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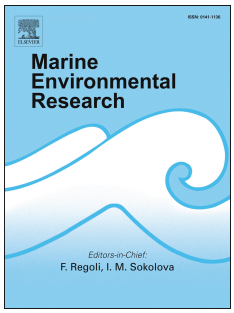

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PII: S0141-1136(17)30266-0

DOI: 10.1016/j.marenvres.2017.07.024

Reference: MERE 4356

To appear in: *Marine Environmental Research*

Received Date: 20 April 2017

Revised Date: 28 July 2017

Accepted Date: 31 July 2017

Please cite this article as: Beyer, J., Green, N.W., Brooks, S., Allan, I.J., Ruus, A., Gomes, Tâ., Bråte, I.L.N., Schøyen, M., Blue mussels (*Mytilus edulis* spp.) as sentinel organisms in coastal pollution monitoring: A review, *Marine Environmental Research* (2017), doi: 10.1016/j.marenvres.2017.07.024.

Blue mussels (*Mytilus edulis* spp.) as sentinel organisms in coastal pollution monitoring: A review

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Abstract:

The blue mussel (*Mytilus* spp.) is widely used as a bioindicator for monitoring of coastal water pollution (mussel watch programs). Herein we provide a review of this study field with emphasis on: the suitability of *Mytilus* spp. as environmental sentinels; uptake and bioaccumulation patterns of key pollutant classes; the use of *Mytilus* spp. in mussel watch programs; recent trends in Norwegian mussel monitoring; environmental quality standards and background concentrations of key contaminants; pollutant effect biomarkers; confounding factors; particulate contaminants (microplastics, engineered nanomaterials); climate change; harmonization of monitoring procedures; and the use of deployed mussels (transplant caging) in pollution monitoring. Lastly, the overall state of the art of blue mussel pollution monitoring is discussed and some important issues for future research and development are highlighted.

Keywords: Blue mussels; sentinels; pollution monitoring

1. Introduction

Blue mussels (Figure 1), are common in temperate seas all around the globe and they are widely used both as seafood and as sentinel[1] organisms in monitoring of anthropogenic pollution trends in coastal waters (Goldberg, 1975, 1980; Farrington et al., 2016). Comprehensive reviews about the biology of blue mussel are made by Bayne (ed.) (1976), and Gosling (ed.) (1992), and many sources of such information can be found online, for example at The Marine Life Information Network (http://www.marlin.ac.uk/). Blue mussels have been important as food for humans for many thousands of years and mussel farming dates back at least to the Ancient Romans. Mussels were also among the

[1] Sentinel species can be defined as biological monitors that accumulate a pollutant in their tissues without significant adverse effects and can be used to measure in a sensitive manner the amount of a pollutant that is biologically available Beeby, A., 2001. What do sentinels stand for? Environmental Pollution 112, 285-298.

first animals to be used by researchers for assessing the environmental quality of seawater, e.g. (Anonymous, 1886). Environmental monitoring with mussels is often termed as mussel watch programs and data from such monitoring is available from more than 50 nations, in some cases with data going back to the 1960s (Cantillo, 1998; Beliaeff et al., 1998). The popularity of *Mytilus* spp. as environmental sentinels stems from their biological and ecological characteristics which make them virtually ideal for pollution monitoring, e.g. as judged by the suitability criteria formulated by the OSPAR commission (2012). Blue mussels are sessile (provide location-specific information), they are medium-sized (one individual may provide enough tissue material for chemical analysis), they form (often large) mussel beds in shallow waters from where they easily can be collected, and as they are hardy creatures they are easy to keep in culture, making them suitable for ecotoxicological laboratory exposure studies as well as *in situ* analysis. They filter-feed on phytoplankton (mainly) by pumping and filtering large volumes of water over their large ciliated gills. This seawater filtration behavior also makes them to efficiently accumulate pollutant chemicals from the seawater, thereby providing an integrative measure of the concentration and bioavailability of seawater pollutants *in situ*. Furthermore, blue mussels are ecologically important as they provide essential ecological services such as food and habitat to a multitude of other species, and as primary consumers they act as vehicles for transfer of anthropogenic pollutants from the abiotic phase and the primary production level to the higher trophic levels in the coastal marine food chain, such as to mussel eating invertebrates (e.g. polychaeta, sea stars, dog whelks and crabs), sea birds (e.g. eiders), sea otters, walrus and seals (Wang and Fisher, 1999; Haukas et al., 2010; Farrell and Nelson, 2013; Larsen et al., 2016).

In this review paper, our aim is to provide an updated overview of the broad study-field of blue mussel ecotoxicology and pollution monitoring. Both potentials and challenges for the use of blue mussels in environmental research and pollution monitoring are summarized and discussed. Trend data from long-term mussel monitoring in Norway are shown and discussed with special reference to the ongoing process driven by the Water Framework Directive on implementation of environmental quality standards (EQSs) of anthropogenic contaminants in marine biota (EC, 2000, 2008, 2013, 2014). The necessity of standardized guidelines for blue mussel pollution monitoring is discussed, with special attention to a transplant mussel caging design and exemplified by a recent mussel caging study in our group (Schøyen et al., this volume). Recent developments in the field of pollutant responsive biomarkers in blue mussels are also discussed to identify markers which are operational for use in pollution effect monitoring. Lastly, the overall state of the art of blue mussel pollution monitoring is discussed and knowledge gaps and some key research needs are highlighted.

Figure 1: Photo of an opened specimen of *Mytilus edulis* seen here from the ventral side with posterior end upwards. Photo source: Wikispecies.

2. Suitability of blue mussels in coastal pollution monitoring

The genus *Mytilus* includes several closely related (congeneric) species (or subspecies) that can interbreed with each other and make fertile hybrids. It is often called the *Mytilus edulis* complex. Although the exact taxonomy within the *Mytilus* genus is not yet fully clarified, recent research indicates there are five species occurring in the Northern Hemisphere (*M. edulis*, *M. galloprovincialis*, *M. trossulus*, *M. californianus* and *M. coruscus*) and two in the Southern Hemisphere (*M. galloprovincialis*, and *M. platensis*), whereas the former *M. chilensis*, the Chilean mussel, is currently considered to be a variant of *M. platensis* (Gaitan-Espitia et al., 2016). The main native distribution range of the different *Mytilus* taxa is: *M. edulis* (North Atlantic region), *M. galloprovincialis* (Mediterranean), *M. trossulus* (northern Pacific and the Baltic Sea), *M. californianus* (coast of the North Eastern Pacific Ocean) and *M. coruscus*, (coasts of the subtropical Western Pacific Ocean) and *M. platensis* (South America). Because of the growing mussel mariculture industry and the global increase in long-range maritime transport, *Mytilus* sub-species have been introduced to areas far outside their native range. This is especially the case for *M. galloprovincialis* which has established itself as an invasive species at widely distributed locations all around the globe; including South America, South Africa, Japan, California, New Zealand, and Australia (Lockwood and Somero, 2011; Briski et al., 2012; Gardner et al., 2016). The spatial distribution of each *Mytilus* species is thought to be controlled by their tolerances of environmental factors (especially temperature and salinity) (Braby and Somero, 2006). The natural habitat requirements of *Mytilus* are described by (Hawkins and Bayne, 1985; Newell, 1989). Overlapping distribution ranges and an ability of congeneric *Mytilus* species to interbreed often leads to development of mixed populations in which genetic hybrids can be phenotypically indistinguishable from the original species (Dias et al., 2008; Brooks and Farmen, 2013). In these mixed (i.e. genetic inhomogeneous) populations, the use of genetic markers is

considered the only certain way for species identification (Rawson et al., 1996; Daguin et al., 2001; Brannock et al., 2009; Fraisse et al., 2016). For example, *M. edulis*, *M. galloprovincialis* and their hybrids can be identified using the Glu-5' gene and the ME15 and ME16 primer sets that distinguish alleles specific to *M. edulis* (180 bp), *M. galloprovincialis* (126 bp) and hybrids (180 bp/126 bp) (Bignell et al., 2008). Whether genetic inhomogeneity represents a significant confounding factor to mussel watch investigations is further discussed later.

Blue mussels are suspension feeders and feed mainly on planktonic microalgae such as *Phaeodacolum* sp., *Isochrysis* sp., and *Rhodomonas* sp. (Rouillon and Navarro, 2003; Riisgard et al., 2013; Fernandez-Reiriz et al., 2015), but they can when necessary also exploit other food sources such as bacteria (Jacobs et al., 2015) and even aquaculture fish feed (Redmond et al., 2010). Each mussel filters food particles from the seawater by means of their large and ciliated gills (Cannuel et al., 2009; Riisgård et al., 2011). If the water contains a suitable concentration of food particles the mussel will continuously pump and filter seawater at a maximum rate by the coordinated action of numerous cilia that are localized at the gill epithelium surface. During active feeding the water pumping rate for one single adult blue mussel is typically about 50 ml of seawater per min (3 liters per hour) (Famme et al., 1986). Also under conditions of food surplus, the mussel will continue to filter seawater at max speed but will now expel the excess food as pseudofaeces particles, which then consist of a mixture of mucus and undigested algae. This pseudofaeces production is ecologically important for many other species but may sometimes lead to development of anoxic sediment conditions underneath dense mussel beds as well as under mussel mariculture facilities. Blue mussels may form large local populations (mussel beds), which in some areas can be several km wide and include an immense number of mussel individuals. Blue mussels represent an important food source for many shell eating animals (including humans) and mussel aquaculture is a growing industry worldwide. Currently, mussel aquaculture accounts for about 80% of the total global production of blue mussels for human consumption (http://www.fao.org/fishery/species/2688/en).

The blue mussel life cycle includes several free-swimming larvae stages (trochophora, veliger, pediveliger) before the larvae after a couple of months undergo metamorphosis (to spat) and eventually attach themselves permanently to a suitable substratum by means of strong byssus threads. Blue mussels are tolerant to a relative broad range of environmental conditions (salinity, temperature, wave exposure) but there are differences among *Mytilus* taxa to which conditions that are optimal for settling, e.g. *M. trossulus* are more tolerant than *M. edulis* to low temperature and low salinity conditions (Wenne et al., 2016). The size and lifespan of *Mytilus* spp. individuals vary considerably depending on the suitability of growth conditions. In favorable conditions, *Mytilus edulis* can grow to a shell-length of >10 cm and have a lifespan of >20 years (Powell and Cummins, 1985; Sukhotin et al., 2007), although specimens larger than 8 cm and older than 10 years are uncommon. *Mytilus edulis* reaches sexual maturity after 1-2 years and the main spawning occurs in the spring (typically in April,

or when the water temperature reaches ~9° C) timed with the main phytoplankton spring bloom, but an opportunistic and less intensive secondary spawning often takes place later in the season (typically late August – September), depending on food availability. *M. edulis* has a high fecundity and a full grown female produces normally around 5,000,000 eggs per main spawning event (Pronker et al., 2008). Gametogenesis occurs mainly throughout the winter season, but also through the summer season in populations which have a second spawning period. Timing of spawning of *Mytilus* populations vary greatly with geographic location, and this is a highly relevant factor to consider when mussels are used for environmental monitoring (see confounding factors later).

Mytilus spp. exhibit strong seasonal growth patterns and the mussel's condition index (CI) is an indicator of the overall favorability of the growth conditions as well as the overall biological status of the individual. The CI is normally understood as the quantitative relationship of the mussels' soft tissue weight (wet or dry) to its overall size; the latter measured as the shell dry weight, the weight of soft tissue + shell, the shell length, the shell volume, or the shell cavity volume. Hence, there are several alternative equations for estimating CI of blue mussel, as discussed by Davenport and Chen (1987). Probably, the easiest and most convenient equation for use in mussel monitoring is: CI = (MW/SW) x 100, wherein MW is the wet meat weight (g) and SW is the shell dry weight (g). Note that this calculation can easily be performed both at the level of the individual mussel and at the level of a composite (pooled) sample. The advantage of taking wet weight is that the mussel can be used for other endpoints after weighing, such as gill and digestive gland analysis. This is not possible for dry weight. However, using the wet weight of the mussel is not as reliable as the dry weight and is more dependent on the sampling method; i.e. some researchers may drain the mussel thoroughly before weighing, whilst other would not, and this may influence the weight significantly. The CI of *Mytilus* spp. vary considerably during the annual cycle depending on the mussels nutritional and reproductive status. To obtain CI data is considered important in mussel monitoring, as it provides key information regarding the overall biological status of the sampled mussels. For example, the shell from a rapidly growing individual is typically thinner compared to those from slow growing individuals and this information can assist in the interpretation of data from the environmental quality parameters which are measured in the mussel sample. It is often found that the mussels CI is negatively correlated with the *in vivo* concentration level of chemical contaminants, as slow-growing mussels will accumulate contaminants for a longer time per weight unit. Many reports have emphasized CI as an important biological value to consider in pollutant fate and effect studies with blue mussels, e.g. (Granby and Spliid, 1995; Mubiana et al., 2006; Benali et al., 2015; Touahri et al., 2016). It is unfortunate that there is apparently not yet established any firm international standard for how to estimate CI in blue mussel monitoring. Some studies, e.g. Giltrap et al. (2016), have even used the Fulton's condition factor formula: $K = 100(W/L^3)$, where W is meat wet weight (g) and L is shell length (cm), although that estimation method is designed for fish and not mussels.

3. Uptake, accumulation, and depuration of anthropogenic contaminants in blue mussels

Marine mussels are known to efficiently absorb and accumulate anthropogenic contaminants from their surroundings and they have a limited biotransformation capacity for pollutants in comparison to for example fish and other vertebrates. Mussels are therefore suitable as animal models in pollutant bioconcentration/bioaccumulation and toxicokinetic studies. *Bioconcentration* is the process in which chemical substances are absorbed by receptor organisms solely through uptake over respiratory and dermal surfaces, i.e. exposure via diet is not included; whereas *bioaccumulation* is the same (as bioconcentration) but includes also chemical exposure and uptake from the diet (Arnot and Gobas, 2006). *Toxicokinetics* of chemical contaminants encompasses all phenomenona related to the chemicals' physicochemical properties and environmental behavior (phase distribution, bioavailability); uptake in receptor organisms; internal transport rates and distribution patterns *in vivo*; the rate of bioconcentration/bioaccumulation, biotransformation (metabolism); elimination/depuration; and trophic transfer (biomagnification) tendencies. An overview of research studies and review papers on toxicokinetics of various anthropogenic contaminant in blue mussels is provided in Table 1.

