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# Using mussel as a global bioindicator of coastal microplastic pollution

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Running title: Mussel as a bio-indicator of microplastics

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40 **Abstract**

41 The ubiquity and high bioavailability of microplastics have an unknown risk on the marine  
42 environment. Biomonitoring should be used to investigate biotic impacts of microplastic  
43 exposure. While many studies have used mussels as indicators for marine microplastic pollution,  
44 a robust and clear justification for their selection as indicator species is still lacking. Here, we  
45 review published literature from field investigations and laboratory experiments on microplastics  
46 in mussels and critically discuss the suitability and challenges of mussels as sentinel organisms  
47 for microplastic pollution. Mussels are suitable sentinel organisms for microplastic pollution  
48 because of their wide distribution, vital ecological niches, susceptibility to microplastic uptake  
49 and close connection with marine predators and human health. Field investigations highlight a  
50 wide occurrence of microplastics in mussels from all over the world, yet their abundance varies  
51 enormously. Problematically, these studies are not comparable due to the lack of a standardized  
52 approach, as well as temporal and spatial variability. Interestingly, microplastic abundance in  
53 field-collected mussels is closely related to human activity, and there is evidence for a positive  
54 and quantitative correlation between microplastics in mussels and surrounding waters.  
55 Laboratory studies collectively demonstrate that mussels may be good model organisms in  
56 revealing microplastic uptake, accumulation and toxicity. Consequently, we propose the use of  
57 mussels as target species to monitor microplastics and call for a uniform, efficient and  
58 economical approach that is suitable for a future large-scale monitoring program.

59

60 **Keywords:** microplastic; mussel; bioindicator; plastic pollution

61 **Capsule:** Mussel is a global bioindicator of microplastic pollution.

62

## 63 1. Introduction

64 Environmental presence and accumulation of plastic debris has become a widespread  
65 scientific and social concern due to the dramatic increase in the production of plastics, with an  
66 estimate of an additional 335 million tonnes of world plastic production in 2016 alone  
67 (PlasticsEurope, 2017). Microplastics (particles less than 5 mm; Arthur et al., 2009) are reported  
68 to account for 92.4% among marine plastic debris (Eriksen et al., 2014) and have been identified  
69 in many environmental matrices globally. This includes surface waters of every major ocean, the  
70 water column, beaches, sea ice, deep sea sediment, marine biota and consumables sourced from  
71 the sea (Nor and Obbard, 2014; Van Cauwenberghe et al., 2013; Ng and Obbard, 2006; Eriksen  
72 et al., 2014; Cózar et al., 2014; Wesch et al., 2016; Yang et al., 2015; Van Sebille et al., 2015;  
73 Lusher et al., 2014, 2015; Browne et al., 2011).

74 Microplastics ingestion has been identified in a range of species from mussels to mammals,  
75 with over 220 species from different trophic levels consuming microplastic debris *in natura*, and  
76 99% of all seabird species are predicted to ingest microplastic by 2050 (Ter Halle et al., 2017;  
77 Lusher et al., 2017a; Wilcox et al., 2015; Hu et al., 2016). Microplastic ingestion by marine  
78 organisms can accelerate microplastics' transference from the sea surface through the water  
79 column to the sea floor via feces and marine snow, or between trophic chains via predation  
80 (Farrell and Nelson, 2013; Santana et al., 2017; Setälä et al., 2014; Katija et al., 2017).

81 Additionally, microplastics are subjected to biofouling leading to colonization by  
82 microorganisms and invertebrates, which in turn can contribute to long-range transport of alien  
83 species, and serve as reservoirs for pathogen transmission, which broadens the risks of  
84 microplastic pollution to marine organisms and ecosystems (Andrady, 2011; Barnes, 2002;  
85 GESAMP, 2015, 2016). In addition, environmental weathering of microplastics may also cause

86 release of harmful monomers and additives from the polymer into the associated media (Gandara  
87 e Silva et al., 2016; Nobre et al., 2015; Rochman et al., 2014). Together, these aspects represent  
88 some of the primary and emerging problems associated with microplastics to date but are by no  
89 means the only issues.

90 Since microplastics are ubiquitous and bioavailable, the associated environmental and  
91 health impacts have received an increasing amount of attention amongst the scientific community,  
92 regulatory agencies, the public, media and policy makers. Nevertheless, consequences of wild  
93 biota interacting with microplastic have not been established, although the current body of  
94 evidence from laboratory studies suggests that microplastic exposure may lead to a suite of  
95 negative health effects for marine biota; including for example, increased immune response,  
96 decreased food intake and growth rate, weight loss, energy depletion, apoptosis, upregulation of  
97 stress and damage repair pathways and negative impacts on subsequent generations (e.g., Von  
98 Moos et al., 2012; Besseling et al., 2013; Canesi et al., 2015; Sussarellu et al., 2016). However,  
99 to date most exposure studies have tested unrealistically high doses, and used plastic polymers  
100 that are less environmentally-relevant (Phuong et al., 2016), making extrapolation challenging in  
101 terms of the microplastic associated risk to the environment. In addition, microplastics' capacity  
102 to adsorb, act as vectors of, and leach toxic substances to marine biota may also pose further  
103 health risks (Frère et al., 2017; Engler, 2012; Browne et al., 2013; Gandara e Silva et al., 2016).

104 Despite uncertainties regarding ecological and health risks of microplastic pollution,  
105 knowledge based on the wide occurrence of microplastics in the environment has led to calls to  
106 classify microplastics as hazardous, and plastic pollution has been compared with climate change  
107 in terms of scale and degree of severity by the United Nations Environment Programme  
108 (Rochman et al., 2013; UNEP, 2016; Borrelle et al., 2017). From a risk assessment perspective, it

109 is necessary to develop a comprehensive and harmonized evaluation method of microplastic  
110 pollution for inclusion in routine monitoring programs. Traditionally, three marine compartments  
111 including water column, sediment and biota could be used to monitor spatial and temporal trends  
112 of microplastic abundance. However, microplastic abundances in water and sediment tend to be  
113 affected by a variety of environmental factors such as biofilms, bioturbation, tides, winds,  
114 currents and wave fronts; all these parameters giving a stochastic pattern, which can complicate  
115 the interpretation of impacts on biota (Gibson and Bowman, 2000; Turra et al., 2014; Eriksen et  
116 al., 2014; GESAMP, 2015; Moreira et al., 2016a,b; Fisner et al., 2017). In addition, sediment is a  
117 more complicated compartment to analyze than water and most biota, including mussels, since  
118 sample processing requires multiple steps, which have not been standardised by the scientific  
119 community, to degrade organic material and separate microplastics from natural particles. In  
120 terms of addressing unknowns regarding risk, biomonitoring, alongside investigations to  
121 understand the relationship between an organism and the polluted environment with respect to  
122 microplastics and their ingestion, can be used (Gibson and Bowman, 2000; Wesch et al., 2016).

123 To have a robust sentinel species for environmental monitoring the following criterias  
124 should be fulfilled: a wide distribution range, a well known biology, immobility, an ability to  
125 provide an early alert, a key function in the ecosystem, a homogeneous response to pollutants,  
126 and the existence of identifiable toxic effects associated with the degree of pollution (Hilty and  
127 Merenlender, 2000; Goodsell et al., 2009). Seabirds and sea turtles have been selected as  
128 bioindicators for monitoring ingestion of plastic debris (>1 mm) for the land-ocean interaction.  
129 For instance, fulmar (*Fulmarus glacialis*) is used as an indicator species in Northern Europe, and  
130 its digestive content is currently utilized as an indicator for regional plastic pollution under the  
131 OSPAR Convention (Van Franeker et al., 2011). Loggerhead turtles (*Caretta caretta*) have been

132 chosen as a target species to monitor litter presence in the Mediterranean Sea under UNEP-  
133 MedPol Convention and Descriptor 10 of the European Union (EU)'s Marine Strategy  
134 Framework Directive (MSFD) (Galgani et al., 2014). The suitability of loggerhead turtles as a  
135 bioindicator for marine litter >1 mm has been confirmed and is widely supported (Campani et al.,  
136 2013; Matiddi et al., 2017; Pham et al., 2017). Although some studies have addressed their  
137 proposal for indicator species in microplastic investigation, a robust and clear justification for  
138 their selection as indicator species is still scarce (Wesch et al., 2016). Furthermore, the methods  
139 currently used are not appropriate for the study of the ingestion of smaller microplastics (<1 mm).

