. Passive dosing (also described as partition controlled delivery) is a method of maintaining stable freely dissolved concentrations (C_{free}) of hydrophobic organic contaminants (HOCs) in aqueous toxicity tests (Mayer *et al.*, 1999; Kiparissis *et al.*, 2003). Classical tests of HOCs in which the test substance is dissolved/diluted in solvent and added to the aqueous test system suffer from sorbtive and evaporative losses and consequently unstable C_{free}. Since it is C_{free} (or more accurately *chemical activity*) that determines the toxicity of a substance, such losses mean that classical aqueous toxicity tests can underestimate the true toxicity of HOCs. In passive dosing, a polymer phase with a large sorbtive capacity is pre-loaded with the test substance(s) and added to the test system to act as a reservoir from which the HOC(s) partition into the water phase and establish stable C_{free} concentrations according to their polymer-water partition coefficients (K_{pw}). Recent review papers (Jahnke *et al.*, 2016a,b; Smith and Schaefer, 2016) describe how to perform toxicological tests using passive dosing approaches, including in combination with passive sampling of environmental matrices. These confirm the utility of passive dosing in maintaining controlled, stable and reproducible exposure concentrations of hydrophobic organic contaminants during *in vitro* toxicity tests and bioassays.

Some authors have attempted to couple passive sampling of environmental matrices with passive dosing in order to assess environmental risks due to the presence of HOCs via bioassay or cell-line toxicity studies. Limitations with this approach, particularly with respect to evaluating toxicity of passively sampled water phases, are recognised; principally these are in relation to whether sampling and dosing are conducted at equilibrium for all substances in the present in the sampled environment: passive sampling in the kinetic uptake phase results in alterations to the mixture exposure concentration scenario during passive dosing due to different K_{pw} values of compounds within the complex mixture of substances found in environmental compartments. Equilibrium conditions are more readily obtained when passive sampling sediments, sediment porewaters or biota than when sampling surface waters and thus the combination of PS and PD may be more useful in assessing the toxicity of HOCs present in these matrices.

To date, most studies have used PD to assess the toxicity of single compounds, or simple mixtures; most frequently using PAHs and often using the approach described by Smith *et al.* (2010) that utilises silicone rubber o-rings as the reservoir for dosing microtitre plates. Those authors demonstrated the approach to investigate oxidative stress and other endpoints in human cells and cell lines following passive dosing with PAHs. Buttler *et al.* (2013) demonstrated that passive dosing using silicone coated vials can be used to assess the toxicity of PAHs in fish embryo toxicity (FET) bioassays. Using silicone rubber sheets to obtain time-integrated samples of the water column in and offshore from 3 Belgian harbours Claessens *et al.* (2015) placed the silicone sheet samplers directly into culture flasks to passively dose marine phytoplankton bioassays and showed that 4 of 17 samples caused severely inhibited diatom growth, and ascribed this to compounds not routinely determined in the passively sampled locations.

Oostingh *et al.* (2015) examined the immunotoxicity of 9 PAHs found in the marine environment to human bronchial epithelial cells using a passive dosing approach. They showed that the most hydrophobic PAHs were the strongest inducers of immunotoxicity and that induction was often higher at lower exposure levels and decreased with increasing concentration, despite an absence of cytotoxicity.

Sediment HOC concentrations determined by classical methods do not inform on bioaccessibility and toxicity. Passive sampling provides information on C_{free} and the chemical activity of contaminants in sediments, allowing more accurate risk assessment. Rojo-Nieto and Perales (2015) demonstrated that the chemical activity of PAHs in three marine sediments with similar contaminant concentrations varied by 10-fold; although concentrations were classified as "moderately polluted", the determined chemical activities were below those at which baseline toxicity occurs. Multiplying C_{free} by biota-sediment accumulation factors (BSAFs) was used to estimate bioaccumulation into flatfish tissues.

Although using a rainbow trout cell-line, the study of Heger *et al.* (2016) of the toxicity of fossil and biofuels is of interest in the marine field when considering that increasing amounts of both fossil and biofuels are being transported around the globe. High volatility and incompatibility of the test substances with the usual test system required modifications to the test protocol; nonetheless, the authors demonstrated that the three tested biofuels did not cause induction of CYP1A (EROD) activity in trout liver cells and appeared to be less cytotoxic than traditional fossil fuels, although continuing issues with the exposure regimes meant that this was not definitive.