In mussels, there are three major mechanisms for the uptake of environmental contaminants: (1) uptake by passive diffusion from the dissolved phase over external surfaces (predominantly gills, but also mantle and gut wall), (2) active uptake by transmembrane ion-pump transport (gills, gut wall), and (3) active uptake by endocytosis of contaminated particles (predominantly gut wall, but also gill surface). The principle route(s) and mechanisms for the uptake of chemicals into the mussel is dependent on a range of factors including: the physicochemical properties of the contaminant substance; the physicochemical conditions of the ambient water; and several biological factors related to the mussel itself. While uptake of hydrophobic (non-polar) organic contaminants, such as polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs), is thought to occur mainly as a passive diffusive process/equilibrium partitioning process, other and more complex mechanisms apply for trace metals, as their accumulation by mussel and other aquatic organisms is influenced by a variety of factors, such as multiple routes of exposure (diet and solution), metal speciation, ligand associations and complexation, chemical composition of the surrounding medium and physiological or biochemical effects on bioavailability (Luoma, 1983; Simkiss and Taylor, 1989; Luoma and Rainbow, 2005). For hydrophobic, organic contaminants, both the uptake and depuration in *Mytilus* are thought to occur predominantly by passive phase equilibrium processes over the external surfaces (mainly gills) and over the gut wall (Goldberg, 1980). The key sources for contaminant uptake in mussels are chemicals dissolved in the ambient seawater and in contaminated food particles (mainly phytoplankton). Counteracting the uptake and bioaccumulation processes, there are several factors/processes controlling the loss/depuration of contaminants in mussels. These include respiratory exchange, fecal egestion, biotransformation (limited), and growth dilution. A complex balance

between contaminant uptake and depuration processes decides whether a pollutant at any given time will concentrate or depurate in the mussel. The kinetics of pollutant uptake and depuration in mussels and other sentinel organisms are most often studied and described in a simplified manner, e.g. by assuming Steady-State (SS) conditions for key factors and the feasibility of employing one-compartment uptake and elimination models (further described later). Diagrammatic representations of key routes for uptake and elimination for such a simplified scheme are shown in Figure 2, and an overview of the key routes for internal transport/distribution of contaminants in mussels is shown in Figure 3. However, it was early realized that toxicokinetic processes are too complex to be correctly described by simple one-compartment uptake and elimination models, e.g. (Stegeman and Teal, 1973). More advanced toxicokinetic models were therefore developed, such as those based on dynamic energy budget (DEB) theory (Vanharen and Kooijman, 1993; Vanharen et al., 1994), to describe how multiple factors related to the pollutant, the environment, and the physiological condition of the sentinel organism may act in concert to influence the bioaccumulation and effect of anthropogenic contaminants. A recent review by Grech et al. (2017) summarizes the present state-of-the-art of toxicokinetic tools and models which are applied in environmental risk assessment of anthropogenic contaminants, including both simple one-compartment and multi-compartment models as well as physiologically-based toxicokinetic models.

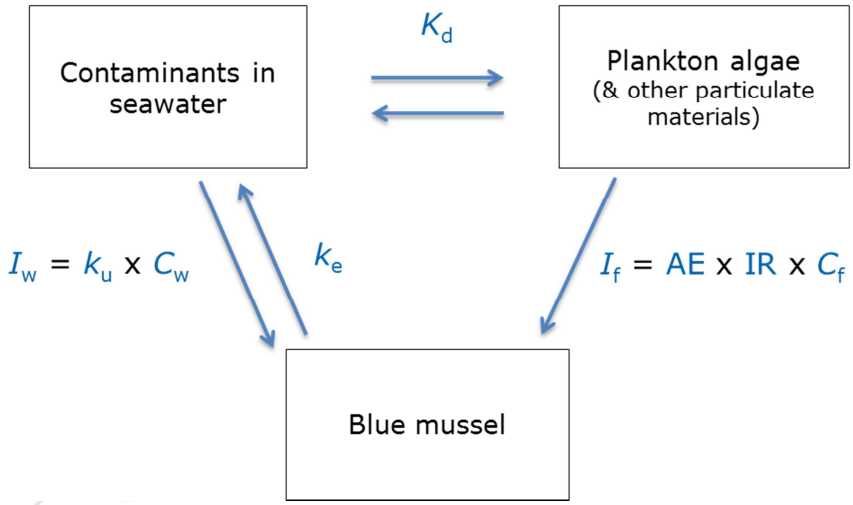
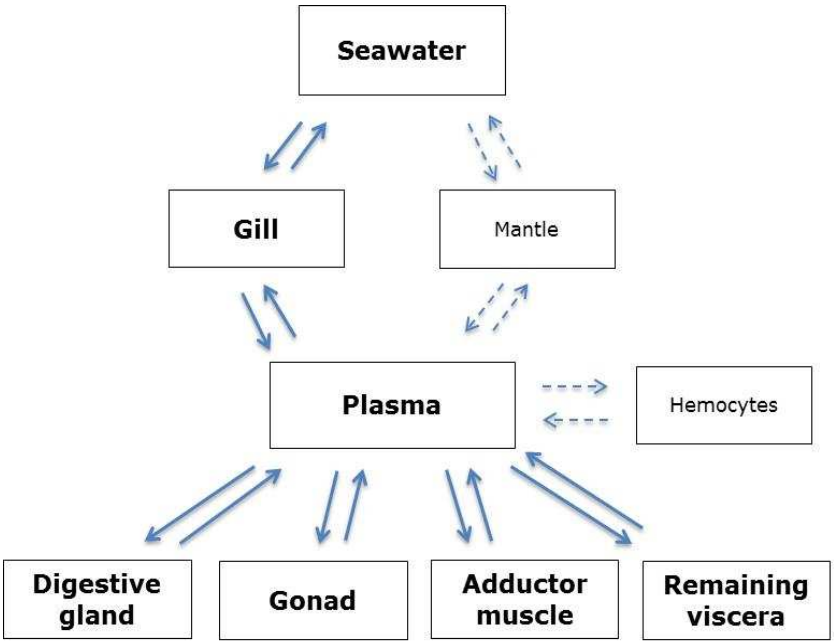


Figure 2: Diagrammatic representation of two of the key pathways of contaminant uptake and elimination in blue mussels (uptake from the aqueous phase and dietary uptake) and the parameters describing the rates of each pathway. K_d: partitioning coefficient between food and water; I_w: contaminant influx rate from the dissolved phase; k_u: contaminant uptake rate constant from the dissolved phase; C_w: contaminant concentration in the dissolved phase; k_e: efflux rate constant; I_f : contaminant influx rate from the food source; AE: contaminant assimilation efficiency from ingested food; IR: ingestion rate of the animal; C_f : contaminant concentration in ingested food;. Illustration adapted from Wang and Fisher (1999).



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223 **Figure 3: Diagrammatic representation of the prominent pathways for internal transport/distribution of**
224 **contaminants in blue mussels. Solid arrows and bold fonts indicate major pathways whereas alternative**
225 **routes are shown as dashed arrows and normal fonts. Illustration adapted from Ricciardi et al. (2016).**

226 As in other species of suspension-feeding bivalves, the very large and complex gills of *Mytilus* spp.
227 have a variety of key functions in feeding, gas exchange, digestion, and evacuation of propagules and
228 wastes (Beninger et al., 1991; Cannuel et al., 2009; Cranford et al., 2011). The coordinated movements
229 of cilia at the gill surface mediates steady pumping of seawater through the mussels' pallial cavity
230 where the gills with high efficiency capture and trap suspended food particles into mucus and
231 subsequently mediates the transport of this mixture to the mussels' mouth and digestive system. The
232 gill system is the dominant site for direct interaction with the environment, with its large surface and
233 thin epithelium, and is therefore a key organ for uptake and elimination of chemical contaminants
234 (Figure 2, Figure 3). For metals, the gill tissue constitutes a key interface for uptake of dissolved
235 metals, for binding of metals to metallothionein (MT), for incorporation of metals into lysosomes, and
236 for further transport in blood plasma and circulating hemocytes (Marigomez et al., 2002). The many
237 mucus cells (mucocytes) located on the mussel gill surface continuously synthesize and secret mucous
238 glycoproteins. This process is important for the capture and transport of food particles from the gills
239 and into the mussels' digestive system and for decreasing the resistance of water flowing across the
240 gills (Beninger and St-Jean, 1997; Beninger et al., 1997). In polluted waters, contaminated mucus acts
241 as a vehicle for trapping contaminants into the dietary uptake. Additionally, the mucus layer is also
242 important for the uptake over the gill epithelium as contaminants trapped in the mucus form a
243 diffusion gradient towards the gill epithelium, which favors uptake (Baker et al., 2014).

244 Active contaminant uptake via the gut wall (e.g. typically involving endocytosis of particulate matter
245 and contaminated food particles) is generally less studied in comparison to uptake processes involving

passive diffusion over the external surfaces. However, several studies have shown that the contaminant load that enters via the digestive tract can be significant for many contaminants, e.g. Björk et al. (1997, 1999) and Axelman et al. (1999). For example, particulate metals are mostly taken up over the digestive surface mediated by endocytosis and then transferred further to lysosomes and residual bodies, especially in the digestive cells of the digestive gland (Marigomez et al., 2002). This dietary uptake pathway is also most likely important for emerging particulate contaminants (engineered nanoparticles (ENPs), microplastics, etc.), e.g. (Moore, 2006; Browne et al., 2008; Koehler et al., 2008; Ward and Kach, 2009; von Moos et al., 2012; Baker et al., 2014; Van Cauwenberghe et al., 2015; Lusher, 2015; Doyle et al., 2015; Vandermeersch et al., 2015). Research on ENPs indicate that nanoparticle aggregation may significantly enhance this uptake (Ward and Kach, 2009) and other studies show that larger particles (<100 nm) such as microplastics can be taken up both in the gills and the digestive system (von Moos et al., 2012). Presently there is therefore a growing awareness concerning the fate and potential effects of ENPs and microplastics in mussels and other commercial seafood (Van Cauwenberghe and Janssen, 2014; Li et al., 2015; Mathalon and Hill, 2014). Unlike fish, that humans normally eat without the digestive system, mussels are eaten whole, making it especially important to evaluate human food safety from these emerging particle contaminants. Therefore, more knowledge is needed on uptake and depuration of nano- and microscale particles in mussels.

Knowledge of pollutant bioaccumulation in mussels is important for risk assessment and reviews concerning this issue are available for many pollutant classes (Table 1). The ratio of contaminant concentration in sentinels to the contaminant concentration in exposure media is under Steady-State conditions (SS) referred to either as the *bioconcentration factor* (BCF) (when the contaminated exposure media is seawater) and as the *bioaccumulation factor* (BAF) (when the exposure media is a combination of contaminated seawater and diet). BCF and BAF data for lipophilic organic contaminants are often normalized to a lipid basis. The uptake of nonpolar non-ionized chemicals into blue mussels occurs mainly by a partitioning process. According to Björk and Gilek (1997) and Endicott et al. (1998) the change in organic contaminant concentration in mussels (C_m) over time can be described as the sum of rates of processes leading to the uptake or losses of contaminants from the mussel, given as:

$$\frac{dC_m}{dt} = k_u C_w + AE \times IR \times C_f - (k_d + k_b + G)C_m \qquad (1)$$

where k_u is the uptake rate from water (L g^{-1} d^{-1}), C_w is the freely dissolved contaminant concentration in water (ng L^{-1}), AE is the assimilation efficiency, IR the food ingestion rate (g g^{-1} d^{-1}), C_f the contaminant concentration in food such as algae (g g^{-1}), k_d the rate of contaminant depuration through gills (d^{-1}), k_b a biotransformation rate (d^{-1}) and G the mussel growth rate (g g^{-1} d^{-1}). When the

concentration of contaminants in the food is at equilibrium with that in water (through a food-water partition coefficient K_{fw}), the equation (1) above can be rewritten as:

$$\frac{dC_m}{dt} = (k_u + AE \times IR \times K_{fw})C_w - (k_d + k_b + G)C_m \qquad (2)$$

which reduces to:

$$\frac{dC_m}{dt} = k_{acc}C_w - k_{loss}C_m \qquad (3)$$

where k_{acc} and k_{loss} are the overall contaminant accumulation and loss rate constants. The k_{loss} rate constant is often termed as the elimination rate constant k_2. With the bioaccumulation factor (BAF) calculated as k_{acc}/k_{loss}, the solution to the equation above is given by:

$$C_m = BAF\ C_w[1 - e^{-k_{loss}t}] \qquad (4)$$

It is often assumed that organic contaminant concentrations in native organisms are at equilibrium/steady-state with the concentrations in the water. For deployed mussels, a six-week exposure has generally been expected to result in an equilibrium (Peven et al., 1996; Björk and Gilek, 1997). Loss rate constants, k_{loss} for PAHs and PCBs in *E. complanata* (a freshwater mussel) and *M. edulis* as summarized in Booij et al. (2006), decrease with increasing contaminant hydrophobicity (log K_{ow}) and range from 0.27 to 0.015 d^{-1}, equivalent to half-lives of 3-46 days. Gewurtz et al. (2002) observed elimination rates for PAHs and PCBs from *E. complanata* in a very similar range and expected passive diffusion through the gills of the mussel to be the principal depuration pathway for PAHs. A different elimination behavior for benzo[a]pyrene (BaP) is found in some *Mytilus* studies, e.g. Magnusson et al. (2000), suggesting that metabolism may be responsible for observed elimination or lack of appreciable accumulation. Exposure and uptake kinetics generally increase with increasing water pumping rates and feeding, but AE has also been shown to be inversely related to mussel filtration and water pumping rates (Björk and Gilek, 1999). These authors demonstrated the increasing relative importance of food as a source for chemical contaminants of increasing hydrophobicity. Overall, the relative contribution of uptake from water and food is difficult to assess but generally depends on food availability and on the hydrophobicity of the chemicals of interest.

An understanding of chemical bioaccumulation factors (BAF) is a prerequisite for the use of mussel as biomonitoring organisms in the aquatic environment. Bioaccumulation factors can be estimated from: (i) the ratio of k_{acc} and k_{loss} through laboratory experiments (e.g. (Björk and Gilek, 1997; Gustafsson et al., 1999)), to estimate both accumulation and depuration kinetics; or (ii) the ratio of contaminant concentration in mussel (at steady-state/equilibrium) and freely dissolved in water. Laboratory experiments designed to expose mussel to constant contaminant concentrations are generally complex to put in place whilst *in situ* measurements of BAFs are more simple to implement. However, *in situ* measurements rely on the assumption that contaminant concentrations in native organisms have reached steady-state. Booij et al. (2006) reviewed paired mussel-passive sampling datasets for a

variety of freshwater and marine mussel species. Across various studies and mussel species, a strong relationship was found between wet-weight BAF values (calculated as ratio of mussel concentration over freely dissolved concentration estimated by using semipermeable membrane devices (SPMDs)) and the compound's hydrophobicity ($logK_{ow}$). LogBAF-$logK_{ow}$ linear relationships for the various studies had similar slope but different intercepts. This relationship was logBAF = 0.84 $logK_{ow}$ + a_0, with a_0 varying from -1.06 to 0.22 and an average of -0.49 (R^2 = 0.89, s = 0.36, n = 68). The reviewed studies encompassed mostly PAHs and alkylated PAHs, PCBs and chlorinated pesticides such as HCHs, DDTs, chlordanes, or cyclic dienes (aldrin, dieldrin). The relationship of logBAF with $logK_{ow}$ for PAHs and PCBs in transplanted blue mussels co-deployed with silicone rubber passive samplers twice a year over a period of 4-5 years at 8 sampling stations (Smedes, 2007) had a slope of 1.1 and an intercept of -2.14. Considering the general variability in logBAF values, the half an order of magnitude higher BAF values for compounds with higher $logK_{ow}$ is not out of proportion. BAFs for PAHs were observed to be higher in the winter than for autumn deployments (by 60 %). Interestingly, much higher BAFs could be observed for BaP in the winter than for autumn exposures and this could indicate lower metabolism of this chemical during winter. Some variability in PAH BAFs could be seen between stations. In general, less variation in BAF was observed for PCBs both between stations and seasons. Axelman et al. (1999) determined BAFs for PAHs that were significantly higher (> 1 log unit of BAF) for blue mussels exposed in recipient waters at an aluminum smelter site compared with data from a reference location or literature values. Under the smelter site conditions, mussels may be substantially exposed to PAHs through filter-feeding on PAH-contaminated particles from the smelter effluent releases. While PAHs strongly sorbed to these black carbon, soot-like particles may not readily partition into water once particles are released into seawater (Allan et al., 2012; Allan et al., 2016), rather they may be more available for desorption while in the gut of blue mussels. Very few BAF values are available for other classes of chemicals including emerging chemicals. Gustafsson et al. (1999) conducted uptake and depuration studies to estimate BAFs for polybrominated diphenyl ethers (PBDEs) in *M. edulis* and found that accumulation rates and BAFs were higher for BDE 47 and BDE 99 than for PCBs with similar hydrophobicity. These data were supported by *in situ* BAFs estimated by Booij et al. (2002) for native blue mussels from the Western Scheldt (The Netherlands). BAFs for BDE congeners 28, 47, 99 and 100 were much larger than those estimated for PCBs with same $logK_{ow}$. Some studies have been conducted to assess uptake and depuration of pharmaceuticals such as carbamazepine by mussels, e.g. Boillot et al. (2015), and have shown limited potential for bioaccumulation and relatively high uptake and depuration kinetics with biological half-lives of a few days. For the compounds mentioned above, bioaccumulation in mussel is expected to be a partition processes and therefore normalization of data to mussel lipid content (most often expressed as extractable organic matter measured gravimetrically) is often undertaken. This also means that the contaminant concentration in mussel will react to changes in contaminant concentration in water and that BAF are independent of concentrations in water. Contradictorily, the uptake of

perfluorochemicals (PFCs) has been shown to be concentration dependent (Liu et al., 2011). Uptake and depuration experiments demonstrated non-linear accumulation of PFCs and the involvement of adsorption processes in the accumulation of PFCs in the green mussel, *Perna viridis* (family Mytilidae).

