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# 1 **Blue mussels (*Mytilus edulis* spp.) as sentinel organisms in coastal pollution**

## 2 **monitoring: A review**

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### 9 **Abstract:**

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

20 **Keywords:** Blue mussels; sentinels; pollution monitoring

21

## 22 **1. Introduction**

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

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<sup>1</sup> 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.

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

50 In this review paper, our aim is to provide an updated overview of the broad study-field of blue mussel  
51 ecotoxicology and pollution monitoring. Both potentials and challenges for the use of blue mussels in  
52 environmental research and pollution monitoring are summarized and discussed. Trend data from  
53 long-term mussel monitoring in Norway are shown and discussed with special reference to the  
54 ongoing process driven by the Water Framework Directive on implementation of environmental  
55 quality standards (EQSs) of anthropogenic contaminants in marine biota (EC, 2000, 2008, 2013,  
56 2014). The necessity of standardized guidelines for blue mussel pollution monitoring is discussed,  
57 with special attention to a transplant mussel caging design and exemplified by a recent mussel caging  
58 study in our group (Schøyen et al., this volume). Recent developments in the field of pollutant  
59 responsive biomarkers in blue mussels are also discussed to identify markers which are operational for  
60 use in pollution effect monitoring. Lastly, the overall state of the art of blue mussel pollution  
61 monitoring is discussed and knowledge gaps and some key research needs are highlighted.



62

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

## 65 **2. Suitability of blue mussels in coastal pollution monitoring**

66 The genus *Mytilus* includes several closely related (congeneric) species (or subspecies) that can  
67 interbreed with each other and make fertile hybrids. It is often called the *Mytilus edulis* complex.  
68 Although the exact taxonomy within the *Mytilus* genus is not yet fully clarified, recent research  
69 indicates there are five species occurring in the Northern Hemisphere (*M. edulis*, *M. galloprovincialis*,  
70 *M. trossulus*, *M. californianus* and *M. coruscus*) and two in the Southern Hemisphere (*M.*  
71 *galloprovincialis*, and *M. platensis*), whereas the former *M. chilensis*, the Chilean mussel, is currently  
72 considered to be a variant of *M. platensis* (Gaitan-Espitia et al., 2016). The main native distribution  
73 range of the different *Mytilus* taxa is: *M. edulis* (North Atlantic region), *M. galloprovincialis*  
74 (Mediterranean), *M. trossulus* (northern Pacific and the Baltic Sea), *M. californianus* (coast of the  
75 North Eastern Pacific Ocean) and *M. coruscus*, (coasts of the subtropical Western Pacific Ocean) and  
76 *M. platensis* (South America). Because of the growing mussel mariculture industry and the global  
77 increase in long-range maritime transport, *Mytilus* sub-species have been introduced to areas far  
78 outside their native range. This is especially the case for *M. galloprovincialis* which has established  
79 itself as an invasive species at widely distributed locations all around the globe; including South  
80 America, South Africa, Japan, California, New Zealand, and Australia (Lockwood and Somero, 2011;  
81 Briski et al., 2012; Gardner et al., 2016). The spatial distribution of each *Mytilus* species is thought to  
82 be controlled by their tolerances of environmental factors (especially temperature and salinity) (Braby  
83 and Somero, 2006). The natural habitat requirements of *Mytilus* are described by (Hawkins and Bayne,  
84 1985; Newell, 1989). Overlapping distribution ranges and an ability of congeneric *Mytilus* species to  
85 interbreed often leads to development of mixed populations in which genetic hybrids can be  
86 phenotypically indistinguishable from the original species (Dias et al., 2008; Brooks and Farnen,  
87 2013). In these mixed (i.e. genetic inhomogeneous) populations, the use of genetic markers is

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

94 Blue mussels are suspension feeders and feed mainly on planktonic microalgae such as *Phaeodacolum*  
95 sp., *Isochrysis* sp., and *Rhodomonas* sp. (Rouillon and Navarro, 2003; Riisgard et al., 2013;  
96 Fernandez-Reiriz et al., 2015), but they can when necessary also exploit other food sources such as  
97 bacteria (Jacobs et al., 2015) and even aquaculture fish feed (Redmond et al., 2010). Each mussel  
98 filters food particles from the seawater by means of their large and ciliated gills (Cannuel et al., 2009;  
99 Riisgård et al., 2011). If the water contains a suitable concentration of food particles the mussel will  
100 continuously pump and filter seawater at a maximum rate by the coordinated action of numerous cilia  
101 that are localized at the gill epithelium surface. During active feeding the water pumping rate for one  
102 single adult blue mussel is typically about 50 ml of seawater per min (3 liters per hour) (Famme et al.,  
103 1986). Also under conditions of food surplus, the mussel will continue to filter seawater at max speed  
104 but will now expel the excess food as pseudofaeces particles, which then consist of a mixture of mucus  
105 and undigested algae. This pseudofaeces production is ecologically important for many other species  
106 but may sometimes lead to development of anoxic sediment conditions underneath dense mussel beds  
107 as well as under mussel mariculture facilities. Blue mussels may form large local populations (mussel  
108 beds), which in some areas can be several km wide and include an immense number of mussel  
109 individuals. Blue mussels represent an important food source for many shell eating animals (including  
110 humans) and mussel aquaculture is a growing industry worldwide. Currently, mussel aquaculture  
111 accounts for about 80% of the total global production of blue mussels for human consumption  
112 (<http://www.fao.org/fishery/species/2688/en>).

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

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

132 *Mytilus* spp. exhibit strong seasonal growth patterns and the mussel's condition index (CI) is an  
133 indicator of the overall favorability of the growth conditions as well as the overall biological status of  
134 the individual. The CI is normally understood as the quantitative relationship of the mussels' soft  
135 tissue weight (wet or dry) to its overall size; the latter measured as the shell dry weight, the weight of  
136 soft tissue + shell, the shell length, the shell volume, or the shell cavity volume. Hence, there are  
137 several alternative equations for estimating CI of blue mussel, as discussed by Davenport and Chen  
138 (1987). Probably, the easiest and most convenient equation for use in mussel monitoring is:  
139  $\text{CI} = (\text{MW}/\text{SW}) \times 100$ , wherein MW is the wet meat weight (g) and SW is the shell dry weight (g).  
140 Note that this calculation can easily be performed both at the level of the individual mussel and at the  
141 level of a composite (pooled) sample. The advantage of taking wet weight is that the mussel can be  
142 used for other endpoints after weighing, such as gill and digestive gland analysis. This is not possible  
143 for dry weight. However, using the wet weight of the mussel is not as reliable as the dry weight and is  
144 more dependent on the sampling method; i.e. some researchers may drain the mussel thoroughly  
145 before weighing, whilst other would not, and this may influence the weight significantly. The CI of  
146 *Mytilus* spp. vary considerably during the annual cycle depending on the mussels nutritional and  
147 reproductive status. To obtain CI data is considered important in mussel monitoring, as it provides key  
148 information regarding the overall biological status of the sampled mussels. For example, the shell from  
149 a rapidly growing individual is typically thinner compared to those from slow growing individuals and  
150 this information can assist in the interpretation of data from the environmental quality parameters  
151 which are measured in the mussel sample. It is often found that the mussels CI is negatively correlated  
152 with the *in vivo* concentration level of chemical contaminants, as slow-growing mussels will  
153 accumulate contaminants for a longer time per weight unit. Many reports have emphasized CI as an  
154 important biological value to consider in pollutant fate and effect studies with blue mussels, e.g.  
155 (Granby and Spliid, 1995; Mubiana et al., 2006; Benali et al., 2015; Touahri et al., 2016). It is  
156 unfortunate that there is apparently not yet established any firm international standard for how to  
157 estimate CI in blue mussel monitoring. Some studies, e.g. Giltrap et al. (2016), have even used the  
158 Fulton's condition factor formula:  $K = 100(W/L^3)$ , where W is meat wet weight (g) and L is shell  
159 length (cm), although that estimation method is designed for fish and not mussels.

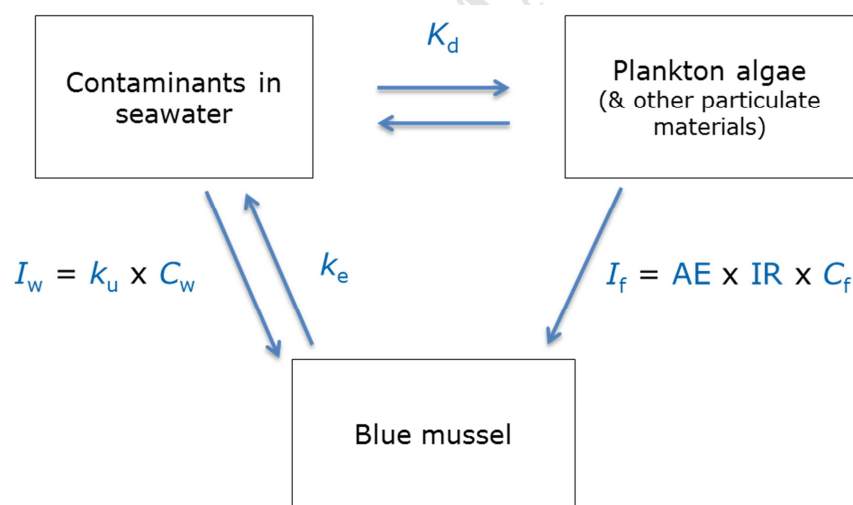


### 3. Uptake, accumulation, and depuration of anthropogenic contaminants in blue mussels

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 phenomena 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.

