

**Microplastics alter multiple biological processes of marine benthic fauna**

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1 **Microplastics alter multiple biological processes of marine benthic fauna**

2
3 **Running page head:** Microplastic impacts on benthic fauna.

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10 ABSTRACT

11 Marine sediments are a sink for microplastics, making seabed organisms particularly
12 exposed. We used meta-analysis to reveal general patterns in a surge in experimental studies
13 and to test for microplastic impact on biological processes including invertebrate feeding,
14 survival and energetics. Using Hedge's effect size (g), which assesses the mean response of
15 organisms exposed to microplastics compared to control groups, we found negative impacts
16 (significant negative g values) across all life stages (overall effect size (g) = -0.57 95% CI [-
17 0.76, -0.38]), with embryos most strongly affected (g = -1.47 [-2.21, -0.74]). Six of seven
18 biological process rates were negatively impacted by microplastic exposure, including
19 development, reproduction, growth and feeding. Survival strongly decreased (g = -0.69 [-
20 1.21, -0.17]), likely due to cumulative effects on other processes such as feeding and growth.
21 Among feeding habits, omnivores and deposit feeders were most negatively impacted (g = -
22 0.93 [-1.69, -0.16] and -0.92 [-1.53, -0.31], respectively). The study incorporated the first
23 meta-analysis to contrast the effects of leachates, virgin, aged and contaminated particles.
24 Exposure to leachates had by far the strongest negative effects (g = -0.93 [-1.35, -0.51]),
25 showing studies of contaminants and leachates are critical to future research. Overall, our

26 meta-analysis reveals stronger and more consistent negative impacts of microplastics on
27 seabed invertebrates than recorded for other marine biota. Seabed invertebrates are numerous
28 and diverse, and crucial to bottom-up processes, including nutrient remineralisation, benthic-
29 pelagic coupling and energy transfer through the ocean food web. Marine sediments will
30 store microplastics over long timescales. The reveal that microplastics impinge on multiple
31 fundamental biological processes of seabed fauna implies plastic pollution could have
32 significant and enduring effects on the functioning of the ocean.

33 Key Words

34 Systematic review • Benthos • Functional traits • Survival • Development • Meta-analysis

35 1.0 INTRODUCTION

36 The problem of plastic pollution is growing, resulting from an average annual increase of 9%
37 in plastic manufacturing between 1950 and 2009 (Hammer et al. 2012). The input of plastics
38 into the marine environment, both directly and indirectly through riverine inputs, is also
39 increasing. An estimated 4.7 to 12.8 million tonnes of plastic enters the marine environment
40 every year (Agamuthu et al. 2019). The fate of much of this plastic is unknown; the term
41 ‘missing plastic’ was coined to describe the shortfall in the estimated volume of plastics
42 found in the water column compared to inputs (Wayman & Niemann 2021). It is thought that
43 deep-water and sediment storage of plastics and microplastics, in particular, make up the
44 majority of this ‘missing plastic’ (Zhang 2017). Here, we assess the impact of accruing
45 microplastics on invertebrate animals of the seafloor.

46

47 The definition of microplastics is inconsistent throughout the existing literature, but most
48 commonly includes plastic particles of any shape from 0.1µm to 5mm (Auta et al. 2017).

49 Within this category exist intentionally manufactured primary microplastics, such as highly

50 prevalent pre-production plastic ‘nurdles’ (Jiang et al. 2021), as well as secondary
51 microplastics, resulting from the UV or physical degradation of marine macroplastics
52 (Efimova et al. 2018). Microplastic prevalence in the ocean was recently estimated at 2.41
53 million tonnes across the Atlantic, Indian and Pacific subtropical gyres (Vazquez & Rahman
54 2021). This prevalence is likely to increase with inputs not only from terrestrial activity, but
55 also from the breakdown of plastics already present in the marine environment (Kooi et al.
56 2017). Microplastics are subject to further change upon entering the marine environment;
57 they may be further broken down into nanoplastic particles ($<0.1\mu\text{m}$) or experience
58 biofouling (Zhang 2017). Biofouling of microplastics occurs predominantly as a result of the
59 attraction of organic substances to the hydrophobic surface of the particle (Kaiser et al.
60 2017). Cózar et al. (2014) showed that the specific density of most microplastics is lower
61 than that of seawater, so particles should remain buoyant. However, settling of microplastics
62 on the seafloor has been documented, with Zhang (2017) suggesting sinking rates of
63 approximately 4mm per day. Sinking is stimulated by the biofouling of microplastic particles
64 which increases the specific density, although studies have also suggested the influence of
65 microplastic shape and size on the sinking rate of a particle (Melkebeke et al. 2020). Using
66 Environmental Risk Assessment modelling, Everaert et al. (2018) found species had varying
67 sensitivity to microplastic, but that sediment concentrations <540 microplastic particles kg^{-1}
68 were ‘safe’ and unlikely to have negative impact. The same study reported a current
69 concentration of 32-144 particles kg^{-1} in marine intertidal sediments, suggesting that the safe
70 threshold is likely to be exceeded in the latter half of the 21st century. Estimates of
71 microplastics in seawater itself vary widely and Xu et al. (2020) reported seawater
72 concentrations ranging from 0.33 to 3252 particles m^{-3} globally. The vast majority ($>90\%$) of
73 marine microplastics have been reported to accumulate on the seafloor (Melkebeke et al.
74 2020). In the southern North Sea, for example, sediment microplastics have been reported to

75 range in concentration from 2.8 to 1188.8 particles kg⁻¹ dry weight (Lorenz et al. 2019).
76 Microplastics are therefore likely to become a ubiquitous component of seabed sediments and
77 thus the influence of microplastics on benthic habitats must be considered.
78
79 Gall and Thompson (2015) reported over 44000 interactions of marine fauna with plastic
80 debris, across 693 species. Larger plastic fragments impact fauna predominantly through
81 ingestion and entanglement. A systematic review of 747 studies quantifying the interactions
82 of plastics with marine megafauna found 701 species had ingested plastics and 354 species
83 had experienced entanglement (Kühn & van Freneker 2020). Microplastics can impact
84 marine organisms through a wider range of mechanisms, as shown in many experimental
85 laboratory studies. Microplastic exposure caused abnormal embryo development in the brown
86 mussel *Perna perna* (Gandara e Silva et al. 2016). The lugworm *Arenicola marina* reduced
87 its feeding rate with increasing microplastic dosage (Besseling et al. 2013). Reduced feeding
88 can be the result of a false sense of fullness, damage or blockages to the digestive tract or
89 confusing microplastics for prey (de Sá et al. 2015). Numerous studies have found cellular
90 level impacts of microplastics, for example, microplastic consumption influenced cellular
91 pathway signalling, diminished growth and induced toxicity and oxidative stress in rotifers
92 (Jeong et al. 2016). Such impacts may lead to behavioural changes, growth inhibition and,
93 ultimately, increased mortality (de Sá et al. 2018). Microplastics also have the potential to
94 cause adverse reactions via persistent organic pollutants (POPs), which adhere to plastic
95 particles. Particularly hazardous are endocrine disruptor chemicals (EDCs), which
96 accumulate in fatty tissues, altering hormone production and potentially causing thyroid
97 problems, reduced reproductive success and hormone-sensitive cancers (Gallo et al. 2018).
98 While the study of POPs so far has focussed primarily on the impacts on human health,
99 effects on marine fauna have been observed, one example being reduced survival rate and

100 jump height in beach hoppers (Tosetto et al. 2016). Microplastics encountered in nature are
101 often contaminated, giving them the potential to be more toxic than virgin microplastics.
102 Abnormal development was found in 23% of brown mussel *Perna perna* embryos from
103 virgin pellets compared to 100% abnormal development from pellets sourced from beach
104 sediments (Gandara e Silva et al. 2016). Despite such indications of impact to benthic
105 organisms, there is no overview of implications to the breadth of seabed organisms. In a
106 systematic review of 220 studies published prior to the year 2010, Ajith et al. (2020) found
107 that 38% of existing studies on the impacts of microplastics had used fish as the study
108 organism, followed by 18% studies targeting molluscs. This leaves a knowledge gap
109 surrounding the majority of benthic invertebrate species. Here, we make use of a rapid
110 increase in publications on marine benthos since 2019 (Figure S2, Supplementary Materials)
111 and new data for a total of 6 taxa to generate a comprehensive overview across seabed
112 taxonomic and functional groups.

113

114 As a means of quantifying the impacts of microplastics on marine fauna, recent studies have
115 considered the impact of microplastics on what was termed the ‘functional traits’ of
116 organisms (Berlino et al. 2021, Salerno et al. 2021), albeit several ‘traits’ are more correctly
117 perceived as the rates of important biological processes like growth, reproduction and
118 survival (See Supplementary Materials, Table S1). Focussing on biological rates offers
119 insights into the impacts of microplastics on wider organismal and ecosystem functioning.
120 Since many impacts of microplastics result directly from the ingestion of particles, feeding
121 strategy in particular may contribute to variation in the magnitude of impacts. Thus, among
122 fish and invertebrates, predators and deposit feeders contained more plastics than filter
123 feeders and, sometimes, deposit feeders (Bour et al. 2018, Naji et al. 2018). It stands to

124 reason that if the ingestion of microplastics varies by feeding strategy, so might the effects on
125 biological processes.

126

127 There is a lack of consensus of the impacts of microplastics on marine benthic fauna,
128 particularly in terms of the range of factors which might be contributing to the variation in
129 effects. Here, we make use of a rapid increase in publications on marine benthos since 2019
130 with new data for a total of 6 phyla to generate a comprehensive overview of the impacts of
131 microplastics across seabed taxonomic and functional groups. Using a systematic review and
132 associated meta-analysis of extracted data we quantify the impacts of microplastics on marine
133 benthic fauna and identify knowledge gaps and potential bias in the current state of the art.
134 We hypothesised that microplastics would have an overall negative effect on the performance
135 of marine benthic fauna, which would increase with exposure concentration. We expected the
136 effects of microplastics to vary amongst feeding habits, with predators at risk of stronger
137 effects resulting from trophic transfer of microplastic particles. Microplastic characteristics,
138 including size, shape, exposure duration and concentration, were expected to be primary
139 drivers of any variation in effect size.

140

141 2.0 MATERIALS AND METHODS

142 2.1 STUDY DESIGN

143 The study used a systematic review and meta-analysis to assess the impacts of experimental
144 exposure to microplastics particles (hereinafter, MPP will refer to microplastic particles) on
145 marine benthic fauna. Only laboratory studies that included a control (no MPP) and one or
146 more MPP exposure levels were included, so that overall mean effect sizes could be
147 determined. Studies focusing on MPP ingestion but not impacts on biological processes were

148 excluded. The review had no geographical or temporal limits. Two search engines, Web of
149 Science and the Wiley online library, were used in order to include papers from a range of
150 sources, including grey literature, and minimise publication bias otherwise arising from
151 restricting search results to peer-reviewed journals favouring studies with significant results
152 (Sterne et al. 2000). Ultimately, all studies included in the analysis were from peer-reviewed
153 journals. The study considered the influence of potential contributing factors, such as
154 phylum, feeding strategy and microplastic composition, on variation in the magnitude of
155 microplastic impacts (see Supplementary Materials, Figure S1).

156

157 2.2 LITERATURE SEARCH AND DATA EXTRACTION

158 The systematic literature search was conducted on the 7th June 2021, following the
159 methodology of Pullin and Stewart (2006) and O’Dea et al. (2021). The search string had
160 three components using the Boolean operators “AND” and “OR”. Each component of the
161 string was designed to address an area (impact, microplastics or biological processes) of the
162 research question and to include studies on any marine benthic fauna. The string of search
163 terms was tested to ensure it delivered relevant literature hits (tested using 10 pre-identified
164 highly relevant key references. Table S2). The final string of search terms was as follows:

165

166 impact* OR response* OR effect* OR interaction* OR consequence* OR implication* OR
167 contamination* OR ingestion* OR consumption* OR consume* OR uptake* OR “taken up”
168 OR accumulation OR contamination OR transfer

169

AND

170 Microplastic* OR “micro plastic” OR “micro-plastic” OR microfilament* OR filament* OR
171 “plastic pellet*” OR nurdle*

172

AND

173 trait* OR “functional trait*” OR growth OR feeding OR reproduction OR fecundity OR
174 behaviour* OR development OR hatching OR health OR survival OR digestion

175

176 A total of 3,650 search results (studies, papers) were identified on Web of Science, with a
177 further 166 from the secondary Wiley Online Library. For each paper, the title, then abstract
178 and then the full-text content were screened for relevance (Table S3) according to the
179 following criteria. Studies that purely addressed the distribution or sources of microplastics
180 were excluded, as were observational work documenting only the ingestion of microplastics,
181 qualitative and systematic reviews. Changes to feeding rates following microplastic
182 consumption were included, but ingestion rates of microplastic particles themselves were not
183 included as a change to a biological process. Experimental studies with a focus on cellular
184 impacts were also excluded, unless the impact could be tied directly to one of the biological
185 processes we evaluated (e.g. O₂ consumption, representing respiration and energy demand).
186 Only studies focussing on marine benthic organisms were considered. Freshwater organisms
187 or those from inland saltwater were excluded, while both intertidal and subtidal marine and
188 estuarine organisms were included, where the species was determined to spend the majority
189 of its lifecycle on, or buried within, the seafloor. Experiments which used microplastics of
190 sizes outside of the predetermined range (0.1µm - 5mm) were excluded. A final list of 72
191 papers (Table S3) was selected for meta-analysis.

