



Baroja, E., Christoforou, E., Lindstrom, J. and Spatharis, S. (2021) Effects of microplastics on bivalves: Are experimental settings reflecting conditions in the field? *Marine Pollution Bulletin*, 171, 112696. (doi: [10.1016/j.marpolbul.2021.112696](https://doi.org/10.1016/j.marpolbul.2021.112696))

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1 **Effects of microplastics on bivalves: are experimental settings reflecting conditions in**  
2 **the field?**

3

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11 **Abstract**

12 Bivalves are the focus of experimental research as they can filtrate a broad size range of  
13 microplastics (MPs) with negative consequences on their physiology. Studies use a range  
14 of MP shapes, materials, sizes and concentrations raising the question on whether these  
15 reflect environmental observations. We review experimental studies on MPs effects on  
16 marine bivalves and contrast the MP characteristics used with corresponding data from  
17 the environment. Mussels were the most common bivalve across experiments which  
18 reflects their high abundance and broad distribution in the field. Although fibres are the  
19 dominant shape of MPs in coastal systems, most studies focus on spherules and beads  
20 whereas MP concentrations are often orders of magnitude higher than environmental  
21 levels. For higher relevance of experimental findings we recommend that maximum  
22 experimental concentrations of MPs are in the range of 100-1000 particles/L, that there  
23 is more focus on microfibers and that concentration is reported in particles/volume.

24

25 Key words: bivalves, polyethylene, polypropylene, fibres, coastal systems

26 **Introduction**

27 Mainly due to their small size (<5mm) (Barnes et al., 2009), widespread use and  
28 improper disposal or leakage, microplastics (MPs) are currently regarded as one of the  
29 most widespread forms of pollution (Napper and Thompson, 2019). Aquatic  
30 environments, and coastal ecosystems in particular, are especially prone to MP pollution  
31 due to intense anthropogenic activities, e.g., inflows from wastewater treatment plants,  
32 coastal landfills, industrial outfall and coastal fisheries (Garcia-Garin et al., 2019; Kazour  
33 et al., 2019; Xue et al., 2020). Investigating the effects of MP pollution on marine biota  
34 *in situ* poses many challenges associated with the effects of confounding factors that are  
35 difficult to control in the field (Lebreton et al., 2017). For this reason, the bulk of  
36 evidence on the impacts of MPs on marine organisms comes from experimental studies  
37 under controlled laboratory conditions. A focal group for such investigations are marine  
38 bivalves due to their susceptibility in ingesting MPs while filter feeding (Ward et al.,  
39 2019) and because of their importance in coastal ecosystem goods and services (van der  
40 Schatte Olivier et al., 2018a). However, to assess the impact of MP pollution on coastal  
41 marine bivalves, it is imperative to understand how relevant the conditions used in  
42 laboratory MP exposure studies are to the conditions and species observed in marine  
43 systems.

44

45 Bivalves play an essential role in ecosystem function (Dame, 1993) providing invaluable  
46 services including carbon sequestration, nutrient remediation and coastal defence (van  
47 der Schatte Olivier et al. 2018). However, different taxonomic groups can have different  
48 contribution to ecosystem goods and services. For example, mussels have the greatest  
49 potential for bioremediation as they remove the most nitrogen and phosphorus per  
50 tonne of shellfish produced (van der Schatte Olivier et al., 2018). On the other hand,  
51 clams, oysters and scallops amount to the highest percentages of global marine bivalve  
52 aquaculture (31%, 27% and 23% respectively) (FAO/ICAC, 2018a). It is thus essential to  
53 understand whether the choice of species -and studied responses- in MP exposure  
54 studies, reflects their importance to ecosystem goods and services, or is instead based  
55 on ease of accessibility and experimentation in laboratory settings.

56

57 The relevance of experimental MP exposure studies should also be assessed with  
58 respect to the characteristics of the MPs used. Field studies have shown that certain MP  
59 shapes and types tend to be more dominant in marine systems (Baldwin et al., 2020;  
60 Ekvall et al., 2019; Galaiduk et al., 2020; O'Connor et al., 2020). For instance, although a  
61 range of shapes can be found in marine ecosystems (e.g. spherical, pellets, grains and  
62 irregular fragments), fibres are the most common (Henry et al., 2019) accounting for up  
63 to 91% of all MPs in the water (Barrows et al., 2018). This is concerning as fibres are  
64 longer than they are wide, therefore, they can enter the digestive system of the bivalves  
65 when penetrating through the narrower width, avoiding the mechanisms of the bivalves  
66 to filter out particles larger than  $\sim 100\mu\text{m}$  (Sendra et al., 2021; Ward et al., 2019).  
67 Establishing whether fibres are well represented in experimental studies on the effects  
68 on bivalves is thus crucial.

69

70 It has been shown that certain bivalve species can distinguish, in the pre-ingestion level,  
71 between particle size, density and physicochemical properties (Brillant & MacDonald  
72 2000, 2002). Similarly, oysters and mussels can reject particles depending on their  
73 surface properties (Rosa et al., 2013). In the case of MP pollution, such selective  
74 mechanisms could moderate the potential effects of MPs on organisms (Sendra et al.,  
75 2021). For experimental inference to be environmentally relevant, it is imperative that  
76 the MP material used in bivalve exposure studies is related to what is encountered as  
77 the dominant material in the field. This is particularly important because, although some  
78 polymer types seem to be dominant globally, (e.g., polypropylene and polyethylene),  
79 others have a more localised presence. For instance, additional to polypropylene and  
80 polyethylene, polystyrene is dominant in the Mediterranean whereas nylon is dominant  
81 in the North-Western Pacific (Pan et al. 2019).

