ICES VIEWPOINT BACKGROUND DOCUMENT: HOW CAN WE QUANTIFY AND MANAGE THE IMPACT OF CHEMICAL POLLUTION IN THE OCEANS? (AD HOC)

VOLUME 3 | ISSUE 102

ICES SCIENTIFIC REPORTS

RAPPORTS
SCIENTIFIQUES DU CIEM

ICES INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA
CIEM COUNSEIL INTERNATIONAL POUR L'EXPLORATION DE LA MER

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

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

ISSN number: 2618-1371

ICES Scientific Reports

Volume 3 | Issue 102

ICES VIEWPOINT BACKGROUND DOCUMENT: HOW CAN WE QUANTIFY AND MANAGE THE IMPACT OF CHEMICAL POLLUTION IN THE OCEANS? (AD HOC)

Recommended format for purpose of citation:

Hylland, K., Bellas, J., Giltrap, M., Brooks, S. 2021. ICES Viewpoint background document: How can we quantify and manage the impact of chemical pollution in the oceans? (Ad Hoc).
ICES Scientific Reports. 3:102. 29 pp. https://doi.org/10.17895/ices.pub.5446

Authors

Ketil Hylland • Juan Bellas • Michelle Giltrap • Steven Brooks

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

Contents

i Executive summary

Chemical pollution is ubiquitous in the world's oceans and affects marine organisms. Pollutants in marine ecosystems include trace metals and organic pollutants such as pesticides, industrial chemicals, pharmaceuticals and personal care products, household chemicals, chemicals released from transport and chemicals associated with solid waste or effluents. The substances of major concern are persistent, bioaccumulative and/or have high or specific toxicity to marine organisms. Although the extent to which contaminants accumulate in organisms is clearly important, not least from the perspective of human consumption, the relationship between the concentration of one or more contaminants in tissues of an organism and health impact is not straightforward. To quantify the impact of chemical pollutants on marine organisms there is therefore a need to quantify their toxicity in the field.

Chemical pollutants are known to cause effects on populations of marine organisms, e.g. endocrine disruption caused by the antifouling agent tributyltin in gastropods, reproductive failure in Baltic grey seal (*Halichoerus grypus*) and in European killer whale (*Orcinus orca*), the latter two associated with accumulation of polychlorinated biphenyls, PCBs. Important mechanisms of toxicity in marine organisms are carcinogenicity, genotoxicity, neurotoxicity, immunotoxicity, cardiotoxicity, endocrine disruption, reproductive toxicity and developmental toxicity. Methods to quantify carcinogenicity, genotoxicity, neurotoxicity, endocrine disruption, reproductive toxicity and developmental toxicity have to varying extents been included in monitoring programmes in European countries over the past decades. The methods in use have been chosen for their ability to distinguish between effects from contaminant exposure and the influence of other environmental processes. For most of them, there is an understanding of the mechanism of toxicity and the relationship between exposure and response is known. Over the past two decades, ICES working groups have developed a list of recommended methods, as well as criteria by which to assess data for the different methods and species. In parallel, activities have been established to ensure the quality and consistency of effect data from national and international monitoring programmes.

The range of organisms used in monitoring programmes does not represent the full marine biodiversity. Progress has however been made to allow intercomparison of effect responses between species through developing species-specific assessment guidelines.

Society needs to know the extent to which chemicals affect the oceans. New substances, for which there are no or limited effects data, are continuously being introduced, and a combination of methods will need to be used to identify and quantify health impacts caused by chemical pollutants.

The following are recommendations for national and international monitoring programmes.

1. Full adoption of the 'biological effects methods' monitoring approach within the integrated chemical-biological monitoring and assessment framework for accurate and realistic assessment of chemical pollution and its impacts, including the effects of chemical mixtures.
2. Inclusion of robust monitoring methods that quantify the most important toxicity mechanisms: carcinogenicity, genotoxicity, neurotoxicity, immunotoxicity, cardiotoxicity, and endocrine disruptiony.
3. Develop and implement quality assurance programs and intercalibration exercises to ensure comparability of data between laboratories, and to promote free accessibility of data.
4. Continue to bring in new evidence of biological effects of chemical pollution into national and international regulatory frameworks.

NOTE: The report takes into account the comments received from the reviewers (Annex 1) and has been updated accordingly.

1 Chemical pollution in the oceans

No part of the oceans is entirely free from chemical pollution caused by human activity. "Chemical pollution" in this context refers to substances that have a potential toxicity to marine organisms. In a marine context they are widely referred to as "environmentally contaminants". The term "contaminant" comprises a wide range of chemicals, both natural and man-made. The two terms will be used interchangeably in this viewpoint. Contaminants in marine ecosystems include trace metals and organic pollutants such as pesticides, industrial chemicals, pharmaceuticals and personal care products, household chemicals, chemicals released because of transport and chemicals associated with different kinds of waste. They can be entirely synthetic, such as polychlorinated biphenyls (PCBs), which would not be present at all in a pristine ocean, or naturally present, such as trace metals, for which concentrations in the environment may be elevated as a result of anthropogenic activity (Selin *et al.*, 2009). There are natural sources for some contaminants, such as polycyclic aromatic hydrocarbons (PAHs), from e.g. forest fires and underwater oil seeps, and mercury, from e.g. volcanic eruptions, but the majority of contaminants in the oceans hail from anthropogenic activity, and their presence in the oceans is the result of both continuous releases and accidental spills (Bayona and Albaigés, 2006; Merian *et al.*, 2004; NRC, 2003).

Legacy contaminants such as polychlorinated biphenyls, dioxins, brominated flame retardants and chlorinated pesticides all score high on persistence and hydrophobicity, which means they will remain in the marine environment for decades or centuries, and will be present at the highest concentrations in long-lived organisms high in food chains, such as seabirds and marine mammals. Commonly grouped under the term persistent organic pollutants (POPs), their toxicological profiles encompass a wide range of mechanisms, from neurotoxic and immunotoxic to carcinogenic and endocrine disrupting. A comparatively recent addition to this list, poly- and perfluorinated substances (PFAS), have very high persistence but are not very hydrophobic, accumulating at all trophic levels (Conder *et al.*, 2008). Due to their biphasic nature, being both water and lipid soluble, their predominant mechanisms of toxicity have been found to be associated with perturbation of lipid metabolism (Gorrochutegai *et al.*, 2014). Another group of widespread marine organic contaminants, polycyclic aromatic hydrocarbons (PAHs), found in oil, atmospheric deposition and combustion-related effluents, have the curious property that they accumulate and biomagnify in invertebrate food chains, but are metabolised in fish and other vertebrates (Wan *et al.*, 2007). In addition to PFAS, there has been an increasing focus on other groups of other contaminants of emerging concern (CECs), such as plasticizers (Mathieu-Denoncourt *et al.*, 2015) and less persistent pollutants with high biological activity, such as pharmaceuticals and personal care products (Arpin-Pont *et al.*, 2016).

All metals and metalloids are naturally present in trace amounts in seawater, so for them the definition of "contaminant" indicates concentrations higher than background. Many trace elements are essential to all living organisms, but some such as lead, gold, silver and mercury have no known biological function. Metals and metalloids differ from the organic contaminants in that they generally carry one or more charges, are hydrophilic and as a rule do not biomagnify in food webs. Mercury is a notable exception to the latter, as it is present in the biosphere in both organic and inorganic forms. The predominant organic form, methyl mercury, will bioaccumulate and biomagnifies in marine food webs (Chen *et al.*, 2008). In addition, organic forms of some metalloids, such as arsenic, are found in comparatively high concentrations in marine organisms, accumulate efficiently and some of them possibly have a role in the physiology of marine species (Amlund *et al.*, 2006; Hoffmann *et al.*, 2017). Even essential trace metals such as copper and zinc

may be present at concentrations in marine ecosystems sufficiently high for them to be contaminants (Rainbow, 2002).

Anthropogenic particles such as nanoparticles and nano-/microplastics are known to be present in marine ecosystems and may affect the health of marine organisms (Xu *et al.*, 2020). They are however not included in this viewpoint as the processes that govern the environmental behaviour of particles, as well as mechanisms for uptake and effects in organisms are entirely different from those of molecular pollutants, such as chemicals.

The toxicity of a contaminant is determined by the concentration that reaches its target within or on the outside of cells (Gregus, 2015). Internal exposure may therefore be equally important in causing toxicity as exposure directly from water or diet. Both internal and external exposure will however be modulated by many factors, including availability of the substance to tissues or cells. For internal exposure this could e.g. imply that the contaminant in question is associated with lipids, which would mean only a minor fraction would be available to tissues under normal metabolism (de Laender *et al.*, 2010), but that internal exposure would increase when lipids are used for energy requirements (Jørgensen *et al.*, 2006). For external exposure, the concentrations of e.g. organic material, ions and particles in water will modulate the bioavailability of contaminants (Luoma, 1983; Thorsen *et al.*, 2004). Although health impairment is the focus for effect measurements, it is still important to quantify contaminant concentrations to aid in the elucidation of mechanism of toxicity and for regulatory purposes. For most contaminants, it is most relevant to measure such concentrations in biota, as that will provide information about the bioavailability of the contaminant or contaminants in question as well as its environmental concentration. There are instances where this may however not be the case: water-soluble, degradable and toxicologically active contaminants such as pharmaceuticals, personal care products, many endocrine disruptors and neurotoxicants may have dramatic effects even with low or undetectable concentrations in tissues because they are metabolised and/or excreted efficiently after causing disruption in tissues.

The chemical characteristics of different contaminants or contaminant groups is a critical factor in determining the extent to which marine organisms are exposed. Following uptake, the inherent ability of different marine species to metabolise the contaminant in question will, directly or indirectly, modulate internal exposure, for better or worse; some contaminants such as PAHs exert their toxicity primarily following biotransformation, but the same processes also render the molecules non-toxic (Hylland, 2006). Lipid soluble and persistent contaminants such as PCBs, dioxins and chlorinated pesticides accumulate over a life-time, can biomagnify and reach health-impacting concentrations in species high in food chains, such as seals, otters, whales and humans (AMAP, 2004a,b).