192

193 Data were extracted directly from paper text, tables and figures, the latter using Automeris
194 WebPlotDigitizer Version 4.4. Types of data extracted from each study were study
195 identifiers, meta-data and data for quantitative synthesis (control and experimental mean,
196 standard deviation, SD, and number of replicates, n (Table S4). Examples of response
197 variables which were used for biological traits are outlined in Table S1. A total of 701 case

198 studies (independent experiments included in the same study. For example, multiple exposure
199 concentrations or species tested) were extracted from the 72 papers.

200

201 2.3 DATA ANALYSIS

202 Data extracted from papers required standardisation before analysis to overcome the use of
203 different units and approaches among studies. The data were standardised to common units of
204 microplastic exposure concentration, duration and particle size in order to allow comparison
205 of the experimental conditions that test animals were exposed to. Microplastic particles were
206 classified into: fibre, fluff (usually derived from clothing fibres), fragment, pellet, square,
207 sphere (including microbeads) or powder, plus leachates and leachates adsorbed to
208 microplastics, according to how they were described by the authors (see e.g. Gray and
209 Weinstein 2017). Microplastic exposure units which could not be standardised into common
210 units (e.g. % sediment weight, fibres per prey individual) were excluded from concentration
211 analysis (18 studies). Remaining microplastic exposure units from 54 studies were
212 standardised into common units of g L^{-1} . Concentrations given in particles L^{-1} were converted
213 using $\text{mass}_{\text{particle}} = \text{density} \times \text{volume}$ (Everaert et al. 2018), using a standard density of marine
214 microplastics of 0.925g cm^{-3} , determined by Van Cauwenberghe (2016). Density of plastic
215 particles was not available for the microplastics used in most studies and using this standard
216 density was the most appropriate approach. Particle volumes were calculated for spheres (and
217 for fragments, with assumptions of largely spherical shape) using $V = 4/3\pi r^3$, where the
218 radius of the particle was provided in the original study. Microplastic concentration was log
219 transformed for analysis to allow patterns to be more clearly seen, since data were skewed
220 towards very small values. Where necessary, medians and interquartile ranges (IQR) were
221 converted into means and standard deviations (SD), where SD was taken to equal $\text{IQR}/1.35$,
222 assuming normal distribution of data (Higgins et al. 2019). Any 95% confidence intervals

223 (CI) were also converted into SD, where $SD=CI/3.92$, multiplied by the square root of the
224 sample size (n) (Higgins et al. 2019). Data were explored for patterns in the number of
225 studies per geographical region, taxa (phylum of organism), feeding strategy (predator,
226 deposit feeder, scavenger, filter feeder, omnivore) and microplastic characteristic (shape,
227 size, polymer type) to generate an overview of the geographical distribution of research and
228 to identify potential bias within the results, such as a high proportion of studies published in
229 one geographic region.

230

231 Effect size for each study was calculated as Hedge's g (Borenstein et al. 2009):

232

$$233 \quad \text{Hedge's } g = \frac{m_c - m_e}{SD_{pooled}} \times J$$

234

235 Where m_c was the control mean, m_e was the experimental mean, SD_{pooled} was the pooled
236 standard deviation across the samples and J was the correction factor used to account for bias
237 arising from variation in sample size.

238

239 Hedge's g values were interpreted using the recommended thresholds from Cohen (2013),
240 where ~ 0.2 indicated a small effect, ~ 0.5 indicated a moderate effect and > 0.8 indicated a
241 larger effect. A negative Hedge's g represents a negative impact of the experimental
242 condition relative to the mean. Directionality of effect sizes was corrected to ensure g values
243 were representative of the effects shown by studies and as described by the authors (Table
244 S5). For example, an increased time to find a new shell (automatically a positive effect) was
245 corrected to be negative, when the authors noted this represented a negative impact on the
246 organism (Crump et al. 2020). We checked for any influence of publication bias by applying
247 the non-parametric trim and fill method (Duval and Tweedie 2000) to an rma.uni model of

248 our data, whereby the number of missing studies at either extreme positive or negative values
249 could be estimated. This showed that publication bias was likely to have had a negligible
250 effect on the outcome of our meta-analysis (Table S6).

251

252 Once an effect size had been determined for each case study ($k = 701$, where k signifies
253 independent experiments, or case studies, included in the same study), a pooled effect size
254 was calculated for all values and each biological process, using a random effects model with
255 the “*rma.mv*” function of the “*metafor*” package (Viechtbauer 2010) in *Rstudio* Version
256 1.3.1093 (*Rstudio* Team 2020). In each model, we included ‘Study ID’ of the published study
257 to account for non-independence of data extracted from the same study (Viechtbauer 2007).
258 To evaluate data compliance with test assumptions, an I^2 value was produced by Wald’s test
259 for heterogeneity of variance between studies (Borenstein et al. 2009) and a Cochran’s Q
260 value determined the level and significance of heterogeneity (Cochran 1954). Since results
261 from the random effects model indicated significant heterogeneity between studies, subgroup
262 analyses (categorical data) and meta-regressions (continuous data) were conducted using
263 random effects models in *metafor* (*R* statistics) to identify moderator variables which may
264 have been driving the variation. Organism traits such as taxa, feeding type and life stage and
265 experimental variables including microplastic size, shape, polymer type and concentration,
266 were investigated for contribution to heterogeneity as well as the pooled effect size for each
267 variable. Effect sizes were given with 95% confidence intervals.

268

269 3.0 RESULTS

270 3.1 SUMMARY AND DISTRIBUTION OF FINDINGS

271 While no temporal limits of publication were implemented, all papers were published from
272 2013 onwards, with 79.2% published since 2018 and nearly half (43.1%) published in the last

273 1.5 years covered by our systematic review (Figure S2). Published findings were from 6
274 continents, leaving only Antarctica absent, with the most research having occurred in Europe
275 (n = 35) and Asia (n = 17) (Figure S3).

276

277 Experiments involved 6 animal phyla and 6 feeding strategies (Figure 1a), with the majority
278 of studies focused on filter feeders (n = 39). A wide range of experimental conditions were
279 used by studies. Exposure concentrations were reported in a multitude of units, of which 'g l⁻¹
280 ' and 'particles l⁻¹' were the most common, with less frequently used units including '% of
281 feed' and '% of sediment weight'. Approaches to reporting microplastic leachates were
282 varied, since some studies used leachates adsorbed to particles and others used leachates
283 independently (reported as concentration in the water column). The majority of studies (n =
284 26) exposed organisms to microplastic spheres, although 30 studies did not state the shape of
285 particles (Figure 1b). Out of 19 types and combinations of polymers used for exposure,
286 polystyrene and polyethylene were the most commonly used (n = 25 and n = 10,
287 respectively).

288

289 3.2 EFFECTS OF MICROPLASTICS ON BIOLOGICAL PROCESSES

290 The effect size for all organisms pooled indicated a moderate, but significant overall negative
291 effect of microplastics on biological processes ($g = -0.57 [-0.76, -0.38]$, $p < 0.001$) (Figure 2).
292 Significant negative effects were also seen for all categories of biological processes, except
293 energy use (e.g. respiration). Large negative effects of microplastic particles (MPP) on
294 animal development, reproduction and survival were seen (Figure 2). A small and non-
295 significant effect of MPP on energy processes was found. Significant heterogeneity of
296 variance was found between studies ($I^2 = 61.4\%$, $Q_{700} = 2668.9$, $p < 0.001$), including within
297 every biological process category (Table 1), indicating unexplained variance beyond the

298 effect of biological process and supporting the need for a sub-group analysis to investigate
299 other drivers of effect size.

300 301 3.3 SUB-GROUP ANALYSIS

302 **3.3.1 Organism Characteristics.** The taxonomic group of organisms explained a significant
303 amount of heterogeneity of variance in the dataset ($Q_{\text{moderators}, 6} = 39.87, p < 0.001$).

304 Microplastic exposure had a large and significantly negative effect on all phyla, with
305 chordates (ascidians) most significantly affected ($g = -1.79 [-3.47, -0.12], p = 0.04$), although
306 this result originated from only one study (Anderson and Shenkar 2021). Echinoderms,
307 crustaceans and molluscs were less, but still significantly, impacted by microplastic exposure,
308 while impacts on annelids and cnidarians were not significant (Figure 3). Species-level
309 effects were also statistically significant ($Q_{\text{moderators}, 61} = 160.81, p < 0.001$). The greatest
310 negative plastics effect on a single species was in the sea urchin *Lytechinus variegatus* ($g = -$
311 $11.57[-16.21, -6.92], p < 0.001, k = 2$), followed by the coral *Acropora formosa* ($g = -4.67 [-$
312 $7.22, -2.11], p < 0.001, k = 5$).

313

314 Feeding strategy of the organism contributed significantly to heterogeneity between studies
315 ($Q_{\text{moderators}, 6} = 42.15, p < 0.001$) (Figure 4). Omnivores and deposit feeders experienced the
316 largest negative effects from MPP ($g = -0.93 [-1.69, -0.16]$ and $-0.92 [-1.53, -0.31]$,
317 respectively), while all other feeding strategies except scavengers were also negatively
318 impacted (Figure 4). Every life stage of organism was significantly negatively impacted by
319 MPP, with earlier life stages most strongly affected, particularly embryos ($g = -1.47 [-2.21, -$
320 $0.74], p < 0.001$) (Figure 5).

321

322 **3.3.2 Microplastic exposure.** Microplastic exposure concentration ranged from 1.21×10^{-11} to
323 1000 g L^{-1} (median = $4.84 \times 10^{-4} \text{ g L}^{-1}$) but did not contribute significantly to between-study
324 heterogeneity ($R^2 = 0.99$, $Q_{\text{moderators}, 1} = 0.0077$, $p = 0.93$, Figure S4). However, analysis of the
325 distribution of data showed higher variability in effect sizes at higher concentrations,
326 particularly for fragments (Figure 6).

327

328 The duration for which organisms were exposed to microplastics ranged from 0.17 to 5760
329 hours, with a median duration of 120 hours. Meta-regression showed that duration of
330 exposure to microplastics did not explain a significant amount of heterogeneity ($R^2 = 0.02$,
331 $Q_{\text{moderator}, 1} = 0.13$, $p = 0.72$) (Figure S5a) and the size of microplastic particle did not
332 contribute to variation in effect size ($R^2 = 0.10$, $Q_{\text{moderator}, 1} = 0.08$, $p = 0.77$) (Figure S5b),
333 although the effects of nanoparticles ($<0.1 \mu\text{m}$) were not explored in this study.

334

335 Microplastic shape accounted for significant heterogeneity in the data (mixed-effect
336 modelling: $Q_{\text{moderators}, 10} = 47.10$, $p < 0.001$), although there was significant residual
337 heterogeneity ($Q_{\text{residual}, 691} = 2543.67$, $p < 0.001$) (Figure 7). Microplastic fibres, fragments,
338 leachates and spheres had significant negative effects (Figure 7). Effects driven by
339 microplastic fluff, leachates adsorbed onto microplastics, pellets, powders and squares were
340 not significant, although there were only 3 effect sizes of leachates adsorbed to particles, all
341 from one study (Gu et al. 2020). The most negative significant effect resulted from leachates
342 (no longer adsorbed onto microplastics) ($g = -0.93 [-1.35, -0.51]$, $p < 0.001$), followed by
343 fragments ($g = -0.70 [-1.14, -0.26]$, $p < 0.001$).

344

345 From all exposure conditions analysed (MPP concentration, size, shape, exposure duration
346 and polymer type), polymer type contributed the most to between-study heterogeneity

347 ($Q_{\text{moderators}, 19} = 68.93, p < 0.001$) (Figure S6). Polybrominated biphenyl ether had the most
348 negative significant effect ($g = -4.69 [-6.88, -2.51], p < 0.001$) (Figure S6).

349

350 4.0 DISCUSSION

351 4.1 BIOLOGICAL PROCESSES

352 This study offers the strongest and most consistent evidence to date of an overridingly
353 negative impact of microplastics on marine invertebrates. We found highly significant
354 negative effects of microplastics on the biological process rates of marine benthic fauna.
355 Every life stage was negatively impacted, with the strongest effects on early life stages,
356 especially embryos. There were negative impacts on six out of seven fundamental biological
357 processes including survival, development, reproduction, growth and feeding. Among
358 feeding habits, omnivores and deposit feeders were particularly hard hit. Our study differs
359 from previous reviews in that it documents substantially stronger and more consistently
360 negative impacts of microplastics on a much greater variety of animal life-processes. For
361 instance, Foley et al. (2018) described more neutral than negative effects of microplastics on
362 growth, consumption, reproduction on the survival of fishes and aquatic invertebrates.
363 Previous studies differed in focal organisms from the present study by including freshwater
364 species or fishes (Foley et al. 2018, Salerno et al. 2021, Berlino et al. 2021). Yet, the primary
365 cause for greater predominance of negative impact in the present meta-analysis is likely that
366 the rapid increase in experimental studies over the past two years has offered greater
367 statistical power for detecting the impacts of microplastics on marine animals; the present
368 study synthesised data from 72 studies compared to 41 studies in the most recent previous
369 review (Berlino et al. 2021). Certainly, the documentation of negative impacts has become
370 more frequent in recent reviews (Foley et al. 2018, Salerno et al. 2020, Berlino et al. 2021).

