WORKING GROUP ON BIOLOGICAL EFFECTS OF CONTAMINANTS (WGBEC)

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i Executive summary

The Working Group on Biological Effects of Contaminants (WGBEC) investigates the biological effects of contaminants in the marine environment. The group provides research and increases the understanding of contaminant interactions and effects, including the development of integrated biological effects monitoring strategies, which are used to support international research and monitoring.

The WGBEC has contributed significantly to the implementation and harmonization of techniques that can be used to evaluate the biological effects of pollutants in national monitoring programmes. An overview of national effect-based monitoring programmes of Member States is provided with the aim to support European countries and Regional Seas Conventions on their implementation. A summary of the national effects-based monitoring programmes has been provided by twelve European countries represented at the WGBEC meetings. The adoption of biological effects monitoring can differ widely and comparisons between approaches and the choice of biological effects methods used acts as an important tool. A summary of the main findings is presented.

Furthermore, OSPAR's Hazardous Substances and Eutrophication Committee (HASEC) has encouraged contracting parties to perform targeted biological effects monitoring to enhance the assessment of contaminants in sediment and biota towards the OSPAR QSR2023. WGBEC members contributed to the integrated biological effects approach assessment by providing data from their national monitoring activities to produce maps and figures to enable interpretations.

Revision of the biological effects methods, including new techniques and developments, and the quality assurance of existing methods are core activities for the WGBEC, which require continuous discussion and evaluation by the group. Activities include the production of new ICES TIMES documents as well as intercalibration exercises to ensure Member States are providing comparable data for national monitoring. To this end, intercalibration exercises were performed under the BEQUALM programme for two of the more commonly used biological effects methods, including micronucleus formation in mussel haemocytes and PAH metabolites in fish bile. These intercalibrations were successful despite identifying some variation in reported values between laboratories. Further intercalibration exercises are planned and the WGBEC strongly support the need for such quality assurance.

In addition to the national monitoring activities and the different methods and approaches for determining the effects of contaminants on biological systems, the WGBEC was interested in discussing some key questions related to the potential impacts of contaminants to marine life. These questions included: the direct and indirect effects of natural and synthetic particles; how climate change and acidification parameters can interact with contaminants and influence bioavailability and effect; whether the structure of marine communities can be used to indicate contaminant exposure; to provide guidance on performing risk assessments for contaminants of emerging concern; and to evaluate the effects of contaminants in marine sediments and whether current sediment toxicity tests are adequate. In addition, and as a wider concept, the linkages between contaminants in the marine environment and human health were also described.

ii Expert group information

Expert group name	Working Group on Biological Effects of Contaminants (WGBEC)
Expert group cycle	Multiannual
Year cycle started	2019
Reporting year in cycle	3/3
Chair(s)	Juan Bellas, Spain
	Steven Brooks, Norway
Meeting venue(s) and dates	11-15 March 2019, Vigo, Spain, 20 participants
	2-6 March 2020, Lisbon, Portugal, 18 participants
	8-12 March 2021, online meeting, 27 participants

1 New developments and innovative methods to study and monitor effects of contaminants

1.1 Background

Since the mid-1990s, the WGBEC has evaluated the suitability of different biological effects methods for national and international monitoring programmes. This effort was initiated by a request from OSPAR concerning methods appropriate for effects monitoring of tributyltin (TBT), selected metals and polycyclic aromatic hydrocarbons (PAHs), as well as chemical pollution in general (OSPAR 1998a, b). Tables of methods for invertebrates and vertebrates were developed and have been regularly revised until the last revision in 2010 (WGBEC, 2010). Assessment criteria were developed as part of the guidelines established for a framework for ecosystem assessment of contaminant impacts (SGIMC, 2011).

Revision of methods, new methods and quality assurance of existing methods are clearly core activities for the WGBEC and needs continuous discussion and evaluation by the group. WGBEC (2010) concluded on requirements for methods, reported in Hylland *et al.* (2017):

i. The method should be able to separate contaminant-related effects from natural processes or the influence of other stressors, including knowledge of confounding factors;
ii. There should be some knowledge of dose-dependency;
iii. The mechanism of toxicity should at least partly be understood;
iv. Quality assurance should be established;
v. Assessment criteria must be established for responses in relevant species.

In addition, there has been a "hidden" requirement that all proposed methods should be available to laboratories in all countries, not limited by the availability of e.g. a specific antibody (as was the case for vitellogenin for some years). The latter supports the role of the WGBEC for facilitating contact between labs, training of labs as well as to promote commercialisation of relevant antibodies or kits. Finally, there is also a requirement to evaluate and adjust assessment criteria for selected methods, established as part of the SGIMC (2011).

For the recommended list of biomarkers, it is important that all laboratories perform these methods to the same high standard and that there is reasonable compatibility between laboratories. To assist in this the BEQUALM programme was established. Recent activity under the biomarker division of BEQUALM includes the micronuclei assessment in mussel haemocytes (2018) as well as PAH metabolites in fish bile (2019). An EROD and protein intercalibration is proposed for 2021/2022 and preparations are currently underway. A liver histopathology intercalibration exercise is also planned for 2021/2022 under the whole organism division of BEQUALM.

The original WGBEC list of recommended methods (biomarkers) primarily focused on single measurements, such as enzymes or metabolites, although it also included cell- or tissue-based metrics such as membrane stability and histopathological endpoints. The methods on the recommended list have remained nearly unchanged for the last decades. While this shows that good choices were made, it also reflects a lack of enthusiasm or resources in the scientific community to investigate other mechanisms of toxicity. We now know much more about the recommended methods, about confounding factors and about species differences, so progress has been made. Progress has also been made in genotoxicity assessment, with micronucleus and comet assays in common use. We still however lack methods to monitor for developmental toxicity, reproductive

toxicity, immunotoxicity and delayed effects. And it is becoming increasingly clear that species differences are a huge challenge if we want to protect "all" marine organisms.

Over the years there has been a discussion of the existing lag between the discovery of new effect methods in the laboratory to their use in monitoring programmes. At this time, new methods are typically derived from transcriptomic, proteomic and metabolomic analyses, producing thousands of values for each sample. Such results can be analysed directly using multivariate analyses, can be used to quantify effects on specific pathways or possible biomarkers. The interpretation of the immense amount of data that are generated from these 'omics' tools, has typically delayed their involvement in monitoring programmes, although software solutions are becoming increasingly available to help with this. It is important to remember the different time scales of 'omics' responses – transcripts respond on the scale of hours, the proteome on the scale of days to weeks, the metabolome on the scale of minutes to hours, and all may also be affected not only be immediate exposure but also by contaminants accumulated in tissues. An additional issue with proteomics is the loss of low-concentration proteins, which may be where effects are manifested. If used carefully, transcriptomics and proteomics data can fulfil the criteria: (i) specificity, (ii) dose-dependency and (iii) mechanism. These types of data will be particularly strong on (i) and (iii) but can also contribute to increased understanding of non-uniform dose-dependency, i.e. (ii). It is more challenging to include metabolomics data into the criteria as they mainly reflect general processes in the cell, although for some substances, metabolites may be detectable.

1.2 New development of existing recommended techniques

A number of new developments in recommended methods for biological effects monitoring have been considered by WGBEC over the last three year term. The continuous development of recommended methods is a core activity of the group.

1.2.1 Development of a liver neoplasm assessment tool using UK monitoring data

The monitoring of fish diseases contributes to the OSPAR Coordinated Environmental Monitoring Programme (CEMP) of the Joint Assessment Monitoring Programme (JAMP) and has been undertaken in the UK since the 1980s as part of national monitoring activities. In the context of national monitoring, the term 'fish diseases' refers to (a) gross diseases and parasites (including the macroscopic assessment of liver neoplasms) and (b) liver histopathology (including toxicopathic conditions and liver neoplasms). Histopathology is used to investigate the health of aquatic organisms and has been successfully applied when investigating relationships between anthropogenic contaminants and toxicopathic liver lesions in several fish species (Stein *et al.*, 1990; Myers *et al.*, 1994; Stentiford *et al.*, 2003; Stehr *et al.*, 2004; Lang *et al.*, 2017). Flatfish are often the target species in biomonitoring programmes resulting from their sedimentary habitat, therefore possessing an increased likelihood of becoming exposed to harmful sediment-associated contaminants.

Several studies have used liver neoplasms investigating hazardous chemicals and their biological effects. Laboratory and mesocosm studies have reported the formation of liver neoplasms following exposure to hazardous chemicals (Hawkins *et al.*, 1990; Vethaak *et al.*, 1996) and several studies in the aquatic environment have shown correlations between anthropogenic contaminants and neoplastic liver lesions. As such, there is a body of evidence suggesting that polycyclic aromatic hydrocarbons (PAHs) and persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs) and organochloride pesticides, result in the formation of toxicopathic liver

conditions including neoplasms (Myers *et al.*, 1990; Myers *et al.*, 1998; Stehr *et al.*, 2004). European field studies have since identified similar toxicopathic, lesions in the European flounder (*Platichthys flesus*) and common dab (*Limanda limanda*) and these are now considered a major bioindicator species in the OSPAR North-east Atlantic region (Köhler, 1990; Lang *et al.*, 2006; Vethaak *et al.*, 2009; Stentiford *et al.*, 2010; Bignell *et al.*, 2020). Whilst there have been demonstrable links between liver neoplasms and environmental pollution, it is important to consider the effects of age, which is often positively correlated to the formation of cancer in several species, including humans. As such, it is a potentially confounding factor when investigating liver neoplasms in fish. Stentiford *et al.* (2010) demonstrated that incidences of liver neoplasms increase with age in dab (*L. limanda*). Whether this relationship is simply an artefact of increasing age or the result of continued long-term chronic exposure to contaminants over time, is particularly challenging to determine. However, that study also demonstrated that whilst liver neoplasm prevalence increases with age, the age of onset appears 'accelerated' at several locations, thus suggesting there are other factors influencing the formation of liver neoplasms e.g. anthropogenic contaminants.

Assessment tools are widely used to assess biomarkers of contaminant exposure and biological endpoints e.g. ethoxyresorufin-O-deethylase (EROD), PAH bile metabolites and imposex, across the OSPAR region and ascertain whether environment quality standards are being met with regards to the biological effects of contaminants. Within this framework, 'gross diseases and parasites' are currently assessed using the ICES Fish Disease Index (FDI) developed by the ICES Working Group for Parasites and Diseases of Marine Organisms (WGPDMO), as an indicator of general fish health. However, there is currently no tool yet established for the dedicated assessment of liver neoplasms within the context of fish diseases. When attempting to assess liver neoplasms between geographical regions, it is important to ensure that comparisons avoid comparing fish populations whose age varies considerably e.g. younger versus older fish populations, since this will undoubtedly skew any wider assessments. The development of a liver neoplasm assessment tool that can adjust for potential age effects is therefore crucial to investigate the true effects of environmental contaminants and ensure true like-for-like spatial and temporal comparisons can be made. ICES guidelines might be updated with a recommendation on the fish age for monitoring and temporal trends assessment.

Monitoring and assessment of liver neoplasms

Liver neoplasms in the flatfish dab (*Limanda limanda*) are monitored in the UK as part of the UK Clean Seas Environment Monitoring Programme (CSEMP) and provide valuable information on the cause-effect relationship between environmental contaminants Their measurement was selected by the UK to supplement the target covering the biological effects of contaminants set out in the MSFD (descriptor 8), which requires that "the intensity of those biological or ecological effects due to contaminants agreed by OSPAR as appropriate for MSFD purposes are below the toxicologically-based standards". To this end, the UK have developed an assessment method utilising a generalised linear mixed (GLM) model to correct for the confounding factors of age and sex, followed by logistic regression to determine spatial and temporal differences including their significance. The development of this method is considered complimentary to the FDI for gross diseases and parasites and aims to allow for an accurate assessment of toxicopathic liver neoplasms in the UK.

The UK MSFD intermediate assessment undertaken for liver neoplasms analysed data collected by Cefas (England and Wales) and Marine Scotland (Scotland). Status and trends assessments were conducted at two geographical levels (a) the MSFD sub-regions (Greater North Sea and Celtic Seas), and (b) UK bio-hydrographic marine regions (Eastern Channel, Irish Sea, Northern North Sea, Southern North Sea, Western Channel and Celtic Sea) set out in the UK national as-

sessment "Charting Progress 2". Dab were collected from 43 CSEMP fishing stations within English, Welsh (E&W) and Scottish coastal and offshore waters between 2004 and 2015 (Scotland data was only collected between 2010 and 2015). Fish livers were processed for histological analysis using laboratory guidelines described by Feist *et al.* (2004). All fish were analysed for neoplastic lesions using diagnostic criteria agreed under the BEQUALM Fish Disease Measurement programme. Individual fish were given a non-accumulative score of 1, if at least one neoplastic lesion was detected (e.g. the presence of two individual lesion types would result in a score of 1). The age of all fish was determined using otolith analysis (Easey and Milner, 2008) and used to establish the prevalence of liver neoplasms for each age class.

Data were split into two periods including a baseline (2004–2010) and assessment (2011–2015) period. This was undertaken resulting from (a) significant changes in the England and Wales national monitoring programme between 2010 and 2011, i.e. changes from annual to biennial monitoring, and (b) the absence of liver neoplasm data in dab sampled from Scottish waters. The treatment of the data in this manner, following a preliminary data analyses, allowed for efficient use of UK data for the purposes of conducting a UK-wide assessment. A generalised linear model (GLM) was used to normalise for the effects of sex and age, and determine any temporal trend increase or decrease in the prevalence of neoplasms between the baseline and assessment periods. Furthermore, the assessment was conducted on fish aged between 3 and 6 years since this represented the interquartile range across the UK population, thus further helping to mitigate any confounding effects of age.

The UK assessment demonstrated significant differences in the prevalence of liver neoplasms between the MSFD and UK bio-hydrographic marine regions, and the baseline and assessment periods (Figure 1.1). There was a significant decrease in the prevalence liver neoplasms in flatfish from both the Celtic Seas and Greater North Sea MSFD sub-regions. A significant decrease in liver neoplasms was also observed across all UK bio-hydrographic marine regions. The assessment results show that this assessment tool can adjust for age and sex related effects and help determine trends in liver neoplasm prevalence in the UK.

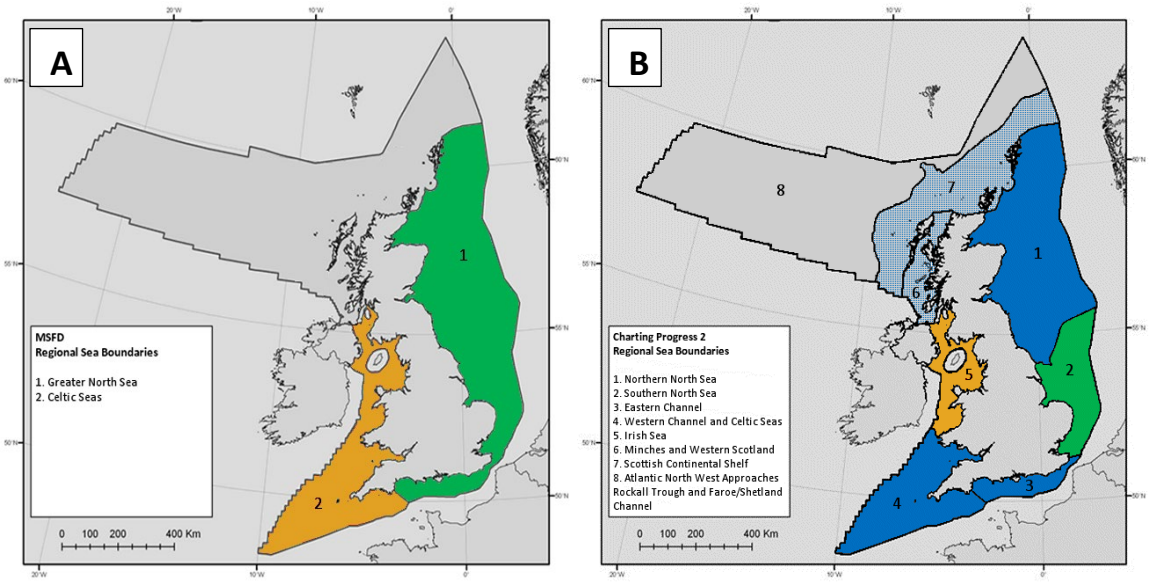

Figure 1.1. (A) Marine Strategy Framework Directive sub-regional status for liver neoplasms in dab. Regions coloured amber indicate a 'significant response' and the green regions indicate a 'background response'. The grey regions in Scotland had insufficient data for incorporation into assessment model for subsequent age and sex normalisation. The Greater North Sea region is coloured resulting from the use of England and Wales data only. (B) UK marine regional status for liver neoplasms in dab. Regions coloured amber indicate 'significant response'; green regions indicate 'elevated response'; and the blue regions indicate a 'background response'. The grey regions had no data collected. Status assessments in regions 6 and 7 used un-normalised data against proposed assessment criteria and are shown as an "indicative assessment" only. The Northern North Sea and Irish Sea UK marine regions (Regions 1 and 5) are coloured based on England and Wales data only due to the absence of normalised data from Scotland. Please see UKMMAS Marine Online Assessment Tool for further information: https://moat.cefas.co.uk/pressures-from-human-activities/contaminants/liver-neoplasms/

However, additional work will be required to test the suitability for its application across the North East Atlantic Regional Seas, since there are still several challenges to overcome particularly with respect to species distribution, age distribution and assessment regions. It is hoped that further refinement will be achieved through consultation with ICES WGPDMO and a future working group responsible for the implementation of liver neoplasms into the OSPAR QSR 2023. Further details on the preliminary data analysis used to develop this assessment tool, the assessment itself and assessment challenges, are available on the UK Marine Monitoring and Assessment Strategy (UKMMAS) Marine Online Assessment Tool: https://moat.cefas.co.uk/pressures-from-human-activities/contaminants/liver-neoplasms/

1.2.2 Micronuclei assay in flatfish – use of DNA specific stain and power analysis to determine the minimum number of cells required to count

The micronuclei assay is a fast and simple method used as a biological effects indicator of genotoxicity. Micronuclei are formed as a result of exposure to genotoxic agents that affect the formation of cells during cell division. They consist of acentric fragments of chromosomes or whole chromosomes which are not incorporated into daughter nuclei during anaphase. This is a useful monitoring tool in mussels and in fish however, the background frequency of micronuclei in fish is considerably lower than mussels. Assessment criteria have been developed for a number of species including blue mussel (*Mytilus edulis*), Mediterranean mussel (*Mytilus galloprovincialis*), bay mussel (*Mytilus trossulus*), flounder (*Platichthys flesus*), dab (*Limanda limanda*), eelpout (*Zoarces viviparous*), cod (*Gadus morhua*) and red mullet (*Mullus barbatus*). The number of micronuclei

per 1000 cells is counted by staining the cells and examining them microscopically. An ICES TIMES protocol is currently being drafted and is a deliverable of this ToR.

Suitability of the DNA specific stain Feulgen for use in the micronuclei assay

In the absence of an ICES TIMES protocol, ICES recommendations have previously been published (Baršienė *et al.* 2012). Although these recommendations do not specify the stain that should be used in the micronuclei assay, they do recommend use of fluorescent staining as very small micronuclei may be hard to detect with light microscopy. Micronuclei assessment has been included in the suite of biological effects monitored in the UK for a number of years and the Giemsa stain is used for the analysis. Giemsa is a non-specific dye suitable for most biological materials and commonly used for many histological practices. Marine Scotland Science (MSS) considered using a DNA specific stain for micronuclei assay in flatfish erythrocytes. They compared two DNA specific stains, Acridine Orange (Çavaş & Ergene-Gözükara 2005) and Feulgen (Bancroft & Gamble 2002), to Giemsa and found Feulgen stain preferable.

To confirm suitability of the Feulgen stain it was used for a small number of sites before use in the entire Scottish national monitoring programme. The Feulgen stain was applied to blood smears prepared from dab or flounder from five locations in the Tayside and Forth area, Scotland, collected between 2017 and 2019. The slides were scored (4000 cells per fish) for micronuclei and the frequency of micronuclei per 1000 cells was calculated. The Feulgen stain has shown to be suitable for the micronucleus assessment conducted on flatfish erythrocytes and analysis of environmental monitoring samples showed a reduction in micronucleus frequency at sites where flounder was sampled. This may indicate that the Giemsa stain used previously gave false positive results in flounder. This was confirmed by re-staining historically stained Giemsa slides with Feulgen which indicated that artefacts from the Giemsa stain may have been mistaken for micronuclei. However full reanalysis of this data has not been carried out so these results are preliminary and further clarification is required.

Other WGBEC labs who run the micronuclei assay in fish use the DNA specific stain Dapi (France, Norway, Italy), Acridine Orange (Spain) or the non-specific Giemsa stain (England, UK). Recommendations in the use of DNA specific stain should be considered in the ICES TIMES protocol for this method.

Power analysis to determine the minimum number of cells required to count in the micronuclei assay

A power analysis was carried out to assess the minimum number of cells required to count in the micronuclei assay in flatfish. This work was initiated following the UK 2018 MSFD assessment of micronuclei in flatfish (Robinson & Bignell, 2018) which identified that different numbers of cells were scored in the two UK labs that provided data for the assessment. MSS counted 4000 cells per fish where Cefas counted 1000 cells per fish however MSS counted fewer fish per site than Cefas. In order to agree the number of cells scored, a review of the incidence and power to detect micronucleus above the Background Assessment Criteria (BAC) was requested.

Statistical analysis was used to estimate the optimum number of fish and cells required to reliably estimate genotoxic micronuclei levels. Variation in micronuclei counts were assumed between fish within locations, a negative binomial distribution was followed and theta was estimated from the available MSS and CEFAS data that was used in the 2018 MSFD assessment. Micronuclei counts were simulated at BAC and typical levels for multiple samples of up to 10 000 cells from up to 20 fish. Flounder (BAC = 0.3 micronuclei per 1000 cells) and dab (BAC =

0.5 micronuclei per 1000 cells) were considered. Sample mean values, standard deviations between mean values and upper 95% quantiles were then estimated. Figure 1.2 shows the coefficient of variation for flounder and dab. It shows the benefit of increasing the number of cells to count from 1000 up to 3000 and thereafter the benefit decreases. It also shows the benefit of increasing the number of fish per site.

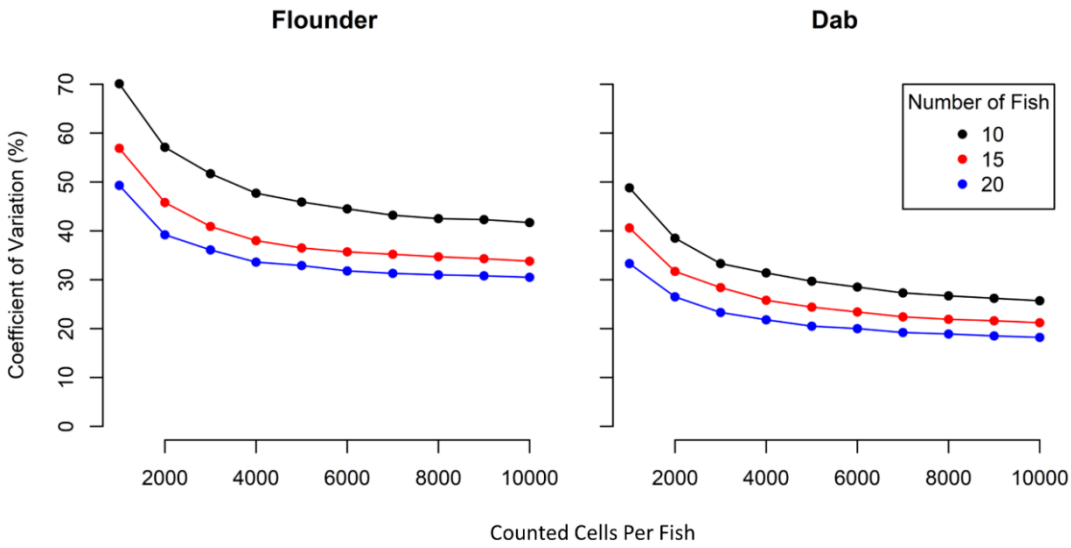

Figure 1.2. Coefficient of variation per number of cells counted per fish in flounder and dab for 10, 15 and 20 fish per site.

For robust assessment of the micronuclei assay upper 90% confidence intervals (CI) should be compared to the BAC (as opposed to mean values). Populations were generated to estimate the upper 90% CI for typical low levels (Baršienė *et al.* 2008, Rybakovas *et al.* 2009) and for levels equivalent to 2/3 of the BAC to check if the upper 90% CI was actually below the BAC. For typical low levels all the CI of the different combinations of fish and cells counted were below the BAC. Table 1.1 shows the results for levels equivalent to 2/3 of the BAC. For flounder at least 20 fish need to be sampled and at least 6000 cells counted in order to be confident that the levels are below the BAC. However, for dab you would be able to count at least 6000 cells from 10 fish and at least 4000 cells from 15 or 20 fish in order to be confident that the levels are below the BAC.

Table 1.1. Upper 90% CI for 2/3 of the BAC. Highlighted cells in bold text indicate that upper 90% CI is above the BAC.

Number of fish	Number of Cells	Flounder	Dab
	1000	**0.40**	**0.60**
	4000	**0.32**	**0.50**
10	6000	**0.32**	0.48
	8000	**0.31**	0.48
	10000	**0.31**	0.47
	1000	**0.33**	**0.53**
	4000	**0.32**	0.47
15	6000	**0.31**	0.45
	8000	**0.30**	0.44
	10000	**0.30**	0.44
	1000	**0.35**	**0.50**
	4000	**0.30**	0.40
20	6000	0.29	0.42
	8000	0.29	0.42
	10000	0.28	0.42

This work is still ongoing. The next approach is to estimate the probability that the mean values of 2/3 of the BAC exceeds the BAC using known variance. MSS also plan to perform a cost benefit analysis and determine the optimum number of fish and cells to count to reliably estimate genotoxic micronuclei levels. This work should feed into the ICES TIMES protocol.

Other WGBEC labs that use the micronuclei assay in fish count 4000 cells (Italy) or 5000 cells (France, Norway and Spain). Norway recommend and are investigating the use of automation for the micronuclei assay as software is now available although requires optimisation. Some stains work better than others for this. Alternatives biomarkers for genotoxicity would be comet or DNA adducts.