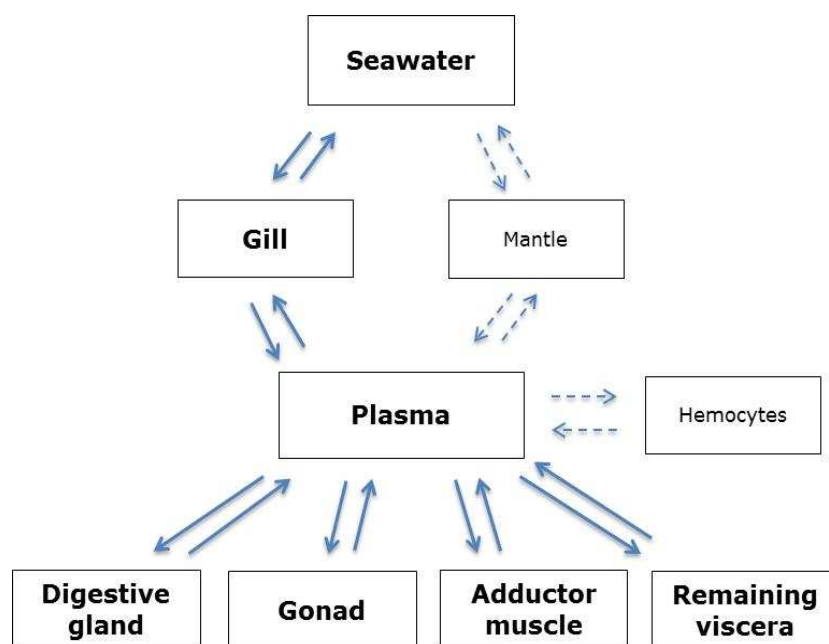
In mussels, there are three major mechanisms for the uptake of environmental contaminants: (1) uptake by passive diffusion from the dissolved phase over external surfaces (predominantly gills, but also mantle and gut wall), (2) active uptake by transmembrane ion-pump transport (gills, gut wall), and (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

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



213  
 214 **Figure 2: Diagrammatic representation of two of the key pathways of contaminant uptake and elimination**  
 215 **in blue mussels (uptake from the aqueous phase and dietary uptake) and the parameters describing the**  
 216 **rates of each pathway.  $K_d$ : partitioning coefficient between food and water;  $I_w$ : contaminant influx rate**  
 217 **from the dissolved phase;  $k_u$ : contaminant uptake rate constant from the dissolved phase;  $C_w$ :**  
 218 **contaminant concentration in the dissolved phase;  $k_e$ : efflux rate constant;  $I_f$ : contaminant influx rate**  
 219 **from the food source; AE: contaminant assimilation efficiency from ingested food; IR: ingestion rate of**  
 220 **the animal;  $C_f$ : contaminant concentration in ingested food; Illustration adapted from Wang and Fisher**  
 221 **(1999).**





222

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 secrete 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

246 passive diffusion over the external surfaces. However, several studies have shown that the  
 247 contaminant load that enters via the digestive tract can be significant for many contaminants, e.g.  
 248 Björk et al. (1997, 1999) and Axelman et al. (1999). For example, particulate metals are mostly taken  
 249 up over the digestive surface mediated by endocytosis and then transferred further to lysosomes and  
 250 residual bodies, especially in the digestive cells of the digestive gland (Marigomez et al., 2002). This  
 251 dietary uptake pathway is also most likely important for emerging particulate contaminants  
 252 (engineered nanoparticles (ENPs), microplastics, etc.), e.g. (Moore, 2006; Browne et al., 2008;  
 253 Koehler et al., 2008; Ward and Kach, 2009; von Moos et al., 2012; Baker et al., 2014; Van  
 254 Cauwenberghe et al., 2015; Lusher, 2015; Doyle et al., 2015; Vandermeersch et al., 2015). Research  
 255 on ENPs indicate that nanoparticle aggregation may significantly enhance this uptake (Ward and  
 256 Kach, 2009) and other studies show that larger particles (<100 nm) such as microplastics can be taken  
 257 up both in the gills and the digestive system (von Moos et al., 2012). Presently there is therefore a  
 258 growing awareness concerning the fate and potential effects of ENPs and microplastics in mussels and  
 259 other commercial seafood (Van Cauwenberghe and Janssen, 2014; Li et al., 2015; Mathalon and Hill,  
 260 2014). Unlike fish, that humans normally eat without the digestive system, mussels are eaten whole,  
 261 making it especially important to evaluate human food safety from these emerging particle  
 262 contaminants. Therefore, more knowledge is needed on uptake and depuration of nano- and microscale  
 263 particles in mussels.

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

$$275 \quad \frac{dC_m}{dt} = k_u C_w + AE \times IR \times C_f - (k_d + k_b + G)C_m \quad (1)$$

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

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

$$282 \quad \frac{dC_m}{dt} = (k_u + AE \times IR \times K_{fw})C_w - (k_d + k_b + G)C_m \quad (2)$$

283 which reduces to:

$$284 \quad \frac{dC_m}{dt} = k_{acc}C_w - k_{loss}C_m \quad (3)$$

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

$$288 \quad C_m = BAF C_w [1 - e^{-k_{loss}t}] \quad (4)$$

289 It is often assumed that organic contaminant concentrations in native organisms are at  
290 equilibrium/steady-state with the concentrations in the water. For deployed mussels, a six-week  
291 exposure has generally been expected to result in an equilibrium (Peven et al., 1996; Björk and Gilek,  
292 1997). Loss rate constants,  $k_{loss}$  for PAHs and PCBs in *E. complanata* (a freshwater mussel) and *M.*  
293 *edulis* as summarized in Booij et al. (2006), decrease with increasing contaminant hydrophobicity (log  
294  $K_{ow}$ ) and range from 0.27 to 0.015 d<sup>-1</sup>, equivalent to half-lives of 3-46 days. Gewurtz et al. (2002)  
295 observed elimination rates for PAHs and PCBs from *E. complanata* in a very similar range and  
296 expected passive diffusion through the gills of the mussel to be the principal depuration pathway for  
297 PAHs. A different elimination behavior for benzo[a]pyrene (BaP) is found in some *Mytilus* studies,  
298 e.g. Magnusson et al. (2000), suggesting that metabolism may be responsible for observed elimination  
299 or lack of appreciable accumulation. Exposure and uptake kinetics generally increase with increasing  
300 water pumping rates and feeding, but AE has also been shown to be inversely related to mussel  
301 filtration and water pumping rates (Björk and Gilek, 1999). These authors demonstrated the increasing  
302 relative importance of food as a source for chemical contaminants of increasing hydrophobicity.  
303 Overall, the relative contribution of uptake from water and food is difficult to assess but generally  
304 depends on food availability and on the hydrophobicity of the chemicals of interest.

