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

16–20 April 2018

Calvi, Corsica, France

ICES
International Council for the Exploration of the Sea

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

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Executive summary

The Working Group on Biological Effects of Contaminants (WGBEC), chaired by Bjørn Einar Grøsvik, Norway, and Ketil Hylland, Norway, met at Stareso marine station, Calvi, Corsica, 16–20 April 2018. There were 12 attendees through the week, including 2 by correspondence, representing 7 countries.

WGBEC aims to address effects of chemical stressors on the marine environment, as well as 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 describing marine environmental quality.

WGBEC is in continuous communication with OSPAR HASEC on the implementation of an integrated monitoring framework by OSPAR HASEC. A simplified reporting format has increased the volume of effect data submitted to the ICES data bank, required for OSPAR assessments.

WGBEC has earlier focused on how contaminants affect marine invertebrates and fish, including pelagic and sediment-dwelling species. WGBEC has expanded its activities to encompass issues relevant to human health, in addition to addressing contaminant effects on seabirds and marine mammals. The overall aim is to address ecosystem-wide impacts of contaminants. Seabirds are important components of marine ecosystems and there are substantial knowledge gaps on how they are affected by contaminants. Marine mammal ecotoxicology was addressed in a dedicated session including invited researchers.

WGBEC members have earlier produced guidelines for monitoring acute oil spills (Martinez-Gomez *et al.*, 2010). WGBEC members are currently working on a review on monitoring of chronic oil spill effects to be submitted by the end of 2018, led by Bjørn Einar Grøsvik (NO).

Pathologies have been associated with apparent changes in vitamin or vitamin-precursor availability or metabolism in marine ecosystems (thiamine, vitamin A). The extent to which contaminant exposure is involved is however not clear and there is a need to further explore links between deficiencies in essential nutrients or vitamins and contaminant exposures.

WGBEC members are heavily involved in research and monitoring of macro- and microplastics. WGBEC members have contributed to a number of scientific papers on the subject through the period and members of the group retain important international positions in the field. Other ICES groups are currently addressing this issue and further involvement of WGBEC needs to take other activities into account.

Environmental interactions is an active research area for WGBEC members and required for a holistic understanding of environmental processes relevant to toxicity. A scientific paper on interactions led by Ketil Hylland and co-authored by WGBEC members will be submitted to a special volume of Frontiers in Marine Science by the autumn 2018.

WGBEC has elicited and produced three TIMES documents during the three-year period. In addition, a full volume of the peer-reviewed journal Marine Environmental Research, 10 papers in total, was dedicated to papers from the ICON project, which originated from

WGBEC with authorship mainly drawn from members of the group. The volume was edited by Ketil Hylland (NO) and Matt Gubbins (Scotland). In addition, members of the group have contributed to a range of research papers on environmental assessment and monitoring, effects of oil and plastics, seabirds and marine mammals during the reporting period.

An intercalibration for selected biological effects methods, i.e. PAH metabolites, EROD, AChE and micronucleus (in mussel and/or fish), is underway and will be performed during the autumn 2018. Coordinators are Bjørn Einar Grøsvik (NO) and Steven Brooks (NO).

1 Administrative details

Working Group name
Working Group on Biological Effects of Contaminants (WGBEC)
Year of Appointment within current cycle
2016
Reporting year within current cycle (1, 2 or 3)
3
Chair(s)
Bjørn Einar Grøsvik, Norway
Ketil Hylland, Norway
Meeting venue(s) and dates
11–12 March 2016, Lisbon, Portugal, (10)
11–12 March 2017, Reykjavik, Iceland, (13)
16–20 April 2018, Calvi, France (10 + 2 by correspondence)

2 Terms of Reference

ToR a - review effects of chronic oil exposure on marine organisms.

ToR b - review available studies on marine seabird ecotoxicology.

ToR c - review available studies on marine mammal ecotoxicology.

ToR d - review effects of contaminants on community composition.

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

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

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

ToR h - review progress with marine plastic ecotoxicity to marine organisms.

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

ToR j - review effects of emerging contaminants on marine organisms.

ToR 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 a status report for ocean acidification.

4 Summary of Achievements of the WG during 3-year term

- Through a decade-long process, WGBEC members in collaboration with OSPAR developed a framework for integrated contaminant monitoring and assessment, then launched the project ICON to test it out in European waters, from Spain to Iceland. The ten scientific papers reporting the results were published in a dedicated volume of the journal Marine Environmental Research in 2017.
- Communication has been maintained with OSPAR on the implementation of the integrated contaminant monitoring and assessment framework developed with significant contributions from WGBEC, including facilitation of effect data entry into ICES databanks for subsequent retrieval for OSPAR evaluations.
- Two TIMES publications to support ongoing development and implementation of effects in monitoring and assessment were produced with contributions from WGBEC members on supporting physiological parameters (Hanson *et al.*, 2017) and a widely used effect method, lysosomal membrane stability (Martinez-Gomez *et al.*, 2016).
- Members of WGBEC has contributed significantly to develop methods to quantify sublethal effects on seabirds and marine mammals. The group has highlighted the very serious contaminant-related population declines of toothed whales in European waters, in particular the killer whale.

5 Final report on ToRs, workplan and Science Implementation Plan

References to the text in each ToR can be found at Annex 5.

5.1 WGBEC and human health

WGBEC is currently extending its activities to also encompass issues relevant to human health.

Dick Vethaak (NL) suggested including the integration and qualification of risks to humans from marine contaminants or particles as a necessary way forward. Several books and scientific papers have been published in recent years suggesting that the marine environment and human health are inextricably linked, primarily through ecosystem health and ecosystem services. Contaminants/pollution is one of the human pressures on marine ecosystem health resulting in human health impacts. In addition to direct effects, chemical pollutants can decrease the resilience of marine ecosystems, affect sea food security production/ resources, and may ultimately contribute to a loss of biodiversity. WGBEC agreed to further explore the opportunities to include the human health link in future work. Several analytical and biological effect methods suggested by the ICES community can be used to establish links with human health. Furthermore, a link between marine contaminants and human health is also apparent from human consumption of marine foodstuffs contaminated with endocrine disrupting chemicals and their contribution to epigenetic diseases in humans.

A position paper is being prepared to clarify the above aspects, to be submitted by the end of 2018, led by Dick Vethaak (NL).

5.2 ToR a) review effects of chronic oil exposure on marine organisms

WGBEC members are involved in a range of activities addressing how oil or oil-related components affect marine organisms. Oil pollution is one of the major chemical and physical challenges for coastal (and to some extent offshore) marine ecosystems. Chronic oil exposure can stem from operational discharges exemplified from the offshore oil and gas exploration in the North Sea or from large oil spills where unrecovered oil is retained in the sediments or in the littoral zone and leak out to the environment over a longer period.

WGBEC has contributed to developing guidelines for OSPAR and individual countries concerning monitoring, as well as performing research (see the BECPELAG special volume, Hylland *et al.*, 2006). Members of WGBEC have earlier produced a guideline for acute oil spill monitoring (Martinez-Gomez *et al.*, 2010). Although it has been shown that early life stages of fish are particularly susceptible to oil pollution, there is limited understanding of the extent to which such pollution impacts natural fish populations and virtually no knowledge of effects on invertebrate early life stages.

A study performed at the Sandgerdi field station (Iceland) highlighted major species differences (Atlantic cod, turbot) in their response to exposure to water soluble components of oil (Holth *et al.*, 2017). A recent study has 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).

Juan Bellas (ES) provided a brief review for the group on pelagic and benthic consequences of oil spills. Natural variability seems to be the main obstacle to accurately determining the effects of oil spills on planktonic organisms (e.g. seasonal variability, mobility of water bodies, patched distribution of plankton). In general, it is assumed that zooplankton is more sensitive to oil pollution than phytoplankton, although there are contradictory results. Phytoplankton recovers rapidly from oil spills due to several factors such as its high regenerative potential, recruitment of adjacent non-contaminated areas, and the decrease in consumption by zooplankton.

