Chronic ingestion of polystyrene microparticles in low doses has no effect on food consumption and growth to the intertidal amphipod *Echinogammarus marinus*?

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ABSTRACT

The ingestion of microplastics (plastic particles <5 mm) has been observed in a range of marine organisms, and adverse effects have been reported in several species after high concentration exposure. However, the long-term effects of low-dose ingestion remains unclear. The aim of this study was thus to assess the chronic effects of low concentrations of polystyrene microparticles to the intertidal amphipod *Echinogammarus marinus*, using food consumption, growth, and moulting as endpoints. Amphipods were fed a gelatinous algal feed spiked with microbeads (8 µm) in concentrations of ~0.9, 9 and 99 microplastics/g for 35 days. *E. marinus* was also analysed for retention of microplastics, and egestion rate was calculated in a separate high-dose feeding experiment. No significant effects were found in the food consumption or growth assays. There was no accumulation of microplastics in the gut, with only one microbead recorded internally in three (8%) of the exposed amphipods. The low number is likely linked to gastrointestinal functions, allowing for easy egestion of indigestible items. This assumption was supported by the observation that after high-dose exposure, 60 % of *E. marinus* egested all microbeads within 24 hours. This study suggests that ingesting low concentrations of 8µm microplastics do not impair the feeding or growth of amphipods along the exposure period. We hope that negative results such as these may further assist in assessing the impact posed by microplastics to marine organisms.

Keywords: Microplastics, ingestion, growth, feeding, *Echinogammarus marinus*, amphipod, chronic, low-dose

1. INTRODUCTION

Plastics are considered to be one of the major current anthropogenic threats to the marine environment and are known to cause harm to a range of aquatic organisms (Bergmann *et al.*, 2015; Gall & Thompson, 2015). Global plastic production exceeded 322 million tons in 2014 (PlasticsEurope, 2016) and an estimated ~8 million tons of plastic entered the oceans in 2010, mainly wind- or waterborne from land based sources (Jambeck *et al.*, 2015). Plastics are not readily broken down in the environment, but rather fragmented through weathering and abrasion into continuously smaller and smaller particles, and if entering the marine realm they can persist for decades as microscopic plastic particles (<5mm), known as microplastics (Andrady, 2011).

Microplastics have been subject of an increasing number of studies over the last decade, both due to their widespread presence in the marine environment (Cole *et al.*, 2011) as well as their potential to affect even microscopic organisms (Cole *et al.*, 2013). The concentration of plastic particles in the aquatic environment is highly variable, generally with higher levels in waterbodies and sediments close to urban settlements (Browne *et al.*, 2011; Mathalon & Hill, 2014). In coastal waters of the southern North Sea, for example, surface samples filtered through a 40 µm mesh contained up to 64±194 plastic particles/L and 88±82 plastic fibres/L (Dubaish & Liebezeit, 2013). and recent studies from around the British Isles show that microplastics are present in close to every sample, both sediment (Blumenröder *et al.*, 2017; Devriese *et al.*, 2015) and surface (Maes *et al.*, 2017). In the estuarine waters around Southampton (UK), microplastics were found in every single surface trawl samples with fibres being the dominant plastic type (Gallagher *et al.*, 2016). However, there are substantial spatial and temporal variations in the reported concentrations (Barnes *et al.*, 2009; Browne *et al.*, 2010; Cole *et al.*, 2011; Lima *et al.*, 2014), and comparison between samples are often challenging due to a lack of standardisation in sampling and quantification methods (Phuong *et al.*, 2016).