Jahnke *et al.* (2016b) recently reviewed approaches to quantitatively maintaining (or re-establishing) the chemical composition of the sampled water, sediment and biota when transferring the contaminants into bioassays using total extraction or polymer-based passive sampling combined with either solvent spiking or passive dosing. This review will provide a good guide to approaches to assessing the toxicity of marine matrices through the use of coupled passive sampling / passive dosing approaches.

Review the status of publications and consider requirements for new publications

Activities stemming from WGBEC has led to the publication of a special volume of Marine Environmental Research (Volume 124), edited by Ketil Hylland (current Chair) and Matthew Gubbins (former Chair). The volume described the ICON project and comprised 12 scientific papers. A TIMES document elicited by the group is currently under publication: Hanson *et al.* "Supporting variables for biological effects measurements in fish and blue mussel" (ICES TIMES 60). WGBEC did not identify requirements for new ICES TIMES documents.

Contact with OSPAR and other ICES WGs

HASEC 2015 granted MIME an opportunity to test the Integrated Guidelines developed during SGIMC and demonstrated as part of ICON (see publications), hence the practical application of biological effects monitoring techniques, including the issue of enhanced access to biological effects measurement data. Trial application of the OSPAR JAMP Integrated Guidelines for the Integrated Monitoring and Assessment of chemical Contaminants and biological effects was validated by HASEC in April 2016 (OSPAR Publication 2016-678). The implementation of the integrated guidelines depends on a regular and combined data transfer of chemical contaminants and biological effects data. The main challenge is to set up a combined data transfer of the mandatory data in chemical contaminants and the voluntary data in biological effects.

Two elements of progress were proposed during the last MIME 2016. First of all, some simplification of the classical ICES format 3.2 were done (e.g.: possibility to send the chemical contaminants analysed in pools of fish or mussels). Secondly, a simplified sheet ICES format 3.2 was widespread with the contribution of the MIME delegates and the chairmen of WGBEC, in 2017.

A presentation was received from Michiel Kotterman (NL) describing research in the Netherlands undertaken on the decline of eel populations and on the output of the joint WGBEC/ WGEEL workshop that took place in 2016. The WKBECEEL meeting discussed reasons for the observed declines in eel populations and in returns of young eels to Europe. The decrease in numbers of returning glass eels is larger than the decrease in the number of adult eels going to sea. Eels are lipid rich and are known to have high concentrations of many hydrophobic organic contaminants, especially in industrialised or heavily populated regions; many of these substances are known to be reproductive toxins.

The WKBECEEL workshop concluded with suggestions of future research (e.g. effect of POPs on reproduction), but with no obvious suggestion as to the cause(s) of declining returns. In discussion, WGBEC noted that it is not currently possible to test many of the hypotheses on why eel numbers are declining, since eels cannot be bred and reared successfully in captivity – no one has yet managed to get larval European eels to feed – and consequently there are no big projects planned looking at eel reproduction. WGBEC has remained in contact with WGEEL and there are loose threads following the WKBECEEL workshop in 2016 that will need to be tied up. Some effort towards that will be done before and during WGBEC 2018.

Action: To compare with other species, WGBEC requests data from the ICES data centre for data for three marine fish species (dab, flounder, cod) for as many years and regions as possible as follows: body wt, liver wt, gonad wt, gender, length, age, liver lipid, muscle lipid.

AQC activities for biological effect methods

An intercalibration for selected biological effects methods, i.e. EROD, AChE and micronucleus, is underway and will be performed early autumn 2017. Co-ordinators are Bjørn Einar Grøsvik (NO) and Steve Brooks (NO).

6 Revisions to the work plan and justification

No paper will be prepared for ToR d – effects of contaminants on community composition. Contributors from WGBEC are already overcommitted with other manuscripts.

7 Next meetings

WGBEC will meet on 9–13 April 2018 in Calvi, Corsica, France.