1.2.3 Method comparison and sample storage time influence on the assessment of acetylcholinesterase inhibition in the muscle tissue of common dab (*Limanda limanda*)

Acetylcholinesterase inhibition (AChE) has been used as a biomarker of effects of organophosphate and carbamate compounds and an ICES TIMES protocol (nr 22) was adopted in 1998 and recommended for contaminant monitoring programmes in the marine environment. However further characterization of the cholinesterase forms present in marine teleost fish is available, and a method comparison study has been carried out at Cefas. In addition, a study to determine the recommended sample storage time, before degradation occurs, when assessing cholinesterases in samples is also underway at Cefas.

For the method comparison study, besides the adopted ICES TIMES protocol, 3 additional methods to measure total cholinesterase (ChE) were tested, including Sturm *et al*. (1999), where total

cholinesterase (ChE), but also AChE and BChE (butyrylcholinesterase) can be assessed separately (via inhibition). Total cholinesterase in marine teleost fish muscle is mostly constituted of AChE and BChE (Sturm, *et al*. 1999). The assessment was carried out in muscle samples of dab (*Limanda limanda*) with the total ChE determined using the 4 methods; additionally, the AChE component was also measured using the Sturm *et al*. (1999) method in the second round of the study. No significant differences were found in the protein content of the muscle samples prepared to assess the different methods. The first round of the method comparison study indicated some difference between total ChE values, with two methods, including Sturm *et al*. (1999), measuring higher levels. All samples however had values below the adopted EAC. The muscle samples used in the first round were frozen at -80°C for 2 years prior use, this therefore raised a concern over potential sample degradation and a second round of the study was conducted, with muscle samples analysed within 2 months of collection and increased replication. The results indicated that total ChE measured using Sturm *et al*. (1999) method showed consistently higher activities then the other methods, with the AChE component measured activity equivalent to the total ChE measured with the other three methods.

The sample storage study preliminary results indicate that dab muscle samples stored frozen at -80°C for 3 months showed minimal decline in total ChE and AChE activities. After 6 months there is a 25% reduction in total ChE and 20% in AChE component. Further analysis beyond 6 months, and up to one year in storage, indicates no significant further reduction on both forms, however final result analysis is ongoing.

This work aims at starting a dialogue with other WGBEC partners already performing the assay, or planning to implement it, and to initiate efforts to harmonize method choice and sample storage time used for the monitoring programmes. These efforts will contribute towards a future intercalibration exercise under BEQUALM.

1.3 Development of new techniques for evaluation

New development and innovative methods for biological effects have been considered by WGBEC at different levels:

i. New ecotoxicological indicators;
ii. New sentinel species and new thresholds;
iii. New synergy between the biological effect tools and the integrated biological and chemical contaminants monitoring;
iv. Mutualization of the scientific cruises between contracting parties.

1.3.1 Toxicity profiling of sediment samples

Since the last years, alternative bioassay tests aimed to reduce whole animal testing in fish toxicity assays is growing. Fish-derived cell lines are being used for basic purposes in the investigation of environmental contaminants such as in ascertaining the mechanisms by which chemical compounds exert their toxicity, in determining the relative toxicity of different chemical contaminants and in evaluating the toxicity of environmental samples (Hamers *et al.*, 2018). Furthermore, more than one hundred different fish cell lines has been developed in the past decade, including fish cell lines of marine species (Lakra *et al.*, 2011; Pandey *et al.*, 2013).

Interesting protocols for evaluating general responses of chemical toxicity at the cellular level using fish cell lines include fluorometric tests that evaluate viability of cells with three indicator dyes and provide a broad overview of the sensitivity of cells to chemical contaminants (Dayeh *et al.*, 2103):

i. Alamar blue for metabolic activity;
ii. 5-carboxyfluorescein diacetate acetoxymethyl ester (cfda-am) for membrane integrity;
iii. Neutral red for lysosomal function.

In vitro methods using cell lines have also been applied to assess the toxic profile of marine sediments in the last years on several studies providing valuable information on the occurrence of bioactive compounds (Hamers *et al.*, 2013; Otte *et al.*, 2013; Vethaak *et al.*, 2017). This approach has been tested on marine surface sediment samples from Mar Menor Lagoon (SE Spain) and results compared with in vivo test (sea urchin embryotoxicity test) (Martínez-Gómez *et al.*, 2020). The occurrence of bioactive compounds (steroids and aryl hydrocarbon receptor inducers) in the surface sediments of the Mar Menor Lagoon was investigated testing sediment samples for (anti)estrogenic, (anti)androgenic and ethoxyresorufin-O-deethylase (EROD) activities using the HER-Luc, AR-EcoScreenTM and fibroblast like RTG-2 cell lines, respectively. In vitro results show the occurrence in extracts of compounds with estrogen antagonism, androgen antagonism and dioxin-like activities. Overall, results pointed to the presence of unknown or unanalyzed biologically-active compounds in the sediments, mostly associated with the extracted polar fraction of the Mar Menor.

On the other hand, *Danio rerio* embryos acute toxicity test have been developed to investigate the toxicological characterization of sediments (Massei *et al.*, 2019). Effect data, were supported by chemical analyses of organic pollutants and trace elements. Endpoints include behavioural biomarkers related with locomotor activity, heartbeat, hatching rate, malformations and inhibition of AChE. Results demonstrated that specifc chemical profiles lead to specifc effect patterns in *Danio rerio* embryos: behavioral endpoints may help detect the exposure to neurotoxins, and the observation of body malformations seems to be a potential tool for the identifcation of site-specifc pollutants as polychlorinated biphenyl (PCBs), brominated flame retardants (BFRs) and several pesticides. Overall, results show the suitability of *Danio rerio* embryos for the fast screening of sediment samples.

1.3.2 Flow-cytometry applied to microalgae

Among the new developments, marine microalgae appear as an indicator still too little developed in ecotoxicology for monitoring the biological effects of chemical contaminants. Located in the first level of the food chain, microalgae are the gateway to many contaminants such as metals and they also have specific herbicide sensitivity. These short-lived single-celled organisms are of particular interest in assessing multigenerational effects and long-term effects of chemical contaminants.

The ability of microalgae to develop stable, long-term resistance to herbicides was studied in marine microalgae *Tetraselmis suecica* (Stachowski-Harberkorn *et al.*, 2013). These microalgae were exposed to the herbicide diuron for a 43-generation exposure period followed by a 12-generation depuration phase. The results provided evidence of culture adaptation to diuron (Dupraz *et al.*, 2016; Stachowski-Haberkorn *et al.*, 2013).

Flow cytometry analyses were used to monitor cell density, viability, morphology, relative chlorophyll content and intracellular reactive oxygen species (ROS) level. This study demonstrates the possible appearance of stable diuron resistance in microalgae in cases of strong, multigenerational chronic exposure to this herbicide in polluted environments. Flow-cytometry was therefore considered as an effective tool for the measurement of multiple biomarker endpoints in microalgae, whereas this method could also be applied to the cells of marine animals such as mussel haemocytes.

1.4 Review of relevant methods of toxicity assessment

Advances in methods by which to detect effects of contaminants in the oceans are both linked to recent technological developments and increased understanding of relevant systems. The increased availability of sequencing tools and tools for the quantification of gene expression, as well as bioinformatics software has made it possible to study whole transcriptomes in fish populations from polluted areas and experimental exposures, as well as the behaviour of specific genes. Interestingly, many of the studies actually pinpoint processes already in use as biomarkers, such as cytochrome P450, metallothionein and vitellogenin. Pathway analyses of transcript-wide analyses have indicated some processes that may be particularly sensitive to contaminant-exposure, such as lipid metabolism (lipidomics), endocrine regulation and immune functions, as well as e.g. cell cycle-related gene expression. Similar, but not as extensive, analyses have been performed for the proteome. For the purpose of quantifying contaminant-related stress, it is however important to remember the time scales of the responses, as mentioned above. If we accept the short time scales represented by transcripts, tools can be developed to analyse transcript-wide data in a quantitative sense. It is however dubious whether transcripts will be accepted as sufficient indication of adverse effects. An alternative is to develop assays reflecting the identified pathways, targeting proteins or enzymes from those analyses. In addition to 'omics' type data, there has been increasing understanding of some pollution-related responses, such as cardiotoxicity following exposure to oil or PAHs (Incardona *et al.*, 2015). Finally, it has become increasingly clear over the past couple of decades that heritable gene expression can be modulated by environmental factors, including contaminants, i.e. epigenetics.

1.4.1 Expression of toxicologically relevant genes

With MSFD requiring robust tools to link cause and effect, there is a pressing need to develop and utilise new assays capable of evaluating both short- and long-term changes in organisms in relation to contaminants. Development of gene expression assays is a first step towards this progression. All cellular/organismal level responses to environmentally toxic chemicals are mediated through modulation of expression of components of specific intracellular signalling pathways, which can significantly alter cellular/tissue physiology and ultimately whole animal health and behaviour.

The degree of such contaminant-induced gene expression modulation can be quantified through genomic approaches (ecotoxicogenomics; Snape *et al.*, 2004; Steinberg *et al.*, 2008) to reveal novel changes in gene expression profiles, while Real-time (RT) PCR has been used for analysis of specific genes within the pathways affected. Giltrap *et al.* (in preparation) validated the use of a quantitative PCR macroarray for use as a biomarker of exposure to contaminants in flounder. Twelve gene targets were assessed for several classic biomarkers covering several prototypical toxicological responses to chemical exposures and compared with traditional biological effects tools (EROD, vitellogenin, PAH metabolites and micronucleus assay) and contaminant levels. The macroarray offers potential to be used as a single assay with multiple endpoints which mirrors changes in traditional biomarker expression in particular, CYP1A activity and endocrine disruption pathways.

1.4.2 Lipidomics

Lipids are irreplacable biological components, involved in critical physiological mechanisms, making them ideal candidates to monitor effects of stress (or exposure) on pathophysiology in organisms (Aristizabal-Henao *et al.*, 2020) Lipids have been used to assess physiological effects in a range of organic contaminants and metals. This includes reporting of altered lipid profiles

after exposure to perfluoroalkyl substances in zebrafish (*Danio rerio*) as a model organism (Yang *et al.*, 2019). Bisphenol A has also been found to stimulate lipid deposition in zebrafish (Gibert *et al.*, 2019). Melvin *et al.* (2019) reported increased oxidative stress and altered lipid profiles in fish residing in metalloid rich wetlands, while Laird *et al.* (2018) reported lower levels of two omega-3 polyunsaturated fatty acids in fresh water fish with increasing mercury levels in the Canadian subarctic. In addition to this, Dreier *et al.* (2020) mention how chemicals can alter lipid metabolism and regulation in largemouth bass (*Micropterus salmoides*) and highlight the need for further research in this area. Approaches for analyses in environmental toxicology include non-targeted high-resolution/accurate mass tandem mass spectrometry lipidomic studies.

1.4.3 Epigenetics

Epigenetic mechanisms are those which do not arise from alterations in DNA nucleotide sequences, but are alterations arising from mitotically or meiotically heritable changes in gene with associated phenotypic traits (Chung and Herceg, 2020). Noncoding RNAs (ncRNAs) can influence chromatin conformation and induce heritable effects. Micro RNAs (MiRNAs) are a type of ncRNAs and are involved in silencing and post-transcriptional regulation of gene expression. MiRNAs have been reported to be responsive to exposures of model compounds such as p,p'-DDE, bisphenol and 2,3,7,8-tetrachloro-dibenzop-dioxin (Chung and Herceg, 2020). MicroRNAs are endogenous regulatory RNA molecules that modulate gene expression and have been identified as important regulators of gene and protein expression (Ruvkun, 2008). Alterations in MiRNA expression have been reported to be involved in response to diseases including cancer (Meng *et al.*, 2006) and some have been determined to be diagnostic biomarkers for disease (Archanioti *et al.*, 2011). Gene expression assays offer both a very sensitive level of detection and a high degree of specificity, e.g. in the case of pharmaceuticals designed to stimulate certain pathways, and so may offer susceptibility or exposure prediction capabilities. MiRNAs have also been reported to show induction of immune, apoptotic pathways, lipid and xenobiotic metabolism in marine mussels (Yu *et al.*, 2021).

1.4.4 Cardiotoxicity

Following the Exxon Valdez and Deepwater Horizon spills, cardiotoxicity was observed in oil-exposed fish, particularly evident for developmental stages (Hicken *et al.*, 2011; Incardona *et al.*, 2015; McGruer *et al.*, 2019). Exposure to oil-derived contaminants, probably dominated by PAHs, affected the morphology of the heart, causing a reduced output, with knock-on effects on swimming ability and endurance (Hicken *et al.*, 2011). PAHs are ubiquitous pollutants in marine ecosystems, not only in areas exposed to oil spills, and this endpoint is a promising endpoint for future effect monitoring programmes.

1.4.5 Neurotoxicity

Neurotoxic agents are widely used in aquaculture, particularly organophosphates and pyrethroids, and there is therefore a large database on acute toxicity and potential environmental consequences. It is not yet known the extent to which neurotoxic agents modulate behaviour in wildlife in addition to direct toxicity through causing reduced swimming activity and disorientation, but this has been comprehensively documented for humans. An additional mechanism which is known from human health studies, but not investigated for marine organisms is delayed toxicity (Grandjean & Landrigan, 2014).

1.4.6 Endocrine disruption

Endocrine disruption is a wide concept that encompasses many mechanisms of toxicity and large range of substances. The main focus in environmental science has been on reproduction-related steroids, particularly oestrogens and androgens, and thyroid hormones. There is evidence that freshwater fish populations throughout Europe are feminised to varying extents, but less data for marine populations. Scott *et al.* (2006) observed increased vitellogenin in male cod from an urban fjord as compared to a pristine reference and early studies on flounder suggested there could be large-scale effects on flounder along UK coasts (Allen *et al.*, 1999). Plasma vitellogenin or zona radiata protein in male or juvenile fish are good markers for oestrogenicity, but we lack sensitive markers for other mechanisms of endocrine disruption.

1.4.7 Immunotoxicity

There is an expectation that exposure to contaminants will lead to disease or increased susceptibility to disease, but there is surprisingly little supporting data. Most studies on immune functions in invertebrates or fish have targeted physiological endpoints such as macrophage functions or morphological properties such as the number of particular cells. Although science has accumulated a substantial database on immune functions in selected fish species as a result of vaccine development for aquaculture, surprisingly little data has made its way to environmental sciences. There is a need to develop robust and sensitive immunotoxicity markers for marine organisms.

1.5 Protocols and Quality Assurance Programmes

The need for quality assurance in biological effects assessments is of the highest importance so that the data collected is of the highest quality and comparable with other leading laboratories. Member States that submit their biological effects data to ICES also need to document participation in quality assurance programmes, such as an intercalibration programme.

Following a simple survey of interest sent out to the laboratories that perform biological effects assessments in the ICES community, three biological effects assessments were selected. These included: 1) micronuclei formation in mussel haemocytes; 2) PAH metabolites in fish bile; and 3) EROD activity in fish livers. Micronuclei formation and PAH metabolites were performed within the BEQULAM (Biological Effects Quality Assurance in Monitoring) programme and the final reports can be found on the BEQUALM website (BEQUALM Biomarkers). A brief summary is provided below.

1.5.1 Micronuclei intercalibration

Nine laboratories participated in the micronuclei intercalibration. A total of 186 photographs containing collections of stained mussel haemocytes were assessed for the number of viable cells, micronuclei, nuclear buds and bi-nucleated cells. The protocol of Bolognesi & Fenech (2012) was recommended as a guideline for the assessment. The results showed differences between the laboratories. Large variations between the number of viable cells, indicated that identifying granular (non-viable) from agranular cells was not achieved by all laboratories. However, despite this, z scores indicated satisfactory results for this assessment. There was reasonable agreement between laboratories in the frequency of micronuclei, nuclear buds and binucleated cells when normalised to 1000 cells, although in all cases one laboratory was found to have a z score marginally outside ±2, indicating questionable results. The results should be used by participating laboratories to assess their internal protocols.

1.5.2 PAH metabolites intercalibration

Eleven laboratories registered to take part in the intercalibration exercise and received samples for analysis. However, for different reasons only 8 laboratories were able to submit their data and are presented in this report. The laboratories were identified by individual laboratory codes in order to keep the intercalibration anonymous. Identical (fully homogenised) aliquots of nine fish bile samples were distributed to the laboratories on dry ice. Each participating laboratory used their own method to determine 1-hydroxypyrene and in some cases 1-hydroxyphenathrene concentrations. Three methods were used including high performance liquid chromatography (HPLC), synchronous fluorescence spectrometry (SFS) and fixed wavelength fluorescence (FF). The results showed that the FF values were two orders of magnitude lower than HPLC and SFS, although between-laboratory variability for the FF method was low. Interlaboratory comparison of 1-hydroxypyrene (6 labs) and 1-hydroxyphenathrene (3 labs) for the nine bile samples using both the HPLC and SFS methods was considered satisfactory with z scores within ± 2 units.

1.5.3 Planned future intercalibration exercises

EROD activity in fish livers intercalibration exercise is planned for 2021 under BEQUALM Biomarkers. Provision of suitable material for this exercise has been difficult, however exposure experiments are planned for spring 2021 and samples should be distributed to participating laboratories later in 2021.

Under the BEQUALM Whole Organism division, a liver neoplasm and histopathology intercalibration exercise is also planned for 2021/22.

1.6 New guidelines for contaminant specific biological effects and general biological effects

In 2018, the OSPAR's Working Group on Monitoring and on Trends and Effects of Substances in the Marine Environment (MIME) requested the WGBEC to initiate the revision of the guidelines of the biological effects of the chemical contaminants drafted in the late 1990s. The WGBEC presented some proposals of revision but considered that the process of revising the new guidelines requires a specific dynamic. This review would be the third review of biological effects in 25 years. Respecting the fundamental OSPAR objectives of monitoring, the new guidelines in the specific and general biological effects should integrate the European methodological evolutions of the MSFD and WFD directives, following the application of new methodologies in monitoring and risk assessment, thresholds in already selected and new sentinels species, transmission of data to the ICES database and the JRC EU recommendations on the harmonisation of WFD and MSFD. In the continuity of the last two joint groups OSPAR/ICES WIKIMON (Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-sea Areas 2007) and SGIMC (Study Group on the Integrated Monitoring of Contaminant and Biological Effects 2010), the WGBEC supports the establishment of a joint OSPAR/ICES group including experts from HELCOM, Barcelona and AMAP to revise the new guidelines between 2022-2025. The WGBEC has agreed to recommend the creation of an expert group for the review of the new guidelines, in order to submit to the OSPAR MIME for validation in November 2021 and then receive the ICES request for the creation of the expert group on the new guidelines for contaminant specific biological effects and general biological effects in 2022.

1.7 EU Chemical Strategy

The European Commission has proposed a new chemicals strategy for sustainability (COM(2020) 667), including a focus on mechanisms of toxicity in protecting humans and the environment, with a particular concern for chemicals causing "cancers, gene mutations", affecting "the reproductive or the endocrine system", or that are "persistent and bioaccumulative". Further assessment of harmful chemicals will include "those affecting the immune, neurological or respiratory systems" and chemicals that are "toxic to a specific organ". With regard to the environment, there is particular emphasis on endocrine disruptors as well as "persistent, mobile and toxic and very persistent and very mobile substances". Pharmaceuticals are particularly highlighted, as are fluorinated substances (PFAS). Finally, the strategy highlights mixture toxicity and different sources of exposure. All in all, the strategy has a very clear effect-oriented focus, potentially indicating a change in direction of EU chemicals policy from concentration- and modelling-based to effect- and mechanism-based.

1.8 Final remarks

If the WGBEC maintains the requirements for methods to be recommended for monitoring, it is clear that there will be many years between the discovery of a potential new method to its implementation. One possible mechanism to avoid this lag was proposed and implemented in the Norwegian offshore monitoring programme, i.e. to include a certain volume of promising methods every year alongside existing methods (recommended by the WGBEC). More recently, the Norwegian offshore monitoring programme has adopted a separate research and development plan to run alongside the existing monitoring programme, enabling established methods to be improved and new methods to be validated for future inclusion.

Recommendations:

- Reviewing 'omics' data from experimental studies with fish or selected invertebrates with an aim to identify specific proteins or enzymes that may be biomarkers for specific contaminants or exposure situations or pathways that appear particularly sensitive (e.g. lipidomics).
- To develop assessment values for multivariate 'omics' endpoints.
- To comprehensively evaluate species differences, particularly for fish, with an aim to identify and, if possible, describe differences between species that explain their sensitivity to particular contaminants or contaminant groups.
- To recommend and support the establishment of a new study group to revise the new biological effects monitoring guidelines between 2022-2025. This should be a joint OSPAR/ICES group including experts from HELCOM, Barcelona Convention and AMAP.
- To update the SGIMC's assessment criteria and establish species specific assessment criteria.
- Continue to support biomarker intercalibration exercises within BEQUALM.

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2 Environmental effects of natural and synthetic particles: direct and interacting effects on marine biota

2.1 Background

With the recent worldwide concern over microplastics (MPs) in the oceans, it is also important to remember other particles that are naturally present in marine ecosystems, which interact directly or indirectly with marine life. These include natural inorganic particles such as clays, natural organic particles such as bacteria, viral particles, algae and other protists, detritus, aggregates and faecal pellets. In addition, natural although anthropogenically derived particles such as drilling muds (from offshore oil and gas activities), mining effluents (mainly mineral particles), metal-based nanoparticles and carbon nanoparticles. In general, natural particles will outnumber synthetic particles in marine coastal and oceanic waters by orders of magnitude. Therefore, despite the real threat of synthetic particles to the marine environment from plastic pollution, the interactions of natural particles with marine organisms should not be overlooked.

There are no data to suggest that MPs have higher affinity for organic or inorganic contaminants than natural particles, as concluded during the joint meeting between WGBEC and MCWG/WGMS in 2018, although they may be preferred by organisms due to their properties, for example: colour, density, shape or charge. It also has to be considered that MPs and NPs that exist in marine ecosystems will always be covered with what has been called an "ecocorona", i.e. organic matter, bacteria, other protists, viruses.

Plastic particles, generally larger than the 5 mm cut-off for MPs, which are classified as meso-(5-20 mm), macro- (>20 mm) and mega-plastics (>100 mm), have been found to accumulate in the stomach of some species of seabirds (e.g. fulmars, albatrosses). Also, MPs have been found in the intestinal system of field-collected marine fish and invertebrates worldwide, but generally at low concentrations. It is considered that the different plastic particle types have different gut uptake rates and pathways within the body, with the potential to internalise smaller nanoparticles and increased chance of entanglement in the gut and intestine of larger and fibrous particles. There is currently no analytical capacity to routinely quantify MPs and nanoplastics (NPs) smaller than around 10 μm in water or organisms, seriously limiting our ability to map and understand MP/NP flows in marine ecosystems. It is further important to quantify aggregation processes in marine coastal ecosystems, with a focus on estuarine areas and at times with high algal production.

To be able to risk assess particles of synthetic origin in coastal waters they must be compared to natural particles. This will require a comparison of the properties of different particles present in coastal ecosystems, including charge, shape, size, colour, density, concentration, hydrophobicity and the presence and properties of their ecocorona. In this context, MPs and NPs differ from other particles in their pattern of weathering and degradation.

All available data suggest that a major fraction of MPs/NPs in marine ecosystems will aggregate along with organic material and be transported to the seafloor. As indicated above, there is a need to confirm these processes. There is limited knowledge of degradation of plastics in marine sediments. Relevant processes are oxygen availability (-), light (-), temperature (-), redox activity (+/-). Aging of plastics in sediments is thus largely unknown, as is the partitioning of contaminants between plastics and natural sediment particles. Studies performed with sediment-dwelling bivalves have shown that even environmentally relevant concentrations of microplastics (such as LDPE) in marine sediments may actually affect organisms living there (Bour *et al.*, 2018).

The observed effects were changes in lipid or protein content of the organism. More data are needed for environmentally relevant species. The nature of their ecocorona will also influence the behaviour of the particle and needs to be accounted for.

Many papers highlight the presence of MPs and NPs in the seas but there remains some debate on the potential biological effects of these plastics on marine life and human health (Borja and Elliott, 2019). Most effects appear to be generated by the particle effect as seen for other natural particles. Typical effects reported include changes in gene expression, inflammation, energy allocation, reproduction, immune system, central nervous system and death (reviewed in Vethaak and Martínez-Gómez, 2020). Furthermore, Chae and An (2017) have reviewed the impact of MPs and NPs in the marine environment, including effects on growth, development, behaviour, reproduction and mortality. Although biological effects are reported it is often not possible to distinguish between the effects caused by the particle as opposed to chemical additives or impurities on the plastic particle itself. In addition, many studies lack a positive particle control.

Relevant knowledge gaps have been indicated from recent reviews including: (i) the lack of chronic experiments at environmental realistic concentrations and comparison to natural particles; (ii) population and ecosystem effects as a result of the seemingly inevitable increasing concentration of synthetic particles in the marine environment; (iii) the role of synthetic particles as transport vectors of pathogens, antibiotic resistance and biotoxins and disease dispersal in marine systems compared to natural particles (Vethaak and Martínez-Gómez, 2020).

2.2 Synthetic particles

2.2.1 Microplastics and nanoplastics

In the last decades, plastic has become important in society, with applications in various sectors such as commerce, industry, and medicine. Plastic debris, tend to accumulate in coastal areas, being a threat to marine organisms and social-economic activities. The continuous flow of plastic litter into our oceans has already led to serious effects on marine mammal, bird and fish populations. MPs, particles smaller than 5 mm (Arthur *et al.*, 2009), represent the most important fraction of marine plastic debris (Ericksen *et al.*, 2014). MPs may enter directly into the aquatic environment or result from the breakdown of larger plastic debris under the influence of UV radiation and mechanical abrasion (Cole *et al.*, 2011). The ecological impact of MPs is still unknown, so it is important to determine the presence of this emergent contaminant in the water and in the organisms and the potential biological effects.

According to a recent SAPEA (Science Advice for Policy by European Academies) report (2018), effects of MPs can be expected to occur in some pockets of benthic fauna in European coastal areas. However, according to this report there are currently no risks of MPs on marine life for open seas (https://www.sapea.info/topics/). It is clear that this preliminary assessment contains many uncertainties. Moreover, it is uncertain if the traditional chemical risk assessment approach used in this case can also be used to assess the risks of polymeric particles. Ecological model approaches using ecosystem-based coupled with Dynamic Energy Budget modelling seem also promising in this area. Recently, a first attempt was published to model the potential impacts of MPs on primary and secondary productivity using reported laboratory findings and a biogeochemical model for the North Sea (Delft3D-GEM); (Troost *et al.*, 2018). Although the model predicted that MPs do not affect the total primary or secondary production of the North Sea as a whole, the spatial patterns of secondary production were altered, showing local changes of ± 10%. However, relevant field data on MPs are scarce, and strong assumptions were required to include the plastic concentrations and their impacts under field conditions into the model.

