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# Microplastics alter multiple biological processes of marine benthic fauna

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

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## ABSTRACT

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

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

## Key Words

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

## 1.0 INTRODUCTION

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

The definition of microplastics is inconsistent throughout the existing literature, but most commonly includes plastic particles of any shape from 0.1µm to 5mm (Auta et al. 2017). Within this category exist intentionally manufactured primary microplastics, such as highly

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

range in concentration from 2.8 to 1188.8 particles kg<sup>-1</sup> dry weight (Lorenz et al. 2019). Microplastics are therefore likely to become a ubiquitous component of seabed sediments and thus the influence of microplastics on benthic habitats must be considered.

Gall and Thompson (2015) reported over 44000 interactions of marine fauna with plastic debris, across 693 species. Larger plastic fragments impact fauna predominantly through ingestion and entanglement. A systematic review of 747 studies quantifying the interactions of plastics with marine megafauna found 701 species had ingested plastics and 354 species had experienced entanglement (Kühn & van Freneker 2020). Microplastics can impact marine organisms through a wider range of mechanisms, as shown in many experimental laboratory studies. Microplastic exposure caused abnormal embryo development in the brown mussel *Perna perna* (Gandara e Silva et al. 2016). The lugworm *Arenicola marina* reduced its feeding rate with increasing microplastic dosage (Besseling et al. 2013). Reduced feeding can be the result of a false sense of fullness, damage or blockages to the digestive tract or confusing microplastics for prey (de Sá et al. 2015). Numerous studies have found cellular level impacts of microplastics, for example, microplastic consumption influenced cellular pathway signalling, diminished growth and induced toxicity and oxidative stress in rotifers (Jeong et al. 2016). Such impacts may lead to behavioural changes, growth inhibition and, ultimately, increased mortality (de Sá et al. 2018). Microplastics also have the potential to cause adverse reactions via persistent organic pollutants (POPs), which adhere to plastic particles. Particularly hazardous are endocrine disruptor chemicals (EDCs), which accumulate in fatty tissues, altering hormone production and potentially causing thyroid problems, reduced reproductive success and hormone-sensitive cancers (Gallo et al. 2018). While the study of POPs so far has focussed primarily on the impacts on human health, effects on marine fauna have been observed, one example being reduced survival rate and

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

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

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

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

## 2.0 MATERIALS AND METHODS

### 2.1 STUDY DESIGN

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

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

## 2.2 LITERATURE SEARCH AND DATA EXTRACTION

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

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

AND

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

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. O<sub>2</sub> 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

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

## 2.3 DATA ANALYSIS

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

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

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

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

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

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

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

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

## 3.0 RESULTS

### 3.1 SUMMARY AND DISTRIBUTION OF FINDINGS

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

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

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

### 3.2 EFFECTS OF MICROPLASTICS ON BIOLOGICAL PROCESSES

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

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

### 3.3 SUB-GROUP ANALYSIS

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

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

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

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

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

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

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

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

## 4.0 DISCUSSION

### 4.1 BIOLOGICAL PROCESSES

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



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

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

## 4.2 ORGANISM CHARACTERISTICS

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

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

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

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

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

#### 4.3 MICROPLASTIC CHARACTERISTICS

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

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

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

Microplastics with adsorbed benzo[a]pyrene and perfluorooctane sulfonic acid cause more

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

#### 4.4 DISTRIBUTION OF LITERATURE USED

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

## 5.0 CONCLUSIONS

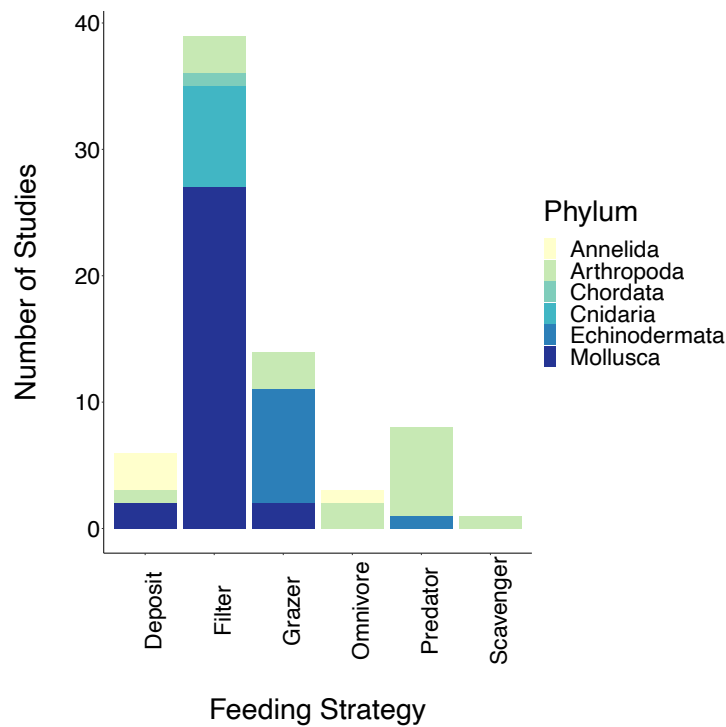
- Microplastic exposure has significant negative impact on multiple biological processes of marine benthic fauna assessed.
- This study provides the first meta-analytical evidence that microplastic leachates have more severe impacts on benthic fauna than microplastic particles themselves. Clearly, microplastic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

510 6.0 FIGURES AND TABLES WITH CAPTIONS

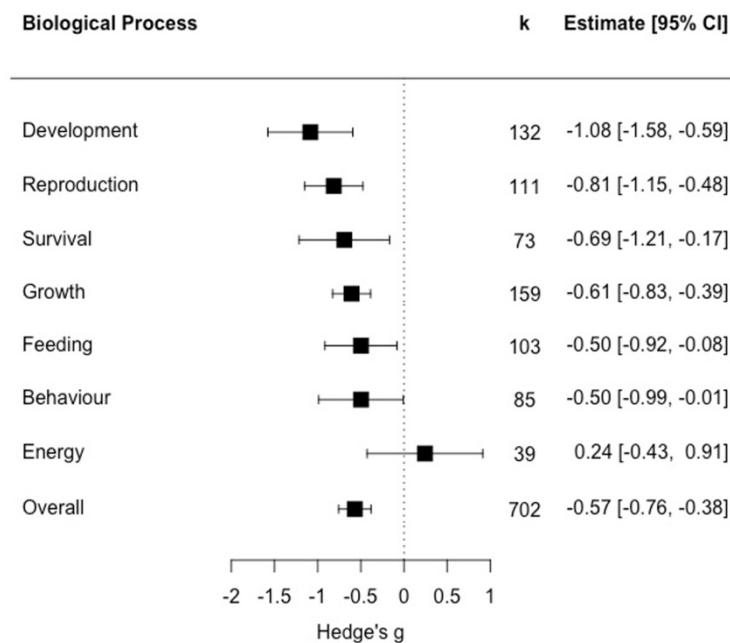
511 **Table 1.** *Heterogeneity of effect sizes of microplastics on marine benthic fauna, given as:*  
 512 *Wald's Value ( $I^2$ ), Cochran's value ( $Q$ ), and the degrees of freedom (DF) and p-value*  
 513 *pertaining to Cochran's  $Q$ .*

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



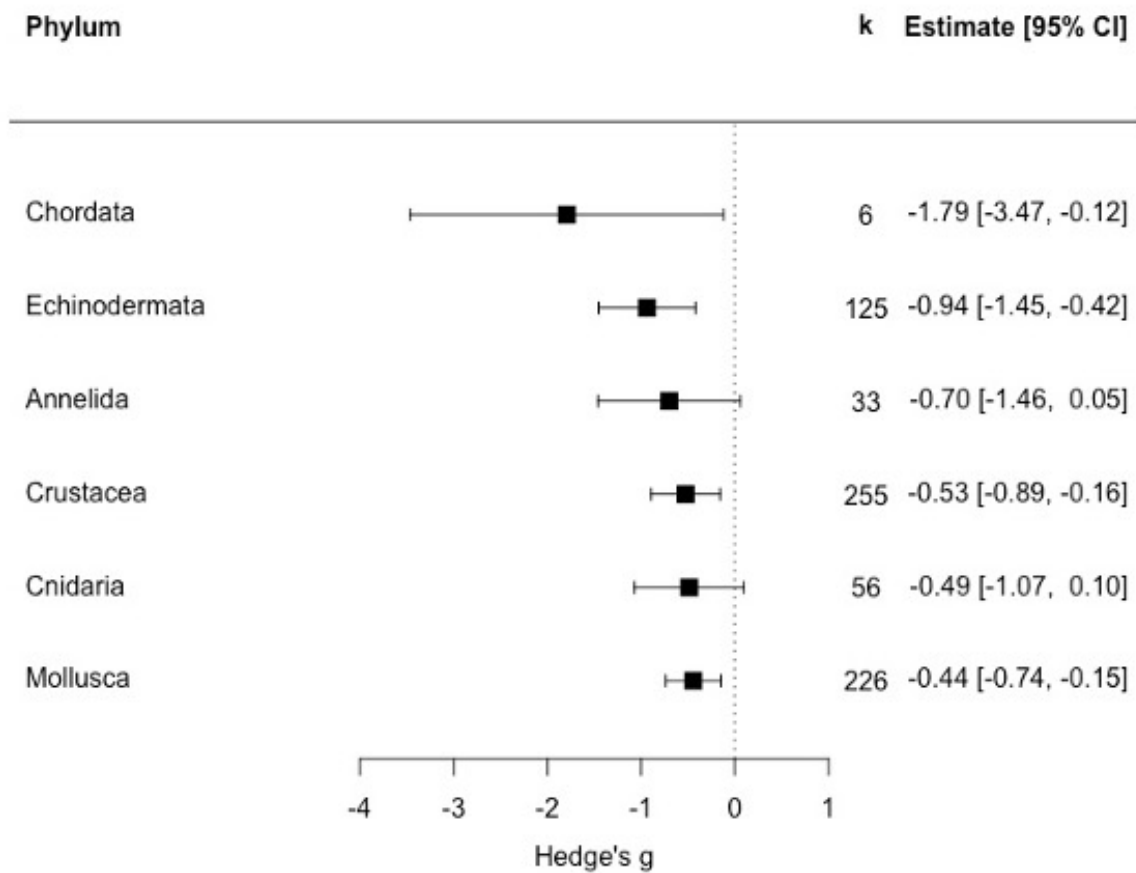
**Figure 1. The frequency of animal feeding strategy by phylum used in 72 experimental studies.**