In general, it is challenging to detect effects in pelagic invertebrate communities after an oil spill, and the structure of the phytoplankton and zooplankton populations are not normally affected. However, not many studies have been published in which biological studies made after an oil spill are contrasted with historical data. For instance, a study carried by Varela *et al.* (2006) compared chlorophyll a and primary production data obtained in the area affected after the *Prestige* oil spill with temporal series. According to this study, no changes in primary production or phytoplankton biomass were observed, compared with previous years. The same study also concludes that no alterations were observed in the monthly variation of the zooplankton biomass on the coast of Vigo and A Coruña, after the *Prestige* accident. In any case, it cannot be assured whether the oil actually affects or not the invertebrate plankton communities through this type of field studies, since the extreme variability of plankton masks the effect of oil components.

The ecotoxicological effects of environmental samples of water and sediments, as well as the fuel itself, collected in affected areas, have been tested. For example, the exposure of mussel (*Mytilus galloprovincialis*) and sea urchin (*Paracentrotus lividus*) embryos to the water-soluble fraction of the *Prestige* fuel shows a decrease in the percentage of normal larvae and in the larval growth at increasing concentrations of water soluble fraction, after 48 hours exposure (Saco-Álvarez *et al.* 2008, Bellas *et al.* 2013). The toxicity of the water-soluble fraction observed in laboratory is comparable to the toxicity of natural samples of seawater collected in affected coastal areas to sea urchin and clam (*Venerupis rhomboideus*) embryos (Mariño-Balsa *et al.* 2003, Beiras and Saco-Álvarez 2006).

Benthic organisms are prone to being coated with oil, leading to asphyxiation and acute toxicity from exposure to its components. This generally leads to high mortalities of macroalgae and invertebrates such as coelenterates, crustaceans, echinoderms and mollusks. The pattern of succession after an oil spill includes the disappearance of the dominant herbivores and the colonization of the substrate by green algae. These changes usually last about 4-5 years. On the other hand, when the oil is buried in the sediment, it can continue to be a source of toxicity for many years, causing chronic effects on the ecosystem, as in Alaska after the Exxon Valdez oil spill (Peterson *et al.* 2003).

Usually, the upper intertidal is the most affected area, with large increases in non-colonized substrate and large mortality of characteristic benthic species. Thus, after the Prestige oil spill, important mortalities of cirripeds, mussels, sea urchins or limpets were observed in the Galician coast, as well as a decrease in the biomass and diversity of macroalgae communities (Penela-Arenaz *et al.* 2009). Studies of biomarkers on mussels showed molecular and cellular alterations after the *Prestige* oil spill, indicating exposure to chemical contaminants, with a certain degree of recovery in the year 2004 associated with a reduction in the concentration of hydrocarbons in mussel tissues (e.g. Orbea *et al.* 2006, Marigomez *et al.* 2006).

de la Huz *et al.* (2005) observed a decline in the number of species in 16 of 18 beaches studied 6 months after the Prestige oil spill, and found a relationship between the degree of pollution and the number of species. The most affected beaches lost about 70% of species richness, compared with data from 1995 and 1996. The authors indicate that differences are not due to seasonal variation. Some species such as bivalves of the genus *Donax* or isopods of the genus *Sphaeroma* have disappeared from beaches where their presence was consistent before the spill.

It must however be kept in mind that many effects are not only caused by oil, but also are due to cleaning operations. In fact, although the beaches usually suffer a strong impact, this is not only caused by the large amount of fuel received, but also for sand and algae removal. This affects species that live on the surface of the sand and/or that use seaweed stranded as food and shelter. For instance, after the Exxon Valdez accident, the beaches that were cleaned took longer to recover than those that were not cleaned.

Sanchez *et al.* (2006) reported a significant reduction in the abundance of the Norway lobster (*Nephrops norvegicus*) and pandalid shrimps (*Plesionika heterocarpus*) in trawling cruises carried out after the Prestige oil spill that were compared to historical series. From 2004 a partial recovery of the populations was observed.

A manuscript is under preparation with the following contributors: Bjørn Einar Grøsvik (IMR, NO), Steven Brooks (NIVA, NO), Juan Bellas (Instituto Español de Oceanografía, IEO, ES), Concepcion Martinez-Gomez (IEO, ES), Dick Vethaak (Deltares, NL), Ketil Hylland (Univ Oslo, NO). WGBEC aims to submit this review to Marine Environmental Research by November 2018.

5.3 ToR b) review available studies on marine seabird ecotoxicology

Ketil Hylland (NO) introduced the subject to WGBEC, referring to earlier studies showing ecologically relevant impacts from contaminants on seabirds, e.g. Bustnes *et al.* (2012, 2015). He presented ongoing studies at the University of Oslo, focusing on bioaccumulation of contaminants and genotoxicity in seabirds, measured using the comet assay (DNA strand breaks). There are clear species differences in baseline DNA strand breaks in different species, i.e. eider duck, two skua species, glaucous gull, kittiwake, but no clear relationship between concentrations of contaminants in blood with DNA strand breaks in lymphocytes (Haarr *et al.*, 2017). A study with herring gull has been performed, comparing the population in the inner Oslofjord (urban) with a population on the Finnmark coast (rural) (Keilen, 2017). The results were somewhat surprising: higher bioaccumulation for a range of organic contaminants in the Finnmark population than in the urban population, but more DNA strand breaks in the urban population. It is not clear which substances or stressors caused the latter effect.

At the Reykjavik meeting, 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, while tern and eider eggs having the lowest contaminant loads.

Dick Vethaak (NL) reminded WGBEC that some seabirds are among the species most at risk from environmental plastic pollution and suggested that WGBEC consider future activity in this area, but also highlighted that many groups are already active in this field internationally.

5.4 ToR c) review available studies on marine mammal ecotoxicology

Ketil Hylland (NO) described a study on genotoxicity in polar bear, in which 47 individuals were sampled in the autumn 2013 in collaboration with the Norwegian Polar Institute. Lymphocytes were isolated and comet analyses (DNA strand breaks) performed in the field. The blood of a volunteer was used as a reference for the analyses. Whole blood was used for contaminant analyses. Contrary to expectations, preliminary results suggest that individuals with high concentrations of contaminants had less DNA damage than individuals with lower concentrations of contaminants. Age, gender and condition did not appear to affect DNA damage in polar bear (Gilmore, 2015).

In the Netherlands, all stranded cetaceans are reported to Naturalis Biodiversity Centre and records are collected in their online database (www.walvisstrandingen.nl). Nowadays, approximately 600 harbour porpoises are annually found death on the Dutch coastline. Since 2008, a subset of all stranded animals is collected and submitted for post mortem investigations, which is conducted at the department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University. This research is funded by the Dutch Government and aims to discriminate natural from anthropogenic causes of death. In addition, data and samples are collected to facilitate a range of additional studies, including diet and contaminant analysis by Wageningen Marine Research WUR. Contaminant analysis is performed in blubber and liver for PCBs, PBDEs, HCB, HCBD and PFOS (amongst others). The influence of size, age, gender and nutritional status on the levels is monitored, and the levels are compared to potential toxic thresholds (see Jepson *et al.* 2016).

As part of a dedicated session during the 2017 WGBEC meeting, Paul Jepson (UK) and Rune Dietz (DK) presented 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 cetacean 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).

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".

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 functions 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 (Desforges *et al.*, 2017). 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).

5.5 ToR d) review effects of contaminants on community composition

The discussion of community was led by and the summary below prepared by Juan Bellas (ES). ‘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, 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) showed that marked changes in communities do not only occur at high levels of impact and that both specific richness and diversity can increase with moderate levels of disturbance. Current approaches include 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 hyper-sedimentation due to mining activities (Olsgard & Hasle, 1993). There have, 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.

WGBEC has been discussing during last years about the appropriateness of including the study of benthic communities in integrated monitoring programs of marine pollution, taking also into account the requirements of the current European legislation (WFD, MSFD), aiming to assess the current state of the marine environment and to identify areas that cannot potentially achieve the desired environmental status.

In this line, more effort would be needed for the validation and application of these techniques in biomonitoring (efficiency, reliability, and cost-effectiveness). For instance, it is important to conduct community ecotoxicological experiments to demonstrate cause-effect and dose-response relationships. Randomization and replication of treatments that characterize an experiment do not take place in natural communities affected by pollution. Appropriate reference sites are essential to demonstrate causality, treatments are not randomly located and reference areas are not true controls, since they must be defined after the pollution event has occurred. As a result, 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.