82

83 Another vital information on the design of environmentally relevant MP exposure  
84 studies on bivalves is the MP size and concentration. Bivalves preferentially select and  
85 feed on particles between  $1\mu\text{m}$  and  $40\mu\text{m}$  in diameter (could reach up to  $400\mu\text{m}$  in  
86 length) (Beecham, 2008). Particles of different sizes have a different ingestion and  
87 retention rate (Møhlenberg and Riisgård, 1978), consequently resulting in a differential  
88 response by bivalves. Bivalves also display sensitivity to the suspended particle

89 concentration, for determining their filtration rate and pseudofaeces production  
90 (Riisgård, 2001), which could also lead to differences in their behaviour and particle  
91 selection capacity (Rosa et al., 2018). Thus the use of environmentally relevant MP sizes  
92 and concentrations in experimental studies is critical in understanding plausible impacts  
93 of MPs on coastal bivalves.

94

95 The aim of the present review is to assess whether experimental settings reflect realistic  
96 exposure conditions faced by bivalves in the field. This will help determine whether our  
97 understanding of the effects of MP pollution is biased by potentially unrealistic study  
98 designs. To address this issue, we performed a systematic review of experimental studies  
99 assessing the impact of MPs on bivalves and extracted data on the species of bivalves  
100 used, the characteristics of MP tested, and the responses monitored during exposure.  
101 Furthermore, we carried out a meta-review (review of review papers) of the MP  
102 characteristics observed in aquatic systems; the findings were compared with the MPs  
103 used in experimental designs and the relevance of the later is discussed. Findings and  
104 recommendations from this study provide a framework for driving future work on the  
105 environmental consequences of MPs, towards settings that are more relevant to the  
106 actual exposure risks to organisms, including both bivalves and other aquatic organisms.

107

## 108 **Methods**

### 109 ***Extracting information from experimental MP exposure studies on bivalves***

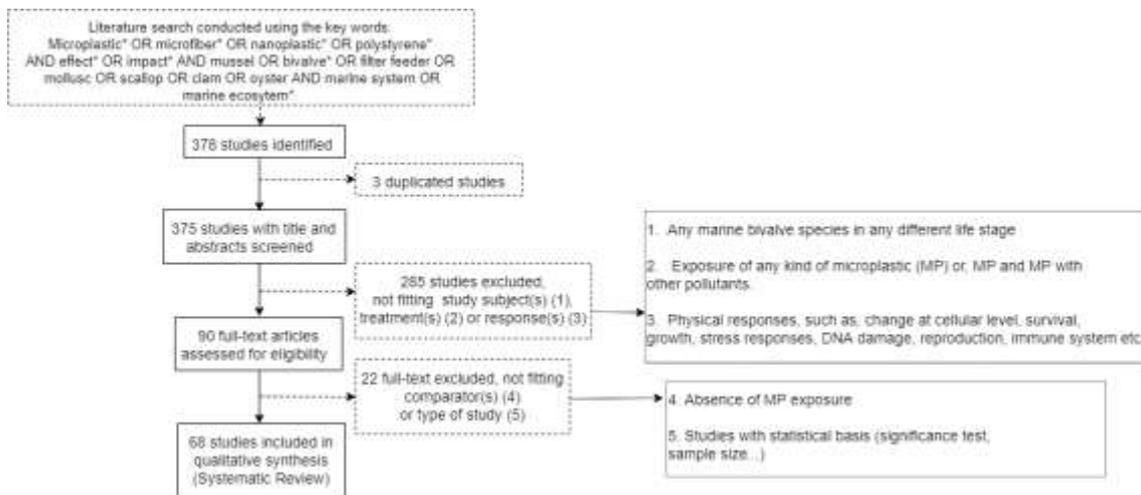
110 To conduct a comprehensive review on the effects of MPs on bivalves, databases and  
111 repositories were searched for relevant studies published in the period between 1989  
112 to 2021 using keywords and applying the Boolean logic. The electronic databases used  
113 were: Web of Science, ScienceDirect and Dissertation Abstracts Online. The search was  
114 carried out by using the following progression of terms: TS= (Microplastic\*OR  
115 microfiber\* OR nanoplastic\* OR polystyrene\*) AND TS= (effect\* OR impact\*) AND TS=  
116 (mussel OR bivalve\* OR filter feeder OR mollusc OR scallop OR clam OR oyster) AND TS=  
117 (marine system OR marine environment\*) (Figure 1). The search was conducted in  
118 March 2020 and updated in September 2020 and February 2021. While we are aware of  
119 the potential publication bias towards statistically significant findings in any area of

120 science, extending the literature search to unpublished research in a systematic fashion  
 121 is challenging, in its own right, and not attempted here.

122

123 The search resulted in 378 publications on effects of MPs on bivalves which were filtered  
 124 in different phases, following inclusion criteria (schematically illustrated in Figure 1). In  
 125 the first phase, after dropping duplicates, titles and abstracts were screened fulfilling  
 126 the first three relevance criteria (see criteria 1-3, Fig.1). The screening procedure was  
 127 carried out by using the R metagear package (Lajeunesse, 2016). In the second phase,  
 128 publications selected were thoroughly read and analysed, ensuring that there was a  
 129 comparator and inclusion criteria 4-5 were met (see criteria 4-5, Figure 1). In the end,  
 130 68 studies which fulfilled all selection criteria were included in this review (Table S1). For  
 131 each publication included in the review, the following data were extracted: year of  
 132 publication (11 years), journal ID (68), organism (4 taxonomic groups), species (22), MP  
 133 concentration (classified in five groups), MP size (classified in four groups), MP type (12  
 134 levels), MP shape (7 levels), and responses (217). In the case where studies investigated  
 135 the combined effects of MPs with other pollutants, only the main effect of MP was  
 136 included, omitting interactions with other pollutants.