Recent research have revealed the environmental presence of microplastics in a number of marine invertebrates, including bivalves (Van Cauwenberghe *et al.*, 2015) and crustaceans (Devriese *et al.*, 2015; Murray & Cowie, 2011). In the latter taxon, adverse effects of plastic uptake such as growth reduction (Au *et al.*, 2015), altered frequency of moulting events (Bergami *et al.*, 2016), and lowered feeding rates (Cole *et al.*, 2015; Watts *et al.*, 2015) have

64 been observed in lab based studies. Translocation of particles to tissues has also been
65 reported (Brennecke *et al.*, 2015; Farrell & Nelson, 2013). Despite the vast number of
66 published studies concerning microplastic uptake and effect, few studies have investigated
67 the chronic impact of microplastic exposure. The majority of studies have focused on acute
68 assessments with concentrations of microplastics orders of magnitude higher than what is
69 typically encountered in the environment (Phuong *et al.*, 2016). Whilst this allows us to gain
70 insight to potential endpoints and pathways of effect, there is a need to better understand the
71 current risks microplastics pose to aquatic organisms in the environment.

72 The aim of this study was thus to assess the chronic effects of ingesting low concentrations of
73 microplastic in an aquatic invertebrate. The intertidal amphipod *Echinogammarus marinus*
74 was chosen as a model species due to its estuarine habitat being at particular risk of plastic
75 pollution (Gallagher *et al.*, 2016; Lima *et al.*, 2014; Mathalon & Hill, 2014), as well as for its
76 central position in the food web, both as predator (Dick *et al.*, 2005) and prey (Múrias *et al.*,
77 1996). Recent evidence suggest that microplastics can adhere to the mucus of *Fucus*
78 *vesiculosus* (Gutow *et al.*, 2015), a favoured seaweed for *E. marinus* (Martins *et al.*, 2014).
79 This may thus represent an important exposure pathway. Furthermore, the gammarids might
80 be exposed to microplastics from its prey, making dietary exposure is a realistic
81 environmental scenario for this gammarid.

82 Based on the findings reported from other studies on crustaceans (Cole *et al.*, 2015; Watts *et*
83 *al.*, 2014), we hypothesised that

- 84 (1) microplastics would be retained in the gut, and disturb food consumption and
85 nutritional uptake, and
- 86 (2) microplastics would translocate to tissues, thereby compromising the animals' energy
87 uptake and growth.

88 To test the hypothesis, two main experiments were conducted. A five-week experiment
89 assessed the effects of microplastic ingestion on growth related endpoints, measured by food
90 consumption, weight increment, and frequency of moulting. We also performed an acute
91 feeding experiment to estimate the retention time of plastic beads in the gut.

93 2. METHODS

2.1 Food and contaminant preparation

Fucus vesiculosus was collected from Langstone Harbour, (Portsmouth, UKGPS coordinates 50.789632, -1.041613) during the first two weeks of January 2016. The collected seaweed was thoroughly cleaned, snap frozen in liquid nitrogen, and freeze dried in a Benchtop Lyophilizer. The *F. vesiculosus* was then powdered in a kitchen blender and stored in an airtight glass jar.

A gelatinous feed was made from the powdered seaweed and Agar, adapting the methodology described in Hämer *et al.* (2014). The ratio was adjusted to 0.8 g seaweed powder, and 0.13 g Agarose per 10 mL glass bead filtered seawater, and the thickness of the food blocks was increased to approximately 3 mm. A stock solution of microbeads (8 µm Fluoro-Max Red fluorescent polystyrene microspheres, Thermo Scientific) was prepared, and quantified to ~10 000 microplastics/g by haemocytometer counts. Plastic particles in concentrations of 1, 10, and 100 particles per mL liquid in the seaweed feed were pipetted into microplastics/mL the food mixture as the Agar was cooling (40-42 °C). The mixture was carefully stirred before allowing to set. Equally sized circular blocks (Ø = 34 mm, mean weight 4.182 ± 1.025) were cut from the food, and refrigerated at 4 C° until it was distributed to the animals within no more than a week. Homogeneity of particles in and between food blocks was confirmed by checking random blocks under the fluorescent microscope. In its set state, the wet weight of the seaweed feed was 1.09 g per mL. The per gram microplastic concentration in the food thus equates to 0.9, 9, and 99 microplastics/g, which will be the unit referred to for this study.