Both health officials and the general public are concerned about contaminants in seafood, and most coastal nations have programmes in place to monitor concentrations of toxic chemicals in commercially relevant species. As briefly touched on above, a pertinent question is why we cannot just estimate the potential toxicity of contaminants to marine organisms from their concentrations in tissues? Although accumulation data have been used previously to infer effects in marine organisms, there are reasons why this approach is not generally adequate. A range of studies and assessments show that there does not necessarily have to be a direct relationship between the concentration of one or more contaminants in the tissues of an organism and health impact (Depledge and Galloway, 2005). There are four reasons why this is the case: (i) bioaccumulated contaminants are generally stored in particular compartments or forms, e.g. in lipids or granules, with limited immediate availability for the biochemical "machinery" of living organisms; (ii) if effects are present they are likely caused by a mixture of contaminants, some of which will probably not have been analysed for; (iii) some contaminants cause effects without accumulating, such as many endocrine disruptors; and finally, (iv) effects arise in the intersection

between contaminant stress and the general health status of the organism. The conclusion is that effects of contaminants in marine ecosystems need to be assessed directly, not through a chemical proxy. Over the past decades, there has been a gradual increase in the use of effect-directed monitoring to assess pollution impacts in marine ecosystems.

This aim of this viewpoint is to review population-relevant mechanisms by which chemical pollutants affect marine organisms and provide recommendations as to how effects of pollutants can be monitored and managed.

2 How may chemical pollution affect the health of marine organisms?

The oceans can be conceived to be so vast that any chemical pollutant would be diluted so much that they would not be toxic to marine organisms. There is nevertheless concern for chemical pollution since: (i) some pollutants are so persistent that they will remain in the marine environment for decades or even centuries (Nizzetto *et al.*, 2010), (ii) lipophilic pollutants will accumulate efficiently in organisms and food chains, resulting in high concentrations in biota even in pristine areas (AMAP, 2014a), (iii) some chemical pollutants, e.g. endocrine disruptors and pharmaceuticals, cause effects at very low concentrations (Tijani *et al.*, 2016), (iv) some compartments in the sea, particularly sediment, are efficient storage sites for subsequent releases of e.g. oil-derived contaminants, such as in the case of the Exxon Valdez spill (Peterson *et al.*, 2003; Esler *et al.*, 2017), and finally, (v) organisms in coastal areas or in the vicinity of point sources may be more or less continuously exposed to a mixture of substances, the individual concentrations of which may be low, but combined exposure can cause effects (Cheng *et al.*, 2020).

There are well-documented cases for the impact of chemical pollutants on marine species. The mechanism of toxicity in each case has been supported by results from experimental studies. One such case is the endocrine disruption caused by the antifouling agent tributyltin in gastropods (Bryan *et al.*, 1988; Gibbs *et al.*, 1986; Oehlmann *et al.*, 1991). Although not evaluated as particularly toxic in short-term acute toxicity tests, tributyltin and its metabolites turned out to have serious ecological consequences through its inhibition of the enzyme that converts testosterone to oestradiol, resulting in an accumulation of the former and a masculinisation of female gastropods (Stange *et al.*, 2012). Many marine mammals are long-lived and at a high trophic level in marine food chains, which lead to them accumulating high concentrations of persistent organic pollutants (POPs), such as PCBs, dioxins, chlorinated pesticides and brominated flame retardants. Population decreases in grey seal (*Halichoerus grypus*) in the Baltic three decades ago was partly caused by reproductive failures associated with accumulation of POPs (Olsson *et al.*, 1994). Causal links were found between PCB accumulation and morphological changes in grey seal uterus. Supporting evidence was provided through an experimental study with mink (Mustela vison), showing the potential for the potential reproductive toxicity of PCBs (Kihlström *et al.*, 1992). In both cases, morphological changes were observed that could explain individual reproductive perturbation and population decreases. More recently, there are reports suggesting accumulation of organic contaminants in marine mammals cause health impairment: in California sea lions (*Zalophus californianus*) there was a correlation between blubber POPs and urogenital cancers, with a possible interaction with herpes virus (Gulland *et al.*, 2020), and reproductive failure in European killer whale (*Orcinus orca*) populations have been linked with blubber accumulation of organic contaminants, particularly PCBs (Desforges *et al.*, 2018; Jepson *et al.*, 2016).

Marine monitoring programmes for contaminants have primarily focused on mussels and fish species, but with some other organism groups included for specific mechanisms of toxicity, such as gastropods for endocrine disruption caused by organotins, selected seabirds species for exposure to plastics and marine mammals for effects of persistent organic pollutants. It is clearly impossible to monitor all marine species groups for contaminant effects, but we need to keep in mind that even closely related species can differ in their sensitivity to contaminant exposure (see e.g. Ploch *et al.*, 1998). In addition to maintaining data series for the species currently used in monitoring programmes, we must therefore also consider species groups not currently included, taking into account both ecological roles and differences in metabolism, physiology and life history traits. Marine food webs are more complex than freshwater food webs and have a higher

number of trophic levels than both freshwater and terrestrial food webs. Marine food webs also have more omnivory at higher trophic levels (Thompson *et al.,* 2007), making it even more challenging to extrapolate between species. The realisation that we are not able to cover all taxa in marine ecosystems has contributed to an increased focus on mechanisms of toxicity, in which we acknowledge that we may not be monitoring the most sensitive species, but where the aim is to quantify the presence of chemical pollutants that singly or in combination cause potentially deleterious effects. When we e.g. monitor for estrogenicity using plasma concentrations of the egg yolk protein vitellogenin in male or juvenile fish, this is not primarily of concern for the health of that individual, but for the potential disruption the oestrogens may cause in more sensitive species or life stages, such as early life stages of fish (which can be very sensitive, see e.g. Andersen *et al.,* 2003).

Our knowledge of the toxicity of any contaminant or mixture of contaminants relies on controlled laboratory experiments. Laboratory studies have shown that the concentrations we find in coastal waters of a range of chemicals are sufficient to affect the health and fitness of marine organisms. The entire concept of effect monitoring is based on sublethal effects, the result of natural exposure to a polluted environment. Direct assessment of effects is the "gold standard" in the sense that the health of organisms in their natural habitat is evaluated. An alternative is to model the potential environmental effects using laboratory data on toxicity, i.e. environmental risk assessment. The risk assessment approach is based on population-relevant endpoints such as mortality or growth, and assume that high concentration exposure in short-term tests will be equivalent to longer term exposure at environmentally relevant concentrations. Also, confounding factors can only be taken into account in risk assessment through modelling.

As referred to above, there is a particular focus on sublethal responses that may have health consequences that can lead to population-relevant effects, which are mortality, reduced growth, reduced reproduction, disease, delayed maturation and changed behaviour. There has been a focus on identifying effects that have links to population-relevant impacts, while at the same time being specific to contaminant exposure. Taking into account the criteria described in Hylland *et al.* (2017a), biological effects methods have been evaluated and appropriate methods identified and recommended for use in marine monitoring. Most of the methods that have been used were originally selected for their specificity to contaminant exposure and response, although some methods directly address population-relevant processes such as reproduction and embryonal development in fish (Vetemaa *et al.*, 1997) and energy budget in mussels (Widdows and Johnson, 1998). The latter group of methods are however to a larger extent affected by non-contaminant factors, such as food availability and ambient oxygen levels. Many of the methods used to quantify sublethal toxicityin marine sciences over the past decades have their origins in medical sciences, and it is a strength that they can use knowledge from the extensive databases of mammalian studies. Similarities and differences between mammalian and fish models have been highlighted in studies comparing transcriptomes and identifying similar cellular pathways (Driessen *et al.,* 2015; Hoeng *et al.,* 2014).

There has been a shift over the past decades towards quantifying effects of contaminants in individuals rather than attempting to quantify population effects. One important reason for this shift has been the superior contaminant-specificity individual assessment can offer compared to population assessments, as well as its direct link to biomedical sciences and human toxicology. The extent to which different methods to quantify sublethal effects can be linked to possible population effects has been and is an important criterion for their selection for monitoring programmes (Lagadic *et al.,* 1994; Lam and Gray, 2003).

Mechanisms of toxicity with a clear potential for population impacts are carcinogenicity, genotoxicity, neurotoxicity, immunotoxicity, cardiotoxicity, endocrine disruption, reproductive toxicity and embryotoxicity. In transcriptomics studies, pathways linked to some of the above are

nearly always identified following exposure to different contaminants, contaminant mixtures or in polluted areas (Ewald *et al.,* 2020; Nacci *et al.,* 2009). In addition, lipid metabolism pathways have been found to be modulated in many studies following exposure to contaminants (Dreier *et al.,* 2020). Transcriptomic analyses of the tissues of marine organisms exposed to contaminants also commonly identify homeostatic processes such as cell signalling and cell cycle regulation as being affected, both of which are less readily incorporated into effect monitoring. Any perturbation that affects growth, as the above effects could, does have the potential to cause population effects, since it will affect ecological position, maturation age and/or reproductive output.

Marine organisms are exposed to chemical mixtures in nature, never single substances. Some of the chemicals in such cocktails will act in concert and some will counteract the effects caused by other chemicals. One of the strengths of the approach described in this viewpoint is exactly its ability to describe the integrated response of an organism following its exposure to a mixture of chemicals. Although many of the mechanisms of toxicity discussed here are primarily triggered by specific molecules, they are by no means substance-specific. Through having knowledge of the mechanisms of toxicity above in a model species, a manager would be able to perform a well-advised assessment.

Carcinogenesis is arguably the most extensively studied mechanism of health impairment in human toxicology (Loeb and Harris, 2008). In marine ecosystems, causality from sediment exposure, through PAH metabolite accumulation in bile, to induction of biotransformation enzymes, DNA adduct accumulation and liver tumour development, was documented in a series of studies of flatfish species in Puget Sound (Johnson *et al*., 1998; Malins *et al*., 1987; Myers *et al*., 1990, 1992, 1998; Stein *et al*., 1993). Induced cytochrome P4501A and increased concentrations of DNA adducts or levels of DNA strand breaks are therefore considered as early indicators of possible carcinogenesis, in addition to causing other physiological perturbations.