In general, the accumulation of non-ionized and nonpolar chemicals into mussels is well understood. In some cases, large variations in BAFs require additional work to understand the reasons for these differences. For substances whose mode of uptake and accumulation in mussels deviate from general partitioning (e.g. PFCs), with possible concentration dependency of the uptake, more work is required to clarify whether body burden data for these chemicals can indeed be useful for biomonitoring purposes.

Table 1: Overview of published studies on toxicokinetics (uptake, accumulation and depuration processes and rates) or field-based concentrations of different contaminant classes in *Mytilus* mussels.

Contaminant class	Toxicokinetics	Field studies (native or transplanted mussels)	Reviews
Metals	(Phillips, 1976; Vanharen et al., 1994; Fisher et al., 1996; Wang and Fisher, 1996; Wang et al., 1996; Wang and Fisher, 1997; Wang et al., 1997; Reinfelder et al., 1998; Wang and Fisher, 1999; Bendell-Young and Arifin, 2004; Pempkowiak et al., 2006; Baines et al., 2006; Casas et al., 2008; Borretzen and Salbu, 2009; Attig et al., 2010; Herve-Fernandez et al., 2010)	(Haynes and Toohey, 1998; Devier et al., 2005)	(Cossa, 1989; Luoma and Rainbow, 2005; Chapman, 2008; Stankovic and Jovic, 2012; Zuykov et al., 2013)
Petroleum hydrocarbons and polycyclic aromatic hydrocarbons (PAHs)	(Vanharen et al., 1994; Peven et al., 1996; Björk and Gilek, 1996; Okay et al., 2000; Baussant et al., 2001; Durand et al., 2002; Pempkowiak et al., 2006; Enwere et al., 2009; Yakan et al., 2011, 2013)	(Förlin et al., 1996; Gilek et al., 1997; Potrykus et al., 2003; Page et al., 2005; Devier et al., 2005; Namiesnik et al., 2008; Leon et al., 2013)	(Kasiotis and Emmanouil, 2015)
Polychlorinated biphenyls (PCBs)	(Vanharen et al., 1994; Bergen et al., 1996; Peven et al., 1996; Gilek et al., 1996a; Gilek et al., 1996b; Björk and Gilek, 1997; Hofelt and Shea, 1997; Björk and Gilek, 1999)	(Gilek et al., 1997; Potrykus et al., 2003; Devier et al., 2005; Namiesnik et al., 2008)	(Arnot and Gobas, 2006)

Polychlorinated and polybrominated dibenzofurans and dibenzo-p-dioxins	(Miyata et al., 1989; Hektoen et al., 1994)	(Miyata et al., 1987; Haynes et al., 1995; Gilek et al., 1997; Malmvarn et al., 2005; Lofstrand et al., 2010)	
Polybrominated diphenyl ethers (PBDEs)	(Gustafsson et al., 1999)	(Johansson et al., 2006; Wang et al., 2009; Fernandes et al., 2009; Hong et al., 2009; Winnberg et al., 2014; Piersanti et al., 2015)	
Organotins (TBT, DBT)	(Page et al., 1995; Folsvik et al., 2002; Devier et al., 2003; Harino et al., 2005)	(Page et al., 1995; Devier et al., 2005; Ruiz et al., 2005; Namiesnik et al., 2008; Furdek et al., 2012)	
Organochlorine pesticides	(Peven et al., 1996; Hofelt and Shea, 1997)	(Milun et al., 2016)	(Katagi, 2010)
Pharmaceuticals (17α-ethinyl estradiol, diflubenzuron, fluoxetine, cypermethrin, etc.)	(Gowland et al., 2002; Gomez et al., 2012; Silva et al., 2016; Ricciardi et al., 2016; Norambuena-Subiabre et al., 2016)	(Maruya et al., 2014)	(Fabbri and Franzellitti, 2016)
Alkylphenols (e.g. 4-nonylphenol)	(Ekelund et al., 1990; Gatidou et al., 2010; Vidal-Linan et al., 2015b; Ricciardi et al., 2016)	(Ferrara et al., 2001; Hong et al., 2009; Dodder et al., 2014; Vidal-Linan et al., 2015b)	(David et al., 2009)
Nanoparticles and engineered nanomaterials	(Koehler et al., 2008; Ward and Kach, 2009; Conway et al., 2012; Gomes et al., 2012; Wegner et al., 2012; Hull et al., 2013; Hu et al., 2014; Doyle et al., 2015; Rocha et al., 2015b; Rocha et al., 2016)		(Baker et al., 2014; Doyle et al., 2015; Rocha et al., 2015c)
Microplastics	(Browne et al., 2008; von Moos et al., 2012;	(Li et al., 2016;	(Wright et al.,

	Farrell and Nelson, 2013; Van Cauwenberghe et al., 2015; Vandermeersch et al., 2015)	Mathalon and Hill, 2014; Van Cauwenberghe and Janssen, 2014)	2013; Ziccardi et al., 2016)

4. Mussel watch programs

A mussel watch program is a systematic repeated analysis of environmental quality parameters (e.g. anthropogenic contaminant concentrations) in natural, cultured, or deployed mussels from a set of defined geographical locations (mussel stations) and over a time-span of several years (Goldberg, 1975, 1980; Goldberg, 1986). The first known mussel surveys were performed in the USA during the late 1960s and early 1970s to monitor spatial and temporal trends of contaminants in coastal and estuarine ecosystems (Goldberg, 1975). From 1986 on, the activity was continued with the US Mussel Watch Program, which by 2008 had extended to include monitoring of approximately 140 prioritized pollutants in *Mytilus* spp., oyster (*Crassostrea virginica*), or zebra mussel (*Dreissena* sp., in freshwater) sentinels from nearly 300 mussel stations (Kimbrough et al., 2008). The mussel watch activities in North America also motivated the initiation of similar systematic programs in many other coastal countries throughout the world, e.g.: Burns and Smith (1981), the MED POL Biomonitoring Programme (Viarengo et al., 2000), the Joint Assessment and Monitoring Programme (JAMP) of the OSPAR Convention (Besada et al., 2002); often organized or supervised by the UN supported International Mussel Watch Committee (UNESCO, 1992). Data from these monitoring programs are presently becoming available to users outside the research community as digitized monitoring reports more often can be found by means of common Internet search engines.

Mussel monitoring and mussel watch programs fall broadly within two major user categories: namely *trend oriented monitoring* and *problem oriented monitoring*. In trend monitoring, the key issue is to describe long-term spatial and temporal trends for pollution oriented quality status in a certain marine region; often involving a large study area, multiple monitoring stations and many anthropogenic contamination sources. Problem monitoring, on the other hand, are more narrowly defined studies; often focused on a single issue (e.g. one industrial discharge or a type of discharge). Problem monitoring activities are typically performed (or funded) by a responsible *problem owner* and are often an integrated part of the problem owner's management of their industrial operation (as in compliance monitoring). Data from both trend monitoring and problem monitoring have relevance for assessing the efficiency of discharge regulations in the industry. For example, in China mussel-based monitoring of trace metal and organic contaminants clearly links a rapidly increasing level of coastal contamination to the intense industrial growth that have occurred in these coastal areas during the recent decades (Fung et al., 2004; Pan and Wang, 2012). In severely contaminated areas, assessing

potential risk to human health due to consumption of mussel seafood is often undertaken as an integrated part of mussel watch programs. Consumer safety thresholds, e.g. maximum acceptable toxicant concentration of key contaminants in seafood mussels, have therefore been established within the environmental legislation of many coastal countries. Mussel watch programs generate spatial and temporal trend data about the locations and regions monitored, i.e. showing whether there are significant site differences and whether the pollution level is stable, increasing, or decreasing. Further integration of data from several programs may generate trend pictures that are representative of a broader regional or even a global scale. As part of the Global Ocean Observing System (GOOS) which was developed under the auspices of the United Nations (Andersen, 1997), the United States National Oceanic and Atmospheric Administration (NOAA) National Status and Trends (NS&T) Program compiled the World Mussel Watch database with data from analyses of marine or estuarine mussels or oysters as far back in time as possible (Cantillo, 1998). Some mussel monitoring studies describe contaminant concentration data and trends from remote and pristine reference locations, e.g. (Jaffe et al., 1998; Green and Knutzen, 2003; Conti et al., 2011). This type of baseline data is valuable as the information is helpful in other mussel monitoring programs for instance in discriminating between non-contaminated and low-contaminated locations, for assessing inputs from long-range pollution transport, and for evaluating the operability/functionality of regulatory Environmental Quality Standards (EQSs). In Europe, such EQSs have been defined for prioritized substances and that have become implemented at the national and international scales as a part of pollution source minimization strategies (EC, 2008, 2013, 2014).

No global harmonized standard exists for how to perform mussel monitoring studies; although several key agencies have published comprehensive instructions, e.g. UNESCO (1992), NOAA (Lanksbury et al., 2010; Lanksbury and West, 2012), ICES (Davies and Vethaak, 2012), OSPARCOM (2012) and the European Commission (EC, 2014). Despite the lack of a single harmonized standard, most ecotoxicologists would agree that good comparability of monitoring data is important for a variety of reasons (data sharing and comparisons, international monitoring collaboration, quality assurance, quality standards, quality improvement, training & competence development, etc.). Chemical contaminant analyses in mussel watch programs are normally performed by using the whole soft tissue of the animal (i.e. the mussel without the shell), and most often by using composite samples, i.e. samples in which a certain number of individual mussels within a certain size-range prior to analysis are mixed into a single pooled sample. The use of pooled mussel samples is rational due to several practical reasons (especially for reducing analysis cost), although studies of toxicant distribution patterns show that different biological compartments (gills, mantle, plasma, digestive gland, gonads, muscle, and other viscera) may contain variable concentrations of contaminants, e.g. (Page et al., 1995; Raftopoulou and Dimitriadis, 2011; Rocha et al., 2015a; Ricciardi et al., 2016), and although a seemingly homogenous group of mussels collected from a field population may contain individuals

that are different with respect to relevant biological factors such as taxonomy, gender, dietary condition and spawning status (see confounding factors section). Furthermore, as different mussel watch programs may vary considerably in content, duration, and other design-oriented factors, it is a question whether different mussel watch programs are comparable with each other. To address this, Cantillo et al. (1998) compared chemical concentration data from US and French mussel watch programs with data from worldwide studies. They found generally good agreement for medians among all three data sets, whereas the upper ends of the worldwide data tended to be higher compared to their US and French counterparts. This difference probably reflects the fact that the latter two programs emphasize collection of mollusks at representative sites rather than within small areas of extreme contamination such as near waste discharges. This exemplifies that technical differences at all levels of the monitoring activity (field work, sample procession, sample analyses, data analysis) may add to the variance and hence hamper comparability of mussel monitoring studies. To achieve better comparability a harmonization of monitoring procedures and/or use of standard materials would be required. In this connection, some studies suggest the use of an active monitoring design by means of mussel deployment (see mussel caging later) and more standardized procedures for mussel monitoring at certain industrial sites, such as around an offshore oil production facility, e.g. Gorbi et al. (2008).

In Norway, mussel monitoring activities with use of *Mytilus edulis* have been a part of the national coastal environmental monitoring program (MILKYS) running since 1981 (Green et al., 2016). In this program, there has been a general lowering trend of most legacy contaminants in mussels from Norwegian coastal waters during the recent 30 years; although there are certain pollutants in some areas that occur either in increasing concentrations or in levels significantly above typical (unpolluted) background level (Figure 4 A&B). By using the whole MILKYS data set, time trend analyses were performed on a selection of 30 representative contaminants or their effect (Vas Deferens Sequence Index, VDSI, a measure of the effect of tributyltin (TBT) in female neogastropods), and included data for 2015 and totalled 829 data series[2]. Of these 829 cases, 52% could be classified and there were 59 cases where median concentrations were in Class II or higher as judged by the Norwegian Environment Agency classification system (Molvær et al., 1997), or above what is expected in only diffusely contaminated areas (collectively termed: “over presumed high background concentrations”). Of the 829 data series recent and significant trends were registered in 98 cases, of which 81 (9.8%) were downward trends and 17 (2.1 %) were upward trends (Figure 4A). Of the 431 cases that could be classified by the system of the Norwegian Environment Agency, 378 (87.7 %) were classified as insignificantly polluted (Class I), 48 (11.1 %) as moderately polluted (Class II), 4 (0.9 %) as markedly polluted (Class III), 1 (0.2 %) as severely polluted (Class IV) and none as extremely polluted (Class V, Figure 4B). The observed downward trends were primarily associated with metals (47.2 %), tributyltin (TBT, 6.6 %) and VDSI (3.3 %) (Figure 4A). The upward trends were also mainly associated with

[2] Consisting of one or more annual medians contrasting earlier reports which tallied only datasets of five or more annual medians.

metals (82.4 %), primarily Hg (29.4 %). There were only five cases that were classified higher than Class II (Figure 4B). In Class III there was one case for arsenic and PCB, two cases for DDT metabolite. In Class IV there was also one case for DDE, which is the degradation product of the pesticide DDT (ibid.).