2.2 Chronic ingestion experiment

E. marinus were collected from Langstone Harbour (Southern England, UK) at the same location as the *F. vesiculosus*, in April 2016. To reduce variation caused by females in different stages of oogenesis and brood development, only adult males were used for the experiment. The animals were acclimatized and accustomed to the artificial feed for 2 weeks prior to the experiment. During the experiment, 60 animals were kept in 30 litre aquarium tanks, with three replicate tanks per concentration, each tank containing 5 replicate test specimens. Natural seawater was filtered through a 4-weir sedimentation system following by glass bead and sand filtration and pumped on a constant flow in to each tank through the Institute of Marine Sciences flow-through seawater systems, reflecting actual environmental

conditions. Water parameters (temperature, pH, salinity and dissolved oxygen) of inflowing water were constantly monitored. The aquarium operated with a 12:12h light-dark cycle.

Prior to the experiment, each individual's weight was recorded. Whilst each animal in each tank was weighed individually, the animals were not marked, and the individual weight gain or loss could thus not be tracked. The recorded weights of each animal in the tank was therefore summarised and the mean weight per tank calculated. Each surviving animal was weighed again upon termination of the experiment.

The growth assay (G) was recorded as the change between measured weight at the beginning and at the end of the experiment, and was determined by:

$$G = W_f - W_s$$

with W_s being the mean start weight and W_f being the mean final weight.

The amphipods were fed once per week. Pre-cut food blocks were chosen at random, and saturated in seawater for a minimum of 30 minutes. Individual beakers were used to avoid cross contamination between concentrations. Each block was weighed before being distributed to the respective tanks. Every 7 days the remaining food from each tank was weighed, and the mean food consumption (C) per animal was calculated as:

$$C = (F_g - F_r) / (N - D)$$

where F_g and F_r represents the weight of food given and food remaining, respectively. N is the initial number of animals, D the number of deaths recorded.

Moult and dead individuals were counted and removed three times per week. In cases of mortality the animal was excluded from subsequent analysis.

Upon terminating the experiment, the amphipods guts and digestive midgut glands were dissected, and a quantitative analysis for retention and translocation of micro-particles was performed using a Leica MZ10F fluorescent microscope.

2.3 Microplastic egestion experiment

To assess microplastic egestion rate, 50 male and female *E. marinus* were kept in a 20 litre bucket of aerated seawater and fed artificial seaweed food spiked with >10 000 microplastics/g for two weeks. The greater concentration used for this experiment was chosen to ensure reliable detection of microplastic particles through the digestive system. Water was

exchanged and fresh food supplied twice per week. After 14 days, six individuals were dissected to confirm ingestion of particles. The animals were then rinsed in running seawater to remove any externally attached particles. To assess the difference in egestion rate with and without the presence of food, the remaining animals were divided into two experimental groups, with 22 individuals in each group. One group was fed uncontaminated food, whilst the other group was starved. Five individuals from each group were dissected and analysed for plastic particles every 24 hours, until no particles were observed in the gut of any of the dissected animals.

2.4 Statistical analysis

Normality and homogeneity of variances of the dataset was first assessed using a one sample Shapiro-Wilk test and Levene's test respectively. A Generalized Linear Model (GzLM) was conducted for the growth assay, comparing the weights at the start and the end of the experiment, taking into account variation amongst the individuals within each replicate tank nested in each concentration. To measure the effects on food consumption, a repeated measures ANOVA was performed.

A Pearson's product-moment correlation coefficient was used to determine the relationship between food consumption and growth, whilst linear regression analysis were performed to assess the relationship between food consumption and measured water parameters. The number of moulting events per replicate and mortality was compared by chi-square analysis, and a Spearman rank-order correlation coefficient was performed to assess the correlation between moulting and death.

Differences in egestion between animals that had been fed uncontaminated food versus those who had been starved following microplastic ingestion, was calculated by chi-square analysis.