Damage to DNA may lead to carcinogenesis, cell death and mutagenesis. DNA damage has been viewed as a serious impact in an organism due to its range of possible implications, both for the health of the individual and for possible effects on its offspring. DNA adduct accumulation has been documented following exposure to e.g. dredged sediment (Sundberg *et al.,* 2007) and oil-related pollution, both in the Puget Sound (Krahn *et al.,* 1986; Myers *et al.,* 1990) and in the North Sea (Balk *et al.,* 2011). Europe-wide field sampling of fish as part of an international initiative also showed increased DNA damage, both as adducts and DNA strand breaks, in polluted estuaries and in an area of offshore oil activities (Hylland *et al.,* 2017c). Fish populations from several demersal species that live in and around offshore oil and gas installations in the North Sea have frequently reported elevated levels of DNA adducts above those levels found in the same species from reference areas (Brooks *et al.,* 2013; 2014; Pampanin *et al.,* 2019).

Neurotoxicity can lead to a range of health-related impacts, including changed behaviour. Neurotoxic agents are widely used in aquaculture, particularly organophosphates and pyrethroids. Sewage and agricultural run-off are other sources. It was earlier considered that neurotoxicants such as organophosphates would degrade so rapidly in marine ecosystems that their environmental effects would be minor, but more recent studies have shown that they have sufficient persistence in seawater to affect natural populations (Sidhu *et al.,* 2019). Neurotoxicity has been studied in both marine fish (Kirby *et al.,* 2000) and invertebrates (Deidda *et al.,* 2021), using cholinesterase inhibition as a biomarker. AChE inhibition has been identified as a dominant mechanism of aquatic neurotoxicity (Busch *et al.,* 2019). In addition to direct toxicity through causing reduced swimming activity, disorientation or other behavioural changes, there is limited understanding of the extent to which neurotoxic agents modulate other types of behaviour in wildlife, although this has been well documented for humans. A particularly serious mechanism which is known from human health studies, but not investigated for marine organisms, is developmental neurotoxicity (cf. Grandjean and Landrigan, 2014). The relevance of neurotoxicity as a

predictor for population impacts, including delayed neurotoxicity, is comprehensively discussed in Legradi *et al.* (2018). AMAP reviews have shown that mercury concentrations in top predators such as polar bears and man are sufficiently high to have a potential to cause neurotoxicity (AMAP, 2004b) and human toxicological studies has shown that prenatal mercury exposure can lead to learning disability in children (Debes *et al.,* 2016).

Increased susceptibility to disease can directly affect survival in marine organisms, and there is therefore clearly important to identify mechanisms that are involved. The immune systems of both vertebrates and invertebrates are complex and comprise to different extents specific and non-specific components. There has been a focus on non-specific responses in environmental toxicology although increased disease susceptibility, which will include both, is the main concern (Martinez-Gomez and Vethaak, 2019; Rehberger *et al.,* 2017). Yang *et al*. (2020) recently reviewed immune responses in fish as a result of exposure to pesticides. Their work shows the complexity of immune system responses, in that some components may be inhibited whereas other responses are induced. Other studies showing effects on immune parameters in fish include Gao *et al.* (2020), Mauri *et al.* (2011), Perez-Casanova *et al.* (2010) and Sueiro *et al.* (2020). Relevant methods were reviewed in Segner *et al*. (2012), but there is still a lack of robust methods by which to assess immune effects in marine fish populations.

Following the Exxon Valdez and Deepwater Horizon spills, cardiotoxicity has been observed in different species of oil-exposed fish, particularly evident for developmental stages (Incardona *et al.,* 2005). The results show that exposure to crude oil or PAHs may affect the morphology of the heart, reducing its output and hence reducing aerobic capacity, even in adult fish (Hicken *et al.,* 2011). The mechanism appears to be effects on calcium cycling, which disrupts excitation-contraction coupling in cardiomyocytes (Brette *et al.,* 2014). This is a subject of some concern, as PAHs are ubiquitous in marine ecosystems, not only in areas exposed to oil spills. In the above studies, cardiotoxicity was observed at concentrations that are also found elsewhere in marine ecosystems, particularly in coastal waters and near oil production and refining facilities. A range of other chemical pollutants have recently been shown to have the potential to cause cardiotoxicity (Meador, 2021).

Endocrine disruption is a very wide concept that includes a range of mechanisms of toxicity and an even larger range of substances. The main focus in environmental science has been on reproduction-related steroids: oestrogens (Sumpter and Jobling, 1995), androgens (Ankley *et al.*, 2020) and thyroid hormones (Knapen *et al.,* 2020). There is convincing evidence that different freshwater fish populations throughout Europe are feminised, but less data is available for marine populations. Scott *et al.* (2006) showed 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 of oestrogens on flounder along UK coasts (Allen *et al.,* 1999). As discussed above, the main concern is elevated concentrations of oestrogens in the water, not necessarily increased vitellogenin in male fish plasma. Further motivation for monitoring effects of sex steroids come from observations that exposure to environmentally relevant concentrations of alkylphenols, widespread chemicals also found in oil-related effluents, affect the timing of gonad maturation (Holth *et al.*, 2010; Meier *et al.*, 2011). Thyroid hormones have different functions, depending on life stage and species (Eales, 2019) and there is limited understanding of how contaminants affect this vital endocrine regulatory pathway in different fish species.

A reduction in reproductive output is critical for any population. As described above, POP accumulation has been implied in observed reproductive dysfunction in marine mammals, one of the most recent examples being killer whales in Europe (Jepson *et al.,* 2016). Reproductive output is presumably not the best marker for contaminant effects, simply because it will be affected by so many other factors. Nevertheless, a model has been developed in northern Europe using the live-bearing eelpout (*Zoarces viviparus*), for which it is possible to perform specific analyses on

all larvae from one female (Korsgaard *et al.,* 1985). In one study using this model species, exposure to pulp and paper mill effluents were found to lead to changes in the sex ratio of larvae developing within exposed females (Larsson and Förlin, 2002).

Embryos and larvae are generally thought to be sensitive life stages and may be exposed to contaminants both through the yolk and surrounding water, and eventually their diet. A monitoring programme for fish embryos was implemented by Germany for the southern North Sea in the 1980s. Correlations were found between an increased frequency of embryonal aberrations in different marine fish species and contaminants in the southern North Sea (Dethlefsen *et al.,* 1996; von Westernhagen *et al.,*1988). In addition, recent studies have shown that embryos of some species such as haddock (*Melanogrammus aeglefinus*) are particularly sensitive to oil pollution since they have "stickier" eggs than closely related species (Sørhus *et al.,* 2015). Early life stages of both marine fish and invertebrates are used in toxicity tests for chemical risk assessment and there is a plethora of studies showing effects from a wide range of contaminants.

There are practical and logistical reasons why there is limited data on how and whether sublethal contaminant-related responses in marine organisms eventually lead to changes in populations, simply because dead or diseased fish will generally not be sampled. Carcinogenicity has been included in this viewpoint because of the importance of this mechanism in human toxicology and evidence of tumour formation. Diseases in marine organisms clearly have consequences for populations and their susceptibility disease will be affected by immune suppression, although direct links between contaminant-related immune suppression and disease are not clear (but see Rehberger *et al.,* 2017). Recent exposure studies with marine fish species have highlighted the importance of lipid metabolism, supporting earlier studies showing changes in lipid composition following exposure to contaminants (cf. Dreier *et al.,* 2021#). Reproductive and developmental toxicity measure population-relevant endpoints directly. Links between the other mechanisms of toxicity and population-relevant endpoints are shown below (Table 1).

Table 1. Cases to illustrate the relationship between mechanisms of toxicity and population-relevant endpoints.

Mechanism of toxicity	Organism(s)	Pollutant(s)	Population-relevant endpoint	Literature reference
carcinogenicity	English sole (*Pleuronectes vetulus*) sea lion (*Zalophus californianus*)	environmental: PAHs environmental: POPs	tumour formation	Myers *et al.*, 2003 Gulland *et al.,* 2020
genotoxicity	nematode, polychaete sea urchin larvae brine shrimp	radiation, phenols oil ethylmethane sulphonate (EMS)	reduced reproductive output development growth, survival, reproduction	Anderson and Wild, 1994 Anderson *et al.,* 1994 Sukumaran and Grant, 2013
neurotoxicity	protists, invertebrates, fish	pesticides	developmental; ecologically relevant change in behaviour	Legradi *et al.* (2018)
immunotoxicity	fish species	EDCs, pesticides, metals	immune suppression	Rehberger *et al.,* 2017
cardiotoxicity	Pacific herring (*Clupea pallasi*), pink salmon (*Oncorynchus gorbuscha*)	crude oil	perturbed development, reduced cardiac output, reduced swimming ability	Incardona *et al.,* 2015

Mechanism of toxicity	Organism(s)	Pollutant(s)	Population-relevant endpoint	Literature reference
endocrine disruption	zebrafish (*Danio rerio*) roach (*Rutilus rutilus*) gastropod (*Ocinebrina aciculata*)	oestrogens, androgens environmental: urban antifouling agent TBT	changed sex ratio reduced reproductive output sterilisation of females, reduced popuation	Holbech *et al.*, 2006 Jobling *et al.*, 2002 Oehlmann *et al.*, 1996

From the above, it will be clear that contaminants can cause health effects in marine species at different trophic levels and that some mechanisms of toxicity are more relevant than others if effects are to be interpreted at a population level. Such implications can only be performed through modelling, but there are clear limitations as to what the most widely used models can use as input. Mechanisms of toxicity that can be used in population models more or less directly are developmental toxicity (mortality), reproductive toxicity (reproductive output), cardiotoxicity (growth, mortality), neurotoxicity (mortality) and endocrine disruption (maturation, reproductive behaviour and reproductive output). Carcinogenicity and immunotoxicity will need another layer of physiological modelling before responses can be extrapolated to reduced growth, increased mortality or reduced reproductive output.