Annex 1: List of participants

Name	Address	Phone/Fax	Email
Steven Brooks	Norwegian Institute for Water Research, Gaustadalléen 21, NO-0349 Oslo, Norway	+47 92696421	sbr@niva.no
Thierry Burgeot	IFREMER, rue de l'Ile d'Yeu, B.P. 21105, F-44311 Nantes, Cédex 03, France	+33 240374051 +33 240374075	tburgeot@ifremer.fr
Bjørn Einar Grøsvik (Chair)	Institute of Marine Research P.O. Box 1870 Nordnes N-5817 Bergen, Norway	+47 93412 859	bjorn.grosvik@imr.no
Halldor P. Halldorsson	The Univ. of Iceland's Research Centre in Sudurnes, Gardvegur 1 245 Sandgerdi, Iceland	354-5255226	halldor@hi.is
A. Dick Vethaak	Deltares, P.O. Box 177, 2600 MH Delft The Netherlands	+31 (0)88 335 80 59	Dick.Vethaak@deltares.nl
Ketil Hylland (Chair)	Department of Biosciences, University of Oslo, PO Box 1066, Blindern, N-0316 Oslo Norway	+47 222857315	ketil.hylland@ibv.uio.no
Andrea Johnson	National Science Foundation, 4201 Wilson Blvd, Arlington, VA 22230, US	703-292-5164	andjohn@nsf.gov
Craig Robinson	Marine Scotland Science Marine Laboratory 375 Victoria Road Aberdeen AB30 1AD, UK	+44(0)1224 295469	craig.robinson@scotland.gsi.gov.uk
Jörundur Svavarsson	Institute of Biology, University of Iceland, 101 Reykjavik, Iceland	354-5254610	jorundur@hi.is
Michiel Kotterman	Wageningen Marine Research PO Box 68 1970 AB Ijmuiden, The Netherlands	+31317487132	michiel.kotterman@wur.nl
Thomas Maes	Centre for Environment Fisheries and Aquaculture Science (Cefas), Pakefield Road NR330HT Lowestoft, UK	+44 (0)1502 524433	thomas.maes@cefas.co.uk

Rune Dietz	Århus University, Frederiksborgvej 399, PO Box 358, DK-4000 Roskilde, Denmark	(+45) 8715-5000	rdi@bios.au.dk
Paul Jepson	Institute of zzology, Zoological Society of London, Regent's Park, London NW1 4RY, UK	+44 (0)20 74449 6691	Paul.jepson@ioz.ac.uk

Annex 2: Recommendations

Recommendation	Adressed to
1. WGBEC are deeply concerned about the PCBs loads on killer whales and other toothed whale populations in the North Atlantic since they appear to have devastating consequences. WGBEC invites the Regional Seas Conventions to take note of these serious findings and encourage member countries to dramatically increase the rate of clean-up of contaminated landfills, rivers, estuaries and coastal areas. Marine mammals and seabirds appear to be sensitive components of marine ecosystems and the conventions are invited to include relevant components in ongoing monitoring programmes.	Regional Seas Conventions
2. WGBEC requests data from the ICES Data Centre for data for three marine fish species (dab, flounder, cod) for as many years and regions as possible as follows: body wt, liver wt, gonad wt, gender, length, age, liver lipid, muscle lipid.	ICES Data Centre

Annex 3: References to ToRs

ToR a

Holth TF, Storset A, Ribeiro AL, Ólafsdóttir Á, Halldórsson HP, Hylland K. Environmentally realistic exposure to weathered North Sea oil: sublethal effects in Atlantic cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*). J Toxicol Environ Health (in press).

Pampanin DM, Brooks S, Grøsvik BE, Le Goff J, Meier S, Sydnes MO. DNA adducts in marine fish as biological marker of genotoxicity in environmental monitoring: The way forward. Marine Environmental Research In press. DO: 10.1016/j.marenvres.2017.01.002.

Sørhus E, Incardona JP, Furmanek T, Goetz GW, Scholz NL, Meier S, Edvardsen RB Jentoft S. 2017. Novel adverse outcome pathways revealed by chemical genetics in a developing marine fish. eLife 2017; 6:e20707.

ToR b

Haarr, A, Eckbo, N, Hylland, K, Herzke, D, Gabrielsen, GW, Bustnes, JO, Borgå, K. DNA damage in Arctic avian predators: baseline, sensitivity to damage and association to contaminant exposure. in prep.

Keilen, E.K. 2017. Levels and effects of environmental contaminants in herring gull (*Larus argentatus*) from an urban and a rural colony in Norway, MSc thesis, Department of Biosciences, University of Oslo, 105 p.

ToR c

De Guise, S., Ross, P.S., Osterhaus, A.D.M.E., Martineau, D., Beland, P., Fournier, M., 1997. Immune functions in beluga whales Delphinupterus Zeucas: evaluation of natural killer cell activity. Vet. Immunol. Immunopathol. 58, 345–354.

