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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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Contents:

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

1. Introduction

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

Microplastics ingestion has been identified in a range of species from mussels to mammals, with over 220 species from different trophic levels consuming microplastic debris *in natura*, and 99% of all seabird species are predicted to ingest microplastic by 2050 (Ter Halle et al., 2017; Lusher et al., 2017a; Wilcox et al., 2015; Hu et al., 2016). Microplastic ingestion by marine organisms can accelerate microplastics' transference from the sea surface through the water column to the sea floor via feces and marine snow, or between trophic chains via predation (Farrell and Nelson, 2013; Santana et al., 2017; Setälä et al., 2014; Katija et al., 2017). Additionally, microplastics are subjected to biofouling leading to colonization by microorganisms and invertebrates, which in turn can contribute to long-range transport of alien species, and serve as reservoirs for pathogen transmission, which broadens the risks of microplastic pollution to marine organisms and ecosystems (Andrady, 2011; Barnes, 2002; GESAMP, 2015, 2016). In addition, envrionmental 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

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

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

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

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

195

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

Species & Location	Digestion method	Identification technique	Classification	Abundance (items/g.ww)	Size (µm)	Environmental media	Reference
Mytilus. edulis							
Canada	30% H_2O_2	visual sorting	fiber	2.79-7.42 [a]	no data	sediments: 2-8 items/g.dw	Mathalon and Hill, 2014
Germany	69% HNO_3	micro-Raman	particle	0.36±0.07	no data	no data	Van Cauwenberghe and Janssen, 2014
Belgium	HNO_3:$HClO_4$	visual sorting	fiber, fragment, film, sphere	0.26-0.51	200-1500	no data	De Witte et al., 2014
France, Belgium, Netherlands	69% HNO_3	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_2O_2	micro-FTIR	fiber, fragment, sphere, flake	0.7-2.9	8-4700	seawater:1.5-6.7 items/L	Li et al., 2018
Netherlands	proteinase K and 30% H_2O_2	Raman	fiber, particle	37 (items/g.dw)	30-2000	seawater: 27 items/L sediments: 48 items/kg.dw	Karlsson et al., 2017
Netherlands	HNO_3, NaOH & 30% H_2O_2	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_3	FTIR	fiber, fragment, pellet	wild:138±202 farmed:259±114	<530	no data	Murphy, 2018
China	30% H_2O_2	micro-FTIR	fiber, fragment, sphere, flake	2.2	5-5000	no data	Li et al., 2016
China	30% H_2O_2	micro-FTIR	fiber, sheet, fragment, sphere,	9.2 [b]	50-5000	no data	Kolandhasamy et al., 2018
China	30% H_2O_2	micro-FTIR	fiber, fragment, pellet	1.52-5.36	5-4000	seawater: 0.68-6.44 items/L	Qu et al., 2018
M. galloprovincialis							
Italy	30% H_2O_2	visual sorting	filament, fragment	0.05 (items/g.dw)	60.01 ±38	no data	Bonello et al., 2018
Italy	30% H2O2	visual sorting	filament	6.2-7.2[c]	750-6000	no data	Renzi et al., 2018
Italy, Portugal, Spain	69% HNO_3	visual sorting	fiber, particle	0.12±0.04	no data	no data	Vandermeersch et al., 2015b

Italy, Portugal, Spain	HNO_3:$HClO_4$	visual sorting	fiber, particle	0.18±0.14	no data	no data	Vandermeersch et al., 2015b
Greece	30% H_2O_2	FTIR	filament, fragment, film	46.25% ingested microplastics	<5000	seawater: 0.41 items/m^2	Digka et al., 2018a
Greece	30% H_2O_2	FTIR	fiber, fragment	wild:5.3±0.5[d] farmed:2.5±0.3[d]	40-737	sediments: 1816.7 items/m^2 no data	Digka et al., 2018b
China	30% H_2O_2	micro-FTIR	fiber, fragment, pellet	2.39 ±1.32	5-5000	no data	Li et al., 2015
M. trossulus							
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^3	Railo et al., 2018
***Mytilus* spp.**							
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_3:$HClO_4$	visual sorting	fiber, particle	0.13±0.14	no data	no data	Vandermeersch et al., 2015b
Modiolus modiolus							
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
Perna perna							
Brazil	22.5 M HNO_3	visual sorting	fiber, irregular particle	75% ingested microplastics	no data	no data	Santana et al., 2016
P. viridis							
Indonesia	30% H_2O_2	SEM/EDX[e]	fiber, fragment, sphere, flake	4-20	51.31-232	no data	Khoironi et al., 2018
China	30% H_2O_2	micro-FTIR	fiber, fragment, pellet	1.52-5.36	5-4000	seawater: 0.68-6.44 items/L	Qu et al., 2018

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

2.2 Characteristics of microplastic pollution

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

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

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

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

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

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

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

For the extraction method, common agents used to digest biotic tissues include acid (HNO_3, HNO_3:$HClO_4$), alkaline (NaOH, KOH), oxidizing (H_2O_2) and enzymatic (trypsin, proteinase K, Corolase 7089) approaches. However, drawbacks of these digestion methods have been widely reported, such as structural damage, dissolution and discoloration caused by acid, basic and H_2O_2; incomplete soft tissue digestion by enzyme; production of foam caused by H_2O_2; expensive price and time-consuming nature of some of the solvents (Table 1, Lusher et al., 2017b). This might lead to underestimations of microplastic loads, especially smaller particles, or limit their adaptability for large scale monitoring. Hence, selection of a digestion solution requires further testing and optimization.