140       Mussels have been utilized extensively as ideal biological indicators in monitoring of  
141 anthropogenic pollution trends in coastal waters due to their special characteristics (Farrington et  
142 al., 2016; Beyer et al., 2017). As one of the first animals used to assess the environmental quality  
143 of seawater (Goldberg, 1975), mussels meet almost all required criteria for a useful indicator  
144 species. Firstly, mussels are globally distributed, easily accessible and have a high tolerance to a  
145 wide range of environmental parameters including salinity, temperature, oxygen levels and food  
146 availability (Bayne, 1976; O'Connor, 1998). Furthermore, as representative benthic filter feeders,  
147 mussels can efficiently accumulate chemical pollutants from seawater to provide an integrative  
148 measure of the concentration and bioavailability of seawater pollutants *in situ* (Beyer et al.,  
149 2017). Mussels provide food (Kautsky, 1981) and habitat (Norling and Kautsky, 2007) to a lot of  
150 other species, forming important links between pelagic and benthic ecosystems (Dame, 1993).  
151 They also act as a transport route of marine pollutants to higher trophic levels in the coastal  
152 marine food chain (Meador et al., 1995; Strand and Jacobsen, 2005). Importantly, mussels have  
153 been an important seafood for humans for thousands of years (Beyer et al., 2017). Hence,  
154 mussels also attract attention regarding assessing human health risks associated with marine



155 pollution (Van Cauwenberghe and Janssen, 2014; UNEP, 2016). Up to now, mussel has been  
156 widely used in many regional environmental monitoring programs such as U.S. Mussel Watch  
157 Project, Assessment and Control of Pollution in the Mediterranean region (MEDPOL), OSPAR's  
158 Coordinated Environmental Monitoring Program (CEMP) (Beyer et al., 2017).

159 In this review, both the suitability and challenges related to mussels as sentinel species for  
160 microplastic pollution will be discussed. We aim to address (i) why mussels lend themselves as  
161 good indicators of microplastics; (ii) the extent to which mussel can provide useful information  
162 regarding microplastics pollution in the marine environment; and (iii) how to improve current  
163 methodology, with an emphasis of standardization of techniques to allow cross calibration  
164 between studies worldwide.

## 165 **2. Global field investigations on microplastic pollution in mussels**

166 Environmental risks associated with microplastics are primarily focused on their suspected  
167 bioavailability for marine organisms (Wright et al., 2013; Desforges et al., 2015). Bivalves are of  
168 particular interest because their extensive filter-feeding activity exposes them directly to  
169 microplastics present in the environment. Globally, microplastic occurrences in wild caught  
170 mussels have been extensively investigated and reported (Table 1).

### 171 **2.1 Selected species and geographic coverage**

172 Blue mussels (*Mytilus* spp.) are currently the dominant species used for field investigations  
173 of microplastics. The genus *Mytilus* has seven subspecies that can interbreed with each other and  
174 are widely distributed around the world (Beyer et al., 2017). For instance, *M. galloprovincialis*  
175 has become an invasive species and is widely spreaded in South America, South Africa, Japan,  
176 California, New Zealand, and Australia (Beyer et al., 2017). Different species within the genus

177 *Mytilus* have different genomic composition and gene expression profiles, which may lead to  
178 differences in the way they deal with stress as well as microplastic uptake (De Witte et al., 2014;  
179 Lusher et al., 2017b). *Mytilus* spp. have been investigated in all the involved countries except  
180 Brazil and Indonesia, which investigated *Perna viridis* and *P. perna* instead (Table 1, Fig. S1).

181 Spatially, field investigations of microplastics in mussels are currently spread over 16  
182 countries (Fig. S1), especially in European countries including Germany, France, Belgium, the  
183 Netherland, Italy, Greece, Portugal, Spain, Denmark, Finland, Norway and the U.K. In addition,  
184 research from China, Indonesia, Canada and Brazil also contribute to the available field data.  
185 Research on microplastic can be traced back to 1970s when the occurrence of small plastic  
186 particles in coastal environment was first reported (Bowmer and Kershaw, 2010). At that time,  
187 small polystyrene beads in New England (Carpenter et al., 1972), Sargasso Sea (Carpenter and  
188 Smith, 1972) and Bristol Channel (Morris and Hamilton, 1974) attracted researchers's attention.  
189 Afterwards, the term "microplastic" were put forward for the first time by Thompson in Europe  
190 (Thompson et al., 2004). Currently, the monitoring of marine litter is required as part of the EU  
191 Marine Strategy Framework Directive (MSFD) (Hanke et al., 2013) and many projects fund  
192 research on microplastic pollution in Europe such as Marine Litter Projects Funded under FP7  
193 and Horizon 2020, which likely accounts for the increased number of studies from Europe.

194

**Table 1. Summary of global field investigations on microplastics in mussels.**

Species & Location	Digestion method	Identification technique	Classification	Abundance (items/g.ww)	Size ( $\mu\text{m}$ )	Environmental media	Reference
<i>Mytilus. edulis</i>							
Canada	30% H <sub>2</sub> O <sub>2</sub>	visual sorting	fiber	2.79-7.42 <sup>a</sup>	no data	sediments: 2-8 items/g.dw	Mathalon and Hill, 2014
Germany	69% HNO <sub>3</sub>	micro-Raman	particle	0.36±0.07	no data	no data	Van Cauwenberghe and Janssen, 2014
Belgium	HNO <sub>3</sub> :HClO <sub>4</sub>	visual sorting	fiber, fragment, film, sphere	0.26-0.51	200-1500	no data	De Witte et al., 2014
France, Belgium, Netherlands	69% HNO <sub>3</sub>	micro-Raman	particle	0.2±0.3	20-90	seawater: 0.4±0.3 items/L sediments: 6±5.7 items/kg.dw	Van Cauwenberghe et al., 2015
UK	trypsin	FTIR	fiber, bead, fragment, film	1.05-4.44	200-10670	no data	Courtene-Jones et al., 2017
UK	Corolase 7089 enzyme	FTIR	fiber, particle, film	2.5	no data	no data	Catarino et al., 2017
UK	30% H <sub>2</sub> O <sub>2</sub>	micro-FTIR	fiber, fragment, sphere, flake	0.7-2.9	8-4700	seawater: 1.5-6.7 items/L	Li et al., 2018
Netherlands	proteïnase K and 30% H <sub>2</sub> O <sub>2</sub>	Raman	fiber, particle	37 (items/g.dw)	30-2000	seawater: 27 items/L sediments: 48 items/kg.dw	Karlsson et al., 2017
Netherlands	HNO <sub>3</sub> , NaOH & 30% H <sub>2</sub> O <sub>2</sub>	FTIR	fibre, sphere, foil	19-105 (items/g.dw)	10-5000	sediments: 100-3600 items/kg.dw	Leslie et al., 2017
France	10% KOH	micro-FTIR	filament, fragment	0.23±0.20	20-400	no data	Phuong et al., 2018a
France	10% KOH	micro-FTIR	fiber, fragment	0.23±0.09	30-200	no data	Phuong et al., 2018b
Canada	68-70% HNO <sub>3</sub>	FTIR	fiber, fragment, pellet	wild: 138±202 farmed: 259±114	<530	no data	Murphy, 2018
China	30% H <sub>2</sub> O <sub>2</sub>	micro-FTIR	fiber, fragment, sphere, flake	2.2	5-5000	no data	Li et al., 2016
China	30% H <sub>2</sub> O <sub>2</sub>	micro-FTIR	fiber, sheet, fragment, sphere,	9.2 <sup>b</sup>	50-5000	no data	Kolandhasamy et al., 2018
China	30% H <sub>2</sub> O <sub>2</sub>	micro-FTIR	fiber, fragment, pellet	1.52-5.36	5-4000	seawater: 0.68-6.44 items/L	Qu et al., 2018
<i>M. galloprovincialis</i>							
Italy	30% H <sub>2</sub> O <sub>2</sub>	visual sorting	filament, fragment	0.05 (items/g.dw)	60.01 ±38	no data	Bonello et al., 2018
Italy	30% H <sub>2</sub> O <sub>2</sub>	visual sorting	filament	6.2-7.2 <sup>c</sup>	750-6000	no data	Renzi et al., 2018
Italy, Portugal, Spain	69% HNO <sub>3</sub>	visual sorting	fiber, particle	0.12±0.04	no data	no data	Vandermeersch et al., 2015b