Another issue to consider is that changes in community structure due to pollution do 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.

WGBEC discussed the relationships between the 'biomarker approach' and community responses. 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 might arise in part from the combined use of biomarkers and community responses, 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, which is not usually taken into account in benthic ecology studies. Also, such integrated approaches may help to establish the link between the presence of pollutants in the environment and their ecological effects, allowing discerning between the impact of pollution on biological communities from natural factors.

A case study from the Ría de Vigo was presented by Juan Bellas to illustrate some of these aspects, and also commented to the group the indicator "Typical species composition (BH1)", that has been proposed by Spain to be adopted within the Coordinated Environmental Monitoring Programme (CEMP), and has finally been considered as 'common indicator' by OSPAR. This indicator relates the survival of 'typical species' with the environmental/conservation status of a given habitat type in the long-term, in comparison to reference conditions. The aim is to measure changes in the proportion of 'typical species' within each habitat type when a disturbance occurs, compared to reference conditions, and the key point is to select a set of those 'typical species'. Therefore, species are classified into groups for a given pressure (e.g. pollution) based on functionality (biological traits). For instance, five groups of sensitivity of soft-bottom macrofauna to increasing stress gradient have been summarized by Grall and Glemarec (1997) as: Group I. Species very sensitive to organic enrichment; Group II. Species indifferent to enrichment; Group III. Species tolerant to excess organic matter enrichment; Group IV. Second-order opportunistic species; Group V. First-order opportunistic species. A way forward discussed by the group is to focus on both sensitive and opportunistic species, and measure bioaccumulation and biomarkers, concurrently with the study of their community structure.

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

This ToR is related to MSFD's descriptor 8 criteria: D8C3 "the spatial extent and duration of significant acute pollution events are minimized" and D8C4 "abundance -number of individuals or other suitable units as agreed at regional or subregional level- per species affected; extent in square kilometres (km^2) per broad habitat type affected".

According to the *EU Commission Decision 2017/848 of 17 May 2017 laying down criteria and methodological standards on good environmental status of marine waters and specifications and standardised methods for monitoring and assessment, and repealing Decision 2010/477/EU*, criteria D8C3 and D8C4 should indicate significant acute pollution events involving polluting substances, as defined in Article 2(2) of Directive 2005/35/EC of the European Parliament and of the Council(1), including crude oil and similar compounds.

Decision 2017/848 states that criterion D8C3 is 'primary', and Member States shall estimate the duration in days, the distribution and the total spatial extent in km^2 of significant acute pollution events per year, and identify the source of these significant acute pollution events. They may use the European Maritime Safety Agency satellite-based surveillance for this purpose.

On the other hand, criterion D8C4 is 'secondary'', and is to be used when a significant acute pollution event has occurred. This criterion relates to the adverse effects of significant acute pollution events on the health of species and on the condition of habitats, providing an estimate of the abundance of each species and an estimate of the extent of each broad habitat type that is adversely affected.

Members of WGBEC have addressed issues relevant to acute spills effects and the group recently produced a guideline for acute oil spill monitoring (Martinez-Gomez *et al.*, 2010). All countries with a coastline have some guideline as to how to monitor acute spills, but there is a scarcity of effect methods. Upon request, Jim Readman (UK) contributed input to this item, summarising methods on water analyses and monitoring strategies.

Acute spills have conceivably different patterns of effects than diffuse, chronic inputs. This is a fundamental question in environmental science, and very important for environmental assessment. Global frequencies of acute accidents related to exploration, production and transport of oil has been presented by Eckle *et al.*, 2012. They show that accidental tanker spills have significantly decreased over the past four decades both in terms of numbers and volumes of spills. Key factors are improvements in navigation technology, the automatic identification system (AIS), double hull as requirements after the Exxon Valdez accident and decrease in average age of the world tanker fleet. Less data are available for acute spills of other types of contaminants.

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 review of the above information, WGBEC came to the conclusion that what is needed is a document of legislative and not ecotoxicological nature. The ToR was therefore not followed up further.

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

Progress with this ToR over the three reporting years was reviewed by Hermann Guls (IS). Recent progress on ocean acidification research has been reported in a special issue of the ICES Journal of Marine Science (2016, Volume 73 issue 3). Over the past few years, another ICES group has addressed issues relating to acidification, SGOA (Study Group on ocean acidification). No clear indicators for acidification have emerged from the work by that group (SGOA, 2014). Possibly that is due to several reasons: the pH decrease so far has been ~ 0.1 unit and the expectation is 0.3 unit until the end of the century. In coastal areas, the pH ranges much more on a diurnal basis, by other influences such as deposition, changes in the catchments and changes in eutrophication status. The observed effects on organisms have not been very clear in an environment with high variability.

The last IPCC report (2014) gives a good overview of the sensitivity and tolerances of different organisms for two IPCC scenarios. Available data shows that the most sensitive taxa are coral, echinoderms (especially larvae), bivalves and gastropods. The effects are reduced calcification and survival.

Dick Vethaak referred to an evaluation that suggested a shortlist of pteropods, coccolithophores and foraminifera as the most sensitive. It indicated that the Arctic pteropod *Limacina helicina* would be most appropriate as an indicator species. The advice from the SGOA was to archive pteropods (in ethanol). Mussels (*Mytilus edulis*) were not included in the shortlist because they do not appear to be sensitive to the pH levels that we can expect by 2100 and they also inhabit waters with higher pH ranges. Brittlestar *Ophiothrix fragilis* larvae appear to be sensitive to acidification, with reduced survival and growth (see Dupont *et al.*, 2008, 2010). So far, most laboratory exposure studies are short-term experiments. They do not allow the investigation of adaptation to reduced pH, which is poorly understood. In areas with naturally low pH or pH gradients, such as around underwater volcanoes, long-term effects can be observed. In general, there is evidence of a decrease of the number of calcifiers and an increase of non-calcifiers near the source (volcano); (Hall-Spencer *et al.*, 2008).

Pelagic habitats experience natural diurnal variation in pH through natural cycles of photosynthesis and respiration of primary producers. It would be interesting to clarify why such variation does not cause damage to e.g. brittlestar larvae.

As referred to above, there is already substantial research on possible effects of acidification on marine organisms and on climate change-associated impacts on marine ecosystems and food webs in general (see Alava *et al.*, 2017).

The main focus of WGBEC will be how pH changes interact with other environmental factors or stressors in affecting marine organisms or processes, integrating this ToR with ToR i.

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

The main focus for this ToR has been on thiamine deficiency and retinol (vitamin A) metabolism. Both could have interactions with contaminant exposure and effects, but there is no clear evidence. WGBEC aimed for a full-day workshop on thiamine deficiency in year 2, but this failed due to a late withdrawal. While there is clearly scope to investigate both above further, there are other aspects of nutritional toxicology that also deserve attention, relating to both aquaculture and pollution.

5.9 ToR h) review progress with marine plastic ecotoxicity to marine organisms

Dick Vethaak (NL) introduced marine plastics with emphasis on recent key studies and Dutch contributions. Marine plastics is a global challenge, with increasing quantities documented in recent decades. It is clear that microplastic ingestion is widespread across marine animals and can be found in food chains. On basis of current knowledge particle toxicity seems to be a major hazard while chemical effects mediated via ingested microplastics seem less important. Microbial contamination and its potential threat to dispersion of pathogens and increasing disease risk seem plausible but will require further study. The latter hazard is well illustrated by Lamb *et al.* (2018), who reported an association between the presence of plastic waste and coral reef disease and suggested that plastic waste can promote microbial colonization by pathogens implicated in outbreaks of disease in the ocean.