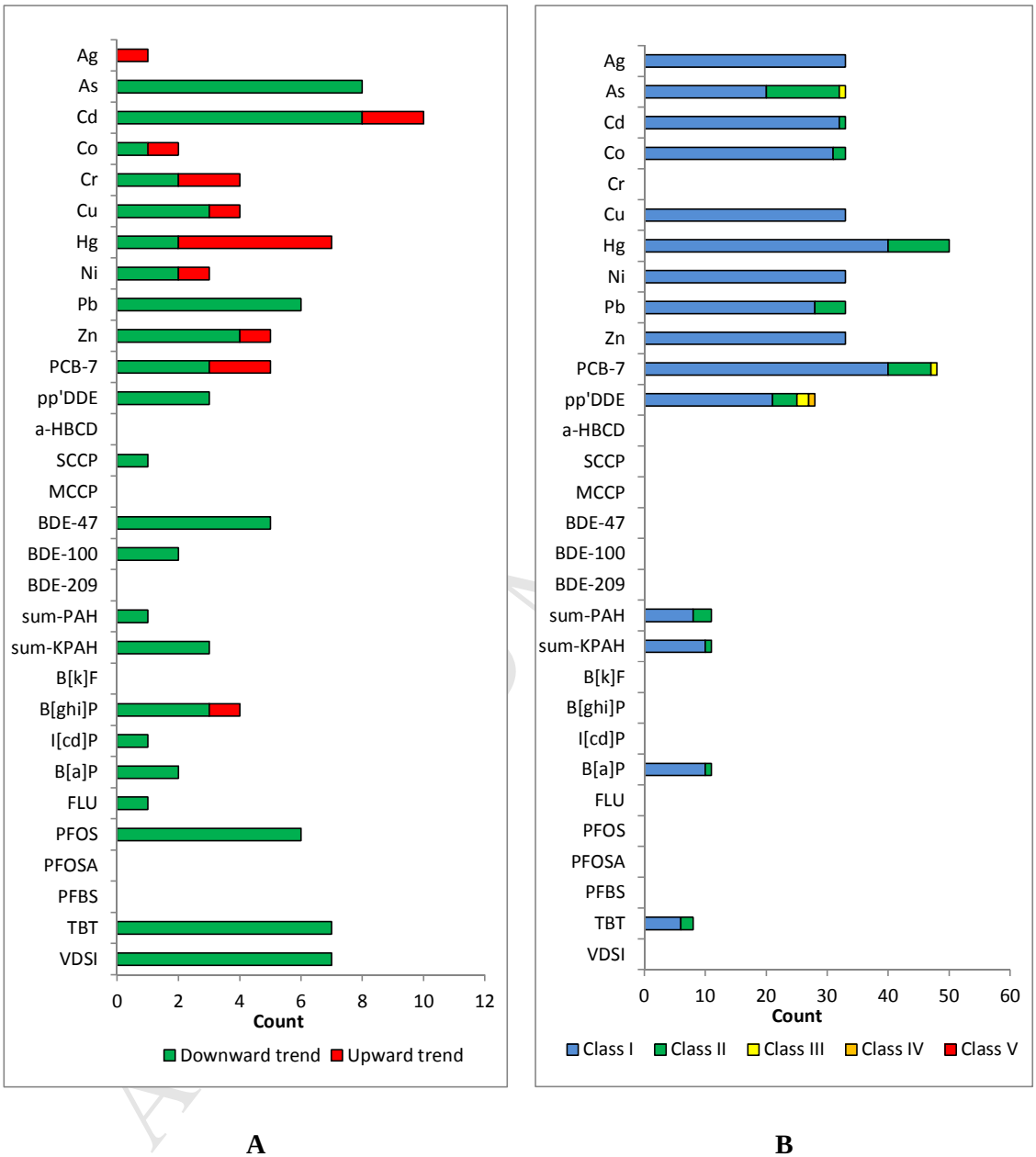

Figure 4: Figure that summarizes 829 recent trends (A) and classification of levels (B) of 30 key contaminants in Norwegian mussel watch activities. Data source: MILKYS trends report (Green et al., 2016).

5. Regulatory monitoring and environmental quality standards

In Europe, the Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC) are two wide-ranging environmental legislation frameworks designed for the protection and restoration of aquatic environments (Borja et al., 2010). The two legislations overlap spatially in coastal waters, as the WFD concerns all water bodies on land and to coastal waters extending 1 nautical mile from the coastline, whereas the MSFD covers all marine waters from the low-water line (baseline of territorial waters) until the 200 nautical mile Exclusive Economic Zone border. These coastal waters are also the key habitats for *Mytilus* spp., hence blue mussel sentinels are relevant for both WFD and MSFD.

A key part of the WFD is the development and use of Environmental Quality Standards (EQSs) of prioritized hazardous substances (PS) in different aquatic media (waters, sediments, water living biota) as described by the EQS Directive (EQSD) (directive 2013/39/EU, replacing directive 2008/105/EC) (EC, 2008, 2013). The biota EQSs under WFD are designed for fish sentinels unless other taxons are specified, e.g. EQS for polyaromatic hydrocarbons (PAHs) are defined for crustacean or shellfish sentinels as fish are considered as an unsuitable monitor for this pollutant class. The EQSs under WFD are set to represent the contaminant concentrations below which no chronic effects are expected to occur (concerning also secondary poisoning and human health effects); see (EC, 2011, 2014) for technical guidance documents for deriving EQSs under WFD. EQSs for 45 PS (or groups of such) are outlined by EQSD for aqueous samples (not shown). The WFD EQSs serve as thresholds for assessing the water body for compliance to Good Environmental Status (GES)[3] and as regulatory benchmarks to decide whether any remediating measures are required. EQSs under WFD are determined by use of a risk-based approach, i.e. incorporating toxicity testing, predicted no effect concentration (PNEC) data and the use of safety factors to encompass for uncertainty. This risk-based approach is different from the earlier used regulatory environmental assessment criteria, which mostly were based on environmental concentration data (assessed in both non-polluted and polluted waters). All marine biota EQSs, which so far have been developed under WFD (EQSD) are shown in Table 2 (first shaded column), and the table also includes other marine biota quality standards that have been developed either by OSPAR or by Norway (two next shaded columns). To allow comparison of these risk-based EQSs to relevant empirical environmental concentration data for mussels, Table 2 also includes background assessment criteria developed by OSPAR and Norwegian environmental classification standards (representing insignificantly polluted (Class I) and as moderately polluted (Class II) situations) as well as information about typical background concentrations of contaminants (10% and

[3] The WFD and the MSFD are two major policies at the EU level, which were designed to achieve “good ecological status" (WFD) or "good environmental and chemical status" (MSFD) (herein jointly termed Good Environmental Status, GES) for all European water-bodies by the year 2015 and 2020. These two directives also set out to ensure the continued protection and preservation of the environment and the prevention of further deterioration.

90% percentiles from background and slightly impacted stations) measured in the Norwegian coastal monitoring program. Several key issues can be highlighted from data shown in Table 2. Most importantly, for the four prioritized substances brominated diphenyl ethers (BDEs), mercury, TBT (formulation based) and PCB7 the risk based marine biota EQS, which currently are valid under WFD, are set considerably lower than those measured in mussels from remote non-polluted coastal sites in Northern Norway. In other words, blue mussels from non-polluted coastal water bodies in Northern Norway failed to be compliant to the EQS set by the WFD for BDEs, mercury, TBT and PCB7. This non-compliant classing of such very remote coastal sites far from any significant anthropogenic pollutant inputs appears illogical and it could possibly indicate that the current EQS assessment criteria for these substances are set too low. It is therefore questionable whether these EQSs are operational for use in industrial compliance monitoring in coastal waters, i.e. to define when there is and when there is not a regulatory demand for source reducing measures.

Several studies have expressed concerns related to the suitability of the current biota EQSs under WFD (Carere et al., 2012; Besse et al., 2012; Lava et al., 2014; Ricci et al., 2016). One concern is related to the biota EQSs for chemical monitoring in fish being designed for whole body samples and not for certain tissues. For fish sentinels, this whole-body-sample approach could be suboptimal not least as toxicants often distribute extremely unevenly *in vivo* (due to large tissue-vise and species-vise variation in lipid content). For mussels, on the other hand, the contaminant analyses are done by using the whole-body as the sample matrix, and this is well established worldwide. An interesting possibility for future revisions of the WFD is therefore to develop a broad set of biota EQSs that are specially adapted for *Mytilus* spp. sentinels, and several reports have emphasized the relevance of doing so, e.g. (Zaldivar et al., 2011; Besse et al., 2012; Maggi et al., 2012; Ricci et al., 2016). It would be appropriate if such EQSs targeted for blue mussels were developed for the priority substances that are already identified under EU WFD, and possibly also supplemented with key substances that are prioritized by various coastal nations because of special national conditions. The selection of priority substances under EU WFD are performed by working groups under the EU commission. The process is principally risk-based, i.e. depending on the quantity of substance released combined with the substance properties for persistence, bioaccumulation, and toxicity. Expert groups at ICES and OSPAR are important for the priority substance selection process. ICES has highlighted key substances of concern regarding marine environments, these include: toxic transition elements (arsenic, cadmium, chromium, copper, mercury, nickel, lead, and zinc); organometallic compounds (TBT); hydrocarbons (PAHs); priority substances listed in Annex II of Directive 2008/105/EC[4]; and synthetic compounds (pesticides, antifoulants, and pharmaceuticals). Environmental assessment

[4] DIRECTIVE 2008/105/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. Official Journal of the European Union, L 348/84.

criteria for mussels can also be applied in a standardized manner to biomonitoring studies using transplanted mussel cages (see caging section later). However, the problems described and discussed above demonstrate that developments of *Mytilus* EQS values cannot be based on the same principles for EQS_{biota} as those now available (trophic transfer and "secondary poisoning"), but that a different approach may be necessary. For instance, using mussels as an organism to reflect water concentrations, i.e. that the base for development of EQS_{mussel} could be a mathematical conversion from $EQS_{seawater}$, based on known bioaccumulation properties of the contaminants in mussels.

The overall objective of the EU Marine Strategy Framework Directive (MSFD) (2008/56/EC) is to achieve or maintain Good Environmental Status (GES) in all European seas by 2020, and eleven qualitative *Descriptors* of GES are defined in Annex I of MSFD, see explanation of descriptors in Borja et al. (2010) and Lyons et al. (2010). The MSFD also requires participating states to develop a robust set of study tools and assessment criteria for providing documentation of all 11 GES Descriptors. The use of blue mussels as sentinels can be highly relevant for several of these MSFD GES, such as Descriptor 8 ‘‘Concentrations of contaminants are at levels not giving rise to pollution effects”, Descriptor 9 “Contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards” and Descriptor 10 “Properties and quantities of marine litter do not cause harm to the coastal and marine environment”. Furthermore, for Descriptor 5, which partly concerns harmful algae blooms, the involvement of blue mussel as test medium for assessing presence and levels of toxic algae in the ambient seawater is highly relevant and could provide a valuable tool. Similarly, as for the WFD EQSs, the MSFD Descriptors 5, 8, 9 and 10 rely on defining environmental stressor levels (e.g. contaminant concentrations) that represent thresholds for quality status compliance. Hence, the quality standards and empirical data shown in Table 2 has relevance also for the development and use of *Mytilus* spp. data as assessment criteria in conjugation with MSFD. The possible use of biological effect parameters in *Mytilus* spp. as tools to meet challenges outlined by MSFD is further addressed in the biomarker section.

564 **Table 2: Risk based (shaded cells) and background-based quality standards for blue mussel (*Mytilus edulis*) unless otherwise specified. Standards are from EU**
565 **(Environmental Quality Standards (EQS), European Commission food standard), OSPAR (Environmental Assessment Criteria (EAC), Background assessment**
566 **criteria (BAC)), and Norway (see footnotes). All concentrations in µg/kg wet weight.**

Name of substance	CAS-no	EQS biota [5]	OSPAR EC/EAC [6]	Nor-wegian [7]	OSPAR BAC [6]	Indi-cator	Norw. Class I	Norw. Class II	Background and slightly impacted stations		
									10 %	90 %	Count [8]
Brominated diphenyl ethers (BDEs) [9]	32534-81-9	0.0085							0.006	0.604	*22*
C10-C13 chloroalkanes (SCCP)	85535-84-8			6000 [10]					1.71	44.48	*9*
DDT, total of four isomers [12]				609 [10]	0.11 [11]	DDE (p,p') [13]	2	5	0.075	0.480	*59*
Decamethylcyclopentasiloxane (D5)	541-02-6			15217							
Di(2-ethylhexyl)-phthalate (DEHP)	117-81-7			2900 [10]							
Dicofol	115-32-2	33									
Diflubenzuron	35367-38-5			730							
Dioxins and dioxin-like compounds [20]	[21]	0.0065 [22]				TCDDN	0.00002	.0005	0.00002	0.00016	*11*
Endosulfan	115-29-7			370 [10]							
Heptachlor and heptachlor epoxide	76-44-8/ 1024-57-3	0.0067									
Hexabromocyclododecane (HBCDD)	[23]	167				□-HBCD			0.020	0.150	*9*
Hexachlorobenzene (HCB)	118-74-1	10			0.11 [11]		0.1	0.3	0.037	0.099	*62*
Hexachlorobutadiene (HCBD)	87-68-3	55									
Hexachlorocyclohexane (HCH)	608-73-1		0.25 [11]	61 [10]	0.17 [11]	□-HCH	1	3	0.050	0.128	*61*
Hexabromocyclododecane (HBCDD)	[23]	167				□-HBCD			0.020	0.150	*9*
Medium chained chloroalkanes (MCCP)	85535-85-9			170					5.56	115	*8*
Metals and organo metals											
Arsenic (As) [17]	7440-38-2						1748 [11]	5244 [11]	1382	3392	*26*
Cadmium (Cd) and its compounds	7440-43-9		1000		168 [11]		349.6 [11]	874.0 [11]	131	344	*65*
Copper (Cu) [17]	7440-50-8				1049 [11]		1748 [11]	5244 [11]	930	1465	*69*
Chromium (Cr) [17]	7440-47-3						524 [11]	1758 [11]	82	404	*31*
Lead (Pb) and its compounds	7439-92-1		1500		227 [11]	Total Pb	524.4 [11]	2622.0 [11]	105	449	*68*
Mercury (Hg) and its compounds	7439-97-6	20	500		15.7 [11]	Total Hg	35.0 [11]	87.4 [11]	8	29	*69*
Nickel (Ni) and its compounds	7440-02-0					Total Ni	874.0 [11]	3496.0 [11]	153	355	*32*
Silver (Ag)	7440-22-4						52.4 [11]	175 [11]	4	13	*32*
Zinc (Zn) [17]	7440-66-6						34960 [11]	69920 [11]	13400	23460	*70*
Tributyltin compounds (Tributyltin cation)	36643-28-4 688-73-3			150 [10]							
TBT formulation based	688-73-3		2.1 [11]				17.5 [11]	87.4 [11]	1.61	31.85	*20*
Triphenyltin	639-58-7			152							
Naphthalene	91-20-3		59.4 [11]	2400 [10]					0.50	15.52	*32*
Nonylphenol (4-Nonylphenol)				3000 [10]		4-t-NP [14]			18.7	233	*10*
Octylphenol (4-(1,1',3,3'-tetramethyl-butyl)-phenol)				0.004 [10]		4-n-OP [15]			1.0	46	*10*
Polycyclic aromatic hydrocarbons (PAH)											
Acenapthene	83-32-9								0.30	2.00	*32*
Acenaphthylene	208-96-8								0.20	0.57	*32*
Anthracene	120-12-7		50.7 [11]	2400 [10]					0.20	2.83	*32*
Benzo[a]anthracene	56-55-3		14.0 [11]	304	1.42 [11]				0.28	4.02	*32*
Benzo[a]pyrene [16]	50-32-8	5	105. [11]		0.24 [11]		1	3	0.20	0.77	*32*
Benzo[ghi]perylene	191-24-2		19.2 [11]		0.44 [11]				0.20	1.90	*32*
Chrysene [17]	218-01-9				0.44 [11]				0.50	8.26	*26*
Dibenzo[ah]anthracene [17]	53-70-3					DBA3A			0.20	0.50	*31*
Fluoranthene	206-44-0	30	19.2 [11]		2.13 [11]				0.77	26.65	*32*
Fluorene [17]	86-73-7								0.43	2.71	*32*
Indeno[1,2,3-cd]pyrene	193-39-5				0.42 [11]				0.23	1.41	*32*
Phenanthrene [17]	85-01-8		297 [11]		1.92 [11]				1.22	11.10	*32*
Pyrene [17]	129-00-0		17.5 [11]		1.57 [11]				0.30	13.96	*32*
Sum PAH15 [18]									3.91	28.57	*28*
Sum PAH16 [19]							50	200			
Sum carcinogen PAHs							10	30	<0.01	7.20	*28*