Statistics were done using SPSS software (Version 22). For all tests results were considered significant if $p < 0.05$.

3. RESULTS

3.1 Chronic effects of microplastic ingestion

After five weeks of exposure, no significant differences were observed in the weight change between treatments when taking into account replicate tanks nested within each concentration ($X = 3.534$, $df = 8$, $p = 0.897$; Figure 1). The highest increase in weight was amongst the control animals who had a $13.6 \% \pm 13.1 \%$ mean weight gain, whilst a slight mean decrease of $-0.6 \% \pm 1.2 \%$ was observed in the 9 microplastics/g microplastic treatment. No significant differences were observed in the overall weight before and after the experiment ($X = 2.027$, $df = 1$, $p = 0.155$) and no interaction between treatment and start/end weights ($p = 0.707$).

Figure 1: Mean weight of *Echinogammarus marinus* recorded at the start of the experiment and at the end of the experiment following diets of microplastics. Data expressed in $g \pm SD$. $n = 11-15$.

On average, the animals consumed 0.736 ± 0.267 g of food per week. No significant difference was found in food consumption between the exposed and control animals (repeated measure ANOVA $F_{3,8} = 1.920$, $p > 0.05$; Figure 2). However, a significant increase (ANOVA, $F_{1,8} = 1883.533$, $p = <0.001$) in consumption rate was observed in all groups throughout the experiment (Figure 3). The increase in consumption was positively, although not significantly, linked to the $5\text{ }^{\circ}\text{C}$ rise in water temperature that was recorded between the first and the last week (Linear Regression, $F = 5.389$, $df = 1$, $p = 0.103$).

Figure 2 The mean amount of seaweed feed consumed per animal per week in the different treatments over the entire 5-week experiment. Data expressed in g per week ± SD. n = 11-15.

Figure 3. Mean consumption rate per animal per treatment each of the five weeks of the experiment. Data expressed in g per week ± SD. n = 11-15.

There were no significant differences in number of moults shed (Figure 4) between any of the treatments ($\chi^2 = 5.50$, $df = 3$, $p = 0.138$). Nor were there any significant differences in the mean number of days between moulting events (Kruskal-Wallis $X=3.024$, $df=3$, $p=0.388$).

Figure 4 Cumulative number of moults per treatment in *Echinogammarus marinus* over 35 days exposure.

Overall mortality was 20 %, with deaths recorded in all groups. Mortality was positively correlated to moulting events (Spearman's, $R_s(60) = 0.295$, $p = 0.021$), and not related to contaminant level (chi-square $X^2(3, N = 60) = 2.40$, $p = 0.494$). Of the 39 surviving animals exposed to plastics, only 3 (8 %) were confirmed to have microbeads in their gut, one from each exposure group. Each individual contained one single particle located in the midgut. One particle was also found in a fourth amphipod, however the particle was found outside the intestinal area and may be an artefact of cross contamination from external appendages during dissection. The observation was therefore excluded. The two animals from the higher concentration groups that were found with microbeads internally, had full guts and the food consumption recorded during the final week in their associated tanks was high. The third animal, which had been exposed to 0.9 microplastics/mL, had less than half full guts.

3.2 Egestion of microplastics

After two weeks of feeding on food spiked with >10 000 microplastics/g of microplastics, all of the six dissected animals had microbeads in their guts (>20 beads per individual). Twenty-four hours after being fed contaminated food for two weeks, 1 from 5 of the amphipods changed to an uncontaminated diet had particles (2) in its gut. All the dissected animals had full guts. In the group that was starved following the initial high-dose microplastic feeding, 3/5 animals had plastic beads (1-4) and small amounts of food in the gut. No particles were found in any of the dissected animals, fed or starved, after 48 hours.