3 How may existing knowledge be used to develop new monitoring methods?

Over the past three decades there has been vital communication between science and management on what is needed for biological effects methods for them to be useful in contaminant monitoring. The result was a list of biological effects methods that can be recommended for monitoring (ICES SGIMC, 2011) and a framework for using the methods in a holistic manner to provide an overall assessment for contaminant impacts (Vethaak *et al.*, 2017). The existing list of recommended biological effects methods for fish include biomarkers for genotoxicity, carcinogenesis (including histology), neurotoxicity, metal toxicity, endocrine disruption (estrogenicity), reproductive toxicity and membrane disruption. The methods for invertebrates lack markers for carcinogenesis and differ for mechanisms of toxicity, but otherwise target the same processes as for fish. Scientific progress over the last decades has contributed to identifying novel mechanisms of toxicity, such as cardiotoxicity, that require the development of methods suitable for monitoring programmes. In addition, there is a lack of robust markers for immunotoxicity.

Method requirements should form a basis for selection of effect methods in the future. In addition to the requirements outlined in Hylland *et al.* (2017a), there is a need for mechanisms for technology transfer and training, as well as a field-derived dataset for each method and relevant species involving institutions from more than one country.

Once a relevant method has been identified, there is still a need to ensure that all laboratories performing the analyses will get the same result for the same sample. This requires quality assurance protocols and intercalibration exercises. For biological effects analyses, both were initiated under the umbrella of the Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM). The programme was designed to provide quality assurance (QA) for all recommended biological effects techniques and is required to ensure laboratories generate high-quality and comparable data in national monitoring programmes.

The next step is to develop assessment criteria, both to allow integration of results for different markers and comparison between different species for each marker (see Hylland *et al.*, 2017b, Robinson *et al.*, 2017). Assessment criteria have been determined for the recommended methods using available data for different species (ICES SGIMC, 2011). This process has been developed over more than two decades, and the mechanisms and know-how are now available in European institutions to be applied to new methods.

There is currently a rapid development of methods for contaminant effect responses that provide large amounts of data for each individual, primarily the transcriptome, proteome and metabolome. In addition, there is an increasing understanding that exposure to contaminants can affect epigenetic regulation of genes, with a potential for transfer to offspring. As referred to in the previous section, data from transcriptome analyses has shown that specific pathways are particularly affected by exposure to different classes of contaminants. While it is important to remember that transcriptomes are transient, with a half-life of hours or at most a few days, and although its relevance in field monitoring is under discussion, such data can clearly be useful in providing information about mechanisms of toxicity as well as pinpointing candidates for new effect monitoring methods (cf. Ankley *et al.*, 2010). Transcriptome and proteome analyses have in many cases pinpointed existing effect methods, supporting existing strategies, but in addition other pathways for which there is limited data and no directly applicable biomarker. As discussed above, such methods need to be specific to exposure to contaminants, and the process of developing new methods should target candidates that have links to population impacts as well as fulfilling the criteria described in Hylland *et al.* (2017a).

The last couple of decades have seen the development of frameworks to develop formalised pathways for different mechanisms of toxicity leading to serious health impairment, termed "adverse outcome pathways" (AOPs; Ankley and Edwards, 2018). The concept of AOP is to include quantifiable events in a sequence, potentially leading to an adverse effect. The data currently available in marine monitoring programmes will not cover a sufficient number of nodes of an AOP for a complete analysis, but the concept clearly has merit for future consideration in marine monitoring, particularly if 'omics data are implemented. A comprehensive overview of both AOPs that have been reviewed and those that are under development can be found at http://www.aopwiki.org.

4 What actions can be recommended, based on the evidence of biological effects, to regulate/manage chemical pollution in the marine environment?

Society needs to know the extent to which chemicals affect the oceans and marine organisms. Studies on effects have shown that legacy contaminants such as PCBs can have dramatic consequences for the reproduction of species high in food chains, such as seals and long-lived toothed whales. The experience with tributyltin, in which effects of the chemical were discovered in field studies, rather than following regulatory testing, showed that such testing does not necessarily provide all the answers that are needed to protect the marine environment. As mentioned above, effect-based monitoring has also been successful in demonstrating that low-level contamination by oil-related PAHs may affect fish populations, e.g. in Puget Sound (Johnson *et al.,* 1998), following the Exxon Valdez spill (Incardona *et al.,* 2013; Marty *et al.,* 2003) as well as in high production areas in the North Sea (Balk *et al.*, 2011).

Monitoring methods must be kept updated to reflect scientific progress, including our understanding of how sublethal effects relate to individual health and populations. There should be a particular focus on how to implement and assess input from data-intensive analytical strategies, such as metabolomics, proteomics and transcriptomics.

As mentioned above, there is a requirement that biological effect methods must be able to separate between exposure to contaminants and other environmental processes. The value of all methods can be improved for environmental management purposes with in-depth knowledge of confounding factors and the baseline response in an unpolluted environment. High-quality data for baseline responses and expected increases under contaminant stress will make it possible to compare responses between different species (cf. Hylland *et al.*, 2017b).

Quality assurance programmes must be implemented and kept active to ensure comparability of results from different laboratories, geographical regions and from surveys performed at different times. Time-series should be established to identify and quantify environmental changes in marine ecosystems.

Contaminant inputs is one of many stressors in marine ecosystems. The strategy recommended in this viewpoint will make it possible to identify the contribution by contaminants in marine ecosystems, but will also increase our understanding of mixed stressor responses in general.

The all-important question is whether current regulatory frameworks for chemicals provides sufficient protection for marine ecosystems and whether in situ monitoring for effects in the sea can provide the additional required knowledge, including species differences, interactions with other environmental factors, effects of mixture toxicity and other stressors. The framework developed under ICES SGIMC (ICES SGIMC, 2011; Vethaak *et al.*, 2017) showed clear promise in being able to assess and compare contaminant effects in coastal and offshore areas of Europe in the large-scale research programme ICON, from the Mediterranean in the south to Iceland in the north (Hylland *et al.*, 2017a, b). There is a need to include new methods as well as refining assessment values and links between responses, but the developed framework was robust and could easily be expanded.

The following is recommended for national and international monitoring programmes.

1. Full adoption of the 'biological effects methods' monitoring approach within the integrated chemical-biological monitoring and assessment framework for accurate and realistic assessment of chemical pollution and its impacts, including the effects of chemical mixtures.
2. Inclusion of robust monitoring methods that quantify the most important toxicity mechanisms: carcinogenicity, genotoxicity, neurotoxicity, immunotoxicity, cardiotoxicity, and endocrine disruption.
3. Develop and implement quality assurance programs and intercalibration exercises to ensure comparability of data between laboratories, and to promote free accessibility of data.
4. Continue to bring in new evidence of biological effects of chemical pollution into national and international regulatory frameworks.

5 Conclusions

Effects of contaminants on marine organisms can only be accurately assessed through sampling and analysis of organisms from natural populations. We will not be able to detect impacts on all species in the sea and it is important to identify and use sensitive species in monitoring and to investigate selected mechanisms of toxicity. New substances, for which there are no or limited effects data, are continuously being introduced into the oceans and their toxicity in marine ecosystems must be assessed. A combination of biological effects methods should be used to provide a holistic assessment. There is a need for continuous development of methods to understand effects of contaminants in marine ecosystems. Previous experience will be critical in identifying and implementing appropriate methods, as well as including them in a holistic framework.

Acknowledgements

The authors would like to acknowledge members of the ICES working group for biological effects of contaminants (WGBEC) for fruitful discussions over the past 20 years. In addition, we are indebted to ICES ADG chair Henn Ojaveer and members Kiyomi Ferguson, Kari Lehtonen, Jasmine Nahrgang, and Paula Sanchez for their assistance in focusing this viewpoint.

References

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

AMAP, 2004a. AMAP Assessment 2002: Persistent Organic Pollutants in the Arctic. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. 310 p.

AMAP, 2004b. Persistent Toxic Substances, Food Security and Indigenous Peoples of the Russian North. Final Report. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. 192 p.

Amlund, H., Ingebrigtsen, K., Hylland, K., Ruus, A., Eriksen, D.Ø., Berntssen, M.H.G. 2006. Disposition of arsenobetaine in two marine fish species following administration of a single oral dose of [14C] arsenobetaine. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 143, 171–178.

Andersen, L., Holbech, H. 2003. Effects of exposure to 17a-ethinylestradiol during early development on sexual differentiation and induction of vitellogenin in zebrafish (Danio rerio). Comparative Biochemistry And Physiology 134C, 365–374.

Anderson, S.L., Hose, J.E., Knezovich, J.P. 1994. Genotoxic and developmental effects in sea urchins are sensitive indicators of effects of genotoxic chemicals. Environmental Toxicology and Chemistry, 13, 1033-1041.

Anderson, S. L., Wild, G. C., 1994. Linking genotoxic responses and reproductive success in ecotoxicology. Environmental Health Perspectives, 102 (suppl 12), 9-12.

Ankley, G. T., Bennett, R. S., Erickson, R. J., Hoff, D. J., Hornung, M. W., Johnson, R. D., Mount, *et al*. 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environmental Toxicology and Chemistry, 29, 730-741.

Ankley, G.T., Blackwell, B.R., Cavallin, J.E., Doering, J.A., Feifarek, D.J., Jensen, K.M., Kahl, M.D., *et al*. 2020. Adverse Outcome Pathway Network–Based Assessment of the Interactive Effects of an Androgen Receptor Agonist and an Aromatase Inhibitor on Fish Endocrine Function. Environmental Toxicology and Chemistry 39, 913–922.

Ankley, G.T., Edwards, S.W., 2018. The adverse outcome pathway: a multifaceted framework supporting 21st century toxicology. Current Opinion in Toxicology 9, 1–7.

Aristizabal-Henao, J., Ahmadireskety, A., Griffin, E., Ferreira Da Silva, B., Bowden, J. 2020. Lipidomics and environmental toxicology: Recent trends. Current Opinion in Environmental Science & Health, 15, 26–31.

Arpin-Pont, L., Bueno, M.J.M., Gomez, E., Fenet, H., 2016. Occurrence of PPCPs in the marine environment: a review. Environmental Science & Pollution Research 23, 4978–4991.