This modelling approach seems to be promising and could be extended to higher trophic levels, but further work is needed in this area, especially for model validation.

Case study I: Microplastics in Portuguese coastal waters

Water samples were collected in transects perpendicular to the coast, along the Portuguese coast with the RV Noruega. The results identified fibres as the predominant category of MPs, followed by fragments, with most of the particles black and blue. A north-south pattern was shown, with higher concentrations of MPs found in the SW. A new sampling cruise is planned that will provide information on the seasonal differences and depth profiles of MP in the water column.

Small pelagic fish species (European sardine, horse mackerel, anchovy, chub mackerel, Atlantic mackerel, and bogue) were also collected along the Portuguese coast. Significant differences between the diet of the studied fish species in terms of prey type and size were found (Lopes *et al.*, 2020). MPs ingestion was significantly related to diet composition. Species with diets that include smaller prey (European sardine, chub mackerel, and bogue) had lower MPs concentration in the stomachs than fish depending on larger mesozooplankton prey. Horse mackerel had the highest proportion of larger prey (> 1000 μm) and the highest MPs abundance in the stomachs, and thus are a suitable bioindicator for MPs monitoring in the pelagic Iberian ecosystem.

MPs contamination and effect biomarkers were investigated in three commercially important fish species from the North East Atlantic Ocean (Barboza *et al.*, 2019). From the 150 analysed fish (50 per species), 49% had MPs. MPs were found in the gastrointestinal tract, gills and dorsal muscle of individuals from the 3 species. Fish with MPs showed an association with higher lipid peroxidation levels in the brain, gills and dorsal muscle, and enhanced brain acetylcholinesterase activity than fish with no MPs. These results suggest lipid oxidative damage in gills and muscle, and neurotoxicity through lipid oxidative damage and acetylcholinesterase induction in relation to MPs and/or MPs-associated chemicals. These studies highlighted the importance of identifying a bioindicator species for MPs monitoring in the environment.

Cast study II: Microplastics in Icelandic coastal waters

The first assessment of MPs (0.1–5 mm) in Iceland was carried out in 2018, funded by the Environment Agency of Iceland. Blue mussels (*Mytilus edulis*), 40–50 mm in size, were collected in the intertidal zone from six stations (n = 20) alongside the west coast of Iceland, ranging from the Sudurnes Peninsula to the Western Fjords. The methodology employed was adapted from the Norwegian Institute for Water Research (NIVA) report (Lusher *et al.*, 2017). In short, soft tissue of mussels were digested in 10% KOH, filtered through 2.4 μm glass fibre filter and filter transferred under a stereoscope for visual identification of MPs. A hot-needle test was implemented for confirmation of plastics.

The results showed that 40–50% of collected mussels contained 0–4 plastic particles per mussel with 1.27 particles on average. Over 90% of all identified particles were fibres 1.1 mm in size, on average. No significant differences were observed in occurrence and size distribution of MPs between the stations, which is of particular interest considering their different geographical location and distance from potential anthropogenic sources such as populated areas. Since these studies were only the first investigations of the presence of microplastics in marine organisms in Iceland, biological effects were not evaluated.

Case study III: Microplastics in Estonian coastal waters

Microplastic samples from surface water as well as from bottom sediments were collected from 15 sampling stations in the Western-Estonian archipelago in the Baltic Sea, in the summer of 2018 (Kreitsberg *et al.*, submitted). A total of 955 particles (626 from the surface water samples and 329 from sediment samples) were identified using microscopy. Surface water in seagrass beds had 0.04–1.2 (median 0.14) MPs/L. The sediments of the seagrass beds had 0–1817 (median 208) MPs/kg dwt. Blue fibrous MPs were prevalent in both the water and sediment. Within all sites, the majority of observed MPs were small (mean = 1351 µm, median 913 µm, min = 72 µm, max = 27635 µm). However, multiple linear regression revealed that the size of MPs differed between water and sediment, with larger MPs identified in the water.

Effects of nanoplastics

Due to their small size, NPs (< 100 nm) possess specific properties which can increase their toxicity towards aquatic organisms, depending on surface characteristics and interactions with the surrounding environment. A recent review of NPs in the marine environment is available that highlights some of their characteristics and toxic effects to marine organisms (Goncalves and Bebianno, 2021). Here NPs are divided into primary or secondary NPs depending on whether they entered the environment as their original nano size or as a consequence of plastic degradation. Studies on the effects of NPs to marine organisms are rare in the scientific literature, particularly at environmental realistic concentrations. Therefore, their full impact it is not known.

The impact of the surrounding environment on nanoparticle effects were demonstrated in an exposure study using plain and carboxylated polymethyl methacrylate (PMMA and PMMA-COOH, 50 nm) material (Gomes *et al.*, 2020). The study reports effects of PMMA exposure on cellular responses of the marine microalgae *Rhodomonas baltica* after 72 h and on developmental and reproductive responses of the copepod *Tisbe battagliai* after a 6 days.

Behaviour of the PMMA particles using dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA) was measured, showing different behaviours of PMMA NPs in exposure media over time. PMMA were found to form micro-scale aggregates (2266 ± 260 nm) while PMMA-COOH maintained its nominal size range (57.1 ± 0.4 nm). Several differences were also seen in terms of toxicity between both PMMA NPs. PMMA exposure caused a significant effect in cell viability, ROS formation, LPO, pigment content and photosynthetic performance in exposed *R. baltica*, probably associated with particle interaction with the cellular membrane. On the other hand, a higher impact of PMMA-COOH was observed in terms of algal growth, photosynthetic performance, cell viability and metabolic activity, with negligible effects seen for ROS formation and pigment content. In *T. battagliai*, surface-dependent effects were observed; PMMA particles affected the early life development of *T. battagliai* while no effects were observed for the PMMA-COOH. A decreased developmental rate and reproductive output was observed at 10 mg PMMA/L.

Overall, surface chemistry and size were key parameters for the interaction and impact of PMMA nanoparticles in the two test organisms. Future experiments will focus on the in-depth characterisation of the mode of action of these particles.

2.3 Natural particles from anthropogenic activities

2.3.1 Mining particles

The discharge of unwanted mine tailings into the sea is a common practice in Norway where large quantities are deposited into coastal fjords (Brooks *et al.*, 2018). There are thought to be some positive impacts such as the creation of new land masses and harbour areas, although there is more concern over the potential impacts on the local marine environment, particularly in regard to local fish and shellfish industries. In Norway, research into the potential impact of mine tailings has been performed under the Norwegian Research Council funded project, NYKOS (New knowledge on Sea Disposal). The following two studies (laboratory and field) were funded by this project.

Laboratory exposures were performed to assess the potential environmental toxicity of three Norwegian mine tailings from Hustadmarmor, Sydvaranger, and Sibelco, which are all released into a seawater recipient (Brooks *et al.*, 2019). The tailings consisted of 1) fine particles with both flotation and flocculation chemicals from processing marble (Hustadmarmor), 2) sharp angular particles with flocculation chemicals from an Iron ore mine (Sydvaranger), and 3) sharp angular particles without process chemicals from nepheline syenite production (Sibelco). Ecotoxicity assessments were performed on the overlying water extracted from the mine tailings, the transformation/dissolution waters obtained from the mine tailings, and whole sediment assessment using a suite of marine organisms including algae, crustacea, and mollusc. Overall, based on the toxicity evaluation of the transformation/ dissolution data, Sibelco tailings resulted in the highest toxicity albeit at relatively high concentrations, followed by Sydvaranger and Hustadmarmor. Sibelco was the only mine where process chemicals were not used. The *Corophium* sediment contact assay revealed a significantly higher toxicity exerted by Hustadmarmor tailings, which was thought to indicate a physical impact of the fine tailings. With the *Corophium* test, the organisms were exposed directly to the tailings, enabling the interaction between organism and particles to be investigated. Interference of the fine particles with the gill epithelia of the crustacean was suggested by the authors to be a contributing factor to the adverse impact on the test organisms.

In field experiments, blue mussels (*Mytilus* sp.) have been used to assess the potential biological effects of the fine discharge effluent from the Hustadmarmor mine, which releases its tailings into the Frænfjord near Molde, Norway (Brooks *et al.*, 2018). Chemical body burden and a suite of biological effects markers were measured in mussels positioned for 8 weeks at known distances from the discharge outlet. The biomarkers used included: condition index (CI); stress on stress (SoS); micronuclei formation (MN); acetylcholine esterase (AChE) inhibition, lipid peroxidation (LPO) and Neutral lipid (NL) accumulation. Methyl triethanol ammonium (MTA), a chemical marker for the esterquat based flotation chemical (FLOT2015), known to be used at the mine, was detected in mussels positioned 1500 m and 2000 m downstream from the discharge outlet. Overall the biological responses indicated an increased level of stress in mussels located closest to the discharge outlet. The same biomarkers (MN, SoS, NL) were responsible for the integrated biological response (IBR/n) of the two closest stations and indicates a response to a common point source. The integrated biological response index (IBR/n) reflected the expected level of exposure to the mine effluent, with the highest IBR/n calculated in mussels positioned closest to the discharge. Principal component analysis (PCA) also showed a clear separation between the mussel groups, with the most stressed mussels located closest to the mine tailing outlet. In this study it was not possible to determine if the effects observed in the field transplanted mussels were a result of particle effects or due to chemical exposure. The study did see slightly elevated concentrations of certain metals and exposure to the flotation chemical, but only at low concentrations that were unlikely to have major impacts. Exposure of the mussels to the fine

particles was thought by the authors to be a contributing factor to the observed biological responses. On collection of the mussels from the field at the two closest stations (1500 and 2000 m) from the discharge, the mussels were coated with a white film of fine tailing particles. Such fine -grained particles, in such high densities, would result in impairment and clogging of respiratory organs and was thought to have contributed to some extent to the effects observed.

2.3.2 Particles of Calcium oxide

Calcium oxide (CaO) is being considered as a possible treatment for both the control of sea urchin populations and treatment against sea lice infestation in salmon farming. When CaO particles make contact with water, an exothermal reaction is produced. Contact of these particles with the outer surface of the organism can create epidermal burns and lesions. In echinoderms, it is thought that these epidermal burns enable bacteria to enter the coelomic fluid creating infection and death within days. In contrast, other species have been found to be less affected by CaO particle exposure. A study was designed to determine the effects of CaO particle (fine and coarse particle size) exposure to a range of Norwegian coastal water species by performing a series of toxicity bioassays on marine organisms from different taxonomic groups (Brooks *et al.*, 2020). The test organisms included two echinoderms (starfish, *Asterias ruben* and sea urchin, *Strongylocentrotus droebachiensis*), two crustaceans (shore crab, *Carcinus maenas* and copepod, *Tisbe battagliai*), two molluscs (blue mussel, *Mytilus edulis* and netted dog whelk, *Hinia reticulata*), a polychaete (slender ragworm, *Nereis pelagica*), a fish (lump sucker, *Cyclopterus* sp.) and a seaweed (bladder wrack, *Fucus vesiculosus*). Exposure of the test organisms to the CaO particles was performed by evenly scattering known concentrations of particles over the surface of the exposure tanks at the start of the test. The concentration range was 3.2, 10, 32, 100 and 320 g CaO particles/m^2, and the survival of the test organisms over a 10-day period was recorded. The toxicity data generated from the exposures were used to generate species sensitivity distributions (SSDs) for both fine and coarse CaO particles. The hazard concentration for 5% of the species (HC5) calculated from the SSDs, based on NOEC values, for the coarse and fine particles was 27.62 and 1.04 g/m^2 respectively. Using a recommended assessment factor of 5, the Predicted No Effect Concentration (PNEC) was calculated as 5.52 and 0.21 g/m^2 for coarse and fine CaO particles respectively.

2.4 Final remarks

During recent years it has been demonstrated that MPs pollution is widespread in the marine environment and this may result in negative effects on aquatic ecosystems and potentially on human health. Despite the scientific progress achieved in this area there are knowledge gaps related to the methods of MPs and NPs quantification, understanding of weathering, degradation and fragmentation processes, and of the interaction of MPs with biota, including the release of plastic additives and sorbed pollutants. Overall results obtained so far using traditional ecotoxicological tools suggest weak toxicity of MPs' environmental concentrations, but long-term effects and subtle responses have not been sufficiently studied. It has also been demonstrated that MPs can modulate the bioaccumulation and the toxicity of metals and many organic pollutants, frequently enhancing their effects, but similar studies indicate other particles of the seston, such as microalgae and many of the natural particles described above, may have similar effects.

Key research questions on the biological effects of MPs and NPs in marine ecosystems are:

- The role of the particle characteristics (e.g. size, shape, polymer characteristics, ecocorona) in modulating their biological effects.
- The need to develop long-term sensitive endpoints for MPs/NPs toxicity assessment.

- The combined effects with other environmental stressors, such as chemical pollution and global change factors.
- The toxicity of lower size particles, such as NPs, and biodegradable polymers.
- The comparison between the effects of synthetic (MPs/NPs) and natural particles and their role as vectors of environmental pollutants.

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3 Direct and indirect effects of ocean contamination to human health

3.1 Background

The World Health Organization (WHO) defines health as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" (https://www.who.int). This short literature overview focuses on the presence of diseases or physiological disorders in humans as a direct or indirect consequence of ocean contamination by chemical compounds and polymeric materials. Because ocean chemical contamination coexists with organic, biological, physical and radioactive contamination along with climate change, global warming and ocean acidification, the direct and indirect effects of the chemical contamination to human health are impacted by all these processes and thus should be considered accordingly (European Marine Board, 2013; Landrigan *et al.*, 2020). Of particular importance is the so-called "toxicity debt" associated with the longer-term consequences of marine plastic litter. The large amounts of plastic waste currently in the marine environment subjected to degradation, will have many more years of decay and release of toxic compounds to follow, consequently resulting in an additional (and increasing) source of toxic chemicals in the oceans over time (Rillig *et al.*, 2021).

3.2 Direct effects

3.2.1 Chemical contaminants in seafood

Most of the direct effects of ocean chemical contamination to human health are linked to the consumption of chemically contaminated seafood and sea by-products (e.g. salt, processed food). The chemical pollutants most often identified in seafood are methylmercury, PCBs, dioxins, brominated flame retardants, perfluorinated substances (such as PFOA and PFOS) and pesticides. However, humans are often exposed to the same chemical contaminants via other non-marine exposure pathways. Accordingly, and with the exception of Hg, the contribution of contaminated seafood to the total chemical load and to the global burden of disease (GBD) remains largely unclear.

Chemical form of contaminants in seafood

The infaunal bivalve species *Dosinia exoleta* naturally presents levels of lead (Pb) that occasionally exceed 1.5 mg/Kg ww, a sanitary threshold established by the EU for bivalve mollusks in the EC regulation 1881/2006. Scientific studies have demonstrated that Pb is accumulated by this mollusk in the form of metal rich granules (MRG) in the kidney (Darriba and Sánchez-Marín, 2012), and that Pb in this form is not available for trophic transfer to invertebrate consumers (Sánchez-Marín & Beiras, 2017). A recent study presented by Paula Sánchez-Marín *et al.* (2019) shows that the oral bioavailability of this Pb to a rat model is low (1% absolute bioavailability) and that the relative bioavailability compared to Pb contained in other bivalve species (*Mytilus galloprovincialis*) is 0.5. On this basis, this study proposed that the maximum permitted level could be increased to the double of the actual level (to 3 mg/Kg ww) for this species without increasing the risk for consumers.

This example shows that the chemical form of the pollutants in the marine products can affect their bioavailability and the degree of assimilation to human consumers, and this could be taken

into account in EC regulations, so that different maximum levels could be established for different products on the basis of the new scientific evidences about the chemical form and bioavailability of pollutants. In the case of metals, subcellular distribution analysis has been applied successfully in trophic transfer studies in the marine environment to define the Trophically Available Metal fraction (TAM) (Rainbow and Smith, 2010; Rainbow *et al.*, 2011). This is an example of how ecotoxicological approaches can be useful for its application to human health issues.

Also, the possibility to trace the origin of the marine products could be used to link the presence of pollutants in these products with anthropogenic inputs. As established in EC regulation 1881/2006, the pollutants should be at levels that are safe and as low as reasonable achievable on the basis of good agriculture/fishery practices. An additional consideration could be that marine products should come from clean areas, with low levels of chemical pollution, and this could be traced for some products on the basis of available information from marine pollution monitoring programs in different member states.

Recently, a comprehensive review was published on the impacts of ocean pollution on human health and the adverse human health outcomes linked to chemical pollutants in the oceans (Landrigan *et al.*, 2020). The study also provided an assessment of adverse health outcomes and the strength of the association assessed (strong, moderate and weak), using systematic reviews and meta-analyses of animal and human data (Table 3.1). Although there is a large variety of chemical contaminants that have the capacity to accumulate and biomagnify in commercial marine organisms, so far, effects to human health caused by methylmercury (MeHg) and PCBs in seafood are the best understood. It is well known that, in the natural environment, organisms including humans are exposed to complex mixtures of chemical contaminants. Understanding whether a single chemical that shows an association with human health outcomes, could be used as a "proxy" for combination effects of specific chemicals, needs further study.

In the case of Hg, the large quantities of legacy Hg in the global environment and the potential for climate change to remobilize it, is of greatest concern for human health. Methyl-Hg bioaccumulates and biomagnifies in the aquatic food chain; consequently, the consumption of fish and marine mammals is the major exposure pathway of MeHg for humans (Landrigan and Goldman, 2011; WHO, 2017). The high concentrations of Hg in the marine food chain's top predators pose risks to infants, children and adults. The brain and the gut biota are the targets in the human body most vulnerable to MeHg (Seky *et al.*, 2021). Once incorporated into the body, MeHg passes the blood-brain barrier and causes damage to the central nervous system, particularly in fetuses. There appears to be no safe level of MeHg exposure in early human development.

The atmospheric transport of manufactured chemicals from land-based sources to the marine environment is affected by the phenomenon named "atmospheric distillation", causing high concentrations of persistent pollutants in marine microorganisms and top predators in the circumpolar regions. The Inuit from Nunavik (Arctic Québec, Canada) are among the most heavily exposed human populations on earth to PCBs and MeHg because of the long-range transport of these chemicals via atmospheric and ocean currents and their bioaccumulation in fish and marine mammals, that are basic food items of the traditional Inuit diet (Muckle *et al.* 2001).

Table 3.1. Selected examples of strong established links between chemical contaminants in the oceans and a range of human health outcomes. Based on: Landrigan *et al.* (2020).

Pollutant associated	Adverse outcome	Type of epidemiological studies
POA PCBs Lead	Impaired somatic development (growth and birth weight)	Cross-sectional; Prospective cohort
Lead PCBs Methylmercury Organophosphates Organolchlorines BPA Phthalates and PBDEs Perchlorate (through thyroid impairment) PFAS	Developmental neurotoxicity, including decreased IQ, learning disabilities, ADHD and autism spectrum disorder (ASD)	Systematic reviews; Prospective cohort
Methylmercury Lead	Adult neurotoxicity, with cognitive and motor impairment	Cross-sectional; Prospective cohort
BPA DES Tribtyltin	Metabolic disorder, including hyperlipidemia, insulin, resistance, obesity and type 2 diabetes	Cross-sectional; Prospective cohort
Phthalates BPA	Male reproductive effects, including testicular dysgenesis syndrome, cryptorchidism, hypospadias, decreased ano-genital distance and decreased male fertility	Cross-sectional; Prospective cohort

Examples of health effects of MeHg and certain POPs among Inuit and other exposed populations are described in more detail below.

- *Effects of losses of cognitive function in children*. A recent estimate reported that between 317 000 and 637 000 babies are born in the United States each year with losses of cognitive function that are the consequence of prenatal exposures to MeHg resulting from consumption of Hg-contaminated fish by their mothers during pregnancy. These losses range in magnitude from 0.2 to 5.13 IQ points depending on the severity of exposure. These authors found additionally that population-wide downward shifts in IQ caused by widespread exposure to MeHg are associated with excess cases of mental retardation (IQ below 70), amounting to 3.2% (range: 0.2–5.4%) of all cases of mental retardation in the United States (Trasande *et al.*, 2005; Gaylord *et al.*, 2020). Studies conducted in Nunavik of child development at age 11 years showed that MeHg exposure in early life is associated with slowed processing of visual information, decreased IQ, diminished comprehension and perceptual reasoning, impaired memory, shortened attention span,

and increased risk of attention deficit/hyperactivity disorder (ADHD) (Boucher *et al.*, 2011, 2012). Other prospective studies have also reported neurobehavioral deficits in children with elevated prenatal exposure to low-levels of MeHg (Karagas *et al.*, 2012).

- *Persistent effects in adults by prenatal exposure*. Prospective epidemiological cohort studies undertaken in the Faroe Islands, where dietary MeHg exposure mainly originates from traditional consumption of meat from the pilot whale, demonstrate that children exposed to MeHg in utero exhibit decreased motor function, shortened attention span, reduced verbal abilities, diminished memory and reductions in other mental functions. In this study, maternal hair-mercury concentrations were measured and the child's prenatal exposure was assessed from the mercury concentration in cord blood. Follow-up of these children to age 22 years indicates that these deficits persist and appear to be permanent (Debes *et al.*, 2016). Elevated concentrations of MeHg in blood and tissues in humans have been associated with arteriosclerosis, thrombosis, accelerated loss of neurocognitive function in adults and cardiovascular diseases, increased risk for acute coronary events, hypertension, myocardial infarction, coronary heart disease and death (Virtanen *et al.*, 2005; Genchi *et al.*, 2017).
- *Effects of losses of cognitive function*. Postnatal PCBs exposure of Inuit children appears to affect processes associated with error monitoring, an aspect of behavioural regulation required to adequately adapt to the changing demands of the environment, which results in reduced task efficiency (Boucher *et al.*, 2011).
- *Effects on glucose metabolism*. An association between exposure to POPs and Hg, and glucose metabolism in Inuit from Nunavik has also been demonstrated. However, global pollution and bioaccumulation leads to simultaneous intake of POPs such as PCBs and metals. Exposure to POPs has been suspected of increasing the risk of type 2 diabetes. In 2004, prevalence of diabetes and glucose metabolism were assessed among 877 Inuit adults in Nunavik. Blood concentrations of POPs, Hg, selenium and omega-3 were measured in samples collected during the survey. Associations between risk of diabetes and exposure to contaminants were analysed using multivariate regression modelling adjusting for sex, obesity and smoking. Interaction with nutrients intake was studied. Results showed high exposure levels and strong correlations between contaminants blood concentrations. Authors found a linear increase in diabetes risk with higher exposure to POPs, especially PCBs and Mirex but no association of diabetes risk with Hg blood concentrations (Cordier *et al.*, 2020).
- *Risk for cancer, metabolic disorders, cardiovascular diseases, and immune system effects*. It has been reported that health risks (based on a quantitative cancer risk assessment) associated with consumption of farmed salmon contaminated with PCBs, toxaphene, and dieldrin were higher than risks associated with exposure to the same contaminants in wild salmon. Consumption of farmed salmon would need to be limited to many fewer meals per month than from wild salmon, to reduce cancer risk to a level near the WHO's "tolerable daily intake" for dioxin-like compounds (Foran *et al.*, 2005). Recent research reported that in the northern and eastern parts of Baltic Sea, herring over 17 cm in length, and in the Baltic proper, herring over 21 cm, is suspected to exceed the maximum; and in general, all Baltic salmon above 2 kg in weight are supposed to exceed the maximum dioxin limits (Commission Recommendation (EU) 2016/688); (Haapassari *et al.*, 2019).

Human exposure to contaminants in a changing ocean scenario

Contaminants stand as a threat to human health, and this may be enhanced by changes predicted for the future of our oceans. Several studies on contaminants and climate change pointed that warming is likely to promote bioaccumulation of persistent contaminants in marine organisms (e.g. MeHg, dechlorans), and compromise the safety of seafood products; whereas acidification generally leads to a reduced bioaccumulation of contaminants.

An attempt to understand the effects of warming in the seafood safety was done by Camacho *et al.* (2020). Mercury concentrations of several fish species of commercial interest were compared to tolerable weekly intake (TWI). A warming scenario was applied and an increasing probability of exceeding the Hg TWI and a higher hazard quotient (HQ) was observed. The consumption of wild seafood, particularly carnivorous and top-predators may lead to an enhanced accumulation of Hg.

3.2.2 Plastic particles in seafood

Global microplastics (MPs) pollution, and their implications for human health, is of increasing concern (Barboza *et al.*, 2018; Vethaak & Legler, 2021). Seafood consumption as well as products made with sea by-products, sea salt or seawater may represent one pathway for human micro-plastic/nanoplastic (MPs/NPs) exposure. Although currently there is no evidence that MPs/NPs concentrations in the marine environment may have a negative impact on fish and shellfish health or on commercial stocks (e.g. Barboza *et al.*, 2018), the presence of MPs in the edible parts of marine organisms typically eaten by humans (bivalves, crustaceans, fish) is widespread (Lusher *et al.*, 2017; Barboza *et al.*, 2018) due to their ubiquity in the environment and to translocation of particles (Rochman *et al.*, 2015; EFSA J, 2016; Smith *et al.*, 2018). However, MPs fibre ingestion by humans via the consumption of contaminated seafood has been found to contribute minimally to the MPs contamination of the total food basket (Catarino *et al.*, 2018). Also, MPs can be likely detected in any food product due to outfall of outdoor and indoor air pollution.

A semi-systematic review of studies investigating the number of MPs found in commercially important organisms of different trophic levels suggests that MPs do not biomagnify, and that organisms at lower trophic levels are more likely to be contaminated by MPs pollution than apex predators (Smith *et al.*, 2018; Walkingshaw *et al.*, 2020), with bivalves being of more concern with regard to transferring MPs to humans since they are mostly consumed whole with the digestive system included. A recent study based on assessment of commonly consumed food items estimates that an average person consumes between 74 000 and 121 000 MPs particles per year (Cox *et al.*, 2019). Barboza *et al.* (2019) showed that fish species of commercial interest such as *Dicentrachus labrax*, *Trachurus trachurus* or *Scomber colias* presented MPs in the dorsal muscle. Around 32% of the individuals presented MPs, with a total mean (± SD) of 0.054 ± 0.099 MP items/g. Based on this value and considering the recommendation of the European Food Safety Authority (EFSA) for fish consumption by adults, human consumers may intake 842 MPs/year through fish consumption only. Using the mean value to calculate the intake per capita in selected European and American countries (EUMOFA, NOAA), the estimated value of MPs ingested through fish consumption ranged from 518 to 3078 MPs/year/capita.