Dietz R, Eulaers I, Desforges JP, Sonne C, 2016. Deeper insights to PCBs in orcas. Science 24 July 2016, E-letter. http://science.sciencemag.org/content/352/6292/1388.e-letters

Jepson, P.D., Deaville, R., Barber, J.L., Aguilar, À., Borrell, A., Murphy, S., Barry, J., Brownlow, A., Barnett, J., Berrow, S., Cunningham, A.A., Davison, N.J., ten Doeschate, M., Esteban, R., Ferreira, M., Foote, A.D., Genov, T., Giménez, J., Loveridge, J., Llavona, Á., Martin, V., Maxwell, D.L., Papachlimitzou, A., Penrose, R., Perkins, M.W., Smith, B., de Stephanis, R., Tregenza, N., Verborgh, P., Fernandez, A., Law, R.J., 2016. PCB pollution continues to impact populations of orcas and other dolphins in European waters. Sci. Rep. 6, 18573.

Jepson, P.D. and Law, R.J. (2016) Persistent pollutants, persistent threats: Polychlorinated biphenyls remain a major threat to marine apex predators such as orcas. *Science* **352**: 1388-1389. **doi**: 10.1126/science.aaf9075

Mori, C., Morsey, B., Levin, M., Nambiar, P.R., De Guise, S., 2006. Immunomodulatory effects of in vitro exposure to organochlorines on T-cell proliferation in marine mammals and mice. J. Toxicol. Environ. Health. A 69, 283–302.

Ross, P.S., 2002. The Role of Immunotoxic Environmental Contaminants in Facilitating the Emergence of Infectious Diseases in Marine Mammals. Hum. Ecol. Risk Assess. An Int. J. 8, 277–292.

ToR d

Gray J.S. (1992). Eutrophication in the sea. In: Colombo G., Ferrari I., Ceccherelli V., Rossi R. (Eds.) Marine eutrophication and population dynamics. Olsen & Olsen, Fredensborg, 25th EMBS, pp. 3-15.

Gray, John S., Torgeir Bakke, Hans Jakob Beck, and Ingunn Nilssen (1999) Managing the Environmental Effects of the Norwegian Oil and Gas Industry: From Conflict to Consensus. Mar Pollut Bull 38: 525–530.

Kolkwitz, R., Marsson, M. (1908). Ökologie per pflanzlichen Saprobien. Festschrift zur Feier des, 25.

Kolkwitz, R., Marsson, M. (1909). Ökologie der tierischen Saprobien. Beiträge zur Lehre von der biologischen Gewässerbeurteilung. Internat. Rev. Ges. Hydrobiol, 2(1-2), 126-152.

Newman, M. C., & Clements, W. H. (2007). Ecotoxicology: a comprehensive treatment. cRc Press.

Olsgard, Frode, and Jon Rytter Hasle. (1993) Impact of Waste from Titanium Mining on Benthic Fauna. J Exp Mar Biol Ecol 172: 185–213.

Pearson, TH, Rosenberg, R (1978). Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr Mar Biol Ann Rev. 16: 229-311.

Reish, DJ (1955). The relation of polychaetous annelids to harbor pollution. Public Health Rep 70: 1168-1174.

Reish, D. J. (1986). Benthic invertebrates as indicators of marine pollution: 35 years of study. Proceed IEEE Oceans '86 Conference, pp. 885-888. Washington.

ToR e

Law, R.J., Kirby, M.F., Moore, J., Barry, J., Sapp, M. and Balaam, J., 2011. *PREMIAM – Pollution Response in Emergencies Marine Impact Assessment and Monitoring: Post-incident monitoring guidelines.* Science Series Technical Report, Cefas, Lowestoft, 146: 164 p.

ToR f

ToR g

ToR h

Maes T, Jessop R, Wellner N, Haupt K, Mayes AG. 2017. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. Scientific Reports 7, Art no: 44501.

ToR i

Albentosa, M., Fernández-Reiriz, M. J., Labarta, U., Pérez-Camacho, A. (2007). Response of two species of clams, *Ruditapes decussatus* and *Venerupis pullastra*, to starvation: physiological and biochemical parameters. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 146, 241-249.

Albentosa, M., Viñas, L., Besada, V., Franco, M.A., González-Quijano, A. (2012). First measurements of the scope for growth (SFG) in mussels from a large scale survey in the North-Atlantic Spanish coast. Sci. Tot. Environ. 435-436, 430-445.