Microplastic contamination was determined in sediments of the Southern North Sea and floating at the sea surface of northwest European waters (Maes *et al.*, 2017). Floating concentrations ranged between 0 and 1.5 microplastic/m^3, whereas microplastic concentrations in sediments ranged between 0 and 3146 particles/kg dry weight sediment. In sediments, mainly fibres and spheres were found, whereas at the sea surface fragments were dominant. At the sea surface, concentrations of microplastics are lower and more variable than in sediments, meaning that larger sample sizes and water volumes are required to find detectable concentrations. Higher concentrations of floating microplastics were found near estuaries. In sediments, estuaries and areas with a high organic carbon content were likely hotspots. Standardization of monitoring methods within marine regions is recommended to compare and assess microplastics pollution over time. According to PlasticsEurope (2017), the annual production for plastic materials in 2016 was around 280 million tons. Jambeck *et al.* (2015) estimated that somewhere between 4.8-12.7 million tons of plastics finds their way in to the world's oceans annually. It is established and accepted worldwide that plastics are a cause for concern. A significant amount of papers has been published concerning the understanding of how plastics behave in the environment. Microplastics and nanoplastics especially cause concern in toxicological science, but not without contradiction (Lohmann, 2017).

Secondary microplastics are the ones which contribute to the cause as a consequence of degradation of larger plastic debris (Galloway *et al.*, 2017). Light-induced degradation appears to be the fastest way by which plastic materials break down to microplastic level, sunlight initiates photo oxidative degradation which then can continue as thermo-oxidation (Andrady, 2011), as opposed to biodegradation. It can be speculated that plastic debris found on beaches around the world is most susceptible to direct sunlight, hence making it a big contributor of MP to intertidal zones (Andrady, 2011).

Different polymers of plastic have varying properties, density for example is a property that directly influences particle and debris behaviour in the environment. Polymers such as polyethylene, polypropylene, polystyrene and foamed polystyrene have low specific gravity are more susceptible to stay afloat and adrift, making it possible for them to be carried over large distance by ocean currents (Andrady, 2011) while higher density polymers tend to sink to the benthos.

Floating particles in the ocean, of course develop fouling, covering the surface of plastics. The sequence of epibionts: bacteria > diatoms > hydroids > ectocarpales > barnacles > bryozoans (Andrady and Song, 1991).

This adds yet another complexity level to the plastic behaviour, since it changes the density of the particles. There is evidence, that once on the surface, a plastic accumulates so called ecocorona, and sinks to the water column, where, given time, the corona dies, and plastic resubmerges on the surface to repeat the process. This effect makes it difficult to model the distribution of MPs and shows that microplastics move through the environment differently than a chemical dissolved in water would. Such fouling also slows down degradation process significantly. Hence plastic particle have the potential to diffuse readily across ocean surface or water column, but may take significantly longer time to reach benthic environment, Clark *et al.* (2016) estimates time range from weeks to years.

Methods

A number of papers have described methods for measuring microplastics in various matrices. Karlsson *et al.* (2017) optimized a method for microplastics in sediment showing an improved recovery with olive oil. For analysis of microplastics in biota an adapted enzymatic digestion protocol using proteinase K performed best, with a 97% recovery of spiked plastic particles and no observed degradation effects on the plastics in subsequent Raman analysis. Microplastics were found in 8/9 invertebrate species on the North Sea coast. MPs were found in 68% of stomach samples of brown trout (*Salmo trutta*) from the Swedish west coast. In another study by Zada *et al.* (2018), a novel method, Stimulated Raman scattering (SRS) microscopy, based on the coherent interaction of 2 different laser beams with vibrational levels in the molecules of the sample, proofed an efficient method for monitoring microplastics in the environment and potentially many other matrices of interest. This method would enable much faster detection and identification of microplastics (a thousand-fold higher speed of mapping with SRS compared with conventional Raman). So far, the method could identify 5 different high production-volume polymer types in microplastics extracted from environmental or consumer product samples. The particles from the extracts were collected on a flat alumina filter, and 6 SRS images were acquired. After density separation of a Rhine estuary sediment sample, the authors scanned 1 cm^{-2} of the filter surface in less than 5 hr and detected and identified 88 microplastics, which corresponds to 12,000 particles per kilogram dry weight.

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).

Ashok Deshpande (US) described pyrolysis GC-MS, used in their lab. In this method, a very small piece of microplastics sample, less than 1 mg in weight is placed in a narrow quartz tube, which is then placed in a platinum coil and heated to 750°C. The intense heat in the pyrolysis chamber breaks down the large polymer chains into smaller fragments that are then analysed by GS/MS. The fragmentation patterns appear to be reproducible and unique to a given polymer type. A pyrolysis GC-MS library of commonly used plastic polymers was created. These included polymers like polyethylene, polypropylene, polystyrene, polyvinylchloride, polymethyl methacrylate, sodium polyacrylate, polyurethane, polyethylene terephthalate, polyamides, etc. Also included in this list were some copolymers. The typical fingerprints of the pyrolysis GC-MS were then used to test the proof-of-concept on the unweathered plastics items used in the household, office, and in the laboratory. Further testing included weathered microplastics samples from the different littoral and aquatic environments.

Pyrolysis GC-MS uses a two-tier approach for the confirmation of the microplastics polymers. Similar to FT-IR or Raman Spectroscopy, the peak fingerprints are also used for the identification of plastic polymers. In addition to the peak fingerprints, the pyrolysis GC-MS technique also allows for a mass spectrometric corroboration of the individual marker compounds within a given GC-MS pyrogram. The pyrolysis GC-MS technique is also useful for the analysis of microplastics copolymers and plastics additives in the same run. The technique is simple and straightforward, and it is not limited by the shape, size, density, surface appearance, or colour.

This technique has been included to test methodologies to characterize potential environmental risk associated with the presence of microplastics in surface waters (Ravit *et al.*, 2017). The goals of the study were to determine whether urban New Jersey freshwaters contained microplastic pollutants, and if so, to test analytic techniques that could potentially identify chemical compounds associated with this pollution. A third objective was to test whether identified associated compounds might have physiological effects on an aquatic organism. Using field collected microplastic samples obtained from the heavily urbanized Raritan and Passaic Rivers in New Jersey, microplastic densities, types, and sizes at 15 sampling locations were determined. Three types of plastic polymers were identified using pyrolysis coupled with gas chromatography (Pyr-GC/MS). Samples were further characterized using solid phase micro extraction coupled with headspace gas chromatography/ion trap mass spectrometry (HS-SPME-GC/ITMS) to identify organic compounds associated with the: (i) solid microplastic fraction, fractions indicated compounds can move between the two phases, potentially available for uptake by aquatic biota in the dissolved phase. Patterns of tentatively identified compounds were similar to patterns obtained in Pyr-GC/MS. Embryonic zebrafish exposed to PyCG/MS- identified pure polymers in the 1–10 ppm range exhibited altered growth and heart defects. Using two analytic methods (SPME GC/MS and Pyr-GC/MS) allows unambiguous identification of compounds associated with microplastic debris and characterization of the major plastic type(s). Specific —fingerprint patterns can categorize the class of plastics present in a waterbody and identify compounds associated with the particles. This technique can also be used to identify compounds detected in biota that may be the result of ingesting plastics or plastic-associated compounds.

Associated chemicals

In a study by de Vriese *et al.* (2017), PCB-loaded microplastics were offered to field-collected Norway lobsters (*Nephrops norvegicus*) during *in vivo* feeding laboratory experiments. Each ingestion experiment was repeated with and without loading a mixture of ten PCB congeners onto plastic microspheres (MS) made of polyethylene (PE) and polystyrene (PS) with diameters of either 500–600 µm or 6 µm. The presence of chemicals adsorbed to ingested microplastics did not lead to significant bioaccumulation of the chemicals in the exposed organisms. There was negligible PCB bioaccumulation observed after ingestion of PCB-spiked polystyrene. The results demonstrated that after 3 weeks of exposure the ingestion of plastic MS themselves did not affect the nutritional state of wild *Nephrops*. The results largely confirm chemical partitioning models that predict that the ingestion of microplastics with adsorbed chemicals in the field will tend not to result in significant net desorption of the chemical to the organism's tissues.

A study by Martínez-Gómez *et al.* (2017) highlighted the necessity to wash or weather virgin microplastics before toxicity testing. It was shown that virgin microplastics are toxic to sea urchin embryo through the leaching of chemicals.