Name of substance	CAS-no	EQS biota [5]	OSPAR EC/EAC [6]	Nor-wegian [7]	OSPAR BAC [6]	Indi-cator	Norw. Class I	Norw. Class II	Background and slightly impacted stations		
									10 %	90 %	Count [8]
Polychlorinated biphenyls (PCB)											
CB28	7012-37-5		1.02 [24]		0.13 [11]				0.050	0.100	*67*
CB52	35693-99-3		1.64 [24]		0.13 [11]				0.050	0.224	*67*
CB101	37680-73-2		1.84 [24]		0.12 [11]				0.073	0.401	*67*
CB105	32598-14-4				0.13 [11]				0.050	0.150	*62*
CB118	31508-00-6		0.38 [24]		0.10 [11]				0.074	0.352	*67*
CB138	35065-28-2		4.82 [24]		0.10 [11]				0.088	0.583	*67*
CB153	35065-27-1		24.1 [24]		0.10 [11]				0.108	0.706	*67*
CB156	38380-08-4				0.10 [11]				0.050	0.090	*62*
CB180	35065-29-3		7.13 [24]		0.10 [11]				0.050	0.088	*67*
Sum PCB7	1336-36-3			1			4	15	0.268	2.170	*65*
Pentachlorobenzene (QCB)	608-93-5			50 [10]					0.030	0.058	*64*
Pentachlorophenol	87-86-5			180 [10]							
Perfluorooctane sulfonic acid (PFOS) and its derivatives	1763-33-1	9.1									
Perfluorooctanoic acid (PFOA)	3825-26-1			91.3							
Teflubenzuron	83121-18-0			609							
Trichlorobenzenes	12002-48-1			490 [10]							
Triclosan	3380-34-5			15217							
Tri(2-chloroethyl)phosphate (TCEP)	115-96-8			7304							

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[5] EQSD - Directive 2013/39/EU EC, 2013. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy, in: Union, T.E.P.a.t.C.o.t.E. (Ed.), Official Journal of the European Union, p. 17.

[6] Refers to «mussels», see http://dome.ices.dk/osparmime/main.html

[7] NEA Norwegian Environment Agency NEA, 2016. Quality standards for water, sediment and biota (report in Norwegian). Norwegian Environment Agency, Oslo, Norway, p. 24., report no. M608 (in Norwegian)

[8] Each count represents the number of station averages the statistic is based on. Each station average is based on all annual medians for the entire monitoring period (1991-2015). Each annual median is based usually on three replicates.

[9] Sum of PBDE congeners: 28, 47, 99, 100, 153 and 154

[10] EU prioritized substance but EQS for biota established in Norway based on EU-TGD.

[11] Converted from the original threshold on the preferred dry weight basis based on 17.38 % dry weight (an average of 4279 samples from Norwegian waters under the MILKYS programme for the period 1981-2015). Values are rounded off.

[12] DDT total comprises the sum of the isomers 1,1,1-trichloro-2,2 bis (p-chlorophenyl) ethane (CAS number 50-29-3); 1,1,1-trichloro-2 (o-chlorophenyl)-2-(p-chlorophenyl) ethane (CAS number 789-02-6); 1,1-dichloro-2,2 bis (p-chlorophenyl) ethylene (CAS number 72-55-9); and 1,1-dichloro-2,2 bis (p-chlorophenyl) ethane (CAS number 72-54-8).

[13] CAS-no. 72-55-9

[14] CAS-no. 84852-15-3

[15] CAS-no. 140-66-9

[16] Benzo(a)pyrene can be considered as a marker for the PAHs: benzo(b)fluoranthene (CAS 205-99-2), benzo(k)fluoranthene (CAS 207-08-9), benzo(g,h,i)perylene (CAS 191-24-2) and indeno(1,2,3-cd)-pyrene (CAS 193-39-5)

[17] Not described in NEA Norwegian Environment Agency NEA, 2016. Quality standards for water, sediment and biota (report in Norwegian). Norwegian Environment Agency, Oslo, Norway, p. 24., report no. M608

[18] Sum of 15 PAHs: acenaphthene (CAS 83-32-9), acenaphthylene (CAS 208-96-8), anthracene (CAS 120-12-7), benzo[a]anthracene (CAS 56-55-3), benzo[a]pyrene (CAS 50-32-8), benzo[b]fluoranthene (CAS 205-99-2), benzo[ghi]perylene (CAS 191-24-2), benzo[k]fluoranthene (CAS 207-08-9), chrysene (CAS 218-01-9), dibenzo[a,h]anthracene (CAS 53-70-3), fluoranthene (CAS 206-44-0), fluorene (CAS 86-73-7), indeno[1,2,3-cd]pyrene (CAS 193-39-5), phenanthrene (CAS 85-01-8) and pyrene (CAS 129-00-0).

[19] Sum of 16 PAHs: PAH15 plus naphthalene (CAS 91-20-3).

[20] Sum of: polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and dioxin like PCBs (PCB-DL, i.e. PCB congeners: 77, 81, 126, and 169)).

[21] Refers to the following compounds: 7 polychlorinated dibenzo-p-dioxins (PCDDs): 2,3,7,8-T4CDD (CAS 1746-01-6), 1,2,3,7,8-P5CDD (CAS 40321-76-4), 1,2,3,4,7,8- H6CDD (CAS 39227-28-6), 1,2,3,6,7,8-H6CDD (CAS 57653-85-7), 1,2,3,7,8,9-H6CDD (CAS 19408-74-3), 1,2,3,4,6,7,8-H7CDD (CAS 35822-46-9), 1,2,3,4,6,7,8,9-O8CDD (CAS 3268-87-9) 10 polychlorinated dibenzofurans (PCDFs): 2,3,7,8-T4CDF (CAS 51207-31-9), 1,2,3,7,8-P5CDF (CAS 57117-41-6), 2,3,4,7,8-P5CDF (CAS 57117-31-4), 1,2,3,4,7,8-H6CDF (CAS 70648-26-9), 1,2,3,6,7,8-H6CDF (CAS 57117-44-9), 1,2,3,7,8,9-H6CDF (CAS 72918- 21-9), 2,3,4,6,7,8-H6CDF (CAS 60851-34-5), 1,2,3,4,6,7,8-H7CDF (CAS 67562-39-4), 1,2,3,4,7,8,9-H7CDF (CAS 55673-89-7), 1,2,3,4,6,7,8,9-O8CDF (CAS 39001-02-0) 12 dioxin-like polychlorinated biphenyls (PCB-DL): 3,3',4,4'-T4CB (PCB 77, CAS 32598-13-3), 3,3',4',5-T4CB (PCB 81, CAS 70362- 50-4), 2,3,3',4,4'-P5CB (PCB 105, CAS 32598-14-4), 2,3,4,4',5-P5CB (PCB 114, CAS 74472-37-0), 2,3',4,4',5-P5CB (PCB 118, CAS 31508-00-6), 2,3',4,4',5'-P5CB (PCB 123, CAS 65510-44-3), 3,3',4,4',5-P5CB (PCB 126, CAS 57465-28-8), 2,3,3',4,4',5-H6CB (PCB 156, CAS 38380-08-4), 2,3,3',4,4',5'-H6CB (PCB 157, CAS 69782-90-7), 2,3',4,4',5,5'-H6CB (PCB 167, CAS 52663-72-6), 3,3',4,4',5,5'-H6CB (PCB 169, CAS 32774-16-6), 2,3,3',4,4',5,5'-H7CB (PCB 189, CAS 39635-31-9).

[22] Value as TEQ: toxic equivalents according to the World Health Organization 2005 Toxic Equivalence Factors Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E., 2006. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicological Sciences 93, 223-241..

[23] Refers to 1,3,5,7,9,11-Hexabromocyclododecane (CAS 25637-99-4), 1,2,5,6,9,10- Hexabromocyclododecane (CAS 3194-55-6), α-Hexabromocyclododecane (CAS 134237-50-6), β-Hexabromocyclododecane (CAS 134237-51-7) and γ-Hexabromocyclododecane (CAS 134237-52-8).

[24] Converted from the original threshold on the preferred lipid weight basis based on 1.52 % lipid weight (an average of 2982 samples from Norwegian waters under the MILKYS programme for the period 1981-2015). Values are rounded off.

6. Biomarkers of exposure and effects of pollutants in blue mussels

Pollutant responsive biomarkers have gained much momentum in marine ecotoxicology, most often with fish as target organisms, for overview see van der Oost et al. (2003). But the many interesting potentials related to biomarker detection in mussels was recognized early on, e.g. Moore (1991), and the number of blue mussel biomarker studies reported in environmental science journals has steadily increased and counts presently to more than 1700 studies, or approximately 10% of all reported biomarker studies. An overview of these sorted per ecotoxicant category is shown in Table 3.

Within the many mussel biomarker reports, oxidative stress is the issue that most frequently has been addressed. Oxidative stress is an adverse condition that results from a prolonged imbalance between the *in vivo* concentration of toxic free radicals derived from oxygen, nitrogen or sulphur molecules and the capacity of the organisms' antioxidant defense system to neutralize these highly active oxidative molecules (Winston and Digiulio, 1991; Lushchak, 2011). Radicals are produced naturally during cellular respiration and metabolism and radicals have also essential roles in cell signaling, apoptosis, gene expression and ion transportation; but excess levels will cause an array of damaging effects to tissues, cells, and subcellular components, e.g. lipid peroxidation, DNA lesions and mutations (Lue et al., 2010). Many environmental contaminant stressors, including transition metals, PAHs, ionization radiation, organochlorine, and organophosphate pesticides, are known to cause oxidative stress. The antioxidant defense systems in *Mytilus* spp. involve both actions of molecular antioxidants such as glutathione (GSH) and the activities of glutathione *S*-transferase (GST), GSH-peroxidase and catalase preferably in gill and digestive gland tissues (Power and Sheehan, 1996; Fitzpatrick et al., 1997; Regoli, 1998; Manduzio et al., 2004; Letendre et al., 2006; Einsporn et al., 2009; Fernandez et al., 2010; Gonzalez-Rey and Bebianno, 2011).

Effect issues related to the gills are addressed in about 25% of all blue mussel biomarker papers reported in the scientific literature, emphasizing the key role of gills in uptake and elimination kinetics and as a highly suitable matrix for studies of oxidative stress effects and histopathological issues. Soldatov et al. (2007) summarized the research on tissue specifics of the enzymatic antioxidant complex in *M. galloprovincialis* and concluded that the gill is the tissue suffering most when environmental toxicants cause oxidative stress, thus rendering the gills suitable for ecological diagnostics on these issues. Multiple studies have employed histological and histopathological markers in mussel gills for assessing pollutant stress effects, e.g. (Bignell et al., 2011; von Moos et al., 2012; Katalay et al., 2016). The mussel gills can also be the source of live gill cells, which can be prepared and used as *in vitro* test models for toxicological studies (Gomez-Mendikute et al., 2005).

DNA is one of the cellular components that are highly susceptible to oxidative stress and the assessment of DNA damage is considered a crucial biomarker for evaluating the genotoxic potential of

environmental stressors. Among the assays available in literature, the comet assay (or single Cell Gel Electrophoresis) is the most widely used to detect DNA damage in mussels in response to several genotoxic agents, either in the hemocytes or in gill cells (Jha, 2008; Almeida et al., 2011; Gomes et al., 2013; Avio et al., 2015). The comet assay is highly sensitive for measuring DNA strand breaks (single and double), and can also be used for quantification of base oxidations when used in concert with endonucleases that have specificity for oxidized bases (i.e. to introduce breaks that subsequently are measured using the comet assay) (Azqueta et al., 2009). The overall cellular DNA damage can be visualized under the microscope and estimated from several measures of the proportions and relationships between the comet head (undamaged DNA) and tail (broken DNA strand fragments), being the size of the tail proportional to the amount of damaged DNA (Dixon et al., 2002; de Lapuente et al., 2015). Another assay that can be used in association with the comet assay to provide a more realistic analysis of genotoxic effects on a higher level in mussels is the micronucleus (MN) test. The MN test allows for the identification of chromosomal DNA damage that result from either chromosomal breakage during cell division or chromosome mis-segregation during mitosis (Bolognesi and Fenech, 2012). Similarly to the comet assay, the frequency of MN can be assessed by microscopic visualization of mussel cells from both laboratory and field studies (Dixon et al., 2002; Bolognesi and Fenech, 2012; Rocha et al., 2014). Both genotoxicity biomarkers are routinely applied in large-scale biomonitoring programs using standardized protocols developed specifically for both hemocytes and gill cells of mussels (Azqueta et al., 2009; Bolognesi and Fenech, 2012).

Pathological changes to the integrity of lysosomal organelles (lysosomal membrane stability) in cells of the mussel immunocompetence system is a key ecotoxicity biomarker in blue mussels. Initially, the earliest studies in this field were focused on digestive cells localized within the mussel digestive gland, e.g. (Moore, 1976; Moore et al., 1978; Lowe et al., 1981; Viarengo and Moore, 1982; Viarengo et al., 1985; Winston et al., 1991), whereas the focus shifted later more over to circulating hemocytes (Coles et al., 1994; Lowe et al., 1995; Grundy et al., 1996a; Grundy et al., 1996b; Moore et al., 1996). Although similar effects can be detected both places, the hemocytes are easier to collect and use for the Neutral Red (NR) retention assay (Lowe et al., 1995), a colorimetric cell test which involves the incubation of cells with the toxic dye NR and that earlier had been developed for the assessment of cytotoxicity of yeasts (Ogawa, 1961). NR added to the cell medium is taken up by cells and accumulates in the cell lysosomes, which are essential for the defense against toxic substances, until the lysosomes eventually leak the dye into the cytosol and the cell dies. The time it takes for greater than 50% of the cells to leak the dye determines the stability of the lysosomal membrane. This stability is often significantly compromised if the cell is already stressed (unhealthy) from exposure to ecotoxicants, hence the NR retention time is decreased in pollutant exposed (and health compromised) mussels. Recent developments with the NR retention assay have occurred to propose a new scoring system that increases the sensitivity of the assay. The new scoring system incorporates a value for the

quality and size of the lysosomes in addition to the time required for the lysosomes to leak from 50% of their cells. Details on the improved scoring system can be obtained from the ICES TIMES (Techniques In Marine Environmental Sciences) document (Martinez-Gomez et al., 2015). Martinez-Gomez et al (2017) recently reported the first field application of this new procedure. Although the new scoring system is a better method and considers the condition of the lysosomes at the start of the assay, it would require a revision of the current assessment criteria and validation therein. However, this will eventually occur as data are obtained from studies that adopt the improved method.