MPs can reach the human gut and have been detected in human stool samples (2 particles per gram stool); (Schwabl *et al.*, 2019) and human colon tissue (Ibrahim *et al.*, 2021). Human health effects depend on exposure concentrations. Considering that fish consumption is only one of the MPs routes of human exposure, there is a general acknowledgment that currently there is still insufficient information to assess the actual amount of MPs humans may be exposed to via seafood, due to data gaps in MPs research. Food safety in the near future may be impacted as new

knowledge will become available including the presence and potential impact of sub-micrometer sizes. Furthermore, larger-size MPs are likely to be excreted through the faeces (Vethaak and Legler, 2021). The physical effects of internalized and accumulated MPs are less understood than the distribution and storage of toxicants in the human body, but preliminary research has demonstrated several potentially concerning impacts, including enhanced inflammatory response, size-related toxicity of plastic particles, chemical transfer of adsorbed chemical pollutants, and disruption of the gut microbiome (Wright *et al.*, 2017).

There is also a need to quantify the total release of toxic chemical contaminants deriving from plastic litter in European waters and to assess their threat to both ocean and human health. Several thousand different additives are used in the plastic production and numerous of them are known to be toxic (e.g. endocrine disrupting and other hazardous properties) to ocean and human life. The majority of these toxic additives can leach relative quickly (within weeks) from the plastic product into the water and could accumulate in marine organisms and food chains potentially affecting living resources, biodiversity and human consumers of sea products. Preliminary assessments have suggested that the contribution of hazardous chemicals released from MPs for top consumers of bivalves is very small compared to other sources. Overall, from the current knowledge on MPs content in seafood, it can be concluded that there is no evidence that the safety of consuming such a highly recommended food is compromised (reviewed by Garrido *et al.*, 2020). However, there are still major knowledge gaps concerning the levels of smaller MPs/NPs that are potentially more dangerous than the larger ones. A novel and understudied hazard to consumers of seafood is the potential effect of the biofilm around the plastic particles as potential reservoirs and vectors of human pathogens, antibacterial resistance genes and biotoxins (Radisic *et al.*, 2020).

3.2.3 Oils spills

Direct effects of marine oil spills to human health include acute respiratory effects by inhalation of volatile petrochemicals, mainly among occupationally workers involved in clean-up efforts (Alexander *et al.*, 2018). As happens with other types of situations (accumulation of marine litter in coastal areas, harmful algal blooms, etc.) serious impacts on mental health on the human populations living in the area of influence of the environmental impact can be expected if such events persist in time.

3.3 Indirect effects

3.3.1 Intake or air exposure to biotoxins

Climate change, sea surface warming and ocean pollution (in particular coastal pollution by nitrogen and phosphorous from industrialized agriculture and human sewage inputs), appear to be increasing the frequency, severity and global geographical extent of harmful algal blooms (HABs), and the occurrence of HABs in previously unaffected areas and in unexposed populations (i.e. circumpolar regions); (Morabito *et al.*, 2018; Wells *et al.*, 2020).

Consumption of fish and shellfish that have ingested toxic algae is the major route of human exposure. Harmful Algal Blooms produce toxins (e.g. saxitoxins, domoic acid, okadaic acid, azaspiracids, yessotoxins, brevetoxins, ciguatoxins, palytoxins, etc.) associated with paralytic shellfish poisonin (PSP), amnesic shellfish poisoning (ASP), diarrheic syndrome dementia poisoning (DSP), neurotoxic shellfish poisoning (NSP) and ciguatera fish poisoning (CFP) and clupotoxism (reviewed by Landrigan *et al.*, 2020).

Effects in humans after intoxication by HABs toxins include gastrointestinal symptoms (diarrhea, nausea, vomiting, abdominal cramps), neurological, (headache, paresthesia, paralysis, convulsions and coma), cardiovascular symptoms, as well weakness, cough, muscle pain amnesia, neurological damage, and rapid death in humans. Harmful Algal Blooms produce potent toxins that accumulate in fish and shellfish. When ingested, these toxins can cause severe neurological impairment and rapid death (Morabito *et al.*, 2018). Many thousands of poisoning episodes occur worldwide each year, with CFP outstanding by affecting 50 000 to 200 000 people per year (Landrigan *et al.*, 2020). Caribbean and Pacific regions are those with important health problems by CFP, but the occurrence of ciguatera is spreading to higher latitudes, posing increasing risk for north European countries and Mediterranean areas (Soliño and Costa, 2020).

Laboratory studies performed with mussels *Mytilus galloprovincialis* in warming and acidification scenarios (current conditions, warming, acidification and interaction of warming with acidification) showed low accumulation/elimination rates in a warming condition but higher rates in an acidification condition. The combined effect of climate change drivers on accumulation/elimination of paralytic shellfish toxins (PSTs) in mussels indicated that warming and acidification may lead to lower toxicity values but longer toxic episodes (details in Braga *et al.*, 2018).

Human exposure to HABs toxins can also occur through skin or inhalation of aerosols via swimming of visiting/residing in coastal areas during HABs events (Morabito *et al.*, 2018; Diaz *et al.*, 2019). Macroalgal bloom decomposition in coastal areas can release fouls-smelling and hazardous gases (hydrogen sulphide, methyl mercaptans and dimetthyl sulphide) causing eye and respiratory tract irritation.

Potential effects of chemical contaminants in HABs events

Polycyclic aromatic hydrocarbons (PAHs) have been found to reduce DNA synthesis and delay cell division in the widespread primary producers *Prochlorococcus* (Cerezo and Agustí, 2015). Toxicity of binary mixtures of pesticides has been also found to cause physiological impacts to the marine microalgae *Tisochrysis lutea* and *Skeletonema marinoi* (Dupraz *et al.*, 2019). PAHs contamination has been found to alter freshwater cyanobacterial blooms by affecting the microcystin production and physiological characteristics (Zhang *et al.*, 2018). Decrease in the abundance and viability of oceanic phytoplankton may result from trace levels of complex mixtures of POPs and PAHs in seawater, as these mixtures may cause a photosynthetic toxicity that exceeds that of single chemicals by as much as three orders of magnitude (Echeveste *et al.*, 2010). Ocean acidification induce changes in the speciation of metals that alter their solubility and bioavailability and may increase or mitigate, in some instances, the toxicity of certain heavy metals and organic pollutants (Zeng *et al.*, 2018; IPCC, 2019; Landrigan *et al.*, 2020). The acidification of the oceans will enhance biotoxicity of Cu to plankton photosynthesis and productivity but at the same time, will increase the concentration of dissolved Fe, which could partially alleviate the inhibitory effect of Cu on photosynthesis (revised in Landrigan *et al.*, 2020).

Some emergent contaminants, as the widely used sunscreen oxybenzone and other organic ultraviolet (UV) filters, may have toxic effects on the larval forms of several coral species and increase coral bleaching (Downs *et al.*, 2016; He *et al.*, 2019). Dead bleached coral provide new surfaces for dinoflagelates implicated in ciguatera fish poisoning (Fleming *et al.*, 2009).

Potential effects of microplastics on the development of HABs

Coastal waters impacted by industrialized agriculture and human sewage usually are also impacted by pesticides and MPs pollution. The contribution of chemical contaminants and MPs pollution to HABs events is unknown but some findings indicate that marine environmental

contaminants and plastic surfaces could also play a role by altering the biodiversity of phytoplankton communities. Since MPs degrade very slowly, they remain in the marine environment for much longer timescales than most natural organic substrates and provide a novel habitat for colonization by bacterial and algal communities (Amaral-Zettler *et al.*, 2013, 2020). In an experimental study conducted with MPs and mussels, *Ostreopsis* cells attached to different plastic materials, indicating an alternate route for toxin transfer to marine fauna via ingestion of biofilm-coated plastic debris (Tibiricá *et al.*, 2019). Cell densities have been found to be equally high on both natural and artificial substrates. Algal species involved in HABs (Masó *et al.*, 2003) and ciliates implicated in coral diseases (Goldstein *et al.*, 2014) have also been found attached to marine MPs. These findings suggest that algae that colonize plastics in the marine environment may use MPs particles to expand their geographical range.

Case study: Increasing blooms of *Ostreopsis ovata* and noxious effects on human health

During the 1st International Symposium on Human Health & the Ocean in a Changing World (December 2020), the Surfrider Foundation Europe presented data concerning blooms of *Ostreopsis ovata*. Results of the monitoring program showed that since 2005 and all around Europe, including the Mediterranean Sea, event blooms of the tropical algae *Ostreopsis ovata* are increasing. Intense blooms of the toxic dinoflagellate *Ostreopsis* have been a recurrent phenomenon along the Mediterranean coasts. Blooms have been associated with noxious effects on human health (such as rhinorrhoea, cough, fever, bronchoconstriction with mild breathing difficulties, wheezing, and, in a few cases, conjunctivitis) and with mortality of marine organisms due to the production of palytoxin-like compounds (Gallitelli e al., 2005; Brescianini *et al.*, 2006). High temperature and balanced nutrient conditions were the optimal environmental conditions to start and sustain blooms as well as to maximize toxin production (Accoroni *et al.*, 2017).

3.3.2 Exposure to pathogens: Seafood intake, skin and air exposure

The small round structured viruses (SRSVs); (Matte *et al.*, 1994) or Norwalk-like viruses are classified as caliciviruses and are common causes of outbreaks of gastrointestinal illness in humans. Since the infectious dose is small, cooking the shellfish does not reliably eliminate the risk of contracting gastroenteritis (McDonnell *et al.*, 1997; Kirkland *et al.*, 1996). Pathogenic marine bacteria may cause gastrointestinal diseases and deep wound infections to humans. Naturally occurring marine bacterial pathogens of great significance for human health include *Vibrio cholera*, *Vibrio vulnificus*, *Vibrio parahaemolyticus* and *Mycobacterium marinum*.

Greater occurrence and expanded geographic range of marine-borne parasitic infections (with particular relevance cryptosporidiosis, giardiasis and salt water schistosomiasis) are linked to climate change (Cohen *et al.*, 2018). Consumption of raw and inadequately cooked seafood, especially in certain ethnic subpopulations, is associated with two emerging human parasitic diseases of particular concern, anisakis (fish parasitic nematode) and *Diphyllobothriasis* (tapeworm). Climate change and rising sea surface temperatures and increasing pollution, are likely to increase the risk of *Vibrio* infections, including cholera, resulting also in greater abundance and expanded geographic ranges of naturally occurring marine pathogens with the potential increases in the frequency of *Vibrio* associated illness (Escobar *et al.*, 2015).

Potential effects of microplastic-mediated pathogens on human health

There is increasing evidence that plastic could act as a vector for the dispersion of exotic species including pathogens (i.e. causing infectious diseases) and antibiotic resistance, affecting marine

stocks and potentially humans. This could result in direct or indirect impact on ecosystems, marine food resources and human coastal communities.

MPs have been shown to harbor marine microbial communities different or similar to the microorganisms in the surrounding water, providing a novel habitat for colonization of hazardous microorganisms, including vectors for human disease (Zettler *et al.*, 2013, 2020). Pathogenic bacteria have been detected on sub-surface MPs comprised of polyethylene fibers, in plastic-containing sea surface films, and in polypropylene fragments sampled in some coastal areas (Kirstein *et al.*, 2016). Similarly, *Escherichia coli* and other potentially pathogenic species have been found on plastics in coastal waters (Curren and Leong, 2018; Littman *et al.*, 2020) and on public beaches (Rodrigues *et al.*, 2019). In a recent study of river water, a selective enrichment of human bacterial pathogens (*Pseudomonas monteilii*, *Pseudomonas mendocina*) and one plant pathogen (*Pseudomonas syringae*) were detected in MPs biofilm, but not in biofilms formed on natural substrates (Wu *et al.*, 2019). Whether this phenomenon also occurs in marine MPs-biofilms is yet unknown. Coastal flooding with water-dispersed plastics could increase the spread of potentially human pathogens and increase infection rate of coastal populations. Further, adhesion to marine plastic may also enable pathogens to increase their anti-microbial resistance thus facilitating their spread to new areas where they may cause disease and death in previously unexposed populations (Kirstein *et al.*, 2016).

Case study: Effects of environmental conditions on the expression of pathogenic factors of *Vibrio parahaemolyticus*

The marine bacterium *Vibrio parahaemolyticus* is an example of an important bacterial pathogen for humans causing gastroenteritis associated with the consumption of seafood (bivalve shellfish species) or via wound exposure to seawater containing these bacteria. It is a free swimming bacterium that may be attached to sediment, fish, shellfish and plastics. An experimental study has demonstrated that viability of *V. parahaemocytes* is double at 27ºC and 31ºC than at 21ºC. Increasing temperature induces over-expression of adhesion virulence factors in free-swimming bacteria. Different plastic (low-density polyethylene –LDPE-, high-density polyethylene –HDPE-, polypropylene –PP-, polycarbonate –PC-, and polystyrene –PS-) and glass, hung from a floating dock, and MPs, collected from the environment, have been recorded as colonization substrates for bacterial communities, with particular focus on *Vibrio* sp., especially the human pathogens, *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* (Laverty *et al.*, 2020). These three pathogenic *Vibrio* species identified from plastics in estuaries, reinforce and expand upon earlier reports of plastic pollution as a habitat for *Vibrio* species (Laverty *et al.*, 2020).

Recent studies indicate that environmental factors govern the development of bacterial communities on plastics, more so than the characteristics of the plastic substrates themselves. *Vibrio* abundance in particles collected from the Baltic Sea has shown a more stable population at the sites with highest nutrients and lowest salinity (Kesy *et al.*, 2021). The antibiotic resistance detected among the isolates, coupled with the longevity of plastics in the aqueous environment, suggests biofilms on plastics have potential to persist and serve as focal points of potential pathogens and horizontal gene transfer.

3.4 Final remarks

- There is increasing awareness that chemical pollution (chemical contaminants and synthetic particles) in the marine environment may contribute to adverse health effects in humans. As such the WGBEC should continue to explore the linkages between marine chemical and other relevant contaminants and human health in its future meetings.

- More research is also needed to investigate the role of plastic debris as a transport vector of pathogens, antibiotic resistance and biotoxins, and their potential for dispersing diseases among marine life including commercial marine stocks and human consumers and users of ocean space (food safety and quality, swimming water quality, etc.).
- To further progress the field of MPs and human health, and in analogy to endocrine disrupting and other chemicals, much can be learned from studies with marine sentinel organisms (e.g. marine mammals, fish, mussels, sea urchins) and such studies should be encouraged to look for commonalities in uptake, fate and effects of plastic particles from marine organisms to humans.
- Studies with marine organisms that serve as alternative models of human diseases and human physiology (such as immunomodulation, endocrine disruption, neurotoxicity, genotoxicity) should be particularly encouraged and the research results should be reported to and analyzed by the WGBEC in future meetings.

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4 National activities on effect-based monitoring: approaches taken, gaps and future avenues

4.1 Background

Regional Seas Conventions (OSPAR, Barcelona Convention, HELCOM) have warranted for decades international cooperation for marine pollution monitoring in European seas. However, there are some differences in the approaches taken and in the degree of parameters measured by national monitoring programs. In this context, the WGBEC contributed significantly to the implementation of techniques that can be used to evaluate the biological effects of pollutants, and has maintained an overview of national effect-based monitoring programs, with the aim to support European countries and Regional Seas Conventions on their implementation.

In recent years, European legislation for the protection of the marine environment, specifically the Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD), demand the harmonization of national monitoring programs, which must consider not only on the analytical chemistry of pollutants, but also their effects on the ecosystems. For instance, MSFD's descriptor 8 indicates that to achieve the good environmental status (GES), 'the concentrations of contaminants [should be] at levels not giving rise to pollution effects', and this directive emphasizes the need to evaluate and keep within acceptable limits the biological effects of contaminants. In this regard, a relevant effort, based on European consensus, has been conducted by the WGBEC to develop an integrated approach for the monitoring of chemical contaminants and biological effects (Davies & Vethaak, 2012), which can serve to establish the link between pollutant levels and their harmful effects on marine ecosystems.

4.2 National activities on effect-based monitoring

4.2.1 Denmark

The biological effects monitoring in Denmark is coordinated with the contaminant monitoring within the framework of the Danish Nationwide Monitoring and Assessment Programme for the Aquatic and Terrestrial Environment (NOVANA) funded by the Ministry for the Environment. The marine monitoring data are reported by the National Centre for Environment and Energy at Aarhus University.

The elements included in NOVANA have been adopted on the basis of OSPAR JAMPs and HELCOM COMBINEs scheme of approach for integrated monitoring of environmental hazardous substances and their effects in the environment. The national method protocols are as the primary references using the relevant ICES TIMES. Spatial and time-trend data from monitoring of different parameters are studied.

The integrated monitoring of biological effects and chemical measurements in NOVANA for the period 2017–2021 includes: 1) TBT-specific effects in marine gastropods: Imposex in whelks (*Neptunea, Buccinum, Nassarius and Nucella*) and intersex in periwinkles (*Littorina*); 2) General stress biomarker lysosomal membrane stability (LMS) in blue mussels; and 3) PAH-specific and general stress biomarkers in coastal fish: CYP1A activity (EROD), PAH-metabolites and reproductive success in eelpout (*Zoarces viviparus*).

The chemical measurements performed in the NOVANA monitoring program 2017-2021 (revision in play from 2022 onwards) consists chemical analyses in: 1) Sediments: Nonylphenols, phthalates with TOC in all samples, only marine strategy (open North Sea) samples are also analyzed for metals, PAH, dioxins etc. once every 6 years; 2) Mussels: Metals, PAH is measured in all mussels in the basic program, TBT and dioxin in extended programs on few selected samples and 3) Fish: Hg, PCBs (incl. chlorine pesticides), PBDE, PFOS, dioxins, all dioxin-like PCBs in flounder, eelpout and gobies. For eelpout TBT have also been measured at few stations.

Recent developments in NOVANA: marine areas covered by MSFD, WFD and Habitat- directives; since 2017, revisions and reduction in number of stations for biological effect monitoring within NOVANA.

Ongoing developmental activities on relevant monitoring indicators are currently including reproductive success in amphipods and interspecies comparisons of PAH-specific biomarkers in eelpout, flounder and round goby.

4.2.2 England and Wales

Cefas is the competent monitoring authority (CMA) for hazardous substances and their biological effects in offshore and coastal locations throughout England and Wales (E&W) and carries out this role under the UK Clean Seas Environmental Monitoring Programme (CSEMP). The CSEMP supports activities under the OSPAR Coordinated Environmental Monitoring Programme (CEMP) of the Joint Assessment Monitoring Programme (JAMP) and the Marine Strategy Framework Directive (MSFD). Cefas operates a biennial monitoring programme encompassing 31 and 15 offshore fishing and sediment stations respectively, whereby the North Sea and East Channel is sampled one year, followed by the Celtic Seas and West English Channel during the next.

The monitoring of hazardous substances in sediments, water and biota includes trace elements (e.g. heavy metals), polychlorinated biphenyls (PCBs), organotins, organochlorine pesticides, polycyclic aromatic hydrocarbons (PAHs), brominated flame retardant (PBDEs, HBCDDs), perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), pharmaceuticals, alternative flame retardants and poly- and perfluorinated compounds. The integrated monitoring of biological effects in common dab (*Limanda limanda*) includes ethoxyresorufin-O-deethylase (EROD), acetylcholinesterase (ACHE), micronuclei formation, PAH bile metabolites, liver neoplasms and external fish diseases. The monitoring of imposex in gastropods is also undertaken and is conducted regularly (≈3 years), although the number of sites surveyed has been reduced significantly since 2010. Monitoring sites include sites at or above (OSPAR) assessment class C in the 2010 or 2007 imposex survey, or where the E&W Environment Agency recorded TBT concentrations in the water column above the EQS.

In 2018, several MSFD intermediate assessments were produced in partnership with several national agencies including Environment Agency, Marine Scotland and Defra. These assessments included several categories of chemical measurements (heavy metals, PCBs, PAHs, PBDEs and radionuclides) in sediment and biota, and biological effects covering different mechanisms of toxicity (PAH bile metabolites, micronuclei, EROD activity, imposex, liver neoplasms and visible fish diseases). These assessments have been published on UK Marine Monitoring and Assessment Strategy (UKMMAS) Marine Online Assessment Tool: (https://moat.cefas.co.uk/pressures-from-human-activities/contaminants/):

The monitoring undertaken by Cefas has historically produced a consistent data time-series with sampling taking place during the summer in accordance with the UK national sampling protocol (The Green Book). This is primarily to avoid the spawning season of the primary indicator species (dab) and to avoid corresponding confounding effects. The Covid-19 pandemic resulted in

the 2020 survey being delayed until December, which resulted in fewer samples being obtained as part of the national monitoring effort. Cefas is currently developing a biological effect monitoring programme using European flounder (*Platichthys flesus*) in estuaries throughout England. This programme will identify whether there have been improvements in the health of estuaries 10 years after biological effects monitoring in estuaries was last conducted. Pending a successful outcome of the UK spending review, the work will be carried out in 2021 in collaboration with the Environment Agency.

4.2.3 Finland

During the past decades, regular biological effects monitoring of the marine environment of Finland (northern Baltic Sea) consisted of examinations of parameters related to the reproduction of top predators, the white-tailed eagle (*Haliaeetus albicilla*), the grey seal (*Halichoerus grypus*), the ringed seal (*Phoca hispida*), and the harbour seal (*Phoca vitulina*). Early warning biomarkers were not considered although some case studies applying (e.g., EROD activity) were carried out.

In 2014, the lysosomal membrane stability (LMS, histochemical method) was included in the national monitoring campaign. The parameter is measured in two species of fish, the Baltic herring (*Clupea harengus membras*) in the open sea area and the perch (*Perca fluviatilis*) in the coastal zone. Catching of herring is carried out every year in October onboard r/v *Aranda* of the Finnish Environment Institute (SYKE) in connection with the annual fish stock assessment cruise of the Nature Resources Institute Finland (Luke). The offshore target areas of catching herring for the LMS measurements include five areas, three in the Bothnian Sea (southern Gulf of Bothnia), one outside the Archipelago Sea (Hanko Peninsula), and one in the central Gulf of Finland. Samples for perch are caught in September with the aid of local fishermen at five sites along the coast of Finland close to some main settlements and industry, in the Quark (Vaasa), central Bothnian Sea (Pori), Archipelago Sea (Parainen), and Gulf of Finland (Helsinki and Kotka). The fish caught during the sampling campaigns are also used for the determination of selected chemical contaminants as part of the national monitoring programme and periodically reported to the EU and the ICES database. Regarding chemicals a rotatory system is in use, i.e., not all the listed ones are measured every year. Also, due to occasional adverse weather conditions (offshore sampling) or the lack of target species (coastal sampling) the data sets are not spatiotemporally complete. In addition to LMS, tissue samples of fish liver and fillet (muscle tissue) are collected and stored at -80°C for possible other biomarker measurements in the future.

However, the LMS analyses are lagging badly behind the schedule. So far, results only from herring collected in 2014 and 2015 have been reported to the national database. Thus, no scientifically sound analyses of spatial trends or potential connections with the observed tissue concentrations of the monitored contaminants can be made. However, the situation is improving. In addition, negotiations with the Finnish Ministry of Environment on funding of the analysis of the collected liver and muscle tissue samples for retrospective analysis of other biomarkers (e.g., AChE, antioxidant defence system enzymes, oxidative damage, many in use during the past decade in various research/case studies) are underway.

Development of biological methods monitoring is included in the recently (2020) published national "Roadmap for marine monitoring" for the period 2020–2026. The plan includes the methodological assessment and implementation of integrated chemical-biological monitoring using (1) caged mussels (tissue concentrations of target chemicals, biomarkers) and passive samplers in coastal areas, (2) perch (coastal areas) and herring (open sea) (tissue concentrations of target chemicals, biomarkers), and (3) testing and applying novel effect-based methods and assessment protocols (i.e., data integration). In 2021, the activity focusing on the implementation of the mussel caging approach (1) will be carried out jointly with a planned HELCOM project aiming at

establishing common approaches and a regional survey through wide-scope screening of contaminants.

4.2.4 France

Biolgical effects monitoring: SELI. Since 2016, France has implemented sea campaigns, so-called SELI, dedicated to the assessment of biological effects of chemical contaminants in fish and mussels (https://doi.org/10.18142/285). SELI addresses voluntary OSPAR monitoring initiative and descriptor 8 criteria 2 requirement (effects of contaminants) of the Marine Strategy Framework Directive (MSFD). SELI was designed based on the ICES ICON project of demonstration on a large geographical scale in the north Atlantic Est initiative (2008–2009). It completes the imposex mandatory monitoring undergoing since 2003 in the CEMP. Flatfishes (soles, flounders, dabs) and mussels are sampled at stations likely to receive contrasted pressures in the Atlantic, i.e. in the bays of Loire and Vilaine in 2017 and 2020, and the Bay of Seine in 2018 (next one in 2021). A core set of biomarkers was measured to target the potential effects on main biological functions: reprotoxicity (intersex), genotoxicity (micronucleus and DNA strand breaks -comet assay-), neurotoxicity (AChE inhibition) and general stress markers (lysosomal membrane stability and structural changes, histological hepatic lesions). This list was completed with: 1) concentrations of PAH metabolites in fish bile; 2) concentrations of metallic and organics contaminants (Hg, MeHg, Cd, Pb, Cu, Zn, Ni, Cr, PCBs, brominated flame retardants (PBDEs, HBCDDs), perfluoroalkyl substances (PFASs) and organochlorinated pesticides (cyclodienes, HCHs, DDTs)) in fish muscle and/or liver. Contaminants levels in the mussels are measured within our mussel watch program (https://wwz.ifremer.fr/pollution/Laboratoires-et-cellules-d-expertise/ROCCH).

The objectives were to investigate: 1) the levels of chemical contamination impregnation (historical and emerging) in marine organisms; 2) the levels of the biological responses; 3) the applicability of BAC/EAC developed for OSPAR species, to other flatfish to support the use of the integrated approach for MSFD GES assessment. Based on these data, the link between chemical contamination and biological effects in areas with different anthropic pressures is studied.