Balk, L., Hylland, K., Hansson, T., Berntssen, M.H., Beyer, J., Jonsson, G., Melbye, A., *et al*. 2011. Biomarkers in natural fish populations indicate adverse biological effects of offshore oil production. PLoS One 6, e19735.

Bayona, J. M., Albaigés, J. 2006. Sources and fate of organic contaminants in the marine environment. In Marine organic matter: biomarkers, isotopes and DNA (pp. 323-370). Springer, Berlin, Heidelberg.

Brette, F., Machado, B., Cros, C., Incardona, J.P., Scholz, N.L., Block, B.A., 2014. Crude oil impairs cardiac excitation-contraction coupling in fish, Science 343, 772–776.

Bryan, G.W., Gibbs, P.E., Burt, G.R., 1988. A comparison of the effectiveness of tri-n-butyltin chloride and five other organotin compounds in promoting the development of imposex in the dog-whelk, *Nucella lapillus*, Journal of the Marine Biological Association of the United Kingdom, 68, 733–744.

Brooks, S.J., Pampanin, D.M., Harman, C.P., Dunaevskava, E. 2013. The Water Column Monitoring Programme 2013: Determining the biological effects of two offshore platforms on local fish populations, NIVA report 6595- 2013, 62 p.

Brooks, S.J., Pampanin, D.M., Harman, C.P., Grung, M. 2015. Water column monitoring 2014. Determining the biological effects of an offshore platform on local fish populations. NIVA report 6735-2014, 75 p.

Busch, W., Schmidt, S., Kühne, R., Schulze, T., Krauss, M., Altenburger, R. 2016. Micropollutants in European rivers: a mode of action survey to support the development of effect-based tools for water monitoring. Environmental Toxicology and Chemistry 35, 1887–1899.

Carls, M.G., Holland, L., Larsen, M., Collier, T.K., Scholz, N.L., Incardona, J.P., 2008. Fish embryos are damaged by dissolved PAHs, not oil particles. Aquatic Toxicology 88, 121–127.

Chen, C., Amirbahman, A., Fisher, N., Harding, G., Lamborg, C., Nacci, D., Taylor, D. 2008. Methylmercury in marine ecosystems: spatial patterns and processes of production, bioaccumulation, and biomagnification, EcoHealth, 5, 399-408.

Cheng, F., Li, H., Brooks, B.W., You, J., 2020. Retrospective Risk Assessment of Chemical Mixtures in the Big Data Era: An Alternative Classification Strategy to Integrate Chemical and Toxicological Data. Environmental Science & Technology 54, 5925–5927.

Conder, J. M., Hoke, R. A., Wolf, W. D., Russell, M. H., Buck, R. C. 2008. Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds, Environmental Science & Technology, 42, 995-1003.

De Laender, F., Van Oevelen, D., Middelburg, J.J., Soetaert, K., 2010. Uncertainties in ecological, chemical and physiological parameters of a bioaccumulation model: Implications for internal concentrations and tissue based risk quotients, Ecotoxicology and Environmental Safety, 73, 240–246.

Debes, F., Weihe, P., Grandjean, P., 2016. Cognitive deficits at age 22 years associated with prenatal exposure to methylmercury, Cortex, 74, 358–369.

Deidda, I., Russo, R., Bonaventura, R., Costa, C., Zito, F., Lampiasi, N., 2021. Neurotoxicity in Marine Invertebrates: An Update. Biology 10, 161.

Depledge, M. H., Galloway, T. S. 2005. Healthy animals, healthy ecosystems. Frontiers in Ecology and the Environment, 3, 251-258.

Desforges, J. P., Hall, A., McConnell, B., Rosing-Asvid, A., Barber, J. L., Brownlow, A., De Guise, S., *et al*. 2018. Predicting global killer whale population collapse from PCB pollution. Science, 361, 1373-1376.

Dethlefsen, V., von Westernhagen, H., Cameron, P. 1996. Malformations in North Sea pelagic fish embryos during the period 1984-1995. ICES Journal of Marine Science 53, 1024–1035.

Dreier, D.A., Bowden, J.A., Aristizabal-Henao, J.J., Denslow, N.D., Martyniuk, C.J., 2020 Ecotoxico-lipidomics: An emerging concept to understand chemical-metabolic relationships in comparative fish models, Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 36, 100742.

Driessen, M., Vitins, A.P., Pennings, J.L.A., Kienhuis, A.S., Water, B. van de, van der Ven, L.T.M. 2015. A transcriptomics-based hepatotoxicity comparison between the zebrafish embryo and established human and rodent in vitro and in vivo models using cyclosporine A, amiodarone and acetaminophen. Toxicology Letters 232, 403–412.

Eales, J.G. 2019. The relationship between ingested thyroid hormones, thyroid homeostasis and iodine metabolism in humans and teleost fish. General and Comparative Endocrinology 280, 62–72.

Esler, D., Ballachey, B.E., Bowen, L., Miles, A.K., Dickson, R.D., Henderson, J.D. 2017. Cessation of oil exposure in harlequin ducks after the Exxon Valdez oil spill: Cytochrome P4501A biomarker evidence: Exxon Valdez oil exposure in harlequin ducks. Environmental Toxicology and Chemistry 36, 1294–1300.

Ewald, J.D., Soufan, O., Crump, D., Hecker, M., Xia, J., Basu, N. 2020. EcoToxModules: Custom Gene Sets to Organize and Analyze Toxicogenomics Data from Ecological Species. Environ. Sci. Technol. 54, 4376–4387.

Gao, Y., Xu, H., Li, L., Niu, C. 2020. Immune defense parameters of wild fish as sensitive biomarkers for ecological risk assessment in shallow sea ecosystems: A case study with wild mullet (*Liza haematocheila*) in Liaodong Bay. Ecotoxicology and Environmental Safety 194, 110337.

Gibbs, P.E., Bryan, G.W. 1986. Reproductive failure in populations of the dog-whelk, *Nucella lapillus*, caused by imposex induced by tributyltin from antifouling paints. Journal of the Marine Biological Association of the United Kingdom 66, 767–777.

Gorrochategui, E., Pérez-Albaladejo, E., Casas, J., Lacorte, S., Porte, C., 2014. Perfluorinated chemicals: Differential toxicity, inhibition of aromatase activity and alteration of cellular lipids in human placental cells. Toxicology and Applied Pharmacology 277, 124–130.

Grandjean, P., Landrigan, P.J. 2014. Neurobehavioural effects of developmental toxicity. The Lancet Neurology 13, 330–338.

Gregus, Z. 2015. Chapter 3. Mechanisms of toxicity. In: Cassarett & Doull's Essentials of Toxicology, 3rd edition, Klaassen, C.D., Watkins, J.B. III, McGraw-Hill.

Gulland, F.M.D., Hall, A.J., Ylitalo, G.M., Colegrove, K.M., Norris, T., Duignan, P.J., Halaska, B., *et al*. 2020. Persistent Contaminants and Herpesvirus OtHV1 Are Positively Associated With Cancer in Wild California Sea Lions (Zalophus californianus). Frontiers in Marine Science 7, 602565.

Hicken, C.E., Linbo, T.L., Baldwin, D.H., Willis, M.L., Myers, M.S., Holland, L., Larsen, M., *et al.* 2011. Sublethal exposure to crude oil during embryonic development alters cardiac morphology and reduces aerobic capacity in adult fish. Proceedings of the National Academy of Sciences 108, 7086–7090.

Hoeng, J., Talikka, M., Martin, F., Sewer, A., Yang, X., Iskandar, A., Schlage, W.K., *et al*. 2014. Case study: the role of mechanistic network models in systems toxicology. Drug Discovery Today 19, 183–192.

Holbech, H., Kinnberg, K., Petersen, G.I., Jackson, P., Hylland, K., Norrgren, L., Bjerregaard, P. 2006. Detection of endocrine disrupters: evaluation of a Fish Sexual Development Test (FSDT). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 144, 57–66.

Hoffmann, T., Warmbold, B., Smits, S.H.J., Tschapek, B., Ronzheimer, S., Bashir, A., Chen, C., *et al*. 2018. Arsenobetaine: an ecophysiologically important organoarsenical confers cytoprotection against osmotic stress and growth temperature extremes. Environmental Microbiology 20, 305-323.

Holth, T.F., Thorsen, A., Olsvik, P.A., Hylland, K. 2010. Long-term exposure of Atlantic Cod (Gadus morhua) to components of produced water: condition, gonad maturation and gene expression. Canadian Journal of Fisheries and Aquatic Sciences 67, 1685–1698.

Hylland, K. 2006. Polycyclic aromatic hydrocarbon (PAH) ecotoxicology in marine ecosystems. Journal of Toxicology and Environmental Health, Part A 69, 109–123.

Hylland, K., Burgeot, T., Martínez-Gómez, C., Lang, T., Robinson, C.D., Svavarsson, J., Thain, J.E., *et al*. 2017a. How can we quantify impacts of contaminants in marine ecosystems? The ICON project. Marine Environmental Research, 124, 2–10.

Hylland, K., Robinson, C.D., Burgeot, T., Martínez-Gómez, C., Lang, T., Svavarsson, J., Thain, J.E., *et al*. 2017b. Integrated chemical and biological assessment of contaminant impacts in selected European coastal and offshore marine areas. Marine Environmental Research, 124, 130–138.

Hylland, K., Skei, B.B., Brunborg, G., Lang, T., Gubbins, M. J., Le Goff, J., Burgeot, T. 2017c. DNA damage in dab (*Limanda limanda*) and haddock (*Melanogrammus aeglefinus*) from European seas. Marine Environmental Research 124, 54–60.

ICES SGIMC. 2011. Report of the Study Group on Integrated Monitoring of Contaminants and Biological Effects (SGIMC), 14-18 March 2011, Copenhagen, Denmark. ICES CM 2011/ACOM:30. 265 pp.

Incardona, J.P., Carls, M.G., Holland, L., Linbo, T.L., Baldwin, D.H., Myers, M.S., Peck, K.A., *et al*. 2015. Very low embryonic crude oil exposures cause lasting cardiac defects in salmon and herring. Science Reports 5, 13499.