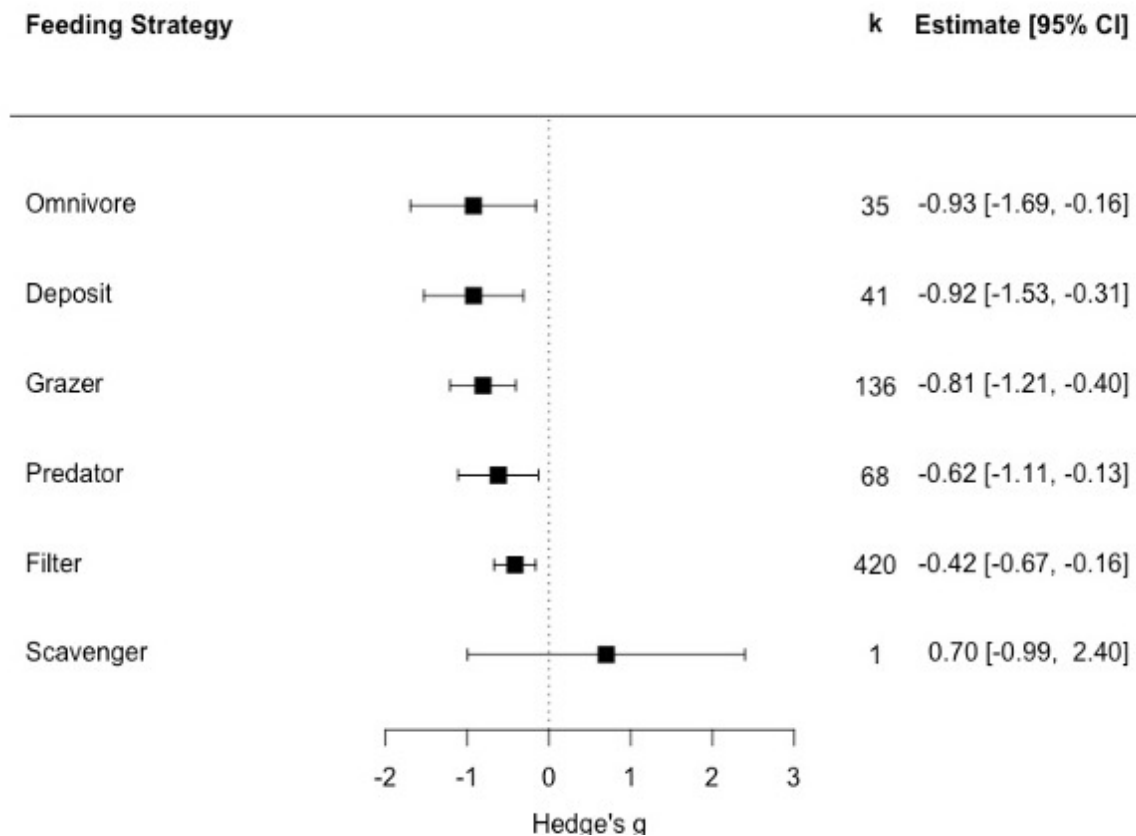
**Figure 2. The effects of microplastic exposure on biological processes of marine benthic fauna. Effects on each of 7 processes and overall, as indicated from random-effects modelling. Boxes and error bars represent pooled Hedge's g values and 95% confidence**



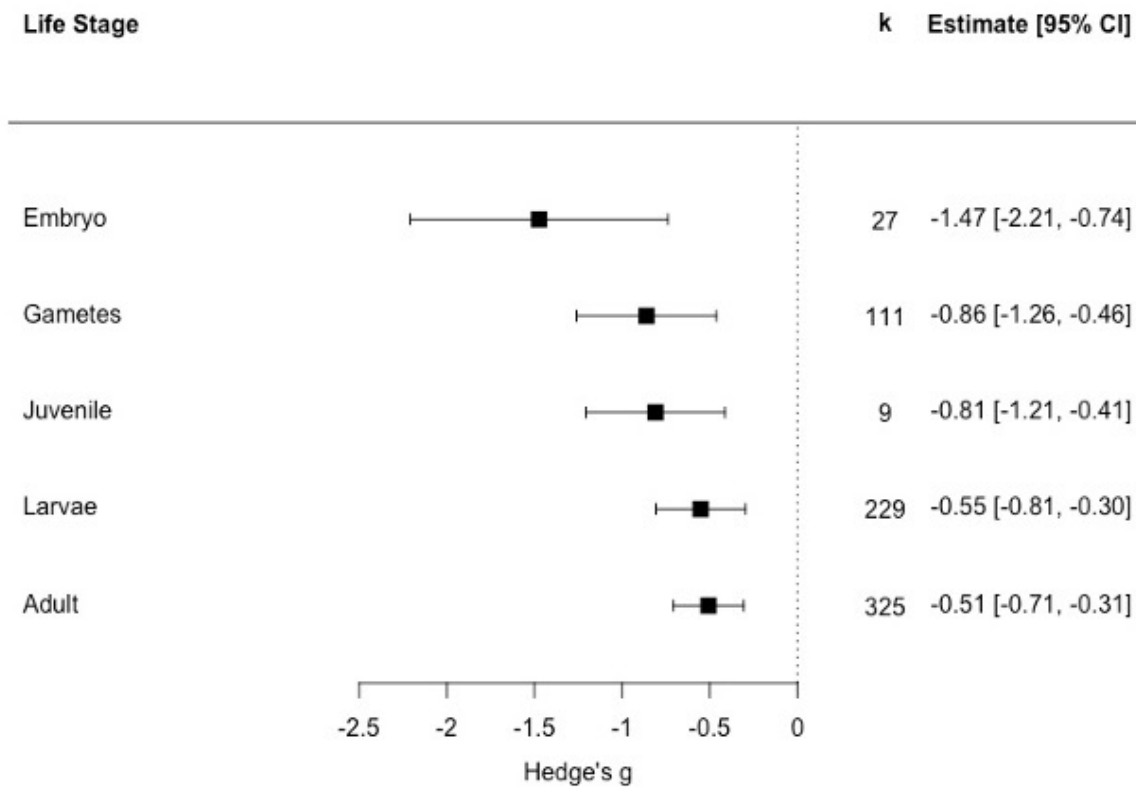
intervals, respectively.  $K$  represents the number of case studies, or independent experiments within the same study. Overlap of confidence intervals with 0 indicate non-significance.



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



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



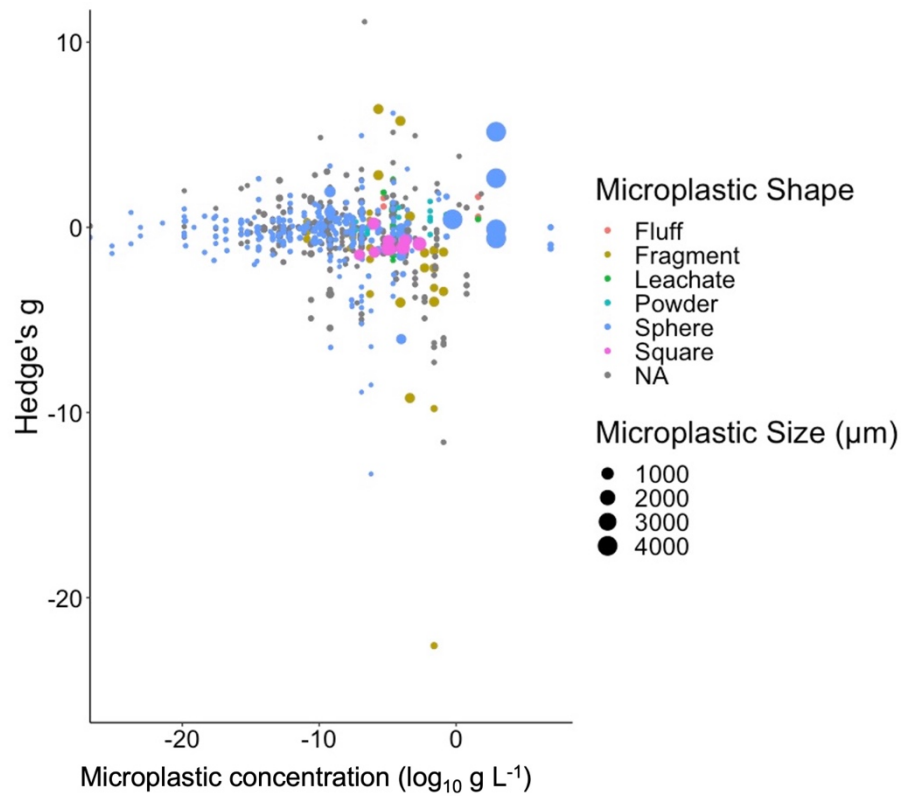
534

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

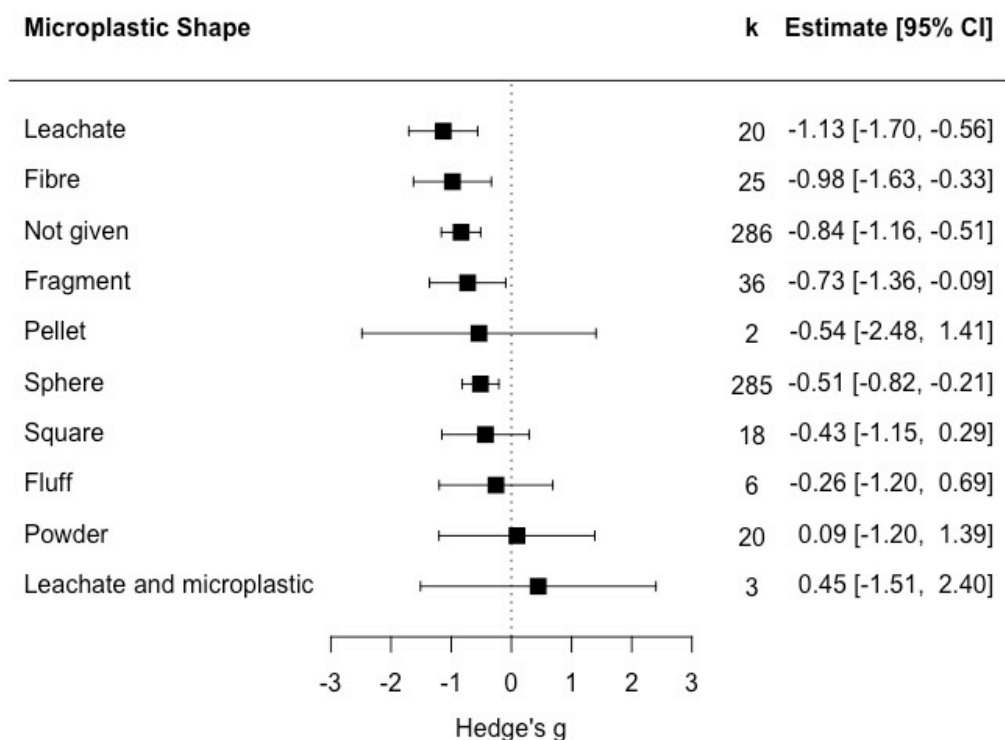
536 *Effects on each of 5 life stages as indicated from mixed-effects modelling. Boxes and error*