371 Our findings of stronger impacts on benthic organisms compared to pelagic and freshwater
372 organisms emphasises the need to improve research efforts in this area.

373

374 The reveal that multiple organismal processes and traits are affected by plastics is not
375 surprising. The biological rates of an organism are intrinsically linked and it is unlikely that
376 the effects of microplastics would act independently on each of these. Figure 8 explores this
377 principle of interlinkages: commencing with the process of feeding, which can be impacted
378 by microplastics as a result of intestinal blockages, false sense of fullness or confusion with
379 prey (Cole et al. 2011), reduced feeding will limit energy availability for morphological
380 change, gonad development and movement. The suppression of feeding indirectly affects
381 somatic growth, development and reproduction (Foley et al. 2018, Salerno et al. 2021), in
382 addition to direct cellular effects or other growth altering processes such as tissue
383 incorporation (Hierl et al. 2021). The observation that survival was significantly negatively
384 impacted indicates a synergistic effect of plastics on the organism as a whole, wherein the
385 impacts on different processes interact to create a larger combined effect than expected from
386 the sum of individual impacts (Figure 8). Energy was the only response not significantly
387 impacted by microplastic exposure, which may in part be due to the methodological
388 difficulties in ascribing effects on energetic processes as either positive or negative (Table
389 S5).

390

391 4.2 ORGANISM CHARACTERISTICS

392 Effects of microplastics on benthic taxonomic groups were universally negative, although not
393 significant for annelids and cnidarians. Across multiple taxonomic groups, a reduction in
394 growth was documented, most likely the result of reduced energy reserves as reported by
395 Wright et al. 2013. In that study, a range of exposure concentrations were used, up to 5%

396 sediment weight. This is likely to be higher than environmentally realistic concentrations of
397 microplastics, perhaps causing more extreme impacts. However, impacts on growth have
398 been seen more widely; previously, 58.8% of nematodes were shown to suffer energy loss
399 from consuming microplastic particles, particularly fibres (Hodgson 2018). Growth inhibition
400 may also have resulted from changes in cellular activity (Prinz & Korez 2020), for instance
401 through cellular modifications (e.g. penetration of microplastics into cell structures) and
402 oxidative stress, although this study focused on organismal level processes rather than
403 cellular. Further research into cellular level effects is therefore strongly recommended.

404

405 For several species the strength of impact can be explained by the life stage investigated,
406 although it was not possible to fully disentangle the effects of life stage from species through
407 meta-analysis. The effects of microplastics tends to increase with decrease in organismal size
408 (Salerno et al. 2021), with earlier life stages (gametes, embryos, larvae and juveniles) more
409 severely affected than adults, as recorded here. Thus, the strongest negative effects we
410 recorded were for the larvae of the sea urchin *Lytechinus variegatus*, where abnormal
411 development increased 58.1-66.5% after microplastic exposure (Nobre et al. 2015). Smaller
412 invertebrates are often numerous and crucial to bottom-up processes in natural ecosystems.
413 Their study is therefore particularly important to predicting the influences of plastic pollution
414 on whole-ecosystem functioning.

415

416 The severity of impact from plastics varied with feeding strategy. Omnivores and deposit
417 feeders were most greatly affected, with filter feeders experiencing weaker, but nonetheless
418 significant, negative impacts. Microplastic ingestion varies by feeding strategy (Bour et al.
419 2018, Naji et al. 2018), with 16% more microplastics ingested by predators and deposit
420 feeders compared to filter feeders (Bour et al. 2018). The greater ingestion of MPP by

421 predators in particular helps explain the larger negative impacts seen on this trophic group.
422 Our findings were in keeping with Berlino et al. (2021), which also found that benthic filter
423 feeders were negatively impacted by microplastics although, in the earlier study, omnivores,
424 predators and grazers were not. The strong effects on grazers in the present meta-analysis
425 likely resulted from high microplastic concentration on the sediment surface or, in
426 experimental conditions, on the tank floor. Microplastics naturally congregate on the
427 seafloor, with the majority of benthic microplastics found in the top 0.5cm sediment (Martin
428 et al. 2017), where grazers (and some omnivores) predominantly feed (Duchêne and
429 Rosenberg 2001). Strong effects of microplastics on predators and omnivores could result
430 from the trophic transfer of microplastics through the food chain, with microplastic fragments
431 being most prone to bioaccumulation (Gray & Weinstein 2017). The majority of our 72
432 studies, however, were short-term laboratory experiments, in which study organisms were
433 purchased from aquaria and exposed directly to microplastics, suggesting that trophic transfer
434 would not have influenced our results and demonstrating a need for more environmentally
435 realistic laboratory experiments.

436

437 4.3 MICROPLASTIC CHARACTERISTICS

438 While organismal characteristics were the primary causes for variation in microplastic
439 impact, microplastic shape and polymer type significantly contributed to variation in effect
440 size. We found no effect of microplastic size, exposure concentration and exposure duration,
441 despite individual studies documenting stronger negative impacts at higher exposures (Green
442 et al. 2016, Lo & Chan 2018). The recorded influence of polymer type conflicted with
443 findings of Lei et al. (2018), where the size of microplastic particle determined toxicity in
444 nematodes and zebrafish and the polymer composition was less important. However, polymer
445 type of a microplastic influences the specific density and hydrophobicity of a particle and

446 thus the biofouling and sinking rates (Kaiser et al. 2017). It is therefore logical that polymer
447 type will influence the availability of both the microplastic itself and its leachates to an
448 organism. In terms of shape, fragments and fibres had larger effects than spheres and squares,
449 potentially, in the case of fragments, due to sharp edges that cause damage following
450 ingestion (Pirsaheb et al. 2020). Fragments and fibres are likely to become the most prevalent
451 microplastics in marine ecosystems, already constituting 48.5% and 31%, respectively, of
452 microplastics in sediment and water (Kooi & Koelmans 2019). The high prevalence of
453 fragments and fibres in marine ecosystems makes the effects of these shapes, compared with
454 spheres, for example, far more environmentally realistic, suggesting that the strong negative
455 impacts of these particle shapes could have widespread implications for benthic ecosystems.
456

457 Microplastic dosage had less influence over impacts than microplastic shape or polymer type.
458 This may in part be due to the focus of meta-analytical techniques on average responses,
459 since the influence of microplastic concentration may be more pronounced at extreme values.
460 However, since extreme values are likely to be less environmentally realistic, we consider the
461 use of average values was not detrimental to our conclusions. Of the polymer types
462 investigated, microplastic leachates which had been separated from their microplastic
463 substrates had the strongest negative impacts on fauna. The impacts of leachates on benthic
464 fauna have not been previously investigated by meta-analyses. Leachates included
465 contaminants such as persistent organic pollutants (POPs) which had adsorbed onto the
466 microplastic surface and later been separated, as well as chemicals which has leached directly
467 from the microplastic. Leachates had negative impacts on reproduction, development and
468 feeding of echinoderms. Leachate endocrine disruptor chemicals (EDCs) can alter hormone
469 production, causing issues such as reduced reproductive success (Gallo et al. 2018).
470 Microplastics with adsorbed benzo[a]pyrene and perfluorooctane sulfonic acid cause more

471 damage to gill tissues and digestive glands compared to non-contaminated microplastics
472 (O'Donovan et al. 2018). On a cellular level, changes to enzyme activity in gobies have been
473 seen following exposure to the antibiotic cefalexin (Fonte et al. 2016), while microplastic
474 associated polychlorinated biphenyls (PCBs) have been shown to contribute to effects such as
475 hepatic stress, tissue accumulation of chemicals, reduced feeding activity and increased
476 mortality (Besseling et al. 2013, Rochman et al. 2013, Herzke et al. 2016). Adsorbed
477 chemicals may therefore have contributed to the negative impacts on feeding activity found
478 by the present study.

479

480 4.4 DISTRIBUTION OF LITERATURE USED

481 There was a skew in the number of studies by geographic location and sampling taxa. Most
482 studies were published in Europe (49%) or Asia (24%), with Africa, North America and
483 South America somewhat underrepresented, resulting in a lack of knowledge surrounding
484 native and commercially important species in these regions. The majority of studies analysed
485 were conducted on molluscs that had relevance to human food supply, usually commercially
486 important bivalve species such as the blue mussel, *Mytilus edulis*. For a comprehensive
487 overview to be representative of global impacts, funding should be directed towards
488 addressing the knowledge gaps surrounding continents such as Africa and less commercially
489 important organisms such as polychaetes, for which there is a lack of data. The numbers of
490 relevant studies are increasing rapidly, indicating an opportunity for these knowledge gaps to
491 be filled. Crucially, for findings to be truly comparable there is a need for standardisation of
492 sampling methodology and units of expression, a point widely made in past papers (Hermsen
493 et al. 2016, Miller et al. 2017, Ajith et al. 2020).

494 5.0 CONCLUSIONS

- 495 • Microplastic exposure has significant negative impact on multiple biological
496 processes of marine benthic fauna assessed.
- 497 • This study provides the first meta-analytical evidence that microplastic leachates have
498 more severe impacts on benthic fauna than microplastic particles themselves. Clearly,
499 microplastic management should consider the fate of microplastic already within the
500 marine system, alongside minimising further input.
- 501 • Significant knowledge gaps remain surrounding certain geographic regions and
502 species without commercial interest. Future research should be directed towards
503 addressing these gaps.
- 504 • A rapid increase in microplastic studies since 2019 caused this study to reveal
505 stronger and more consistently negative effects of microplastics than previous meta-
506 analyses. There is an undeniable and urgent call to address the microplastic crisis
507 within waste management systems globally.

508

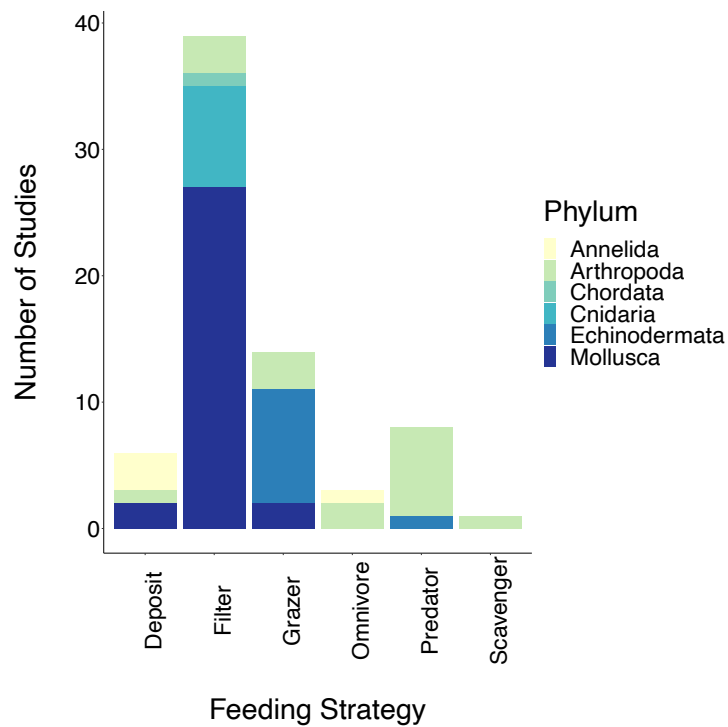
509

510 6.0 FIGURES AND TABLES WITH CAPTIONS

511 **Table 1.** *Heterogeneity of effect sizes of microplastics on marine benthic fauna, given as:*
 512 *Wald's Value (I^2), Cochran's value (Q), and the degrees of freedom (DF) and p-value*
 513 *pertaining to Cochran's Q .*

Process	I^2 (%)	Q	DF	p-value
All	61.4	2668.9	700	<0.001
Survival	75.2	658.3	72	<0.001
Feeding	74.4	368.5	102	<0.001
Development	59.4	278.4	131	<0.001
Reproduction	34.2	179.7	109	<0.001
Growth	47.0	602.2	158	<0.001
Energy	80.3	149.4	38	<0.001
Behaviour	73.9	304.2	84	<0.001

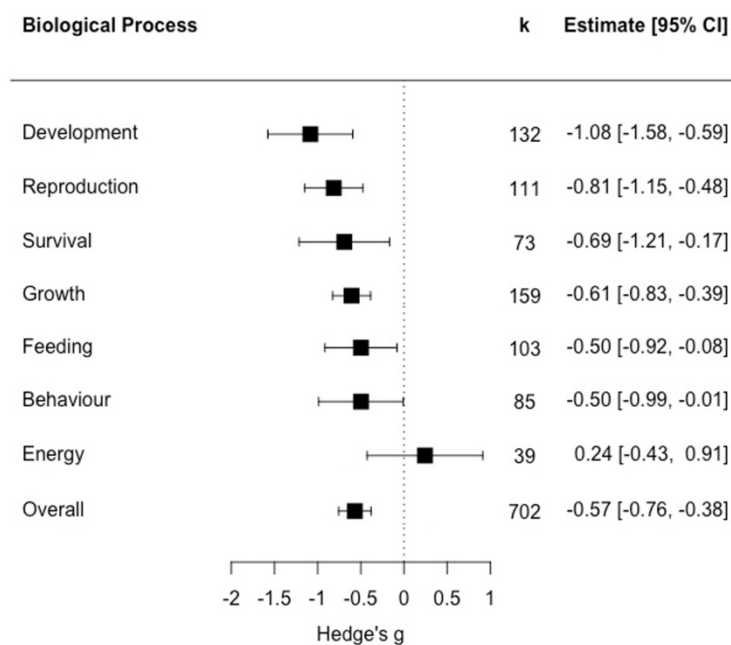
514



515

516 *Figure 1. The frequency of animal feeding strategy by phylum used in 72 experimental*