137



138

139 **Figure 1.** Schematic representation of the selection process. Numbers in brackets  
 140 represent the requirements for inclusion criterion summarized on the right.

141

142 Upon extraction of the information from the selected studies, the reported 217  
 143 responses were categorised in 18 broader groups for clarity: oxidative damage,

144 immunotoxicity, antioxidant capacity, feeding behaviour, genotoxicity, structural  
145 damage, growth, neurotoxicity, apoptosis, mortality, bioaccumulation, larval  
146 development, metabolism, fecundity, behaviour, homeostasis, microbiota and  
147 malformations (Table S2). As MP concentration was reported in particles/volume or  
148 weight/volume, a comparison between the two units of measure was not possible.  
149 However, to enable interpretation of findings, the MP concentrations of each unit type  
150 were transformed to MP/L and mg/L as appropriate and were classified in five groups  
151 (<1; 1-100; 100 to 10<sup>4</sup>; 10<sup>4</sup> to 10<sup>6</sup>; > 10<sup>6</sup>).

152

153 This review presents the number of publications for each focal variable: bivalve species,  
154 the characteristics of microplastics tested and the responses recorded after exposure.  
155 Some studies tested the impact of MPs using multiple species, responses, and MP  
156 characteristics. As a result, the mentioned publications include multiple data entries  
157 identified with the same code.

158

### 159 ***Meta-review on environmental MPs***

160 To enable a representative comparison and discussion on the relevance of MPs in  
161 experimental studies and MPs observed in the marine environment we conducted a  
162 literature search on review studies reporting environmental MPs published during the  
163 period 2016-2021. The reason we focused on a meta-review of existing reviews on  
164 environmental MPs was due to the high number of original research papers on the topic  
165 as well as the fact that these have already been summarised by a high number of reviews  
166 in a manner that provided useful baseline info on MP characteristics for our study (eg  
167 info on shape, size, material, concentration). The search was carried out in Web of  
168 Science using the following progression of search terms: TS= (Microplastic\*) AND TS=  
169 (size OR shape OR abundance\* OR composition\*) AND TS= (marine environment). The  
170 search resulted in 196 reviews, from which 13 were selected according to the following  
171 criteria: (1) reviews needed to include marine surface or seawater studies and (2)  
172 reviews had to include studies on marine systems reporting MP abundance values in  
173 particles per volume. After selecting the reviews, the following data were extracted:  
174 number of publications per review, number of publications per environment within a  
175 given review, highest MP concentration (MP/L) and corresponding geographical location

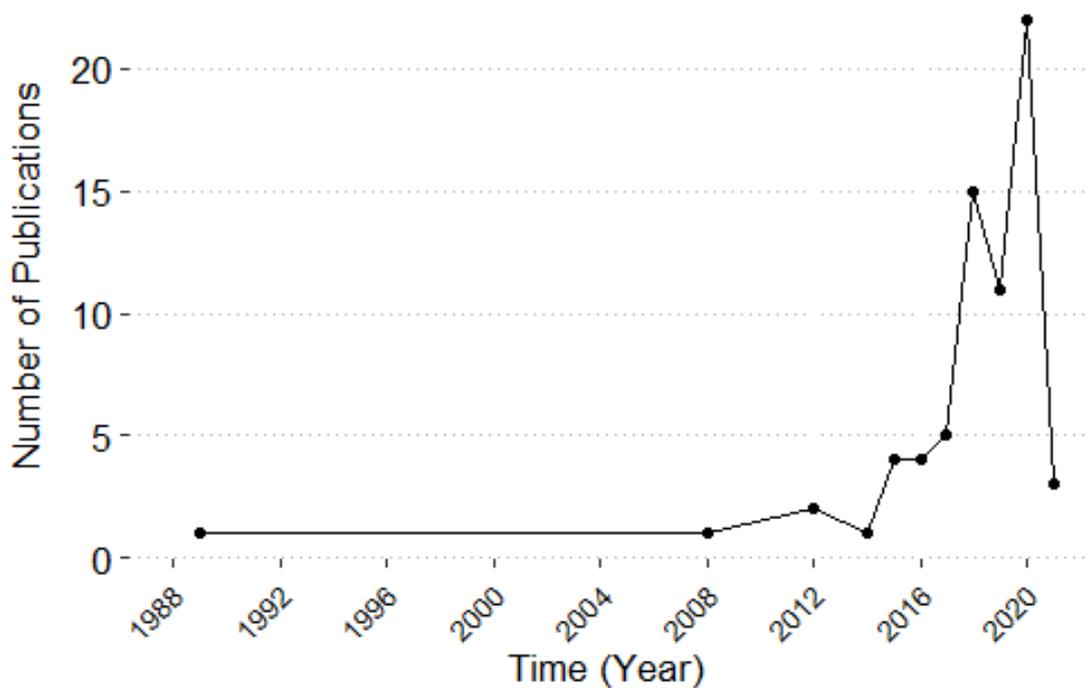
176 reported within a review, dominant polymers, shape and size range ( $\mu\text{m}$ ) reported  
177 within a review. It has been demonstrated that most of the marine MPs are coming from  
178 riverine inflows (Lebreton et al., 2017; Meijer et al., 2019; Schmidt et al., 2017). In  
179 reviews on environmental MPs, using studies from both marine and freshwater systems,  
180 the highest concentrations reported are often from rivers. We have reported these  
181 concentrations in our meta-review table since they provide an indication of the upper  
182 threshold that MP concentration can reach in the coastal environment.

183

## 184 **Results**

### 185 ***Data from MP exposure studies on bivalves***

186 The analysis of the responses of bivalves to MP exposure was assessed with a group of  
187 68 publications out of which 67 were published from 2008 onwards (Figure 2).