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

538 *represents the number of case studies.*

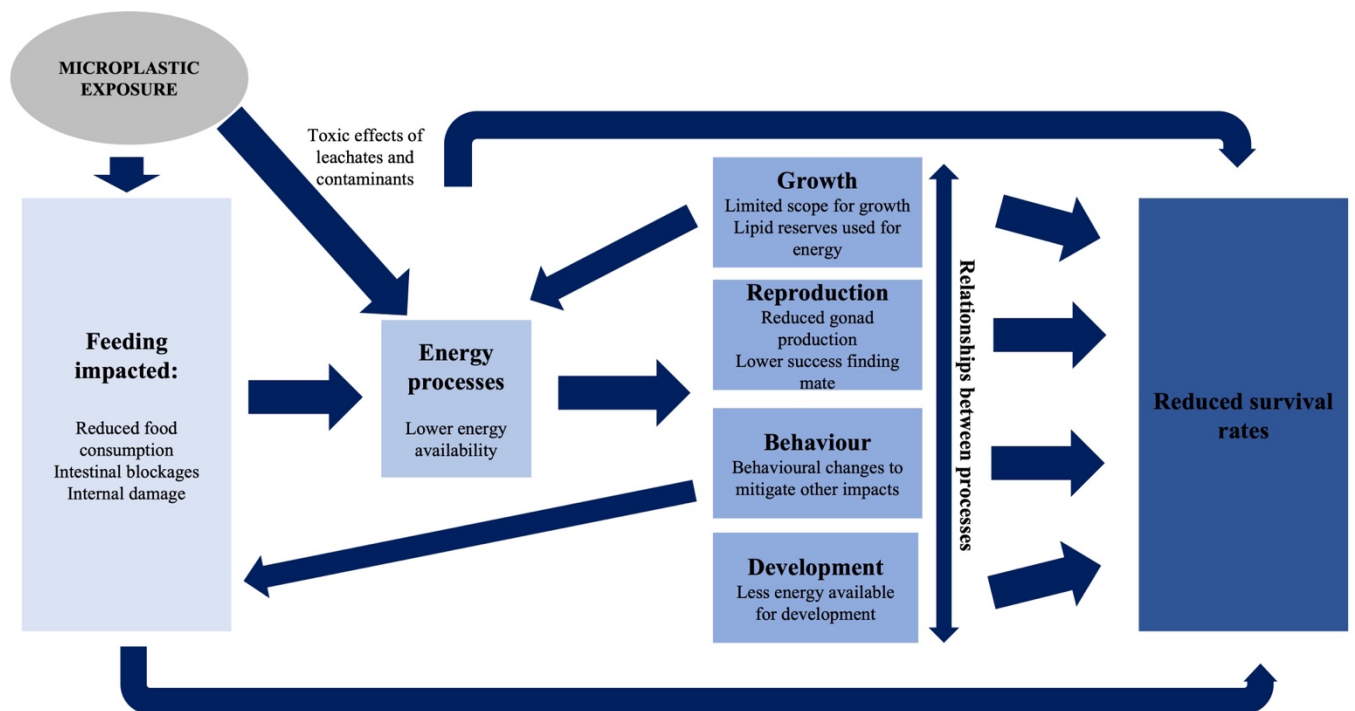


**Figure 6. Effect of microplastic exposure concentration on the biological processes of marine benthos.** Effect size indicated by Hedge's g value. Point size is indicative of microplastic particle size, while colour represents the shape of the particle.



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

Responses indicated from mixed effects modelling. Boxes and error bars represent pooled Hedge's g values and 95% confidence intervals, respectively. K represents the number of case studies. 'Leachate and microplastic' refers to microplastic particles with adsorbed leachates, while 'leachate' refers to leachate which is not adsorbed to a particle.



**Figure 8. Interactions of impacts on different biological processes of marine benthic fauna, as a result of microplastic exposure. Interactions demonstrated by arrows, culminating in a synergistic effect and overall reduction in survival rate.**

## 7.0 ACKNOWLEDGEMENTS

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## 563 8.0 REFERENCES

- 564 Agamuthu P, Mehran SB, Norkhairah A, Norkhairiyah A (2019) Marine debris: A review of  
565 impacts and global initiatives. *Waste Manag Res* 37:987–1002
- 566 Ajith N, Arumugam S, Parthasarathy S, Manupoori S, Janakiraman S (2020) Global  
567 distribution of microplastics and its impact on marine environment—a review. *Env*  
568 *Sci Pollut Res* 27:25970–25986
- 569 Anderson G and Shenkar N (2021) Potential effects of biodegradable single-use items in the  
570 sea: Polylactic acid (PLA) and solitary ascidians. *Environ Pollut* 268:115364
- 571 Auta HS, Emenike CU, Fauziah SH (2017) Distribution and importance of microplastics in  
572 the marine environment A review of the sources, fate, effects, and potential solutions.  
573 *Environ Int* 102:165–176
- 574 Berlino M, Mangano MC, De Vittor C, Sarà G (2021) Effects of microplastics on the  
575 functional traits of aquatic benthic organisms: A global-scale meta-analysis. *Environ*  
576 *Pollut* 285
- 577 Besseling E, Wegner A, Foekema EM, Van Den Heuvel-Greve MJ, Koelmans AA (2013)  
578 Effects of microplastic on fitness and PCB bioaccumulation by the lugworm  
579 *Arenicola marina* (L.). *Environ Sci Technol* 47:593–600
- 580 Borenstein M, Hedges LV., Higgins JPT, Rothstein HR (2009) Introduction to Meta-  
581 Analysis. John Wiley and Sons
- 582 Bour A, Avio CG, Gorbi S, Regoli F, Hylland K (2018) Presence of microplastics in benthic  
583 and epibenthic organisms: Influence of habitat, feeding mode and trophic level.  
584 *Environ Pollut* 243:1217–1225
- 585 Cochran WG (1954) The Combination of Estimates from Different Experiments. *Biometrics*  
586 10:101

587 Cohen J (2013) Statistical Power Analysis for the Behavioral Sciences (2<sup>nd</sup> edition).  
588 Routledge

589 Cole M, Lindeque P, Halsband C, Galloway TS (2011) Microplastics as contaminants in the  
590 marine environment: A review. *Mar Pollut Bull* 62:2588–2597

591 Cózar A, Echevarría F, González-Gordillo J, Irigoien X, Ubeda B, Hernández-León S, Palma  
592 A, Navarro S, García-de-Lomas J, Ruiz A, Fernández-de-Puelles M, Duarte C (2014)  
593 Plastic debris in the open ocean. *Proc Natl Acad Sci U S A* 111:10239–10244

594 Crump A, Mullens C, Bethell EJ, Cunningham EM, Arnott G (2020) Microplastics disrupt  
595 hermit crab shell selection. *Biol Lett* 16

596 de Sá LC, Luís LG, Guilhermino L (2015) Effects of microplastics on juveniles of the  
597 common goby (*Pomatoschistus microps*): Confusion with prey, reduction of the  
598 predatory performance and efficiency, and possible influence of developmental  
599 conditions. *Environ Pollut* 196:359–362

600 de Sá LC, Oliveira M, Ribeiro F, Rocha TL, Futter MN (2018) Studies of the effects of  
601 microplastics on aquatic organisms: What do we know and where should we focus  
602 our efforts in the future? *Sci Total Environ* 645:1029–1039

603 DerSimonian R, Laird N (1986) Meta-Analysis in Clinical Trials\*. *Control Clin Trials*  
604 7:177–188

605 Duchêne JC and Rosenberg R (2001) Marine benthic faunal activity patterns on a sediment  
606 surface assessed by video numerical tracking. *Mar. Ecol. Prog. Ser.* 223:113-119.

607 Duval S and Tweedie R L (2000) Trim and fill: A simple funnel-plot-based method of testing  
608 and adjusting for publication bias in meta-analysis. *Biometrics* 56:455-463



609 Efimova I, Bagaeva M, Bagaev A, Kileso A, Chubarenko IP (2018) Secondary Microplastics  
 610 Generation in the Sea Swash Zone With Coarse Bottom Sediments: Laboratory  
 611 Experiments. *Front Mar Sci* 0:313

612 Everaert G, Van Cauwenberghe L, De Rijcke M, Koelmans AA, Mees J, Vandegehuchte M,  
 613 Janssen CR (2018) Risk assessment of microplastics in the ocean: Modelling  
 614 approach and first conclusions. *Environ Pollut* 242:1930–1938

615 Foley CJ, Feiner ZS, Malinich TD, Höök TO (2018) A meta-analysis of the effects of  
 616 exposure to microplastics on fish and aquatic invertebrates. *Sci Total Environ* 631–  
 617 632:550–559

618 Fonte E, Ferreira P, Guilhermino L (2016) Temperature rise and microplastics interact with  
 619 the toxicity of the antibiotic cefalexin to juveniles of the common goby  
 620 (*Pomatoschistus microps*): Post-exposure predatory behaviour, acetylcholinesterase  
 621 activity and lipid peroxidation. *Aquat Toxicol* 180:173–185

622 Gall SC, Thompson RC (2015) The impact of debris on marine life. *Mar Pollut Bull* 92:170–  
 623 179

624 Gallo F, Fossi C, Weber R, Santillo D, Sousa J, Ingram I, Nadal A, Romano D (2018) Marine  
 625 litter plastics and microplastics and their toxic chemicals components: the need for  
 626 urgent preventive measures. *Environ Sci Eur* 30:1–14

627 Gandara E Silva PP, Nobre CR, Resaffe P, Pereira CDS, Gusmão F (2016) Leachate from  
 628 microplastics impairs larval development in brown mussels. *Water Res.* 106:364-370

629 Gray A, Weinstein J (2017) Size- and shape-dependent effects of microplastic particles on  
 630 adult daggerblade grass shrimp (*Palaemonetes pugio*). *Environ Toxicol Chem*  
 631 36:3074–3080

632 Green DS, Boots B, Sigwart J, Jiang S, Rocha C (2016) Effects of conventional and  
633 biodegradable microplastics on a marine ecosystem engineer (*Arenicola marina*) and  
634 sediment nutrient cycling. *Environ Pollut* 208:426–434

635 Gu H, Wei S, Hu M, Wei H, Wang X, Shang Y, Li I, Shi H, Wang Y (2020) Microplastics  
636 aggravate the adverse effects of BDE-47 on physiological and defense performance in  
637 mussels. *J Hazard Mater* 398:122909

638 Hammer J, Kraak MHS, Parsons JR (2012) Plastics in the Marine Environment: The Dark  
639 Side of a Modern Gift

640 Hermesen E, Mintenig SM, Besseling E, Koelmans AA (2018) Quality Criteria for the  
641 Analysis of Microplastic in Biota Samples: A Critical Review. *Environ Sci Technol*  
642 52:10230–10240

643 Herzke D, Anker-Nilssen T, Nøst TH, Götsch A, Christensen-Dalsgaard S, Langset M,  
644 Fangel K, Koelmans AA (2016) Negligible Impact of Ingested Microplastics on  
645 Tissue Concentrations of Persistent Organic Pollutants in Northern Fulmars off  
646 Coastal Norway. *Environ Sci Technol* 50:1924–1933

647 Hierl, F., Wu, H.C. and Westphal, H., 2021. Scleractinian corals incorporate microplastic  
648 particles: identification from a laboratory study. *Environmental Science and Pollution*  
649 *Research*, pp.1-12.

650 Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors)  
651 (2019) *Cochrane Handbook for Systematic Reviews of Interventions*. 2nd Edition.  
652 Chichester (UK): John Wiley & Sons

653 Hodgson DJ (2018) The impacts of microplastic ingestion on marine polychaete worms.  
654 MRes Dissertation. University of Exeter, UK

655 Jeong C, Won E, Kang H, Lee M, Hwang D, Hwang U, Zhou B, Souissi S, Lee S, Lee J  
 656 (2016) Microplastic Size-Dependent Toxicity, Oxidative Stress Induction, and p-JNK  
 657 and p-p38 Activation in the Monogonont Rotifer (*Brachionus koreanus*). Environ Sci  
 658 Technol 50:8849–8857

659 Jiang X, Lu K, Tunnell JW, Liu Z (2021) The impacts of weathering on concentration and  
 660 bioaccessibility of organic pollutants associated with plastic pellets (nurdles) in  
 661 coastal environments. Mar. Pollut. Bull. 170:112592.