Assessment criteria have been developed for many of the mussel biomarkers in the ICES framework, including so-called background assessment criteria (BAC) and environmental assessment criteria (EAC). The 90th percentile of reference site data is used to calculate the BAC for each biomarker, with the reliability dependent on the quality and quantity of biomarker data available. The BAC calculation is therefore a continuing process as more data becomes available for the different endpoints. It is recognized that background response levels have an important role in integrating biological effects parameters into environmental risk assessments. For example, an elevated increase in one biomarker, when compared to a background response, may indicate that a chemical substance has caused an unacceptable level of biological harm. However, species differences in contaminant uptake and biomarker responses are known to occur between mussels, e.g. (Brooks et al., 2015a; Barsiene et al., 2012; Burgeot et al., 2012) (see also confounding factor section), and this has led to the development of species-specific assessment criteria for certain biomarker responses, such as micronuclei formation. For the use of biomarkers in *Mytilus* in monitoring it is important to emphasize the relevance of good quality assurance (QA) routines in the analytical laboratories. For example, the MED POL programme was the first to develop a systematic laboratory intercalibration exercise for ecotoxicological biomarkers in blue mussels (Viarengo et al., 2000).

In natural populations, mussels are exposed to complex mixtures of chemical pollutants of varying concentrations and other anthropogenic pressures that may cause a diverse range of effects, especially for mussels that live close to populated or industrialized areas. Various kinds of additive, synergistic, or antagonistic effect phenomena may develop when sentinels are exposed to several (and often multiple) toxic stressors at the same time (Beyer et al., 2014). To assess how mussels and other sentinel species in such exposed ecosystems respond to complex stresses, the so-called *integrated ecosystem assessment* (IEA) has been developed in recent years as a holistic integrated approach to biological effects monitoring. ICES suggested an IEA approach with an integrative mussel monitoring strategy as a key component, involving the monitoring of biological responses in mussels at different levels of biological complexity, including subcellular responses, tissue responses and whole organism responses, combined with contaminant concentrations measured in the whole soft tissue of the mussel (Davies and Vethaak, 2012). The recommended list of biomarkers and chemical analysis within the ICES integrative scheme is shown below (Figure 5). Although there are many more biomarkers

available, these comprise of validated methods that may be considered as more reliable and have assessment criteria associated with them. They measure a range of responses from general health to more specific effects such as neurotoxicity and genotoxicity. Sub-cellular responses are often relatively rapid following chemical exposure and can be used as an early warning signal. The response time is typically short and the effects reversible. In contrast, tissue level responses are formed following longer exposure durations and develop into structural changes within key tissues such as the digestive gland, gonad, and gills. Whole organism responses, such as scope for growth or the survival in air (stress on stress) assays, are simple but important endpoints when assessing population fitness and linking to environmental status. By combining biomarkers from all three categories and considering these responses with chemical body burden data, a holistic integrative approach may be achieved that can indicate the health of an environment or the impact a chemical mixture may be having on mussels within a certain water body. Countries participating in the OSPAR agreement are oblige to perform integrative monitoring as part of their national monitoring programs. However, due to low pressure applied on member states and constrains of monitoring budgets, very few of the countries fully comply to this demand. A more typical approach in general monitoring has been to employ a smaller number of the recommended biomarkers in mussels and focus the study more towards measuring chemical body burden concentrations. However, limiting the number of biomarkers within the integrative scheme would reduce the effectiveness of the IEA approach that could lead to under-evaluation of the state of the environment. The selection of biomarkers within an integrated framework is dependent on the type of chemical contaminant expected to dominate the water body. A recommended framework has been suggested for metal and organic exposure, selecting a combination of general and more specific biomarkers that would most likely respond to the different chemical groups (Gubbins et al., 2012).

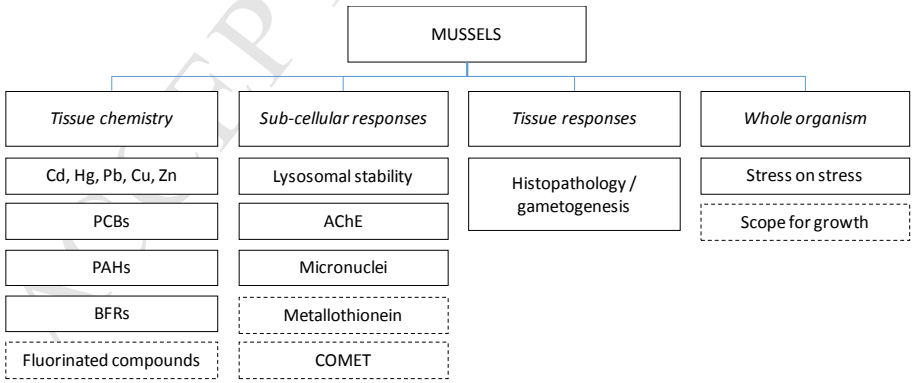

Figure 5: Recommended biological effects methods included in the mussel component of the ICES integrated ecosystem assessment. Solid lines are the core methods, whilst dotted lines represent additional methods. PCBs, polychlorinated biphenyls; PAH, polycyclic aromatic hydrocarbon; BFRs, brominated flame retardants; AChE, acetylcholinesterase. Figure adapted from Davies and Vethaak (2012).

The use of a multi-biomarker approach will often produce complex response data that can be hard to interpret, not to say integrate in environmental policy frameworks. To encompass this problem, several ways for simplifying complex data have been developed, among which the so-called *Integrated Biomarker Response* (IBR) index (Beliaeff and Burgeot, 2002) is one of the most frequently used, e.g. (Bocquene et al., 2004; Dagnino et al., 2007; Damiens et al., 2007; Hagger et al., 2008; Pytharopoulou et al., 2008; Brooks et al., 2011a; Brooks et al., 2012; Marigomez et al., 2013a; Marigomez et al., 2013b; Devin et al., 2014; Turja et al., 2014). A key element of IBR is the use of biomarker star plots as a tool for combining multiple biomarker responses into one simplified response index value. Although the IBR index is met with skepticism for possible oversimplification, misuse, and calculation bias (Devin et al., 2014), others consider it to be a useful tool that correlated well with known chemical hotspots and sources of contamination, e.g. Hagger et al. (2009). The IBR method has several times been updated and improved e.g. by including the number of biomarker observations in its calculation (Broeg and Lehtonen, 2006) and by developing an easier index calculation formula that considers all the permutation procedures (Devin et al., 2014). The latter improvement was implemented to remove possible bias in the calculation due to relative positioning of biomarkers within the star plots. However, as emphasized by Broeg and Lehtonen (2006), the IBR method represents a simplification of very complex exposure situations in the field, and the response index should not be taken at "face value" but rather as a tool to direct further actions. For instance, since the biomarker data is normalized with respect to the other mussel groups, a biomarker which is low and unresponsive in all groups, may find significant contribution to the IBR in the group where the biomarker is high relative to the other groups. It is always advised therefore to look behind the IBR calculation to ascertain which of the biomarkers are contributing and whether this is a true reflection or individual biomarker response.

Recently, a more integrated and multidisciplinary approach, the weight of evidence approach (WOE model, Sediqualsoft), has been developed and applied to different multidisciplinary studies for the characterization of highly complex and heterogeneous environments by using blue mussels e.g. (Regoli et al., 2014; Bebianno et al., 2015). This model combines different lines of evidence, including not only biomarker responses at the cellular and molecular level but also chemical characterization of environmental compartments (water or sediment), assessments of chemicals bioavailability, and data from ecotoxicological laboratory bioassays at the organism level, which all are integrated and weighted into a quantitative WOE evaluation (Piva et al., 2011). The use of these weighted criteria allows the summarization of large datasets of complex data into integrative indices that provide a decision-support tool for monitoring and management protocols (Piva et al., 2011). Even though the WOE model has proven effective in assessing the health status of blue mussels in impacted areas (e.g. harbor areas or the Costa Concordia shipwreck) (Bebianno et al., 2015; Regoli et al., 2014), the approach is still not frequently implemented in biomonitoring programs. The use of conventional

biomarkers in mussels requires a deep knowledge of toxicity mechanisms as a mean to reduce the possible bias of focusing on only few responses (e.g. specific proteins or enzymes) and overlooking others of unknown relation to the contaminant, but which could be more ecotoxicological significant. On the other hand, the use of novel molecular tools, as the –omics technologies, is thought to have a great potential for identifying new and unbiased biomarkers of exposure and effect without any previous knowledge of the toxic mechanisms of contaminants, e.g. Gomes et al. (2014a). Molecular profiling technologies such as transcriptomics, proteomics and metabolomics are used in an increasing number of laboratory and field studies with *Mytilus* and other marine sentinels to identify molecular signature profiles indicative of different environmental stressors and for identifying the mode of action (MoA) of toxic chemicals and their mixtures (Veldhoen et al., 2012; Tomanek, 2014). Today, such systems toxicology tools represent the research frontier within ecotoxicological biomarker discovery and for the study of possible links to effects at higher level of biological organization, including the effects to human health.

Table 3: This table shows an overview of reported biomarker effect studies in blue mussels in relation to the type of chemical stressors addressed.

Chemical stressor	Reported biomarker effect studies
Metals	(Romeo et al., 2003b; St-Jean et al., 2003; Knigge et al., 2004; Geffard et al., 2005; Lehtonen et al., 2006; Schiedek et al., 2006; Wepener et al., 2008; Attig et al., 2010; Kopecka-Pilarczyk, 2010; Dragun et al., 2010; Gomes et al., 2011; Hoeher et al., 2012; Gomes et al., 2012; Dabrowska et al., 2013; Gomes et al., 2014a; Gomes et al., 2014b; Poynton et al., 2014; Turja et al., 2014; Della Torre et al., 2015; Brooks et al., 2015a; Brooks et al., 2015b; Le et al., 2016)
Petroleum hydrocarbons and PAHs	(Eertman et al., 1995; Grundy et al., 1996b; St-Jean et al., 2003; Olsson et al., 2004; Brooks et al., 2011b; Brooks et al., 2011a; Sundt et al., 2011; Chatel et al., 2011; Hoeher et al., 2012; Brooks et al., 2012; Turja et al., 2014; Farkas et al., 2015)
PCBs	(Romeo et al., 2003b; Kopecka et al., 2004; Edgar et al., 2006; Vidal-Linan et al., 2016)
Dioxines	(Canesi et al., 2014; Banni et al., 2016)
Polybrominated diphenyl ethers, PBDEs	(Apraiz et al., 2006; Barsiene et al., 2006; Jonsson et al., 2006; Ji et al., 2013; Vidal-Linan et al., 2015a)
Organotins	(Lundebye et al., 1997; Smith et al., 2000; Pempkowiak et al., 2001; St-Jean et al., 2002; Devier et al., 2003; Devier et al., 2005; Hagger et al., 2005; Halldorsson et al.,

	2005; Rank, 2009; Chatel et al., 2010; Chatel et al., 2011; Turja et al., 2014; Mazzei et al., 2015; Okoro et al., 2015)
Natural toxins (e.g. algae toxins)	(Dizer et al., 2001; Kankaanpaa et al., 2007; Gorbi et al., 2012; Farcy et al., 2013; Qiu et al., 2013)
Pharmaceuticals	(Ait Ayad et al., 2011; Franzellitti et al., 2013; Gonzalez-Rey and Bebianno, 2013; Gonzalez-Rey et al., 2014; Gowland et al., 2002; Lacaze et al., 2015; Peters and Granek, 2016; Silva et al., 2016)
Pesticides	(Radenac et al., 1998; Galloway et al., 2002; Lehtonen and Leinio, 2003; Lionetto et al., 2003; Rickwood and Galloway, 2004; La Porte, 2005; Canty et al., 2007; Dondero et al., 2010; Kopecka-Pilarczyk, 2010; Ait Ayad et al., 2011; Canesi et al., 2011; Karagiannis et al., 2011; Patetsini et al., 2012; Patetsini et al., 2013; Turja et al., 2014; Kovacic and Medic, 2016)
Nanoparticles	(Koehler et al., 2008; Tedesco et al., 2008; Canesi et al., 2010; Gomes et al., 2010; Tedesco et al., 2010b; Gomes et al., 2011; Gomes et al., 2012; Barmo et al., 2013; Gomes et al., 2013; Canesi et al., 2014; Gomes et al., 2014b; Della Torre et al., 2015; Farkas et al., 2015; Rocha et al., 2015a; Banni et al., 2016; Taze et al., 2016)
Microplastics	(Browne et al., 2008; von Moos et al., 2012)

7. Confounding factors in blue mussel monitoring

In ecotoxicology, confounding factor influence are situations when one or more variable influence the status of one or more other variables in such a way that obscures the relationships of independent and dependent variables in the study, typically leading to Type I or Type II errors. Type I error (false positive) means that an "effect" is found in the observed data although there *is* no such effect around. Type II error (false negative) means that a "no effect" conclusion is drawn even though there *is* an effect. It has been recognized in both early and recent studies with mussels that several non-target factors can exert significant confounding influence and a possible masking effect on biomarker effect signals, e.g. (Hole et al., 1992; Bellas et al., 2014). Factors that exert confounding influence in *Mytilus* spp. can be the organisms' taxonomy, health, gender, age, nutritional status, condition index, metabolism, reproductive and developmental status, population density, seasonal fluctuations, ambient temperature, pollution heterogeneity, etc. (Table 4), and some key issues of this are discussed in this chapter.

The various taxa in the *Mytilus* genus can be difficult distinguish from each other morphologically, and hybrids can sometimes be phenotypically indistinguishable from the pure species. It is therefore

relevant to know whether contaminant bioaccumulation patterns, biological traits and pollution stress responses differ significantly among the genetic variants of blue mussels. The answers on this issue tend to be mixed. For example, some studies have found that *M. trossulus* accumulate certain metals to a higher extent than *M. edulis* (Lobel et al., 1990), whereas others have found that metal uptake and clearance rates vary only little between *Mytilus* species and between different climatic zones of collection, as long as the influence of different body-size is corrected for (Blackmore and Wang, 2003). Brooks et al. (2015a) found differences in bioaccumulation and biological responses to copper in the three *Mytilus* species *M. galloprovincialis*, *M. edulis* and *M. trossulus* exposed to water-borne copper at three concentrations, whereas Arnold et al. (2009), on the other hand, found no statistically different sensitivity to copper toxicity in *M. galloprovincialis* and *M. edulis*, but rather that the copper toxicity in both species was a function of organic matter concentration in the test water, a parameter which often is not reported. In a recent study from Greenland (Wenne et al., 2016) found that in a metal affected fjord *M. trossulus* coped significantly better with the low-salinity and low-temperature conditions in the inner fjord locations in comparison to *M edulis* which was more dominant in the outer fjord locations. This difference in site preferences may also have implications for differences in the metal uptake and bioaccumulation pattern. Bignell et al. (2008) investigated possible species effects between *M. edulis*, *M. galloprovincialis* and their hybrids at different points during the annual cycle at two uncontaminated field sites in UK. Twenty-nine histological health parameters were measured and overall for the annual cycle only insignificant species differences were detected. However, greater differences were observed between species during the autumn and winter than during the spring and summer, thus indicating that season may exacerbate species differences in monitoring programs.