Bellas, J., Albentosa, M., Vidal-Liñán, L., Besada, V., Franco, M. Á., Fumega, J., González-Quijano, A., Viñas, L., Beiras, R. (2014). Combined use of chemical, biochemical and physiological variables in mussels for the assessment of marine pollution along the N-NW Spanish coast. Marine Environmental Research, 96, 105-117.

Canesi L., Viarengo, A. (1997). Age-related differences in glutathione metabolism in mussel tissues (*Mytilus edulis* L.). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 116, 217-221.

Coulaud, R., Geffard, O., Xuereb, B., Lacaze, E., Quéau, H., Garric, J., & Chaumot, A. (2011). *In situ* feeding assay with *Gammarus fossarum* (Crustacea): modelling the influence of confounding factors to improve water quality biomonitoring. Water Research, 45, 6417-6429.

Davies, I.M., Vethaak, A.D. (Eds.) (2012). Integrated monitoring of chemicals and their effects. Chapter 30. ICES Cooperative Research Report 315, 209-212.

Eggens, M., Bergman, A., Vethaak, D. (1995). Seasonal variation of hepatic EROD activity in flounder (Platichthys flesus) in the Dutch Wadden Sea. Marine Environmental Research, 39, 231-234.

Giese A.C., Pearse J.S. (1974). Introduction: General principles. Reproduction of Marine Invertebrates Vol. 1, Acoelomate and Pseudocoelomate Metazoans (A. C. Giese and J. S. Pearse, eds.), pp. 1-49. Academic Press, New York.

González-Fernández C., Albentosa M., Campillo J.A., Viñas L, Romero D., Franco M.A., Bellas J. (2015a). Effect of nutritive state on *Mytilus galloprovincialis* pollution biomarkers: Implications for large-scale monitoring programmes. Aquatic Toxicology. 167, 90-105.

González-Fernández, C., Lacroix, C., Paul-Pont, I., Le Grand, F., Albentosa, M., Bellas, J., Viñas, L., Campillo, J.A., Hégaret, H., Soudant, P. (2016a). Effect of diet quality on mussel biomarker responses to pollutants. Aquat. Toxicol. 177, 211-225.

González-Fernández, C., Albentosa, M., Campillo, J. A, Viñas, D., Franco, A., Bellas, J. (2016b). Effect of mussel reproductive status on pollution biomarkers: Implications for large-scale monitoring programs. Aquat. Toxicol. 177, 380-394.

Hines, A., Oladiran, G. S., Bignell, J. P., Stentiford, G. D., Viant, M. R. (2007). Direct sampling of organisms from the field and knowledge of their phenotype: key recommendations for environmental metabolomics. Environmental Science & Technology, 41, 3375-3381.

Hylland, K., Haux, C., & Hogstrand, C. (1992). Hepatic metallothionein and heavy metals in dab Limanda limanda from the German Bight. *Marine Ecology Progress Series*, 91, 89-96.

Hylland, K., Nissen-Lie, T., Christensen, P. G., Sandvik, M. (1998). Natural modulation of hepatic metallothionein and cytochrome P4501A in flounder, *Platichthys flesus* L. Marine Environmental Research, 46(1-5), 51-55.

Jarque, S., Prats, E., Olivares, A., Casado, M., Ramón, M., Piña, B. (2014). Seasonal variations of gene expression biomarkers in *Mytilus galloprovincialis* cultured populations: Temperature, oxidative stress and reproductive cycle as major modulators. Science of The Total Environment, 499, 363-372.

Jiménez, B.D., Oikari, A., Adams, S.M., Hinton, D.E., McCarthy, J.F. (1990). Hepatic enzymes as biomarkers: interpreting the effects of environmental, physiological, and toxicological variables. In: McCarthy, J.F., Shugart, L.R. (Eds.), Biomarkers of Environmental Contamination. Lewis Pubishers, pp. 123-142.

Jørgensen, E.H., Bye, B.E., Jobling, M. (1999). Influence of nutritional status on biomarker responses to PCB in the arctic charr (*Salvelinus alpinus*). Aquat. Toxicol. 44, 233-244.

Kiørboe, T. (1993). Turbulence, phytoplankton cell size, and the structure of pelagic food webs. Advances in Marine Biology, 29, 1-72.