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

The pore size of the filter, the magnification times and resolution of microscopy employed determine the observed microplastic size lower limit. ICES has recommended the use of filter with 5 μm pore size for mussel (Vandermeersch et al., 2015b). In the current literature, 5 μm 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 μm) or bigger (12, 20 μm) size. Among all the given size ranges of microplastics
317 detected in mussels, 5 μm is the minimum size (Table 1). Although smaller sizes of microplasctic
318 undoubtably occur in mussels, their observation and identification are still limited by current
319 instrumentation and method. For example, 20 μm seems to be the smallest size that could be
320 identified using μFTIR in the reflection mode under manual inspection (Phuong et al., 2018b).
321 Hence, 5 μm 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 microplatic 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 spectroscopy (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

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

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

The variability in the way the results are characterized further hampers the comparision among different studies. These factors such as reporting units, classification of type and size range should be standardized in the future studies. Both items individual^{-1} and items gram^{-1} as reporting units are required. The latter is a more appropriate unite to compare different studies and it has been used most commonly in current research (Table 1). For the classification of type, four kinds including fiber (filament), fragment, sphere (pellet, bead), film (flake, sheet) could be adopted which almost covers all the types in current studies (Table 1). An optimal classification of size range still requires more research to determine. In addition, contamination control is a crucial factor during the whole procedure. Procedural blanks must be carried out to monitor 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 airbore 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 μm 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 μm) in comparison to surrounding water (mean size, 0.6 μm). Van
386 Cauwenberghe et al. (2015) found that larger sized (15-500 μm) microplastics were detected in
387 mussel's faeces compared to mussel tissue (20-90 μm). 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 μm 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 (~ 1 μm)
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 μm) were egested completly, whilst
428 smaller particles (5-20 μm) 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 μm)
433 was detected in mussles although three sizes (10 μm, 30 μm, 90 μm) of microplastics were used
434 in the exposure experiment. Furthermore, the size of microplastics reported to occur in
435 haemolymph (e.g., 0.1-1 μm, 3 μm, 9.6 μm, 10 μm, 20-25 μm, 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

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

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				
Mytilus edulis						
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	1.3×10^4 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 µm (beads) or 23 x 3000 µm (fibers)	50 beads/ml or 0.1 fibers/ml	60 min	whole soft tissue	Porter et al., 2018
M. galloprovincialis						
PS, PE	powders	<100 µm	1.5 g/L	7 d	haemolymph, gill digestive gland	Avio et al.,2015
LDPE	particles	20-25 µm	2.34×10^7 particles/L	28 d	hemolymph, gills, digestive glands, intestine	Pittura et al., 2018
PE	fragments (derived from toothpaste)	50-590 µm	0.01 g/L	21 d	digestive tract, whole body	Bråte et al., 2018a
PS	spheres	3 µm	$50\text{-}1\times10^4$ particles /ml	24 h-192 h clearance	gut of larva	Capolupo et al., 2018
***Mytilus* spp.**						
PS	beads	2, 6 µm	32 µg/L/day =2000 beads/ml/day	7 d-7 d clearance	digestive tract intestine, gills	Paul-Pont et al., 2016
Dreissena polymorpha						
PS	beads	1, 10 µm	1×10^6 or 4×10^6 particles/L	6 d	gut, digestive gland, haemolymph	Magni et al., 2018
Geukensia demissa						
PS, PE	spheres	5, 250-300 µm	3.467 g/L	2 h-24 h clearance	stomach, digestive tubules, intestine	Khan and Prezant, 2018
Perna perna						
PVC	spheres	0.1-1 µm	0.5 g/L	3 h-12 d clearance	gut, haemolymph	Santana et al., 2017

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

3.2 Toxic effects of microplastics

In terms of toxicity, a number of adverse effects associated with microplastic ingestion have been reported. Notable histological changes in mussel digestive cells, strong inflammatory responses with formation of granulocytomas, and lysosomal destabilization which increases with exposure time, have all been observed (Von Moos et al., 2012). Avio et al. (2015) demonstrated 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-NH2 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

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

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

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

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

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

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

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Legend of figures and tables

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

Table 2. Uptake and accumulation of microplastics by mussels in laboratory exposures. Abbreviations: PS, polystyrene; PE, polyethylene; HDPE, high-density polyethylene; LDPE, low-density polyethylene; PP, polypropylene; PVC, polyvinyl chloride.

Supplementary materials

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

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