662 Kaiser D, Kowalski N, Waniek JJ (2017) Effects of biofouling on the sinking behavior of  
 663 microplastics. Environ Res Lett 12:124003

664 Kooi M, Koelmans AA (2019) Simplifying Microplastic via Continuous Probability  
 665 Distributions for Size, Shape, and Density. Environ Sci Technol Lett 6:551–557

666 Kooi M, Nes EH van, Scheffer M, Koelmans AA (2017) Ups and Downs in the Ocean:  
 667 Effects of Biofouling on Vertical Transport of Microplastics. Environ Sci Technol  
 668 51:7963

669 Kühn S, van Franeker JA (2020) Quantitative overview of marine debris ingested by marine  
 670 megafauna. Mar Pollut Bull 151

671 Lei L, Wu S, Lu S, Liu M, Song Y, Fu Z, Shi H, Raley-Susman K, He D (2018) Microplastic  
 672 particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio*  
 673 and nematode *Caenorhabditis elegans*. Sci Total Environ 619–620:1–8

674 Lo H K A, Chan K Y K (2018) Negative effects of microplastic exposure on growth and  
 675 development of *Crepidula onyx*. Environ pollut 233:588-595.

676 Lorenz C, Roscher L, Meyer M S, Hildebrandt L, Prume J, Löder M G, Primpke S and  
 677 Gerdt G (2019) Spatial distribution of microplastics in sediments and surface waters  
 678 of the southern North Sea. Environ Poll 252:1719-1729.

679 Martin J, Lusher A, Thompson R C and Morley A (2017) The deposition and accumulation  
680 of microplastics in marine sediments and bottom water from the Irish continental  
681 shelf. *Sci. Rep.* 7:1-9.

682 Melkebeke M Van, Janssen C, De Meester S (2020) Characteristics and Sinking Behavior of  
683 Typical Microplastics Including the Potential Effect of Biofouling: Implications for  
684 Remediation. *Environ Sci Technol* 54:8680

685 Miller ME, Kroon FJ, Motti CA (2017) Recovering microplastics from marine samples: A  
686 review of current practices. *Mar Pollut Bull* 123:6–18

687 Naji A, Nuri M, Vethaak AD (2018) Microplastics contamination in molluscs from the  
688 northern part of the Persian Gulf. *Environ Pollut* 235:113–120

689 Nobre CR, Santana MFM, Maluf A, Cortez FS, Cesar A, Pereira CDS, Turra A (2015)  
690 Assessment of microplastic toxicity to embryonic development of the sea urchin  
691 *Lytechinus variegatus* (Echinodermata: Echinoidea). *Mar Pollut Bull* 92:99–104

692 O’Dea RE, Lagisz M, Jennions MD, Koricheva J, Noble DWA, Parker TH, Gurevitch J, Page  
693 MJ, Stewart G, Moher D, Nakagawa S (2021) Preferred reporting items for systematic  
694 reviews and meta-analyses in ecology and evolutionary biology: a PRISMA  
695 extension. *Biol Rev*

696 O’Donovan S, Mestre NC, Abel S, Fonseca TG, Carteny CC, Cormier B, Keiter SH,  
697 Bebianno MJ (2018) Ecotoxicological Effects of Chemical Contaminants Adsorbed to  
698 Microplastics in the Clam *Scrobicularia plana*. *Front Mar Sci* 0:143

699 Pirsheh M, Hossini H, Makhdoumi P (2020) Review of microplastic occurrence and  
700 toxicological effects in marine environment: Experimental evidence of inflammation.  
701 *Process Saf Environ Prot* 142:1–14

702 Prinz N, Korez Š (2020) Understanding How Microplastics Affect Marine Biota on the  
 703 Cellular Level Is Important for Assessing Ecosystem Function: A Review. In:  
 704 *YOUMARES 9 - The Oceans: Our Research, Our Future*. Springer, Cham, p 101–120  
 705 Pullin AS, Stewart GB (2006) Guidelines for systematic review in conservation and  
 706 environmental management. *Conserv Biol* 20:1647–1656  
 707 Rochman CM, Hoh E, Kurobe T, Teh SJ (2013) Ingested plastic transfers hazardous  
 708 chemicals to fish and induces hepatic stress. *Sci Rep* 3:1–7  
 709 RStudio Team (2020) RStudio: Integrated Development Environment for R. RStudio,  
 710 PBC, Boston, MA URL <http://www.rstudio.com/>  
 711 Salerno M, Berlino M, Mangano MC, Sarà G (2021) Microplastics and the functional traits of  
 712 fishes: A global meta-analysis. *Glob Chang Biol* 27:1–11  
 713 Sterne JAC, Gavaghan D, Egger M (2000) Publication and related bias in meta-analysis:  
 714 Power of statistical tests and prevalence in the literature. *J Clin Epidemiol* 53:1119–  
 715 1129  
 716 Tosetto L, Brown C, Williamson JE (2016) Microplastics on beaches: ingestion and  
 717 behavioural consequences for beachhoppers. *Mar Biol* 163  
 718 Van Cauwenberghe L (2016) Occurrence, effects and risks of marine microplastics. PhD  
 719 dissertation. Ghent University, Belgium  
 720 Vazquez O, Rahman S (2021) An ecotoxicological approach to microplastics on terrestrial  
 721 and aquatic organisms: A systematic review in assessment, monitoring and biological  
 722 impact. *Environ Toxicol Pharmacol* 84:103615  
 723 Viechtbauer W (2007) Accounting for heterogeneity via random-effects models and  
 724 moderator analyses in meta-analysis *J. Psychol. Psychology* 215:104.

725 Viechtbauer W (2010) Conducting meta-analyses in R with the metafor package. *Journal of*  
726 *Statistical Software* 36:1-48. <https://doi.org/10.18637/jss.v036.i03>

727 Wayman C, Niemann H (2021) The fate of plastic in the ocean environment – a minireview.  
728 *Environ Sci Process Impacts* 23:198–212

729 Wright SL, Rowe D, Thompson RC, Galloway TS (2013) Microplastic ingestion decreases  
730 energy reserves in marine worms. *Curr Biol* 23:R1031–R1033

731 Xu S, Ma J, Ji R, Pan K, Miao AJ (2020) Microplastics in aquatic environments: Occurrence,  
732 accumulation, and biological effects. *Sci Total Environ* 703:1–14

733 Zhang H (2017) Transport of microplastics in coastal seas. *Estuar Coast Shelf Sci* 199:74–86

## 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

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



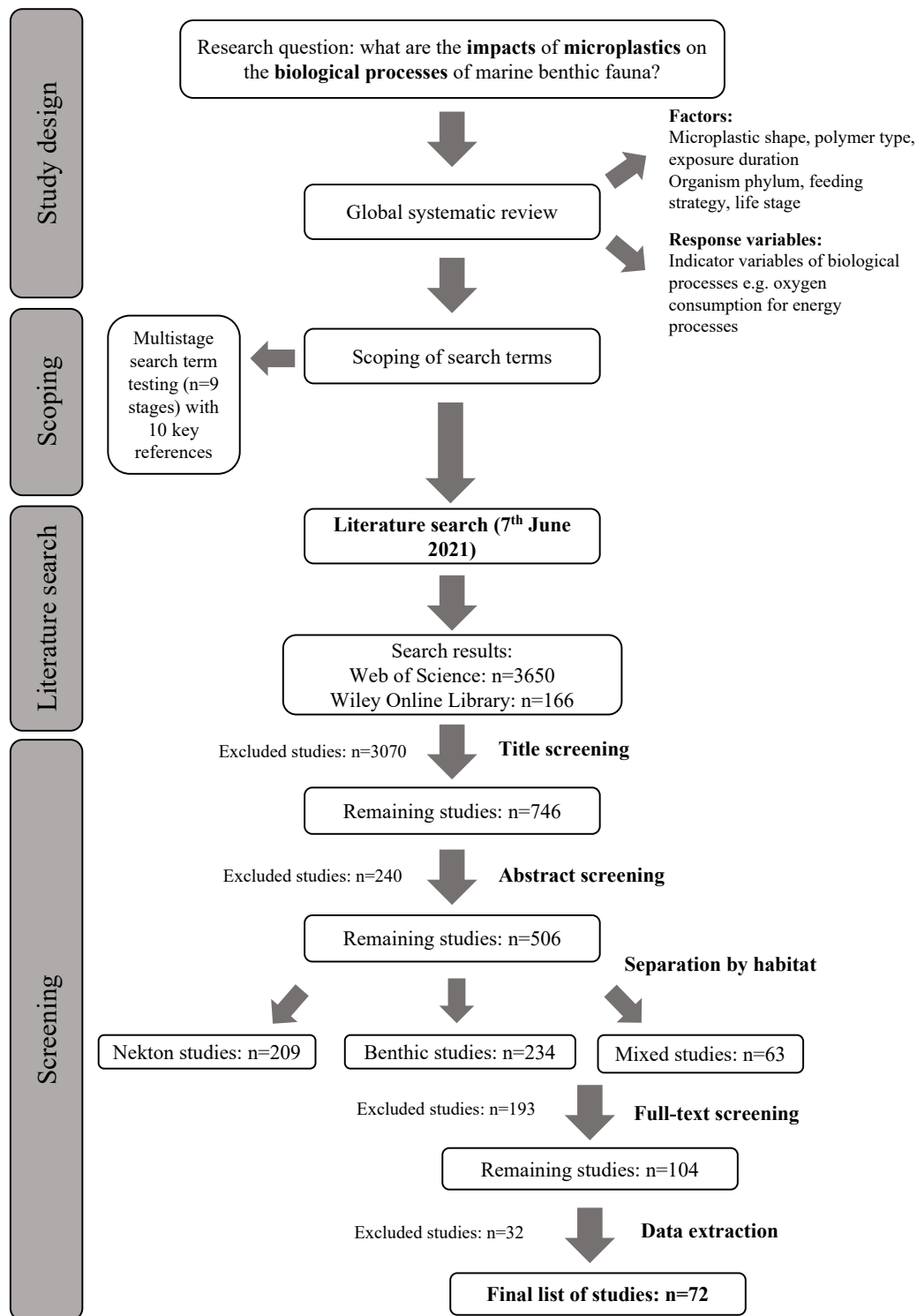
## 39 1.0 INTRODUCTORY TABLES

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

## 43 2.0 SYSTEMATIC REVIEW METHODOLOGY



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.