Incardona, J.P., Carls, M.G., Teraoka, H., Sloan, C.A., Collier, T.K., Scholz, N.L. 2005. Aryl hydrocarbon receptor-independent toxicity of weathered crude oil during fish development. Environmental Health Perspectives, 113, 1755–1762.

Incardona, J.P., Swarts, T.L., Edmunds, R.C., Linbo, T.L., Aquilina-Beck, A., Sloan, C.A., Gardner, L.D., *et al*. 2013. Exxon Valdez to Deepwater Horizon: Comparable toxicity of both crude oils to fish early life stages. Aquatic Toxicology 142–143, 303–316.

Jepson, P.D., Deaville, R., Barber, J.L., Aguilar, À., Borrell, A., Murphy, S., Barry, J., *et al*. 2016. PCB pollution continues to impact populations of orcas and other dolphins in European waters. Scientific Reports 6, 18573.

Jobling, S., Coey, S., Whitmore, J.G., Kime, D.E., Van Look, K.J.W., McAllister, B.G., Beresford, N., *et al*. 2002. Wild Intersex Roach (*Rutilus rutilus*) Have Reduced Fertility. Biology of Reproduction 67, 515–524.

Johnson, L.L., Landahl, J.T., Kubin, L.A., Horness, B.H., Myers, M.S., Collier, T.K., Stein, J.E. 1998. Assessing the effects of anthropogenic stressors on Puget Sound flatfish populations. Journal of Sea Research 39, 125–137.

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

Korsgaard, B., Andersen, F.O. 1985. Embryonic nutrition, growth and energetics in Zoarces viviparus L. as indication of a maternal-fetal trophic relationship. Journal of Comparative Physiology 155B, 437–444.

Krahn, M.M., Rhodes, L.D., Myers, M.S., Moore, L.K., MacLeod, W.D., Malins, D.C. 1986. Association between metabolites of aromatic compounds in bile and the occurrence of hepatic lesions in English sole (*Parophrys vetulus*) from Puget Sound, Washington. Archives of Environmental Contamination and Toxicology 15, 61–67.

Kihlström, J.E., Olsson, M., Jensen, S., Johansson, Å, Ahlbom, J., Bergman, Å. 1992. Effects of PCB and different fractions of PCB on the reproduction of the mink (Mustela vison). Ambio 21, 563-569.

Kirby, M.F., Morris, S., Hurst, M., Kirby, S.J., Neall, P., Tylor, T., Fagg, A. 2000. The use of cholinesterase activity in flounder (*Platichthys flesus*) muscle tissue as a biomarker of neurotoxic contamination in UK estuaries. Marine Pollution Bulletin 40, 780–791.

Knapen, D., Stinckens, E., Cavallin, J.E., Ankley, G.T., Holbech, H., Villeneuve, D.L., Vergauwen, L. 2020. Toward an AOP Network-Based Tiered Testing Strategy for the Assessment of Thyroid Hormone Disruption. Environmental Science & Technology 54, 8491–8499.

Lagadic, L., Caquet, T., Ramade, F. 1994. The role of biomarkers in environmental assessment (5). Invertebrate populations and communities. Ecotoxicology 3, 193-208.

Laird, M.J., Aristizabal Henao, J.J., Reyes, E.S., Stark, K.D., Low, G., Swanson, H.K., Laird, B.D. 2018. Mercury and omega-3 fatty acid profiles in freshwater fish of the Dehcho Region, Northwest Territories: informing risk benefit assessments. Science of the Total Environment 637–638, 1508–1517.

Lam, P. K., Gray, J. S. 2003. The use of biomarkers in environmental monitoring programmes. Marine Pollution Bulletin 46, 182-186.

Larsson, D.J., Förlin, L. 2002. Male-biased sex ratios of fish embryos near a pulp mill: temporary recovery after a short-term shutdown. Environmental Health Perspectives 110, 739–742.

Legradi, J.B., Di Paolo, C., Kraak, M.H.S., van der Geest, H.G., Schymanski, E.L., Williams, A.J., Dingemans, M.M.L., *et al*. 2018. An ecotoxicological view on neurotoxicity assessment. Environmental Sciences Europe 30, 46.

Loeb, L.A., Harris, C.C. 2008. Advances in Chemical Carcinogenesis: A Historical Review and Prospective. Cancer Research 68, 6863-6872.

Luoma, S.N. 1983. Bioavailability of trace metals to aquatic organisms - a review. Science of the Total Environment 28, 1–22.

Malins, D.C., McCain, B.B., Myers, M.S., Brown, D.W., Krahn, M.M., Roubal, W.T., Schiewe, M.H., *et al*. 1987. Field and laboratory studies of the etiology of liver neoplasms in marine fish from Puget Sound. Environmental Health Perspectives 71, 5–16.

Martínez-Gómez, C., Vethaak, A.D. 2019. Understanding the impact of chemicals on marine fish populations: the need for an integrative approach involving population and disease ecology. Current Opinion in Environmental Science & Health 11, 71–77.

Marty, G.D., Hoffmann, A., Okihiro, M.S., Hepler, K., Hanes, D. 2003. Retrospective analysis: bile hydrocarbons and histopathology of demersal rockfish in Prince William Sound, Alaska, after the Exxon Valdez oil spill. Marine Environmental Research 56, 569–584.

Mathieu-Denoncourt, J., Wallace, S.J., de Solla, S.R., Langlois, V.S., 2015. Plasticizer endocrine disruption: Highlighting developmental and reproductive effects in mammals and non-mammalian aquatic species. General and Comparative Endocrinology 219, 74–88.

Mauri, I., Romero, A., Acerete, L., MacKenzie, S., Roher, N., Callol, A., Cano, I., *et al*. 2011. Changes in complement responses in Gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) under crowding stress, plus viral and bacterial challenges. Fish & Shellfish Immunology 30, 182–188.

Meier, S., Morton, H.C., Andersson, E., Geffen, A.J., Taranger, G.L., Larsen, M., Petersen, M., *et al.* 2011. Low-dose exposure to alkylphenols adversely affects the sexual development of Atlantic cod (*Gadus morhua*): Acceleration of the onset of puberty and delayed seasonal gonad development in mature female cod. Aquatic Toxicology 105, 136–150.

Melvin, S.D., Lanctôt, C.M., Doriean, N.J.C., Bennett, W.W., Carroll, A.R. 2019. NMR-based lipidomics of fish from a metal(loid) contaminated wetland show differences consistent with effects on cellular membranes and energy storage. Science of the Total Environment, 654, 284–291.

Merian, E., Anke, M., Ihnat, M., Stoeppler, M. 2004. Elements and their compounds in the environment: occurrence, analysis and biological relevance. 2nd Ed. Wiley-VCH Verlag GmbH.

Myers, M.S., Johnson, L.L., Collier, T.K. 2003. Establishing the Causal Relationship between Polycyclic Aromatic Hydrocarbon (PAH) Exposure and Hepatic Neoplasms and Neoplasia-Related Liver Lesions in English Sole (Pleuronectes vetulus). Human and Ecological Risk Assessment, 9, 67–94.

Myers, M.S., Johnson, L.L., Hom, T., Collier, T.K., Stein, J.E., Varanasi, U. 1998. Toxicopathic hepatic lesions in subadult English sole (*pleuronectes vetuls*) from Puget Sound, Washington, USA: relationships with other biomarkers of contaminant exposure. Marine Environmental Research 45, 47–67.

Myers, M.S., Landahl, J.T., Krahn, M.M., Johnson, L.L., McCain, B.B. 1990. Overview of studies on liver carcinogenesis in English sole from Puget Sound; evidence for a xenobiotic chemical etiology I: pathology and epizootiology. Science of the Total Environment 94, 33–50.

Myers, M.S., Olson, O.P., Johnson, L.L., Stehr, C.S., Hom, T., Varanasi, U. 1992. Hepatic lesions other than neoplasms in subadult flatfish from Puget Sound, Washington: relationships with indices of contaminant exposure. Marine Environmental Research 34, 45–51.

Nacci, D., Huber, M., Champlin, D., Jayaraman, S., Cohen, S., Gauger, E., Fong, A., *et al*. 2009. Evolution of tolerance to PCBs and susceptibility to a bacterial pathogen (*Vibrio harveyi*) in Atlantic killifish (*Fundulus heteroclitus*) from New Bedford (MA, USA) harbor. Environmental Pollution 157, 857–864.

Nizzetto, L., Macleod, M., Borgå, K., Cabrerizo, A., Dachs, J., Guardo, A.D., Ghirardello, D., *et al.* 2010. Past, Present, and Future Controls on Levels of Persistent Organic Pollutants in the Global Environment. Environmental Science & Technology 44, 6526–6531.

NRC 2003. Oil in the sea III. Inputs, fates, and effects. Washington, The National Academies Press.

Oehlmann, J., Fioroni, P., Stroben, E., Markert, B. 1996. Tributyltin (TBT) effects on *Ocinebrina aciculata* (Gastropoda: Muricidae): imposex development, sterilization, sex change and population decline. Science of the Total Environment 188, 205–223.

Oehlmann, J., Stroben, E., Fioroni, P. 1991. The morphological expression of imposex in *Nucella lapillus* (Linnaeus)(Gastropoda: Muricidae). Journal of Molluscan Studies, 57, 375–390.

Olsson, M., Karlsson, B., Ahnland, E. 1994. Diseases and enviromental contaminants in seals from the Baltic and the Swedish west coast. Science of the Total Environment, 154, 217–227.

Pampanin, D., Brooks, S., Grøsvik, B., Sanni, S. 2019. Water Column Monitoring 2017. Environmental monitoring of petroleum activities on the Norwegian continental shelf 2017. NORCE-Environment Report 007.

Pérez-Casanova, J.C., Hamoutene, D., Samuelson, S., Burt, K., King, T.L., Lee, K. 2010. The immune response of juvenile Atlantic cod (*Gadus morhua* L.) to chronic exposure to produced water. Marine Environmental Research 70, 26–34.