Seasonal fluctuations in temperature, salinity, oxygen, and nutrition concentration are important confounding factors in blue mussels, not least since these mussels are generally robust and tolerant to a broad range of environmental conditions. Many studies have demonstrated significant seasonal effects in the expression levels of different pollution stress biomarkers, e.g. (Cancio et al., 1999; Shaw et al., 2004; Caricato et al., 2010; Nahrgang et al., 2013; Schmidt et al., 2013). Seasonal variation in food availability is most likely a key explaining factor in both these connections. Samples of similar sized mussels from two sites can be significantly different in age due to differences in the favorability of growth conditions between the two sites. Lobel et al. (1991) investigated confounding effects of five biological variables (sex, soft tissue dry weight, condition index, width to height ratio, chronological age) on the concentrations of 24 elements in *M. edulis* and proposed also a protocol for collecting mussels for biological monitoring programs. They found that mussel age did not significantly influence the element concentration, although a strong negative association between element concentration and both condition index and soft tissue dry weight was observed, possibly due to growth rate (dilution effect) differences. Many studies have shown seasonal fluctuations in feeding

and growth intensity as well as gonad development and spawning can influence contaminant bioaccumulation and biological effect endpoints in blue mussels (Table 4). For example, Schmidt et al (2013) examined seasonal variations for a suite of biomarkers (glutathione S-transferase, vitelline-like proteins, lipid peroxidation and DNA damage) over a 12-month period in a hybrid blue mussel population from a pristine area, and found season effects for all the biomarkers examined apparently linked most strongly to the mussel's reproductive cycle.

Blue mussels have strong seasonal growth patterns linked to food availability and reproductive development. In the Spanish mussel monitoring program, food availability and nutritional status were found to be the main parameters influencing biomarker variability in blue mussels (Gonzalez-Fernandez et al., 2015b; Gonzalez-Fernandez et al., 2015a; Gonzalez-Fernandez et al., 2016). Mussel biomarkers such as anti-oxidant enzymes, lipid peroxidation and scope for growth were significantly correlated (positively or negatively) with mussel nutritional status. The authors found that variability in food availability was a significant confounding factor which can mask the effects of contaminants on the biomarker responses. A similar concern was expressed by (Knights, 2012) who demonstrated that mussels positioned at the edge (or margin) of a mussel bed are systematically larger and in a better reproductive condition than the individuals located in the center of the mussel bed, implicating that the spatial positioning within the bed is a confounding factor that should be controlled for in mussel monitoring. During a main spawning event, blue mussels will normally lose up to 40% of their biomass (Cossa, 1989). This sudden loss of biomass represents a significant physiological challenge for the mussel, a challenge that significantly influences both chemical and biological markers in mussel monitoring. The gender is also found to be a significant confounding factor, suggesting that it sometimes could be necessary to separate male and female specimens before contaminant analyses, although this is rarely done. Monitoring protocols may suggest that all mussels collected from different sites should be of similar *relative* shell length, e.g. within 70-90% of the maximum potential length observed at the given mussel collection site, and that all mussel specimens should be collected subtidally, if available, and preferably prior to significant spawning activity.

Variability of natural oceanographic processes such as upwelling of oceanic waters near the coast may lead to significant variations in background concentrations of contaminants, for example trace metals, in mussel sentinels (OSPAR, 2016). That type of natural variability of background concentrations should be considered in the interpretation of monitoring data, and local conditions should be accounted for when assessing the significance of any exceedance of established quality standards. Measures to minimize the influence of confounding factor in mussel monitoring should most importantly involve the development and use of harmonized/standardized procedures for sample collection and sample preparation. This is further discussed in one of the following chapters, with special attention to the use of a transplant caging approach.

Table 4: Overview of factors that may exert a confounding influence to assessments of pollution responsive markers in blue mussels, and examples of relevant literature information sources.

Confounding factor	Studies reporting confounding factor influence in *Mytilus* spp.
genetic differences of *Mytilus* spp. subspecies	(Gardner and Thompson, 2001; Brooks et al., 2015a);
seasonal fluctuations in natural environmental factors (temperature, salinity, oxygen, nutrition concentrations)	(Tremblay and PellerinMassicotte, 1997; Björk and Gilek, 1997; Gardner and Thompson, 2001; Westerbom et al., 2002; Orban et al., 2002; Bodin et al., 2004; Pfeifer et al., 2005; Leinio and Lehtonen, 2005; Nesto et al., 2007; Wepener et al., 2008; Farcy et al., 2013; Schmidt et al., 2013; Mugica et al., 2015)
age, size, soft-tissue weight, growth, nutrition status and condition index	(Lobel et al., 1991; Blackmore and Wang, 2003; Bodin et al., 2004; Dragun et al., 2010; Albentosa et al., 2012; Bellas et al., 2014; Gonzalez-Fernandez et al., 2015a; Gonzalez-Fernandez et al., 2015b; Lehtonen et al., 2016)
gender, sexual maturity and spawning	(Lobel et al., 1991; Bodin et al., 2004; Farcy et al., 2013; Schmidt et al., 2013)
earlier exposure to contaminants	(Leung et al., 2008)
disturbing presence of predating, parasitic or fouling species	(Honkoop et al., 2003; Cuevas et al., 2015)
stress in connection with handling or transport	(Chandurvelan et al., 2013)

8. Mussel transplant caging in pollution monitoring

An advantage of *Mytilus* spp. is the feasibility of adopting active monitoring with use of controlled deployment (transplant caging) *in situ* (Lake et al., 1981; Widdows et al., 1981; Dekock, 1983). The principle approach in mussel caging is to obtain a homogenous group of (native or farmed) mussels matched for taxonomy, size/age, and physiological state, split it by random in sub-groups and deploy these in the sea at pre-decided stations placed along a predicted pollution gradient. Subsequently, after a certain period of caging (weeks/months/years), the mussels are collected and analyzed for chemical and/or biological endpoints of relevance to the study problem addressed. The first field studies that employed *Mytilus* transplant caging in coastal pollution monitoring started in the late eighties in US, e.g. (Salazar and Salazar, 1991), and in Europe, e.g. (Martincic et al., 1992). Subsequently, more extensive mussel caging projects were performed in the Western Mediterranean with the RINBIO

project from 1998 (Andral et al., 2004) and the MYTILOS project from 2004 (Benedicto et al., 2011; Galgani et al., 2011; Scarpato et al., 2010).

A key question in mussel caging is how long mussels should be deployed *in situ* for the contaminant concentrations in the mussels' tissue to reach a steady-state and for the deployed mussels to be equalized with native mussels. The *Mytilus* literature is very variable with respect to this issue, i.e. with reported caging periods lasting for 3 weeks (Peters et al., 1998; Zorita et al., 2006; Marigomez et al., 2013b), 4 weeks/one month (Stien et al., 1998; Utvik et al., 1999; Mauri and Baraldi, 2003; Romeo et al., 2003a; Regoli et al., 2004; Camus et al., 2004; Frenzilli et al., 2004; Gorbi et al., 2008; Schintu et al., 2008; Taleb et al., 2009; Giarratano et al., 2010; Zorita et al., 2015; Cappello et al., 2015; Turja et al., 2015), 5-6 weeks (Nasci et al., 2002; Booij et al., 2002; Ericson et al., 2002; Pampanin et al., 2005a; Orbea and Cajaraville, 2006; Regoli et al., 2014; Greenfield et al., 2014; Brooks et al., 2015b), 8 weeks/two months (Sole et al., 1998; Mauri and Baraldi, 2003; Milun et al., 2016), 12-13 weeks/three months (Salazar and Salazar, 1991; Radenac et al., 1997; Folsvik et al., 2002; Shaw et al., 2002; Oros and Ross, 2005; Pampanin et al., 2005b; Galgani et al., 2011; Benedicto et al., 2011; Scarpato et al., 2010; Beyer et al., 2013; Turja et al., 2014; Moschino et al., 2016), 18 weeks (Giltrap et al., 2013), up to 6 months (Touahri et al., 2016; Schøyen et al., this volume), or up to 2 years (Bodin et al., 2004). Comparisons of temporal bioaccumulation patters of key contaminants (e.g. trace metals, PCBs, PAHs) demonstrate that the issue of what is an optimal deployment time for blue mussels is dependent on which contaminant being targeted, generally with trace metals in deployed mussels reaching steady-state and a concentration comparable to native mussels considerably faster than the non-polar, hydrophobic organic contaminants. This suggest that in caging studies with short term deployments, there is a need for employing time-specific and contaminant-specific recalculation factors for the non-polar, hydrophobic organic contaminants to render contaminant data from deployed specimens more comparable to assumed steady-state levels in native blue mussels.

Some reported studies have employed a cross-transplantation approach, i.e. transplanting clean mussels at polluted sites at the same time as polluted mussels are deployed at clean locations, e.g. (Okumus and Stirling, 1998; Maria et al., 2009; Serafim et al., 2011; Lopes et al., 2012). An important plus of mussel caging is the opportunity for producing mussel samples from sites where native mussels are scarce or absent, and the approach also allows for improved control of confounding factors as well as opportunities of controlled manipulations of relevant factors (e.g. spatial positioning, cross-transplantation, etc.). As transplant caging offers possibility of improved experimental control the approach is sometimes recommended also when native mussel are available at the study sites (Honkoop et al., 2003; Besse et al., 2012; Bolognesi and Cirillo, 2014; Lacroix et al., 2015). The use of caging is particularly suitable when key natural factors (salinity, temperature, depth, nutrition, etc.) are within the tolerable range for the mussel, and especially when there is no significant variance for these factors between the different caging stations. However, if natural mussel populations are totally

absent from an area, that could well be for natural reasons, and this issue should thus be critically considered, although blue mussel beds are often patchily distributed, also in areas with generally favorable conditions.

Mussel monitoring procedure documents, e.g. from UNESCO (1992), NOAA (Lanksbury et al., 2010; Lanksbury and West, 2012), ICES (Davies and Vethaak, 2012), OSPARCOM (2012) and European Commission (EC, 2014), often also describe mussel deployment procedures, but as for studies on native mussel, there is no internationally harmonized guideline for how to conduct mussel caging in marine environmental monitoring. The Norwegian Environment Agency (NEA) has previously developed monitoring guidelines for the offshore oil and gas industry with mussel caging as a key element, e.g. Iversen et al. (2015). Recently NEA initiated a process to develop a national standard for the field application of mussel monitoring, including both collection of native mussels and transplant caging procedures, with planned completion in 2017. When established, that standard should include recommended procedures for how to produce mussel samples in a quality suitable for any type of monitoring endpoint. The standard should will some level of standardization for the following elements:

- Definition of key monitoring issue (e.g. metals, PAHs, organochlorine pesticides (OCPs), veterinary medicines, etc.)
- Design of station net (depending on issue and local recipient factors)
- Recommended equipment list
- Preparation of mussel deployment groups (source, biotic factors, statistical power issues)
- Caging rig design (vertical positioning in WC, parallel samples, avoiding feeding differences, etc.)
- Timing and duration of deployment
- Required supportive data of mussels (condition, reproduction status)
- General preparation procedures for biological samples

Other elements may subsequently be attached as addendums to the standard, such as: recommended suites of exposure and/or effect markers to analyze; recommended analytical procedures; procedures for possible supportive measures (e.g. passive samplers); recommended procedures for data treatment and data reporting; and recommended Quality Assurance and Quality Control measures.

9. Emerging issues in blue mussel monitoring

Microplastic particles is presently an important emerging issue in marine ecotoxicology, and ICES has suggested blue mussels as suitable sentinels for monitoring of microplastic contamination (Vandermeersch et al., 2015). However, microplastics are different from most other pollutants as they are not metabolized and they cover a range of different polymers with highly different characteristics,

some being bioavailable to mussels like the positively buoyant polyethylene, whilst the denser polymers, such as polyvinyl chloride, are considered not to be bioavailable (Wang et al., 2016). Microplastics consist of different shapes as seen for polymers found in wild mussels, although several studies have also seen a predominance of synthetic fibers in the environment (Li et al., 2015; Mathalon and Hill, 2014). At high concentration doses, microplastics may induce significant histological alterations in *Mytilus* spp. (von Moos et al., 2012), but it is unknown whether such effects will be induced at more environmentally realistic exposure levels. Microplastic effect studies are often hampered by the lack of appropriate control exposures. As mussels are constantly exposed to natural particles with the same size range as microplastics, effect tests should include control treatments to natural materials to unravel whether it is the particle *size* or the particle *material* which is the effect causing factor. When considering microplastics as an emerging contaminant, it is also important to include the debate on whether microplastic particles could act as vectors for the transport of environmental contaminants into mussels (Ziccardi et al., 2016). The ability of microplastic to act as a vector depends on a variety of factors including polymer type, octanol-water partition coefficient (K_{ow}) of the contaminant, salinity, embrittlement of the polymer, biofouling on the polymer, concentration of background contamination in the tissue of the organism exposed, pH, etc. (Nerland et al., 2014).

Another group of emerging contaminants with increasing focus in the research community are engineered nanoparticles (ENPs). Among bivalve species, *Mytilus* spp. represents so far the most used model for predicting the impact of ENPs on the health of the marine environment (Rocha et al., 2015c; Canesi and Corsi, 2016). As filter-feeders, mussels remove ENPs from the water column independently of their form (individual particles, homo- and/or hetero-aggregates), being their bioavailability and uptake dependent on their peculiar features (e.g. size, shape, surface charge), type and composition of dispersing media, presence of normal organic matter, behavior, and fate (Corsi et al., 2014). Special attention has been given to the sub-lethal effects of different types of ENPs on immune function, embryo development and the main tissues involved in nanoparticle uptake and accumulation in mussels (i.e. gills and digestive gland), and several reviews about these issues are available (Canesi et al., 2012; Rocha et al., 2015c; Canesi et al., 2015). Consensus has been achieved that as the first barrier with the surrounding water, mussel gills can take up and break down ENPs aggregates into smaller particles that can be further transported to the digestive gland. Gills are also susceptible to interaction with individual particles and ionic metal forms released from the ENPs. On the other hand, the digestive system is apparently the main target for ENPs uptake and accumulation either through translocation from the gills or directly via endocytosis, mainly in the form of aggregates, e.g. (Gomes et al., 2011; Gomes et al., 2012; Rocha et al., 2015c). A smaller role on ENPs toxicokinetics has been given to the hemolymph and circulating hemocytes, as well as to mucus, fecal pellets and gametes produced by mussels (Canesi et al., 2012; Gomes et al., 2013; Della Torre et al., 2015; Rocha et al., 2015c). Immunotoxicity, oxidative stress (e.g. lipid peroxidation), cell injury in

proteins (e.g. protein carbonylation and ubiquitination), membrane (e.g. LMS) and DNA damage are considered as the main modes of action underlying the potential toxicity of ENPs in mussels, mostly related to the direct and indirect formation of reactive oxygen species (ROS) (Rocha et al., 2015c). However, the production of radical species is not specific to all nanoparticles, as responses may differ depending on particle size, composition and concentration, type and time of exposure, as well as target tissue, being the oxidative stress responses seen commonly associated with exposure to metal nanoparticles (e.g. Ag NPs, CuO NPs, Au NPs and Fe NPs) (Tedesco et al., 2010b, a; Gomes et al., 2012; Rocha et al., 2015c; Canesi and Corsi, 2016). Similar as for microplastics, ENPs can also possibly interact with other contaminants present in the aquatic environment and cause further biological responses via synergetic, antagonistic, or Trojan horse effects, e.g. Canesi et al. (2014). Overall, a multi-biomarker approach used for evaluating the biological responses to conventional contaminants has also proven effective for the screening of sub-lethal effects of ENP ecotoxicants in mussel. The combined use of oxidative stress, lysosomal, genotoxicity, immunotoxicity and physiological biomarkers, together with toxicokinetic data (uptake, accumulation, and depuration processes) is an interesting approach for the characterization of effects and modes of action of different ENPs in mussels and it is possible that biological and toxicological responses to ENPs are common to both invertebrate and mammalian systems (Canesi et al., 2012; Rocha et al., 2015c; Canesi and Corsi, 2016). However, there are still challenges that must be overcome for mussel biomonitoring programs to fully incorporate the emerging issue of ENP pollutants and their fate and effects in coastal environments.