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

314 variety of freshwater and marine mussel species. Across various studies and mussel species, a strong  
315 relationship was found between wet-weight BAF values (calculated as ratio of mussel concentration  
316 over freely dissolved concentration estimated by using semipermeable membrane devices (SPMDs))  
317 and the compound's hydrophobicity ( $\log K_{ow}$ ).  $\log BAF$ - $\log K_{ow}$  linear relationships for the various  
318 studies had similar slope but different intercepts. This relationship was  $\log BAF = 0.84 \log K_{ow} + a_0$ ,  
319 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  
320 studies encompassed mostly PAHs and alkylated PAHs, PCBs and chlorinated pesticides such as  
321 HCHs, DDTs, chlordanes, or cyclic dienes (aldrin, dieldrin). The relationship of  $\log BAF$  with  $\log K_{ow}$   
322 for PAHs and PCBs in transplanted blue mussels co-deployed with silicone rubber passive samplers  
323 twice a year over a period of 4-5 years at 8 sampling stations (Smedes, 2007) had a slope of 1.1 and an  
324 intercept of -2.14. Considering the general variability in  $\log BAF$  values, the half an order of  
325 magnitude higher BAF values for compounds with higher  $\log K_{ow}$  is not out of proportion. BAFs for  
326 PAHs were observed to be higher in the winter than for autumn deployments (by 60 %). Interestingly,  
327 much higher BAFs could be observed for BaP in the winter than for autumn exposures and this could  
328 indicate lower metabolism of this chemical during winter. Some variability in PAH BAFs could be  
329 seen between stations. In general, less variation in BAF was observed for PCBs both between stations  
330 and seasons. Axelman et al. (1999) determined BAFs for PAHs that were significantly higher ( $> 1$  log  
331 unit of BAF) for blue mussels exposed in recipient waters at an aluminum smelter site compared with  
332 data from a reference location or literature values. Under the smelter site conditions, mussels may be  
333 substantially exposed to PAHs through filter-feeding on PAH-contaminated particles from the smelter  
334 effluent releases. While PAHs strongly sorbed to these black carbon, soot-like particles may not  
335 readily partition into water once particles are released into seawater (Allan et al., 2012; Allan et al.,  
336 2016), rather they may be more available for desorption while in the gut of blue mussels. Very few  
337 BAF values are available for other classes of chemicals including emerging chemicals. Gustafsson et  
338 al. (1999) conducted uptake and depuration studies to estimate BAFs for polybrominated diphenyl  
339 ethers (PBDEs) in *M. edulis* and found that accumulation rates and BAFs were higher for BDE 47 and  
340 BDE 99 than for PCBs with similar hydrophobicity. These data were supported by *in situ* BAFs  
341 estimated by Booij et al. (2002) for native blue mussels from the Western Scheldt (The Netherlands).  
342 BAFs for BDE congeners 28, 47, 99 and 100 were much larger than those estimated for PCBs with  
343 same  $\log K_{ow}$ . Some studies have been conducted to assess uptake and depuration of pharmaceuticals  
344 such as carbamazepine by mussels, e.g. Boillot et al. (2015), and have shown limited potential for  
345 bioaccumulation and relatively high uptake and depuration kinetics with biological half-lives of a few  
346 days. For the compounds mentioned above, bioaccumulation in mussel is expected to be a partition  
347 processes and therefore normalization of data to mussel lipid content (most often expressed as  
348 extractable organic matter measured gravimetrically) is often undertaken. This also means that the  
349 contaminant concentration in mussel will react to changes in contaminant concentration in water and  
350 that BAF are independent of concentrations in water. Contradictorily, the uptake of

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

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

361  
 362 **Table 1: Overview of published studies on toxicokinetics (uptake, accumulation and depuration processes  
 363 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 (17 $\alpha$ -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)
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364

#### 365 4. Mussel watch programs

366 A mussel watch program is a systematic repeated analysis of environmental quality parameters (e.g.  
367 anthropogenic contaminant concentrations) in natural, cultured, or deployed mussels from a set of  
368 defined geographical locations (mussel stations) and over a time-span of several years (Goldberg,  
369 1975, 1980; Goldberg, 1986). The first known mussel surveys were performed in the USA during the  
370 late 1960s and early 1970s to monitor spatial and temporal trends of contaminants in coastal and  
371 estuarine ecosystems (Goldberg, 1975). From 1986 on, the activity was continued with the US Mussel  
372 Watch Program, which by 2008 had extended to include monitoring of approximately 140 prioritized  
373 pollutants in *Mytilus* spp., oyster (*Crassostrea virginica*), or zebra mussel (*Dreissena* sp., in  
374 freshwater) sentinels from nearly 300 mussel stations (Kimbrough et al., 2008). The mussel watch  
375 activities in North America also motivated the initiation of similar systematic programs in many other  
376 coastal countries throughout the world, e.g.: Burns and Smith (1981), the MED POL Biomonitoring  
377 Programme (Viarengo et al., 2000), the Joint Assessment and Monitoring Programme (JAMP) of the  
378 OSPAR Convention (Besada et al., 2002); often organized or supervised by the UN supported  
379 International Mussel Watch Committee (UNESCO, 1992). Data from these monitoring programs are  
380 presently becoming available to users outside the research community as digitized monitoring reports  
381 more often can be found by means of common Internet search engines.

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

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

415 No global harmonized standard exists for how to perform mussel monitoring studies; although several  
416 key agencies have published comprehensive instructions, e.g. UNESCO (1992), NOAA (Lanksbury et  
417 al., 2010; Lanksbury and West, 2012), ICES (Davies and Vethaak, 2012), OSPARCOM (2012) and  
418 the European Commission (EC, 2014). Despite the lack of a single harmonized standard, most  
419 ecotoxicologists would agree that good comparability of monitoring data is important for a variety of  
420 reasons (data sharing and comparisons, international monitoring collaboration, quality assurance,  
421 quality standards, quality improvement, training & competence development, etc.). Chemical  
422 contaminant analyses in mussel watch programs are normally performed by using the whole soft tissue  
423 of the animal (i.e. the mussel without the shell), and most often by using composite samples, i.e.  
424 samples in which a certain number of individual mussels within a certain size-range prior to analysis  
425 are mixed into a single pooled sample. The use of pooled mussel samples is rational due to several  
426 practical reasons (especially for reducing analysis cost), although studies of toxicant distribution  
427 patterns show that different biological compartments (gills, mantle, plasma, digestive gland, gonads,  
428 muscle, and other viscera) may contain variable concentrations of contaminants, e.g. (Page et al.,  
429 1995; Raftopoulou and Dimitriadis, 2011; Rocha et al., 2015a; Ricciardi et al., 2016), and although a  
430 seemingly homogenous group of mussels collected from a field population may contain individuals

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

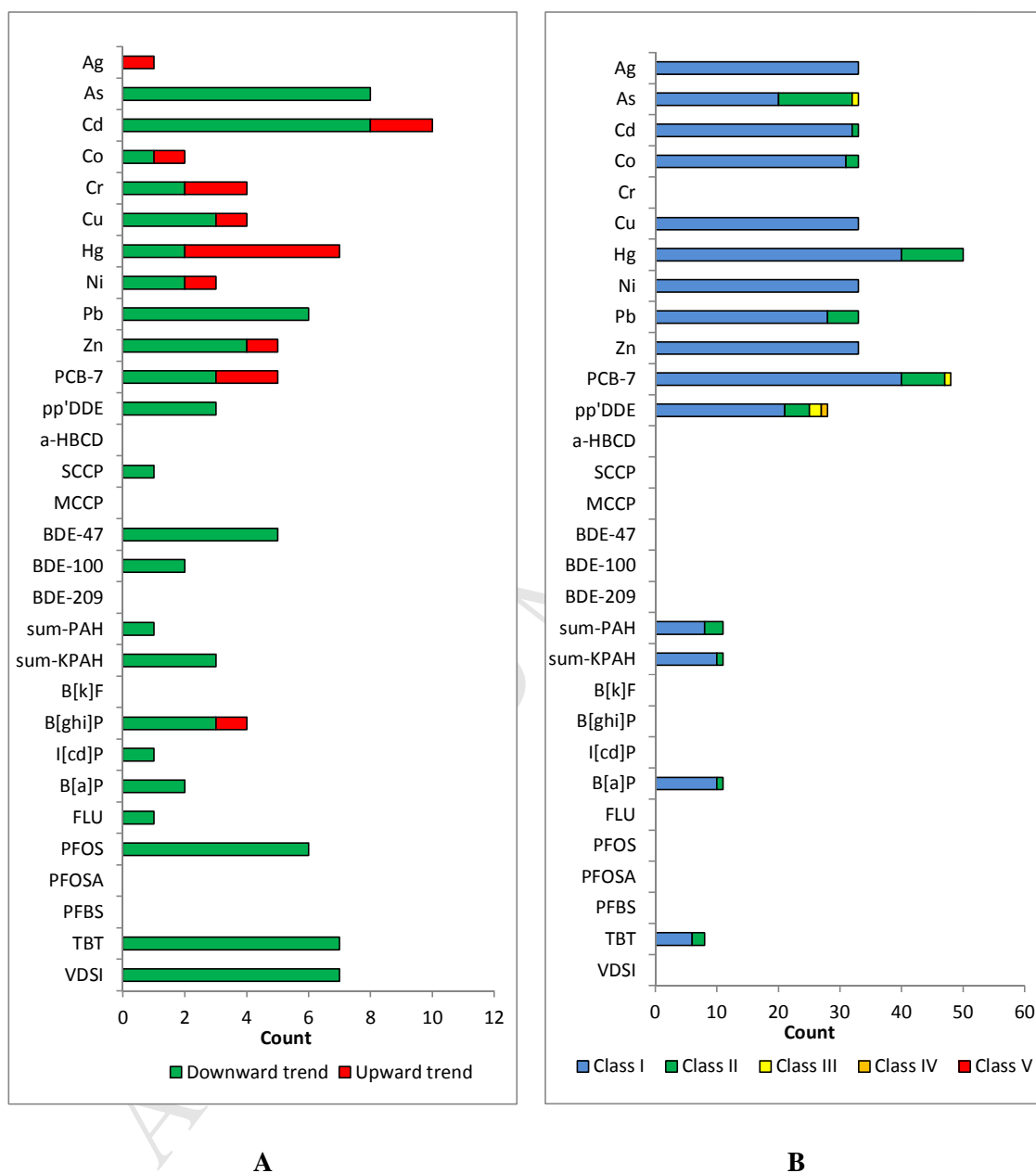
447 In Norway, mussel monitoring activities with use of *Mytilus edulis* have been a part of the national  
448 coastal environmental monitoring program (MILKYS) running since 1981 (Green et al., 2016). In this  
449 program, there has been a general lowering trend of most legacy contaminants in mussels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

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<sup>2</sup> Consisting of one or more annual medians contrasting earlier reports which tallied only datasets of five or more annual medians.