188

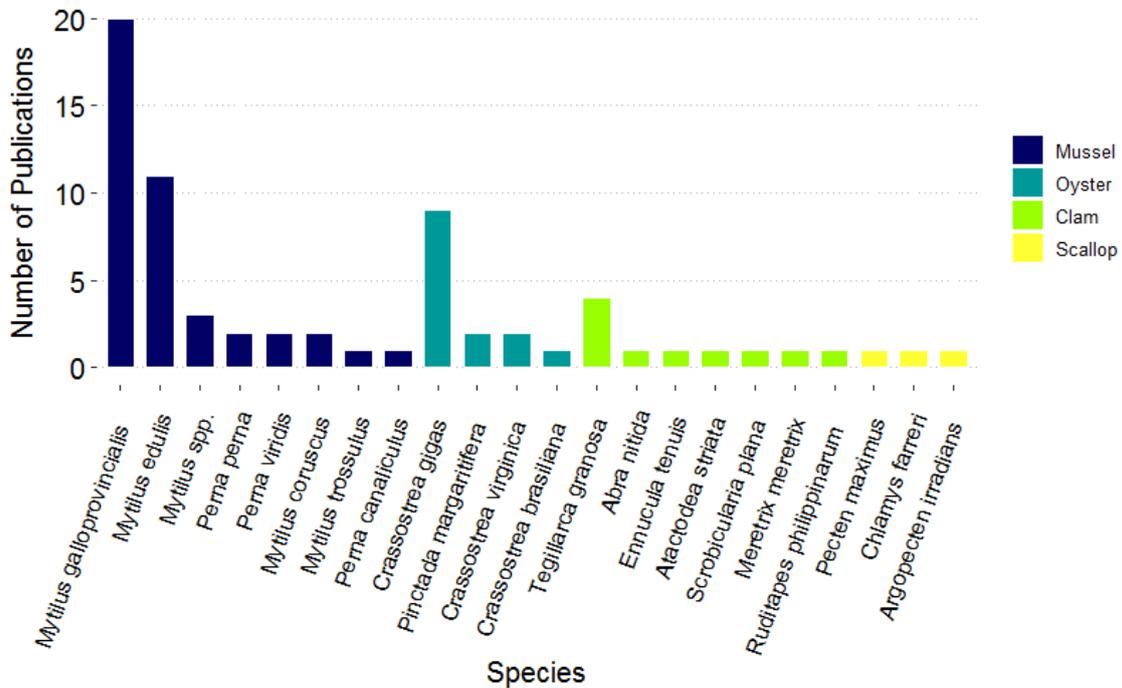
189 **Figure 2.** Number of publications included in this review that investigate the effects of  
190 MPs on bivalves in laboratory settings from 1989 to 2021.

191

192 The most frequently used taxonomic group in experimental studies were mussels ( $n=42$ ,  
193 62%) followed by oysters ( $n=13$ , 19%), clams ( $n=11$ , 16%) and scallops ( $n=3$ , 3%). Overall,  
194 22 species, from the 4 taxonomic groups were used in the experiments. Specifically,  
195 36.4% of species were mussels, 31.8% clams, 18.2% oysters and 13.6% scallops (Figure

196 3). The most abundant species within each group were the mussel *Mytilus*  
 197 *galloprovincialis*, oyster *Crassostrea gigas*, clam *Tegillarca granosa*, and scallops  
 198 *Chlamys farreri*, *Argopecten irradians* and *Pecten maximus*.

199

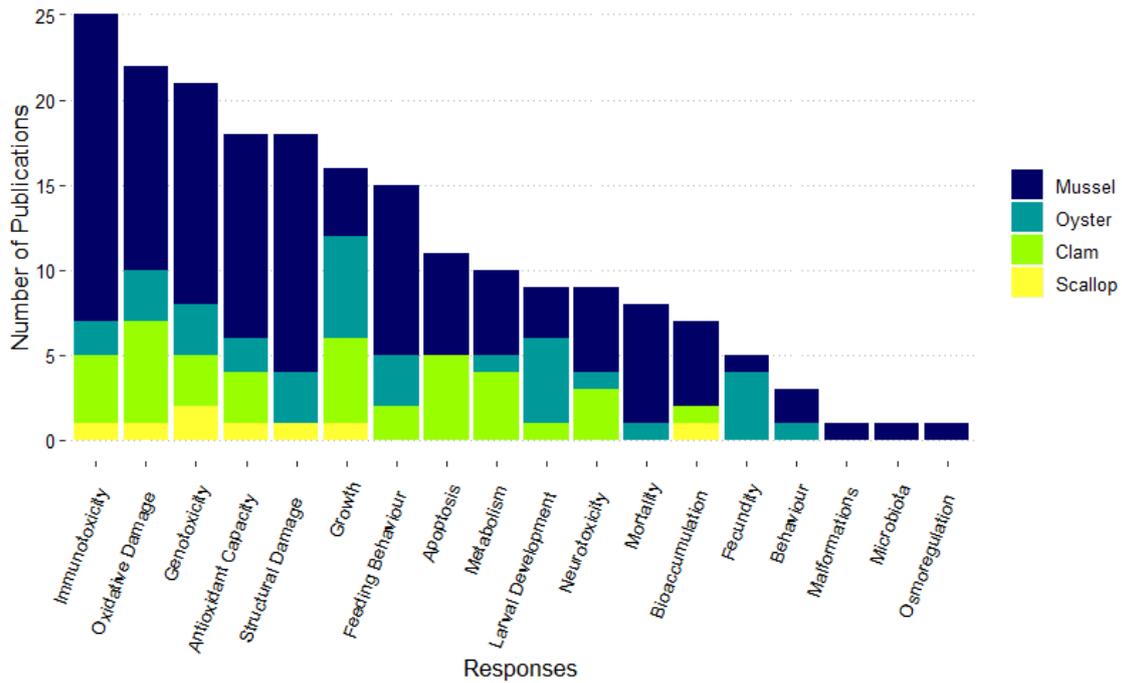


200

201 **Figure 3.** Number of publications, from 1989 to 2021, investigating the effects of MPs  
 202 on bivalves in laboratory settings across the different bivalve species used in the  
 203 experiments.