**Table S2.** Key references used when scoping potential search terms to assess for relevance of results. Studies given with author, publication date, study organism and the number of citations. Number of citations as given by Web of Science on 27<sup>th</sup> May 2021 (benthic studies) and 4<sup>th</sup> June 2021 (nekton studies). Studies selected for relevance, range of study organisms 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
	Van Cauwenberghe et al.	2015	<i>Mytilus edulis</i>	429
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 (7<sup>th</sup> 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 harrisii</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.2020.111280	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.2021.1116649	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.2020.111703	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; Soyeux, 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

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		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

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1010	Bringer, A; Thomas, H; Prunier, G; Dubillot, E; Bossut, N; Churlaud, C; Clerandau, 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.env pol.2020.1139 78	15
1028	Bringer, A; Cachot, J; Prunier, G; Dubillot, E; Clerandau, 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.che mosphere.202 0.126793	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.jhaz mat.2018.07.1 01	3
1794	Beiras, R; Tato, T	Microplastics do not increase toxicity of a hydrophobic organic chemical to marine plankton	2019	10.1016/j.mar polbul.2018.1 1.029	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.env pol.2020.1154 29	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.mar polbul.2020.1 11850	20

508	Li, ZC; Zhou, H; Liu, Y; Zhan, JJ; Li, WT; Yang, KM; Yi, XL	Acute and chronic combined effect of polystyrene microplastics and dibutyl phthalate on the marine copepod <i>Tigriopus japonicus</i>	2020	10.1016/j.che mosphere.202 0.127711	6
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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,	Biological rate indicator (e.g.
Case study (a, b, c etc.)	region)	growth rate, respiration rate):
Author	Date of experiment	Control group (mean, standard
Publication Type	Study organism (phylum,	deviation, number of replicates,
Publication Year	species, life stage, feeding	units)
	strategy)	Experimental group (mean,
	Exposure conditions (duration,	standard deviation, number of
	microplastic concentration,	replicates, units)
	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 <sup>-1</sup> )

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 (%)

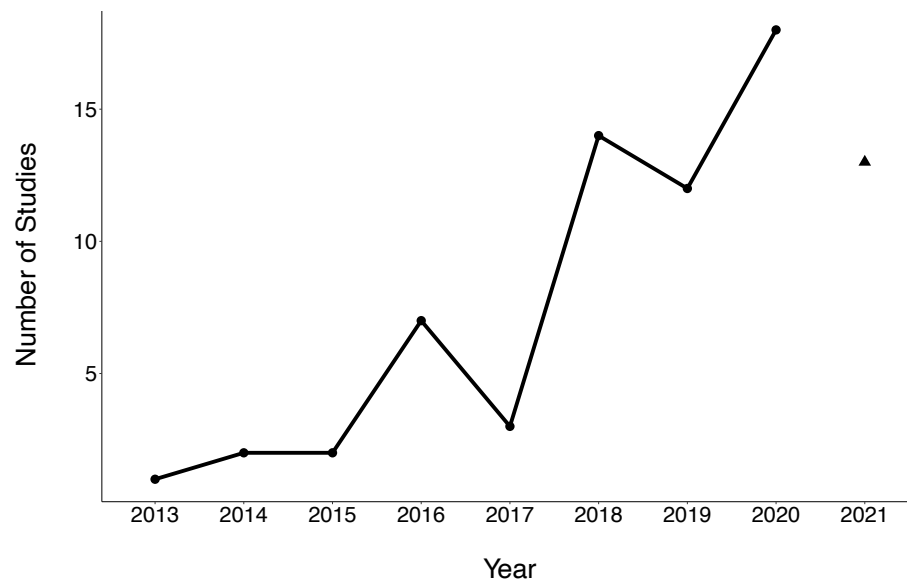


### 68 3.0 RESULTS

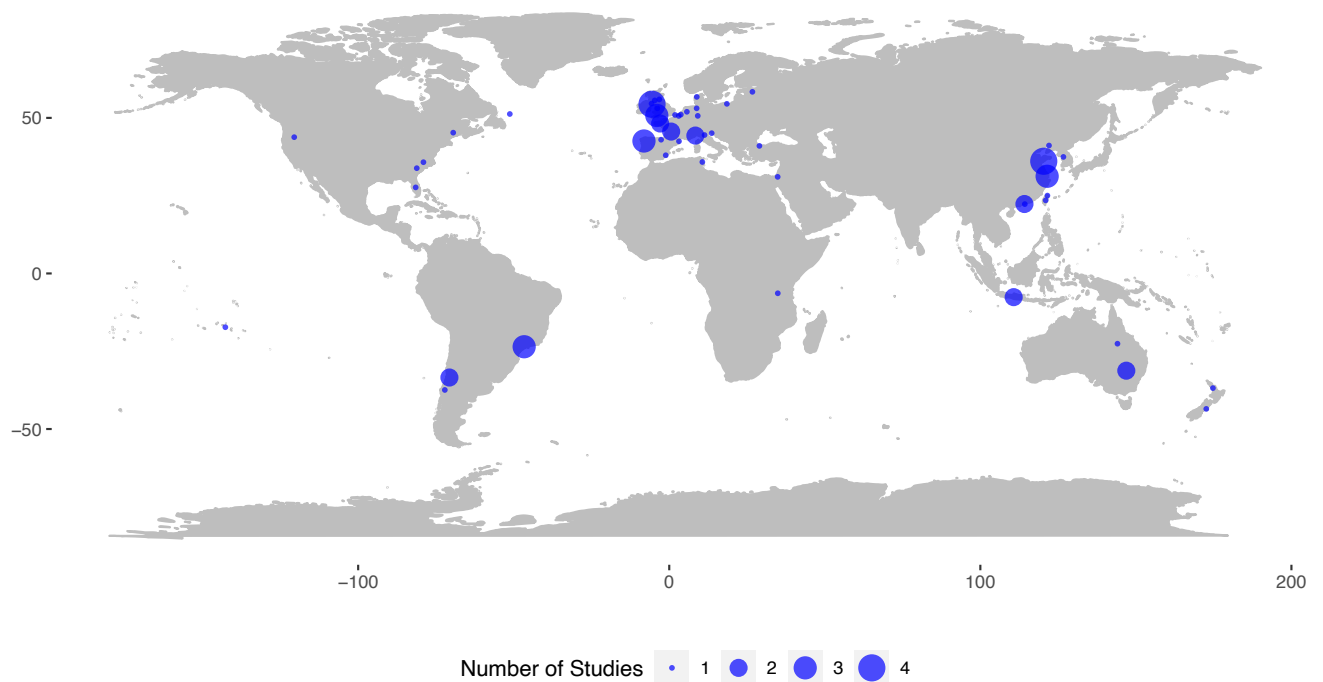
69 **Table S6.** Results of testing for publication bias using the ‘trim and fill’ method on rma.uni  
70 model. Result indicates assessment of balance of positive and negative effect size studies.  
71 Estimated effect size (**in bold**) indicates overall pooled Hedge’s g effect size of microplastics  
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	<b>-0.61</b>	Moderate negative	Duval and Tweedie (2000)
Rma.mv model		< 0.0001	<b>-0.57</b>	Moderate negative	Viechtbauer (2010)

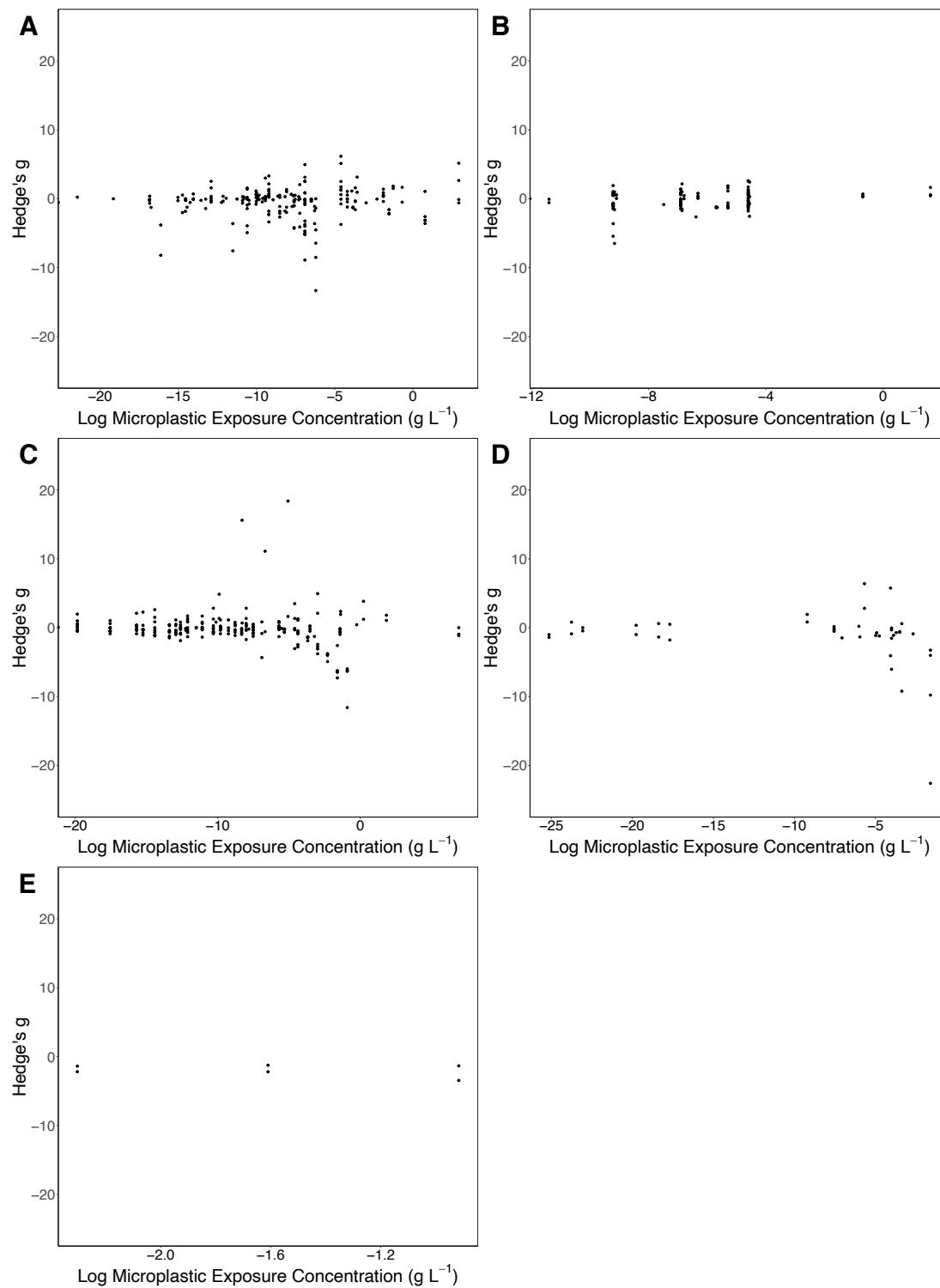
74



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

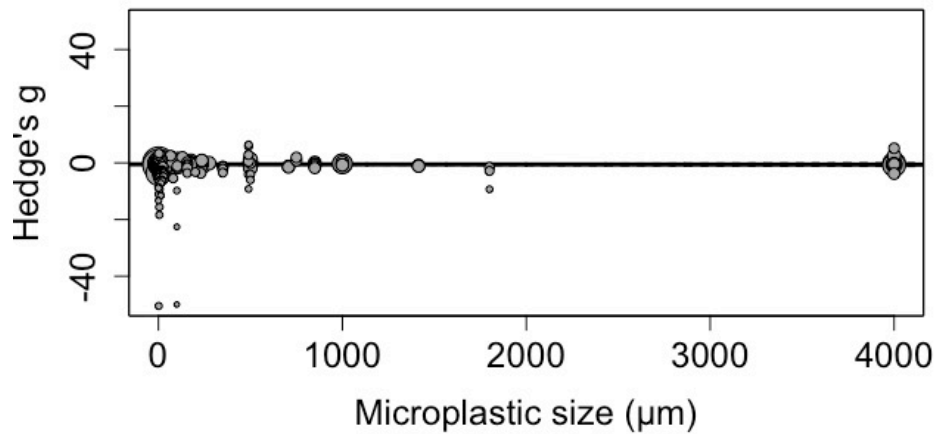
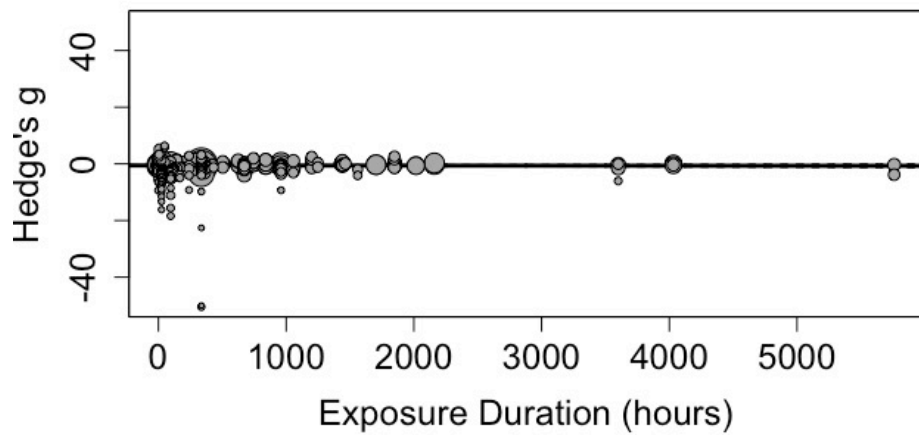


**Figure S3.** World map showing the number of publications related to the impact of microplastics on the functional traits of marine benthic fauna in each region. Circle size is proportional to the number of studies. Studies represented were published from 2013-21.