Peterson, C. H., Rice, S. D., Short, J. W., Esler, D., Bodkin, J. L., Ballachey, B. E., Irons, D. B. 2003. Long-term ecosystem response to the Exxon Valdez oil spill. Science, 302, 2082-2086.

Ploch, S.A., King, L.C., Di Giulio, R.T. 1998. Comparative time-course of benzo [a] pyrene-DNA adduct formation, and its relationship to CYP1A activity in two species of catfish. Marine Environmental Research 46, 345–349.

Rainbow, P. S. 2002. Trace metal concentrations in aquatic invertebrates: why and so what? Environmental pollution, 120, 497-507.

Rehberger, K., Werner, I., Hitzfeld, B., Segner, H., Baumann, L. 2017. 20 Years of fish immunotoxicology – what we know and where we are. Critical Reviews in Toxicology 47, 516–542.

Scott, A.P., Katsiadaki, I., Witthames, P.R., Hylland, K., Davies, I.M., McIntosh, A.D., Thain, J., 2006. Vitellogenin in the blood plasma of male cod (*Gadus morhua*): A sign of oestrogenic endocrine disruption in the open sea? Marine Environmental Research 61, 149–170.

Segner, H., Wenger, M., Möller, A.M., Köllner, B., Casanova-Nakayama, A. 2012. Immunotoxic effects of environmental toxicants in fish — how to assess them? Environmental Science and Pollution Research 19, 2465–2476.

Sidhu, G.K., Singh, S., Kumar, V., Dhanjal, D.S., Datta, S., Singh, J. 2019. Toxicity, monitoring and biodegradation of organophosphate pesticides: a review. Critical Reviews in Environmental Science and Technology 49, 1135–1187.

Sørhus, E., Edvardsen, R.B., Karlsen, Ø., Nordtug, T., van der Meeren, T., Thorsen, A., Harman, C., *et al*. 2015. Unexpected Interaction with Dispersed Crude Oil Droplets Drives Severe Toxicity in Atlantic Haddock Embryos. PLOS ONE 10, e0124376.

Stange, D., Sieratowicz, A., Oehlmann, J., 2012. Imposex development in *Nucella lapillus* – Evidence for the involvement of retinoid X receptor and androgen signalling pathways in vivo. Aquatic Toxicology 106–107, 20–24.

Stein, J.E., Collier, T.K., Reichert, W.L., Casillas, E., Hom, T., Varanasi, U. 1993. Bioindicators of contaminant exposure and sublethal effects in benthic fish from Puget Sound, WA, USA. Marine Environmental Research 35, 95–100.

Sueiro, M.C., Awruch, C., Gilardoni, C., Demetrio, M., Palacios, M.G. 2020. Immunity and health of two wild marine fishes naturally exposed to anthropogenic pollution. Science of The Total Environment 726, 138303.

Sukumaran, S., Grant, A., 2013. Effects of genotoxicity and its consequences at the population level in sexual and asexual Artemia assessed by analysis of inter-simple sequence repeats (ISSR). Mutation Research/Genetic Toxicology and Environmental Mutagenesis 757, 8–14.

Sumpter, J.P., Jobling, S. 1995. Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment, Environmental Health Perspectives, 103, 173–178.

Sundberg, H., Hanson, M., Liewenborg, B., Zebühr, Y., Broman, D., Balk, L. 2007. Dredging associated effects: maternally transferred pollutants and DNA adducts in feral fish. Environmental Science & Technology, 41, 2972–2977.

Tijani, J.O., Fatoba, O.O., Babajide, O.O., Petrik, L. F. 2016. Pharmaceuticals, endocrine disruptors, personal care products, nanomaterials and perfluorinated pollutants: a review. Environmental Chemistry Letters, 14, 27-49.

Thompson, R.M., Hemberg, M., Starzomski, B.M., Shurin, J.B. 2007. TROPHIC LEVELS AND TROPHIC TANGLES: THE PREVALENCE OF OMNIVORY IN REAL FOOD WEBS. Ecology 88, 612–617.

Thorsen, W.A., Cope, W.G., Shea, D. 2004. Bioavailability of PAHs: Effects of Soot Carbon and PAH Source. Environmental Science & Technology 38, 2029–2037.

Vetemaa, M., Sandström, O., Förlin, L. 1997. Chemical industry effluent impacts on reproduction and biochemistry in a North Sea population of viviparous blenny (Zoarces viviparus). Journal of Aquatic Ecosystem Stress and Recovery 6, 33–41.

Vethaak, A.D., Davies, I.M., Thain, J.E., Gubbins, M.J., Martínez-Gómez, C., Robinson, C.D., Moffat, C.F., *et al.* 2017. Integrated indicator framework and methodology for monitoring and assessment of hazardous substances and their effects in the marine environment. Marine Environmental Research 124, 11–20.

von Westernhagen, H., Dethlefsen, V., Cameron, P., Berg, J., Fürstenberg, G., 1988. Developmental defects in pelagic fish embryos from the western Baltic. Helgoländer Meeresuntersuchungen 42, 13–36.

Wan, Y., Jin, X., Hu, J., Jin, F., 2007. Trophic Dilution of Polycyclic Aromatic Hydrocarbons (PAHs) in a Marine Food Web from Bohai Bay, North China. Environmental Science & Technology 41, 3109–3114.

Widdows, J., Johnson, D. (1988). Physiological energetics of Mytilus edulis: scope for growth. Marine Ecology Progress Series, 46, 113-121.

Xu, S., Ma, J., Ji, R., Pan, K., Miao, A.-J., 2020. Microplastics in aquatic environments: Occurrence, accumulation, and biological effects. Science of The Total Environment 703, 134699.

Yang, Y., Deng, J., Liu, Y., He, K., Xiang, Z., Luan, T. 2019. A microscale solid-phase microextraction probe for the in situ analysis of perfluoroalkyl substances and lipids in biological tissues using mass spectrometry. Analyst, 144, 5637–5645.

Annex 1: Reviewers' reports

Review 1

I think this report should be substantially rewritten before it is adopted by ICES. While nothing about the report is wrong per se, the report could be improved and made more original, as explained in the four sections below.

To conduct this review, I added line numbers to the text. In the examples I provide, I refer to these each page and line numbers.

1. I agree with the central tenet

On page 3, and again on page 17, you write: The following are recommendations for national and international monitoring programmes.

1) Effects of contaminants on marine organisms should be assessed using field-based monitoring.

2) Effect methods need to be contaminant-specific.

3) Monitoring programmes should address genotoxicity, neurotoxicity, immunotoxicity, cardiotoxicity, endocrine disruption and reproductive toxicity.

4) Robust markers must be established for immunotoxicity and cardiotoxicity.

5) The recommended methods needs to be evaluated regularly.

I wholeheartedly agree. Too often impact assessment is based on the results of laboratory toxicity tests, or on chemical monitoring compared to guideline values.

2. Needs to be better organised – subheadings and conceptual diagrams would help the reader understand the flow. Also – the paragraphs jump all over the place and should be grouped so that the authors arguments are easier to follow

For example – a diagram of the steps being proposed in section 3 would be helpful

3. Needs to be updated – much of the literature is more than 10 years out of date, much of the recent stuff is self-referential, many missing references

Page 4, lines 1-11 are missing references

Page 4, lines 19-22 " "

Page 4, lines 27-29 "

Page 4 line 32- Page 5 line 3 " "

Page 5 lines 11-17 " "

Page 5 lines 22-30 " "

Page 6 line 34-Page 7 line 8 " "

Page 7 line 14-18 " "

Page 7 line 25 " "

Page 8 line 15-22 " "

Page 9 line 4-17 " "

Page 10 – line 16-20	"	"
Page 14 – line 5-14	"	"
Page 15 – line 14-20	"	"
Page 15 – line 27-30	"	"

Page 4, lines 14-15: All references are more than 10 years old

Page 5 lines 11-30:	"	"	"
Page 7 line 26-page 8 line 9	"	"	"
Page 8 line 15- page 9 line 3	"	"	"
Page 10 line 21-28	"	"	"

4. Needs to have clear cut recommendations – what do you want from us – as the scientific community; as regulators, etc. – and what will the consequences be if you don't get it

For example – Page 8 lines 25-26 you write: "we must therefore increase the scientific focus on species groups not currently included" Why? What would the benefit be?

Page 9 lines 30-32 you write "Many of the biological effects methods for sublethal toxicity identified and used in marine sciences over the past decades hail from medical sciences…." What are you suggesting as an alternative?

Section 4 is all recommendations – for each, what is the benefit, and what are the consequences of inaction?

5. Needs to be more pragmatic – what can we do today with the science we have? Also, what science tools do we need to build? For example:

Page 2 line 22: you write "Effect methods to be used in monitoring need to be contaminant-specific," yet the endpoints you discuss (reproductive toxicity, genotoxicity, etc) are often caused by a large suite of chemicals. How would specificity be possible?

Page 2 line 28: You write "organisms used in monitoring programmes or for toxicity testing does not in any way represent the full marine biodiversity" and that we should "ensure that society provides adequate protection for all species in marine ecosystems" There are 20 000 fish species that we know of, let alone the invertebrates. Many parts of the world have a vast number of undescribed species. What are you suggesting?

Other, more minor comments:

Page 2, lines 6-8 and page 5 line 11-13: terrestrial organisms rely on air for temperature maintenance, and pH maintenance – please rethink this sentence

Page 2, line 13: consider rephrasing "effect methods" as "methods to quantify toxic effect or impact"

Page 4 line 2: The statement "No part of the oceans is entirely free from chemical pollution caused by human activity." can be moved to after the definition of the contaminants.

Page 4 line 27: avoid ableist language – use biphasic instead of "somewhat schizophrenic"

Review 2

Comments to the document "Viewpoint of ICES"

How can we quantify and manage the impact of chemical pollution in the oceans?

This document has been written by Ketil Hylland. Juan Bellas. Michelle Giltrap and Steven Brooks and the objective is to summarize the viewpoint of ICES about this topic. The authors have summarized the knowledge about t

The document is divided into an Executive summary and four sections:

a) Effects of chemical pollution in the ocean

b) How may chemical pollution affect the health of marine organisms?

c) How may existing knowledge to be used to develop new monitoring methods?

d) What actions can be recommended, based on the evidence of biological effect, to regulate/manage chemical pollution in the marine environment?