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

470



471 **Figure 4: Figure that summarizes 829 recent trends (A) and classification of levels (B) of 30 key**  
 472 **contaminants in Norwegian mussel watch activities. Data source: MILKYS trends report (Green et al.,**  
 473 **2016).**

## 5. Regulatory monitoring and environmental quality standards

474

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

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

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<sup>3</sup> 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.

506 90% percentiles from background and slightly impacted stations) measured in the Norwegian coastal  
507 monitoring program. Several key issues can be highlighted from data shown in Table 2. Most  
508 importantly, for the four prioritized substances brominated diphenyl ethers (BDEs), mercury, TBT  
509 (formulation based) and PCB7 the risk based marine biota EQS, which currently are valid under WFD,  
510 are set considerably lower than those measured in mussels from remote non-polluted coastal sites in  
511 Northern Norway. In other words, blue mussels from non-polluted coastal water bodies in Northern  
512 Norway failed to be compliant to the EQS set by the WFD for BDEs, mercury, TBT and PCB7. This  
513 non-compliant classing of such very remote coastal sites far from any significant anthropogenic  
514 pollutant inputs appears illogical and it could possibly indicate that the current EQS assessment  
515 criteria for these substances are set too low. It is therefore questionable whether these EQSs are  
516 operational for use in industrial compliance monitoring in coastal waters, i.e. to define when there is  
517 and when there is not a regulatory demand for source reducing measures.

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

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<sup>4</sup> 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.



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

546 The overall objective of the EU Marine Strategy Framework Directive (MSFD) (2008/56/EC) is to  
547 achieve or maintain Good Environmental Status (GES) in all European seas by 2020, and eleven  
548 qualitative *Descriptors* of GES are defined in Annex I of MSFD, see explanation of descriptors in  
549 Borja et al. (2010) and Lyons et al. (2010). The MSFD also requires participating states to develop a  
550 robust set of study tools and assessment criteria for providing documentation of all 11 GES  
551 Descriptors. The use of blue mussels as sentinels can be highly relevant for several of these MSFD  
552 GES, such as Descriptor 8 "Concentrations of contaminants are at levels not giving rise to pollution  
553 effects", Descriptor 9 "Contaminants in fish and other seafood for human consumption do not exceed  
554 levels established by Community legislation or other relevant standards" and Descriptor 10 "Properties  
555 and quantities of marine litter do not cause harm to the coastal and marine environment". Furthermore,  
556 for Descriptor 5, which partly concerns harmful algae blooms, the involvement of blue mussel as test  
557 medium for assessing presence and levels of toxic algae in the ambient seawater is highly relevant and  
558 could provide a valuable tool. Similarly, as for the WFD EQSs, the MSFD Descriptors 5, 8, 9 and 10  
559 rely on defining environmental stressor levels (e.g. contaminant concentrations) that represent  
560 thresholds for quality status compliance. Hence, the quality standards and empirical data shown in  
561 Table 2 has relevance also for the development and use of *Mytilus* spp. data as assessment criteria in  
562 conjugation with MSFD. The possible use of biological effect parameters in *Mytilus* spp. as tools to  
563 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 <sup>5</sup>	OSPAR EC/EAC <sup>6</sup>	Norwegian <sup>7</sup>	OSPAR BAC <sup>6</sup>	Indicator	Norw. Class I	Norw. Class II	Background and slightly impacted stations		
									10 %	90 %	Count <sup>8</sup>
Brominated diphenyl ethers (BDEs) <sup>9</sup>	32534-81-9	0.0085							0.006	0.604	22
C10-C13 chloroalkanes (SCCP)	85535-84-8			6000 <sup>10</sup>					1.71	44.48	9
DDT, total of four isomers <sup>12</sup>				609 <sup>10</sup>	0.11 <sup>11</sup>	DDE (p,p') <sup>13</sup>	2	5	0.075	0.480	59
Decamethylcyclpentasiloxane (D5)	541-02-6			15217							
Di(2-ethylhexyl)-phthalate (DEHP)	117-81-7			2900 <sup>10</sup>							
Dicofol	115-32-2	33									
Diflubenzuron	35367-38-5			730							
Dioxins and dioxin-like compounds <sup>20</sup>		0.0065 <sup>22</sup>				TCDDN	0.00002	.0005	0.00002	0.00016	11
Endosulfan	115-29-7			370 <sup>10</sup>							
Heptachlor and heptachlor epoxide	76-44-8/ 1024-57-3	0.0067									
Hexabromocyclododecane (HBCDD)		167				□-HBCD			0.020	0.150	9
Hexachlorobenzene (HCB)	118-74-1	10			0.11 <sup>11</sup>		0.1	0.3	0.037	0.099	62
Hexachlorobutadiene (HCBd)	87-68-3	55									
Hexachlorocyclohexane (HCH)	608-73-1		0.25 <sup>11</sup>	61 <sup>10</sup>	0.17 <sup>11</sup>	□-HCH	1	3	0.050	0.128	61
Hexabromocyclododecane (HBCDD)		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) <sup>17</sup>	7440-38-2						1748 <sup>11</sup>	5244 <sup>11</sup>	1382	3392	26
Cadmium (Cd) and its compounds	7440-43-9		1000		168 <sup>11</sup>		349.6 <sup>11</sup>	874.0 <sup>11</sup>	131	344	65
Copper (Cu) <sup>17</sup>	7440-50-8				1049 <sup>11</sup>		1748 <sup>11</sup>	5244 <sup>11</sup>	930	1465	69
Chromium (Cr) <sup>17</sup>	7440-47-3						524 <sup>11</sup>	1758 <sup>11</sup>	82	404	31
Lead (Pb) and its compounds	7439-92-1		1500		227 <sup>11</sup>	Total Pb	524.4 <sup>11</sup>	2622.0 <sup>11</sup>	105	449	68
Mercury (Hg) and its compounds	7439-97-6	20	500		15.7 <sup>11</sup>	Total Hg	35.0 <sup>11</sup>	87.4 <sup>11</sup>	8	29	69
Nickel (Ni) and its compounds	7440-02-0					Total Ni	874.0 <sup>11</sup>	3496.0 <sup>11</sup>	153	355	32
Silver (Ag)	7440-22-4						52.4 <sup>11</sup>	175 <sup>11</sup>	4	13	32
Zinc (Zn) <sup>17</sup>	7440-66-6						34960 <sup>11</sup>	69920 <sup>11</sup>	13400	23460	70
Tributyltin compounds (Tributyltin cation)	36643-28-4 688-73-3			150 <sup>10</sup>							
TBT formulation based	688-73-3		2.1 <sup>11</sup>				17.5 <sup>11</sup>	87.4 <sup>11</sup>	1.61	31.85	20
Triphenyltin	639-58-7			152							
Naphthalene	91-20-3		59.4 <sup>11</sup>	2400 <sup>10</sup>					0.50	15.52	32
Nonylphenol (4-Nonylphenol)				3000 <sup>10</sup>		4-t-NP <sup>14</sup>			18.7	233	10
Octylphenol (4-(1,1',3,3'-tetramethyl-butyl)-phenol)				0.004 <sup>10</sup>		4-n-OP <sup>15</sup>			1.0	46	10
Polycyclic aromatic hydrocarbons (PAH)											
Acenaphthene	83-32-9								0.30	2.00	32
Acenaphthylene	208-96-8								0.20	0.57	32
Anthracene	120-12-7		50.7 <sup>11</sup>	2400 <sup>10</sup>					0.20	2.83	32
Benzo[a]anthracene	56-55-3		14.0 <sup>11</sup>	304	1.42 <sup>11</sup>				0.28	4.02	32
Benzo[a]pyrene <sup>16</sup>	50-32-8	5	105.1 <sup>11</sup>		0.24 <sup>11</sup>		1	3	0.20	0.77	32
Benzo[ghi]perylene	191-24-2		19.2 <sup>11</sup>		0.44 <sup>11</sup>				0.20	1.90	32
Chrysene <sup>17</sup>	218-01-9				0.44 <sup>11</sup>				0.50	8.26	26
Dibenzo[ah]anthracene <sup>17</sup>	53-70-3					DBA3A			0.20	0.50	31
Fluoranthene	206-44-0	30	19.2 <sup>11</sup>		2.13 <sup>11</sup>				0.77	26.65	32
Fluorene <sup>17</sup>	86-73-7								0.43	2.71	32
Indeno[1,2,3-cd]pyrene	193-39-5				0.42 <sup>11</sup>				0.23	1.41	32
Phenanthrene <sup>17</sup>	85-01-8		297 <sup>11</sup>		1.92 <sup>11</sup>				1.22	11.10	32
Pyrene <sup>17</sup>	129-00-0		17.5 <sup>11</sup>		1.57 <sup>11</sup>				0.30	13.96	32
Sum PAH15 <sup>18</sup>									3.91	28.57	28
Sum PAH16 <sup>19</sup>							50	200			
Sum carcinogen PAHs							10	30	<0.01	7.20	28