Italy, Portugal, Spain	HNO <sub>3</sub> :HClO <sub>4</sub>	visual sorting	fiber, particle	0.18±0.14	no data	no data	Vandermeersch et al., 2015b
Greece	30% H <sub>2</sub> O <sub>2</sub>	FTIR	filament, fragment, film	46.25% ingested microplastics	<5000	seawater: 0.41 items/m <sup>2</sup>	Digka et al., 2018a
Greece	30% H <sub>2</sub> O <sub>2</sub>	FTIR	fiber, fragment	wild:5.3±0.5 <sup>d</sup> farmed:2.5±0.3 <sup>d</sup>	40-737	sediments: 1816.7 items/m <sup>2</sup> no data	Digka et al., 2018b
China	30% H <sub>2</sub> O <sub>2</sub>	micro-FTIR	fiber, fragment, pellet	2.39 ±1.32	5-5000	no data	Li et al., 2015
<b><i>M. trossulus</i></b>							
Finland	Sodium Dodecyl Sulphate (SDS) and detergent enzymes	FTIR	fiber, fragment, sphere, flake	0.4 ± 1.9	>20	seawater: 11.4-23.5 items/m <sup>3</sup>	Railo et al., 2018
<b><i>Mytilus spp.</i></b>							
Norway	10% KOH	micro-FTIR	fiber, foam, fragment, film	1.85±3.74	150-8010	no data	Lusher et al., 2017b
Norway	10% KOH	micro-FTIR	fiber, foam, fragment, film	0.97±2.61	70-3870	no data	Bråte et al., 2018b
UK	Corolase® 7089 enzyme	Nile Red staining and FT-IR	fiber, film, sphere, other particle	3±0.9	200-2000	no data	Catarino et al., 2018
Italy, Netherlands France, Denmark, Spain, Portugal	HNO <sub>3</sub> :HClO <sub>4</sub>	visual sorting	fiber, particle	0.13±0.14	no data	no data	Vandermeersch et al., 2015b
<b><i>Modiolus modiolus</i></b>							
UK	Corolase® 7089 enzyme	Nile Red staining and FT-IR	fiber, film, sphere, other particle	0.086±0.031	200-2000	no data	Catarino et al., 2018
<b><i>Perna perna</i></b>							
Brazil	22.5 M HNO <sub>3</sub>	visual sorting	fiber, irregular particle	75% ingested microplastics	no data	no data	Santana et al., 2016
<b><i>P. viridis</i></b>							
Indonesia	30% H <sub>2</sub> O <sub>2</sub>	SEM/EDX <sup>e</sup>	fiber, fragment, sphere, flake	4-20	51.31-232	no data	Khoironi et al., 2018
China	30% H <sub>2</sub> O <sub>2</sub>	micro-FTIR	fiber, fragment, pellet	1.52-5.36	5-4000	seawater: 0.68-6.44 items/L	Qu et al., 2018

197

198 <sup>a</sup> The microplastic level was transferred by dividing total microplastics per individual by the shelled weight. <sup>b</sup> The abundance of microplastics in intestine. <sup>c</sup> The  
199 abundance of microplastics in hepatopancreas and gills. <sup>d</sup> The abundance of microplastics in digestive glands and gills. <sup>e</sup> Scanning Electron Microscopy/ Electron  
200 Dispersive X-Ray.

## 201 **2.2 Characteristics of microplastic pollution**

202 It is indisputable that microplastics are widespread in both wild and farmed mussels in  
203 many countries (Table 1). Regarding the morphotypes of microplastics observed in such mussels,  
204 fibers are dominant in 13/27 of the current filed investigations compared with fragments which  
205 account for 5/27. Only one paper reported the prevalence of pellets (Murphy, 2018). The  
206 remaining studies counted one type of microplastics due to methodological limitations or omitted  
207 to report the proportion of different types. Polyethylene, polypropylene, polystyrene, polyester,  
208 polyethylene terephthalate, polyamide, polyvinyl chloride and cellophane were the most reported  
209 polymers. Out of the studies conducted, nine of them did perform a corresponding investigation  
210 of the microplastic level in the associated sediment or seawater (Table 1). From these, it appears  
211 that the main morphotype and polymeric composition in mussels tend to be consistent with their  
212 surrounding environmental media (Li et al., 2018; Leslie et al., 2017; Qu et al., 2018; Digka et al.,  
213 2018a; Railo et al., 2018). Furthermore, Qu et al. (2018) observed consistency of their proportion  
214 in mussels and in seawater. These results suggest that the microplastics in mussels can reflect the  
215 real pollution status in the environment in terms of morphotype and polymer types.

216 For the size range of microplastics, the current working minimum limit is 5 $\mu$ m, yet some  
217 studies fail to provide information on the minimum size of the detected microplastics (Table 1).  
218 The minimum limit depends methodology employed by research teams. Selected research to date  
219 have adopted a classified size range approach and in doing so have highlighted a dominant  
220 smaller size range (e.g., 5-250  $\mu$ m, 10-300  $\mu$ m, 50-100  $\mu$ m, 50-250  $\mu$ m, 100-500  $\mu$ m, 0.25-1 mm)  
221 that reveals mussel's uptake incidences for specific size ranges (Li et al., 2018; Leslie et al., 2017;  
222 Phuong et al., 2018a; Kolandhasamy et al., 2018; Qu et al., 2018; Digka et al., 2018b). However,  
223 the lack of a unified classification standard for reporting the size range complicates efforts to

224 compare these results. In addition, smaller size of microplastics seems to take up a larger  
225 proportion in mussels compared to the surrounding environmental medium (Li et al., 2018; Qu et  
226 al., 2018; Digka et al., 2018a). For example, the smaller microplastics (<1 mm) account for  
227 62.3%, 96.9%, 100% in seawater, sediments and mussels from the Northern Ionian Sea  
228 respectively (Digka et al., 2018a) and the mussels from U.K. contained 44%-83% of smaller  
229 microplastics (less than 250  $\mu\text{m}$ ) compared to seawater with only 30%-40% (Li et al., 2018).  
230 Another interpretation is thus that the microplastics in mussels indicates the size range in the  
231 surrounding environment partially as a factor of their selective feeding behavior (Ward and  
232 Shumway, 2004).