Koehler, A. (1989). Cellular effects of environmental contamination in fish from the River Elbe and the North Sea. Responses to marine organisms to pollutants. Mar. Environ. Res. 28, 417-424.

Leon, V.M., Gómez-Parra, A., González-Mazo, E. (2004). Biodegradation of linear alkylbenzene sulfonates and their degradation intermediates in seawater. Environ. Sci. Technol. 38, 2359–67.

Lesser, M. P. (2006). Oxidative stress in marine environments: biochemistry and physiological ecology. Annu. Rev. Physiol., 68, 253-278.

Lindström-Seppä, P., Stegeman, J. J. (1995). Sex differences in cytochrome P4501A induction by environmental exposure and β-naphthoflavone in liver and extrahepatic organs of recrudescent winter flounder. Marine Environmental Research, 39(1), 219-223.

Moore, M. N. (2004). Diet restriction induced autophagy: a lysosomal protective system against oxidative-and pollutant-stress and cell injury. Marine Environmental Research, 58(2), 603-607.

Moriarty, F. (1999). Ecotoxicology e the Study of Pollutants in Ecosystems, third ed. Academic Press, London.

Nahrgang, J., Brooks, S.J., Evenset, a, Camus, L., Jonsson, M., Smith, T.J., Lukina, J.,Frantzen, M., Giarratano, E., Renaud, P.E. (2013). Seasonal variation inbiomarkers in blue mussel (*Mytilus edulis*), Icelandic scallop (*Chlamys islandica*) and Atlantic cod (*Gadus morhua*): implications for environmental monitoringin the Barents Sea. Aquat. Toxicol. 127, 21-35.

OSPAR Commission (2010). JAMP guidelines for monitoring contaminants in biota, (agreement 1999–2).

Rantamäki, P. (1997). Release and retention of selected polycyclic aromatic hydrocarbons (PAH) and their methylated derivatives by the common mussel (*Mytilus edulis*) in the brackish water of the Baltic Sea. Chemosphere, 35(3), 487-502.

Regoli, F. (1998). Trace metals and antioxidant enzymes in gills and digestive gland of the Mediterranean mussel *Mytilus galloprovincialis*. Arch. Environ. Contam. Toxicol. 34, 48-63.

Sheehan, D., Power, A. (1999). Effects of seasonality on xenobiotic and antioxidant defence mechanisms of bivalve molluscs. Comp. Biochem. Physiol. Part C Pharmacol. Toxicol. Endocrinol. 123, 193–199.

Sleiderink, H.M., Boon, J.P. (1996). Temporal induction pattern of hepatic cytochrome P450 1A in thermally acclimated dab (*Limanda limanda*) treated with 3,3’,4,4’-tetrachlorobiphenyl (CB77). Chemosphere 32, 2335-2344.

Sleiderink, H.M., Beyer, J., Scholtens, E., Goksøyr, A., Nieuwenhuize, J., van Liere, J.M., Everaarts, J.M., Boon, J. (1995). Influence of temperature and polyaromatic contaminants on CYP1A levels in North Sea dab (*Limanda limanda*). Aquat. Toxicol. 32, 189-209.

Sokolova, I. M., Lannig, G. (2008). Interactive effects of metal pollution and temperature on metabolism in aquatic ectotherms: implications of global climate change. Climate Research, 37 (2-3), 181-201.

Stegeman, J. J., Woodin, B. R. (1984). Differential regulation of hepatic xenobiotic and steroid metabolism in marine teleost species. Marine Environmental Research, 14(1-4), 422-425.

Sukhotin, A.A., Abele, D., Pörtner, H.O. (2002). Growth, metabolism and lipid peroxidation in *Mytilus edulis* L.: age and size effects. Mar. Ecol. Prog. Ser. 226, 223-234.

Sukhotin, A.A., Lajus, D.L., Lesis, P.A. (2003). Influence of age and size on pumping activity and stress resistance in the marine bivalve *Mytilus edulis* L. J. Exp. Mar. Biol. Ecol. 284, 129-144.

Thain, J. E., Vethaak, A. D., Hylland, K. (2008). Contaminants in marine ecosystems: developing an integrated indicator framework using biological-effect techniques. ICES Journal of Marine Science: Journal du Conseil, 65(8), 1508-1514.