Name of substance	CAS-no	EQS biota <sup>5</sup>	OSPAR EC/EAC <sup>6</sup>	Norwegian <sup>7</sup>	OSPAR BAC <sup>6</sup>	Indicator	Norw. Class I	Norw. Class II	Background and slightly impacted stations		
									10 %	90 %	Count <sup>8</sup>
Polychlorinated biphenyls (PCB)											
CB28	7012-37-5		1.02 <sup>24</sup>		0.13 <sup>11</sup>				0.050	0.100	67
CB52	35693-99-3		1.64 <sup>24</sup>		0.13 <sup>11</sup>				0.050	0.224	67
CB101	37680-73-2		1.84 <sup>24</sup>		0.12 <sup>11</sup>				0.073	0.401	67
CB105	32598-14-4				0.13 <sup>11</sup>				0.050	0.150	62
CB118	31508-00-6		0.38 <sup>24</sup>		0.10 <sup>11</sup>				0.074	0.352	67
CB138	35065-28-2		4.82 <sup>24</sup>		0.10 <sup>11</sup>				0.088	0.583	67
CB153	35065-27-1		24.1 <sup>24</sup>		0.10 <sup>11</sup>				0.108	0.706	67
CB156	38380-08-4				0.10 <sup>11</sup>				0.050	0.090	62
CB180	35065-29-3		7.13 <sup>24</sup>		0.10 <sup>11</sup>				0.050	0.088	67
Sum PCB7	1336-36-3			1			4	15	0.268	2.170	65
Pentachlorobenzene (QCB)	608-93-5			50 <sup>10</sup>					0.030	0.058	64
Pentachlorophenol	87-86-5			180 <sup>10</sup>							
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 <sup>10</sup>							
Triclosan	3380-34-5			15217							
Tri(2-chloroethyl)phosphate (TCEP)	115-96-8			7304							

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<sup>5</sup> 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.

<sup>6</sup> Refers to «smussels», see <http://domc.ices.dk/osparmime/main.html>

<sup>7</sup> 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)

<sup>8</sup> 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.

<sup>9</sup> Sum of PBDE congeners: 28, 47, 99, 100, 153 and 154

<sup>10</sup> EU prioritized substance but EQS for biota established in Norway based on EU-TGD.

<sup>11</sup> 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.

<sup>12</sup> 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).

<sup>13</sup> CAS-no. 72-55-9

<sup>14</sup> CAS-no. 84852-15-3

<sup>15</sup> CAS-no. 140-66-9

<sup>16</sup> 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)

<sup>17</sup> 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

<sup>18</sup> 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).

<sup>19</sup> Sum of 16 PAHs: PAH15 plus naphthalene (CAS 91-20-3).

<sup>20</sup> 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)).

<sup>21</sup> 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',4,4'-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).

<sup>22</sup> 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.

<sup>23</sup> Refers to 1,3,5,7,9,11-Hexabromocyclododecane (CAS 25637-99-4), 1,2,5,6,9,10- Hexabromocyclododecane (CAS 3194-55-6),  $\alpha$ -Hexabromocyclododecane (CAS 134237-50-6),  $\beta$ -Hexabromocyclododecane (CAS 134237-51-7) and  $\gamma$ -Hexabromocyclododecane (CAS 134237-52-8).

<sup>24</sup> 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.

## 569 **6. Biomarkers of exposure and effects of pollutants in blue mussels**

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

576 Within the many mussel biomarker reports, oxidative stress is the issue that most frequently has been  
577 addressed. Oxidative stress is an adverse condition that results from a prolonged imbalance between  
578 the *in vivo* concentration of toxic free radicals derived from oxygen, nitrogen or sulphur molecules and  
579 the capacity of the organisms' antioxidant defense system to neutralize these highly active oxidative  
580 molecules (Winston and Digiulio, 1991; Lushchak, 2011). Radicals are produced naturally during  
581 cellular respiration and metabolism and radicals have also essential roles in cell signaling, apoptosis,  
582 gene expression and ion transportation; but excess levels will cause an array of damaging effects to  
583 tissues, cells, and subcellular components, e.g. lipid peroxidation, DNA lesions and mutations (Lue et  
584 al., 2010). Many environmental contaminant stressors, including transition metals, PAHs, ionization  
585 radiation, organochlorine, and organophosphate pesticides, are known to cause oxidative stress. The  
586 antioxidant defense systems in *Mytilus* spp. involve both actions of molecular antioxidants such as  
587 glutathione (GSH) and the activities of glutathione *S*-transferase (GST), GSH-peroxidase and catalase  
588 preferably in gill and digestive gland tissues (Power and Sheehan, 1996; Fitzpatrick et al., 1997;  
589 Regoli, 1998; Manduzio et al., 2004; Letendre et al., 2006; Einsporn et al., 2009; Fernandez et al.,  
590 2010; Gonzalez-Rey and Bebianno, 2011).

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

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

603 environmental stressors. Among the assays available in literature, the comet assay (or single Cell Gel  
604 Electrophoresis) is the most widely used to detect DNA damage in mussels in response to several  
605 genotoxic agents, either in the hemocytes or in gill cells (Jha, 2008; Almeida et al., 2011; Gomes et al.,  
606 2013; Avio et al., 2015). The comet assay is highly sensitive for measuring DNA strand breaks (single  
607 and double), and can also be used for quantification of base oxidations when used in concert with  
608 endonucleases that have specificity for oxidized bases (i.e. to introduce breaks that subsequently are  
609 measured using the comet assay) (Azqueta et al., 2009). The overall cellular DNA damage can be  
610 visualized under the microscope and estimated from several measures of the proportions and  
611 relationships between the comet head (undamaged DNA) and tail (broken DNA strand fragments),  
612 being the size of the tail proportional to the amount of damaged DNA (Dixon et al., 2002; de Lapuente  
613 et al., 2015). Another assay that can be used in association with the comet assay to provide a more  
614 realistic analysis of genotoxic effects on a higher level in mussels is the micronucleus (MN) test. The  
615 MN test allows for the identification of chromosomal DNA damage that result from either  
616 chromosomal breakage during cell division or chromosome mis-segregation during mitosis (Bolognesi  
617 and Fenech, 2012). Similarly to the comet assay, the frequency of MN can be assessed by microscopic  
618 visualization of mussel cells from both laboratory and field studies (Dixon et al., 2002; Bolognesi and  
619 Fenech, 2012; Rocha et al., 2014). Both genotoxicity biomarkers are routinely applied in large-scale  
620 biomonitoring programs using standardized protocols developed specifically for both hemocytes and  
621 gill cells of mussels (Azqueta et al., 2009; Bolognesi and Fenech, 2012).

622 Pathological changes to the integrity of lysosomal organelles (lysosomal membrane stability) in cells  
623 of the mussel immunocompetence system is a key ecotoxicity biomarker in blue mussels. Initially, the  
624 earliest studies in this field were focused on digestive cells localized within the mussel digestive gland,  
625 e.g. (Moore, 1976; Moore et al., 1978; Lowe et al., 1981; Viarengo and Moore, 1982; Viarengo et al.,  
626 1985; Winston et al., 1991), whereas the focus shifted later more over to circulating hemocytes (Coles  
627 et al., 1994; Lowe et al., 1995; Grundy et al., 1996a; Grundy et al., 1996b; Moore et al., 1996).  
628 Although similar effects can be detected both places, the hemocytes are easier to collect and use for  
629 the Neutral Red (NR) retention assay (Lowe et al., 1995), a colorimetric cell test which involves the  
630 incubation of cells with the toxic dye NR and that earlier had been developed for the assessment of  
631 cytotoxicity of yeasts (Ogawa, 1961). NR added to the cell medium is taken up by cells and  
632 accumulates in the cell lysosomes, which are essential for the defense against toxic substances, until  
633 the lysosomes eventually leak the dye into the cytosol and the cell dies. The time it takes for greater  
634 than 50% of the cells to leak the dye determines the stability of the lysosomal membrane. This stability  
635 is often significantly compromised if the cell is already stressed (unhealthy) from exposure to  
636 ecotoxicants, hence the NR retention time is decreased in pollutant exposed (and health compromised)  
637 mussels. Recent developments with the NR retention assay have occurred to propose a new scoring  
638 system that increases the sensitivity of the assay. The new scoring system incorporates a value for the