233 Microplastic abundance varies between different studies, ranging from 0.05 items/g to 259  
234 items/g (Bonello et al., 2018; Murphy, 2018). This is mainly due to the differences in levels of  
235 background contamination and the diversity of methods used amongst different research groups  
236 as well as regional variations in microplastic content. On a broad scale, research has  
237 demonstrated a positive correlation between coastal microplastic concentrations and human  
238 population density (Browne et al., 2010, 2011). Furthermore, microplastic abundance in mussels  
239 is closely related to human activity, and mussels from areas with intensive human activities  
240 contain significantly higher numbers (Li et al., 2016), or in areas suggested to have accumulation  
241 zones of microplastics such as the Barents Sea (Lusher et al., 2017b). There are indications that  
242 microplastics can accumulate because significantly higher concentrations have been found in  
243 mussels ( $3.7 \times 10^4$  items/kg dry weight) compared to surrounding sediment (48 items/kg dry  
244 weight) and seawater (27 items/L) (Karlsson et al., 2017). When we unify the units of the  
245 abundance in mussels as items/g.w and in seawater as items/L, similar abundances can be found  
246 in mussels and ambient seawater (Table 1, Van Cauwenberghe et al., 2015; Li et al., 2018;

247 Karlsson et al., 2017; Qu et al., 2018), which is further supported by a recent study that showed a  
248 positive and quantitative correlation of microplastics in mussels and in their surrounding waters  
249 (Qu et al., 2018). This indicates that microplastic pollution in mussels is closely correlated with  
250 the degree of pollution in coastal habitats and can reflect the real abundance of microplastics in  
251 the environment within certain size range. However, one study does not show the quantitative  
252 correlation between microplastics in mussels and their ambient seawaters (Li et al., 2018), this  
253 may be due to limited sampling sites and outliers derived from contingency. More studies are  
254 still needed to verify this outcome.

### 255 **2.3 Methodological challenges**

256 Procedures for investigating microplastic pollution in mussels involve a series of steps and  
257 details that must be taken into consideration including: sampling sites and strategy, sample size  
258 (number of individuals per site), individual condition, sample storage, digestion solution, filter  
259 pore size, chemical identification techniques, classification of microplastics, reporting units, and  
260 contamination control. Although many reviews have systematically and critically discussed  
261 existing microplastic extraction methods and identification techniques, there is still a lot of  
262 debate and many knowledge gaps surrounding choices of an optimal method (Hidalgo-Ruz et al.,  
263 2012; Lusher et al., 2017a,b; Elert et al., 2017; Shim et al., 2017). Variations in methods make it  
264 hard to compare microplastic contamination among different studies and locations  
265 (Vandermeersch et al., 2015b).

266 Hence, a major challenge for monitoring microplastic pollution within mussels is the lack of  
267 uniform methods from extracting to identifying microplastics. Call for the standardization or  
268 harmonization of methods are repeatedly highlighted by the International Council for the  
269 Exploration of the Sea (ICES) and researchers working within the field (ICES, 2015; Hidalgo-

270 Ruz et al., 2012; Wesch et al., 2016; Lusher et al., 2017a, b; Rochman et al., 2017). Since these  
271 methods always have a tension between accuracy, precision and feasibility, different approaches  
272 should be chosen according to the sampling sites, media, equipment, replicates request and the  
273 specific scientific questions of interest (Rochman et al., 2017). In this situation, we suggest that  
274 both standardization and intercalibration of different methods should be adopted at the same time  
275 for improving the comparability of different studies. Some factors could be united while other  
276 variables should be intercalibrated and selected according to the actual situation in the specific  
277 procedure.

278         Sampling strategy represents a challenge in designing a representative and adequately  
279 replicated monitoring scheme. Patchiness of microplastics in different spatial (Browne et al.,  
280 2011; Moreira et al., 2016a; Fisner et al., 2017) and temporal (Moreira et al., 2016b) scales may  
281 lead to variable amounts within mussels. Phuong et al. (2018a) showed the season was not a  
282 relevant influencing factor on the quantitative and qualitative analysis of microplastics in  
283 mussels. However, a different conclusion revealed the similarity of microplastic types and  
284 significant differences of abundance in mussels collected in different seasons (Catarino et al.,  
285 2018). That is to say, some factors changing with season (e.g., wind, currents, rainfall,  
286 temperature, human activity) may affect microplastic distribution. The extent to which these  
287 factors change microplastic abundance or type in the environment varies with sampling sites.  
288 Sampling time and sites should be variable factors considered during the investigation; such that  
289 harmonization of sampling strategy should take these complex environmental and anthropogenic  
290 factors that shows temporal and spatial differences into consideration. Additional factors such as  
291 sampling number and preservation method must also be standardized. Both ICES and MSFD  
292 recommend 50 individuals per species, although research suggest 20 individuals could also be a



293 suitable number for large-scale spatial investigations (Lusher et al., 2017b). Finally, but definitely  
294 most importantly, is to minimise contaminaton as much as possible during the sample  
295 preservation and identification processes.

296 For the extraction method, common agents used to digest biotic tissues include acid ( $\text{HNO}_3$ ,  
297  $\text{HNO}_3\text{:HClO}_4$ ), alkaline ( $\text{NaOH}$ ,  $\text{KOH}$ ), oxidizing ( $\text{H}_2\text{O}_2$ ) and enzymatic (trypsin, proteinase K,  
298 Corolase 7089) approaches. However, drawbacks of these digestion methods have been widely  
299 reported, such as structural damage, dissolution and discoloration caused by acid, basic and  $\text{H}_2\text{O}_2$ ;  
300 incomplete soft tissue digestion by enzyme; production of foam caused by  $\text{H}_2\text{O}_2$ ; expensive price  
301 and time-consuming nature of some of the solvents (Table 1, Lusher et al., 2017b). This might  
302 lead to underestimations of microplastic loads, especially smaller particles, or limit their  
303 adaptability for large scale monitoring. Hence, selection of a digestion solution requires further  
304 testing and optimization.

305 In the future investigations, different digestion agents could be chosen under the premise  
306 that the selected agent does not destroy the main polymer types in the objective environment,  
307 which requires consulting literature or preliminary research. In addition, the digestion efficiency  
308 and recovery rate should be provided in order for the intercalibration of methods. However, only  
309 ten published studies report corresponding recovery rate and five tested polymer alterations by  
310 digestion treatment (Table 1). Low digestion efficiency and recovery rate may lead to  
311 underestimations of microplastics, therefore, a threshold for both efficiency is required.

312 The pore size of the filter, the magnification times and resolution of microscopy employed  
313 determine the observed microplastic size lower limit. ICES has recommended the use of filter  
314 with 5  $\mu\text{m}$  pore size for mussel (Vandermeersch et al., 2015b). In the current literature, 5  $\mu\text{m}$   
315 pore size of filter has been the most frequently used (9/27). Other studies had finer (0.45, 0.7, 0.8,

316 1.2, 2.5, 2.7  $\mu\text{m}$ ) or bigger (12, 20  $\mu\text{m}$ ) size. Among all the given size ranges of microplastics  
317 detected in mussels, 5  $\mu\text{m}$  is the minimum size (Table 1). Although smaller sizes of microplastic  
318 undoubtedly occur in mussels, their observation and identification are still limited by current  
319 instrumentation and method. For example, 20  $\mu\text{m}$  seems to be the smallest size that could be  
320 identified using  $\mu\text{FTIR}$  in the reflection mode under manual inspection (Phuong et al., 2018b).  
321 Hence, 5  $\mu\text{m}$  is a good choice for the unity of pore size of filter. The detection limit of current  
322 methods will not hamper the use of mussels as a bioindicator of microplastic pollution since a  
323 quantitative correlation of microplastics within certain size range in mussels and in their  
324 surrounding waters has been demonstrated (Qu et al., 2018).