New data were reported on the contamination of used as sea food such as mussel, oysters from the Dutch coastal waters (Leslie *et al.*, 2017, Karlsson *et al.*, 2018) and in the Persian Gulf (Naji *et al.*, 2018) and in brown trout (Karlsson *et al.*, 2018). Levels in blue mussels from the Dutch coast contained 2.3–13.2 particles/g ww (whole organism) while 68% of individual trout from the Swedish coast were found to contain microplastics. The importance of QA in MP sampling and analysis was emphasized. Recently, it was demonstrated that the number of MPs for human ingestion of fibres resulting from household dust is higher than the ingestion of fibres via mussel consumption (Catarino *et al.*, 2018), illustrating that the contribution of contaminated marine sea food to total dietary exposure seems less important and should be placed in context of analysis and exposure to microplastics from the total food basket.

Chemical toxicity

Plastics are often produced as a mixture of polymers and additives, microplastics may for example make phenolic additive chemicals available for uptake by organisms (Teuten *et al.*, 2009). However, studies (Koelmans *et al.*, 2014; Rochman *et al.*, 2014) have found that microplastics are irrelevant for the uptake of compounds such as alkylphenols or bisphenol-A.

Tanaka et. al., 2013, linked brominated diphenyl ethers concentrations in seabirds to plastic ingestion in seabirds. Lohmann (2017), speculates that these chemicals could be bound to nanoplastic particles content in animal soft tissue, since no abovementioned chemicals was found in their prey items.

Studies have shown that in marine environment, POPS tend to bind to plastic particles and become highly concentrated in them. POPS such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) have very high affiliation for polymers (Andrady, 2011). The concern here is that microplastics might act as vectors for pollutants carrying them to marine animals. Lohmann, 2017 argues that there is not enough microplastic pollution to outcompete the partitioning of POPS to water and natural matter, backed up by Gouin et. al., 2011; Koelmans et. al., 2016; Zarfl & Matthies, 2010.

Galloway *et al.*, 2017 presents evidence for interaction between plastic particles and organic compounds in marine environment. Some of which are a part of "infochemicals" she specifically mentions dimethylsulfide (DMS), produced by phytoplankton, which induces feeding behaviour of many marine species. Savoca & Nevitt, 2016 show interaction of DMS and plastics, and that plastics particles in marine environment acquire DMS signature relatively quickly, in their studies they show that effect on animals is observed at very low concentrations. Positive relationship was found between DMS responsiveness and plastic ingestion in a study on 13000 seabirds.

Monitoring

The distribution and abundance of marine litter on the seafloor off the United Kingdom's (UK) coasts were quantified during 39 independent scientific surveys conducted between 1992 and 2017 (Maes *et al.* 2018). There was no significant temporal trend in the percentage of trawls containing any or total plastic litter items across the long-term datasets. Statistically significant trends were observed in specific plastic litter categories only. These trends were all positive except for a negative trend in plastic bags in the Greater North Sea - suggesting that behavioural and legislative changes could reduce the problem of marine litter within decades.

An overview of NIVA's projects on plastics were presented by Steven Brooks (NO): Baseman, JPI Oceans (Microplastics analyses in European waters): the purpose of the interdisciplinary and international project BASEMAN is to define a baseline for microplastic analyses through validation and harmonization of analytical methods, as well as establishing and testing standards for microplastic analyses in European waters. The project will provide tools to identify and quantify the amount and distribution of microplastic in the environment. The project has 24 partners and is led by Alfred Wegener Institute, Germany. NIVA participates in two of the work packages and contributes to method development and harmonization of methods for microplastic analysis in sediment and biota.

MIME (Micro and Nano plastic impacts in the marine environment): The purpose of the project is to investigate the presence of microplastic in Norwegian biota and possible sources of microplastic contamination, as well as how microplastics can affect marine organisms. So far, plastics have been identified in Atlantic cod from the coast of Norway (Bråte *et al.*, 2016), controlled laboratory studies for mussel exposure to polyethylene particles have been conducted (Bråte *et al.*, 2018) and methods have been tested to investigate microplastics in wild and commercial mussels. The project is a collaboration between NIVA, University of Oslo, Akvaplan-NIVA, Deltares, University of Gothenburg and CEFAS.

IMPASSE (Impacts of microplastics in agrosystems and stream environments): A JPI-Water funded international project which aims to identify the transport pathways and possible ecological impacts of microplastics, and to develop management solutions which will protect agricultural sustainability, economic goals, and human and animal health. The purpose of the project is to provide improved estimates of the supply of microplastic to soil via sludge from wastewater treatment plants and the impact on soil and terrestrial ecosystems. The project will concentrate on the assessment of fluxes and strains of microplastic in agricultural areas and will develop scaled-up computational tools including a dynamic model for the fate and transport of the microplastic through the drainage system. The project is in collaborations with partners including: IMDEA (Spain), Windsor University (Canada), SLU (Sweden) VU University Amsterdam (Netherlands). The project has produced three publications to date: Nizzetto *et al.*, 2016a, b, Hurley & Nizzetto, 2018.

NanoP – Plastics: Does size matter: This project will investigate the impact of different size plastics, micro- and nano-plastics, in different aquatic organisms, as well as their potential transfer along the aquatic food chain.

ILC-Japan: Interlaboratory comparison for the Government of Japan. NIVA is participating in ILC for standardizing and harmonizing microplastic monitoring. Results were discussed at expert working group in Tokyo, February 2018. A forthcoming publication will present the results and the working group will be conducting further investigations.

Microplastics in drinking water: a national project which aims to determine the extent of microplastics in drinking water by sampling and analysing raw water, treated water and tap water from a variety of waterworks. The project is funded by Norsk Vann and several waterworks from different municipalities around Norway are participating in the project.

Aquatic and urban microplastics: This project aims to develop methods for separation and identification of microplastics from aquatic locations to investigate the effect of populated urban areas and road run off. Collaborators: NIVA (Norway), University of Copenhagen (Denmark), University of Aalborg and The University of Queensland (AUS).

Testing of methodology for measuring microplastics in blue mussels (*Mytilus* spp) and sediments, and recommendations for future monitoring of microplastics: This study aimed to optimise analysis of environmental samples for microplastic monitoring, focusing specifically on blue mussels and sediments. This project included methods testing and data analysis of environmental samples (Lusher *et al.*, 2017).

Micro- and macro-plastics in marine species from Nordic waters: The aim of this desk based project was to compile current knowledgebase on plastic ingestion in commercial and ecologically important marine biota such as fish and invertebrates (Bråte *et al.*, 2017). This project was led by NIVA with collaboration from Aquaplan NIVA (Norway), DTU Aqua (Denmark) and UGOT (Sweden).

Mapping Microplastics in Sludge (MICROSLUDGE): This project was designed as an initial screening program of sludge from different waste water treatment plants around Norway. NIVA researchers developed a method that was suitable to extract plastics for samples with high organic matter content and described the results based on differences between wastewater treatments plants (Lusher *et al.*, 2018).

Microplastics in road dust – characteristics, pathways and measures. The project was a desktop study of the contribution of road dust particles to microplastics in the environment (Vogelsang *et al.*, 2018).

Microplastics in marine environments: Occurrence, distribution and effects. Desk based literature study of reviewing the current understanding of the occurrence, distribution and effects of microplastics on the marine environment (Nerland Bråte *et al.*, 2014). The purpose of the project was to provide the Norwegian Environment Agency with a thorough report on the knowledge base of micro-plastic in the marine environment.

A large-scale monitoring of marine litter performed in the joint Norwegian–Russian ecosystem monitoring surveys in the period from 2010 to 2016 and contribute to documentation of the extent of marine litter in the Barents Sea (Grøsvik *et al.* 2018). The distribution and abundance of marine litter were calculated by recordings of bycatch from the pelagic trawling in upper 60 m, from bottom trawling close to the sea floor, and floating marine debris at surface by visual observations. The study is comprehensive regarding coverage and number with registrations from 2265 pelagic trawls and 1860 bottom trawls, in addition to surface registration between the stations. Marine litter has been recorded from 301 pelagic and 624 of the bottom trawl catches. In total, 784 visual observations of floating marine debris were recorded during the period. Marine litter has been categorized according to volume or weight of the material types plastic, wood, metal, rubber, glass, paper, and textile. Marine litter is observed in the entire Barents Sea and distribution vary with material densities, ocean currents and depth. Plastic dominated number of observations with marine litter, as 72% of surface observations, 94% of pelagic trawls, and 86% of bottom trawls contained plastic. Observations of wood constituted 19% of surface observations, 1% of pelagic trawls, and 17% of bottom trawls with marine litter. Materials from other categories such as metal, rubber, paper, textile, and glass were observed sporadically.