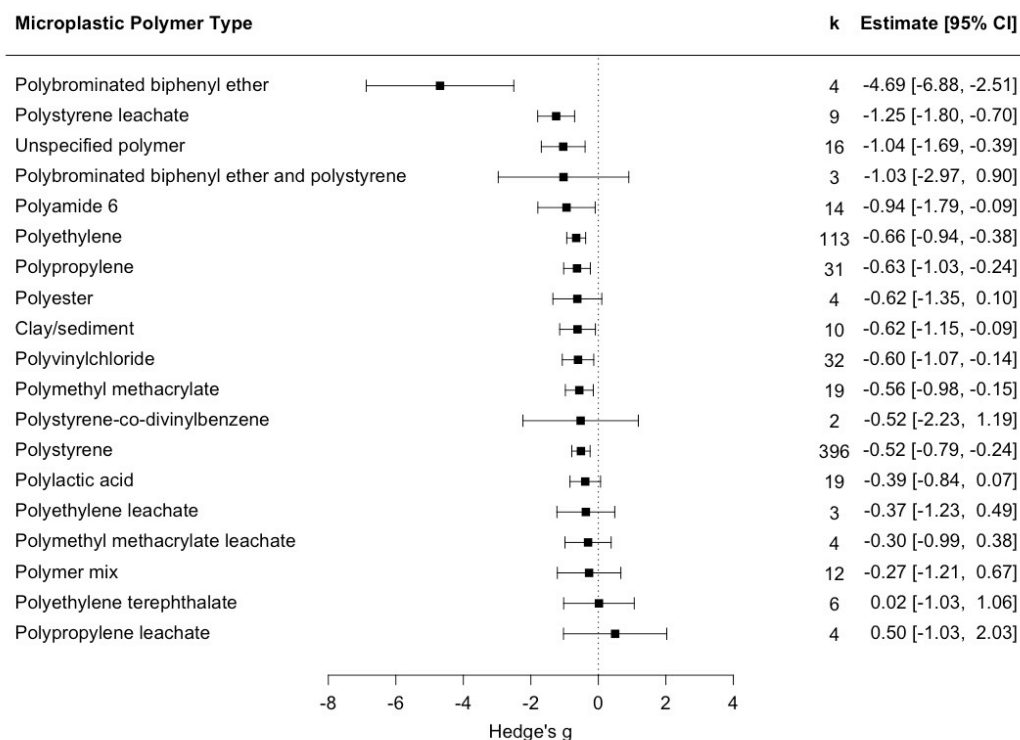
83

84 **Figure S4.** Relationship between log transformed microplastic exposure concentration ( $\text{g L}^{-1}$ )  
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.



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

## 4.0 SUPPLEMENTARY MATERIALS REFERENCES

Albano M, Panarello G, Paola D Di, Capparucci F, Crupi R, Gugliandolo E, Spanò N, Capillo G, Savoca S (2021) The Influence of Polystyrene Microspheres Abundance on Development and Feeding Behavior of *Artemia salina* (Linnaeus, 1758). *Appl Sci* 2021, Vol 11, Page 3352 11:3352

105 Anderson G, Shenkar N (2021) Potential effects of biodegradable single-use items in the sea:  
 106 Polylactic acid (PLA) and solitary ascidians. *Environ Pollut* 268:115364

107 Beiras R, Bellas J, Cachot J, Cormier B, Cousin X, Engwall M, Gambardella C, Garaventa F,  
 108 Keiter S, Le Bihanic F, López-Ibáñez S, Piazza V, Rial D, Tato T, Vidal-Liñán I (2018)  
 109 Ingestion and contact with polyethylene microplastics does not cause acute toxicity on  
 110 marine zooplankton. *J Hazard Mater* 360:452–460

111 Beiras R, Tato T (2019) Microplastics do not increase toxicity of a hydrophobic organic  
 112 chemical to marine plankton. *Mar Pollut Bull* 138:58–62

113 Berry KLE, Epstein HE, Lewis PJ, Hall NM, Negri AP (2019) Microplastic contamination  
 114 has limited effects on coral fertilisation and larvae. *Diversity* 11:1–13

115 Bertucci JJ, Bellas J (2021) Combined effect of microplastics and global warming factors on  
 116 early growth and development of the sea urchin (*Paracentrotus lividus*). *Sci Total*  
 117 *Environ* 782:146888

118 Besseling E, Wegner A, Foekema EM, Van Den Heuvel-Greve MJ, Koelmans AA (2013)  
 119 Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola*  
 120 *marina* (L.). *Environ Sci Technol* 47:593–600

121 Bour A, Haarr A, Keiter S, Hylland K (2018) Environmentally relevant microplastic  
 122 exposure affects sediment-dwelling bivalves. *Environ Pollut* 236:652–660

123 Bourdages MPT, Provencher JF, Sudlovenick E, Ferguson SH, Young BG, Pelletier N,  
 124 Murphy MJJ, D’Addario A, Vermaire JC (2020) No plastics detected in seal (Phocidae)  
 125 stomachs harvested in the eastern Canadian Arctic. *Mar Pollut Bull* 150

126 Bringer A, Cachot J, Prunier G, Dubillot E, Clérandeau C, Hélène Thomas (2020a)  
 127 Experimental ingestion of fluorescent microplastics by pacific oysters, *Crassostrea*  
 128 *gigas*, and their effects on the behaviour and development at early stages. Chemosphere  
 129 254:1–10

130 Bringer A, Thomas H, Prunier G, Dubillot E, Bossut N, Churlaud C, Clérandeau C, Le  
 131 Bihanic F, Cachot J (2020b) High density polyethylene (HDPE) microplastics impair  
 132 development and swimming activity of Pacific oyster D-larvae, *Crassostrea gigas*,  
 133 depending on particle size. Environ Pollut 260

134 Bruck S, Ford AT (2018) Chronic ingestion of polystyrene microparticles in low doses has no  
 135 effect on food consumption and growth to the intertidal amphipod *Echinogammarus*  
 136 *marinus*? Environ Pollut 233:1125–1130

137 Capolupo M, Franzellitti S, Valbonesi P, Lanzas CS, Fabbri E (2018) Uptake and  
 138 transcriptional effects of polystyrene microplastics in larval stages of the Mediterranean  
 139 mussel *Mytilus galloprovincialis*. Environ Pollut 241:1038–1047

140 Carrasco A, Pulgar J, Quintanilla-Ahumada D, Perez-Venegas D, Quijón PA, Duarte C  
 141 (2019) The influence of microplastics pollution on the feeding behavior of a prominent  
 142 sandy beach amphipod, *Orchestoidea tuberculata* (Nicolet, 1849). Mar Pollut Bull  
 143 145:23–27

144 Chapron I, Peru E, Engler A, Ghiglione JF, Meistertzheim AL, Pruski AM, Purser A, Vétion  
 145 G, Galand PE, Lartaud F (2018) Macro- and microplastics affect cold-water corals  
 146 growth, feeding and behaviour. Sci Rep 8:1–8

147 Cole M, Galloway TS (2015) Ingestion of Nanoplastics and Microplastics by Pacific Oyster  
148 Larvae. *Environ Sci Technol* 49:14625–14632

149 Corinaldesi C, Canensi S, Dell’Anno A, Tangherlini M, Di Capua I, Varrella S, Willis TJ,  
150 Cerrano C, Danovaro R (2021) Multiple impacts of microplastics can threaten marine  
151 habitat-forming species. *Commun Biol* 4

152 Cormier B, Gambardella C, Tato T, Perdriat Q, Costa E, Veclin C, Le Bihanic F, Grassl B,  
153 Dubocq F, Kärman A, Van Arkel K, Lemoine S, Lagarde F, Morin B, Garaventa F,  
154 Faimali M, Cousin X, Bégout ML, Beiras R, Cachot J (2021) Chemicals sorbed to  
155 environmental microplastics are toxic to early life stages of aquatic organisms.  
156 *Ecotoxicol Environ Saf* 208:111665

157 Critchell K, Hoogenboom MO (2018) Effects of microplastic exposure on the body condition  
158 and behaviour of planktivorous reef fish (*Acanthochromis polyacanthus*). *PLoS One*  
159 13:e0193308

160 Crump A, Mullens C, Bethell EJ, Cunningham EM, Arnott G (2020) Microplastics disrupt  
161 hermit crab shell selection. *Biol Lett* 16

162 de Barros M, Dos Santos Calado T, de Sá Leitão Câmara de Araújo M (2020) Plastic  
163 ingestion lead to reduced body condition and modified diet patterns in the rocky shore  
164 crab *Pachygrapsus transversus* (Gibbes, 1850) (Brachyura: Grapsidae). *Mar Pollut Bull*  
165 156

166 Détrée C, Gallardo-Escárate C (2018) Single and repetitive microplastics exposures induce  
167 immune system modulation and homeostasis alteration in the edible mussel *Mytilus*  
168 *galloprovincialis*. *Fish Shellfish Immunol* 83:52–60



169 Diana Z, Sawickij N, Rivera NA, Hsu-Kim H, Rittschof D (2020) Plastic pellets trigger  
170 feeding responses in sea anemones. *Aquat Toxicol* 222:105447

171 Doyle D, Frias J, Nash R, Gammell M (2020) Current environmental microplastic levels do  
172 not alter emergence behaviour in the intertidal gastropod *Littorina littorea*. *Mar Pollut*  
173 *Bull* 151

174 Duval S and Tweedie R L (2000) Trim and fill: A simple funnel-plot-based method of testing  
175 and adjusting for publication bias in meta-analysis. *Biometrics* 56:455-463

176 Egbeocha CO, Malek S, Emenike CU, Milow P (2018) Feasting on microplastics: Ingestion  
177 by and effects on marine organisms. *Aquat Biol* 27:93–106

178 Eom H-J, Nam S-E, Rhee J-S (2020) Polystyrene microplastics induce mortality through  
179 acute cell stress and inhibition of cholinergic activity in a brine shrimp. *Mol Cell*  
180 *Toxicol* 2020 163 16:233–243

181 Farrell P, Nelson K (2013) Trophic level transfer of microplastic: *Mytilus edulis* (L.) to  
182 *Carcinus maenas* (L.). *Environ Pollut* 177:1–3

183 Gambardella C, Morgana S, Bramini M, Rotini A, Manfra I, Migliore I, Piazza V, Garaventa  
184 F, Faimali M (2018) Ecotoxicological effects of polystyrene microbeads in a battery of  
185 marine organisms belonging to different trophic levels. *Mar Environ Res* 141:313–321