Sole was the most commonly trawled flatfish in the studied area. Sole showed lower levels of Hg, Cd, Zn, PCB, OCP, PBDE and HBCDD than flounder and dab; however levels of Ag, Cu and perfluorinated compounds were higher in sole than in flounder and dab. Levels of hydroxypyrene were higher in flounder than in dab and sole, possibly due to different metabolic capabilities between flatfish species or different habitats, with flounder more estuarine than the two other species. Although sole does not show the highest chemical concentrations for several chemical families, it showed the most altered responses regarding DNA strand breaks and AChE (together with dab), showing: 1) the complexity of the relationship between chemical contamination and biological effects, and/or the complementarity of both approaches; 2) sole is a sensitive species and a species commonly found in the studies areas, therefore likely to be a suitable candidate as sentinel species. LMS was similar between studied areas and flatfish species, supporting the fact that LMS is evolutionarily highly conserved. On the other side, LMS was higher in mussels than in flatfish, putting in perspective its conservation through evolution.

Several individuals (the large majority for some biomarkers) did not meet EAC or BAC for early warning biomarkers (DNA strand breaks, micronucleus, AChE inhibition, LMS). However, intersex was not observed in male fish (sole -n = 72 males-, dab -n = 16-, flounder (n = 21-), neither neoplasma (benign nor malign) in male or females (n=273 flatfishes). The general conclusion of these two campaigns was that there were several early alerts of chemical impact at sub-individual level in Loire, Vilaine and Seine, but the effect at the population level was not observed with the used biomarkers. An integrated approach, using the ICES recommendations, completed with a proposal when no EAC was available and the consideration of % of studied parameters (and not studied parameters), showed that chemical concentration in biota and sediment decrease

with distance from the Seine river mouth "faster" than biomarkers, with more than 30-50% of the biomarkers still "red" (>EAC)+"orange"(>3*BAC) at the most distant stations. The use of such observatory for GES assessment requires to complete the development of BAC and EAC of the core and new biomarkers and bioassays in fish sentinel species.

Fish contamination monitoring: COREPH. Separately, to complete the actual contaminant monitoring in sediment and mussels to higher trophic levels and to extent the spatial coverage of the monitoring to off-shore and deep-sea, fish contamination (dioxins, PCB, PBDE, HBCD, PFC, As, Cd, Hg and Pb) is assessed together with trophic indicator (e.g. C/N isotopes) since 2014 in fishes. Fishes were obtained from optimized French fisheries surveys in three different marine systems: the Eastern English Channel (EEC), the Bay of Biscay (BoB), and the Western Mediterranean (Med).

The objectives were to: 1) assess MSFD good environmental status (GES) regarding environmental (D8) and human (D9) health; 2) compare fish contamination between the three French marine subregions; and 3) assess the contribution of trophodynamics (D4) to explain or drive fish contamination.

Top predator contamination monitoring. Contamination of mammals (strained dolphins and porpoise) is assessed in samples from the strained mammal bank at Pelagis (La Rochelle, France). Implementing an approach to monitor contamination of birds with contrasted habitats and feeding habits is under-testing.

4.2.5 Iceland

Monitoring of trace metals and organochlorines in marine biota around Iceland was initiated in 1989 to fulfill the OSPAR and AMAP agreements. Blue mussels (*Mytilus edulis*) and cod (*Gadus morhua*) have been collected annually for chemical analyses.

Since 1993 the University of Iceland and the Environment Agency have monitored tributyltin (TBT) pollution along the west coast of Iceland every five years by means of imposex evaluation in dogwhelks (*Nucella lapillus*). Fifteen sites in SW and W Iceland were sampled in 2018 and thereof three new harbour sites in Reykjanes peninsula, SW Iceland, were added to the previous twelve monitoring sites. Imposex levels (VDSI and RPSI) and organotin concentrations were generally low showing continuing improvement in impact and concentrations of TBT in Iceland. However, high imposex levels were observed at the new sites, particularly in Njarðvík harbour (VSDI: 4,11; RPSI: 4.36) with elevated concentrations of TBT (12 µg/kg dw) and triphenyltin (7.3 µg/kg dw) in dogwhelk tissues compared to the other sites. Although organotin pollution in Iceland has decreased over the past 25 years these results highlight the need for continuing monitoring of organotins in the marine environment in Iceland.

EROD responses in three spined sticklebacks: A case study in ponds in Reykjavík was carried out subsequently to the chemical analysis in sediment from Reykjavíkurtjörn. Total EPA 16 PAH concentrations were 12.851 ng/g dw and 18.417 ng/g dw in northern and southern parts of the pond, respectively, with some PAH´s reaching a category of "frequent effect level" according to guidelines adopted from Canada (Environment Canada, 2007), indicating high pollution levels in each site. Investigation of EROD in hepatic tissue of *Gasterosteus aculeatus* (n=20 per. pond), collected post-spawn, showed significantly elevated activity in fish from polluted, northern and southern sites in the Reykjavíkurtjörn in comparison to reference ponds Urriðakotsvatn and Sandgerðistjörn. Fish from Reykjavíkurtjörn showed up to 100 fold increase in EROD activity compared to fish from reference ponds, indicating that fraction of pollutants in the sediment are bioavailable and are being transferred to sticklebacks, likely through their predation on sediment dwelling organisms.

4.2.6 Ireland

Monitoring of contaminants and biological effects in the Irish marine environment is carried out by the Marine Institute. Contaminants are monitored for contribution of data to the coordinated environmental monitoring program (CEMP). Temporal trend and spatial monitoring is carried out at fixed points. The data is also used in Marine Strategy Framework Directive (MSFD) and Water Framework Directive (WFD) assessments.

Previously in 2010, a wide range of biological effects techniques were measured in fish, mussels, gastropods and sediments as well as fish disease. With the focus of the MSFD, and linking cause and effects, there is currently only one biological effects tool used in Irish monitoring.

Biological effects monitoring is primarily restricted to a historic integrated chemical and biological effects monitoring project (Giltrap *et al.* 2014) and to the cyclical (generally 5 to 6 yrs) measurement of imposex (VDSI and VDS) in dog whelks (*Nucella lapillus*) around the coast of Ireland. The national monitoring plan is currently under review and the role of (mainly risk based) imposex monitoring within this should be available by WGBEC 2022.

4.2.7 Italy

The Italian Institute for Environmental Protection and Research (ISPRA) is in charge of the implementation of the MSFD, defining the Monitoring programme in its territorial waters and carrying out monitoring activities together with other Research Institutes and Regional Environmental Agencies. Based on an Initial Assessment undertaken in 2012, in 2014 a first Italian Monitoring Program was drawn up, followed in 2020 by a second MP.

While procedures for hazardous substances monitoring in water, sediments and biota have already been defined under other national monitoring programmes (i.e. WFD), for biological effects monitoring a consistent initial effort was addressed to standardize monitoring activities. Different issues such as target species, type of biomarkers, protocols and assessment criteria have been thoroughly discussed and some of these aspects were finally defined.

To assess contaminants effects within the actual monitoring framework, *Mytilus galloprovincialis*, *Mullus barbatus*, *Merluccius merluccius* have been chosen as potential target species. Three "core" methods such as LMS, MN and AChE and two "additional" ones such as EROD and metallothionein (the first only in fishes) are included in the Monitoring Program, according to the IMAP-UNEP recommendations. Organisms are sampled out of their reproductive period and some "supplementary parameters" are also collected for fish species, such as total length and weight (condition factor), gender and, when possible, also liver and gonadal weight (HSI and GSI), reproductive status, together with seawater temperature, salinity and dissolved oxygen data. Biomarkers have to be analysed using standard protocols such as UNEP MedPol and/or ICES TIMES.

As for sampling strategy, Italian territorial waters have been divided into three sub-regions (Adriatic Sea, Ionian-Central Medit. Sea, West Medit. Sea). Biological monitoring is carried out in 15 sampling sites in all the sub-regions, with a sampling frequency of one sub-region every year, so that each site is sampled once every 3 years. Biomarkers results are compared with existing threshold values (BACs defined by UNEP and ICES) and with data from scientific literature for Reference Areas if threshold BACs have not yet been defined. Effect-based monitoring is performed in a coordinated way with the chemical monitoring on biota.

To assess biological effects of contaminants some preliminary monitoring activities started in 2016; a new six years cycle monitoring activity will begin in 2021 (autumn). Preliminary assessment of biomarker analysed in mussels and red mullet evidenced some differences between sub-

regions, data not always comparable to the available corresponding BAC (different units of measurement or target tissue) and the lack of a defined threshold for MT in red mullet.

Gaps and critical issues. Based on these preliminary results some main critical issues need still to be considered: methodological protocols for biomarker analyses are not harmonized at national level; threshold values for fish are not outlined for all biomarkers assessed at national level; BACs (mussels and fishes) perhaps need to be defined at sub-regional level; data collected so far are not enough for a robust assessment of the health status of marine organisms.

Suitable solutions and future avenues. ISPRA together with other Italian experts is working on the harmonization of methodological protocols at national level toward the elaboration of a "Methodological manual for analyses of biomarkers in marine organisms" taking into account IMAP-UNEP and ICES TIMES indications. Ring tests and training courses will also be carried out for each biomarker. To provide a better definition of assessment criteria a greater amount of data comparable between years and sites needs to be collected. In the future, the implementation of the monitoring plan could include other marine species as well as new effect-based measurements (i.e. liver pathologies) and a greater number of sampling stations, involving actively other institutes (i.e. Italian Regional Environmental Agencies) in biomarker assessment. These actions will allow to confirm the existing thresholds (BAC) at national level or to extrapolate eventual new thresholds at regional (Mediterranean Sea) or sub-regional level.

4.2.8 Norway

The national monitoring programmes in Norway can be divided into the Coastal Monitoring Programme, which represents the Norwegian contribution to the Coordinated Environmental Monitoring Programme (CEMP), and the Offshore Monitoring Programme. The Norwegian coastal monitoring programme determines the concentration levels, trends and effects of contaminants. The contaminants comprise of over 100 different compounds including metals, tributyltin, organochlorines, PAHs, polybrominated diphenyl ethers (PDBE), per fluorinated alkylated substances (PFAS), hexabromcyclododecane (HBCDs), chlorinated paraffin's (SCCP, MCCP), phosphorous flame retardants (PFRs), bisphenol A, tetrabrombisphenol A and alkylphenols. The biological effects parameters include imposex (VDSI) measured in dog whelks (*Nucella lapillus*) at eight locations, and 1-OH pyrene metabolites, ALA-D and EROD in cod (*Gadus morhua*) from 3 locations.

The offshore monitoring programme in Norway has been running for over 25 years and was designed to ensure that the discharge regulations set by the Norwegian Environment Agency (NEA) are suitable for providing adequate environmental protection from produced water discharges. The programme has evolved over the years and currently comprises of a sediment monitoring programme, where the distribution and abundance of benthic communities are investigated with respect to proximity to oil and gas installations and sediment chemical concentrations; as well as the Water Column Monitoring programme, which is an effects based programme using chemical concentrations and biological effects measurements in wild fish populations and caged mussels.

For the wild fish, a minimum of 3 species (n = 15-30) are sampled from within 500 m of an offshore oil and gas platform and compared to populations of the same species in two other regions of the North Sea (Greater Ekofisk and Egersund bank). Chemical concentrations (PAH in liver, PFAS in blood) and biological effects markers (PAH metabolites, liver histology, EROD, CYP1A, DNA adducts, comet and acetylcholine esterase inhibition) are measured. Mussels are cage at distances from the offshore platform at 8 stations and 2 depths (15 and 40 m). Chemical bioaccumulation (PAH, metals) and biological effects (condition index, lysosomal membrane stability, stress on stress, micronuclei and histology of the gill, digestive gland and gonad) were measured

after a 6-week exposure duration. Passive samplers are also used alongside the mussels to measure PAH, alkylphenol and naphthenic acid concentrations.

Biological effects monitoring of an offshore oil and gas installation in the North Sea.

4.2.9 Scotland

Monitoring of contaminants and their biological effects in the Scottish marine environment is undertaken by Marine Scotland Science (MSS) as part of the UK Clean Seas Environment Monitoring Programme (CSEMP). Historically, the main focus of the CSEMP was to meet the temporal trend monitoring requirements of the OSPAR Convention. However the data is also used in Marine Strategy Framework Directive (MSFD) assessments and in Scotland's Overall assessment, which was recently updated and published in 2020 (https://marine.gov.scot/sma/assessment-theme/biological-effects-contaminants).

Different geographic scales are used depending on the assessment. Biota samples are typically taken at fixed locations. Following Scotland's Overall Assessment 2020 a number of new stations have been added to the sampling programme to improve regional assessment capability. Biological effects are measured in fish, mussels and gastropods.

Marine Scotland Science run an annual winter sampling survey programme (November – January) with 23 fishing sites; however not all sites are sampled annually. The sampling frequency varies dependent upon the site-specific risk(s) from hazardous substances (1, 3 or 6 years). Sites are monitored annually if above the EAC/EAC equivalent, every 3 years if between the BAC and EAC/EAC equivalent and every 6 yearly if below BAC. A new 6-year sampling plan was started in 2020/21. Sampling takes place in winter to avoid spawning season and to maintain consistent time-series. Species sampled is dependent on site and includes dab (*Limanda limanda*), plaice (*Pleuronectes platessa*), flounder (*Platichthys flesus*) and cod (*Gadus morhua)*. Biological effects that MSS currently measure in fish include: EROD (males only), bile metabolites, external fish disease, liver histopathology and micronucleus. The determination of acetylcholinesterase activity will hopefully be introduced in the next few years.

Twenty four mussel (*Mytilus edulis*) sites are sampled as part of a sampling plan that rotates around the Scottish coast. Sampling is undertaken in collaboration with the Scottish Environment Protection Agency (SEPA)'s mussel watch programme of contaminant monitoring. Sampling takes place in winter (February) to avoid the spawning season and to maintain consistent

time-series. Biological effects that MSS have measured in mussels include: stress-on-stress, lysosomal membrane stability, genotoxicity (Comet assay), histopathology and condition index. In 2020 SEPA experienced problems with population declines at 5 out of 21 sites.

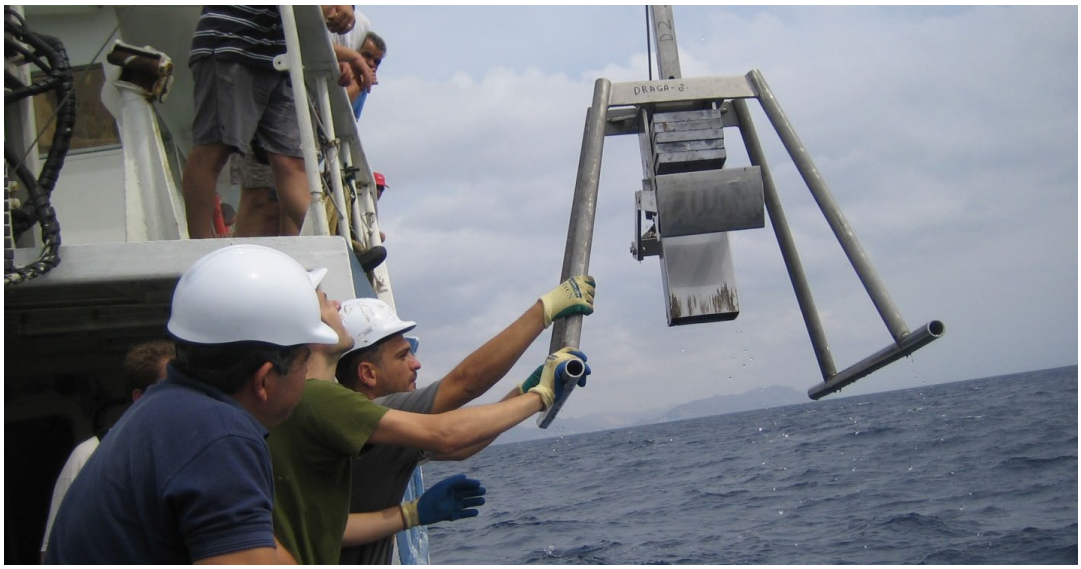
Imposex in dog whelks (*Nucella lapillus*) is monitored in Scotland by a national survey which takes place every three years. This was last done in 2017 and was due in 2020, however the 2020 survey has been delayed due to the Covid-19 pandemic. Twenty five sites are included in the next sampling campaign. Scotland's Overall Assessment 2020 included a regional assessment of imposex in gastropods (https://marine.gov.scot/sma/assessment/imposex-dog-whelks).

4.2.10 Spain

As described in the ICES WGBEC Report 2015, the Spanish Oceanographic Institute (Instituto Español de Oceanografía, IEO) is the scientific institution in charge of carrying out the Spanish Marine Pollution Monitoring Program and the institution in charge of the scientific implementation of the MSFD in Spanish territorial waters. Effect-based monitoring activities are performed by the IEO in two different marine regions following a similar approach but with peculiarities mainly associated to biogeographical differences and recommendations of OSPAR/Barcelona Regional Conventions, such as sampling time and fish target species (detailed information in ICES WGBEC Report 2015). Effect-based monitoring (biomarkers in target species) is performed in a coordinated way with the chemical monitoring.

The effect-based monitoring has been conducted in the Spanish Atlantic region since 2005. Imposex in gastropods populations has been monitored since 2005 and Scope for Growth in mussel populations has been monitored since 2007. Chemical contaminant concentrations (mussels, fish and sediments), sea-urchin (*Paracentrotus lividus*) elutriate embryo-larval bioassays, and biomarker responses (gastropods and mussels) are analysed in selected areas. For chemical concentration and biomarkers in mussels (*Mytilus galloprovincialis*), the temporal monitoring programme comprises a number of locations that are sampled yearly, while the spatial monitoring programme comprises a larger number of locations that are sampled once every 5 years. Gastropods (*Nassarius reticulatus* and *Nucella lapillus*) are sampled for imposex measurements once every 3 years. For chemical concentrations in fish (*Merluccius merluccius*), the temporal monitoring programme comprises a number of locations that are sampled every year. For chemical concentrations and embryo-larval bioassays in sediments, the temporal monitoring programme is established to be conducted once every 5 years on selected areas.

Sampling of wild mussels (*Mytilus galloprovincialis*) with shell length from 3.5 to 4.5 cm for the study temporal trends of pollutants and biological responses is conducted on an annual basis in areas of special relevance and in reference areas (22 sampling stations) and sampling for spatial distribution are conducted every 5 years in 41 sampling stations. During the years 2016, 2017, 2018 and 2020, the sampling campaigns were not performed because economic reasons. The study of temporal trends of pollutants is carried out in the liver and muscle of fish (*Merluccius merluccius*) obtained in 3 areas. The sampling season is the period of October-November. The size range is 30–35 cm. The study of the specific biological effects of tributyltin (Imposex) in gastropod populations (*Nassarius reticulatus* and *Nucella lapillus*) is conducted in 20 sampling stations of the Spanish North Atlantic coast. Biomarkers analyses: SFG, lysosomal membrane stability, micronuclei frequency and acetylcholinesterase in mussels, imposex in gastropods, PAHs metabolites in fish, sea-urchin embryo-larval bioassays with sediment elutriates.



Box corer used to collect undisturbed sediment samples for chemical and biological monitoring.

An integrated chemical-biological effect assessment approach is being conducted in selected areas since 2006 along the Spanish Mediterranean coast. Chemical contaminant concentrations (mussels, fish and sediments) and biomarker responses (mussels and fish) are analysed in selected areas. For chemical concentration and biomarkers in mussels (*Mytilus galloprovincialis*), the temporal monitoring programme comprises a number of locations that are sampled yearly, while the spatial monitoring programme comprises a larger number of locations that are sampled once every 5 years. For chemical concentration and biomarkers in fish (*Mullus barbatus*), the temporal monitoring programme comprises a number of locations that are sampled every two years. For chemical concentration in sediments, the temporal monitoring programme is established to be conducted once every 5 years on selected areas. Sediment and fish sampling are coordinated.

Sampling of wild mussels (*Mytilus galloprovincialis*) with shell length from 3.5 to 4.5 cm are sampled for the study temporal trends of pollutants and biological responses on an annual basis in areas of special relevance and in reference areas (10-12 sampling stations). Unfortunately, during the years 2016, 2018 and 2020, the sampling campaigns were not performed because economic reasons. During the monitoring the following biomarkers were analysed: Stress on Stress, lysosomal membrane stability in hemocytes, micronuclei frequency in agranulocytes, acetylcholinesterase in gills, metallothionein in digestive gland. In addition, condition index of sample population, size, temperature and salinity of surface environmental water were recorded.

Red mullet (*Mullus barbatus*) between 12 and 18 cm length are sampled in the Mediterranean region for chemical and biological measurements. Fish sampling is conducted with research trawling vessels, and the campaigns are conducted in October-November in the Spanish Mediterranean coasts (8 sampling stations). Unfortunately, during the years 2017 and 2018, 2019 and 2020 the sampling campaigns were not performed. The following biomarkers were analysed: Bile metabolites, hepatic EROD activity (microsomal fraction), micronuclei frequency in peripheral erythrocytes, acetylcholinesterase activity in brain. In addition, the presence/absence of nematodos (*Anisakis* sp.) in liver, gonadosomatic index, hepatosomatic index, Fulton´s K index, temperature and salinity of bottom environmental water were recorded.

Blood sampling in *Mullus barbatus* for biological effects monitoring.

4.2.11 Sweden

In the 1960s, the Baltic Sea was found to be severely polluted by POPs and heavy metals. These discoveries were the starting point of a continuous Swedish national monitoring program including contaminant measurements in biological matrices and biological effect monitoring of contaminants to cover the full mix of pollutants present in the environment. The monitoring program builds on a systematic approach of collecting, analysing and assessing environmental data designed to follow up impacts on the environment and changes and trends to estimate effects of measures taken as well as detecting large scale spatial differences. The monitoring is mainly conducted at reference sites not known to be affected by any local pollution. Reasons for using reference sites are for example the aim of assessing the diffuse/overall load of contaminants in the monitored area, that the national monitoring should work as a reference for regional monitoring which normally is conducted at more contaminated sites but also to reduce the variation as much as possible in the monitoring data to increase the ability to detect trends. An increased variation in the data set is often one of the first signs of an increased pressure of contaminants at monitoring site.

Today's monitoring program for biological effects of contaminants is administered by the Swedish Environmental Protection Agency and includes, coastal fish health (e.g. reproductivity, endocrine disruption, blood status), seal health (e.g. blubber thickness, pregnancy rate), reproduction of amphipod and sea eagle and imposex in snails (e.g. VDSI, OTCs). At a few sites integrated monitoring of perch and eelpout is performed were fish health (based on biological effect measurements), contaminant levels (e.g. metals and POPs) and population data (e.g. demography, abundance) are assessed on an annual basis. The Environmental Specimen Bank (ESB) at the Swedish Museum of Natural History in Stockholm is an important part of the national monitoring and contains frozen (-30°C, -80°C, liquid nitrogen) and dry samples dated as far back as the end of the 19th century allowing for retrospective studies. The samples are available for external researchers. The ESB as well as the monitoring program is financed by the Swedish Environmental Protection Agency (SEPA).

The monitoring framework also includes a screening program which is a program that annually reviews numerous emerging substances and new methods to assess effects of contaminants. The

screening program is an important tool that can be used to improve the regular monitoring program.

In 2017–2018, the Swedish Environmental Protection Agency commissioned the survey Effect screening, to investigate biological effects of environmental contaminants in eight polluted hot spot areas along the Swedish coast using established test species. The effect-based methods included health status of fish (perch and eelpout), embryo aberrations and health biomarkers (benthic amphipods), lysosomal membrane stability (blue mussels), and imposex frequency (marine snails).

The main objectives of the Effect screening were: 1) to provide an overview of the severity of the impacts exerted by environmental pollutants in the polluted areas compared to the reference sites; 2) to compare different methods for measuring biological effects of environmental contaminants and 3) to compare how different test organisms respond to the pollution load.

Taken together, the results for all test organisms, i.e., fish, amphipods, blue mussels and snails, used in the Effect screening campaign suggest moderate to severe impacts on the animal health in all areas studied. Moreover, assessments based on different methods generally agreed with each other. This is, probably, because all screened areas have very complex pollution situations, and most organisms inhabiting these areas, regardless of the habitat, are exposed to environmental pollutants. The results indicate that coastal areas act as sources of environmentally harmful substances to the aquatic environment. Screening surveys in such polluted areas can serve as a complement to the regular monitoring of biological effects of exposure conducted by the Swedish national effect-monitoring program. To strengthen the understanding of the relation between levels of contaminants and their effects in the environment contaminants were analysed in the same samples as previously analysed for biological effects. A number of substances were analysed e.g. PCDD/Fs, metals, OTCs and PFASs. A report will be finalised in spring 2021 but preliminary results show a good correlation between the load of contaminants in an area and the effects on population limiting variables.

4.3 OSPAR Quality Status Report – QSR 2023

The next OSPAR Quality Status Report is to be published in 2023 (QSR 2023). The objective of the QSR 2023 is to assess the environmental status of the North East Atlantic against the objectives of the North East Atlantic Environmental Strategy 2010-2020 (NEAES, 2020), evaluate any updated or additional objectives from NEAES 2020-2030, and identify the priority elements for actions to achieve OSPAR's objectives for a clean, healthy, biologically diverse sea, used sustainably. In addition, the QSR 2023 may be used by Contracting Parties that are also EU Member States to support their reporting obligations under the Marine Strategy Framework Directive.

4.3.1 Biological effects contribution to the QSR 2023

Agreement was achieved at the HASEC 2018 meeting to encourage Contracting Parties to carry out targeted biological effects of contaminants towards the OSPAR QSR (HASEC 18/9/1 §4.11). During the 2020 meeting, following a request from HASEC and MIME, the WGBEC discussed the initial specifications for an integrated biological effects approach contribution to the QSR 2023, leaded by France. Contributing countries are: Denmark, England, France, Iceland, Ireland, Norway, Scotland, Spain and Sweden.

The work started in March 2020 and a first draft was presented during the MIME meeting in November 2020, including long term and spatiotemporal local studies resulting from several national initiatives. A WebEx progress meeting will be organised in June 2021 by Thierry Burgeot and an advanced report will be submitted to the QSR2023 committee and the MIME meeting in

November 2021. During the WGBEC 2021 meeting Thierry Burgeot, member of MIME, presented the planning of the OSPAR work to be carried out by 2023, and solicited an editorial team within the WGBEC. Steven Brooks, Juan Bellas and Hannah Anderson proposed their contribution.