Floating marine debris were widely distributed in the Barents Sea, while highest volume of marine litter was observed in the central, eastern and northern areas. Wood dominated the floating marine debris observations (61.9 ± 21.6% by volume), while plastic constituted 34.6 ± 22.3% by volume. Metal, rubber and paper were recorded sporadically.

Pelagic marine litter were observed in 13% of all pelagic trawls with a mean of 58 gram per trawl catch. Marine litter from pelagic trawls distributed wider in the Barents Sea. Plastic was the bulk (85.1%) of pelagic marine litter observations with mean 0.011 mg m^{-3}. Paper (9.4%) and textile (3.9%) were observed more seldom, while other materials only sporadically. Pelagic plastic was significantly correlated with latitude and longitude some years, and indicated north-eastern distribution in 2010, and northern distribution in 2011 and 2014.

Marine litter as bycatch from bottom trawling were observed in 33.5% of all bottom trawl hauls with a mean of 772 g per haul. Marine litter from bottom trawls distributed wider in the Barents Sea, while the highest catches were taken in the western, south eastern, north eastern, and around Svalbard. Plastic were observed from the entire Barents Sea, processed wood in the eastern and northern parts, and metal and rubber in the south east. Processed wood dominated the amount of marine litter from bottom trawls with a mean of 66% of the weight of all catches with any type of marine litter. Plastic constituted 11.4% of the weight, but dominated the number of observations. Metal and rubber consisted ~10% of the weight but from few numbers of observations. On average, 26 kg km^{-2} of marine litter was found in the Barents Sea, with an average of 2.9 kg km^{-2} of plastics-only (Grøsvik *et al.* 2018).

Video recording of marine litter Buhl-Mortensen and Buhl-Mortensen (2017) reported litter from 1778 video transects from the MAREANO project. Each video transect is 700 m long and the average field of view is 3 m. Video recording of the seabed was performed with a tethered video platform that is equipped with a high definition color video camera (Sony HDC-X300) tilted forward at an angle of 45° during transect survey mode. It also has two analog CCD video cameras, one forward-looking for navigation and one for surveillance of the cable. Two lazer beams (10 cm apart) are used for determining the width of the field view. The video rig is towed by the survey vessel at a speed of 0.7 knots and manually controlled by a winch operator at a height of around 1.5 m above the seabed.

The percentage of video transects with litter is comparable for the Barents Sea and Norwegian Sea, with 27 and 29% respectively. The mean density of litter for the Barents Sea and Norwegian Sea were 202 and 279 items/km^2. The mean density of litter near the coast and offshore in the Barents Sea was 268 and 194 items/km^2. A conservative estimate of total amount of litter in the Barents Sea south of Svalbard (523 600 km^2), using mean litter densities in offshore areas (194 items/km^2), is around 101 million litter items corresponding to 79 thousand tonnes (Buhl-Mortensen and Buhl-Mortensen, 2017).

Dividing observations of litter into three density groups, at 23% of the video transects were found low densities of litter (> 0–1000 items/km^2), at 3.0% the video transects were found medium densities (1000–2000 items/km^2) and only on 1.9% of the video transects were found high densities (> 2000 items/km^2) of litter.

The abundance and composition of litter and the density of trawl marks (TM) varied with depth, and type of sediments and marine landscapes (Buhl-Mortensen and Buhl-Mortensen, 2018). Lost or discarded fishing gear (especially lines and nets), and plastics (soft and hard plastic and rubber) were the dominant types of litter. The distribution of litter reflected the distribution of fishing intensity (density of Vessel Monitoring System (VMS) records) and density of TM at a regional scale, with highest abundance close to the coast and in areas with high fishing intensity, indicated from the VMS data. Also, deliberate dumping of discarded fishing gear is likely to occur away from good fishing grounds. Extreme abundance of litter, observed close to the coast is probably caused by such discarded fishing gear, but the contribution from aggregated populations on land is also indicated from the types of litter observed (Buhl-Mortensen and Buhl-Mortensen, 2018).

Barbara Catalano (IT) reported on the ISPRA contribution to two co-founded European projects. The INDICIT (Implementation of the Indicator of marine litter on sea turtles and biota in regional Sea Conventions and Marine Strategy Framework Directive areas, https://indicit-europa.eu), project is based on a 10 partners consortium from the public sector established in EU and non-EU countries, being all contracting parties of the OSPAR and/or Barcelona Conventions. The INDICIT actions aim to obtain a precise definition of this indicator (e.g. threshold values, biological criteria, temporal and spatial scales of use). Starting from the Fulmar EcoQO and the MSFD guidelines, a harmonized procedure of collection and analysis of plastic ingested by loggerhead turtles *Caretta caretta* have been elaborated. Marine litter is subdivided in categories and sub-categories, counted and weighed. Data are collected according to a specific datasheet with basic and optional parameters in order to better understand the biological constraints. The analyses are performed both on dead turtles and on hospitalized ones. Moreover, local training has been performed in each participating country with the aim of creating national networks. Similar activity enlarged to the Mediterranean basin was performed with a special training course held in Italy, involving UNEP/Map delegates from the South Mediterranean Countries. Mediterranean and European researchers and sea turtle rescue centres are invited to contact INDICIT partners in order to join the international network.

The MEDSEALITTER (Developing Mediterranean-specific protocols to protect biodiversity from litter impact at basin and local MPAs scales, *https://medsealitter.interreg-med.eu*) project is based on a 9 partners consortium which aims to develop and validate, within the Mediterranean basin, protocols to monitor marine litter distribution and its impact on key species. Guideline will be provided for conducting surveys of floating macro litter

(>30 cm) by unifying different monitoring approaches, in order to ensure data comparability between surveys and across regions. Plastic presence and distribution will be investigated using different observation platforms including visual (with the use of sailing boat and ferries) and aerial techniques (drones and planes equipped with fixed cameras) at a local and large geographical scales. Guidelines will also be provided to outline best extraction methodologies for microplastics detection in fish and invertebrates. Selected techniques will be tested on target species respectively *Boops boops* and *Sabella spallanzanii*, collected either inside and outsite MPAs areas or in selected sites, including harbour and marine fish farms.

5.10 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). 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 is 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.

Environmental interactions is an active research area for WGBEC members and a critical component of a holistic understanding of environmental processes relevant to toxicity. An outline for a scientific review was presented and discussed at the final meeting in the three-year cycle. A review co-authored by WGBEC members will be submitted to a special volume of Frontiers in Marine Science by the end of 2018. The preparation of the review will be led by Ketil Hylland (NO).

5.11 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.

The status for emerging contaminants was reviewed by Barbara Catalano (IT). Emerging contaminants has been given lower priority by WGBEC this reporting cycle than in the past and was addressed mainly during the first year meeting (2016). WGBEC continued though to receive updates on inputs, concentrations and effects of emerging contaminants but it become more evident that the term "emerging" was often misused to address substances that have been present in the environment for decades.

WGBEC suggests that it would be more appropriate for most of these substances to use the terminology “compounds of emerging concern”. “Emerging” may reflect detection in new matrices, in new organisms or new effects. The groups of substances should generally be referred to by their pattern of use or source e-g. flame retardants, pharmaceuticals, antifouling agents, pesticides, household chemicals, cosmetics, munitions and industrial chemicals. Most of these compounds are used at low concentrations and concentrations in the environment are generally low.

In order to identify substances of emerging concern for wider consideration, a list of compounds was recently revised by ADGHAZ (2017), following an advice request by OSPAR, which included dechlorane, alternative brominated flame retardants, phosphorous flame retardants, antifoulants, per- and polyfluorinated substances (with the exclusion of PFOS, PFOA), benzotriazoles, siloxanes, anticorrosion agents, especially to identify data gaps. A similar process has been conducted under the Water Framework Directive through the Watch List process.