204

205 Bivalve responses to MP exposure that were more frequently investigated included  
 206 immunotoxicity (n=25), oxidative damage (n=23), genotoxicity (n=21), structural  
 207 damage (n=19) and antioxidant capacity (n=19). Mussels were the most dominant  
 208 taxonomic group across the different response groups (Figure 4). Regarding the other  
 209 bivalve taxonomic groups, clams were used to study 12, oysters 13 and scallops 7 out of  
 210 the 18 response groups (Figure 4). Six out of the 18 response groups have been tested  
 211 with all bivalve taxonomic groups and these were growth, genotoxicity, antioxidant  
 212 capacity, oxidative damage, structural damage and immunotoxicity.

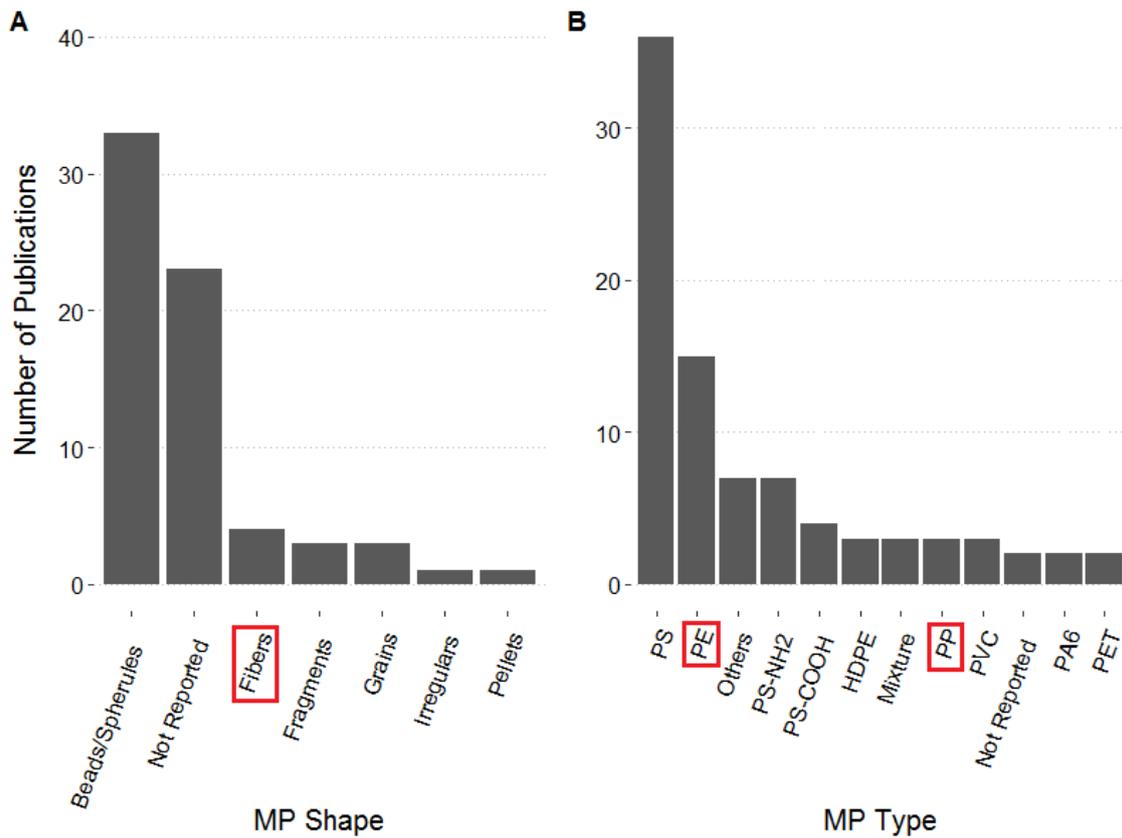


213

214 **Figure 4.** Number of publications, from 1989 to 2021, investigating the effects of MPs  
 215 on bivalves in laboratory settings corresponding to the responses of bivalves to MPs,  
 216 categorised in 18 groups, and the preponderance of different bivalve taxonomic groups  
 217 that the responses were studied on.

218

219 In terms of the MP used in exposure experiments, bead/spherules (n=33) and unknown  
 220 shapes (n=23) were the most used, whereas only 4 trials used fibres (Figure 5A).  
 221 Regarding the composition of the MPs used, polystyrene (PS, n=36) and polyethylene  
 222 (PE, n=15) were the most common types (Figure 5B).