And it is included a section 5 with the Conclusion and the cited references.

Executive summary

In the first paragraph, a relation of contaminants in the marine environment are mentioned, but the occurrence of micro/nano plastics is not mentioned, although they represent a big scientific issue, and society is very sensitized by it.

In the last paragraph about recommendations, I did not find a comment about implementing new methodologies (as omic techniques), that the authors mentioned in the four sections. Finally, I suggest including the 5th recommendation the word updated. The sentence will be "The recommended methods need to be updated and evaluated regularly". I did not find any recommendation for widening the sensitive species selection despite it is mentioned several times in the document.

Section 1. Effects of chemical pollution in the oceans

As I mentioned, previously., I suggest including micro/nano plastics in the relationship of pollutants.

I suggest removing the psychological term "schizophrenic" nature" for referring to amphiphilic characteristics.

Some sentences in this section can be repetitive. In fact, the last sentences about the viewpoint and the two previous sentences are similar.

Section 2. How many chemical pollution affect the health of marine organisms?

This section is the core of this document. In the first paragraph, the authors mentioned that the dilution of chemicals cannot avoid their effect. And to reinforce the idea, they mention in the first place the persistence, but this concept only is not the opposite to limit the effect and it should be associated as result of an increasing of levels as a consequence of the lack of biodegradation. An important point (it is mentioned by the authors) is the mixture effect. This is a real challenge

aspect to assess the pollution effect and to find a causal relationship between chemicals and effects.

A key point mentioned in the document is the extrapolation from individual responses at the population level. However, the responses related to significant effects at population levels are not related on many occasions with specific-contaminant responses. I suggest including in Table 1, besides the endpoints the specific biomarkers employed, in an additional column. And if the information is available to cofounding factors in the responses. Concderning the cardiotoxicity, only this response is mentioned for oil spills and PAHs, but probably it will a more general response. Is there any additional information about it? Can it be considered a contaminant-specific response?

Section 3. How may existing knowledge be used to develop new monitoring methods?

The authors mention the need to transfer technology and training and to build field-derived datasets for relevant species. I suggest mentioning what is the point of view of ICES about the access to these data set for the scientific community.

The authors pointed out relevant methods for future biomonitoring using omics techniques. This is a challenging task finding news and specific biomarkers, but the implementation and I understood that it will be the approach to get candidate contaminant-specific biomarkers for the future and should be linked to AOP approach. This is a very relevant issue, and it should be included in the Conclusions.

Section 4 What actions can be recommended, based on the evidence of biological effects to regulate/manage chemical pollution in the marine environment?

I suggest mentioning that "the mixed stressor responses" should be understood in a global change scenario.

It is mentioned, that "new method as refining assessment and links between responses…" and it should be included specifications about it.

Regarding the list of recommendations, as I mentioned before, in the fifth I will include "methods updated…."

Conclusions

I suggest considering that besides combination biological methods and understanding the toxicity mechanisms it will be necessary to produce new massive data that can be managed using deep learning or AI to improve the knowledge and to identify contaminant-specific responses.

In summary, this viewpoint document is a very interesting document that is written for scientist with a depth knowledge about the topic and wide experience in the frame of ICES WGBEC and in the implementation and development of MFSD. However, my comments have tried to identify or underlined aspects that I have considered to be relevant for this viewpoint of ICES.

Review 3

The viewpoint written by Hylland, Bellas, Giltrap and Brooks deals with the quantification and management of impact of chemical pollution in the oceans. This is a very important viewpoint that describes the science needed to understand and monitor effects of contaminants on marine organisms. The viewpoint is structured in four main sections with a conclusion and an executive summary. This viewpoint gives 5 recommendations for national and international monitoring programmes.

This is an important document and I hope that my comments can be seen as constructive input to improve the final result. I believe that this viewpoint can be used for ICES advice. I made a number of general and more specific comments that I hope can help improving the overall robustness, clarity and scope of the document.

In general, the document is easy to read for a relatively general audience. It is large in scope, providing a short but broad review of pollutants in marine environments and the challenges related to their identification and impact. Although the viewpoint is relatively descriptive, I feel it lacks a more explicit critical evaluation of current state of the art methods/strategies in biological monitoring. Results of decades of biological monitoring programmes across europe, including lessons learned seem to be only briefly mentioned. Thus, the document does not give the impression to be built on the experience from the past 30 years of effect marker research and monitoring programmes (success stories and pitfalls), but rather on the general ecotoxicological principles described in section 1. The authors comment (page 9) "It is outside the scope of this text to provide a full discussion of the pros and cons of environmental health assessment compared to risk assessment based on short-term laboratory tests», but a somewhat more direct discussion of environmental health assessment (especially based on existing programmes and databases) and the suitability of existing methods (e.g contaminant-specific markers) would help to better identify the novelty of the recommendations made. I realize that there may be a trade-off between length and scientific depth, but I think a revision of section 1 and its primary focus could highlight to a larger degree the past 30 years of experience in the field.

This viewpoint highlights five central recommendations. The second recommendation related to the use of contaminant-specific method seems utopic considering the reality of today´s ecosystem states with multiple pollutant sources, mixture toxicities and multiple stressors, where impacts are not necessarily the sum of all contaminants, but the result of interactions and given the knowledge that different contaminant classes can affect similar metabolic pathways. The authors argue that certain pollutant classes could be identified through specific mechanisms of toxicity (Table 1). The mechanisms of toxicity highlighted have direct implications for an organism "fitness" and thus a relevance to population level effects. However, these mechanisms of toxicity are not contaminant specific. Neurotoxicity is a mechanism of toxicity also well known for heavy metals and other organic contaminants other than pesticides. Another example is that of cardiotoxicity, which is a common endpoint in acute fish embryo toxicity tests in response to a large number of chemicals and may be caused through different mode of actions depending on internal dose (receptor based or baseline toxicity, also see Meador and Nahrgang 2019). Thus, a more in-depth description of how the proposed strategy can be used successfully would be needed.

Based on these mechanisms of toxicity relevant for a number of pollutant classes, and the statement made by the authors that "if effects are present, they are likely caused by a mixture of contaminants", how can the authors argue for contaminant-specific methods? The mechanisms of toxicity are very broad and can encompass a number of effect markers. Do the authors think that effect markers can be selected in a way that they would provide information on specific compounds? Example of such markers (and success stories) could be mentioned. Also, would it be relevant to evaluate if other strategies may be reasonable to develop/apply in a multi-stressor, mixture toxicity environment?

Another complex aspect of biological monitoring is the extrapolation of individual data to population level impacts. The authors argue for a range of contaminant specific methods to provide a holistic toxicological profile and data that can be extrapolated to potential population and community impact. Extrapolation of data to population and community levels would demand the understanding of the species 'ecology (life history, life cycle strategies) and population dynamics in a multiple stressor context, not only from a toxicological understanding. It would be nice if the authors could provide a more in-depth description of how far science has come in terms of data extrapolation and what is further required in terms of knowledge and transdisciplinary research to achieve this goal in the future.

In general, the clarity of the text could be improved, especially in the first few pages (executive summary and section 1). The objectives are presented quite late, at the very end of section 1 (page 7) and remain vague. It is not clear what knowledge, experience, or current practices the viewpoint is building on, except general ecotoxicological principles (see my main comment above).

The scope of the first section "Effects of chemical pollution in the oceans" and the second section "How may chemical pollution affect the health of organisms" are somewhat difficult to hold apart both in terms of section title and section content. Section 1 is giving a very general introduction to pollutant classes (persistent organic pollutants, trace metals etc) found in the oceans, their source, distribution and possible toxicities to organisms, with some general descriptions of pollutant behavior depending on their properties. This section highlights the need to study and understand biological effects of pollutants rather than simply quantifying their presence in the environment and concentration in organisms. This section concludes (page 7) by highlighting the focus of this viewpoint: the science needed to understand and monitor the effects of contaminants on marine organisms and ecosystems. In the second section, the authors describe how contaminants can affect populations, highlighting species differences and certain mechanisms of toxicity that are important to focus on in a biological monitoring context as they link to population level impacts. The scope and title of section 1 and 2 could be improved to provide a clearer red thread and differentiation between section 1 and 2. The executive summary content will need to be revised accordingly.

There is a mismatch between the title of the viewpoint and the focus of this viewpoint announced page 7, as the authors indicate that management and decision making are not part of the viewpoint while the title indicates the management of impacts. "Other contributors have addressed management and decision-making processes involved with biomonitoring (e.g. Makiola *et al.*, 2020)" this statement is important and should be made earlier in the document, to define the frame and delimitations of this viewpoint. Also, the title seems somewhat misleading because the viewpoint does not really discuss the quantification of impact (assessment criteria briefly mentioned p15?) but rather an identification of the source of impact using contaminant-specific

mechanisms of toxicity. The objectives of this viewpoint may thus be reformulated to be more accurate.

There is a need to provide a more precise timescale and purpose of the biological monitoring discussed. Are monitoring methods discussed here dealing with early warning effect methods or effect markers indicating health impacts such as measurable loss in individual reproductive success (e.g. reduction in fecundity)? Maybe both? The text seems to move from one to the other unclearly.

Baseline data and confounding factors are only very briefly mentioned in section 4. The authors state: "The strategy recommended in this viewpoint will make it possible to identify the contribution by contaminants in marine ecosystems, but will also increase our understanding of mixed stressor responses in general". The authors should explain how mixed stressors can be better understood through the proposed strategy.

Relatively recent technological advances (-omics) and the AOP framework are briefly mentioned. While I agree with the opinion presented in terms of omics and AOPs, omics and the AOP framework can also be used to identify new biomarkers in research and to discover potentially new toxicity pathways or identify relevant endpoints to individual fitness, rather than being a direct tool used by biological monitoring programmes. Maybe this aspect could be included too.

Finally, and if found relevant, it could be interesting to include a comment regarding data management and data sharing across countries in terms of biological monitoring. This may also be an important aspect that could be part of the recommendations.