Viarengo, A., Abele, D., Burlando, B. (1998). Effects of low temperature on prooxidant processes and antioxidant defence systems in marine organisms. Cold ocean physiology (HO Pörtner, R Playle, eds) Cambridge Univ. Pr, Cambridge, 212-235.

Viarengo A., Canesi L., Pertica M., Livingstone D. R., Orunesu M. (1991). Age-related lipid peroxidation in the digestive gland of musselssThe role of the antioxidant defense systems. Experientia, 47, 454-457.

Viarengo, A., Lowe, D., Bolognesi, C., Fabbri, E., Koehler, A. (2007). The use of biomarkers in biomonitoring: A 2-tier approach assessing the level of pollutant induced stress syndrome in sentinel organisms. Comp. Biochem. Phys. C, 146, 281-300.

Vidal-Liñán, L., Bellas, J. (2013). Practical procedures for selected biomarkers in mussels, *Mytilus galloprovincialis* - Implications for marine pollution monitoring. The Science of the Total Environment, 461, 56-64.

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

Watkins, B., Simkiss, K. (1988). The effect of oscillating temperatures on the metal ion metabolism of *Mytilus edulis*. Journal of the marine biological association of the United Kingdom, 68, 93-100.

Winzer, K., Winston, G. W., Becker, W., Van Noorden, C. J., & Köehler, A. (2001). Sex-related responses to oxidative stress in primary cultured hepatocytes of European flounder (Platichthys flesus L.). Aquatic Toxicology, 52, 143-155.

Zippay, M. L., Helmuth, B. (2012). Effects of temperature change on mussel, *Mytilus*. Integrative Zoology, 7, 312-327.

ToR j

ToR k

Buttler, J.D., Parkerton, T.F., Letinski, D.J., Bragin, G.E., Lampi, M.A., and Cooper, K.R. 2013. A novel passive dosing system for determining the toxicity of phenanthrene to early life stages of zebrafish. *Science of the Total Environment,* **463–464,** 952–958

Claessens, M., Monteyne, E., Wille, K., Vanhaecke, L., Roose, P., and Janssen, C.R. 2015. Passive sampling reversed: Coupling passive field sampling with passive lab dosing to assess the ecotoxicity of mixtures present in the marine environment. *Marine Pollution Bulletin,* **93,** 9–19.

Jahnke, A., Mayer, P., Schäfer, S., Witt, G., Haase, N., and Escher, B.I. 2016a. Strategies for transferring mixtures of organic contaminants from aquatic environments into bioassays. *Environ. Sci. Technol.*, ***50*,** 5424–5431.

Jahnke, A., Witt, G., Schäfer, S., Haase, N., and Escher, B.I. 2016b. Combining passive sampling with toxicological characterization of complex mixtures of pollutants from the aquatic environment. *Advances in Biochemical Engineering/Biotechnology,* August 2016. DOI: 10.1007/10_2015_5014

Kiparissis, Y., Akhtar, P., Hodson, P. V. and Brown, R. S., 2003. Partition-controlled delivery of toxicants: A novel in vivo approach for embryo toxicity testing. *Environmental Science & Technology,* **37,** 2262-2266.

Mayer, P., Wernsing, J., Tolls, J., de Maagd, P. G. J. and Sijm, D., 1999. Establishing and controlling dissolved concentrations of hydrophobic organics by partitioning from a solid phase. *Environmental Science & Technology,* **33,** 2284-2290.

Oostingh, J.G., Smith, K.E.C., Tischler, U., Radauer-Preiml, I., and Mayer, P. 2015. Differential immunomodulatory responses to nine polycyclic aromatic hydrocarbons applied by passive dosing. *Toxicology in Vitro,* **29,** 345–351

Rojo-Nieto, E. and Perales, J.A. 2015. Estimating baseline toxicity of PAHs from marine chronically polluted sediments and bioaccumulation in target organs of fish hypothetically exposed to them: a new tool in risk assessment. *Environmental Science: Processes & Impacts,* **17,** 1331-1339.

Smith, K. E. C., Oostingh, G. J. and Mayer, P., 2010. Passive dosing for producing defined and constant exposure of hydrophobic organic compounds during *in vitro* toxicity tests. *Chemical Research in Toxicology,* **23,** 55-65

Smith, K.E.C. and Schaefer, S. 2016. Defining and controlling exposure during in vitro toxicity testing and the potential of passive dosing. *Advances in Biochemical Engineering/Biotechnology*, July 2016. DOI: 10.1007/10_2015_5017.