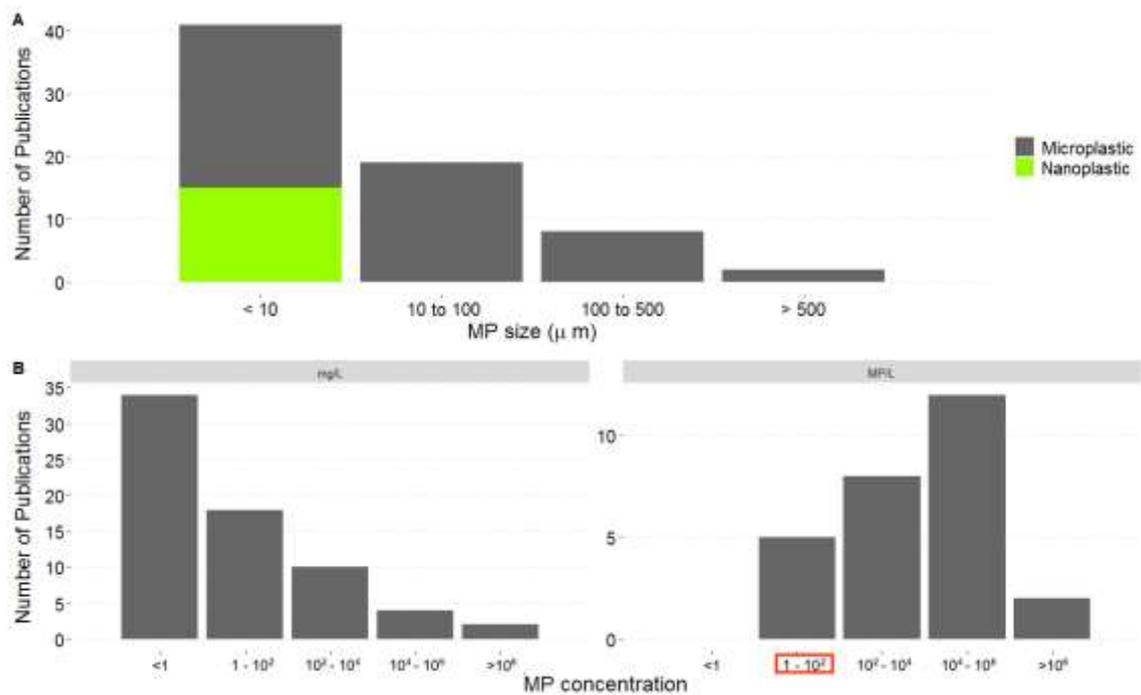


223

224 **Figure 5.** Number of publications, from 1989 to 2021, investigating the effects of MPs  
 225 on bivalves in laboratory settings by microplastic shape (panel A) and material (panel B).  
 226 Red rectangles represent the dominant MP groups in the marine environment based on  
 227 our meta-review (see Table1). Meaning of acronyms: polystyrene (PS), polyethylene  
 228 (PE), amino-functionalized polystyrene (PS-NH<sub>2</sub>), carboxyl-functionalized polystyrene  
 229 (PS-COOH), high-density polyethylene (HDPE), polypropylene (PP), polyvinyl chloride  
 230 (PVC), Nylon 6 (PA6), polyethylene terephthalate (PET).

231

232 The most common size group of MPs used in laboratory studies was <10µm (n=41) from  
 233 which 13 were nanoplastics (0.002-1µm). Only a single study exposed bivalve to particles  
 234 >500µm (Figure 6A). Experimental studies reported concentration of microplastics in  
 235 two different ways: particles per volume (n=27) or weight per volume (n=67), which  
 236 prevent direct comparisons between them. In relation to particles per volume, most  
 237 studies used concentrations between 10<sup>4</sup> to 10<sup>6</sup>MP/L (n=12) whereas the next most  
 238 used concentration group was that of the lower range, 10<sup>2</sup> – 10<sup>4</sup>MP/L (n=8). The most  
 239 used concentrations of weight per volume were <1mg/L (n=33) followed by 1 to  
 240 100mg/L (n=18) (Figure 6B).



241

242 **Figure 6.** Number of publications, from 1989 to 2021, investigating the effects of MPs  
 243 on bivalves in laboratory settings by MP size (panel A) and concentration based on both  
 244 units (mg/L and MP/L) reported in articles (panel B). Red rectangle in panel B represents  
 245 the dominant MP concentration class in the marine environment based on data  
 246 compiled from our meta-review (see Table 1).

247

248 ***Data from review papers on environmental MPs***

249 In terms of MPs presence in the natural ecosystems, a summary of the meta-review  
 250 articles revealed that the most common MP polymers found in marine systems were  
 251 polypropylene (PP) and polyethylene (PE). Regarding shape, fibres were the dominant  
 252 group in marine ecosystems (Table 1). In terms of the MP dominant size, this varied from  
 253 1.2 to 5000μm (Table 1). With respect to the abundance of MPs in the environment  
 254 reported in these reviews, the highest MP concentration was 102 items/L located in  
 255 Stenungsund Harbour, Sweden (Table 1).

256

257 **Table 1.** Summary of different MPs characteristics reported within the 13 review papers  
 258 selected from our systematic meta-review. Location refers to the place where the  
 259 highest MP concentration [MP] was found. Polymers, shape, and size range refer to the  
 260 dominant MP group(s) identified by each review. Note that highest concentrations

261 reported for a freshwater system e.g., a canal, indicate the upper threshold of  
 262 concentration of marine papers examined within the same review.

263

Nº of publications per review	Nº of publications per ecosystem		Highest [MP] (MP/L)	Geographical location of highest [MP]	Polymers	Shape	Size range (µm)	Reference of review paper
	Marine	Freshwater						
59	9	6	1.215	South Easter bays of South Africa			1.2-5000	(Alimi et al., 2021)
18	7	-	0.0035	Bay of Biscay, Spain	PE>PA>PES	Fibres	250-2000	(Mendoza et al., 2020)
12	6	-	6.6	Yangtze Estuary, China		Films>Fibres		(Tang et al., 2020)
>61	23	38	21.839	Rhine-Ruhr, Germany	PP>PE>PVC>PS>PTFE	Fibres		(Xu et al., 2020)
>200	<113	<57	8.369	Lake Yenogoa, Africa	PE>PP>PS		1.2-5000	(Akdogan and Guven, 2019)
180	-	-	100	Canals of Amsterdam				(Cunningham and Sigwart, 2019)
12	7	-	6.6	Yangtze Estuary, China				(Laskar and Kumar, 2019)
38	12	12	102	Skagerrak, Norway/Denmark	PE>PP>PS	Fibres>Fragments		(Wu et al., 2019)
109	58	10	100	Canals of Amsterdam	PE>PET>PA>PP>PS>PVC>PVA	Fibres>Fragments>Beads>Spherules>Films>Foam		(Burns and Boxall, 2018a)
52	43	-	2.4	Swedish West Coast	PP>PE		500-1000	(Gago et al., 2018)
>70	-	-	2.8	Hong Kong	PE>PP>PS			(Shahul Hamid et al., 2018)
13	13	-	10.2	Yangtze Estuary, China		Fibres>Fragments>Beads		(Salvador Cesa et al., 2017)
-	-	-	102	Stenungsund Harbour, Sweden				(Norén, 2017)