4.4 Data submission to ICES

The WGBEC recommends promoting the submission of national monitoring of biological effect data to ICES. Firstly, access to the data (validated data) is essential to support the WGBEC's work for e.g. threshold revision, identification of gaps and new development on national/sub regional levels. Then (and separately), for OSPAR contracting parties, the collection of biological effect data within the ICES database will strongly support the illustration of the data on comprehensive assessment tool (OHAT) developed by ICES at OSPAR level (https://ocean.ices.dk/ohat/?assessmentperiod=2021). This input could be a key argument to defend biological effects as common indicator at OSPAR level.

At the OSPAR MIME meeting 2020 (November 2020), an intersessional contact group of experts supported by the Secretariats of AMAP, HELCOM, ICES and OSPAR[1] was established to explore ways of operationalising the data and assessment processes including the code and developing the three linked Hazardous Assessments Tools (HATs) that are hosted by ICES: AHAT (https://ocean.ices.dk/ahat/?assessmentperiod=2021, only developed for Hg at the moment), HHAT and OHAT. It was suggested to propose MEDPOL to join the discussion. The Mediterranean contracting parties do not usually use the ICES database, apart from Spain and France. But it was recognised that this should not represent a limitation to the discussion for better harmonization of assessment tools and procedures.

4.5 Developments in the HELCOM region

HELCOM is preparing its third holistic assessment (HOLAS III), which includes also the assessment on hazardous substances. Biological effects data from the assessment period (2016–2021) contained in the ICES database (for which there is an approved indicator) will be included in the CHASE integrative tool.

Besides participating in HOLAS III, the HELCOM Expert Network for Hazardous Substances (EN HZ) subgroup for Biological Effects (BE) has continued to work on the development of biological effects monitoring by updating the current use of the methodologies identified earlier by the HELCOM CORESET project in the member countries. In addition, an initiative aiming at a more efficient use of all available data (monitoring, grey literature, case studies, research projects) from all species and the application of different data integration methodologies (incl. those used in OSPAR) was launched, starting with a selection of the subregions for data collection and then followed by analysis of the collected data. The results of this pilot study will be reported to the HELCOM State and Conservation working group in September 2021 and, if considered useful, continuation of the pilot project in a larger scale is planned for 2022. The results will provide alternative approaches to be compared with the present one adopted by the member countries and is expected to bring new ideas for the development of hazardous substances assessment. The project will be carried out in contact with related OSPAR activities and the results will be presented and discussed in the meetings of WGBEC.

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[1] AMAP (Frank Riget), HELCOM Co-chairs of EN-HZ (Berit Brockmeyer & Jaakko Mannio), OSPAR Chair of MIME (Dag Hjermann), OSPAR-France (Aourell Mauffret), OSPAR-Portugal (Mario Milhomens), OSPAR-Sweden (Anne Sørensen) OSPAR-UK (Rob Fryer), and the Secretariats of AMAP (Simon Wilson), HELCOM (Owen Rowe), ICES (Hans Mose Jensen) and OSPAR (Jo Foden).

4.6 Harmonisation and coordination of biological effects monitoring across regional seas

4.6.1 Background

Hazardous substances in the marine environment can lead to numerous subcellular, tissue and whole organism responses in aquatic organisms that can be measured. These measurements are commonly termed 'biological effects' and indicate exposure to either specific contaminants e.g. imposex in relation to TBT, or exposure to contaminant groups e.g. PAH bile metabolite measurement in relation to PAHs. The application of biological effect measurements that cover several modes of action e.g. including but not limited to neurotoxicity and genotoxicity, also have value in determining potentially harmful effects associated with the exposure of aquatic organisms to chemicals of emerging concern, and those chemical measurements that are not yet incorporated into national monitoring programmes. Whilst biological effects tools therefore have value, large scale regional assessments have previously been challenging to coordinate.

The OSPAR Quality Status Report 2020 reported that, whilst several countries undertake biological effects monitoring, "*It is not yet possible in most cases to link chemical monitoring with observations of effects in species in such a way that conclusions can be drawn about the impact of contaminants on the functioning of ecosystems at a regional level. OSPAR countries have not yet implemented a fully coordinated biological effects monitoring programme. This will be needed to support the regional assessment of hazardous substances. Efforts on biological effects monitoring and assessment should therefore continue and be enhanced*".

Numerous standardised biological effect protocols (particularly thorough the ICES TIMES Series) have been produced, although the relative absence of data, notably within the ICES International Databank, is evident, making high level regional assessments difficult to achieve. It is not fully understood why relatively little data are available, although several potential reasons that are not mutually exclusive have been discussed by the WGBEC:

a) The submission of data to ICES may be viewed as technically challenging to individuals wishing to submit their data.

b) There may be a lack of human resources within available national monitoring budgets to address any potential technical challenges.

c) Biological effects data from Regional Seas Conventions (RSC) other than OSPAR e.g. HELCOM, MEDPOL, may be submitted to other national and international databases.

d) Biological effects tools may not be incorporated into many national monitoring activities - this information is not known.

These knowledge gaps must be understood before the scientific community can successfully address those challenges associated with harmonisation and coordination of biological effects monitoring across regional seas.

4.6.2 WGBEC national activities exercise

There were several discussions at the WGBEC 2021 meeting concerning the application of biological effects tools within national monitoring activities and the subsequent submission of their data to ICES and other national/regional databases. This was discussed in the wider context of how the biological effects scientific community might attempt to coordinate and harmonise the application of these techniques for the purposes of international assessment across and between

RSCs. During the meeting, WGBEC members first identified those biological effects data that have previously been submitted to ICES between 1986 and 2020. Whilst this provided a good indication of those data previously submitted to ICES, it did not provide an accurate indication of those biological effect tools that are used in national monitoring activities for which data is 'not' submitted. Furthermore, it was not fully understood which biological effects tools are applied outside of the OSPAR assessment region.

The WGBEC 2021 meeting conducted an exercise amongst members to better understand national monitoring activities within the context of biological effects. To assist in the gathering of this information, attempts were made to obtain summary information on the biological effect tools being used by countries across the numerous RSCs e.g. OSPAR, HELCOM, MEDPOL. This was achieved through the distribution of a table that WGBEC members populated with information regarding those biological effects tools they have used from 2010–2020. Specifically, information was gathered concerning those (a) data collected and submitted to ICES, (b) data collected and submitted to other national/regional databases, and (c) data collected, although not submitted. Whilst the exercise collected information at the level of individual species tissue and database, due to the quantity of information provided and for ease of presentation within this report, it is presented here by (a) animal group e.g. fish, bivalve, gastropod, etc., and (b) submission to ICES or other database.

Table 4.1 presents the information grouped by country, which allows for easy visibility of those biological effect tools used by individual countries between 2010–2020. Table 4.2 presents the information grouped and ranked by biological effect measurement, which allows for easy visibility of those biological effect tools that have been commonly used between 2010–2020. This information will help WGBEC and the wider biological effects community to understand where the gaps and challenges exist regarding quality assurance, coordination, and harmonisation. It is hopeful that the information obtained will help to identify those biological effects techniques that are likely to become common indicators within and across RSCs. Furthermore, it is anticipated that this information will be presented and discussed in a peer-reviewed viewpoint manuscript.

Table 4.1. Heat map table demonstrating biological effects tools utilised in national monitoring activities, grouped by country ranked by alphabetical order. Green indicates that data have been collected and submitted to ICES or other national/regional databases. Red indicates that data are collected, although not yet submitted. White indicates that no data were collected, although were collected at some point within the specified timeframe of 2010–2020. The table was compiled in March 2021 before routine submission of the 2020 data for some member countries.

Country	Biological Effect Measurement	Organism	2020	2019	2018	2017	2016	2015	2014	2013	2012	2011	2010
Denmark	Imposex/intersex	Gastropod											
Denmark	Lysosomal membrane stability (NRR assay)	Bivalve											
Denmark	PAH Bile Metabolites	Fish											
Denmark	Ethoxyresorufin-O-deethylase (EROD)	Fish											
Denmark	Gonadal somatic index (GSI - testis weight in ODA)	Fish											
Denmark	Liver somatic index (LSI)	Fish											
Denmark	Reproductive success	Fish											
Finland	Nutritional status of seals	Pinniped											
Finland	Reproductive status of seals	Pinniped											
Finland	White-tailed eagle productivity	Bird											
Finland	Lysosomal membrane stability (cytochemical assay)	Fish											
France	Acetylcholine esterase activity (ACHE)	Fish											
France	COMET assay	Fish											
France	Fish Disease: Liver histopathology	Fish											
France	Gonadal somatic index (GSI)	Fish											
France	Imposex	Gastropod											
France	Liver somatic index (LSI)	Fish											
France	Lysosomal membrane stability (cytochemical assay)	Fish											
France	Micronuclei	Fish											
France	PAH Bile Metabolites	Fish											
France	Lysosomal membrane stability (cytochemical assay)	Bivalve											
France	Micronuclei	Bivalve											
France	Acetylcholine esterase activity (ACHE)	Bivalve											
France	COMET assay	Bivalve											
Germany	PAH Bile Metabolites	Fish											
Germany	Fish Disease: Gross diseases and parasites	Fish											
Germany	Fish Disease: Liver histopathology	Fish											
Iceland	Imposex	Gastropod											
Ireland	Acetylcholine esterase activity (ACHE)	Fish											
Ireland	COMET assay	Bivalve											
Ireland	Ethoxyresorufin-O-deethylase (EROD)	Fish											
Ireland	Fish Disease: Gross diseases and parasites	Fish											
Ireland	Fish Disease: Liver histopathology	Fish											
Ireland	Imposex	Gastropod											
Ireland	Lysosomal membrane stability (NRR assay)	Bivalve											
Ireland	PAH Bile Metabolites	Fish											
Ireland	Stress on Stress	Bivalve											
Ireland	Acetylcholine esterase activity (ACHE)	Bivalve											
Ireland	Scope for growth	Bivalve											
Ireland	Toxicity Bioassays	Amphipod											
Ireland	Toxicity Bioassays	Polychaete											
Ireland	Toxicity Bioassays	Copepod											
Ireland	Toxicity Bioassays	Diatom											
Ireland	Toxicity Bioassays	Bacteria											
Ireland	Histopathology	Bivalve											
Ireland	Metallothionein	Bivalve											
Ireland	Vitellogenin	Fish											
Italy	Lysosomal membrane stability (cytochemical assay)	Fish											
Italy	Micronuclei	Fish											
Italy	Acetylcholine esterase activity (ACHE)	Fish											
Italy	Ethoxyresorufin-O-deethylase (EROD)	Fish											
Italy	Metallothionein	Fish											
Italy	Liver somatic index (LSI)	Fish											
Italy	PAH Bile Metabolites	Fish											
Italy	Lysosomal membrane stability (NRR assay)	Bivalve											
Italy	Micronuclei	Bivalve											
Italy	Acetylcholine esterase activity (ACHE)	Bivalve											
Italy	Metallothionein	Bivalve											
Latvia	Acetylcholine esterase activity (ACHE)	Bivalve											
Latvia	Glutathionine transferase	Bivalve											
Latvia	Catalase activity	Bivalve											
Latvia	Glutathione reductase	Bivalve											
Latvia	Metallothionein	Bivalve											
Latvia	Reproductive succsess	Amphipod											
Latvia	Fish Disease: Gross diseases and parasites	Fish											
Latvia	Liver somatic index (LSI)	Fish											
Latvia	Gonadal somatic index (GSI)	Fish											
Norway	Aminolevulinic acid dehydratase (ALA-D)	Fish											
Norway	Ethoxyresorufin-O-deethylase (EROD)	Fish											
Norway	PAH Bile Metabolites	Fish											
Norway	Imposex	Gastropod											
Norway	Lysosomal membrane stability (cytochemical assay)	Bivalve											
Norway	Lysosomal membrane stability (NRR assay)	Bivalve											

Country	Biological Effect Measurement	Organism	2020	2019	2018	2017	2016	2015	2014	2013	2012	2011	2010
Norway	Neutral lipid accumulation	Bivalve											
Norway	Lipofuscin accumulation	Bivalve											
Norway	Histology	Bivalve											
Norway	Condition index (CI)	Bivalve											
Norway	Micronuclei	Bivalve											
Norway	DNA adducts	Fish											
Norway	Lysosomal membrane stability (cytochemical assay)	Fish											
Norway	Liver somatic index (LSI)	Fish											
Norway	Gonadal somatic index (GSI)	Fish											
Norway	Condition index (CI)	Fish											
Norway	Histology	Fish											
Norway	COMET assay	Fish											
Norway	Acetylcholine esterase activity (ACHE)	Fish											
Norway	PAH Bile Metabolites	Fish											
Norway	Vitellogenin	Fish											
Spain	Acetylcholine esterase activity (ACHE)	Bivalve											
Spain	Glutathione transferase	Bivalve											
Spain	Glutathione peroxidase	Bivalve											
Spain	Imposex	Gastropod											
Spain	Scope for growth	Bivalve											
Spain	Toxicity Bioassays	Echinoderm											
Spain	Acetylcholine esterase activity (ACHE)	Fish											
Spain	Catalase activity	Bivalve											
Spain	Condition index (CI)	Bivalve											
Spain	Ethoxyresorufin-O-deethylase (EROD)	Fish											
Spain	Gonadal somatic index (GSI)	Fish											
Spain	Liver somatic index (LSI)	Fish											
Spain	Lysosomal membrane stability (NRR assay)	Bivalve											
Spain	Metallothionein	Fish											
Spain	Metallothionein	Bivalve											
Spain	Micronuclei	Fish											
Spain	Micronuclei	Bivalve											
Spain	PAHs metabolites	Fish											
Spain	Stress on Stress	Bivalve											
Sweden	Malformed embryos	Amphipod											
Sweden	Reproductive success	Amphipod											
Sweden	Imposex	Gastropod											
Sweden	Reproductive success	Bird											
Sweden	Reproductive success	Pinniped											
Sweden	Nutritional status	Pinniped											
Sweden	Lysosomal membrane stability	Bivalve											
Sweden	Sex ratio (% female larvae in brood)	Fish											
Sweden	% granulocytes (basophils, eosinophils plus neutrophils) of total blood cells	Fish											
Sweden	hematocrit - volume percentage of red blood cells in blood	Fish											
Sweden	% immature red blood cells of total blood cells	Fish											
Sweden	% lymphocytes (T cells, B cells plus natural killer cells) of total blood cells	Fish											
Sweden	% thrombocytes of total blood cells	Fish											
Sweden	% white blood cells (leucocytes) of total blood cells	Fish											
Sweden	Acetylcholine esterase activity (ACHE)	Fish											
Sweden	Calcium	Fish											
Sweden	Catalase activity	Fish											
Sweden	Chloride ion	Fish											
Sweden	Ethoxyresorufin-O-deethylase (EROD)	Fish											
Sweden	Glucose (Matrx blood)	Fish											
Sweden	Gonadal somatic index (GSI)	Fish											
Sweden	Glutathione reductase	Fish											
Sweden	Glutathionine transferase	Fish											
Sweden	Hemoglobin	Fish											
Sweden	Potassium	Fish											
Sweden	Liver somatic index (LSI)	Fish											
Sweden	Condition factor (length-weight relationship)	Fish											
Sweden	Sodium	Fish											
Sweden	Vitellogenin	Fish											
UK	Acetylcholine esterase activity (ACHE)	Fish											
UK	COMET assay	Bivalve											
UK	Ethoxyresorufin-O-deethylase (EROD)	Fish											
UK	Fish Disease: Gross diseases and parasites	Fish											
UK	Fish Disease: Liver histopathology	Fish											
UK	Gonadal somatic index (GSI)	Fish											
UK	Imposex	Gastropod											
UK	Liver somatic index (LSI)	Fish											
UK	Lysosomal membrane stability (cytochemical assay)	Fish											
UK	Lysosomal membrane stability (NRR assay)	Bivalve											
UK	Micronuclei	Fish											
UK	PAH Bile Metabolites	Fish											
UK	Stress on Stress	Bivalve											
UK	Vitellogenin	Fish											

Table 4.2. Heat map table demonstrating biological effects tools utilised in national monitoring activities, grouped and ranked by biological effect measurement. Green indicates that data have been collected and submitted to ICES or other national/regional databases. Red indicates that data are collected, although not yet submitted. White indicates that no data were collected, although were collected at some point within the specified timeframe of 2010–2020. The table was compiled in March 2021 before routine submission of the 2020 data for some member countries.

Country	Biological Effect Measurement	Organism	2020	2019	2018	2017	2016	2015	2014	2013	2012	2011	2010
France	Acetylcholine esterase activity (ACHE)	Fish											
France	Acetylcholine esterase activity (ACHE)	Bivalve											
Ireland	Acetylcholine esterase activity (ACHE)	Fish											
Ireland	Acetylcholine esterase activity (ACHE)	Bivalve											
Italy	Acetylcholine esterase activity (ACHE)	Fish											
Italy	Acetylcholine esterase activity (ACHE)	Bivalve											
Latvia	Acetylcholine esterase activity (ACHE)	Bivalve											
Norway	Acetylcholine esterase activity (ACHE)	Fish											
Spain	Acetylcholine esterase activity (ACHE)	Bivalve											
Spain	Acetylcholine esterase activity (ACHE)	Fish											
Sweden	Acetylcholine esterase activity (ACHE)	Fish											
UK	Acetylcholine esterase activity (ACHE)	Fish											
Denmark	PAH Bile Metabolites	Fish											
France	PAH Bile Metabolites	Fish											
Germany	PAH Bile Metabolites	Fish											
Ireland	PAH Bile Metabolites	Fish											
Italy	PAH Bile Metabolites	Fish											
Norway	PAH Bile Metabolites	Fish											
UK	PAH Bile Metabolites	Fish											
Spain	PAH Bile Metabolites	Fish											
Denmark	Imposex	Gastropod											
France	Imposex	Gastropod											
Iceland	Imposex	Gastropod											
Ireland	Imposex	Gastropod											
Norway	Imposex	Gastropod											
Spain	Imposex	Gastropod											
Sweden	Imposex	Gastropod											
UK	Imposex	Gastropod											
France	Micronuclei	Fish											
France	Micronuclei	Bivalve											
Italy	Micronuclei	Fish											
Italy	Micronuclei	Bivalve											
Norway	Micronuclei	Bivalve											
Spain	Micronuclei	Fish											
Spain	Micronuclei	Bivalve											
UK	Micronuclei	Fish											
Denmark	Liver somatic index (LSI)	Fish											
France	Liver somatic index (LSI)	Fish											
Italy	Liver somatic index (LSI)	Fish											
Latvia	Liver somatic index (LSI)	Fish											
Norway	Liver somatic index (LSI)	Fish											
Spain	Liver somatic index (LSI)	Fish											
Sweden	Liver somatic index (LSI)	Fish											
UK	Liver somatic index (LSI)	Fish											
Finland	Lysosomal membrane stability (cytochemical assay)	Fish											
France	Lysosomal membrane stability (cytochemical assay)	Fish											
France	Lysosomal membrane stability (cytochemical assay)	Bivalve											
Italy	Lysosomal membrane stability (cytochemical assay)	Fish											
Norway	Lysosomal membrane stability (cytochemical assay)	Bivalve											
Norway	Lysosomal membrane stability (cytochemical assay)	Fish											
UK	Lysosomal membrane stability (cytochemical assay)	Fish											
Denmark	Ethoxyresorufin-O-deethylase (EROD)	Fish											
Ireland	Ethoxyresorufin-O-deethylase (EROD)	Fish											
Italy	Ethoxyresorufin-O-deethylase (EROD)	Fish											
Norway	Ethoxyresorufin-O-deethylase (EROD)	Fish											
Spain	Ethoxyresorufin-O-deethylase (EROD)	Fish											
Sweden	Ethoxyresorufin-O-deethylase (EROD)	Fish											
UK	Ethoxyresorufin-O-deethylase (EROD)	Fish											
France	Gonadal somatic index (GSI)	Fish											
Latvia	Gonadal somatic index (GSI)	Fish											
Norway	Gonadal somatic index (GSI)	Fish											
Spain	Gonadal somatic index (GSI)	Fish											
Sweden	Gonadal somatic index (GSI)	Fish											
UK	Gonadal somatic index (GSI)	Fish											
Denmark	Lysosomal membrane stability (NRR assay)	Bivalve											
Ireland	Lysosomal membrane stability (NRR assay)	Bivalve											
Italy	Lysosomal membrane stability (NRR assay)	Bivalve											
Norway	Lysosomal membrane stability (NRR assay)	Bivalve											
Spain	Lysosomal membrane stability (NRR assay)	Bivalve											
UK	Lysosomal membrane stability (NRR assay)	Bivalve											
Ireland	Toxicity Bioassays	Amphipod											
Ireland	Toxicity Bioassays	Polychaete											
Ireland	Toxicity Bioassays	Copepod											
Ireland	Toxicity Bioassays	Diatom											

Country	Biological Effect Measurement	Organism	2020	2019	2018	2017	2016	2015	2014	2013	2012	2011	2010
Ireland	Toxicity Bioassays	Bacteria											
Spain	Toxicity Bioassays	Echinoderm											
France	COMET assay	Fish											
France	COMET assay	Bivalve											
Ireland	COMET assay	Bivalve											
Norway	COMET assay	Fish											
UK	COMET assay	Bivalve											
Germany	Fish Disease: Gross diseases and parasites	Fish											
Ireland	Fish Disease: Gross diseases and parasites	Fish											
Latvia	Fish Disease: Gross diseases and parasites	Fish											
UK	Fish Disease: Gross diseases and parasites	Fish											
France	Fish Disease: Liver histopathology	Fish											
Germany	Fish Disease: Liver histopathology	Fish											
Ireland	Fish Disease: Liver histopathology	Fish											
Norway	Fish Disease: Liver histopathology	Fish											
UK	Fish Disease: Liver histopathology	Fish											
Ireland	Metallothionein	Bivalve											
Italy	Metallothionein	Fish											
Italy	Metallothionein	Bivalve											
Latvia	Metallothionein	Bivalve											
Spain	Metallothionein	Fish											
Spain	Metallothionein	Bivalve											
Denmark	Reproductive success	Fish											
Sweden	Reproductive success	Amphipod											
Sweden	Reproductive success	Bird											
Sweden	Reproductive success	Pinniped											
Latvia	Reproductive succsess	Amphipod											
Ireland	Vitellogenin	Fish											
Norway	Vitellogenin	Fish											
Sweden	Vitellogenin	Fish											
UK	Vitellogenin	Fish											
Spain	Glutathione transferase	Bivalve											
Latvia	Glutathionine transferase	Bivalve											
Sweden	Glutathionine transferase	Fish											
Ireland	Stress on Stress	Bivalve											
Spain	Stress on Stress	Bivalve											
UK	Stress on Stress	Bivalve											
Latvia	Catalase activity	Bivalve											
Spain	Catalase activity	Bivalve											
Sweden	Catalase activity	Fish											
Norway	Condition index (CI)	Bivalve											
Norway	Condition index (CI)	Fish											
Spain	Condition index (CI)	Bivalve											
Norway	Histopathology	Bivalve											
Ireland	Histopathology	Bivalve											
Latvia	Glutathione reductase	Bivalve											
Sweden	Glutathione reductase	Fish											
Ireland	Scope for growth	Bivalve											
Spain	Scope for growth	Bivalve											
Denmark	Gonadal somatic index (GSI - testis weight in ODA)	Fish											
Denmark	Imposex/intersex	Gastropod											
Finland	Nutritional status of seals	Pinniped											
Finland	Reproductive status of seals	Pinniped											
Finland	White-tailed eagle productivity	Bird											
Norway	Aminolevulinic acid dehydratase (ALA-D)	Fish											
Norway	DNA adducts	Fish											
Norway	Lipofuscin accumulation	Bivalve											
Norway	Neutral lipid accumulation	Bivalve											
Spain	Glutathione peroxidase	Bivalve											
Sweden	Calcium	Fish											
Sweden	Chloride ion	Fish											
Sweden	Condition factor (length-weight relationship)	Fish											
Sweden	Glucose (Matrx blood)	Fish											
Sweden	hematocrit - volume percentage of red blood cells in blood	Fish											
Sweden	Hemoglobin	Fish											
Sweden	Lysosomal membrane stability	Bivalve											
Sweden	Malformed embryos	Amphipod											
Sweden	Nutritional status	Pinniped											
Sweden	Potassium	Fish											
Sweden	Sex ratio (% female larvae in brood)	Fish											
Sweden	Sodium	Fish											
Sweden	% granulocytes (basophils, eosinophils plus neutrophils) of total blood cells	Fish											
Sweden	% immature red blood cells of total blood cells	Fish											
Sweden	% lymphocytes (T cells, B cells plus natural killer cells) of total blood cells	Fish											
Sweden	% thrombocytes of total blood cells	Fish											
Sweden	% white blood cells (leucocytes) of total blood cells	Fish											

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5 Interaction of contamination effects with those of climate change and acidification

5.1 Background

Climate change and acidification are two global challenges facing the world that could potentially have a major impact on the survival and distribution of marine species (Hoegh-Guldberg *et al.*, 2010; Byrne, 2011; Doney *et al.*, 2020). Ocean acidification and warming have been proven to cause several alterations in marine invertebrates. Available data show that calcifying organisms, such as echinoderms, bivalves, gastropods, corals, pteropods, coccolithophores or foraminifera, are the most sensitive to acidification. For instance, pteropods are ubiquitous planktonic calcifiers that have been used as a model to assess the impact of climate change and acidification. A recent study developed thresholds of ocean acidification impacts of pteropods for a variety of responses, such as shell dissolution, calcification, growth, survival and egg development, based on experimental and field data. Thresholds of aragonite saturation state ranged from 0.9 to 1.5 (Bednaršek *et al.*, 2019). Sea urchins, that present calcareous structures both in larval and adult stages, have been used also as a recurrent model to assess the impact of climate change and acidification. In general, ocean warming and acidification have been reported to reduce calcification and to inhibit growth in these organisms, as seen in smaller larval and adult skeletons (e.g. Cohen-Rengifo *et al.*, 2019; Foo *et al.*, 2020). However, the molecular mechanisms behind these effects remain unclear. RNA sequencing technology was used to identify gene expression patterns in sea urchin embryos at different ocean warming scenarios, and the results pointed to complex molecular mechanisms of transgenerational effects, where several genes related to development and growth were dysregulated (Shi *et al.*, 2020). Transcriptomic analyses also showed that sea urchins respond to water acidification by upregulating genes of the Krebs cycle, electron transport chain and fatty acid oxidation, in order to redirect ATP towards maintaining acid-base homeostasis (Evans *et al.*, 2020). These results suggest that natural selection may enhance tolerance to ocean acidification, but reducing the energy available for other fitness-related functions, such as responses to additional pollution stress.