517 *studies.*



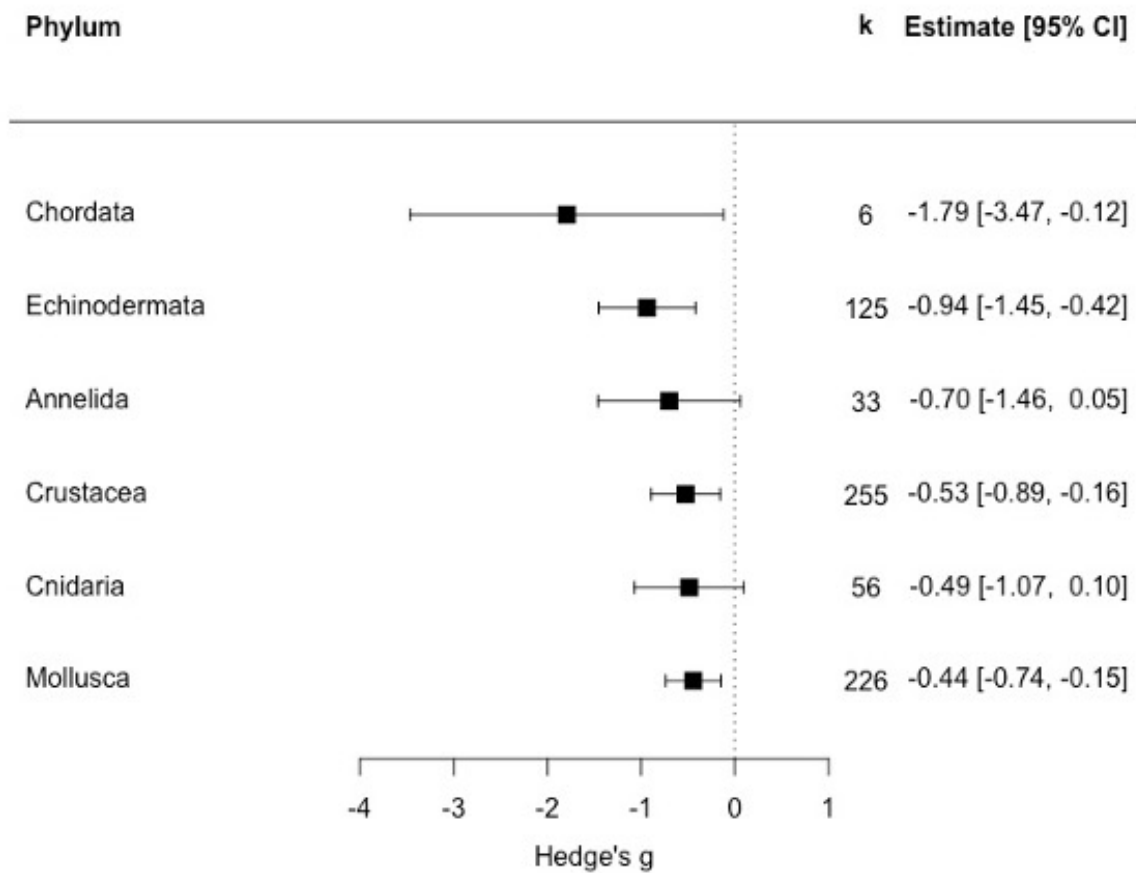
518

519 *Figure 2. The effects of microplastic exposure on biological processes of marine benthic*

520 *fauna. Effects on each of 7 processes and overall, as indicated from random-effects*

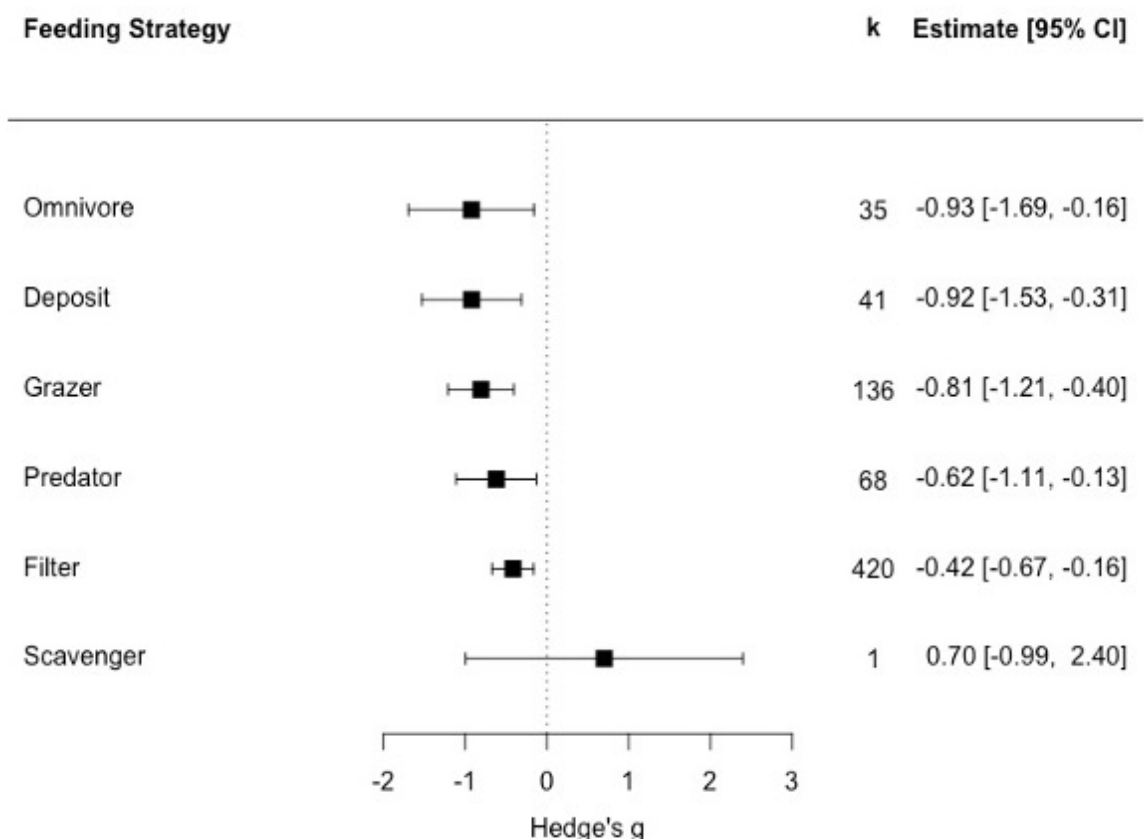
521 *modelling. Boxes and error bars represent pooled Hedge's g values and 95% confidence*

522 intervals, respectively. K represents the number of case studies, or independent experiments
 523 within the same study. Overlap of confidence intervals with 0 indicate non-significance.



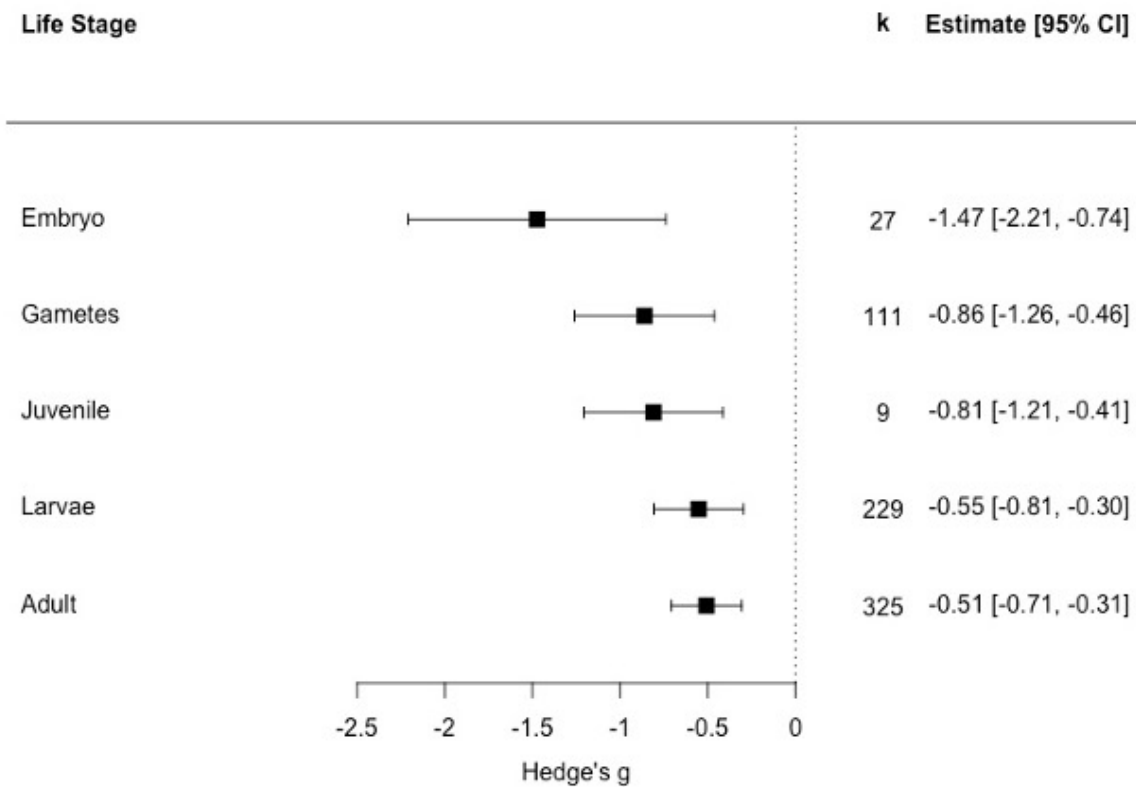
524

525 **Figure 3. The effects of microplastic exposure on phyla of marine benthic fauna.** Effects on
 526 each of 6 phyla as indicated from mixed effects modelling. Boxes and error bars represent
 527 pooled Hedge's g values and 95% confidence intervals, respectively. K represents the
 528 number of case studies.



529

530 **Figure 4. The effects of microplastic exposure on feeding strategies of marine benthic**
 531 **fauna.** Effects on each of 6 feeding strategies, as indicated from mixed-effects modelling.
 532 Boxes and error bars represent pooled Hedge's g values and 95% confidence intervals,
 533 respectively. K represents the number of case studies.



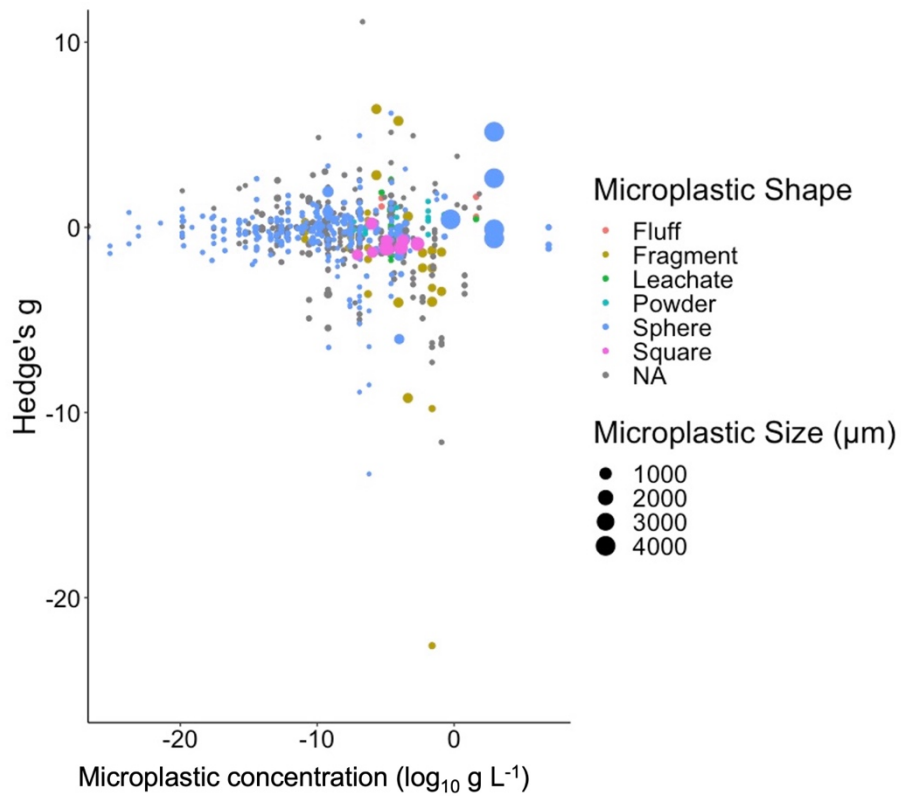
534

535 ***Figure 5. The effects of microplastic exposure on life stages of marine benthic fauna.***