186 Gandara E Silva PP, Nobre CR, Resaffe P, Pereira CDS, Gusmão F (2016) Leachate from  
187 microplastics impairs larval development in brown mussels. *Water Res.* 106:364-370

188 Gardon T, Reisser C, Soyeux C, Quillien V, Le Moullac G (2018) Microplastics Affect Energy  
 189 Balance and Gametogenesis in the Pearl Oyster *Pinctada margaritifera*. Environ Sci  
 190 Technol 52:5277–5286

191 González-Soto N, Hatfield J, Katsumiti A, Duroudier N, Lacave JM, Bilbao E, Orbea A,  
 192 Navarro E, Cajaraville MP (2019) Impacts of dietary exposure to different sized  
 193 polystyrene microplastics alone and with sorbed benzo[a]pyrene on biomarkers and  
 194 whole organism responses in mussels *Mytilus galloprovincialis*. Sci Total Environ  
 195 684:548–566

196 Gray A, Weinstein J (2017) Size- and shape-dependent effects of microplastic particles on  
 197 adult daggerblade grass shrimp (*Palaemonetes pugio*). Environ Toxicol Chem 36:3074–  
 198 3080

199 Green DS (2016a) Effects of microplastics on European flat oysters, *Ostrea edulis* and their  
 200 associated benthic communities. Environ Pollut 216:95–103

201 Green DS, Boots B, O'Connor NE, Thompson R (2017) Microplastics Affect the Ecological  
 202 Functioning of an Important Biogenic Habitat. Environ Sci Technol 51:68–77

203 Green DS, Boots B, Sigwart J, Jiang S, Rocha C (2016b) Effects of conventional and  
 204 biodegradable microplastics on a marine ecosystem engineer (*Arenicola marina*) and  
 205 sediment nutrient cycling. Environ Pollut 208:426–434

206 Green DS, Colgan TJ, Thompson RC, Carolan JC (2019) Exposure to microplastics reduces  
 207 attachment strength and alters the haemolymph proteome of blue mussels (*Mytilus*  
 208 *edulis*). Environ Pollut 246:423–434

209 Gu H, Wei S, Hu M, Wei H, Wang X, Shang Y, Li I, Shi H, Wang Y (2020) Microplastics  
 210 aggravate the adverse effects of BDE-47 on physiological and defense performance in  
 211 mussels. *J Hazard Mater* 398:122909

212 Hämer J, Gutow I, Köhler A, Saborowski R (2014) Fate of Microplastics in the Marine  
 213 Isopod *Idotea emarginata*. *Environ Sci Technol* 48:13451–13458

214 Han X, Zheng Y, Dai C, Duan H, Gao M, Ali MR, Sui I (2020) Effect of polystyrene  
 215 microplastics and temperature on growth, intestinal histology and immune responses of  
 216 brine shrimp *Artemia franciscana*. *J Oceanol Limnol* 2020 393 39:979–988

217 Hankins C, Moso E, Lasseigne D (2021) Microplastics impair growth in two atlantic  
 218 scleractinian coral species, *Pseudodiploria clivosa* and *Acropora cervicornis*. *Environ*  
 219 *Pollut* 275:116649

220 Hariharan G, Purvaja R, Anandavelu I, Robin RS, Ramesh R (2021) Accumulation and  
 221 ecotoxicological risk of weathered polyethylene (wPE) microplastics on green mussel  
 222 (*Perna viridis*). *Ecotoxicol Environ Saf* 208

223 Hope JA, Coco G, Thrush SF (2020) Effects of Polyester Microfibers on Microphytobenthos  
 224 and Sediment-Dwelling Infauna. *Environ Sci Technol* 54:7970–7982

225 Horn D, Granek E, Steele C (2019) Effects of Environmentally Relevant Concentrations of  
 226 Microplastic Fibers on Pacific Mole Crab (*Emerita analoga*) Mortality and  
 227 Reproduction. *Limnol Oceanogr Lett* 5:74–83

228 Hu I, Chernick M, Lewis AM, Ferguson PL, Hinton DE (2020) Chronic microfiber exposure  
 229 in adult Japanese medaka (*Oryzias latipes*). *PLoS One* 15:e0229962

230 Kaposi KL, Mos B, Kelaher BP, Dworjanyn SA (2014) Ingestion of microplastic has limited  
 231 impact on a marine larva. *Environ Sci Technol* 48:1638–1645

232 Korez Š, Gutow I, Saborowski R (2019) Feeding and digestion of the marine isopod *Idotea*  
 233 *emarginata* challenged by poor food quality and microplastics. *Comp Biochem Physiol*  
 234 Part - C Toxicol Pharmacol 226:108586

235 Langlet D, Bouchet VMP, Delaeter C, Seuront I (2020) Motion behavior and metabolic  
 236 response to microplastic leachates in the benthic foraminifera *Haynesina germanica*. *J*  
 237 *Exp Mar Bio Ecol* 529

238 Le Bihanic F, Clérandeau C, Cormier B, Crebassa JC, Keiter SH, Beiras R, Morin B, Bégout  
 239 ML, Cousin X, Cachot J (2020) Organic contaminants sorbed to microplastics affect  
 240 marine medaka fish early life stages development. *Mar Pollut Bull* 154

241 Leads RR, Burnett KG, Weinstein JE (2019) The Effect of Microplastic Ingestion on  
 242 Survival of the Grass Shrimp *Palaemonetes pugio* (Holthuis, 1949) Challenged with  
 243 *Vibrio campbellii*. *Environ Toxicol Chem* 38:2233–2242

244 Lee DH, Lee S, Rhee JS (2021) Consistent exposure to microplastics induces age-specific  
 245 physiological and biochemical changes in a marine mysid. *Mar Pollut Bull* 162:111850

246 Leung J, Chan KYK (2018) Microplastics reduced posterior segment regeneration rate of the  
 247 polychaete *Perinereis aibuhitensis*. *Mar Pollut Bull* 129:782–786

248 Li Z, Zhou H, Liu Y, Zhan J, Li W, Yang K, Yi X (2020) Acute and chronic combined effect  
 249 of polystyrene microplastics and dibutyl phthalate on the marine copepod *Tigriopus*  
 250 *japonicus*. *Chemosphere* 261:127711

251 Lo HKA, Chan KYK (2018) Negative effects of microplastic exposure on growth and  
 252 development of *Crepidula onyx*. Environ Pollut 233:588–595

253 Luan I, Wang X, Zheng H, Liu I, Luo X, Li F (2019) Differential toxicity of functionalized  
 254 polystyrene microplastics to clams (*Meretrix meretrix*) at three key development stages  
 255 of life history. Mar Pollut Bull 139:346–354

256 Martínez-Gómez C, León VM, Calles S, Gomáriz-Olcina M, Vethaak AD (2017) The  
 257 adverse effects of virgin microplastics on the fertilization and larval development of sea  
 258 urchins. Mar Environ Res 130:69–76

259 Mendrik FM, Henry TB, Burdett H, Hackney CR, Waller C, Parsons DR, Hennige SJ (2021)  
 260 Species-specific impact of microplastics on coral physiology. Environ Pollut  
 261 269:116238

262 Messinetti S, Mercurio S, Parolini M, Sugni M, Pennati R (2018) Effects of polystyrene  
 263 microplastics on early stages of two marine invertebrates with different feeding  
 264 strategies. Environ Pollut 237:1080–1087

265 Messinetti S, Mercurio S, Scari G, Pennati A, Pennati R (2019) Ingested microscopic plastics  
 266 translocate from the gut cavity of juveniles of the ascidian *Ciona intestinalis*. Eur Zool J  
 267 86:189–195

268 Missawi O, Bousserhine N, Zitouni N, Maisano M, Boughattas I, De Marco G, Cappello T,  
 269 Belbekhouche S, Guerrouache M, Alphonse V, Banni M (2021) Uptake, accumulation  
 270 and associated cellular alterations of environmental samples of microplastics in the  
 271 seaworm *Hediste diversicolor*. J Hazard Mater 406:124287

272 Mohsen M, Zhang I, Sun I, Lin C, Wang Q, Liu S, Sun J, Yang H (2021) Effect of chronic  
 273 exposure to microplastic fibre ingestion in the sea cucumber *Apostichopus japonicus*.  
 274 *Ecotoxicol Environ Saf* 209:111794

275 Mohsen M, Zhang I, Sun I, Lin C, Wang Q, Yang H (2020) Microplastic fibers transfer from  
 276 the water to the internal fluid of the sea cucumber *Apostichopus japonicus*. *Environ*  
 277 *Pollut* 257:113606

278 Mouchi V, Chapron I, Peru E, Pruski AM, Meistertzheim AL, Vétion G, Galand PE, Lartaud  
 279 F (2019) Long-term aquaria study suggests species-specific responses of two cold-water  
 280 corals to macro-and microplastics exposure. *Environ Pollut* 253:322–329

281 Murray F, Cowie P (2011) Plastic contamination in the decapod crustacean *Nephrops*  
 282 *norvegicus* (Linnaeus, 1758). *Mar Pollut Bull* 62:1207–1217

283 Nobre CR, Santana MFM, Maluf A, Cortez FS, Cesar A, Pereira CDS, Turra A (2015)  
 284 Assessment of microplastic toxicity to embryonic development of the sea urchin  
 285 *Lytechinus variegatus* (Echinodermata: Echinoidea). *Mar Pollut Bull* 92:99–104

286 Oliviero M, Tato T, Schiavo S, Fernández V, Manzo S, Beiras R (2019) Leachates of  
 287 micronized plastic toys provoke embryotoxic effects upon sea urchin *Paracentrotus*  
 288 *lividus*. *Environ Pollut* 247:706–715

289 Opitz T, Benítez S, Fernández C, Osorio S, Navarro JM, Rodríguez-Romero A, Lohrmann  
 290 KB, Lardies MA (2021) Minimal impact at current environmental concentrations of  
 291 microplastics on energy balance and physiological rates of the giant mussel  
 292 *Choromytilus chorus*. *Mar Pollut Bull* 162:111834

293 Peixoto D, Amorim J, Pinheiro C, Oliva-Teles I, Varó I, de Medeiros Rocha R, Vieira MN  
 294 (2019) Uptake and effects of different concentrations of spherical polymer  
 295 microparticles on *Artemia franciscana*. *Ecotoxicol Environ Saf* 176:211–218

296 Piccardo M, Provenza F, Grazioli E, Anselmi S, Terlizzi A, Renzi M (2021) Impacts of  
 297 Plastic-Made Packaging on Marine Key Species: Effects Following Water Acidification  
 298 and Ecological Implications. *J Mar Sci Eng* 2021, Vol 9, Page 432 9:432

299 Reichert J, Arnold AL, Hoogenboom MO, Schubert P, Wilke T (2019) Impacts of  
 300 microplastics on growth and health of hermatypic corals are species-specific. *Environ*  
 301 *Pollut* 254:113074

302 Reichert J, Schellenberg J, Schubert P, Wilke T (2018) Responses of reef building corals to  
 303 microplastic exposure. *Environ Pollut* 237:955–960

304 Rendell-Bhatti F, Paganos P, Pouch A, Mitchell C, D’Aniello S, Godley BJ, Pazdro K,  
 305 Arnone MI, Jimenez-Guri E (2020) Developmental toxicity of plastic leachates on the  
 306 sea urchin *Paracentrotus lividus*. *Environ Pollut* 269

307 Rist S, Baun A, Almeda R, Hartmann NB (2019) Ingestion and effects of micro- and  
 308 nanoplastics in blue mussel (*Mytilus edulis*) larvae. *Mar Pollut Bull* 140:423–430