325 Current methods for microplastic identification involve visual sorting (with the aid of  
326 polarized light microscopy), Nile Red staining, Fourier transformed infrared spectrometry (FT-  
327 IR), attenuated total reflectance (ATR), Raman spectrometry, pyrolysis-gas chromatography  
328 combined with mass spectrometry (Pyr-GC-MS), high temperature gel-permeation  
329 chromatography (HT-GPC) with IR detection, SEM-EDS, thermal extraction desorption gas  
330 chromatography mass spectrometry (TED-GC-MS) and liquid extraction. FT-IR is the most  
331 commonly used technique in recent literature (Table 1). Each applied technique has some  
332 drawbacks including size limitations, time constraints and interference factors and we refer the  
333 readers to published literature on the advantages and limitations of these methods (Lusher et al.,  
334 2017b; Elert et al., 2017; Shim et al., 2016, 2017). Since no single method is able to obtain the  
335 physical (size, shape and colour) and chemical (polymer type) characteristics of particles in a  
336 single step, the combination of several parallel approaches should be applied and considered in  
337 future research. Meanwhile, intercalibration between different methods is necessary to

338 understand the extent to which each method differs and compare the data already collected with  
339 that in future studies.

340 Preliminary visual sorting is still needed for a fast quantification analysis. Nevertheless, the  
341 result is largely dependent on personal experience which may result in underestimation or  
342 overestimation of real results to different degree. A library matching the photos of environmental  
343 samples with their spectrograms should be established to help reduce error rates and  
344 misidentification and improve this method. For future, small-scale investigations, FTIR and  
345 Raman are strongly recommended with 70% match rate as a standard threshold which has been  
346 applied in most research. However, spectra libraries still require intercalibration. For future  
347 large-scale investigation, Nile Red staining and thermo-analytical technique could be combined  
348 to obtain both qualitative and quantitative information efficiently. However, the accuracy of Nile  
349 Red staining should be calibrated using spectroscopy methods simultaneously.

350 The variability in the way the results are characterized further hampers the comparison  
351 among different studies. These factors such as reporting units, classification of type and size  
352 range should be standardized in the future studies. Both items individual<sup>-1</sup> and items gram<sup>-1</sup> as  
353 reporting units are required. The latter is a more appropriate unite to compare different studies  
354 and it has been used most commonly in current research (Table 1). For the classification of type,  
355 four kinds including fiber (filament), fragment, sphere (pellet, bead), film (flake, sheet) could be  
356 adopted which almost covers all the types in current studies (Table 1). An optimal classification  
357 of size range still requires more research to determine. In addition, contamination control is a  
358 crucial factor during the whole procedure. Procedural blanks must be carried out to monitor  
359 contamination and correct the empirical data. Most of the current investigations (25/27) set

360 procedural blanks. Two studies even tested limit of detection of airborne fibers (De Witte et al.,  
361 2014).

### 362 **3. Laboratory exposures of microplastics in mussels**

#### 363 **3.1 Uptake, accumulation and clearance of microplastics**

364 In addition to field studies, mussels have been widely used in laboratory exposure  
365 experiments to study uptake, accumulation, clearance characteristics and impact of microplastics.  
366 Microplastic uptake has been demonstrated in all exposure concentrations (Table 2), and  
367 egestion as feces and pseudofeces has also been observed (Ward and Kach, 2009; Wegner et al.,  
368 2012; Khan and Prezant, 2018; Santana et al., 2018). During active feeding, mussels can  
369 continuously pump and filter seawater through coordinated action of cilia localized at the gill  
370 epithelium surface, at a rate of 50 ml of seawater per minute (Famme et al., 1986).

371 According to mussel feeding strategies and laboratory exposure studies, we can hypothesize  
372 pathways of microplastics intake and accumulation as follows. When microplastics in seawater  
373 encounter gill surfaces, they may be captured and trapped into mucus and subsequently  
374 assimilated over the gill epithelium or transported into the mouth and digestive system (Von  
375 Moos et al., 2012; Beyer et al., 2017; Bråte et al. 2018a; Kolandhasamy et al., 2018). Not every  
376 particle captured by gills is ingested (Santana, 2015; Santana et al., 2018) since mussels are able  
377 to separate and reject nonnutritive particles as pseudofeces as a way to defend organisms against  
378 high quantities of suspended particulate matter (Ward and Shumway, 2004).

379 Von Moos et al. (2012) demonstrated that mussels can ingest and accumulate microplastics  
380 (0-80 µm) in digestive system epithelial cells within hours. It appears that smaller particles are  
381 ingested and retained in mussels more easily compared to the larger particles (Van

382 Cauwenberghe et al., 2015). However, behavior of PVC particles in an  
383 emulsion/microsuspension (E/M PVC; size range of 0.1 to 1.0  $\mu\text{m}$  in diameter; Rodolfo et al.,  
384 2006) was different, with larger particles proportionally better represented in mussel digestive  
385 glands (0.8 to 0.96  $\mu\text{m}$ ) in comparison to surrounding water (mean size, 0.6  $\mu\text{m}$ ). Van  
386 Cauwenberghe et al. (2015) found that larger sized (15-500  $\mu\text{m}$ ) microplastics were detected in  
387 mussel's faeces compared to mussel tissue (20-90  $\mu\text{m}$ ). These findings indicate mussel's  
388 selection for a specific size range of microplastics during ingestion and egestion process, which  
389 is consistent with the results of the field investigations discussed in section 2.2. However, this  
390 selectivity characteristic poses an obstacle to the reflection of size distribution of microplastics in  
391 the environment through biomonitoring. More research is needed to test selectivity of mussels  
392 for larger scope and more gradient sizes of microplastics.

393 In addition to size variation, environmentally aged microplastics are differentially ingested  
394 with pre-weathered microplastic ingested to a higher extent by mussels compared with virgin  
395 microplastic (Bråte et al., 2018a). In most exposure studies, only particles or spheres were used  
396 for the exposure (Table 2), which ignores the selectivity of mussels for microplastics of different  
397 shapes. Qu et al. (2018) showed fibers were dominant in mussels from field investigation while  
398 beads were most ingested by mussels after five-day indoor exposure. One explanation is that  
399 fibers in mussels result from a long-term accumulation process in the marine environment while  
400 beads are more easily ingested by mussels in short time periods. Once ingested, beads could be  
401 egested more quickly than fibers. The delay in egestion of synthetic fibers has been addressed  
402 since only fibers were detected in mussels after gut clearance period (De Witte et al., 2014).  
403 Moreover, fibers trapped into gills and hepatopancreas cannot be easily removed by individuals  
404 (Renzi et al., 2018).

405 It has been suggested that microplastics accumulating in mussels will achieve a dynamic  
406 balance between ingestion and clearance and become stable (Li et al., 2016; Setälä et al., 2016).  
407 Although mussels selectively ingest microplastics and there are differences in intestinal retention  
408 times for microplastics of different characteristics during this process (Farrell and Nelson, 2013;  
409 Ward and Kach, 2009), a stable abundance in mussels make it easier to build relationship with  
410 that in the environment media. Not only has a positive and quantitative correlation of  
411 microplastics in mussels and in their surrounding waters from field investigations been reported  
412 (Qu et al., 2018), but similar results from laboratory exposure experiments have been found. The  
413 abundance of microplastics in mussels was significantly higher in the high concentration  
414 exposure group than that in low concentration group (Qu et al., 2018) and a significant and linear  
415 increase of microplastic uptake in mussel larva with increasing exposure concentrations was  
416 observed (Capolupo et al., 2018).