This topic has previously been discussed by the WGBEC, including the possibility of sampling organisms for monitoring low water pH, and the need for selecting a set of responses (at the molecular, morphological and/or community level), that allow accumulating evidence of acidification effects. Major challenges were also identified, such as the adaptation of organisms to the natural variation in the pH of marine water due to the variation of photosynthetic activity, especially in coastal productive waters, and the interaction with other environmental stressors that may interfere with the biological responses to acidification. It was agreed that the main focus of the WGBEC on this issue would be to study the interaction of the effects of climate change and acidification with those of contamination. One of the motivations was that, despite climate change and acidification have been recognized as global problems, research on their interaction with other stressors on marine organisms, populations or communities is still very limited. In fact, the IPCC's Fifth Assessment Report (AR5) acknowledged that the ecological impacts of climate change and ocean acidification could become aggravated by complex interactions with other stressors such as pollution (IPCC, 2014). However, as highlighted by the Arctic Monitoring and Assessment Programme (AMAP, 2016), the combined effect of climate change and acidification on chemical pollution is difficult to predict due, among other factors, to the seasonal variation in pollutant bioaccumulation by marine organisms, caused by the seasonality in lipid content, which is greater than any predicted variation in future climate change scenarios.

Climate change and acidification are likely to interact with many chemical types, through their impact on the general fitness of the organism, which can render the organism more sensitive to the contaminant exposure, and/or the direct impact on the contaminants themselves. Both temperature increase and lowering pH can influence the chemistry of the contaminants, which may cause them to become more bioavailable and toxic, or affect their environmental transport and distribution pathways, affecting their fate in marine food webs (e.g. AMAP, 2016). Therefore, further understanding on these multiple stressor scenarios is needed.

5.2 Interaction of climate change and acidification with chemical pollution

Only a few studies have assessed the combined effects of climate change and acidification with chemical pollution. Considering that organisms are simultaneously exposed to multiple stressors in the natural environment, this knowledge gap can result in the underestimation of the effects of ocean acidification and warming and chemical pollution on marine ecosystems (Nikinmaa, 2013; Alava *et al.*, 2017). Therefore, there is a need to better understand such interactions in order to assess the impact of these stressors on marine ecosystems.

It is known, for instance, that acidification changes speciation of certain metals, influencing their bioavailability and toxicity. In this way, the toxicity of Cu to early life stages of kelps (Leal *et al.*, 2018) or polychaetes (Campbell *et al.*, 2014) has been found to be enhanced under near-future ocean acidification conditions. However, although it has been reported that warming conditions enhance Hg accumulation in fish, acidification can decrease Hg accumulation and reduce the oxidative stress and heat shock responses induced by warming and Hg (Sampaio *et al.*, 2017).

Studies have also been conducted combining exposure to lanthanum, one of the most abundant rare earth element (REE), with a scenario of increased water temperatures (Figueiredo *et al.*, 2020). The selected test species was the European eel (*Anguilla anguilla*) due to the vulnerability of its early life stages to contaminants and its endangered conservation status and economic relevance. Previous work reported on impaired glass eel physiology following exposure to lanthanum (Figueiredo *et al.*, 2018). Glass eels were exposed through water to an ocean warming scenario (OW; Δ +4ºC; 18ºC and 22ºC) and to an environmentally relevant concentration (1.5 μg L^{-1}) of lanthanum (La) for 5 days (plus 5 days of depuration). The aim of this study was to assess the bioaccumulation and elimination of La in glass eel's body parts (head, viscera and body) under a warming scenario and to evaluate lipid peroxidation, heat-shock response, DNA damage (body) and the quantification of acetylcholinesterase (head). Obtained results indicate that bioaccumulation and toxicity of La were enhanced with increasing temperature and that elimination was less effective in warming conditions. Also, lipid peroxidation peaked after five days of exposure to La and warming and La exposure suppressed the expression of heat shock proteins, at both tested temperatures. These results suggest that, when exposed to La, glass eels were unable to efficiently prevent cellular damage, with a particularly dramatic setup in a near-future scenario. Further planned work includes assessing how the combined impact of ocean warming (OW; Δ +4ºC; 18ºC and 22ºC), acidification and hypoxia with lanthanum and cerium (REE) exposure affect survival and early fish development (eggs and larvae) in sea bream (*Sparus aurata*).

The accumulation and toxicity of pharmaceuticals, such as venlafaxine, sulfamethoxazole, sotalol, methylparaben or diclofenac, has also been found to be altered by warming and acidification, which may affect metabolization, gene expression profiles and functional biological responses at the cellular level in mussels (Serra-Compte *et al.*, 2018; Mezzelani *et al.*, 2021) and molecular and behavioural responses in fish populations (Maulvault *et al.*, 2018a,b).

Veterinary medicines currently used in salmon aquaculture in Norway can affect non-target shrimp populations under predicted future environmental conditions. The ectoparastic salmon

lice (*Lepeophtheirus salmonis*) has become a major problem within the marine aquaculture industry. Chemical methods are employed in Norway and elsewhere to control levels of infestation. The chiton synthesis inhibitor diflubenzuron (DFB) is one of the veterinary medicines used, where the chemical interferes with moulting during the development of the crustacean. However, DFB from fish feed may also affect non-target crustaceans such as the Northern shrimp (*Pandalus borealis*), which is an economically and ecologically important species. Laboratory experiments demonstrated that both larval and adult shrimp exposed to DFB through medicated fish feed had reduced survival (Bechmann *et al.*, 2018, 2017). Additionally, the effects of DFB exposure are more severe under future climate conditions over higher temperature and reduced pH (ocean acidification); (Wallhead *et al.*, 2017). These laboratory studies were used to predict the impact of DFB and climate change at the population level. A density-dependent age-structured population model was developed representing a Northern shrimp population located in a hypothetical Norwegian fjord containing a fish farm, under both ambient and future climate conditions. A set of model scenarios representing different DFB application schemes and different degrees of exposure for the shrimp populations were performed. These model predictions demonstrate how the risk of DFB to shrimp populations can be enhanced by factors such as the timing (season) of DFB applications, the percentage of the population affected, future climatic conditions and environmental fluctuations. Discussion followed on how additional knowledge on ecological factors affecting the natural shrimp population dynamics could improve the modelled ecological risk estimates for these populations; these included effects of environmental fluctuation (natural variability) on larval survival, effects of predation and fishing on adult abundance, density dependence mechanisms and incorporating the shrimp moulting phase into the season scenarios tested.

The combined effect of a multi-stressor environment, including global change factors (acidification, temperature increase) and microplastics (MPs) pollution, on embryos and larvae of the sea urchin *P. lividus*, was recently studied by Bertucci & Bellas (2021). Embryo-larval bioassays were conducted to determine growth and morphology alterations after 48 h incubation with MPs (1000–3000 particles/mL); with filtered sea water at pH = 7.6; and with their combinations. Furthermore, the effect of pH and MPs in combination with a temperature increase of 4 °C, was studied. Growth inhibition in larvae resulting from embryos reared at pH = 7.6 was around 75% and embryos incubated at 3000 MPs particles/mL yielded larvae 20% smaller than controls. Exposure to low pH and to MPs also induced morphological alterations such as the increase in the postoral arm separation or rounded vertices. The combined exposure to pH 7.6 and MPs caused a significant decrease of larval growth in comparison to controls, to MPs and to pH 7.6 treatments. Morphological alterations were also observed in these treatments, including the development of only two arms. The increase in temperature resulted in an increased growth in controls, in pH 7.6 and pH 7.6 + MP3000 treatments, but the relative stomach volume decreased. However, when growth parameters were expressed per Degree-Days, the lower growth provoked by the thermal stress was evidenced in all treatments. Thus it was demonstrated that MPs could aggravate the effect of water acidification and that an increase in water temperature caused an additional stress on *P. lividus* larvae, manifested in a lower growth and an altered development. It was concluded that the combined stress caused by ocean warming, ocean acidification, and MPs pollution, could threaten sea urchin populations leading to a potential impact on coastal ecosystems. These effects are considered, though, to be non-additive (see e.g. Darling *et al.*, 2010), because the pH decrease alone is the dominant stressor and has a greater impact than MPs or temperature.

Ongoing research has been performed at the Portuguese Institute of the Sea and the Atmosphere (Instituto Português do Mar e da Atmosfera, IPMA) in the last years with emerging contaminants that can be found in marine organisms (MeHg, iAs, venlafaxine, diclofenac, triclosan, TBBPA,

PFCs and dechlorans), to know how warming and acidification can affect fish and shellfish bioaccumulation, elimination, ecotoxicological responses and consumer safety (e.g. Sampaio *et al.*, 2017; Maulvault *et al.*, 2018a,b; Serra-Compte *et al.*, 2018). Warming is likely to promote bioaccumulation of persistent contaminants in marine organisms (e.g. MeHg, dechlorans), and compromise the safety of seafood products, whereas acidification generally leads to a reduced bioaccumulation of contaminants. The lack of available information further strengths the need to support research in this area (e.g. assessing the effects of other emerging contaminants, understanding contaminants biotransformation processes, integrating other environmental abiotic changes, developing adaptive measures).

5.3 Harmful algal blooms, warming and ocean acidification: toxicokinetics and physiological vulnerabilities in mussels

Knowing that harmful algal blooms (HABs) have been increasing in frequency and intensity most likely due to changes on global conditions, a set of studies were conducted to evaluate: 1) the impact of increasing seawater temperature and acidification on the accumulation/elimination dynamics of HABs-toxins in mussels; and 2) the effects in mussels in terms of oxidative stress and genotoxic responses. Mussels *Mytilus galloprovincialis* were acclimated to four environmental conditions simulating different climate change scenarios: i) current conditions, ii) warming, iii) acidification, and iv) interaction of warming with acidification, and then exposed to the paralytic shellfish toxins (PSTs)-producing dinoflagellate *Gymnodinium catenatum*. Warming-acclimated mussels showed lower accumulation/elimination rates, while acidification-acclimated mussels showed higher capability to accumulate toxins, but also a higher elimination rate preventing high toxicity levels. The combined effect of climate change drivers on accumulation/elimination of PSTs, in mussels, indicated that warming and acidification may lead to lower toxicity values but longer toxic episodes (details in Braga *et al.*, 2018). Results on physiological and genotoxic responses highlight that simultaneous exposure to warming, acidification and PSTs impairs mussel DNA integrity, compromising the genetic information due to synergetic effects, and raises evidences of the increasing ecological risk of HABs to *M. galloprovinciallis* populations (Braga *et al.*, 2020).

5.4 Final remarks

Climate change is likely to affect underlying factors in biota-contaminant interactions e.g. ocean chemistry, biological processes, ecosystem structure and physico-chemical interactions of contaminants. As such behaviour of current contaminants is changing in the marine food webs (e.g. Alava *et al.*, 2017), and a large knowledge gap still exists concerning trends and effects of emerging contaminants. These changes add a level of uncertainty to future predictions on contaminant transport within- and effects thereof on biota. Current efforts to fill the knowledge gap on this subject largely build on experimental research, and as such are highly specific. Furthermore, effects of climate change can vary greatly among regions, creating isolated scenarios.

The main conclusions reached by the WGBEC in this matter are the following:

- Future research in this field should incorporate current knowledge about contaminant interactions on a holistic scale, relevant for environmental applications.
- There is a general need to obtain scientific evidence on the joint effects of acidification, warming and contaminants, to better understand their impact on marine ecosystems.
- There is also a need to understand the mechanisms of action and ecotoxicological effects of the interactions between acidification, warming and environmental toxicants (metals,

legacy POPs or emerging substances), under realistic near-future ocean acidification and warming conditions.

- New approaches are needed to evaluate contamination considering the complexities related to multiple stressor scenarios.
- A selection of robust, sensitive biological indicators, suitable for the assessment of multiple stressor scenarios, need to be developed.

Due to limited direct contributions of the group it was agreed to exclude this particular ToR in the next period. Nevertheless, huge interest exists within the group on the topic and it is likely that future contribution to other ToRs concerning emerging contaminants and development of new methods will touch on the subject and be reported within those ToRs.

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6 Effects of contaminants of emerging concern

6.1 Background and discussed approaches

The review and assessment of contaminants of emerging concern (CECs) by the WGBEC during this reporting cycle considered the list of identified substances of emerging concern, requested by OSPAR, and prepared by the Marine Chemistry Working Group (MCWG) and the Working Group on Marine Sediments (WGMS) during the previous reporting cycle ending in 2018. A concerted effort to bring the three groups together resulted in a combined session in Lisbon, March 2020, where the assessment of biological effects of contaminants of emerging concern was discussed.

The traditional general risk assessment approach, as well as toxicity through structurally similar substances (structure-activity relationship, SAR), were discussed by the WGBEC as potential ways to assess the toxicity of CECs, and as a result a standard suite of suitable chronic ecotoxicological tests was evaluated.

The general risk assessment of chemicals requires at least three aquatic tests with an algae, an invertebrate (commonly a crustacean) and a fish. Depending on the number of tests, whether the tests are acute or chronic and which endpoint is chosen, assessment factors from 10 to 1000 (sometimes lower) are used to arrive at an estimate for a concentration in the environment, called predicted no effect concentration (PNEC), that will not cause harm to organisms. Although this procedure aims to protect aquatic life, it usually relies on lethal toxicity, whilst sublethal responses that may lead to long-term effects, such as endocrine disruption, immunotoxicity or genotoxicity, will often not be covered. Hence, the risk assessment relies on the assessment factor to cover for the other mechanisms of toxicity, a very uncertain and generally incorrect assumption.

Emerging substances with a specific mode of action (e.g. estrogenicity or anti-estrogenicity) will not be identified as problematic if not tested specifically for (Fuhrmana *et al.*, 2015). Many of the suggested tests within the suite can be performed *in vitro*, thus reducing the cost (Table 6.1). One possible option to provide a suggestion for toxicity through specific mechanisms is to read-across from structurally similar substances, i.e. (quantitative) structure-activity relationships, (Q)SAR. (Q)SAR should not replace the expanded toxicity assessment described below, but can be used as screening tool to tentatively identify substances for further testing.

Mechanisms of toxicity that are needed to complement the basic set (the top three lines in Table 6.1) are indicated in bold; they will not ensure full coverage, but represent mechanisms that to some extent includes other mechanisms of toxicity.

This approach for screening CECs should be integrated for the more general prioritisation of the emerging chemical contaminants in the field. The selection of the individual emerging chemical compounds, considered most relevant for marine monitoring, can be considered as a first step. However, it should integrate a wider reflection on the monitoring and their combined biological effects into the marine environment (Nilsen *et al.*, 2019).

The OSPAR's Hazardous Substances & Eutrophication Committee (HASEC) requested a review of new guidelines for monitoring biological effects in 2018. Furthermore the OSPAR's Working Group on Monitoring and on Trends and Effects of Substances in the Marine Environment (MIME) proposed a new approach to the biological effects guidelines in 2019, harmonizing the modes of action and biological responses. France is currently leading on drafting a new biological effects guideline.

In addition, it is acknowledged that international efforts to find a suitable framework for CECs needs to be included for both risk assessment and monitoring purposes (Drewes *et al.*, 2013).

Table 6.1. Tests required for identification of emerging substances. Methods in bold comprise a minimum set of tests.

Mechanism of toxicity	Suggested species/method	Reference
Phytotoxicity	**Planktonic algae, e.g. Skeletonema**	OECD, ISO 10253:2016
Survival aquatic	**(I) Crustacean, e.g. *Acartia*, *Nitocra*** **(II) Fish, e.g. stickleback, turbot**	OECD, ISO 14699:1996 (copepods)
Neurotoxicity	AChE in mussel gills (*in vivo*) Acyl CoA oxidases	ICES TIMES 22
Estrogen Receptor alpha (ER-α)	Cell-line (*in vitro*)	
Aryl hydrocarbon Receptor (AhR) "dioxin-like"	Cell-line	
Endocrine - (anti)estrogenic	Cell-line	
Endocrine - thyroid		
Genotoxicity	**Comet in mussel haemocytes (*in vitro*), fish white blood cells (WBC) (*in vitro*)** **Micronucleus test in mussels/fish**	ICES TIMES 58; Sahlmann *et al.*, 2017
Membrane disruption	**Lysosomal stability in blue mussel haemocytes (*in vivo* or *in vitro*)**	ICES TIMES 56
Ion regulation disruption	**Fish embryo**	Alsop and Wood, 2013
Cytotoxicity	AB, CFDA-AM in mussel haemocytes (*in vitro*)	
Oxidative stress	GSH + fluorescent probes in mussel haemocytes (*in vitro*)	
Immunotoxicity	Fish lymphocytes, qPCR cytokine expression (*in vitro*)	
Reproductive toxicity	*Tisbe* sp.	
Developmental toxicity	**Oyster/mussel embryo-larvae** **Echinoderm embryo-larvae** **Fish embryo**	ICES TIMES 54, ISO 17244: 2015, ICES TIMES 51, OECD236,

WGBEC drafted the guidelines (JAMP OSPAR agreement 2009) to assess the general biological effects of chemical contaminants and the specific effects of a few families of contaminants (PAHs, heavy metals, TBT, endocrine disruptors). Methodological developments over the past 20 years for the application of marine biomarkers and bioassays require a revision of the biological effects guidelines. In particular, two elements need to be reviewed:

1) The redundancy of the same biomarkers and bioassays applied for both an assessment of general biological effects and specific biological effects; this may be confusing for stakeholders.

2) The implementation of biological effects monitoring methodologies initiated in the European Water Framework Directive (WFD) (Brack *et al.*, 2017) and MSFD (Davies and Vethaak, 2012) should be aligned with the monitoring of the biological effects of hazardous substances in the Regional Seas Conventions.

A first step in methodological harmonisation could be based on the combination of effect-based Monitoring (EBM), developed under the Norman Network on emerging contaminants (Brack *et al.*, 2017), and towards the review of the WFD with the WGBEC recommendations (Davies and Vethaak, 2012). Progress on the development of biomarker and bioassay thresholds (BAC, EAC) applied in the integrated chemistry and biological effects approach for OSPAR areas (Hylland and Gubbins, 2017) is unique. They can be combined with an analysis of EBM (Brack *et al.*, 2017). This combination of the two initiatives would also address the question of identifying families of the most toxic contaminants to take regulatory action during the 6-year WFD and MSFD assessment cycles. The European technical report led by the Joint Research Centre (Napieska *et al.*, 2018) recommends a convergence of these two initiatives between the integrated chemistry and biological effects approach and the development of EBM focused on modes of action (MOA) or types of effects. There are indeed methodological similarities between the two ecotoxicological approaches as the same biomarkers and bioassays are recommended for the study of the mechanisms of chronic effects. In this context, it has been identified a need for rationalising the effort at the international level, bringing together different initiatives for scientific support of the environmental policy (WFD, MSFD, ICES, RSC).

The current research in understanding the potential effects of CECs at the molecular level will help define the most suitable biomarkers for early detection of exposure and onset of sublethal adverse effects. Preference could be given to endpoints which have already been established as molecular initiating events (MIEs) or key events (KEs) within Adverse Outcome Pathways (AOPs).

6.2 Reported examples of CECs

During this reporting cycle the effects of CECs were discussed, with special attention to technology-critical elements, which are often disregarded in terms of their environmental impact. Evidence exists for an increase in the concentrations of some of these elements in the marine environment due to their use in current technologies, but their ecotoxicological impact is widely unknown at present. Examples reported by members of the WGBEC include:

Less-Studied Trace Metals: Contaminants of Emerging Concern?

Several trace metals and their compounds (e.g. Pb, Cd, Ni, Hg, Sn-TBT) are included as priority substances in the EU's WFD and must be routinely measured by each country through monitoring programmes for the evaluation of the progress made toward achieving Good Environmental Status in EU's waters.

For most of the chemical elements, however, no regulations regarding their concentrations in the marine environment exist at present. Some of these less-studied trace metals, which were considered just as laboratory curiosities in the past due to the absence of significant anthropogenic use, are now however key components for the development of new technologies thus leading to an increasing use and potential release to the environment. These elements include Nb, Ta, Ga, In, Ge, Tl, Te, the platinum group elements (PGE: Pt, Os, Ru, Rh, Pd and Ir), and most of the rare earth elements (REE: Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Yb, Lu); (Figueiredo *et al.*, 2018).

The present understanding of the concentrations, transformation and transport of these less-studied, technology-critical elements, in the environment is scarce, and their impact on their biogeochemical cycles (local/global) and potential biological and human health threats needs to be further explored. Currently, due to their use in current technologies, evidence exists of an increase in the concentrations of some of these elements in the marine environment (e.g. Gd, Pt). However, their ecotoxicological impact is widely unknown at present.

Sea-dumped chemical and conventional warfare materials

Environmental effects of hundreds of thousands of tons of chemical munitions dumped at sea after the World War II are causing global concern. At many sites the warfare materials are in an advanced state of corrosion, making them a potential risk to local ecosystems. Increased deep sea activities (pipelines, cables, drilling, scuba diving, trawling, research) have increased the risk of disturbing the dumped munitions, and the risk of a massive release to the marine environment remains a distinct possibility. Chemical warfare agents (CWA) such as mustard gas, Lewisite and nerve agents are the most frequently dumped CWA while organoarsenicals, blood agents, choking agents and lacrimators were dumped in smaller volumes. Also, chemicals commonly used in explosives such as 2,4,6-trinitrotoluene (TNT) and cyclotrimethylenetrinitramine (RDX) are highly toxic to organisms. No alarming amounts of CWA or their breakdown products have been found in tissues of marine organisms, but studies have shown evidence of possible chronic toxicity. Laboratory experiments have shown both bioaccumulation of degradation and oxidation products of these chemicals in organisms as well as responses in various biomarkers. Risk posed by seafood consumption is believed to be minimal and acute exposure is still considered as the major human health risk. The current recommendation by experts is to leave the dumped CWs untouched; however, monitoring is considered important. Concerning conventional munitions, clearance activities are ongoing in various areas, either by destructing on land or exploding at the sea bottom.

6.3 Planned and ongoing initiatives on CECs monitoring across all regional organisations

Method approaches currently in use, and planned for the future, within the different regional areas, were presented to the WGBEC during this three-year term and include:

- Presence, behaviour and risk assessment of pharmaceuticals in marine ecosystems (PHARMASEA), funded by EU (joint call between Water JPI, JPI Ocean JPI AMR (Antimicrobial resistance).
- Monitoring Strategies for CECs in Recycled Water (Southern California Coastal Water), funded by the California State Water Board, USA.
- OSPAR CECs and threat in the marine environment (CONnECT) project.
- HELCOM EN-HZ NT screening project partly funded by the Nordic Environment Finance Corporation (NEFCO).

The **PHARMASEA project**, starting in 2021 till 2024, integrates international expertise to answer key research questions on fate and biological effects of active pharmaceutical ingredients (APIs), well recognized CECs for marine ecosystems (Mezzelani *et al.*, 2018). In-depth studies on APIs distribution, effects and risks in four European coastal areas are carried out, targeting occurrence, uptake and trophic transfer along regional marine food webs; bioaccumulation/excretion kinetics, potential ecotoxicological effects from molecular to individual levels, and characterization of modes-of-action in model and selected marine species; development of specific risk assessment procedures for APIs.

The scientific consortium of PHARMASEA is constituted by research institutions from four European countries (Germany, Italy, Norway and Spain). One of the aims of PHARMASEA is to measure (Theme 1 of the EU joint transnational call) the presence, distribution, fate of APIs in marine ecosystems, the transfer from inland sources and their behaviour, unravelling relationships between their occurrence in the water column, sediments as well as biota and the trophic transfer along food webs. These aspects will be investigated at a European-wide geographical scale including the Mediterranean Sea, the Atlantic Ocean and the North Sea. The project also characterises the main factors that modulate bioavailability and fate of APIs, including uptake/excretion kinetics, biotransformation and tissue translocation, biological effects and modes-of-action. Such studies will allow the development of an integrated risk assessment procedure with identification of priority APIs, long-term adverse alterations and cumulative effects of API mixtures.

The communication and connection with industries, private and public stakeholders as well as citizen engagement are an important part of this project, aiming to promote public awareness, pre-normative research and implementation into European Directives. The results could influence prescribing and disposal practices of domestic medicines and increase market opportunities and competitiveness of European pharma industries investing on environmental sustainability of their products.

Monitoring Strategies for CECs in Recycled Water have been discussed and carried out in California Coastal Water since 2009, and the activity is funded by the State Water Board. California has a long history of water reclamation and reuse due to its geographical location and weather conditions. The water recycling practices require appropriate treatment barriers and monitoring strategies to minimise exposure to a wide range of CECs that may be harmful to human health and the ecosystem. In 2010, a first risk-based framework was proposed for prioritising and selecting CECs for recycled water monitoring programmes. At present, there is intense activity updating the list of CECs (containing now 489 CECs, an increased number compared to 418 in 2010), using new data from measured environmental (or effluent) concentrations. Bioanalytical screening tools (including for example the oestrogen receptor alpha (ER-α) and the aryl hydrocarbon receptor (AhR) bioassays) will be used to respectively assess estrogenic and dioxin-like biological activities and non-targeted analysis are highly recommended. The importance data collection for antibiotic resistance is also highlighted. In general, it is suggested to have a flexible and responsive programme to update CECs monitoring. Recommendations in response to rapidly emerging science, technological advances and improved collection of monitoring (screening) data need to be updated (Drewes *et al.*, 2018).

The pilot project **CONnECT (CONtaminants of Emerging Concern and Threat in the marine environment)** relates to a proposal by OSPAR Contracting Parties (CPs) to complete an OSPAR wide collaboration with the NORMAN Research network aiming to complete a pilot target and suspect contaminant screening project to evaluate the extent and range of CECs in OSPAR marine matrices.