309 Rist SE, Assidqi K, Zamani NP, Appel D, Perschke M, Huhn M, Lenz M (2016) Suspended  
 310 micro-sized PVC particles impair the performance and decrease survival in the Asian  
 311 green mussel *Perna viridis*. *Mar Pollut Bull* 111:213–220

312 Rocha R, Rodrigues A, Campos D, Cícero I, Costa A, Silva D, Oliveira M, Soares A, Patrício  
 313 Silva A (2020) Do microplastics affect the zoanthid *Zoanthus sociatus*? *Sci Total*  
 314 *Environ* 713

315 Rotjan RD, Sharp KH, Gauthier AE, Yelton R, Lopez EMB, Carilli J, Kagan JC, Urban-Rich  
 316 J (2019) Patterns, dynamics and consequences of microplastic ingestion by the  
 317 temperate coral, *Astrangia poculata*. *Proc R Soc B* 286

318 Santana MFM, Moreira FT, Pereira CDS, Abessa DMS, Turra A (2018) Continuous  
 319 Exposure to Microplastics Does Not Cause Physiological Effects in the Cultivated  
 320 Mussel *Perna perna*. *Arch Environ Contam Toxicol* 74:594–604

321 Sendra M, Sparaventi E, Blasco J, Moreno-Garrido I, Araujo C (2020) Ingestion and  
 322 bioaccumulation of polystyrene nanoplastics and their effects on the microalgal feeding  
 323 of *Artemia franciscana*. *Ecotoxicol Environ Saf* 188

324 Setälä O, Fleming-Lehtinen V, Lehtiniemi M (2014) Ingestion and transfer of microplastics  
 325 in the planktonic food web. *Environ Pollut* 185:77–83

326 Seuront I (2018) Microplastic leachates impair behavioural vigilance and predator avoidance  
 327 in a temperate intertidal gastropod. *Biol. Lett.* 14:20180453.2018045

328 Seuront I, Nicastro KR, McQuaid CD, Zardi GI (2021) Microplastic leachates induce species-  
 329 specific trait strengthening in intertidal mussels. *Ecol Appl* 31:1–10

330 Sıkdokur E, Belivermiş M, Sezer N, Pekmez M, Bulan ÖK, Kılıç Ö (2020) Effects of  
 331 microplastics and mercury on manila clam *Ruditapes philippinarum*: Feeding rate,  
 332 immunomodulation, histopathology and oxidative stress. *Environ Pollut* 262:114247

333 Suckling CC (2021) Responses to environmentally relevant microplastics are species-specific  
 334 with dietary habit as a potential sensitivity indicator. *Sci Total Environ* 751:142341



335 Suman TY, Jia PP, Li WG, Junaid M, Xin GY, Wang Y, Pei DS (2020) Acute and chronic  
 336 effects of polystyrene microplastics on brine shrimp: First evidence highlighting the  
 337 molecular mechanism through transcriptome analysis. *J Hazard Mater* 400:123220

338 Sussarellu R, Suquet M, Thomas Y, Lambert C, Fabioux C, Pernet MEJ, Goïc N Le, Quillien  
 339 V, Mingant C, Epelboin Y, Corporeau C, Guyomarch J, Robbens J, Paul-Pont I, Soudant  
 340 P, Huvet A (2016) Oyster reproduction is affected by exposure to polystyrene  
 341 microplastics. *Proc Natl Acad Sci U S A* 113:2430–2435

342 Syakti AD, Jaya JV, Rahman A, Hidayati NV, Raza'i TS, Idris F, Trenggono M, Doumenq P,  
 343 Chou LM (2019) Bleaching and necrosis of staghorn coral (*Acropora formosa*) in  
 344 laboratory assays: Immediate impact of LDPE microplastics. *Chemosphere* 228:528–  
 345 535

346 Tallec K, Huvet A, Di Poi C, González-Fernández C, Lambert C, Petton B, Le Goïc N,  
 347 Berchel M, Soudant P, Paul-Pont I (2018) Nanoplastics impaired oyster free living  
 348 stages, gametes and embryos. *Environ Pollut* 242:1226–1235

349 Maes T, Barry J, Stenton C, Roberts E, Hicks R, Bignell J, Vethaak AD, Leslie HA and  
 350 Sanders M (2020) The world is your oyster: low-dose, long-term microplastic exposure  
 351 of juvenile oysters. *Heliyon* 6:e03103

352 Thomas PJ, Oral R, Pagano G, Tez S, Toscanesi M, Ranieri P, Trifuoggi M, Lyons DM  
 353 (2020) Mild toxicity of polystyrene and polymethylmethacrylate microplastics in  
 354 *Paracentrotus lividus* early life stages. *Mar Environ Res* 161:105132

355 Torn K (2020) Microplastics uptake and accumulation in the digestive system of the mud  
 356 crab *Rhithropanopeus harrisii*. *Proc Est Acad Sci* 69:35–42

357 Tosetto I, Brown C, Williamson JE (2016) Microplastics on beaches: ingestion and  
 358 behavioural consequences for beachhoppers. *Mar Biol* 163

359 Trifuoggi M, Pagano G, Oral R, Pavičić-Hamer D, Burić P, Kovačić I, Siciliano A, Toscanesi  
 360 M, Thomas PJ, Paduano I, Guida M, Lyons DM (2019) Microplastic-induced damage in  
 361 early embryonal development of sea urchin *Sphaerechinus granularis*. *Environ Res*  
 362 179:108815

363 Ugolini A, Ungherese G, Ciofini M, Lapucci A, Camaiti M (2013) Microplastic debris in  
 364 sandhoppers. *Estuar Coast Shelf Sci* 129:19–22

365 Urban-Malinga B, Jakubowska M, Białowas M (2021) Response of sediment-dwelling  
 366 bivalves to microplastics and its potential implications for benthic processes. *Sci Total*  
 367 *Environ* 769:144302

368 Van Cauwenberghe I, Claessens M, Vandegehuchte MB, Janssen CR (2015) Microplastics  
 369 are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in  
 370 natural habitats. *Environ Pollut* 199:10–17

371 Van Cauwenberghe I, Janssen CR (2014) Microplastics in bivalves cultured for human  
 372 consumption. *Environ Pollut* 193:65–70

373 Van Colen C, Vanhove B, Diem A, Moens T (2020) Does microplastic ingestion by  
 374 zooplankton affect predator-prey interactions? An experimental study on larviphagy.  
 375 *Environ Pollut* 256:113479

376 Viechtbauer W (2010) Conducting meta-analyses in R with the metafor package. *Journal of*  
 377 *Statistical Software*, 36(3), 1-48. <https://doi.org/10.18637/jss.v036.i03>

378 Violle C, Navas M-L, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E (2007) Let the  
 379 concept of trait be functional! *Oikos* 116:882–892

380 Wakkaf T, Allouche M, Harrath AH, Mansour I, Alwasel S, Mohamed Thameemul Ansari  
 381 KG, Beyrem H, Sellami B, Boufahja F (2020) The individual and combined effects of  
 382 cadmium, polyvinyl chloride (PVC) microplastics and their polyalkylamines modified  
 383 forms on meiobenthic features in a microcosm. *Environ Pollut* 266:115263

384 Wang M, Wang X, Luo X, Zheng H (2017) Short-term toxicity of polystyrene microplastics  
 385 on mysid shrimps *Neomysis japonica*. *IOP Conf Ser Earth Environ Sci Pap* 61:012136

386 Wang S, Zhong Z, Li Z, Wang X, Gu H, Huang W, Fang JKH, Shi H, Hu M, Wang Y (2021)  
 387 Physiological effects of plastic particles on mussels are mediated by food presence. *J*  
 388 *Hazard Mater* 404:124136

389 Wang X, Liu I, Zheng H, Wang M, Fu Y, Luo X, Li F, Wang Z (2020) Polystyrene  
 390 microplastics impaired the feeding and swimming behavior of mysid shrimp *Neomysis*  
 391 *japonica*. *Mar Pollut Bull* 150:110660

392 Wang Y, Zhang D, Zhang M, Mu J, Ding G, Mao Z, Cao Y, Jin F, Cong Y, Wang I, Zhang  
 393 W, Wang J (2019) Effects of ingested polystyrene microplastics on brine shrimp,  
 394 *Artemia parthenogenetica*. *Environ Pollut* 244:715–722

395 Watts AJR, Urbina MA, Corr S, Lewis C, Galloway TS (2015) Ingestion of Plastic  
 396 Microfibers by the Crab *Carcinus maenas* and Its Effect on Food Consumption and  
 397 Energy Balance. *Environ Sci Technol* 49:14597–14604

398 Webb S, Gaw S, Marsden ID, McRae NK (2020) Biomarker responses in New Zealand  
 399 green-lipped mussels *Perna canaliculus* exposed to microplastics and triclosan.  
 400 *Ecotoxicol Environ Saf* 201:110871

401 Welden NAC, Cowie PR (2016) Long-term microplastic retention causes reduced body  
 402 condition in the langoustine, *Nephrops norvegicus*. *Environ Pollut* 218:895–900

403 Woods MN, Hong TJ, Baughman D, Andrews G, Fields DM, Matrai PA (2020)  
 404 Accumulation and effects of microplastic fibers in American lobster larvae (*Homarus*  
 405 *americanus*). *Mar Pollut Bull* 157:111280

406 Wright SL, Rowe D, Reid MJ, Thomas K V., Galloway TS (2015) Bioaccumulation and  
 407 biological effects of cigarette litter in marine worms. *Sci Rep* 5

408 Xu XY, Lee WT, Chan AKY, Lo HS, Shin PKS, Cheung SG (2017) Microplastic ingestion  
 409 reduces energy intake in the clam *Atactodea striata*. *Mar Pollut Bull* 124:798–802

410 Yap VHS, Chase Z, Wright JT, Hurd CL, Lavers JL, Lenz M (2020) A comparison with  
 411 natural particles reveals a small specific effect of PVC microplastics on mussel  
 412 performance. *Mar Pollut Bull* 160:111703

413 Yu J, Tian JY, Xu R, Zhang ZY, Yang GP, Wang XD, Lai JG, Chen R (2020) Effects of  
 414 microplastics exposure on ingestion, fecundity, development, and dimethylsulfide  
 415 production in *Tigriopus japonicus* (Harpacticoida, copepod). *Environ Pollut* 267:115429

416 Yu P, Liu Z, Wu D, Chen M, Lv W, Zhao Y (2018) Accumulation of polystyrene  
 417 microplastics in juvenile *Eriocheir sinensis* and oxidative stress effects in the liver.  
 418 *Aquat Toxicol* 200:28–36

419 Yu SP, Chan BKK (2020b) Effects of polystyrene microplastics on larval development,  
420 settlement, and metamorphosis of the intertidal barnacle *Amphibalanus amphitrite*.  
421 Ecotoxicol Environ Saf 194:110362

422 Yu SP, Chan BKK (2020c) Intergenerational microplastics impact the intertidal barnacle  
423 *Amphibalanus amphitrite* during the planktonic larval and benthic adult stages. Environ  
424 Pollut 267:115560

425 Zhang C, Wang S, Sun D, Pan Z, Zhou A, Xie S, Wang J, Zou J (2020) Microplastic  
426 pollution in surface water from east coastal areas of Guangdong, South China and  
427 preliminary study on microplastics biomonitoring using two marine fish. Chemosphere  
428 256:127202

429

- 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