239 4. Discussion

240 The aim of this study was to determine the effect of diets containing low quantities of
241 microplastics on the feeding and growth of an ecologically important intertidal grazing
242 amphipod. Our results revealed, despite the control group having the highest overall weight
243 gain and lowest food consumption, no significant differences compared to the groups
244 exposed to diets with microplastics for 5 weeks. Although the overall consumption
245 significantly increased during the course of the experiment, no significant differences were
246 observed in the consumption rates between any of the groups.

247 We originally hypothesised that the ingested microbeads would accumulate in the
248 gastrointestinal tract or translocate to the tissues, and consequently reduce nutritional uptake.
249 A lower weight gain amongst the exposed animals compared to control was therefore
250 expected. However, we observed no evidence of any gastrointestinal accumulation of
251 microplastics or translocation which could indicate a hindrance to normal digestion.

252 Increases in feeding rates in animals exposed to contamination, such as the elevated yet not
253 significant food consumption we observed in this experiment, are rarely reported, and the
254 mechanism behind this response is uncertain (Weis, 2015). It has been suggested that a slight
255 temporary increase in ingestion rate during microplastic feeding trials can be explained by a
256 need to consume higher quantities of food to meet nutritional requirements, as the ingested
257 microplastics are of no nutritional value (Watts *et al.*, 2015). The observed increase in food
258 consumption combined with the respective lower weight gain of all microplastic exposed
259 animals compared to controls (see Figures 1 and 2) is curious and could imply that the
260 microplastic contaminated feed may not have provided sufficient nutrition to supplement
261 growth. It is unclear whether the effects may have become more apparent had the experiment
262 persisted beyond 5 weeks, as not all animals moulted during the experiment, and those who
263 did moult may not have had sufficient time to gain weight. Indeed, no significant differences
264 were observed between the start and end weights although the mean weights of individuals
265 did increase across all treatments apart from the 10 particles per gram. This high variability
266 indicates the potential need to greater replication and longer term exposures in these kinds of
267 studies. Having 5 individual per tank reduced our capacity to monitor inter-individual
268 changes in weights and moulting and thus the statistical power of the analyses.

The overall consumption rate of *E. marinus* in the present study was markedly higher than previously recorded for the species. For example, Martins *et al.* (2014) recorded an average consumption of fresh *F. vesiculosus* of 0.29 g per g body mass per day in winter. In comparison, when similarly mass adjusting the daily consumption values for the present experiment using the overall mean consumption divided by the amphipods' mean final weight, the average food consumption was 0.86 g per g body weight. A possible explanation for this discrepancy could be a lower nutritional value of the artificial feed compared to the unprocessed algae which induced increased consumption.. According to literature, a peak in consumption in the spring time is common for the species (Maranhão *et al.*, 2001). This might further explain the elevated consumption rate, as well as the observed weekly increase in feeding relative to water temperature (see figure 3). Furthermore, the degradation of agar in seawater, as well as fragmentation of provided food through shredding by the amphipods during feeding and sheltering may have caused increased disintegration of the food blocks. Particulate matter in the tanks was not filtered to include fragments in the weight of the remaining food. Regrettably, we did not include an additional 'control' tank without gammarids to estimate the rate of food degradation. Consequently, the ingestion rate may have been overestimated although food loss would have been relatively standardised across treatments.

We observed no significant differences in the proportions of animals that moulted during the course of the experiment, or in the time between moults. The results vary between treatments with animals in the 0.9 microplastics/g exposure group moulting more frequently (16 times), yet gained comparatively less weight than the controls which moulted only 9 times in total. These discrepancies could suggest a biological response to the microplastics, as pollutants may alter the moulting cycle (Jimenez & Kinsey, 2015) or simply chance variation. Moulting is however also affected by both natural temporal and biological changes, as well as other external stressors (Maranhão & Marques, 2003; Marques & Nogueira, 1991). The negative results may have been influenced by the limitations in our experimental design. We could not track each individual's moulting cycle, and can thus not be certain of any correlations with the growth and feeding assays and exactly how these are influenced by the ingestion of microplastics. Moreover, moulting may occur in intervals of 4 weeks in older individuals, and several amphipods may naturally have been due to moult only once during the experiment. Therefore, experiments which monitor several intermoult periods would be able to better ascertain the true impact of moulting.