264 \*PE – polyethylene; PA – polyacrylamide; PP – polypropylene; PVC – polyvinylchloride; PS – polystyrene; PET –  
 265 polyethylene terephthalate; PES- polyester; PVA – vinyl alcohol; PTFE– polytetrafluoroethylene.

266 **Discussion**

267

268 This is the first systematic review evaluating the environmental relevance of laboratory  
269 studies assessing the effects of MPs on bivalves. Our findings and suggestions draw an  
270 outline for future, environmentally meaningful, studies on the impact of MP on bivalves.  
271 Moreover, this review identifies issues, such as unit disagreement between published  
272 works and the lack of reported information when reporting results, which limit our  
273 ability to evaluate the extent of the marine bivalves' vulnerability to MP pollution as well  
274 as hinder our ability to interpret and compare results between studies.

275

276 ***Relevance of bivalve species and responses tested in exposure studies***

277 Our findings show that 62% of the reviewed studies were focused on assessing the  
278 impacts of MP on mussels. Within this taxonomic group, species from *Mytilus* and *Perna*  
279 genera predominated in experimental studies. Preference for this group was also  
280 evident in the responses to MP exposure tested as mussels were the only bivalve  
281 taxonomic group present in all responses. Moreover, 17% of the responses were  
282 investigated solely on mussels. We can consider the use of mussels as environmentally  
283 relevant since they are the most dominant bivalve group in marine ecosystems globally  
284 (Gosling, 2003) and were shown to have the greatest potential of bioremediation (van  
285 der Schatte Olivier et al., 2018). Species of the genus *Mytilus* predominate in  
286 communities with cool water in the northern and southern hemispheres whereas  
287 species of the genus *Perna* have a tropical to subtropical distribution in the southern  
288 hemisphere (Gosling, 2003).

289

290 After mussels, oysters and clams were the most common bivalve groups in studies, used  
291 in 72% and 61% of all responses tested, respectively. Effects of MPs on growth were  
292 tested on both organisms, while oysters predominated in larval development and  
293 fecundity testing. This is presumably because of their greater economic importance  
294 (Gosling, 2003), as in 2018 it was estimated that the aquaculture of marine oysters and  
295 clams together was worth \$7.27 billion (FAO/ICAC, 2018b). Despite the great economic  
296 value of scallops - \$5.84 billion in 2018 (FAO/ICAC, 2018b) - they were the least studied  
297 group, present in 39% of all responses tested. A reason for their lower presence could

298 be that most scallop species are found at depths between 10 and 100m in bays and open  
299 coast sites (Gosling, 2003) and are thereby harder to access. That fact could also  
300 complicate their maintenance in laboratories (Lusher et al. 2017, Gosling 2003) and  
301 therefore causing bias against their selection for experimental studies.

302

### 303 ***Environmental relevance of MPs used in exposure studies***

304 To evaluate the environmental relevance of experimental studies, we conducted a  
305 meta-review of recent reviews assessing the characteristics and concentrations of MPs  
306 in nature. Since 48% of the studies included in this review do not report the MP shape  
307 used in experimental trials, drawing conclusions is challenging. Among studies reporting  
308 shape, beads/spherules were the most common MP debris. One of the main reasons,  
309 for the preference of spherical MPs is their uniform size and shape which facilitates  
310 particle identification and quantification compared to irregular debris, such as fibres  
311 (Ward et al., 2019). Furthermore, microfibers are not readily available in the market  
312 and their generation, within a consistent size range, is strenuous and time-consuming  
313 (Christoforou et al., 2020; Cole, 2016). However, there are clear arguments beyond  
314 convenience for including different shapes of MPs in experiments, such as, abundance  
315 in coastal environments or noxious capacity. According to our meta-review of 13 review  
316 papers of environmental MP characteristics, fibres are the most common shape, as  
317 concluded in 5 of the reviews, constituting up to 80-90% of all global water samples  
318 (Barrows et al. 2018). A reason for their abundance could be the increase in their  
319 demand: the latest World Apparel Fibre Consumption Survey reveals that the use of  
320 synthetic fibre for textile industry has increased substantially from 2005 to 2008  
321 (FAO/ICAC 2005). These fibres enter the aquatic environment through garment  
322 washing and improper filtration of wastewaters (De Falco et al., 2019). Fibres are not  
323 only abundant in aquatic environments but also inside bivalves collected from the wild  
324 (Sendra et al., 2021). In addition to their abundance, fibres have proven to be more  
325 damaging than other shapes due to their elongated shape and thus their capacity to be  
326 ingested by bivalves despite their length (Beecham, 2008). Studies reveal that fibres are  
327 more toxic compared to spherical and fragmented MPs (Gray and Weinstein, 2017;  
328 Lehtiniemi et al., 2018). Therefore, we emphasise the urgent need for more studies  
329 focusing on the effect of microfibers on bivalves.