Although EU REACH Directive impose by May 2018 the registration of all substances manufactured or imported (>1 tonne/year per producer or importer) which should include information on risk assessment potentially performed (e.g. PEC/PNEC), a paucity of data on the effects of many substances on marine organisms has been reported (Tornero & Hanke, 2016).

The ICES Advice Drafting Group on Hazardous Substances (ADGHAZ) met on 15 November 2017 at the ICES Headquarters in Copenhagen, with the aim of handle the request made by OSPAR to ICES to receive advice on the selection and deselection of hazardous substances of concern to coastal and marine waters. A member of WGBEC, Juan Bellas, participated in this ADGHAZ as an expert nominated by Spain.

This OSPAR request arises from the interest on emerging pollutants in Europe, and OSPAR recognizes the concern about this type of pollutants. OSPAR would like to ensure that emerging hazardous substances that are of general interest to coastal and marine waters are identified, so that appropriate action can be taken. HASEC is aware that a similar exercise has already been established under the Water Framework Directive through the 'Watch List' process and, therefore, work for the marine environment should be based on this process and coordinated with it.

The work done for this advice results from the activity of the ICES working groups: Marine Chemistry Working Group (MCWG) and Working Group on Marine Sediments (WGMS). In order to identify substances of emerging concern for wider consideration by OSPAR, volunteer experts of these groups have drawn up sheets for groups of pollutants that had been previously considered by the MCWG. These groups of pollutants are: dechlorane+, alternative brominated flame retardants, phosphorous flame retardants, antifoulants, per- and polyfluorinated substances (not PFOS, PFOA), benzotriazoles, siloxanes, anticorrosion agents, especially those applied in windmill parks. For two of these groups (siloxanes and benzotriazoles), no volunteers were found, while for a third group (antifouling substances), the results arrived too late to be included in the advice.

There were some contacts of the Chairmen of the MCWG and WGMS groups with WGBEC members, but WGBEC has not been directly involved in this advice. The WGBEC might have contributed to evaluating these groups of substances if they had been involved in this advice. Moreover, one of the main objectives of this OSPAR request was to identify relevant data gaps for selection and deselection of these substances and, precisely, one of the main data gaps for the pollutants of emerging concern is the knowledge of their biological effects on marine ecosystems.

5.12 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.

5.13 Review the status of publications and consider requirements for new publications

A TIMES document on thiamine deficiency was elicited from Lennart Balk (SE) in 2017 and the TIMES document by Hanson *et al.* "Supporting variables for biological effects measurements in fish and blue mussel" (ICES TIMES 60) has been published. WGBEC requests the publication of a second TIMES document, on micronucleus aberrations, by 2019. The work on this document will be led by Steven Brooks (NO) with contributions from colleagues in Spain, Italy and Lithuania.

5.14 AQC activities for biological effect methods

An intercalibration for selected biological effects methods, i.e. EROD and AChE is underway and samples will be distributed early autumn 2018. Co-ordinators are Bjørn Einar Grøsvik (NO) and Steve Brooks (NO).

6 Cooperation

ICES working groups

WGBEC has regular contact with MCWG and WGMS. In 2016, a joint workshop between WGBEC and WGEEL was undertaken (WKBECEEL). WKBECEEL 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.

WKBECEEL 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. This could not be resolved during this reporting cycle.

OSPAR

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 MIME 2016. First of all, some simplification of the classical ICES format 3.2 were implemented, i.e. a possibility to send the chemical contaminants analysed in pools of fish or mussels. Secondly, a simplified sheet ICES format 3.2 was distributed with the contribution of the MIME delegates and the chairmen of WGBEC, in 2017.

7 Summary of Working Group self-evaluation and conclusions

WGBEC has a broad membership and involves influential European marine researchers. The group has been the single most important scientific body to develop, implement and quality assure effect methods in marine ecosystems in Europe. This process has been documented through ICES publications (e.g. CRR 315, a range of TIMES documents) and international publications, as the special volume of Marine Environmental Research (124; 2017), presenting results from the ICON workshop, initiated, executed and published by

WGBEC members. WGBEC has established itself as the most important European body for marine ecotoxicology and effect monitoring and assessment.

WGBEC took on a very ambitious work programme for the three-year period. In this period, the group has widened its scope of interest to include marine seabirds and mammals, as well as links to human health. WGBEC has maintained its activity to lead in quality assurance of effect methods and performing effect-based marine monitoring. The group will continue to update and facilitate development and implementation of science-based effect-methods. WGBEC fulfils a role which is not found elsewhere in Europe (or the world).

Annex 1: List of participants

Name	Institute	Country (of institute)	Email
Juan Bellas	IEO	Spain	juan.bellas@ieo.es
Steven Brooks	NIVA	Norway	sbr@niva.no
Thierry Burgeot	IFREMER	France	thierry.burgeot@ifremer.fr
Barbara Catalano	ISPRA	Italy	barbara.catalano@isprambiente.it
Bjørn Einar Grøsvik	IMR	Norway	bjorn.grosvik@imr.no
Herman Dreki Guls	Univ Iceland	Iceland	herman@hi.is
Halldór P Halldórsson	Univ Iceland	Iceland	halldor@hi.is
Ketil Hylland	Univ Oslo	Norway	ketilhy@ibv.uio.no
Michiel Kottermann	WUR	Netherlands	michiel.kottermann@wur.nl
A Dick Vethaak	Deltares	Netherlands	dick.vethaak@deltares.nl

by correspondence			
Ashok Deshpande	NOAA	USA	ashok.deshpande@noaa.gov
Aourell Mauffret	IFREMER	France	aourell.mauffret@ifremer.fr

Annex 2: Recommendations

Recommendation	Adressed to
1. MCWG are encouraged to inform WGBEC of substances of emerging concern.	MCWG
2. High priority should be given to field-based monitoring in European waters.	SCICOM
3. There is a general need for baseline studies on sublethal responses to contaminant exposure in European waters.	SCICOM
4. There is a need to develop and update assessment criteria for hazardous substances in marine matrices.	WGMS, MCWG
5. Produce a manual for the TIMES series on micronucleus analysis, to be published in 2019	ICES Secretariat

Annex 3: WGBEC draft resolution 2019–2021

The **Working Group on Biological Effects of Contaminants** (WGBEC), chaired by Juan Bellas, Spain, and Steven Brooks, Norway, will work on ToRs and generate deliverables as listed in the Table below.

	MEETING DATES	VENUE	REPORTING DETAILS	COMMENTS (CHANGE IN CHAIR, ETC.)
Year 2019	11–15 March	Vigo, Spain	Interim report by 1 May	
Year 2020			Interim report by DATE	
Year 2021			Final report by DATE to SCICOM	

ToR descriptors

ToR	Description	Background	Science Plan topics addressed	Duration	Expected Deliverables
a	Review and report new developments and innovative methods to study and monitor effects of contaminants	There is a continuous development of new techniques by which to monitor effects of contaminants. The use of "old" methods needs evaluation and development. For 20 years, WGBEC has maintained a list of recommended methods for marine monitoring, ensured that there are protocols available (mainly through TIMES publications) and developed quality assurance programmes. WGBEC competence has been used to develop programmes elsewhere, e.g. the Baltic, and contributed to the development of MSFD (descriptor 8).	3, 5	year 2	Annual report to ICES, TIMES manuscript
b	Review and synthesise environmental effects of natural and synthetic particles and evaluate their direct effects and interacting effects on marine biota	Particles are critical to understand the behaviour of contaminants in marine ecosystems. Some anthropogenic activity leads to increased input of particles, some of which are associated with chemicals, others providing surfaces for adsorption. Particles will also affect organisms per se. Anthropogenically derived particles include micro- and nanoplastics, nanoparticles, mining dischages and discharges from offshore drilling.	3, 6	year 3	Annual report to ICES, scientific paper
c	Investigate and synthesise the direct and indirect effects of ocean contamination to human health	Contaminants/pollution is one of the human pressures on marine ecosystem health resulting in human health impacts. In addition to direct effects, chemical pollutants can decrease the resilience of marine ecosystems, affect sea food security production/ resources, and may ultimately contribute to a loss of biodiversity. Several analytical and biological effect methods suggested by the ICES community can be used to establish links with human health.	1, 3, 6, 7	year 3	Scientific paper