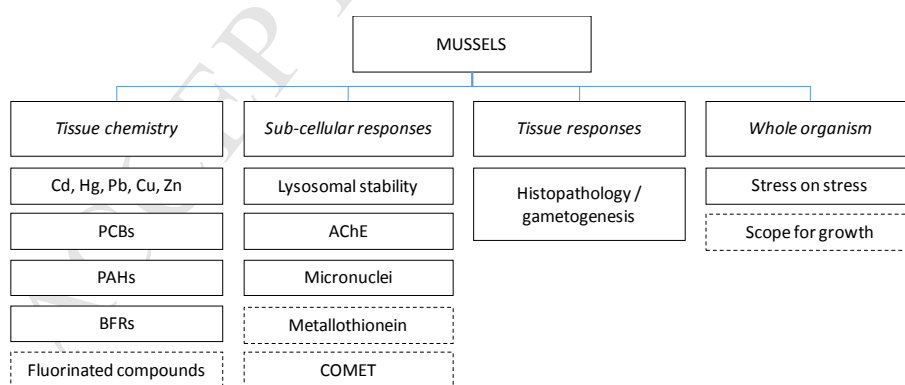
639 quality and size of the lysosomes in addition to the time required for the lysosomes to leak from 50%  
640 of their cells. Details on the improved scoring system can be obtained from the ICES TIMES  
641 (Techniques In Marine Environmental Sciences) document (Martinez-Gomez et al., 2015). Martinez-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

646 Assessment criteria have been developed for many of the mussel biomarkers in the ICES framework,  
647 including so-called background assessment criteria (BAC) and environmental assessment criteria  
648 (EAC). The 90<sup>th</sup> percentile of reference site data is used to calculate the BAC for each biomarker, with  
649 the reliability dependent on the quality and quantity of biomarker data available. The BAC calculation  
650 is therefore a continuing process as more data becomes available for the different endpoints. It is  
651 recognized that background response levels have an important role in integrating biological effects  
652 parameters into environmental risk assessments. For example, an elevated increase in one biomarker,  
653 when compared to a background response, may indicate that a chemical substance has caused an  
654 unacceptable level of biological harm. However, species differences in contaminant uptake and  
655 biomarker responses are known to occur between mussels, e.g. (Brooks et al., 2015a; Barsiene et al.,  
656 2012; Burgeot et al., 2012) (see also confounding factor section), and this has led to the development  
657 of species-specific assessment criteria for certain biomarker responses, such as micronuclei formation.  
658 For the use of biomarkers in *Mytilus* in monitoring it is important to emphasize the relevance of good  
659 quality assurance (QA) routines in the analytical laboratories. For example, the MED POL programme  
660 was the first to develop a systematic laboratory intercalibration exercise for ecotoxicological  
661 biomarkers in blue mussels (Viarengo et al., 2000).

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



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



698  
 699 **Figure 5: Recommended biological effects methods included in the mussel component of the ICES**  
 700 **integrated ecosystem assessment. Solid lines are the core methods, whilst dotted lines represent additional**  
 701 **methods. PCBs, polychlorinated biphenyls; PAH, polycyclic aromatic hydrocarbon; BFRs, brominated**  
 702 **flame retardants; AChE, acetylcholinesterase. Figure adapted from Davies and Vethaak (2012).**

703 The use of a multi-biomarker approach will often produce complex response data that can be hard to  
704 interpret, not to say integrate in environmental policy frameworks. To encompass this problem, several  
705 ways for simplifying complex data have been developed, among which the so-called *Integrated*  
706 *Biomarker Response* (IBR) index (Beliaeff and Burgeot, 2002) is one of the most frequently used, e.g.  
707 (Bocquene et al., 2004; Dagnino et al., 2007; Damiens et al., 2007; Hagger et al., 2008; Pytharopoulou  
708 et al., 2008; Brooks et al., 2011a; Brooks et al., 2012; Marigomez et al., 2013a; Marigomez et al.,  
709 2013b; Devin et al., 2014; Turja et al., 2014). A key element of IBR is the use of biomarker star plots  
710 as a tool for combining multiple biomarker responses into one simplified response index value.  
711 Although the IBR index is met with skepticism for possible oversimplification, misuse, and  
712 calculation bias (Devin et al., 2014), others consider it to be a useful tool that correlated well with  
713 known chemical hotspots and sources of contamination, e.g. Hagger et al. (2009). The IBR method has  
714 several times been updated and improved e.g. by including the number of biomarker observations in  
715 its calculation (Broeg and Lehtonen, 2006) and by developing an easier index calculation formula that  
716 considers all the permutation procedures (Devin et al., 2014). The latter improvement was  
717 implemented to remove possible bias in the calculation due to relative positioning of biomarkers  
718 within the star plots. However, as emphasized by Broeg and Lehtonen (2006), the IBR method  
719 represents a simplification of very complex exposure situations in the field, and the response index  
720 should not be taken at “face value” but rather as a tool to direct further actions. For instance, since the  
721 biomarker data is normalized with respect to the other mussel groups, a biomarker which is low and  
722 unresponsive in all groups, may find significant contribution to the IBR in the group where the  
723 biomarker is high relative to the other groups. It is always advised therefore to look behind the IBR  
724 calculation to ascertain which of the biomarkers are contributing and whether this is a true reflection  
725 or individual biomarker response.

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

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

752

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

<b>Chemical stressor</b>	<b>Reported biomarker effect studies</b>
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; Hoehner 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; Hoehner 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)

755

## 756 **7. Confounding factors in blue mussel monitoring**

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

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

771 relevant to know whether contaminant bioaccumulation patterns, biological traits and pollution stress  
772 responses differ significantly among the genetic variants of blue mussels. The answers on this issue  
773 tend to be mixed. For example, some studies have found that *M. trossulus* accumulate certain metals to  
774 a higher extent than *M. edulis* (Lobel et al., 1990), whereas others have found that metal uptake and  
775 clearance rates vary only little between *Mytilus* species and between different climatic zones of  
776 collection, as long as the influence of different body-size is corrected for (Blackmore and Wang,  
777 2003). Brooks et al. (2015a) found differences in bioaccumulation and biological responses to copper  
778 in the three *Mytilus* species *M. galloprovincialis*, *M. edulis* and *M. trossulus* exposed to water-borne  
779 copper at three concentrations, whereas Arnold et al. (2009), on the other hand, found no statistically  
780 different sensitivity to copper toxicity in *M. galloprovincialis* and *M. edulis*, but rather that the copper  
781 toxicity in both species was a function of organic matter concentration in the test water, a parameter  
782 which often is not reported. In a recent study from Greenland (Wenne et al., 2016) found that in a  
783 metal affected fjord *M. trossulus* coped significantly better with the low-salinity and low-temperature  
784 conditions in the inner fjord locations in comparison to *M. edulis* which was more dominant in the  
785 outer fjord locations. This difference in site preferences may also have implications for differences in  
786 the metal uptake and bioaccumulation pattern. Bignell et al. (2008) investigated possible species  
787 effects between *M. edulis*, *M. galloprovincialis* and their hybrids at different points during the annual  
788 cycle at two uncontaminated field sites in UK. Twenty-nine histological health parameters were  
789 measured and overall for the annual cycle only insignificant species differences were detected.  
790 However, greater differences were observed between species during the autumn and winter than  
791 during the spring and summer, thus indicating that season may exacerbate species differences in  
792 monitoring programs.

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

807 and growth intensity as well as gonad development and spawning can influence contaminant  
808 bioaccumulation and biological effect endpoints in blue mussels (Table 4). For example, Schmidt et al  
809 (2013) examined seasonal variations for a suite of biomarkers (glutathione S-transferase, vitelline-like  
810 proteins, lipid peroxidation and DNA damage) over a 12-month period in a hybrid blue mussel  
811 population from a pristine area, and found season effects for all the biomarkers examined apparently  
812 linked most strongly to the mussel's reproductive cycle.