536 *Effects on each of 5 life stages as indicated from mixed-effects modelling. Boxes and error*

537 *bars represent pooled Hedge's g values and 95% confidence intervals, respectively. K*

538 *represents the number of case studies.*

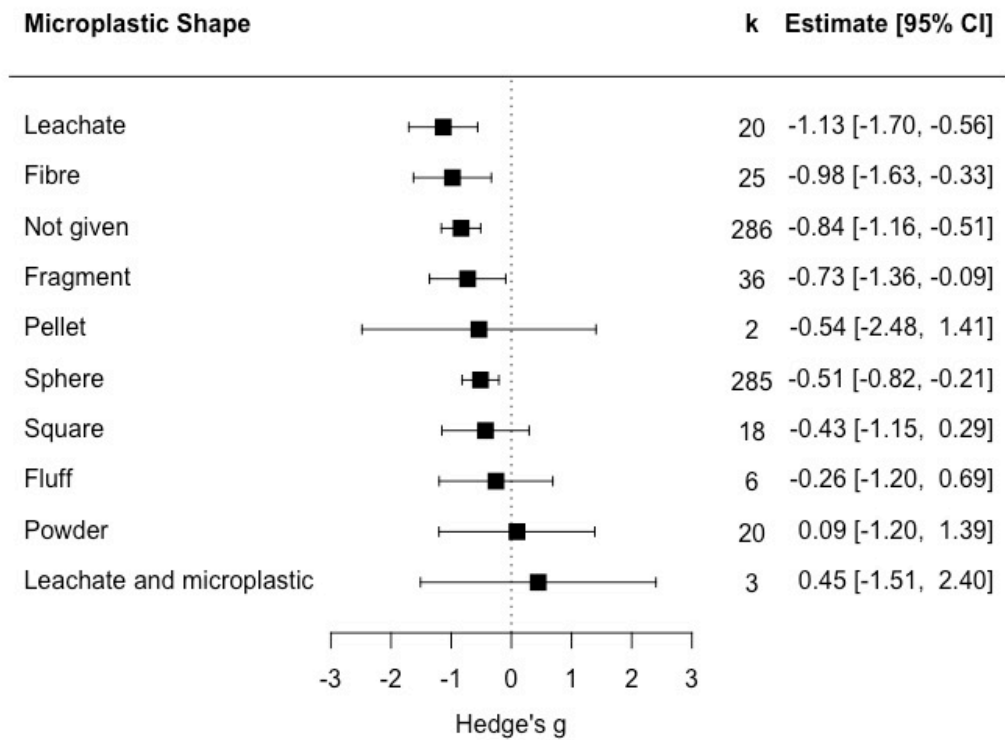


539

540 *Figure 6. Effect of microplastic exposure concentration on the biological processes of*

541 *marine benthos. Effect size indicated by Hedge's g value. Point size is indicative of*

542 *microplastic particle size, while colour represents the shape of the particle.*



543

544 **Figure 7. Responses of benthic fauna to the shape of microplastics used by experiments.**

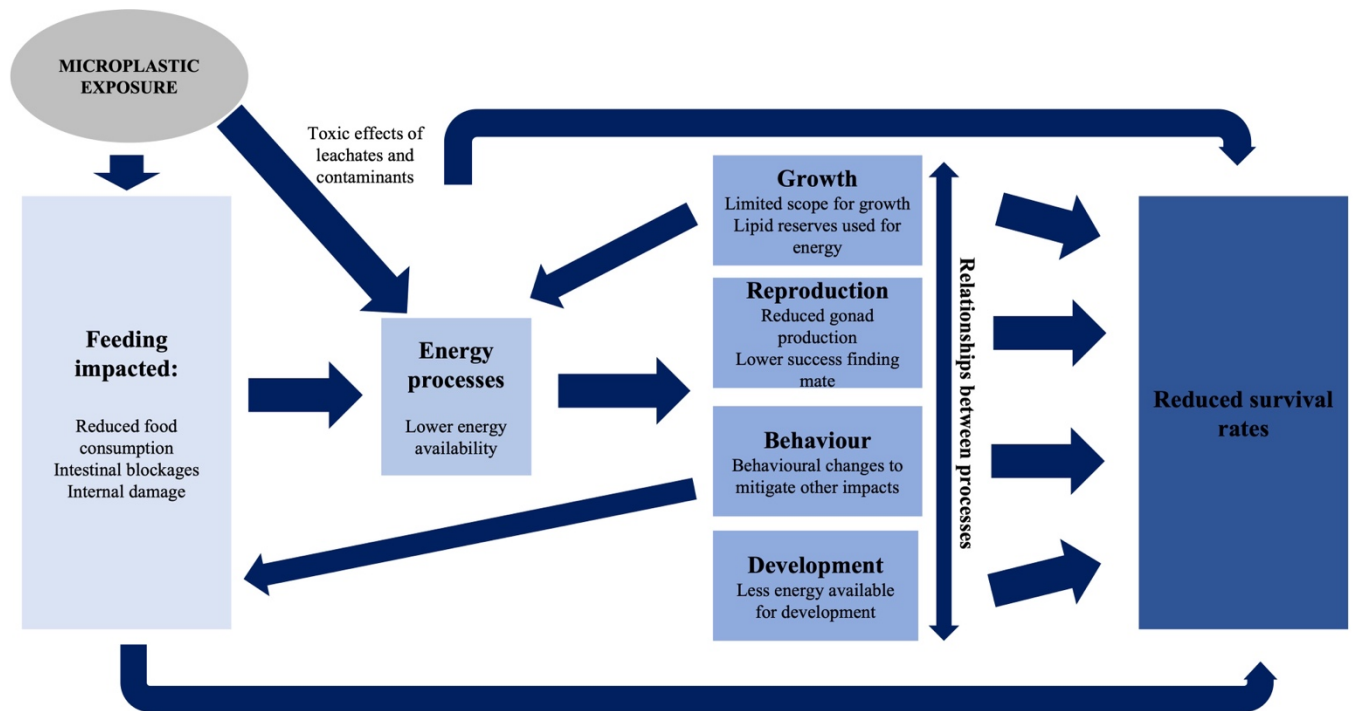
545 Responses indicated from mixed effects modelling. Boxes and error bars represent pooled

546 Hedge's g values and 95% confidence intervals, respectively. K represents the number of

547 case studies. 'Leachate and microplastic' refers to microplastic particles with adsorped

548 leachates, while 'leachate' refers to leachate which is not adsorped to a particle.

549



550

551 **Figure 8. Interactions of impacts on different biological processes of marine benthic**

552 **fauna, as a result of microplastic exposure. Interactions demonstrated by arrows,**

553 **culminating in a synergistic effect and overall reduction in survival rate.**

554 7.0 ACKNOWLEDGEMENTS

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562

563 8.0 REFERENCES

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Appendix: Supplementary Materials

Microplastics alter multiple biological processes of marine benthic fauna

Running page head: Microplastic impacts on benthic fauna.

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OVERVIEW OF CONTENT:

A systematic review and meta-analysis were conducted to quantify the impacts of microplastics on the biological processes (Table S1) of marine benthic fauna (Figure S1). The influence of organism and microplastic characteristics were also investigated. Search terms for the systematic review were scoped using 9 test searches, where the relevance of hits was evaluated based on the inclusion of 10 pre-determined key reference studies (Table S2). Studies were then screened by title, abstract and full text to produce a final list of 72 publications (Table S3). Data were extracted from the final 72 studies (Table S4). Reference numbers were recorded and included for each study to allow tracing through the stages and identification of any replicate studies. Hedge's g value was calculated to quantify the effect size in each study, using the data extracted (Table S4). The directionality of effect was changed from positive to negative for study results where an increase in a response variable represented a negative impact on the organism (Table S5). Number of studies published over

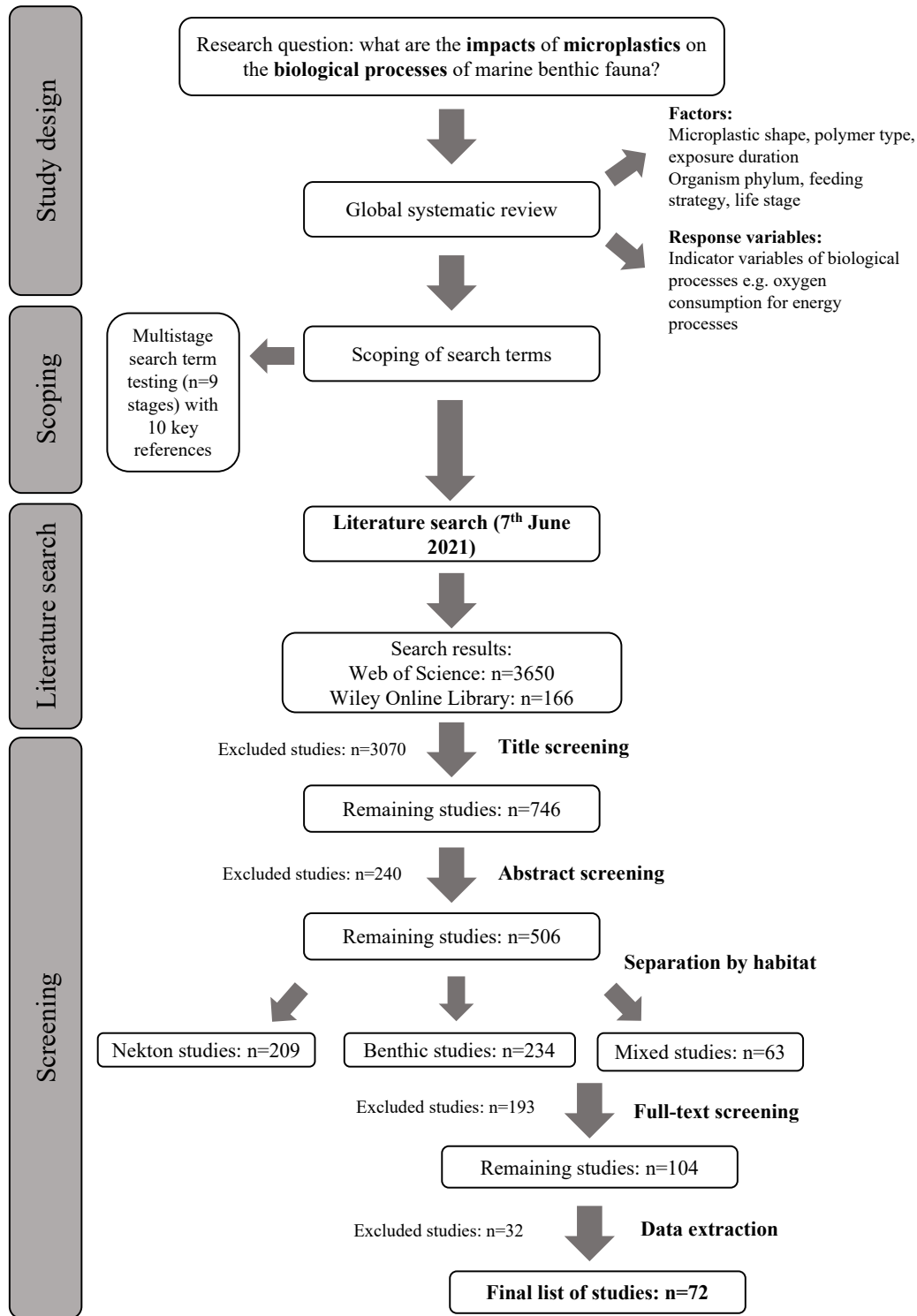
26 time and by region were plotted to visualise the distribution of the data temporally and
27 spatially (Figure S2, S3). The potential effect of publication bias was assessed using the ‘trim
28 and fill’ method (Duval and Tweedie 2000), with the results shown in Table S6. Adjusting
29 the estimated pooled effect size in our study had little effect on the overall outcome and
30 indicated that publication bias was likely to have had a negligible effect on our results.
31 Random effects modelling was then used to analyse the influence of drivers such as phylum,
32 life stage and microplastic exposure characteristics. The most significant results were found
33 from phylum, feeding strategy, microplastic duration, shape and polymer type, as outlined in
34 the main text. Further, less significant results such as the influence of microplastic size and
35 duration were included in these supplementary materials (Figure S4), as well as a sub-group
36 relationships of effect size in each taxonomic group with microplastic exposure concentration
37 (Figure S5). Effect sizes for exposure to different polymer types are shown in Figure S6.
38

39 1.0 INTRODUCTORY TABLES

40 **Table S1.** *Biological rates used in this study, with trait type, indicator variables and source.*

41 *Based on definitions by Violle et al. (2007).*

Biological rate	Definition	Examples of indicator variables
Survival	Number of individuals surviving over time with exposure to microplastic treatment	Mortality rate, survival rate, number/% of live individuals
Growth	Physical increase in body size of an organism (somatic growth)	Somatic growth rate, length increase, weight increase
Reproduction	Ability of an organism to successfully produce viable young	Reproductive success, % live young, sperm velocity, oocyte number, fecundity
Development	The development of specific body parts or progression of an organism through life stages	% normal development, % larval abnormalities, development time, segment regeneration time
Behaviour	Characteristics of organism behaviour relating to movement, boldness and activity	Righting time, byssal thread production, cirral beating frequency, swimming speed
Feeding	Ability of an organism to successfully consume food sources or capture prey	Prey consumption rate, algal clearance rate, % ingestion success
Energy consumption	Processes involving the generation of energy in an organism, usually respiration	Respiration rate (oxygen consumption), energy consumption



44

45 **Figure S1.** Flow chart depicting study design and methodology of the present study through
 46 the scoping literature search and screening processes. One scoping stage refers to one test
 47 search of the search string.