d	Update and summarise national activities on effect-based monitoring, evaluate different approaches taken and identify gaps and future avenues	WGBEC members have contributed significantly to the development and implementation of effect-based monitoring programmes in European countries, as well as OSPAR and MSFD. Monitoring is being harmonised throughout Europe as a result of WFD and MSFD, but there are still differences in take-up and implementation. Through its membership, WGBEC is uniquely placed to maintain an overview of national programmes and discuss pros and cons for different approaches.	3, 5, 6	3 years	Annual report to ICES
e	Describe and evaluate interaction of contamination ettects with those of climate change and acidification	Contaminant exposure is not the only stressor in marine ecosystems and it is important for WGBEC to review effects of climate change and acidification-related stressors and how their presence interact with contaminant stress.	2, 3, 4, 5, 6	year 3	Scientific paper
f	Review and assess effects of contaminants of emerging concern	WGBEC originally requested MCWG to inform about substances of emerging concern since they generally would appear in chemical analyses. The definition of "emerging" has been so wide and important effects have been observed in marine organisms following exposure to e.g. pesticides, so WGBEC have included the item on the work programme.	3, 5, 6	year 2	Annual report to ICES
g	Investigate and report effects of individual contaminants on marine communities	There is an ongoing discussion as to whether community analyses can detect effects of contaminants; they are definitely not the most sensitive in this respect. Since biodiversity, i.e. community analyses, is an important component of WFD and MSFD effect programmes, there is a clear need to develop complementary analytical methods that are specific to effects of contaminants and not influenced by other ecological factors.	2, 3, 4, 5, 6	year 2	Scientific paper
h	Review and evaluate effects of contaminants on sediment-dwelling organisms, together with critical analysis of the sensitivity of the methodologies applied	The highest concentrations of contaminants in marine ecosystems are found in sediments. The standardised toxicity tests for sediments are unfortunately not very sensitive to contaminant exposure, at least partly because the organisms that are used are those amenable to lab culture. This item was on the work programme for WGBEC 20 years ago, but there is still limited progress. New analytical techniques alongside "traditional" methods bear promise for improved methods.	2, 3, 4, 5, 6	year 2	Scientific paper

Summary of the Work Plan

Year 1	Update and review national monitoring programmes.
Year 2	Review effects of contaminants, including baseline studies and risk assessment; Review effects of contaminants of emerging concern; Review the study of individual effects in community studies (scientific paper) Review effects of contaminants on sediment-dwelling organisms (scientific paper) Update ToRs a, b, c, d.
Year 3	Review effects of natural and synthetic particles (scientific paper); Review progress with concepts regarding the oceans and human health (scientific paper) Review interactions of contamination effects with those of climate change and acidification (scientific paper)

Supporting information

Priority	The current activities of this Group will lead ICES into issues related to the ecosystem effects of fisheries, especially with regard to the application of the Precautionary Approach. Consequently, these activities are considered to have a very high priority.
Resource requirements	The research programmes which provide the main input to this group are already underway, and resources are already committed. The additional resource required to undertake additional activities in the framework of this group is negligible.
Participants	The Group is normally attended by some 10–15 members and guests.
Secretariat facilities	None.
Financial	No financial implications.
Linkages to ACOM and groups under ACOM	There are no obvious direct linkages.
Linkages to other committees or groups	There is a working relationship with WGMS, WGEEL and WGIBAR. It is also very relevant to the Marine Chemistry Working Group (MCWG).
Linkages to other organizations	OSPAR MIME/HASEC, HELCOM, EEA

Annex 4: WGBEC self-evaluation

1) **Working group name**: Working Group on the Biological Effects of Contaminants (WGBEC)

2) **Year of appointment**: 2016

3) **Current chairs**: Bjørn Einar Grøsvik (NO), Ketil Hylland (NO)

4) **Meeting dates and venues**:

Lisbon, Portugal, 7-11 March 2016, 10 participants;

Reykjavik, Iceland, 13-17 March 2017, 13 participants;

Stareso station, Corsica, France, 16-20 April 2018, 12 participants (including 2 by correspondence).

WG Evaluation

5) Achievements of the WGBEC since last evaluation:

- Through a decade-long process, WGBEC members in collaboration with OSPAR developed a framework for integrated contaminant monitoring and assessment, then launched the project ICON to test it out in European waters, from Spain to Iceland. The ten scientific papers reporting the results were published in a dedicated volume of the journal Marine Environmental Research in 2017 (volume 124).

- Communication has been maintained with OSPAR on the implementation of the integrated contaminant monitoring and assessment framework developed with significant contributions from WGBEC, including facilitation of effect data entry into ICES databanks for subsequent retrieval for OSPAR evaluations.

- Two TIMES publications to support ongoing development and implementation of effects in monitoring and assessment were produced with contributions from WGBEC members on supporting physiological parameters (Hanson *et al.*, 2017) and a widely used effect method, lysosomal membrane stability (Martinez-Gomez *et al.*, 2016).

- Members of WGBEC has contributed significantly to develop methods to quantify sublethal effects on seabirds and marine mammals. The group has highlighted the very serious contaminant-related population declines of toothed whales in European waters, in particular the killer whale.

6) Recommendation to the Regional Sea Conventions (2017)

- 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.

Recommendation to SCICOM (2018)

- High priority should be given to field-based monitoring in European waters.
- There is a general need for baseline studies on sublethal responses to contaminant exposure in European waters.

Recommendation to WGMS and MCWG (2018):

- There is a need to develop and update assessment criteria for hazardous substances in marine matrices.

7) Please list any specific outreach activities of the WG outside the ICES network (unless listed in question 6). For example, EC projects directly emanating from the WG discussions, representation of the WG in meetings of outside organizations, contributions to other agencies' activities.

- Participation in EU JPI-Oceans projects on microplastics was developed largely around networks stemming from WGBEC.
- Special sessions were instigated at the 2017 SETAC meeting in Brussels to combine ecotoxicology and fisheries/aquaculture science.

8) Please indicate what difficulties, if any, have been encountered in achieving the workplan.

- The workplan was ambitious with regard to the number of reviews to be published. No scientific papers were prepared for ToRs e and f, contributions for ToRs a and i are in the pipeline and expected to be submitted this year (2018).

Future plans

9) 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 effect methods and frameworks by which to evaluate environmental quality.

WGBEC is extending its activities to also encompass issues relevant to human health, with an aim to investigate ecosystem-wide impacts of contaminants. The marine environment and human health are inextricably linked, primarily through ecosystem health and ecosystem services. Contaminants/pollution is one of the human pressures on marine ecosystem health resulting in human health impacts. In addition, chemical pollutants can decrease resilience of marine ecosystems, affect sea food security production/ resources, and ultimately may contribute to loss of biodiversity.

10) WGBEC does consider that our expertise is valuable for society as a whole and also within ICES. Monitoring environmental status in sea and coastal regions are dominated by chemical measurements. Inclusion of biological effect measurements may integrate effects from several compounds and increase the understanding of how exposures impact individuals, populations and ecosystems. Ecosystem health and human health are strongly tied together and the group's broad focus from invertebrates, fish, sea mammals to birds make possible important findings and future advice on how we better can manage human pressures. WGBEC keeps fully updated on national monitoring activities, regularly review techniques to detect effects of contaminants as well as the use of biological effects in risk assessment, review baseline studies of effects, review interaction of climate change and acidification with contaminants, effects of contaminants of emerging concern, individual effects in community studies and effects of contaminants on sediment-dwelling organisms.

11) WGBEC already has members with an expertise relevant to its aims. It would benefit the work if we could have active members from more of the member countries within ICES, mainly to give a broader coverage and to increase the use of appropriate and quality-assured techniques.

12) WGBEC has competence to give advice on aspects connected with assessments of Good Environmental Status for the MSFD, in particular descriptor 8, but also descriptors 9 and 10.

Annex 5: References to specific ToRs

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