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

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

842



843

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

Confounding factor	Studies reporting confounding factor influence in <i>Mytilus</i> spp.
genetic differences of <i>Mytilus</i> 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; Westerbomb 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)

846

## 847 **8. Mussel transplant caging in pollution monitoring**

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

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

860 A key question in mussel caging is how long mussels should be deployed *in situ* for the contaminant  
861 concentrations in the mussels' tissue to reach a steady-state and for the deployed mussels to be  
862 equalized with native mussels. The *Mytilus* literature is very variable with respect to this issue, i.e.  
863 with reported caging periods lasting for 3 weeks (Peters et al., 1998; Zorita et al., 2006; Marigomez et  
864 al., 2013b), 4 weeks/one month (Stien et al., 1998; Utvik et al., 1999; Mauri and Baraldi, 2003; Romeo  
865 et al., 2003a; Regoli et al., 2004; Camus et al., 2004; Frenzilli et al., 2004; Gorbi et al., 2008; Schintu  
866 et al., 2008; Taleb et al., 2009; Giarratano et al., 2010; Zorita et al., 2015; Cappello et al., 2015; Turja  
867 et al., 2015), 5-6 weeks (Nasci et al., 2002; Booij et al., 2002; Ericson et al., 2002; Pampanin et al.,  
868 2005a; Orbea and Cajaraville, 2006; Regoli et al., 2014; Greenfield et al., 2014; Brooks et al., 2015b),  
869 8 weeks/two months (Sole et al., 1998; Mauri and Baraldi, 2003; Milun et al., 2016), 12-13  
870 weeks/three months (Salazar and Salazar, 1991; Radenac et al., 1997; Folsvik et al., 2002; Shaw et al.,  
871 2002; Oros and Ross, 2005; Pampanin et al., 2005b; Galgani et al., 2011; Benedicto et al., 2011;  
872 Scarpato et al., 2010; Beyer et al., 2013; Turja et al., 2014; Moschino et al., 2016), 18 weeks (Giltrap  
873 et al., 2013), up to 6 months (Touahri et al., 2016; Schøyen et al., this volume), or up to 2 years (Bodin  
874 et al., 2004). Comparisons of temporal bioaccumulation patterns of key contaminants (e.g. trace metals,  
875 PCBs, PAHs) demonstrate that the issue of what is an optimal deployment time for blue mussels is  
876 dependent on which contaminant being targeted, generally with trace metals in deployed mussels  
877 reaching steady-state and a concentration comparable to native mussels considerably faster than the  
878 non-polar, hydrophobic organic contaminants. This suggest that in caging studies with short term  
879 deployments, there is a need for employing time-specific and contaminant-specific recalculation  
880 factors for the non-polar, hydrophobic organic contaminants to render contaminant data from deployed  
881 specimens more comparable to assumed steady-state levels in native blue mussels.

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

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

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

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

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

## 923 **9. Emerging issues in blue mussel monitoring**

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

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

944 Another group of emerging contaminants with increasing focus in the research community are  
945 engineered nanoparticles (ENPs). Among bivalve species, *Mytilus* spp. represents so far the most used  
946 model for predicting the impact of ENPs on the health of the marine environment (Rocha et al., 2015c;  
947 Canesi and Corsi, 2016). As filter-feeders, mussels remove ENPs from the water column  
948 independently of their form (individual particles, homo- and/or hetero-aggregates), being their  
949 bioavailability and uptake dependent on their peculiar features (e.g. size, shape, surface charge), type  
950 and composition of dispersing media, presence of normal organic matter, behavior, and fate (Corsi et  
951 al., 2014). Special attention has been given to the sub-lethal effects of different types of ENPs on  
952 immune function, embryo development and the main tissues involved in nanoparticle uptake and  
953 accumulation in mussels (i.e. gills and digestive gland), and several reviews about these issues are  
954 available (Canesi et al., 2012; Rocha et al., 2015c; Canesi et al., 2015). Consensus has been achieved  
955 that as the first barrier with the surrounding water, mussel gills can take up and break down ENPs  
956 aggregates into smaller particles that can be further transported to the digestive gland. Gills are also  
957 susceptible to interaction with individual particles and ionic metal forms released from the ENPs. On  
958 the other hand, the digestive system is apparently the main target for ENPs uptake and accumulation  
959 either through translocation from the gills or directly via endocytosis, mainly in the form of  
960 aggregates, e.g. (Gomes et al., 2011; Gomes et al., 2012; Rocha et al., 2015c). A smaller role on ENPs  
961 toxicokinetics has been given to the hemolymph and circulating hemocytes, as well as to mucus, fecal  
962 pellets and gametes produced by mussels (Canesi et al., 2012; Gomes et al., 2013; Della Torre et al.,  
963 2015; Rocha et al., 2015c). Immunotoxicity, oxidative stress (e.g. lipid peroxidation), cell injury in

964 proteins (e.g. protein carbonylation and ubiquitination), membrane (e.g. LMS) and DNA damage are  
965 considered as the main modes of action underlying the potential toxicity of ENPs in mussels, mostly  
966 related to the direct and indirect formation of reactive oxygen species (ROS) (Rocha et al., 2015c).  
967 However, the production of radical species is not specific to all nanoparticles, as responses may differ  
968 depending on particle size, composition and concentration, type and time of exposure, as well as target  
969 tissue, being the oxidative stress responses seen commonly associated with exposure to metal  
970 nanoparticles (e.g. Ag NPs, CuO NPs, Au NPs and Fe NPs) (Tedesco et al., 2010b, a; Gomes et al.,  
971 2012; Rocha et al., 2015c; Canesi and Corsi, 2016). Similar as for microplastics, ENPs can also  
972 possibly interact with other contaminants present in the aquatic environment and cause further  
973 biological responses via synergetic, antagonistic, or Trojan horse effects, e.g. Canesi et al. (2014).  
974 Overall, a multi-biomarker approach used for evaluating the biological responses to conventional  
975 contaminants has also proven effective for the screening of sub-lethal effects of ENP ecotoxicants in  
976 mussel. The combined use of oxidative stress, lysosomal, genotoxicity, immunotoxicity and  
977 physiological biomarkers, together with toxicokinetic data (uptake, accumulation, and depuration  
978 processes) is an interesting approach for the characterization of effects and modes of action of  
979 different ENPs in mussels and it is possible that biological and toxicological responses to ENPs are  
980 common to both invertebrate and mammalian systems (Canesi et al., 2012; Rocha et al., 2015c; Canesi  
981 and Corsi, 2016). However, there are still challenges that must be overcome for mussel biomonitoring  
982 programs to fully incorporate the emerging issue of ENP pollutants and their fate and effects in coastal  
983 environments.

984 The possible role of mussel sentinels in monitoring of pharmaceuticals and veterinary medicine  
985 pollutants has attracted increasing attention recently. One relevant example is the need for monitoring  
986 chemicals used to control sea lice pests in marine fish farming facilities, including chitin inhibitors  
987 such as diflubenzuron, teflubenzuron and neurotoxicants such as deltamethrin, cypermethrin,  
988 emamectin, ivermectin, and azamethiphos (Roth et al., 1993; Davies et al., 1997; Gowland et al.,  
989 2002; Canty et al., 2007; Ayad et al., 2011; Langford et al., 2014). In Norway, persistence and  
990 possible unintended effects of these toxic agents on non-target species (such as lobsters, crabs, and  
991 shrimps) is a concern. Local fishermen have claimed that smaller prawn catches and occurrence of  
992 lobster mortalities are possibly linked to usage of sea lice pesticides. The ability of mussels to filter  
993 large volumes of seawater and the accumulation of organic lipophilic compounds into their tissues  
994 make them an ideal species for the monitoring of the persistence and potential effects of these  
995 pharmaceutical compounds.

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

1000 and Helmuth, 2012; Cahill et al., 2013), and several recent studies show that general warming of the  
1001 seas is already affecting natural blue mussel populations; especially by shifting the biogeographical  
1002 distribution range of the different *Mytilus* taxa closer to the poles e.g. (Berge et al., 2005; Sorte et al.,  
1003 2011; Wenne et al., 2016), and by causing the more warm-water tolerant *M. galloprovincialis* to  
1004 invade regions which earlier have been dominated by *M. edulis*, *M. trossulus* or other more cold-water  
1005 tolerant mussel taxa, e.g. (Braby and Somero, 2006; Lockwood and Somero, 2011; Gardner et al.,  
1006 2016). Other studies have focused on the possible impact on *Mytilus* populations by ocean  
1007 acidification (OA) (Gazeau et al., 2010; Bechmann et al., 2011) as OA caused by an increased pCO<sub>2</sub>  
1008 in seawater is expected to decrease calcification rates of bivalves. There is also a concern that ocean  
1009 warming and OA could cause various kinds of interactive stress effects in *Mytilus* (Duarte et al., 2014;  
1010 Beyer et al., 2014; Kroeker et al., 2014; Eads et al., 2016) including aggravation of heavy metal  
1011 pollution and enhancing trace metal toxicity (Han et al., 2014). However, the most significant climate  
1012 change effect on *Mytilus* could well be through alteration of food availability, as shown by Thomsen et  
1013 al. (2013).