No accumulation of microplastics was observed. Ingested microbeads were only recorded in three of the surviving 39 animals exposed, each of these containing one single bead. This is low compared to other microplastic exposure experiments (see e.g. Cole *et al.*, 2015; Hämer *et al.*, 2014; Watts *et al.*, 2014). However, the concentrations used in the present experiment were considerably lower than in other studies, and microbead ingestion would consequently occur less frequently. Our results are nonetheless compatible to other chronic studies. For example, Au *et al.* (2015) exposed the amphipod *Hyaella Azteca* to microplastics in concentration between 5000 and 20 000 microplastics/mL. After 42 days of exposure, approximately one ingested microbead per individual was recorded. It is of concern that miniscule plastic particles may be within the same size fraction as particulate digestive matter and thus be absorbed by intestinal cell linings to the organism's tissues along with nutrients. However, we did not observe translocation of microplastics from the digestive tract to the midgut glands in any of the individuals, indicating that the epithelial cell membranes of *E. marinus* efficiently blocks particles 8 µm and larger. Thus far, translocation of ingested microplastics in the µm scale has not been demonstrated in other amphipods (Au *et al.*, 2015; Blarer & Burkhardt-Holm, 2016), nor in isopods (Hämer *et al.*, 2014). The phenomena has in fact only been reported in bivalves (Browne *et al.*, 2008) and crabs (Brennecke *et al.*, 2015; Farrell & Nelson, 2013), although the exact mechanisms behind the displacement in these species remains unclear. In our high dose particle egestion experiment we observed that the overall majority of the amphipods (60%) had no microplastics remaining in their guts after 24 h post feeding with particle contaminated diets, and all animals had eliminated the plastics after 48 h. This rapid passing of microplastics is consistent with the egestion times recorded in other amphipods. For example, *G. fossarum*, was found to eliminate micro-fibres within 16 hours (Blarer & Burkhardt-Holm, 2016). Although it is unclear whether particle concentration may influence the speed of passage, the efficient elimination of food and indigestible items may still serve as a further explanation to the low number of microplastics observed following the chronic experiment. Furthermore, the proportion of *E. marinus* that had not egested all microplastics was lower in the animals that were fed uncontaminated food after the initial two weeks of ingesting 10 000 microplastics/g compared to the starved individuals (1/5 and 3/5, respectively) after 24 h. It thus appears that the egestion of microbeads is slightly accelerated in the group that continued to feed. A more rapid egestion of microplastics in the presence of food has been reported from other experiments to crustaceans (Cole *et al.*, 2015), and is possibly owing to a mechanism that allows crustaceans to self-regulate gut passage depending on food availability, making them likely to slow their

egestion rate when food is scarce (Murtaugh, 1984). Considering the high consumption rates recorded in the last week of the chronic experiment, egestion rates would have been correspondingly high, adding to the understanding of the low number of microplastics retained in the exposed amphipods.