48 **Table S2.** Key references used when scoping potential search terms to assess for relevance of
 49 results. Studies given with author, publication date, study organism and the number of
 50 citations. Number of citations as given by Web of Science on 27th May 2021 (benthic studies)
 51 and 4th June 2021 (nekton studies). Studies selected for relevance, range of study organisms
 52 and number of citations.

	Authors	Year of Publication	Study Organism	Number of Citations
Benthic	Murray and Cowie	2011	<i>Nephrops norvegicus</i>	448
	Farrell and Nelson	2013	<i>Mytilus edulis</i>	569
	Setälä et al.	2014	<i>Macoma balthica</i>	149
			<i>Mytilus trossolus</i>	
			<i>Gammarus</i> spp.	
			<i>Mysid</i> shrimps	
			<i>Monoporeia affinis</i>	
			<i>Marenzelleria</i> spp.	
	Van Cauwenberghe and Janssen	2014	<i>Crassostrea gigas</i>	653
			<i>Mytilus edulis</i>	
	Van Cauwenberghe et al.	2015	<i>Mytilus edulis</i>	429
			<i>Arenicola marina</i>	
Nekton	Bourdages et al.	2020	Seals (range)	6
	Egbeocha et al.	2018	Range	20
	Hu et al.	2020	<i>Oryzias latipes</i>	7
	Le Bihanic et al.	2020	<i>Oryzias melastigma</i>	12
	Critchell and Hoogenboom	2018	<i>Acanthochromis polyacanthus</i>	66

54 **Table S3.** Final list of papers (n=72) from which data were extracted for meta-analysis,
 55 following title, abstract and full text screening. Papers are given with reference number from
 56 the original search results (7th June 2021), title, authors, publication year and DOI.

Ref No	Authors	Article Title	Year	DOI	Number of Observations
11	Berry, KLE; Epstein, HE; Lewis, PJ; Hall, NM; Negri, AP	Microplastic Contamination Has Limited Effects on Coral Fertilisation and Larvae	2019	10.3390/d11120228	30
21	Reichert, J; Arnold, AL; Hoogenboom, MO; Schubert, P; Wilke, T	Impacts of microplastics on growth and health of hermatypic corals are species-specific	2019	10.1016/j.envpol.2019.113074	4
29	Horn, DA; Granek, EF; Steele, CL	Effects of environmentally relevant concentrations of microplastic fibers on Pacific mole crab (<i>Emerita analoga</i>) mortality and reproduction	2020	10.1002/lo12.10137	2
31	Seuront, I	Microplastic leachates impair behavioural vigilance and predator avoidance in a temperate intertidal gastropod	2018	10.1098/rsbl.2018.0453	2
43	Tosetto, I; Brown, C; Williamson, JE	Microplastics on beaches: ingestion and behavioural consequences for beachhoppers	2016	10.1007/s00227-016-2973-0	3
58	Crump, A; Mullens, C; Bethell, EJ; Cunningham, EM; Arnott, G	Microplastics disrupt hermit crab shell selection	2020	10.1098/rsbl.2020.0030	1
69	Santana, MFM; Moreira, FT; Pereira, CDS; Abessa, DMS; Turra, A	Continuous Exposure to Microplastics Does Not Cause Physiological Effects in the Cultivated Mussel <i>Perna perna</i>	2018	10.1007/s00244-018-0504-3	2

93	Corinaldesi, C; Canensi, S; Dell'Anno, A; Tangherlini, M; Di Capua, I; Varrella, S; Willis, TJ; Cerrano, C; Danovaro, R	Multiple impacts of microplastics can threaten marine habitat-forming species	2021	10.1038/s420 03-021- 01961-1	6
108	Sussarellu, R; Suquet, M; Thomas, Y; Lambert, C; Fabioux, C; Pernet, MEJ; Le Goic, N; Quillien, V; Mingant, C; Epelboin, Y; Corporeau, C; Guyomarch, J; Robbens, J; Paul-Pont, I; Soudant, P; Huvet, A	Oyster reproduction is affected by exposure to polystyrene microplastics	2016	10.1073/pnas. 1519019113	1
110	Torn, K	Microplastics uptake and accumulation in the digestive system of the mud crab <i>Rhithropanopeus harrisi</i>	2020	10.3176/proc. 2020.1.04	2
161	Yu, P; Liu, ZQ; Wu, DL; Chen, MH; Lv, WW; Zhao, YL	Accumulation of polystyrene microplastics in juvenile <i>Eriocheir</i> <i>sinensis</i> and oxidative stress effects in the liver	2018	10.1016/j.aqu atox.2018.04. 015	4
239	Seuront, I; Nicastro, KR; McQuaid, CD; Zardi, GI	Microplastic leachates induce species- specific trait strengthening in intertidal mussels	2021	10.1002/eap.2 222	4
252	Welden, NAC; Cowie, PR	Long-term microplastic retention causes reduced body condition in the langoustine, <i>Nephrops norvegicus</i>	2016	10.1016/j.env pol.2016.08.0 20	2
254	Xu, XY; Lee, WT; Chan, AKY; Lo, HS; Shin, PKS; Cheung, SG	Microplastic ingestion reduces energy intake in the clam <i>Atactodea striata</i>	2017	10.1016/j.mar polbul.2016.1 2.027	6

266	Kaposi, KL; Mos, B; Kelaher, BP; Dworjanyn, SA	Ingestion of Microplastic Has Limited Impact on a Marine Larva	2014	10.1021/es404295e	8
289	Green, DS; Boots, B; Sigwart, J; Jiang, S; Rocha, C	Effects of conventional and biodegradable microplastics on a marine ecosystem engineer (<i>Arenicola marina</i>) and sediment nutrient cycling	2016b	10.1016/j.envpol.2015.10.010	18
291	Mouchi, V; Chapron, I; Peru, E; Pruski, AM; Meistertzheim, AL; Vétion, G; Galand, PE; Lartaud, F	Long-term aquaria study suggests species-specific responses of two cold-water corals to macro-and microplastics exposure	2019	10.1016/j.envpol.2019.07.024	4
303	Green, DS; Colgan, TJ; Thompson, RC; Carolan, JC	Exposure to microplastics reduces attachment strength and alters the haemolymph proteome of blue mussels (<i>Mytilus edulis</i>)	2019	10.1016/j.envpol.2018.12.017	2
314	Opitz, T; Benitez, S; Fernandez, C; Osores, S; Navarro, JM; Rodriguez-Romero, A; Lohrmann, KB; Lardies, MA	Minimal impact at current environmental concentrations of microplastics on energy balance and physiological rates of the giant mussel <i>Choromytilus chorus</i>	2021	10.1016/j.marpolbul.2020.111834	6
360	Besseling, E; Wegner, A; Foekema, EM; van den Heuvel-Greve, MJ; Koelmans, AA	Effects of Microplastic on Fitness and PCB Bioaccumulation by the Lugworm <i>Arenicola marina</i> (L.)	2013	10.1021/es302763x	6
402	Gambardella, C; Morgana, S; Bramini, M; Rotini, A; Manfra, I; Migliore, I; Piazza, V; Garaventa, F; Faimali, M	Ecotoxicological effects of polystyrene microbeads in a battery of marine organisms belonging to different trophic levels	2018	10.1016/j.marenvres.2018.09.023	3

532	Silva, PPGE; Nobre, CR; Resaffe, P; Pereira, CDS; Gusmao, F	Leachate from microplastics impairs larval development in brown mussels	2016	10.1016/j.watres.2016.10.016	3
562	Woods, MN; Hong, TJ; Baughman, D; Andrews, G; Fields, DM; Matrai, PA	Accumulation and effects of microplastic fibers in American lobster larvae (<i>Homarus americanus</i>)	2020	10.1016/j.marpolbul.2020.11280	3
570	Leung, J; Chan, KYK	Microplastics reduced posterior segment regeneration rate of the polychaete <i>Perinereis aibuhitensis</i>	2018	10.1016/j.marpolbul.2017.10.072	5
586	Webb, S; Gaw, S; Marsden, ID; Mcrae, NK	Biomarker responses in New Zealand green-lipped mussels <i>Perna canaliculus</i> exposed to microplastics and triclosan	2020	10.1016/j.ecoenv.2020.110871	4
588	Hankins, C; Moso, E; Lasseigne, D	Microplastics impair growth in two atlantic scleractinian coral species, <i>Pseudodiploria clivosa</i> and <i>Acropora cervicornis</i>	2021	10.1016/j.envpol.2021.116649	2
591	Trifuoggi, M; Pagano, G; Oral, R; Pavicic-Hamer, D; Buric, P; Kovacic, I; Siciliano, A; Toscanesi, M; Thomas, PJ; Paduano, I; Guida, M; Lyons, DM	Microplastic-induced damage in early embryonal development of sea urchin <i>Sphaerechinus granularis</i>	2019	10.1016/j.envres.2019.108815	15
621	Yap, VHS; Chase, Z; Wright, JT; Hurd, CL; Lavers, JL; Lenz, M	A comparison with natural particles reveals a small specific effect of PVC microplastics on mussel performance	2020	10.1016/j.marpolbul.2020.11703	18
662	Cole, M; Galloway, TS	Ingestion of Nanoplastics and Microplastics by Pacific Oyster Larvae	2015	10.1021/acs.est.5b04099	10

676	Luan, LP; Wang, X; Zheng, H; Liu, LQ; Luo, XX; Li, FM	Differential toxicity of functionalized polystyrene microplastics to clams (<i>Meretrix meretrix</i>) at three key development stages of life history	2019	10.1016/j.mar polbul.2019.0 1.003	26
717	Missawi, O; Bousserhine, N; Zitouni, N; Maisano, M; Boughattas, I; De Marco, G; Cappello, T; Belbekhouche, S; Guerrouache, M; Alphonse, V; Banni, M	Uptake, accumulation and associated cellular alterations of environmental samples of microplastics in the seaworm <i>Hediste diversicolor</i>	2021	10.1016/j.jhaz mat.2020.124 287	4
721	Rist, SE; Assidqi, K; Zamani, NP; Appel, D; Perschke, M; Huhn, M; Lenz, M	Suspended micro-sized PVC particles impair the performance and decrease survival in the Asian green mussel <i>Perna viridis</i>	2016	10.1016/j.mar polbul.2016.0 7.006	11
729	Nobre, CR; Santana, MFM; Maluf, A; Cortez, FS; Cesar, A; Pereira, CDS; Turra, A	Assessment of microplastic toxicity to embryonic development of the sea urchin <i>Lytechinus variegatus</i> (Echinodermata: Echinoidea)	2015	10.1016/j.mar polbul.2014.1 2.050	2
766	Leads, RR; Burnett, KG; Weinstein, JE	The Effect of Microplastic Ingestion on Survival of the Grass Shrimp <i>Palaemonetes pugio</i> (Holthuis, 1949) Challenged with <i>Vibrio campbellii</i>	2019	10.1002/etc.4 545	5
776	Green, DS	Effects of microplastics on European flat oysters, <i>Ostrea edulis</i> and their associated benthic communities	2016a	10.1016/j.env pol.2016.05.0 43	10
787	Wang, X; Liu, LQQ; Zheng, H; Wang, MX; Fu, YX; Luo, XX; Li, FM; Wang, ZY	Polystyrene microplastics impaired the feeding and swimming behavior of mysid shrimp <i>Neomysis japonica</i>	2020	10.1016/j.mar polbul.2019.1 10660	24

791	Rist, S; Baun, A; Almeda, R; Hartmann, NB	Ingestion and effects of micro- and nanoplastics in blue mussel (<i>Mytilus edulis</i>) larvae	2019	10.1016/j.mar polbul.2019.0 1.069	12
802	Tallec, K; Huvet, A; Di Poi, C; Gonzalez-Fernandez, C; Lambert, C; Petton, B; Le Goic, N; Berchel, M; Soudant, P; Paul-Pont, I	Nanoplastics impaired oyster free living stages, gametes and embryos	2018	10.1016/j.env pol.2018.08.0 20	16
812	Carrasco, A; Pulgar, J; Quintanilla-Ahumada, D; Perez-Venegas, D; Quijon, PA; Duarte, C	The influence of microplastics pollution on the feeding behavior of a prominent sandy beach amphipod, <i>Orchestoidea tuberculata</i> (Nicolet, 1849)	2019	10.1016/j.mar polbul.2019.0 5.018	2
827	Syakti, AD; Jaya, JV; Rahman, A; Hidayati, NV; Raza'i, TS; Idris, F; Trenggono, M; Doumenq, P; Chou, LM	Bleaching and necrosis of staghorn coral (<i>Acropora formosa</i>) in laboratory assays: Immediate impact of LDPE microplastics	2019	10.1016/j.che mosphere.201 9.04.156	5
881	Bertucci, JI; Bellas, J	Combined effect of microplastics and global warming factors on early growth and development of the sea urchin (<i>Paracentrotus lividus</i>)	2021	10.1016/j.scit otenv.2021.14 6888	2
930	Watts, AJR; Urbina, MA; Corr, S; Lewis, C; Galloway, TS	Ingestion of Plastic Microfibers by the Crab <i>Carcinus maenas</i> and Its Effect on Food Consumption and Energy Balance	2015	10.1021/acs.e st.5b04026	3
971	Capolupo, M; Franzellitti, S; Valbonesi, P; Lanzas, CS; Fabbri, E	Uptake and transcriptional effects of polystyrene microplastics in larval stages of the Mediterranean mussel <i>Mytilus galloprovincialis</i>	2018	10.1016/j.env pol.2018.06.0 35	6