1014 Markedly decline and even extinction of local *Mytilus* mussel beds can also be associated with  
1015 outbreaks of adverse mussel deceases and parasite infections, such as the unicellular parasite *Marteilia*  
1016 *refringens*, which is known to disrupt the digestive system of marine bivalves (Villalba et al., 1993;  
1017 Villalba et al., 1997; Fuentes et al., 2002; Carrasco et al., 2015) and which, according to news bulletins  
1018 from the Institute of Marine Research (Bergen, Norway), recently was detected for the first time in  
1019 Norwegian coastal waters. The risk of spreading mussel deceases should be taken into consideration in  
1020 mussel transplant studies (Brenner et al., 2014), and this emphasizes the importance of only using  
1021 healthy and decease free mussel populations to be the source for sentinel specimens to mussel caging  
1022 studies.

## 1023 **10. Discussion**

1024 In this review, we provide an extract of the knowledge related to blue mussels in environmental  
1025 sciences. Blue mussels are among the most frequently used sentinels in marine environmental  
1026 monitoring in temperate seas worldwide, with data reported in tens of thousands of reports. Many key  
1027 issues are broadly investigated in *Mytilus*, but, as indicated by the table summaries included in this  
1028 paper, there are still many issues that yet are not or only little studied; such as toxicokinetics of dioxins  
1029 and PBDEs or field studies of the exposure, accumulation, and effect of nanoscale particulate  
1030 contaminants.

1031 The great suitability of *Mytilus* spp. as environmental sentinels becomes evident when considering the  
1032 criteria for a good monitoring species set by OSPAR commission (2012), i.e. a suitable monitoring  
1033 species should:



- 1034 • Reflect changes in the concentration of contaminants in the surrounding environment
- 1035 • Have similar bioconcentration factors throughout the monitored maritime area
- 1036 • Accumulate contaminants without being seriously affected by the concentrations typically
- 1037 encountered in the marine environment
- 1038 • Be representative of the study area
- 1039 • Be abundant throughout the study area
- 1040 • Be of reasonable size so it can provide adequate amounts of tissue for chemical, biochemical,
- 1041 and physiological analyses
- 1042 • Be easy to sample and hardy enough to survive in the laboratory, thus allowing defecation
- 1043 before analysis (if desired), laboratory studies of contaminant uptake, and laboratory studies
- 1044 for verifying biological field observations.

1045 Because blue mussels are stationary filter feeders that generally are tolerant to handling, they are  
1046 highly suitable for use in transplant caging experiments at marine field locations. The mussel caging  
1047 concept is an important supplement to the use of native mussel as it opens for better opportunities of  
1048 study control as well as facilitating the involvement of experimental factor manipulations in field  
1049 studies. The mussel caging approach has gained increasing popularity in recent years. Already,  
1050 roughly 10 % of all the reported blue mussel ecotoxicity studies have used transplant caging of  
1051 mussels as the design of study, and we foresee this could further increase in the coming years.

1052 Although the present review mainly focuses on the many positive reasons for using blue mussels as  
1053 environmental sentinels, it is also important to realize the most important challenges. For example,  
1054 influence from natural confounding factors or adoption to a suboptimal study design may rapidly  
1055 lessen the information value of *Mytilus* field studies. It is therefore important that investigators pursue  
1056 a high degree of study control in field studies both to optimize the quality of the investigation and to  
1057 minimize the risk for Type I and Type II errors (see confounding factor section). Indeed, there are  
1058 plentiful of examples in the blue mussel literature of studies which have not succeeded to detect any  
1059 clear effect signal in sentinel mussels from waters that evidently were significantly polluted by  
1060 anthropogenic sources. Most of all, this illustrates the need for critical attention among environmental  
1061 researches on key aspects of study quality. After the introduction of the EU WFD in year 2000 there  
1062 has been intensified collaboration among European countries regarding the environmental protection  
1063 of water bodies, and with the subsequent introduction of MSFD in 2008 these efforts have been further  
1064 focused and broadened on marine environments. Important tools in both these regulations are  
1065 Environmental Quality Standards (EQSs), which are developed for different environmental matrices,  
1066 i.e. defined concentrations of prioritized hazardous substances that defines the thresholds for  
1067 compliance to regulatory demands. In this review, we have highlighted the issue of anthropogenic  
1068 contaminants in blue mussels and discussed whether appropriate Environmental Assessment Criteria  
1069 (benchmarks for assessing degrees of anthropogenic pollution) are available for chemical monitoring

1070 with blue mussel sentinels, or whether such tools should be further established or improved. In this  
1071 connection, we emphasize that knowledge about the typical variability of hazardous anthropogenic  
1072 substances in mussels living in non-polluted and in polluted waters is important. Indeed, it is our  
1073 opinion that marine mussels possibly should acquire an extended role as sentinels for marine  
1074 monitoring. As described by the WFD regulation, each member state can choose to establish their own  
1075 quality standards if these provide an equally good level of environmental protection (2013/39/EU §17)  
1076 and guidelines for how to carry out harmonized chemical monitoring of sediment and biota under  
1077 WFD have been published under the WFD Common Implementation Strategy (EC, 2010). There are  
1078 several key advantages by using sediments or biota as monitoring matrices as alternatives to water  
1079 samples. For example, monitoring organic micro-contaminants in water is often hampered with  
1080 analytical detection problems and a need for a higher frequency of sampling and analysis.  
1081 Furthermore, in many countries there is a long tradition for monitoring sediment and biota, thus to  
1082 maintain time series data, continued practice is of importance. Directive 2013/39/EU also emphasize  
1083 the importance of time series data, as it is stated that “member States shall determine the frequency of  
1084 monitoring in sediment and/or biota to provide sufficient data for a reliable long-term trend analysis”.

1085 As blue mussels so widely are used in chemical monitoring of coastal waters, it is unfortunate that the  
1086 WFD biota EQSs are generally not adapted for mussels. Scientists who are using mussel sentinels to  
1087 perform compliance monitoring in coastal waters must from a regulatory standpoint use fish-based  
1088 biota EQSs as assessment criteria for classifying their mussel-based monitoring data, as these are the  
1089 standards that are embedded in the law. However, to use EQSs adapted for fish to assess PS  
1090 concentration data in suspension feeders such as blue mussels may most often not be suitable, unless  
1091 appropriate conversions are employed. However, such conversions may be questionable. In a recent  
1092 study, OSPAR conducted an assessment criteria comparison (EAC/EQS) for mercury in marine biota  
1093 (OSPAR, 2016). The study revealed that with an EQS conversion as described by EU’s technical  
1094 guidance documents (EC, 2011, 2014) 99 % of the OSPAR data exceeded the biota EQS and would  
1095 not be compliant. Furthermore, they concluded that a goal to reduce this portion significantly would  
1096 not be feasible. It was also concluded that “even in the absence of trophic adjustment elements (i.e.  
1097 direct comparison of data to the EQS<sub>biota</sub>) a significant number of OSPAR time series data would fail  
1098 the EQS<sub>biota</sub> threshold”.

1099 Above certain concentration levels, hazardous substances are expected to cause measurable toxic  
1100 stresses to the sentinel organism itself, or to the animals that eat it (secondary poisoning). A key idea  
1101 of the EQSs for marine biota established under WFD is to define the concentration thresholds of  
1102 prioritized toxicants below which no adverse effects are expected in marine sentinels (regardless of  
1103 primary or secondary poisoning). Hence, these benchmarks are key tools for interpretation of chemical  
1104 monitoring data in marine sentinels and for quality classing of the body of seawater in which the  
1105 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 facilitates 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 constraints (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

1142 there could be a concentration dependency of the uptake (e.g. for PFCs). Nevertheless, blue mussels  
1143 have played a crucial role for both regional and local trend monitoring of key pollutants and for  
1144 compliance monitoring of industries that release hazardous chemicals into coastal water bodies. This  
1145 calls for a development of internationally harmonized assessment criteria for prioritized contaminants  
1146 specially adapted for blue mussel sentinels. Such assessment criteria must, in addition to being adapted  
1147 to substance toxicity issues, also be environmentally realistic, i.e. in comparison to the concentrations  
1148 levels which occur in coastal waters far away from major pollutant sources. The regulatory  
1149 benchmarks established for mussels, should be operative as triggers for counteracting and source-  
1150 reducing measures (i.e. towards industries and other parties who are responsible for the release of  
1151 prioritized contaminants). At the present, there is apparently an issue for several key PSs targeted by  
1152 the WFD EQS regulations in Europe (i.e. brominated diphenyl ethers, mercury, TBT and PCB7), and  
1153 these need urgent attention. Significant progress has been made regarding development and use of  
1154 pollutant responsive biomarkers in blue mussel sentinels, and for emerging issues, such as micro- and  
1155 nano-scale particulate contaminants, climate change and ecotoxicity of mixed pollution situations,  
1156 continued progress in the knowledge is expected in the years to come. However, it is also important to  
1157 further clarify and minimize the influence of confounding non-target factors in mussel monitoring, e.g.  
1158 by adopting international harmonization and standardization of study conditions and program designs.  
1159 Such developments could call for an increased use of mussel transplant caging as discussed herein.

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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.