330

331 Apart from the shape, the composition of MPs was another characteristic evaluated for  
332 environmental relevance. Our findings showed that 43% and 18% of exposure studies  
333 used polystyrene (PS) and polyethylene (PE) respectively. However, according to the  
334 environmental MP reviews, PE and polypropylene (PP) have been considered as major  
335 sources of MP pollution in the aquatic ecosystems investigated, and these materials  
336 constituted 36.4% of the European plastic demand in 2017 (Association of Plastic  
337 Manufacturers, 2017). Nevertheless, a recent meta-analysis of distribution of plastic  
338 types suggested that the relative abundance of polymer types is not uniform across  
339 ocean zones. The study points out that low density polymer types, such as PE, are more  
340 common in surface waters, whereas PP and polyester are more dominant in intertidal  
341 areas and polyamide and acrylic in subtidal areas (Erni-Cassola et al., 2019). With the  
342 exception of few deep-sea species, most marine bivalves used in the exposure studies  
343 reviewed were shallow estuarine and coastal species (Dame, 1993). Therefore, to enable  
344 more relevant inferences of bivalve exposure to the dominant polymer we recommend  
345 a shift of focus from PS and PE to PP in experimental studies.

346

347 Finally, we determined the environmental relevance of MP size and concentration used  
348 in bivalve exposure studies. Particles  $<10\mu\text{m}$  were used in 69.4% of the studies from  
349 which 32% were nanoplastics ( $<1\mu\text{m}$ ). Importantly, there is currently no consensus on  
350 the most common size range of MP in aquatic habitats and this could be attributed to  
351 the limitation in collection and quantification methodologies of smaller MPs (Covernton  
352 et al., 2019). However, Cai et al. (2018) observed that MP smaller than  $300\mu\text{m}$   
353 contributed 92% of the total MP quantified in South China Sea and last estimates  
354 relating to the abundance of MP in the marine environment suggest that the amount of  
355 nanoplastics could be much higher than previously thought (Lindeque et al., 2020). In  
356 terms of bivalve retention efficiency a study on 13 species of bivalves showed that all  
357 particles larger than  $4\mu\text{m}$  were ingested but the retention efficiency of any smaller  
358 particles was decreasing the smaller the particles were (Møhlenberg and Riisgård, 1978;  
359 Rosa et al., 2018). This is attributed to the selection properties of the bivalve gills as  
360 small particles can pass through the intrafilamentary gaps, and are thus not retained on  
361 the surface of the ctenidium from where particles are usually directed to the food grove

362 (Christoforou et al., 2020; Rosa et al., 2018). However, in this review it was evident that,  
363 in 16 studies, nanoplastics were still able to induce responses from the exposed bivalves.  
364 Thus, regarding the environmental and biological relevance of the MP sized used, we  
365 conclude that the focus of exposure studies on nanoplastics is relevant.

366

367 During the last years, one of the most controversial topics in MP research (Burns & Boxall  
368 2018, Haegerbaeumer et al. 2019) has been the use of high MP dosages in laboratory  
369 exposure studies. Our meta-review regarding the environmental levels of MPs, revealed  
370 that the highest MP concentration recorded in aquatic environments was 102  
371 particles/L, in Skagerrak, at the coastal waters of Norway and Denmark. Similar values  
372 to this level have been used as a reference to environmentally relevant values for MP  
373 concentration in other studies (Bour et al. 2018, Woods et al. 2018). Based on this  
374 threshold, 81.5% of the bivalve exposure studies (reported in particles/volume)  
375 reviewed here, would be above realistic limits for environmental concentrations. MP  
376 concentrations that are orders of magnitude higher than the maximum reported  
377 concentrations from the field could exacerbate the MP impacts and have a questionable  
378 biological meaning. On the other hand, using in bivalve exposure studies, the maximum  
379 concentration reported from the marine environment and perhaps up to one order of  
380 magnitude higher (100-1000 particles/volume) might be sensible given that MPs  
381 accumulate much faster than they biodegrade in the marine environment thus  
382 concentrations in coastal ecosystems are expected to rise.

383

#### 384 ***Consensus regarding MP research***

385 Another issue faced by researchers in the MP research field is the inconsistency in the  
386 units for reporting MP concentrations. In exposure studies, the MP concentrations are  
387 reported in particles/volume or weight/volume while environmental concentrations are  
388 typically reported in items/area or items/volume. Unfortunately, there is no direct  
389 conversion between these units which prevents us from assessing the environmental  
390 relevance of studies and prevents the comparison between findings and conclusions.  
391 Hence, we highlight the need for consensus in reporting units for future research.  
392 Specifically, we recommend using particles/volume as this is the most frequently

393 reported unit and also enables – when the material and the size are provided - to  
394 calculate the weight/volume but not vice versa.

395

396 Furthermore, 23% of bivalve exposure studies did not report critical information on the  
397 MP characteristics like shape and type. Due to the now established impact of shape and  
398 type on the response of exposed bivalves but also for allowing for replicability of  
399 experimental procedures, it is essential that MP characteristics are provided in detail.

400

401

#### 402 **Acknowledgements**

403 We would like to thank Nissad Development Company and The A.G. Leventis Foundation  
404 for their financial support (sponsors had no further involvement in this review). We also  
405 would like to thank Professor Colin Selman for his help in categorising the response  
406 variables in ecotoxicological papers into similar response groups.

407

#### 408 **Author Contributions**

409 EB: Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation,  
410 Writing-Original Draft, Writing-Review & Editing, Visualization

411 EC: Conceptualization, Methodology, Writing-Original Draft, Writing-Review & Editing,  
412 Visualization, Funding acquisition

413 JL: Conceptualization, Methodology, Writing-Review & Editing, Supervision

414 SS: Conceptualization, Methodology, Writing-Review & Editing, Supervision, Project  
415 administration

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