1036	Urban-Malinga, B; Jakubowska, M; Bialowas, M	Response of sediment-dwelling bivalves to microplastics and its potential implications for benthic processes	2021	10.1016/j.scitotenv.2020.144302	5
1124	Gardon, T; Reisser, C; Soyez, C; Quillien, V; Le Moullac, G	Microplastics Affect Energy Balance and Gametogenesis in the Pearl Oyster <i>Pinctada margaritifera</i>	2018	10.1021/acs.est.8b00168	6
1132	Mohsen, M; Zhang, LB; Sun, LN; Lin, CG; Wang, Q; Liu, SL; Sun, JC; Yang, HS	Effect of chronic exposure to microplastic fibre ingestion in the sea cucumber <i>Apostichopus japonicus</i>	2021	10.1016/j.env.2020.111794	6
1209	Detree, C; Gallardo-Escarate, C	Single and repetitive microplastics exposures induce immune system modulation and homeostasis alteration in the edible mussel <i>Mytilus galloprovincialis</i>	2018	10.1016/j.fsi.2018.09.018	1
1224	Thomas, PJ; Oral, R; Pagano, G; Tez, S; Toscanesi, M; Ranieri, P; Trifuoggi, M; Lyons, DM	Mild toxicity of polystyrene and polymethylmethacrylate microplastics in <i>Paracentrotus lividus</i> early life stages	2020	10.1016/j.envres.2020.105132	58
1247	Mendrik, FM; Henry, TB; Burdett, H; Hackney, CR; Waller, C; Parsons, DR; Hennige, SJ	Species-specific impact of microplastics on coral physiology	2021	10.1016/j.pol.2020.116238	2
1321	Sikdokur, E; Belivermis, M; Sezer, N; Pekmez, M; Bulan, OK; Kilic, O	Effects of microplastics and mercury on manila clam <i>Ruditapes philippinarum</i> : Feeding rate, immunomodulation, histopathology and oxidative stress	2020	10.1016/j.pol.2020.114247	2

1362	Green, DS; Boots, B; O'Connor, NE; Thompson, R	Microplastics Affect the Ecological Functioning of an Important Biogenic Habitat	2017	10.1021/acs.est.6b04496	8
1393	Wang, SX; Zhong, Z; Li, ZQ; Wang, XH; Gu, HX; Huang, W; Fang, JKH; Shi, HH; Hu, MH; Wang, YJ	Physiological effects of plastic particles on mussels are mediated by food presence	2021	10.1016/j.jhazmat.2020.124136	4
1441	Anderson, G; Shenkar, N	Potential effects of biodegradable single-use items in the sea: Polylactic acid (PLA) and solitary ascidians	2021	10.1016/j.envpol.2020.115364	6
1457	Gonzalez-Soto, N; Hatfield, J; Katsumiti, A; Duroudier, N; Lacave, JM; Bilbao, E; Orbea, A; Navarro, E; Cajaraville, MP	Impacts of dietary exposure to different sized polystyrene microplastics alone and with sorbed benzo[a]pyrene on biomarkers and whole organism responses in mussels <i>Mytilus galloprovincialis</i>	2019	10.1016/j.scitotenv.2019.05161	8
1474	Hope, JA; Coco, G; Thrush, SF	Effects of Polyester Microfibers on Microphytobenthos and Sediment-Dwelling Infauna	2020	10.1021/acs.est.0c00514	3
1479	Martinez-Gomez, C; Leon, VM; Calles, S; Gomariz-Olcina, M; Vethaak, AD	The adverse effects of virgin microplastics on the fertilization and larval development of sea urchins	2017	10.1016/j.marenvres.2017.06.016	24
1506	Gu, HX; Wei, SS; Hu, MH; Wei, H; Wang, XH; Shang, YY; Li, LA; Shi, HH; Wang, YJ	Microplastics aggravate the adverse effects of BDE-47 on physiological and defense performance in mussels	2020	10.1016/j.jhazmat.2020.122909	7
1539	Suckling, CC	Responses to environmentally relevant microplastics are species-specific with	2021	10.1016/j.scitotenv.2020.142341	4

		dietary habit as a potential sensitivity indicator			
1590	Diana, Z; Sawickij, N; Rivera, NA; Hsu-Kim, H; Rittschof, D	Plastic pellets trigger feeding responses in sea anemones	2020	10.1016/j.aquatox.2020.105447	3
60	Korez, S; Gutow, I; Saborowski, R	Feeding and digestion of the marine isopod <i>Idotea emarginata</i> challenged by poor food quality and microplastics	2019	10.1016/j.cbpc.2019.108586	1
79	Yu, SP; Chan, BKK	Effects of polystyrene microplastics on larval development, settlement, and metamorphosis of the intertidal barnacle <i>Amphibalanus amphitrite</i>	2020b	10.1016/j.ecoenv.2020.110362	47
83	Bruck, S; Ford, AT	Chronic ingestion of polystyrene microparticles in low doses has no effect on food consumption and growth to the intertidal amphipod <i>Echinogammarus marinus</i> ?	2018	10.1016/j.envpol.2017.10.015	6
181	Lo, HKA; Chan, KYK	Negative effects of microplastic exposure on growth and development of <i>Crepidula onyx</i>	2018	10.1016/j.envpol.2017.10.095	8
342	Yu, SP; Chan, BKK	Intergenerational microplastics impact the intertidal barnacle <i>Amphibalanus amphitrite</i> during the planktonic larval and benthic adult stages	2020c	10.1016/j.envpol.2020.115560	96
392	Van Colen, C; Vanhove, B; Diem, A; Moens, T	Does microplastic ingestion by zooplankton affect predator-prey interactions? An experimental study on larviphagy	2020	10.1016/j.envpol.2019.113479	9

1010	Bringer, A; Thomas, H; Prunier, G; Dubillot, E; Bossut, N; Churlaud, C; Clerandeanu, C; Le Bihanic, F; Cachot, J	High density polyethylene (HDPE) microplastics impair development and swimming activity of Pacific oyster D-larvae, <i>Crassostrea gigas</i> , depending on particle size	2020	10.1016/j.envpol.2020.113978	15
1028	Bringer, A; Cachot, J; Prunier, G; Dubillot, E; Clerandeanu, C; Thomas, H	Experimental ingestion of fluorescent microplastics by pacific oysters, <i>Crassostrea gigas</i> , and their effects on the behaviour and development at early stages	2020	10.1016/j.chemosphere.2020.126793	9
1153	Beiras, R; Bellas, J; Cachot, J; Cormier, B; Cousin, X; Engwall, M; Gambardella, C; Garaventa, F; Keiter, S; Le Bihanic, F; Lopez-Ibanez, S; Piazza, V; Rial, D; Tato, T; Vidal-Linan, I	Ingestion and contact with polyethylene microplastics does not cause acute toxicity on marine zooplankton	2018	10.1016/j.jhazmat.2018.07.101	3
1794	Beiras, R; Tato, T	Microplastics do not increase toxicity of a hydrophobic organic chemical to marine plankton	2019	10.1016/j.marpolbul.2018.11.029	2
13	Yu, J; Tian, JY; Xu, R; Zhang, ZY; Yang, GP; Wang, XD; Lai, JG; Chen, R	Effects of microplastics exposure on ingestion, fecundity, development, and dimethylsulfide production in <i>Tigriopus japonicus</i> (Harpacticoida, copepod)	2020a	10.1016/j.envpol.2020.115429	28
498	Lee, DH; Lee, S; Rhee, JS	Consistent exposure to microplastics induces age-specific physiological and biochemical changes in a marine mysid	2021	10.1016/j.marpolbul.2020.111850	20

508 Li, ZC; Zhou, H; Liu, Y; Zhan, JJ; Acute and chronic combined effect of 2020 10.1016/j.che 6
Li, WT; Yang, KM; Yi, XL polystyrene microplastics and dibutyl mosphere.202
phthalate on the marine copepod 0.127711
Tigriopus japonicus

58

59

60 **Table S4.** Data extracted from the final list of papers (n=72) for meta-analysis of the impacts
 61 of microplastics on the functional traits of marine benthic fauna, categorised by study
 62 identifiers, meta-data and data for quantitative synthesis.

Study Identifier	Meta-data	Data for quantitative synthesis
Reference number	Location (continent, country, region)	Biological rate indicator (e.g. growth rate, respiration rate):
Case study (a, b, c etc.)		
Author	Date of experiment	Control group (mean, standard deviation, number of replicates, units)
Publication Type	Study organism (phylum, species, life stage, feeding strategy)	Experimental group (mean, standard deviation, number of replicates, units)
Publication Year	Exposure conditions (duration, microplastic concentration, polymer type, microplastic shape, microplastic size, added contaminants)	

63 **Table S5.** Measured response variables of biological rates for which the units measured were
 64 converted from a positive to a negative value in this study (prior to calculation of effect size)
 65 in order to signify a negative impact on fauna. For example, mortality was measured as a
 66 positive value, but converted into a negative value as it was deemed negative for the animal.

Biological rate	Study	Year	Measured response and units
Survival	Wang et al.	2020	Mortality (%)
	Lo and Chan	2018	Mortality (individuals day ⁻¹)

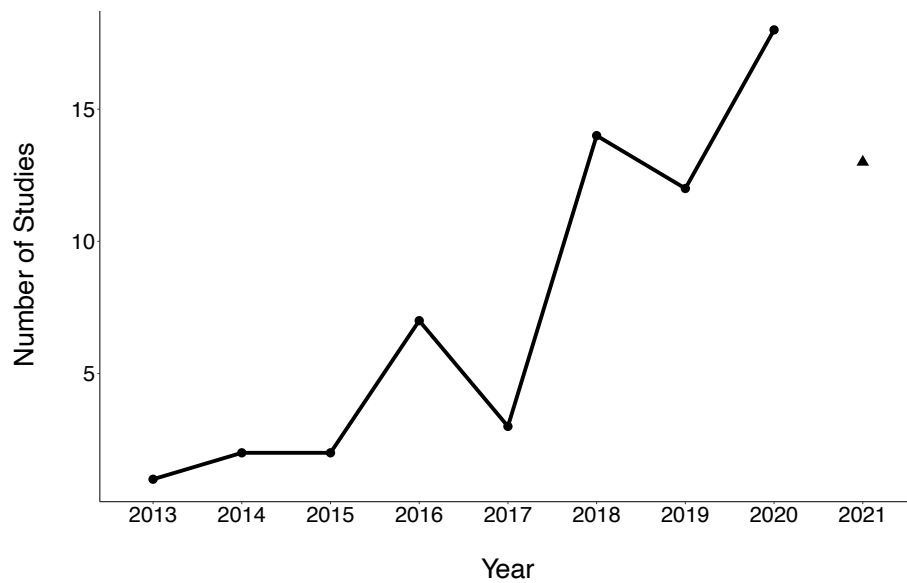
Development	Berry et al.	2019	Embryo abnormality (%)
	Gandara e Silva et al.	2016	Abnormal larvae (%)
	Rist et al.	2019	Malformations (individuals/10)
	Thomas et al.	2020	Developmental defects (%)
	Martínez-Gómez et al.	2017	Abnormality (%)
	Bringer et al.	2020	Larval abnormalities (%)
	Yu et al.	2020	Development time (days)
Behaviour	Seuront	2018	Righting time (minutes)
	Crump et al.	2020	Time to enter shell (seconds)
	Gambardella et al.	2018	Swimming speed change (%)
	Hope et al.	2020	Burial time (hours)
	Suckling	2021	Righting time (seconds)
Growth	Wang et al.	2020	Growth inhibition (%)

68 3.0 RESULTS

69 **Table S6.** Results of testing for publication bias using the ‘trim and fill’ method on rma.uni
 70 model. Result indicates assessment of balance of positive and negative effect size studies.
 71 Estimated effect size (**in bold**) indicates overall pooled Hedge’s g effect size of microplastics
 72 on biological processes of benthic fauna, with Hedge’s g adjusted for potential publication
 73 bias (trim and fill) and with our data (random effects model).