417 Microplastics can be taken up over the digestive surface of mussels gastrointestinal tracts by  
418 endocytosis and granulocytomas and then transferred to lysosomes and circulatory system or  
419 eliminated as pseudofaeces particles, which contributes to microplastic adherence to the foot and  
420 mantle (Browne et al., 2008; Von Moos et al., 2012; Wegner et al., 2012; Beyer et al., 2017;  
421 Kolandhasamy et al., 2018; Khana and Prezant, 2018). Browne et al. (2008) showed the ability  
422 of mussel to ingest polystyrene microspheres between 3 and 10  $\mu\text{m}$  in size and to transfer them to  
423 the circulatory system, where smaller particles appeared to undergo translocation more readily  
424 than larger ones. Assimilation of very small particles of emulsion/microsuspension PVC ( $\sim 1 \mu\text{m}$ )  
425 was also recorded for *P. perna* (Santana 2015; Santana et al., 2017). Assimilation of small  
426 particles contributes to their accumulation in mussels relatively steadily. This may explain why,

427 after a three day gut clearance, only larger particles ( $> 20 \mu\text{m}$ ) were egested completely, whilst  
428 smaller particles ( $5\text{-}20 \mu\text{m}$ ) were still present (Van Cauwenberghe and Janssen, 2014).

429 Theoretically, small particles or beads should account for a larger proportion due to their  
430 assimilation. However, fibers were always dominant in field investigations as mentioned in  
431 section 2.2. This could be explained by the limitation of current methodology. Van  
432 Cauwenberghe et al. (2015) demonstrated that only microplastics of the smallest size ( $10 \mu\text{m}$ )  
433 was detected in mussels although three sizes ( $10 \mu\text{m}$ ,  $30 \mu\text{m}$ ,  $90 \mu\text{m}$ ) of microplastics were used  
434 in the exposure experiment. Furthermore, the size of microplastics reported to occur in  
435 haemolymph (e.g.,  $0.1\text{-}1 \mu\text{m}$ ,  $3 \mu\text{m}$ ,  $9.6 \mu\text{m}$ ,  $10 \mu\text{m}$ ,  $20\text{-}25 \mu\text{m}$ , Table 2) tend to be close to or  
436 smaller than the detection limit of field investigation method. Therefore, a large proportion of  
437 these small particles are unlikely to be detected in field surveys. Even so, laboratory exposures of  
438 these smaller microplastics contribute to our understanding of accumulation of microplastics in  
439 mussels and relative toxicology effects.

440 The total body burden of microplastics in mussels goes beyond ingestion. Besides uptake  
441 through the gut and across the gills, microplastics adhere to mussel's soft tissue (mantle, gonad,  
442 adductor, visceral tissue and foot) can further contribute to microplastic presence within  
443 individuals. This has been verified in both field and laboratory environments (Von Moos et al.  
444 2012; Kolandhasamy et al., 2018). Since mussels are eaten whole by both animals and humans,  
445 Microplastics can also be passed to higher trophic levels following predation, as demonstrated in  
446 laboratory exposure experiments (Farrell and Nelson 2013; Watts et al., 2014; Santana et al.,  
447 2017).

448 At present, however, the microparticles behaviour within the mussels tissue is still largely  
449 unknown; this includes translocation into, and from, haemolymph to other tissues as well as

450 depuration and egestion rates. Studies have shown that microplastics may be retained for  
 451 extended periods of time, for example, complete clearance of microplastics was not achieved  
 452 after a seven-days depuration period under laboratory conditions with microbeads (2,6 µm) being  
 453 retained within the digestive tracts (Paul-Pont et al., 2016). In addition, microplastics were  
 454 retained in the haemolymph of *M. edulis* 48 days after exposure (Browne et al., 2008), however,  
 455 there was a reduce in microplastic numbers over time which suggested egestion was occurring.  
 456 These results suggest that mussels are effective indicators of recent exposure. Although efficient  
 457 gut clearance and selective feeding behavior of mussels limit their quantitative ability as  
 458 indicators of microplastic. For example, the only available data on retention refers to those that  
 459 have been selected by mussels, especially in terms of size. Microplastics in mussels can still  
 460 reflect the abundance, polymer type and morphotype of microplastics in the environment when  
 461 sampling and thereby come a bit closer to the risk assessment.

462

463 **Table 2. Uptake and accumulation of microplastics by mussels in laboratory exposures**

Exposure microplastic			Exposure concentration	Exposure time	Uptake and accumulation organs	Reference
Types	Shapes	Sizes				
<i>Mytilus edulis</i>						
PS	spheres	3, 9.6 µm	42 particles/L	3 h-48 d clearance	gut, haemolymph	Browne et al., 2008
PS	particles, beads	100 nm, 10 µm	1.3×10 <sup>4</sup> particles/ml and 1000 beads/ml	45 min-72 h clearance	digestive gland	Ward and Kach, 2009
HDPE	powders	0-80 µm	2.5 g/L	96 h	gill, stomach, digestive gland	Von Moos et al., 2012
PS	beads	30 nm	0.1,0.2,0.3 g/L	8 h	foot	Wegner et al., 2012
PS	spheres	10, 30, 90 µm	110 particles/ml	14 d-24 h clearance	whole soft tissue	Van Cauwenberghe et al., 2015
	beads, fragments and fibers		100,1000 particles/L	5 d	whole soft tissue	Qu et al., 2018
	fibers		2000 microfibers/L	48 h-48 h clearance	gill, intestine, foot, stomach, mantle, gonad, adductor	Kolandhasamy et al., 2018



					visceral tissue	
PS, PE, PP	beads, fibers	7-30 $\mu\text{m}$ (beads) or 23 x 3000 $\mu\text{m}$ (fibers)	50 beads/ml or 0.1 fibers/ml	60 min	whole soft tissue	Porter et al., 2018
<b><i>M. galloprovincialis</i></b>						
PS, PE	powders	<100 $\mu\text{m}$	1.5 g/L	7 d	haemolymph, gill digestive gland	Avio et al., 2015
LDPE	particles	20-25 $\mu\text{m}$	$2.34 \times 10^7$ particles/L	28 d	hemolymph, gills, digestive glands, intestine	Pittura et al., 2018
PE	fragments (derived from toothpaste)	50-590 $\mu\text{m}$	0.01 g/L	21 d	digestive tract, whole body	Bråte et al., 2018a
PS	spheres	3 $\mu\text{m}$	$50-1 \times 10^4$ particles /ml	24 h-192 h clearance	gut of larva	Capolupo et al., 2018
<b><i>Mytilus spp.</i></b>						
PS	beads	2, 6 $\mu\text{m}$	32 $\mu\text{g/L/day}$ =2000 beads/ml/day	7 d-7 d clearance	digestive tract intestine, gills	Paul-Pont et al., 2016
<b><i>Dreissena polymorpha</i></b>						
PS	beads	1, 10 $\mu\text{m}$	$1 \times 10^6$ or $4 \times 10^6$ particles/L	6 d	gut, digestive gland, haemolymph	Magni et al., 2018
<b><i>Geukensia demissa</i></b>						
PS, PE	spheres	5, 250-300 $\mu\text{m}$	3.467 g/L	2 h-24 h clearance	stomach, digestive tubules, intestine	Khan and Prezant, 2018
<b><i>Perna perna</i></b>						
PVC	spheres	0.1-1 $\mu\text{m}$	0.5 g/L	3 h-12 d clearance	gut, haemolymph	Santana et al., 2017

464 Abbreviations: PS, polystyrene; PE, polyethylene; HDPE, high-density polyethylene; LDPE, low-density  
465 polyethylene; PP, polypropylene; PVC, polyvinyl chloride.