The possible role of mussel sentinels in monitoring of pharmaceuticals and veterinary medicine pollutants has attached increasing attention recently. One relevant example is the need for monitoring chemicals used to control sea lice pests in marine fish farming facilities, including chitin inhibitors such as diflubenzuron, teflubenzuron and neurotoxicants such as deltamethrin, cypermethrin, emamectin, ivermectin, and azamethiphos (Roth et al., 1993; Davies et al., 1997; Gowland et al., 2002; Canty et al., 2007; Ayad et al., 2011; Langford et al., 2014). In Norway, persistence and possible unintended effects of these toxic agents on non-target species (such as lobsters, crabs, and shrimps) is a concern. Local fishermen have claimed that smaller prawn catches and occurrence of lobster mortalities are possibly linked to usage of sea lice pesticides. The ability of mussels to filter large volumes of seawater and the accumulation of organic lipophilic compounds into their tissues make them an ideal species for the monitoring of the persistence and potential effects of these pharmaceutical compounds.

Global warming caused by human release of greenhouse gases is expected to affect sea surface temperatures, ocean acidification (OA), ocean currents, ocean bio-geochemistry and other large-scale processes that in sum will have wide-scale ecological implications for coastal systems in all temperate seas. For blue mussels, significant alterations on local and regional population are expected (Zippay

and Helmuth, 2012; Cahill et al., 2013), and several recent studies show that general warming of the seas is already affecting natural blue mussel populations; especially by shifting the biogeographical distribution range of the different *Mytilus* taxa closer to the poles e.g. (Berge et al., 2005; Sorte et al., 2011; Wenne et al., 2016), and by causing the more warm-water tolerant *M. galloprovincialis* to invade regions which earlier have been dominated by *M. edulis*, *M. trossulus* or other more cold-water tolerant mussel taxa, e.g. (Braby and Somero, 2006; Lockwood and Somero, 2011; Gardner et al., 2016). Other studies have focused on the possible impact on *Mytilus* populations by ocean acidification (OA) (Gazeau et al., 2010; Bechmann et al., 2011) as OA caused by an increased pCO2 in seawater is expected to decrease calcification rates of bivalves. There is also a concern that ocean warming and OA could cause various kinds of interactive stress effects in *Mytilus* (Duarte et al., 2014; Beyer et al., 2014; Kroeker et al., 2014; Eads et al., 2016) including aggravation of heavy metal pollution and enhancing trace metal toxicity (Han et al., 2014). However, the most significant climate change effect on *Mytilus* could well be through alteration of food availability, as shown by Thomsen et al. (2013).

Markedly decline and even extinction of local *Mytilus* mussel beds can also be associated with outbreaks of adverse mussel deceases and parasite infections, such as the unicellular parasite *Marteilia refringens*, which is known to disrupt the digestive system of marine bivalves (Villalba et al., 1993; Villalba et al., 1997; Fuentes et al., 2002; Carrasco et al., 2015) and which, according to news bulletins from the Institute of Marine Research (Bergen, Norway), recently was detected for the first time in Norwegian coastal waters. The risk of spreading mussel deceases should be taken into consideration in mussel transplant studies (Brenner et al., 2014), and this emphasizes the importance of only using healthy and decease free mussel populations to be the source for sentinel specimens to mussel caging studies.

10. Discussion

In this review, we provide an extract of the knowledge related to blue mussels in environmental sciences. Blue mussels are among the most frequently used sentinels in marine environmental monitoring in temperate seas worldwide, with data reported in tens of thousands of reports. Many key issues are broadly investigated in *Mytilus*, but, as indicated by the table summaries included in this paper, there are still many issues that yet are not or only little studied; such as toxicokinetics of dioxins and PBDEs or field studies of the exposure, accumulation, and effect of nanoscale particulate contaminants.

The great suitability of *Mytilus* spp. as environmental sentinels becomes evident when considering the criteria for a good monitoring species set by OSPAR commission (2012), i.e. a suitable monitoring species should:

- Reflect changes in the concentration of contaminants in the surrounding environment
- Have similar bioconcentration factors throughout the monitored maritime area
- Accumulate contaminants without being seriously affected by the concentrations typically encountered in the marine environment
- Be representative of the study area
- Be abundant throughout the study area
- Be of reasonable size so it can provide adequate amounts of tissue for chemical, biochemical, and physiological analyses
- Be easy to sample and hardy enough to survive in the laboratory, thus allowing defecation before analysis (if desired), laboratory studies of contaminant uptake, and laboratory studies for verifying biological field observations.

Because blue mussels are stationary filter feeders that generally are tolerant to handling, they are highly suitable for use in transplant caging experiments at marine field locations. The mussel caging concept is an important supplement to the use of native mussel as it opens for better opportunities of study control as well as facilitating the involvement of experimental factor manipulations in field studies. The mussel caging approach has gained increasing popularity in recent years. Already, roughly 10 % of all the reported blue mussel ecotoxicity studies have used transplant caging of mussels as the design of study, and we foresee this could further increase in the coming years.

Although the present review mainly focuses on the many positive reasons for using blue mussels as environmental sentinels, it is also important to realize the most important challenges. For example, influence from natural confounding factors or adoption to a suboptimal study design may rapidly lessen the information value of *Mytilus* field studies. It is therefore important that investigators pursue a high degree of study control in field studies both to optimize the quality of the investigation and to minimize the risk for Type I and Type II errors (see confounding factor section). Indeed, there are plentiful of examples in the blue mussel literature of studies which have not succeeded to detect any clear effect signal in sentinel mussels from waters that evidently were significantly polluted by anthropogenic sources. Most of all, this illustrates the need for critical attention among environmental researches on key aspects of study quality. After the introduction of the EU WFD in year 2000 there has been intensified collaboration among European countries regarding the environmental protection of water bodies, and with the subsequent introduction of MSFD in 2008 these efforts have been further focused and broadened on marine environments. Important tools in both these regulations are Environmental Quality Standards (EQSs), which are developed for different environmental matrices, i.e. defined concentrations of prioritized hazardous substances that defines the thresholds for compliance to regulatory demands. In this review, we have highlighted the issue of anthropogenic contaminants in blue mussels and discussed whether appropriate Environmental Assessment Criteria (benchmarks for assessing degrees of anthropogenic pollution) are available for chemical monitoring

with blue mussel sentinels, or whether such tools should be further established or improved. In this connection, we emphasize that knowledge about the typical variability of hazardous anthropogenic substances in mussels living in non-polluted and in polluted waters is important. Indeed, it is our opinion that marine mussels possibly should acquire an extended role as sentinels for marine monitoring. As described by the WFD regulation, each member state can choose to establish their own quality standards if these provide an equally good level of environmental protection (2013/39/EU §17) and guidelines for how to carry out harmonized chemical monitoring of sediment and biota under WFD have been published under the WFD Common Implementation Strategy (EC, 2010). There are several key advantages by using sediments or biota as monitoring matrices as alternatives to water samples. For example, monitoring organic micro-contaminants in water is often hampered with analytical detection problems and a need for a higher frequency of sampling and analysis. Furthermore, in many countries there is a long tradition for monitoring sediment and biota, thus to maintain time series data, continued practice is of importance. Directive 2013/39/EU also emphasize the importance of time series data, as it is stated that "member States shall determine the frequency of monitoring in sediment and/or biota to provide sufficient data for a reliable long-term trend analysis".

As blue mussels so widely are used in chemical monitoring of coastal waters, it is unfortunate that the WFD biota EQSs are generally not adapted for mussels. Scientists who are using mussel sentinels to perform compliance monitoring in coastal waters must from a regulatory standpoint use fish-based biota EQSs as assessment criteria for classifying their mussel-based monitoring data, as these are the standards that are embedded in the law. However, to use EQSs adapted for fish to assess PS concentration data in suspension feeders such as blue mussels may most often not be suitable, unless appropriate conversions are employed. However, such conversions may be questionable. In a recent study, OSPAR conducted an assessment criteria comparison (EAC/EQS) for mercury in marine biota (OSPAR, 2016). The study revealed that with an EQS conversion as described by EU's technical guidance documents (EC, 2011, 2014) 99 % of the OSPAR data exceeded the biota EQS and would not be compliant. Furthermore, they concluded that a goal to reduce this portion significantly would not be feasible. It was also concluded that "even in the absence of trophic adjustment elements (i.e. direct comparison of data to the EQS_{biota}) a significant number of OSPAR time series data would fail the EQS_{biota} threshold".

Above certain concentration levels, hazardous substances are expected to cause measurable toxic stresses to the sentinel organism itself, or to the animals that eat it (secondary poisoning). A key idea of the EQSs for marine biota established under WFD is to define the concentration thresholds of prioritized toxicants below which no adverse effects are expected in marine sentinels (regardless of primary or secondary poisoning). Hence, these benchmarks are key tools for interpretation of chemical monitoring data in marine sentinels and for quality classing of the body of seawater in which the sentinels live. As blue mussels are suitable for and so widely used in environmental monitoring, it is

1106 therefore unfortunate that internationally agreed pollution assessment criteria for mussel sentinels are
1107 largely lacking, although mussel watch programs and research surveys performed in many countries
1108 already provide much of the information required, and although assessment criteria based on
1109 background concentrations have been used in many countries. The shortage of internationally
1110 harmonized assessment criteria for mussels can partly be explained by the toxicity-based and risk
1111 based focus for the EQSs which so far have been developed for marine biota (e.g. in conjugation with
1112 the EU WFD); a focus which facilities for the use of organisms higher up in the marine food chain as
1113 target sentinels for EQSs. With basis in the knowledge reviewed in this paper, an increased use of
1114 marine *Mytilus* spp. mussels as sentinels for chemical monitoring is rational for many reasons, and that
1115 development of environmental assessment criteria specially adapted for these sentinels is a
1116 strategically important endeavor. Already, the different EU Member States are allowed flexibility to
1117 apply EQSs for alternative matrices or, where relevant, an alternative biota taxon, if the level of
1118 protection afforded by the EQS and the monitoring system applied is as good as that provided by the
1119 EQS and matrix laid down in Directive 2013/39/EU. For some PSs, the use of fish sentinels is already
1120 regarded as unsuitable, such as for assessments of PAHs, and for these contaminants the Directive
1121 2013/39/EU recommend the use of crustaceans or mollusks as sentinels.

1122 The biota EQSs under WFD are defined at the whole body level of the sentinel organism (which is *fish*
1123 unless other sentinel is indicated), i.e. the EQS standard value is not specified to a type of tissue
1124 matrix. Analyses of whole body samples of fish may often have practical constrains (e.g. large body
1125 size of fish). It may also be complicated by relevant biological factors (such as variable fat index
1126 between tissues, biotransformation etc.). Another practical issue with fish sentinels is the catch
1127 uncertainty which can make it difficult to obtain a required number of specimens in field monitoring,
1128 and in marine situations their exposure history will be generally uncertain simply because fish move
1129 around and are not sessile as blue mussels are. With respect to these issues, the increased application
1130 of *Mytilus* spp. as sentinels for chemical status assessments in coastal waters may seem more
1131 appropriate. Several recent studies point to the relevance of using *Mytilus* spp. or other marine mussels
1132 as sentinels in pollution monitoring and compliance checking against biota EQS established by WFD,
1133 e.g. (Zaldivar et al., 2011; Maggi et al., 2012; Besse et al., 2012; Helmholz et al., 2016). As suggested
1134 herein, a set of EQSs specially targeted for blue mussels should be developed for those PSs which
1135 already are included under EU WFD but also for anthropogenic contaminants that are nationally
1136 prioritized because of special national conditions.

1137 In summary, blue mussels are almost ideal as sentinels for chemical pollutant monitoring in coastal
1138 waters. They are among the most studied marine species in ecotoxicology and the toxicokinetic
1139 features of a broad range of key anthropogenic contaminants are well described in blue mussel taxa,
1140 although there are still remaining knowledge gaps calling for further research and clarification, such as
1141 for substances whose mode of uptake and accumulation deviate from general partitioning and when

there could be a concentration dependency of the uptake (e.g. for PFCs). Nevertheless, blue mussels have played a crucial role for both regional and local trend monitoring of key pollutants and for compliance monitoring of industries that release hazardous chemicals into coastal water bodies. This calls for a development of internationally harmonized assessment criteria for prioritized contaminants specially adapted for blue mussel sentinels. Such assessment criteria must, in addition to being adapted to substance toxicity issues, also be environmentally realistic, i.e. in comparison to the concentrations levels which occur in coastal waters far away from major pollutant sources. The regulatory benchmarks established for mussels, should be operative as triggers for counteracting and source-reducing measures (i.e. towards industries and other parties who are responsible for the release of prioritized contaminants). At the present, there is apparently an issue for several key PSs targeted by the WFD EQS regulations in Europe (i.e. brominated diphenyl ethers, mercury, TBT and PCB7), and these need urgent attention. Significant progress has been made regarding development and use of pollutant responsive biomarkers in blue mussel sentinels, and for emerging issues, such as micro- and nano-scale particulate contaminants, climate change and ecotoxicity of mixed pollution situations, continued progress in the knowledge is expected in the years to come. However, it is also important to further clarify and minimize the influence of confounding non-target factors in mussel monitoring, e.g. by adopting international harmonization and standardization of study conditions and program designs. Such developments could call for an increased use of mussel transplant caging as discussed herein.

Acknowledgments

This study is funded by the Norwegian Research Council through NIVA's institutional funding and the Norwegian Environmental Agency via the National Function programme. The authors want to acknowledge the reviewers of this journal for their valuable recommendations.

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Highlights

- An overview of the study-field of blue mussel ecotoxicology and pollution monitoring was provided.
- Factors that render blue mussels favorable as environmental sentinels were discussed.
- Challenges related to influence from different confounding factors were discussed.
- There is a need for standardization and harmonization of blue mussel monitoring techniques.