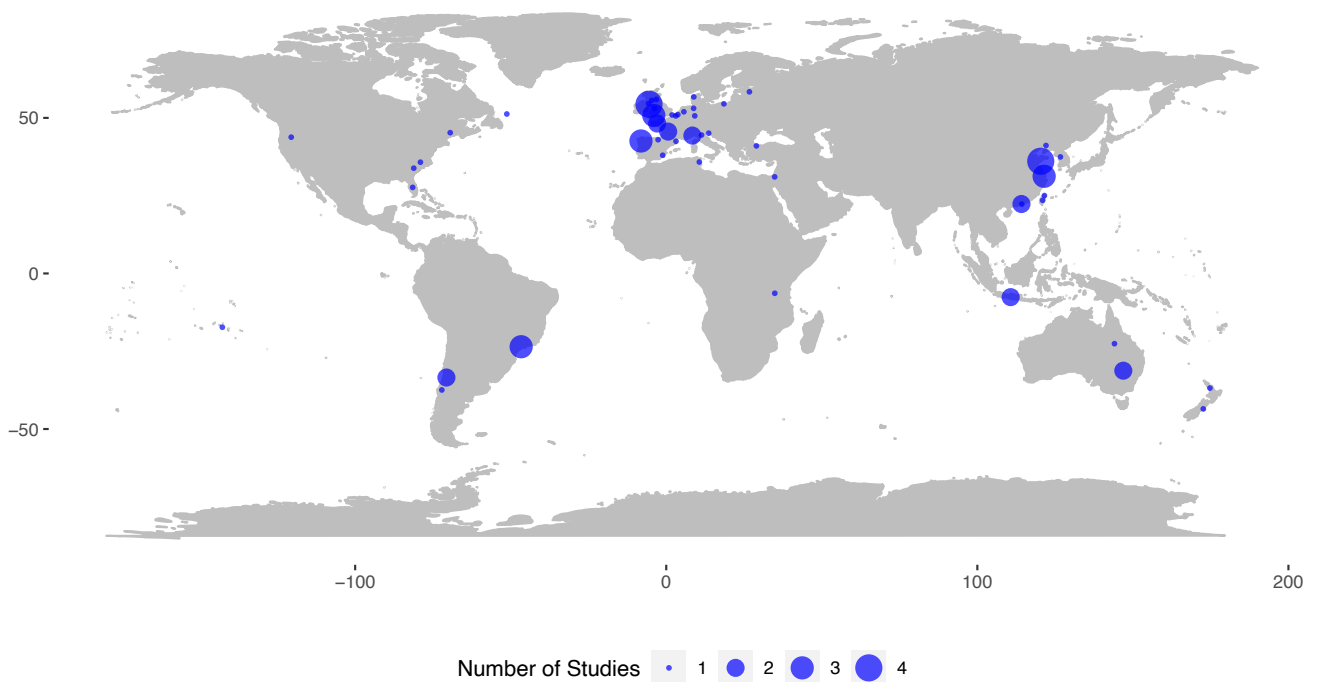
Test	Result	p-value	Estimated effect size (Hedge’s g)	Effect type	Model Reference
Trim and fill with random effects model	17 positive effect studies filled in (SE = 6.00)	< 0.0001	-0.61	Moderate negative	Duval and Tweedie (2000)
Rma.mv model		< 0.0001	-0.57	Moderate negative	Viechtbauer (2010)

74



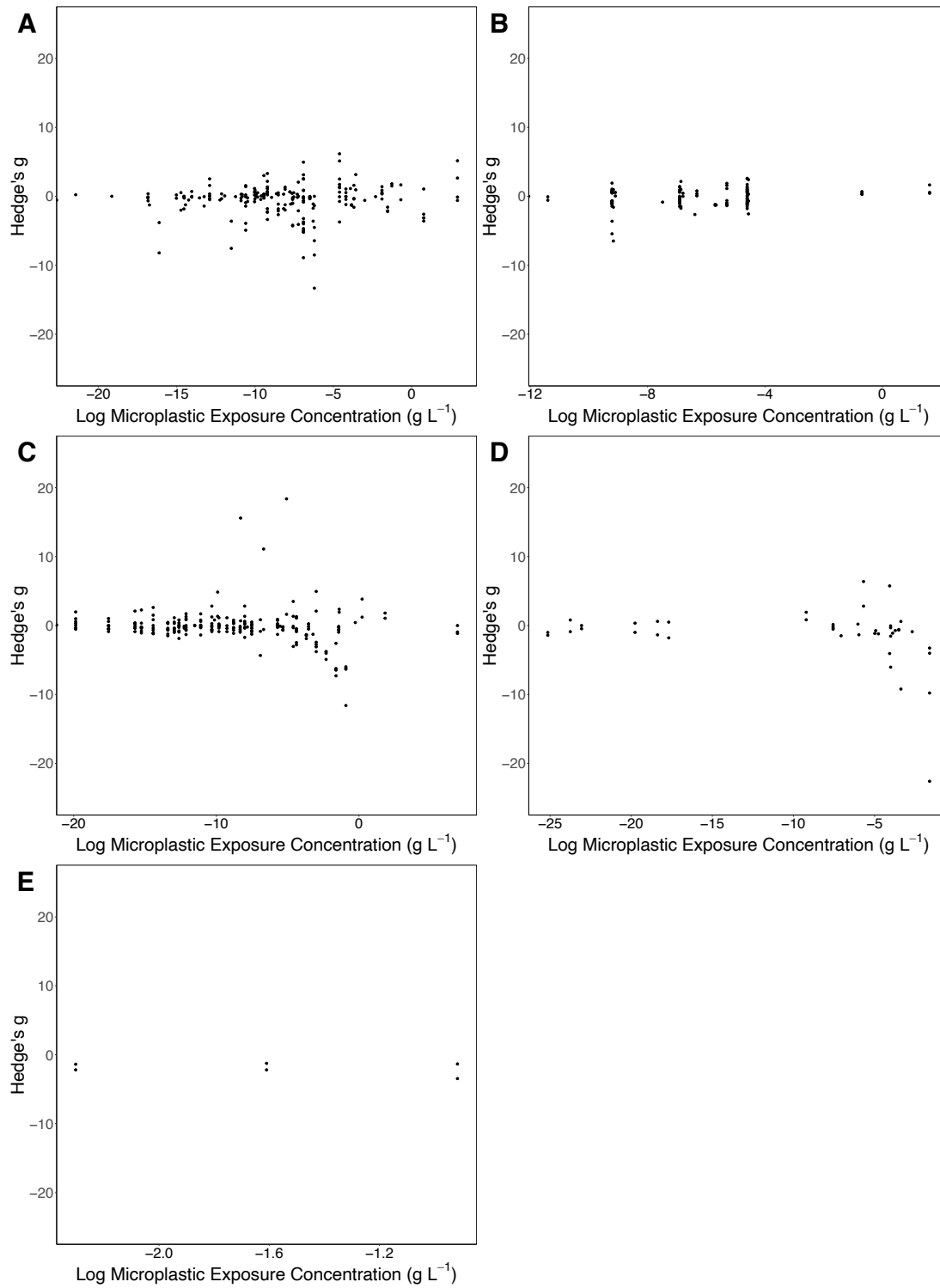
75

76 **Figure S2.** Number of studies related to the impact of microplastics on the biological rates of
 77 marine benthic fauna per publication year, from 2013-20. The triangle represents studies
 78 published in 2021 up until date of final search (7th June 2021).



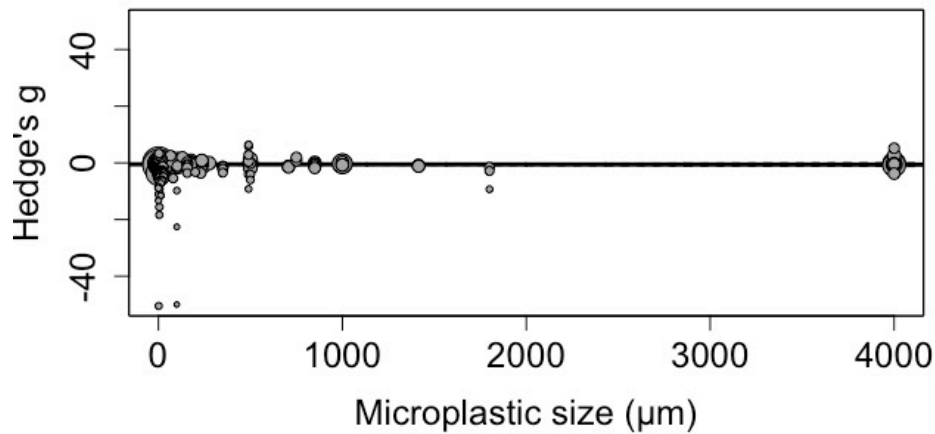
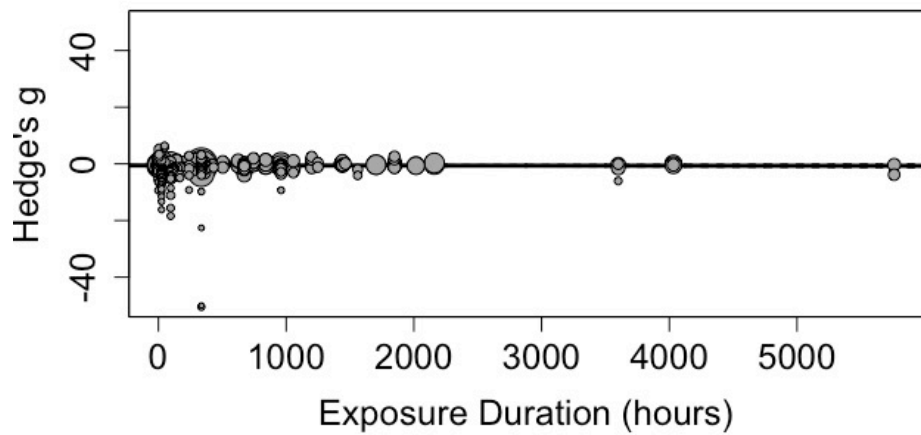
79

80 **Figure S3.** World map showing the number of publications related to the impact of
 81 microplastics on the functional traits of marine benthic fauna in each region. Circle size is
 82 proportional to the number of studies. Studies represented were published from 2013-21.



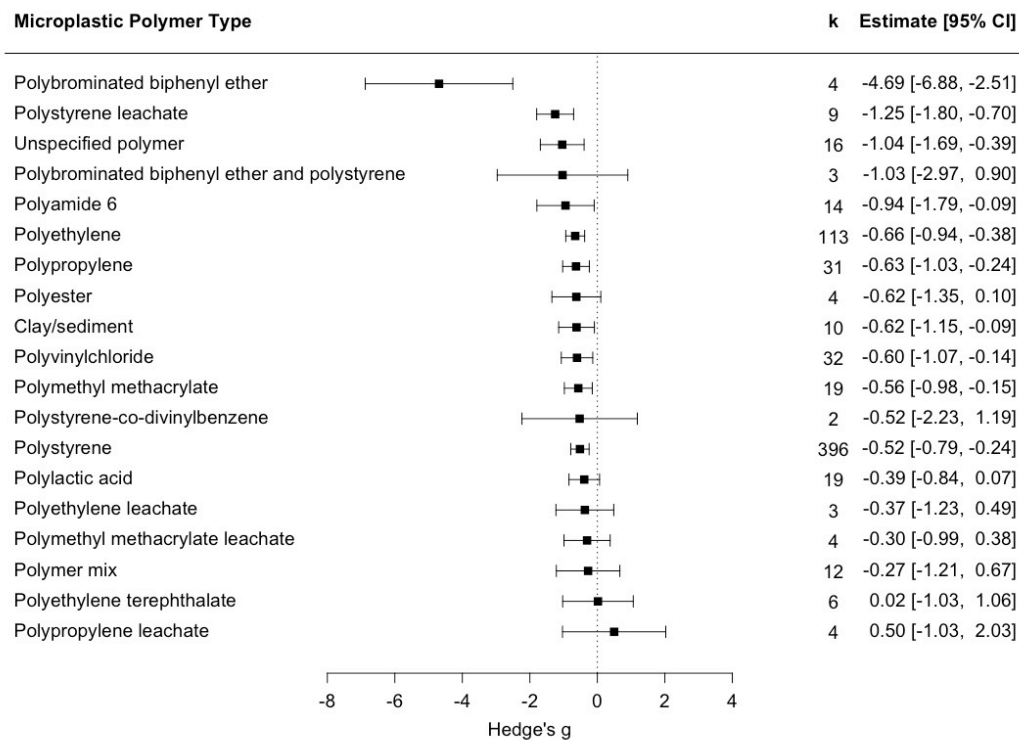
83

84 **Figure S4.** Relationship between log transformed microplastic exposure concentration ($g L^{-1}$)
 85 and effect size on marine benthic fauna (Hedge's g) for a) molluscs, b) echinoderms, c)
 86 crustaceans, d) cnidarians and e) chordates using studies from 2013-2021 which reported
 87 standardisable exposure concentration units ($n = 54$).



88

89 **Figure S5.** Meta-regression of a) exposure duration and b) microplastic size with Hedge's g
 90 effect size. The size of each point is proportional to the weight of the study (studies with
 91 larger sample sizes given greater weight), with smaller points given less weight. Regressions
 92 were produced based on the results of mixed-effects modelling using a) exposure duration
 93 and b) microplastic size as moderators.



94

95 **Figure S6. Influence of microplastic polymer type on marine benthic fauna. Influence**
 96 *indicated from mixed effects modelling, clay/sediment represents control. Boxes and error*
 97 *bars represent pooled Hedge's g values and 95% confidence intervals, respectively. K*
 98 *represents the number of case studies.*

99

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- Meta-analysis revealed microplastics weaken multiple processes fundamental to seabed life
- Surge in research helps establish that plastic impacts are stronger than thought
- Severity of impact depends on feeding strategy, life stage and taxonomic group
- Early life stages are most strongly impacted by microplastic exposure
- Leaking chemicals generate stronger responses than plastic particles themselves