466

### 467 3.2 Toxic effects of microplastics

468 In terms of toxicity, a number of adverse effects associated with microplastic ingestion have  
469 been reported. Notable histological changes in mussel digestive cells, strong inflammatory  
470 responses with formation of granulocytomas, and lysosomal destabilization which increases with  
471 exposure time, have all been observed (Von Moos et al., 2012). Avio et al. (2015) demonstrated  
472 cellular effects including alterations of immunological responses, lysosomal

473 compartmentalisation, peroxisomal proliferation, antioxidant system, neurotoxic effects, onset of  
474 genotoxicity, and changes in gene expression profile associated with microplastic exposure.  
475 Bråte et al. (2018a) found histological alterations in gills and digestive tissue, and hemocytic  
476 aggregates in the digestive gland following exposure to PE fragments (ranging from 50-590 µm)  
477 extracted from toothpaste. On a nanoplastic scale, mussels showed reduced filtering activity, and  
478 the total weight of the feces and pseudofeces increased with the increase of nano PS (30 nm,  
479 Wegner et al., 2012). Furthermore, PS-NH<sub>2</sub> particles stimulated increase in extracellular reactive  
480 oxygen species and nitric oxide production and induced apoptotic process of hemocytes (Canesi  
481 et al., 2015). Finally, Gandara e Silva et al. (2016) showed the toxic effect of leachates of virgin  
482 PP and beached plastics pellets caused mortality and abnormal embryos of *P. perna*.

483 In summary, the reported effects of microplastic uptake include histological changes,  
484 inflammatory response, lysosomal membrane destabilization, reduced filtering activity,  
485 neurotoxic effects, alterations of antioxidant system, increase in hemocyte mortality, dysplasia,  
486 genotoxicity and transcriptional responses (Table S1). These research results lay a good  
487 foundation for the exploration of specific biomarkers for microplastic pollution.

### 488 **3.3 Optimization of laboratory exposures**

489 It should be highlighted that in many laboratory studies, organisms are exposed to  
490 unrealistically high doses of microplastics with uniform size or shape, in virgin condition, and  
491 for relatively short time frames (Rochman et al., 2016; Koelmans et al., 2017; Lambert et al.,  
492 2017). Whereas, environmentally exposed plastics are subject to weathering, abrasion and  
493 photodegradation, therefore comprising of a broad size distribution and various shapes (Phuong  
494 et al., 2016; Lambert et al., 2017). In addition, weathering processes may weaken the plastic

495 surface, enhance chemical leaching and change the outcome of toxicological investigations of  
496 microplastic particles (Ogonowski et al.2016; Lambert et al., 2017; Potthoff et al., 2017).

497 In some studies, mussels were caged in specific areas for extended periods to investigate the  
498 microplastic pollution related to specific anthropogenic activity, such as the removal of wreck or  
499 to assess seasonal changes in plastic pollution (Catarino et al., 2018; Avio et al., 2017). To  
500 mimic environmental weathering, some studies exposed organisms to microplastics collected  
501 from beaches or deployed in a bay for a period time (Gandara e Silva et al., 2016; Nobre et al.,  
502 2015; Rochman et al., 2014; Bråte et al., 2018a). Furthermore, a photo-oxidative degradation of  
503 plastic pellets incubated in seawater, ultrapurewater and air with UV irradiation over a three-  
504 month period observed some changes in hydroxyl groups, carbonyl groups and surface textures  
505 which provides a good foundation for making environmental microplastics under laboratory  
506 conditions (Cai et al., 2018).

507 A recent study using weathered PE particles from toothpaste showed that following a  
508 chronic exposure (21 days) with lower dose than normally tested (~ 1 particle per ml), still  
509 induces tissue alterations in mussels (Bråte et al., 2018a). In contrast, a relative longterm  
510 exposure (90 days) of *P. perna* to a less extreme concentration compared with previous studies  
511 (0.125 g/L) indicated no behavioral and physiological effects of microplastics (Santana et al.,  
512 2018). Calls for more testing on toxicological effects of long-term exposure to environmentally  
513 realistic concentrations and shapes are repeatedly made by the scientific community (Van  
514 Cauwenberghe et al., 2015; Phuong et al., 2016; Koelmans et al., 2017). Furthermore, Connors et  
515 al. (2017) and Karami (2017) provide guidance which should be considered to improve the  
516 quality and reliability of ecotoxicological studies of microplastics. This includes the  
517 characterization (physical and chemical properties) and quantification of microparticles in future

518 laboratory exposure studies to facilitate a comprehensive understanding of the causal links  
519 between physical-chemical properties of microplastic particles and toxic effects (Connors et al.,  
520 2017).

#### 521 **4. Scope of mussels as global bioindicators of microplastic**

##### 522 **4.1 Advantages of utilizing mussel**

523 There is a consensus that mussels make good biological indicators for monitoring many  
524 anthropogenic pollutants (Beyer et al., 2017). Besides the advantages discussed above, mussels  
525 also have specific advantages as sentinel organisms for microplastic pollution. Feeding type  
526 affects microplastic ingestion, for example, filter-feeding makes bivalves ingest more  
527 microplastics (Setälä et al., 2016). Mussels as species susceptible to microplastic uptake have  
528 been documented widely (e.g., Browne et al., 2008; Van Moos et al., 2012; Mathalon and Hill,  
529 2014; Santana et al., 2016; Li et al., 2016). Furthermore, potential contamination during  
530 sampling and laboratory processing is a key problem in microplastic research, mussel's hard  
531 shells and easy handling minimize contamination risk (Beyer et al., 2017; Setälä et al., 2016).  
532 Bivalves are likely the largest source of microplastics from seafood to humans because they are  
533 consumed whole (Lusher et al., 2017c). This adds to their selection as ideal indicators for  
534 microplastic pollution monitoring.

535 Furthermore, a vast amount of field data shows that microplastics are widespread in mussels  
536 around the world, and laboratory exposure studies have demonstrated that mussels can be good  
537 model organisms in understanding uptake, accumulation and toxicity of microplastic (Tables 1, 2,  
538 S1). This highlights the feasibility and advantages of mussels as indicator species for monitoring  
539 of microplastics from an implementation perspective.

540 Practically, the quantification of pollutant levels in bioaccumulator organisms and a specific  
541 response to a toxic substance by an organism provide two frequently employed pathways for  
542 monitoring environmental quality (Reguera et al., 2018). The suitability of the first approach  
543 relies on the relationship of pollutant level between the organism and ambient environment.  
544 Based on laboratory studies, mussels show selection for particles including microplastics (Ward  
545 and Shumway, 2004). Nevertheless, there are diverse ways for mussels to take microplastics  
546 (Kolandhasamy et al., 2018), and various microplastics exist in real environments. Though not  
547 all the properties of microplastics in mussels can exactly match those in their environment,  
548 quantitative correlations of abundance between microplastics in mussels and in surrounding  
549 seawaters makes it practicable to deduce environmental microplastic pollution levels from that in  
550 mussels (Qu et al., 2018). Since the concentration of pollutants including microplastics in  
551 mussels tend to remain stable after obtaining a balance between intake, assimilation in tissues  
552 and defecation/eggestion, this method can effectively mitigate or avoid error rates and  
553 misinterpretation stemming from contingency in environmental medium (Setälä et al., 2016;  
554 Beyer et al., 2017).

555 As for the other pathway, efforts have been taken to reveal the toxic effects resulting from  
556 microplastic intake, translocation and accumulation in mussels. Most biomarkers such as  
557 lysosomal membrane stability, inflammatory response, antioxidant enzymes are sensitive to other  
558 pollutants as well (Brooks et al., 2011; González-Fernández et al., 2016; Burgeot et al., 2017).  
559 Utilising these toxicological studies will provide evidence and scientific basis for the selection of  
560 specific biomarkers for the early warning and monitoring of microplastic pollution and related  
561 ecological risk assessment.