Routine monitoring programmes such as those completed under the Water Framework Directive and the Regional Seas Conventions, include the provisions against pollution of marine waters by chemical substances. Additionally, the Marine Strategy Framework Directive (MSFD, 2008/56/EC) aims to provide an integrative marine environment status assessment and considers both coastal and offshore environment. The identification of substances that are not listed as WFD PS (priority substances) or RBSP (river basin-specific pollutants), but that entail a significant risk to the marine environment is part of the provisions under the MSFD (Commission Decision 2017/848/EU). Monitoring programmes to support these mandates are generally much targeted in nature. While targeted programmes provide valuable information on the presence and

often the potential for deleterious effects relative to a variety of Environmental Criteria/Threshold values for specific lists of priority substances, they cannot deliver information on the myriad range of substances that may be present in the environment. Many of these chemicals and chemical mixtures have now been characterised as CECs.

This pilot project seeks to complete the first coordinated target and suspect screening of CECs in the OSPAR region. Some key aims of this pilot study include:

a) Completion of the first (geographically extensive) comprehensive wide scope target and suspect screening analysis of pollutants of emerging concern in biota from OSPAR marine waters.
b) Generating lists of potential contaminant threats at both a local Contracting Party level but also on an OSPAR regional basis.
c) Establishment of collaboration with the NORMAN network to complete a prioritisation listing integrating levels measured to their potential for harmful effects.
d) Generation of CECs data to support OSPAR activities in the area of future prioritisation.
e) Provision of "baseline" data for a range of CECs for future OSPAR work.
f) Feeding into OSPAR's vision of a clean, healthy and biologically diverse North-East Atlantic.
g) Identification of substances not listed as WFD PS or RBSP that may entail a significant risk to the marine environment (as provided for under the MSFD).
h) Supporting future assessment of marine waters.

First results of target analysis and suspect screening will be available during 2021.

The **HELCOM EN-HZ NT screening project** is a wide-scope project across the Baltic Sea, which will take place during 2021 (sampling and data generation). The project is also well aligned with ongoing work the OSPAR CONnECT project. The project aims to establish a common approach and a regional survey through wide-scope and suspect screening. The selection of samples (spatial distribution and type) is determined by national decisions (e.g. the resources available and thus the total number of samples that can be analyzed). In this project individual Contracting Parties carry out the sample collection. The additional sample analysis, not funded by NEFCO, is paid for by each Contracting Party.

The screening approach will provide an overview of an extensive number of hazardous and potentially hazardous (e.g. substances of concern) substances. Currently wide-scope target substances (>2400 substances) can be determined in addition to suspect substances (>65000 substances) that can be screened for their semi-quantitative presence/absence in each sample. The number of substances is also steadily increasing. The list of substances covers a large number of priority and concern substances identified through policy initiatives and research studies. The assessment will also therefore address an extensive number of substances that are not covered by the existing selection of 11 HELCOM indicators on the topic of hazardous substances. This approach has the ability to support overall status assessments (e.g. HOLAS III) and support work in relation to other policies (e.g. the Marine Strategy Framework Directive). All samples are run by the same laboratory at the University of Athens to ensure identical methodology and conditions. This aspect is key to an effective quantitative (wide-scope target screening) and semi-quantitative (suspect screening). Priority selection and sampling matrix options are provided in Table 6.2. Where bivalves (i.e. blue mussels, zebra mussels or limecola –macoma-) are not possible to sample, it is proposed that selected fish species are the secondary target (flounder, eelpout or, if required, perch). The proposed selection is based on discussion within the EN-HZ sub group and the logic that maintaining a biota aspect as the secondary category (especially selecting less dispersive/ migratory fish species) as this maintains the samples within a single ecosystem component and also increases likely policy application and relevance (i.e. biota are a common target

of policy initiative for hazardous substances). Where such samples are not available or should Contracting Parties select additional samples on top of the primary core spatially distributed samples, then sediments are also considered the tertiary option.

Table 6.2. Sample priority selection.

Priority selection	Sampling matrix	Details
First choice*	Biota	Blue mussel (*Mytilus edulis*); Macoma/Limecola (*Limecola balthica*); Zebra mussel (*Dreissena polymorpha*)
Second choice	Biota	Flounder (*Platichthys flesus*), Eelpout (*Zoarces viviparus*), Perch (*Perca fluviatilis*)
Third choice	Sediments in marine environment	
Fourth choice	WWTP effluents (focus on large WWTPs and ones that discharge close or direct to the sea)	Whole water or passive samplers
	Water in marine environment	Whole water or passive samplers
Fifth choice	River water	Whole water or passive samplers
	River sediment(ation)	Sediment traps
Sixth choice	WWTP sludges	

***First choice category should be selected by all Contracting Parties if taking just a single sample. Multiple first choice samples can also be taken to give spatial coverage. Other selections (Second, Third or Fourth) should only be taken in addition to a First-choice selection (i.e. to support an understanding of a causal framework) for those Contracting Parties wishing to expand their assessment.**

6.4 Final remarks and recommendations

Considering the effort over the last year to bring together the 3 working groups involved with the common topic of CECs, this common ToR will continue to be addressed over the next three-year term. Additional recommendations include:

- WGBEC to review the revised list of CECs after prioritisation of substances is carried out by MCWG and WGMS.
- WGBEC to prioritise further collaboration with MCWG and WGMS on this common ToR for the next three-year term identifying gaps.
- WGBEC to review effect methods for identification of CECs, combining a mode of action approach with risk assessment.

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7 Effects of contaminants on marine communities

7.1 Background

The species composition of different communities is for all purposes the same as biodiversity. Different communities, from microbial, through protist and meiofauna to macrofauna and fish, describe the biotic part of ecosystems. Any change in any community is clearly ecologically relevant.

Criteria have been developed for methods to be recommended for contaminant monitoring (Hylland *et al.*, 2017): (i) the method should be able to separate contaminant-related effects from natural processes or the influence of other stressors, including knowledge of confounding factors; (ii) there should be some knowledge of dose-dependency; (iii) the mechanism of toxicity should be at least partly understood; (iv) quality assurance should be established; (v) assessment criteria must be established for responses. The most important criterion is clearly (i), since it is crucial that any method to be used for monitoring contaminants need to be specifically affected by contaminants and the contribution of confounding factors must be quantifiable. Under natural conditions, community structure is affected by many factors that are not contaminants, both abiotic and ecological. The aim of this section is to evaluate whether observed changes in the structure of marine communities can be clearly associated with exposure to one or more contaminants, isolated or in combination with other stressors.

The different communities in marine ecosystems have been investigated to varying extents for their sensitivity to contaminant stress and hence their suitability as targets for environmental monitoring of chemicals. Although there is by far most knowledge concerning sediment macrofauna communities, other investigated sediment or benthic communities are bacteria, protists/foraminifera and meiofauna. It is by nature more challenging to use pelagic communities for field assessment, but experimental studies indicate a potential. Marine fish communities inhabit large areas and are challenging to sample in a representative manner.

7.2 Benthic macrofaunal communities

Benthic macrofaunal community composition were originally used to investigate effects of eutrophication (see e.g. Gray and Pearson, 1982), but have more recently also been used routinely to monitor impacts in Norwegian offshore oil and gas production areas (Olsgard and Gray, 1995). Although there is a component of chemical stress in the latter inputs, the main factors causing community changes from drilling discharges are presumably oxygen deficiency and smothering/sedimentation. The meta-review by Johnston and Roberts (2009) indicated effects in a majority of studies by different groups of contaminants on (primarily) sediment communities. They did not evaluate the co-occurrence of other factors, however, except noting mixed exposures. Close to all studies in the review were field-based, with few controlled-exposure studies.

Filter-feeding mussels are among the most extensively studied organisms for contaminant effects, but there are few available studies on contaminant effects on rocky-shore communities. Concentrations of contaminants are clearly higher in sediments, but that does not mean that the rocky shore community will not be exposed, as indeed is indicated through accumulation in e.g. mussels. A reason for the few available studies on rocky shores is possibly sampling techniques employed for community analyses, which is predominantly based on visual methods (photography, video, visual registration).

Sediment meiofauna and benthic foraminifera have been used previously to evaluate contaminant impacts (Dijkstra *et al.*, 2017; Gyedu-Ababio and Baird, 2006; Laroche *et al.*, 2012). Advantages of using the diversity of smaller organism assemblages rather than macrofaunal diversity to evaluate sediment toxicity is the need for much smaller samples, ease of experimentation and an apparent lower spatial variability.

7.3 Microbial communities

Studies on microbial diversity have mainly focused on the short-term effects of crude oil spills, with the aim to assess bioremediation possibilities (usually involving the addition of nutrients) and the extent of hydrocarbon biodegradation (Head *et al.*, 2006). Fewer studies report chronic or long-term effects (Allison & Martiny, 2008; Paissé *et al.*, 2008; Alonso-Gutiérrez *et al.*, 2009). Unlike nutrient or hydrocarbon pollution, which seems to stimulate bacterial growth, heavy metals such as Cd, Cu, Zn and Pb have a negative effect on total prokaryotic cell numbers (Gillan *et al.*, 2005). More recently, metagenomic analysis have revealed that Zn, Pb, total volatile solids, and ammonia nitrogen were correlated with microbial diversity and function, and several microbes have been proposed as candidates to reflect pollution concentration (Chen *et al*., 2019).

Pollution-induced community tolerance (PICT), another interesting approach based on short-term toxicity tests, that was initially applied in freshwater periphyton (see e.g. Pesce *et al.*, 2010), has also been used to establish causal linkages between contaminants and effects on microbial communities (reviewed by Blanck, 2002). An increase in community tolerance compared to the baseline tolerance at reference sites suggests that the community has been adversely affected by toxicants.

7.4 Pelagic communities

Pelagic community structure has been shown to be affected by contaminants, e.g. in experimental studies (Vestheim *et al.*, 2010), but the implementation of pelagic approaches in monitoring remains a challenge, not least because of exposure uncertainties. In their review of contaminant effects on ecosystem functioning, however, Johnston *et al.* (2015) concluded that respiration and primary productivity were the two indicators that showed the clearest response for pelagic communities.

7.5 Final remarks and recommendations

As will be apparent, there are challenges in separating contaminant-specific impacts on communities to other environmental influences, including ecological interactions (predation, competition). There is a clear need for more controlled exposures.

With the current state of knowledge, marine communities do not fulfil the requirements to be recommended for contaminant monitoring: (i) it does not appear to be feasible to separate effects due to contaminant stress from effects due to other factors such as oxygen availability, organic loading or particle size distribution in sediment; (ii) dose-response relationships have not been adequately described between contaminant concentrations and community changes; (iii) there is limited understanding of mechanisms of toxicity, other than the disappearance or appearance of species or taxonomic groups, and (iv) the duration from exposure to observable effects is longer than sublethal responses for most communities.

Contaminant-specificity may however be addressed through one or more of the following:

- Use multivariate analyses to "filter" non-contaminant influence on communities; this is the "traditional" method to address the issue, but requires a sampling matrix that has gradients in all relevant factors, which is rarely found in nature.
- Combine community data with sublethal responses in selected species, preferably species or taxa that appear to be sensitive to the contaminant(s) in question; this approach can be combined with identification of indicator species (Ugland *et al*., 2008), some of which are robust and some sensitive; it is however an open question whether indicator species, e.g. the polychaete Chaetozone setosa, may be used across different contaminants and stressors.
- Perform mesocosm studies with intact communities, exposed to selected contaminants or combined stressors; this approach requires expertise, equipment (box-corer) and large-scale facilities; typically there will be few replicates. These kind of studies have been performed and include exposure to capping materials (Lillicrap *et al.*, 2015), mining particles (Trannum *et al*., 2019, 2020) and emamectin exposure (Stomperudhaugen *et al*., in prep). An additional parameter would be to add selected test species, e.g. bivalves or polychaetes, but the community composition would then not be very relevant.
- Use large-scale community effect of contaminants following major environmental contamination events, such as oil-spills or leakages, as exposure experiments (as e.g. in the Exxon Valdez infaunal community study by Fukuyama *et al*., 2014).

7.6 References

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8 Effects of contaminants on sediment-dwelling organisms: critical analysis of the sensitivity of the methodologies applied

8.1 Introduction

Due to inputs of contaminants from run-off from agricultural land or urban areas, riverine inputs, releases from leisure craft and commercial vessels or industrial and municipal point sources, sediments in coastal areas can be highly polluted. Dredging operations may further release contaminants locally as well as distribute sediment-bound contaminants to a larger area.

The highest concentrations of both legacy and emerging contaminants in the marine environment are found in sediments. Although initially a sink for particle-associated contaminants in the water column, contaminants may accumulate in food chains or be released to the overlying water through resuspension of particles. Sediment-bound contaminants may be released back to marine food chains through food webs, resuspended sediment may release contaminants to the water column and dredging operations may both release contaminants locally and distribute contaminants to a wider area.

There is a need for sensitive tests for the toxicity of contaminants in marine sediments. The currently available tests are not sufficiently sensitive, ecologically relevant or contaminant-specific. Issues that need to be considered to detect and quantify sediment toxicity are: (i) sampling and treatment of sediment, (ii) selection of test system and matrix, (iii) defaunation, (iv) selection of species, (v) choice of effect endpoints, and (v) whether to use single-species, multiple species or mesocosm studies. The above has been reviewed earlier, e.g. by Simpson *et al.* (2005).

8.2 Methodology

8.2.1 Sampling, treatment and storage of sediment prior to testing

The design for sampling programmes to represent a given area appropriately is crucial and needs to take the patchiness of contaminants in marine sediments into consideration. The depth of the sediment to be sampled need to be decided, depending on sedimentation rate, bioturbation and the duration of any inputs to be assessed. It may be relevant to characterise the sediment in situ, e.g. pH, redox profile, AVS. Care should be taken to avoid stressing the organisms as much as possible with regard to temperature and salinity changes during collection, e.g. through using temperature-controlled water from the depth organisms are sampled from.

Storage of spiked sediment prior to testing was previously evaluated (Cole *et al.*, 2000; Geffard *et al.*, 2004; Hartz *et al.*, 2018). Procedures for homogenising and setting up the experimental system are described in Ruus *et al.* (2006).

8.2.2 Test system and matrix

To test sediment toxicity, there is an option to use sediment extracts, elutriates, pore water or whole sediment. Extracts generally represent a worse-case scenario since bioavailability issues are eliminated. The toxicity of extracts can be evaluated using cell-free systems, cell cultures or

sensitive water toxicity assays (see e.g. Bakke *et al.*, 2010). Testing with extracts does lack ecological relevance. Sediments need to be at least partly defaunated prior to testing - this is generally done by sieving (which will only remove macrofauna). Sediment can be frozen prior to testing, but then all structure and aggregation of particles will be broken up. The sediment must be comprehensively homogenised prior to use (Ruus *et al.*, 2006).

8.2.3 Selection of organisms

When selecting organisms, tests should be performed with organisms that actually interact with sediment. In some contexts the term "sediment reworkers" have been used for desirable species although that may not be entirely appropriate as surface-feeding species may be equally relevant. Filter-feeding species are presumably not appropriate if the exposure system does not include a resuspension component. Intertidal species such as *Arenicola*, *Hediste* and *Corophium* have been widely used. Although useful for many purposes, intertidal species are not found in the most polluted sediments (fine-grained sediment in the deepest areas of coastal waters). Feeding mode should be considered when selecting species, whether a surface deposit feeder, subsurface deposit feeder, filter-feeder, scavenger, omnivore or predator. Some species are useful in the sense that they are amenable to collection and maintenance in the laboratory, e.g. bivalves and some echinoderms, as well as species that can be more or less hand-picked, such as intertidal organisms. Ecologically relevant species in the North Atlantic include sediment-dwelling bivalves such as *Abra* or *Ennucula* (Bour *et al.*, 2018), sea urchins such as *Brissopsis* or *Echinocardium* (Stomperudhaugen *et al.*, 2014), the polychaete *Hediste diversicolor* (Hylland *et al.*, 1996), gastropods such as netted dog whelk, *Hinia reticulata* (Ruus *et al.*, 2006) or brittle stars such as *Amphiura* (Hylland *et al.*, 1996). Whichever species is chosen, there is a need for good understanding of its physiology and autoecology.

The common ragworm *Hediste diversicolor* (Nereidae) is a keystone species of shallow marine and estuarine waters, is an important food resource for different fish species, has a relatively long-life spam (2-3 years) and is semelparous. There is a substantial scientific basis for the use of this species to assess sediment toxicity, by metals, organic compounds, pharmaceuticals, microplastics, nanoparticles contaminants both *in situ* (Ait Alla *et al.*, 2006; Berthet *et al.*, 2003; Bouraoui *et al.*, 2010, 2014; Durou *et al.* 2007; Fossi Tankoua *et al.*, 2012; Geffard *et al.*, 2005; Solé *et al.*, 2009; Mouneyrac *et al.*, 2003; Moreira *et al.*, 2006; Muhaya *et al.*, 1997; Nunes *et al.*, 2016; Poirier *et al.*, 2006; Sizmur *et al.*, 2013), and *ex situ* (Hylland *et al.*, 1996; Banni *et al.*, 2009; Buffet *et al.*, 2013; Cardoso *et al.*, 2009; Gomiero *et al.*, 2018; Maranho *et al.*, 2014; Pires *et al.*, 2016).

Recent studies have suggested using fish species to test sediment toxicity (Costa *et al.*, 2012; Di Domenico *et al.*, 2013). The advantages with fish as a model are more knowledge of physiological processes (compared to invertebrates), dietary exposure and relevance to society. Disadvantages are migration, lack of location specificity and limited direct sediment exposure.

8.2.4 Developing appropriate endpoints

Current protocols either have very coarse endpoints, i.e. mortality, or have used whole-organism approaches such as energy allocation (Bour *et al.*, 2018; Stomperudhaugen *et al.*, 2009). Growth and reproduction are sub-lethal endpoints utilised in aquatic toxicity test, which may be explored for sediment dwelling organisms. Furthermore, transcriptomes and metabolomes of selected species may now easily and inexpensively be characterised and could provide indicators for useful endpoints in future development of sediment toxicity tests.

Standard toxicity tests with sediment have used mortality as endpoint (e.g. *Corophium* tests). Behavioural endpoints include activity (*Corophium*), burial (bivalves, polychaetes, echinoderms),

feeding rate (*Arenicola*, *Abra*), bioturbation (*Brissopsis*, *Ennucula*). Physiological endpoints include cellular energy allocation (CEA) (Stomperudhaugen *et al.*, 2009), arm regeneration in brittle stars, growth, biomass change (Hylland *et al.*, 1996).

Responses of *H. diversicolor* were studied following 10-day exposure to mercury (Hg) (10 and 50 µg/L) benzo(a)pyrene (BaP) (0.1 and 0.5 mg/L). Bioaccumulation and biological responses included neurotoxicity, oxidative stress, genotoxicity and cytochemical biomarkers (Catalano *et al.*, 2012; Moltedo *et al.*, 2019). Mercury and BaP were accumulated by the polychaetes in a dose-response manner and caused biological effects with no significant mortality. Biological responses included oxidative stress (content of glutathione, enzymatic activities of catalase, glutathione S-transferases, glutathione reductase, glutathione peroxidases, total oxyradical scavenging capacity toward hydroxyl radicals), cellular damages at membrane level (lipofuscin and neutral lipid accumulations and levels of Ca2+-ATPase activity). However, only Hg caused neurotoxicity (decrease in acetylcholinesterase activity), and only BaP caused genotoxicity (comet assay, micronuclei frequency).

8.2.5 Single-species, multiple-species or mesocosm

As mentioned above, sediment will have to be treated in one way or another prior to testing with added organisms. An alternative approach is to test entire sediment communities in mesocosms. The sediment is then collected using a box corer, transferred to the laboratory and maintained under appropriate temperature and salinity conditions, generally using flow-through systems. Exposure can then be through sedimentation of contaminants or other stressors (e.g. mine effluents) on the sediment surface or exposure through water. The strong points of this approach are the opportunity to assess changes in the community and quantify biogeochemical processes, the weak point that it is not known which species are available for other types of analyses.

The only way to achieve a "true" representation of natural sediment is to take box cores and transport the intact core to an experimental facility with facilities to maintain flow-through seawater of appropriate temperature and salinity. Box cores require a substantial infrastructure and have the inherent limitation lack of representativity and low replication. Such mesocosm studies have been used previously to test added chemicals or stressors using biogeochemical processes and community change as endpoints, but may be extended to include organisms added to the system. Experience does however suggest that ecological interactions may strongly impact the results (the added organism may thrive or be consumed by other species present).

8.3 Case study with Hediste diversicolor

Hediste diversicolor has been used to test contaminated sediment from three areas: 1) Concordia wreckage (Tyrrhenian sea); 2) offshore oil and gas platforms (Adriatic Sea); and 3) lagoons (Grado and Marano and Boicerbus-IT), following the ASTM E1611-00(2007) methodological protocol, with some modifications.

Tests were carried out for 10 days using 10 chambers (800 mL) with 5 individuals each (for biomarker analyses) and 3 chambers (2 L) with 15 individual each (for bioaccumulation analyses) for each sampling site, with a sediment-water ratio of 1/5. A case study testing sediment from 2 offshore platforms confirmed that contaminants were bioaccumulated from the sediment by *H. diversicolor* with no lethal effects. Genotoxicity was the only sublethal effect observed, and coelomocytes produced the most sensitive response. Moreover, biomarker results were integrated with other biological and chemical parameters using a weight of evidence approach (WOE) to provide a sediment hazard assessment. Several lines of evidence were defined (LOE1: sediment chemistry; LOE2: bioconcentration; LOE3: biomarkers; LOE4: bioassays) for each sampling site

located at increasing distance from platform along a transect in the direction of the main current. Despite a "moderate", "major" or "severe" hazard measured by the chemistry, bioassays and bioconcentration LOEs, the biomarker LOE showed only "slight" sediment quality alteration in some stations, mainly related to genotoxicity test outputs (increase of DNA fragmentation and of micronucleus frequency).

The low toxicity observed, even with apparently high chemical stress, suggests some critical issues for future studies. Tests using *H. diversicolor* require longer periods of exposure (e.g. 28 d) and a different selection of biomarkers, possibly focusing on the coelomic fluid compartment. Moreover, the use of cultured populations of polychaetes may be preferable, since individuals from natural populations could interfere with responses to contaminants.

Further studies are needed to define the baseline levels of the biological responses in *H. diversicolor*, similarly to what was done by Barrick *et al.* (2016, 2018) in order to develop appropriate assessment criteria for this species.

8.4 Sediment quality assessment

Example from Italy

Ecotoxicology has a prominent role in sediment quality assessment within the Italian legislation (Italian Decree No.173 of July 15 2016). The decree provides the technical procedures to grant dumping permit of dredged sediments. Criteria and procedures for characterising marine and brackish sediments to be dredged are described in a technical annex, as well as their classification and description of management options and monitoring. The key principle is that sediments must undergo a physical, chemical and ecotoxicological characterisation, followed by an evaluation using a Weight of Evidence approach.

All sediment samples are required to go through an ecotoxicological evaluation which has to be carried out using at least three bioassays using three different species belonging to different taxa and chosen in order to test both liquid-phase and solid-phase/whole sediment matrices as well as different end points (mortality, larval development, fertility and growth, etc.). Chronic bioassays are mandatory. Species that can be used in the bioassays are *Vibrio fisheri* (Bacteria), *Dunaliella tertiolecta*, *Pheodactylum tricornutum*, *Skeletonema costatum* (Algae), *Amphibalanus amphitrite*, *Corophium* spp, *Acartia tonsa*, *Tigriopus fulvus* (Crustacea), *Crassostrea gigas*, *Mytilus galloprovincialis* (Mollusca) and *Paracentrotus lividus* (Echinoderma). The ecotoxicological classification is based on the Ecotoxicological Hazard Quotient provided by the weighted integration of the results of the bioassays. The HQ is calculated taking into account the statistical significance of results (compared to control conditions), the biological relevance of the measured effects, the sensitivity of organisms, the assay conditions in terms of tested matrix and duration of exposure. The ecotoxicological HQ is normalised to a 0-10 range corresponding respectively to the no effect and to the maximum effect observed by the battery and therefore to the minimum and the maximum ecotoxicological hazard class assessment (absent (<1), slight (≥1-1.5), moderate (≥1.5-3), high (≥ 3-6), severe (≥ 6-10)).

Similarly, the chemical classification is based on the development of a Chemical Hazard Quotient (HQC) which considers the number of chemicals exceeding action levels. Six classes of HQC are used: Absent (0-0.7), Negligible (0.7-1.3), Slight (1.3-2.6), Moderate (2.6-6.5), High (6.5-13), Severe (≥ 13).

Quality Assurance procedures are required for both ecotoxicological and chemical analyses.

Chemical Action Levels for trace metals and organic pollutants, as well as ecotoxicological results, are processed using weighted criteria, which allow abandoning the pass-to-fail or the worst result approaches, respectively.

Six classes of sediment quality, each corresponding to a management option, can be obtained: A (sands mostly for beach nourishment, coastal habitat restoration, or dumping at sea); B (mostly for dumping at sea with environmental monitoring); C (disposal in confined facilities or capping, with environmental monitoring); D (disposal in completely sealed confined facilities, with environmental monitoring); E (material subjected to special environmental safety procedures).

The Decree also allows the possibility of using a simplified procedure for sediment quality characterisation of recreational harbours, harbour entrances (< 40000 m^2) and costal or river areas for which chemical parameters needed to be analysed are defined following ecotoxicological assessment.

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Annex 2: Resolutions

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		
Year 2020	2–6 March	Lisbon, Portugal		Joint meeting with MCWG and WGMS
Year 2021	8–12 March	Online meeting	Final report by 1 May to SCICOM	

ToR descriptors

ToR	Description	Background	Science Plan codes	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).	4.4	year 2–3	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.	3.1; 3.2; 6.1	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	5.8; 6.1; 6.4	year 3	Scientific paper

		biodiversity. Several analytical and biological effect methods suggested by the ICES community can be used to establish links with human health.			
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.	3.1; 3.2; 6.1	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.	2.1; 2.2	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.	2.1; 2.2; 4.5	year 2–3	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.	2.1; 2.2; 6.1	year 2–3	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.	2.2	year 2–3	Scientific paper

i	Contribute to ICES Ecosystem overviewes according to the request	Ecosystem overviews have been advanced significanly during the past years and several ICES EGs have been very active to provide input. However, there is a room for further development through adding new components on issues where ICES has expertise, such as the biological effects of contaminants, and which are essentially relevant in marine ecosystem management and policy context.	6.5	3 years	Contribution to Ecosystem overviews according to the provided guidelines/template

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) Continue work on ToRs a, f, g, h

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