In our study, the amphipods were each week offered a block of food weighing 4.182 ± 1.025 g. With a particle concentration of ~0.9, 9, and 99 microplastics/g, the animals would have had < 4, 40 and 400 microplastics each throughout the experiment in the low, intermediate and high treatments, respectively. This makes the probability of consumption very low, especially for the amphipods in the lowest exposure group. It could thus be argued that the concentrations of microplastics offered to the amphipods were at level too low to alter feeding rate and cause disturbances to digestive functions or energy balance, as reflected in the results. However, our experimental doses do likely hold relevance. In a study by Gutow *et al.* (2015) adherence of microplastics to *F. vesiculosus* was assessed by exposing the seaweed to seawater containing 10 μ m microbeads in concentrations exceeding reported environmental levels by 3-4 orders of magnitude. The microplastic particles adhered to the surface in densities of < 0.1 particles per mm². As the current field sampling methodology most often excludes microplastic particles < 300 micron, the actual environmental concentrations of microplastics below this range is largely unknown and could exceed the concentrations estimated here. Nevertheless, assuming that the density of microplastics would be reduced with reduced particle concentration, we calculated that in a more ‘realistic’ exposure scenario 3 orders of magnitude lower, the particle adherence to *F. vesiculosus* would amount to ~ 0.38 microplastics/g. Based on *E. marinus*’ consumption rate of fresh *F. vesiculosus* (Martins *et al.*, 2014) and the amphipod weights recorded in our study, an average adult amphipod would in this scenario ingest around 0.14 plastic particles per week. Whilst the calculations above are hypothetical, it is less than the numbers ingested by the gammarids in this study’s lowest exposure. Nonetheless, it could be argued that even one single particle lodged in the gastrointestinal cell lining or translocated to tissues has the potential to trigger a negative response. Longer term exposures (full life cycle) with greater replication albeit logistically challenging would no doubt help answer some of these questions.

Overall, the results from this study indicate that *E. marinus*’ digestive system is capable of efficiently processing indigestible items, a capacity required for their opportunistic and varied feeding habit of ingesting small crustaceans, sand and sediment particles adhered to algae, as

well as their own cuticle (Dick *et al.*, 2005). The finding that microplastics did not impact the growth in *E. marinus* is in agreement with other chronic microplastic effect studies to crustaceans. For example, Hämer *et al.* (2014) fed *Ideotea emarginata* artificial food, similar to the feed provided in the present study, spiked with plastic beads, fragments, or fibres. No evidence of accumulation or translocation of plastic fragments was recorded, nor was any significant effects on growth rate or intermoult duration observed. Similarly, *G. fossarum* showed no significant reductions in weight after being exposed to high doses of plastic beads or fibres over a period of 28 days, although a significant reduction in metabolic efficiency was observed in the animals exposed to fibres (Blarer & Burkhardt-Holm, 2016). Metabolic efficiency was however elevated in the microbead exposure group. Conversely, in an environmentally realistic eight month experiment, plastic fibres were found to be retained in quantities reflecting environmental observations in the crustacean *Nephrops norvegicus*. The animals fed plastic fibres had lower food consumption, body mass, and metabolic rate than the controls, compromising their overall health (Welden & Cowie, 2016).

Despite disparities in experimental design, the results of the aforementioned chronic studies reflect the interspecies variability in susceptibility to ingested microplastics on the longer term. This highlights the importance of publishing all results of well-designed studies, whether confirming or rejecting the hypothesis. Undoubtedly, we need to understand how microplastic exposure adversely affects aquatic organisms, but it is also useful to eliminate the scenarios in which no significant consequence was detected. Through an unbiased and non-selective decision to publish results, a more holistic understanding of the topic can be obtained, along with a representative depiction of the impact. Access to the full range of studies performed, including the ones with a ‘negative’ outcome will furthermore provide other scientists a more comprehensive background on which to base their own experimental designs, consequently saving them time, effort, and money (Weintraub, 2016). In summary, the results reject our original hypothesis that microplastics are retained in the gut and translocated to tissues, which consequently would interfere with digestive functions and compromise the animals energy budget. Only 8 % of the animals had one single microbead retained in their gut, and the beads were readily egested. No beads were observed translocated to the tissues. There was no evidence of alteration to the animals feeding rate or growth. Within the parameters set for this study, we therefore find no evidence that 8 µm microbeads has any physiological effects on *E. marinus*. Impact may however be influenced by the size and shape of the plastics, and intraspecific sensitivity might diverge along the life

cycle. Taking into account that marine plastic pollution is predicted to increase (Jambeck *et al.*, 2015), it is important to continue elucidating the impact of plastic pollution to aquatic organisms for a comprehensive assessment of environmental impact and risk.

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