562 Recently, Fossi et al. (2018) proposed to use a threefold monitoring approach to assess the  
563 impact of ingested marine litter including microplastics on marine organisms. It combines an  
564 accurate measure of microplastic levels in target organisms, the concentrations of plastic  
565 additives and other persistent organic pollutants (POPs) in tissues and the corresponding  
566 toxicological effects. According to this new concept, mussels correspond to ideal biological  
567 models because they have been widely used as bioindicators of POPs in coastal environments  
568 (Aznar-Alemany et al., 2017; Martinović et al., 2016; Liu et al., 2014; Chiesa et al., 2018; Gagné  
569 et al., 2017; Chiu et al., 2018; Cunha et al., 2017; Politakis et al., 2018).

#### 570 **4.2 Current regional and national proposals**

571 Recently, mussels have been proposed as suitable indicator organisms of microplastic  
572 pollution by research groups from several geographic locations (Van Cauwenberghe et al., 2015;  
573 Wesch et al., 2016; Li et al., 2016; Lusher et al., 2017b; Qu et al., 2018). Uptake and  
574 accumulation of microplastics in mussels from Belgium has been selected as a marine health  
575 status parameter, and microplastic levels in mussels have been included in European databases  
576 regarding contaminants of emerging concern in seafood (De Witte et al., 2014; Vandermeersch  
577 et al., 2015a). The possibilities of using mussels as monitoring species for microplastics in  
578 Norway and the Nordic marine environment is also supported (Bråte et al., 2017; Lusher et al.,  
579 2017b) since they have been used in other regional, national and international monitoring  
580 programmes. Lusher et al (2017b) suggests that mussel (*Mytilus* spp.) can be a promising  
581 bioindicator of the smallest sized microplastic (<1 mm) in the water column.

582 In a recent workshop on “*Distribution, source, fate and impact of marine microplastics in*  
583 *Asia and the Pacific*” organized by the IOC Sub-Commission for the Western Pacific  
584 (WESTPAC), mussels were recommended as bioindicator species to monitor marine

585 microplastic pollution (WESTPAC., 2017). At the European level, the MSFD has defined marine  
586 litter and microplastics as a full descriptor of the Good Environmental Status (Galgani et al.,  
587 2014). OSPAR have recommended blue mussels as suitable monitoring species because of their  
588 large stocks for repeated sampling and the ability to reflect the local conditions (OSPAR, 2012).  
589 Due to advantages of mussels as traditional biological indicators and mounting evidence of  
590 microplastics in mussels, ICES have advised to use mussel as a indicator of microplastic  
591 pollution (Vandermeersch et al., 2015b; Beyer et al., 2017; ICES, 2015). However, there are  
592 currently no standard monitoring procedures outlined by any of the regulatory bodies (inc.  
593 OSPAR, MSFD, NOAA, UNEP). These monitoring protocols should follow recommendations  
594 from international experts and are expected to be produced in the near future, as the GESAMP  
595 Working Group 40 is currently formulating a report to harmonise monitoring and assessmemnt  
596 of plastics and microplastics globally.

### 597 **4.3 Future developments**

598 Based on the analysis above, we propose to use mussels as bioindicator species for  
599 monitoring microplastics in marine environments. Nevertheless, some questions require further  
600 clarification, and additional factors should be taken into consideration when it comes to building  
601 an efficient and economical approach suitable for future large-scale monitoring program using  
602 mussels.

603 Firstly, it is necessary to develop a global working group investigating microplastics in  
604 mussels under some international organization such as UNEP, including underlying  
605 physiological and behavioral processes and responses to microplastics. Already, mussels have  
606 been proposed to be used as bioindicators in some local or regional areas. It is time to form a  
607 working group globally so that researchers from different areas share and discuss the protocol of

608 monitoring as well as future plans. One possible arena to advertise and promote this discussion is  
609 the Ad Hoc Open-Ended Expert Group on Marine Litter and Microplastics composed by  
610 representatives from member states to support the implementation of the United Nations  
611 Environmental Assembly resolution on marine litter and microplastics (UNEP/EA.3/L.20).

612 Secondly, a uniform protocol should be developed and adopted, at least on a comparable  
613 regional monitoring basis. Uniform protocols and harmonized monitoring methods are need to  
614 allow spatial and temporal comparisons and to enable assessment of the presence of  
615 microplastics and their effects in mussels at a global level (Fossi et al., 2018). Such a detailed  
616 methodology for measuring microplastics in blue mussels has also been described by Lusher et al.  
617 (2017b) which supplies a potential baseline standard to conform too. Future inter-calibration  
618 exercises will help validate and harmonize methods used across different research groups. The  
619 development and use of an internal reference sample(s), one for each matrices, might also help  
620 facilitate inter-laboratory and global validation of results.

621 Finally, monitoring should be practicely conducted regionally or globally. To date,  
622 comparable data of microplastic pollution characteristics in mussels from different parts of the  
623 world is scarce. Ideally, researchers should be encouraged to combine microplastic monitoring  
624 into the existing monitoring projects using mussels. A global picture of microplastic should be  
625 obtained, and the potential ecological and health risk should be assessed.

## 626 **5. Conclusions**

627 Current evidence on microplastic abundance in all parts of the marine environment  
628 including wild biota call for establishing a suitable indicator species for microplastic pollution, to  
629 monitor spatial and temporal trends internationally. Mussels have been widely used as  
630 bioindicators for monitoring of coastal water pollution and their susceptibility to microplastic



631 uptake and assimilation has been well documented. Field investigations have shown that  
632 microplastic abundance in mussels is closely related to human activity and, in some studies, there  
633 has been a positive and quantitative correlation of microplastics in mussels and their surrounding  
634 waters. Laboratory exposure studies demonstrate that mussels can be good model organisms  
635 when investigating uptake, accumulation and toxicity of microplastics. Therefore, we strongly  
636 propose the use of mussels as indicator species for monitoring of microplastics in the marine  
637 environment. We also urge the international organizations (e.g., UNEP) to facilitate the  
638 formation of an international workgroup of microplastics in mussels to develop an internationally  
639 accepted protocol to monitor and collect preliminary data comparing coastal mussels from  
640 around the world.

641

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- 1039

1040 **Legend of figures and tables**

1041

1042 Table 1. Summary of global field investigations on microplastics in mussels. <sup>a</sup>The microplastic  
1043 level was transferred by dividing total microplastics per individual by the shelled weight. <sup>b</sup>The  
1044 abundance of microplastics in intestine. <sup>c</sup>The abundance of microplastics in hepatopancreas and  
1045 gills. <sup>d</sup>The abundance of microplastics in digestive glands and gills. <sup>e</sup> Scanning Electron  
1046 Microscopy/ Electron Dispersive X-Ray.

1047

1048

1049 Table 2. Uptake and accumulation of microplastics by mussels in laboratory exposures.

1050 Abbreviations: PS, polystyrene; PE, polyethylene; HDPE, high-density polyethylene; LDPE,  
1051 low-density polyethylene; PP, polypropylene; PVC, polyvinyl chloride.

1052

1053 **Supplementary materials**

1054

1055 Figure S1. Area of field investigations on microplastics in mussels around the world. Roundness,  
1056 5-point star and triangle represent the investigation region. Each of them include one or more  
1057 sampling sites.

1058

1059 Table S1. The effects of microplastics on mussels in laboratory exposures. Abbreviations: PS,  
1060 polystyrene; PE, polyethylene; PP, polypropylene; HDPE, high-density polyethylene; LDPE,  
1061 low-density polyethylene; PVC, polyvinyl chloride; PLA, polylactic acid.

1062