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The Impact of Polystyrene Microplastics on Feeding, Function and Fecundity in the Marine Copepod *Calanus helgolandicus*

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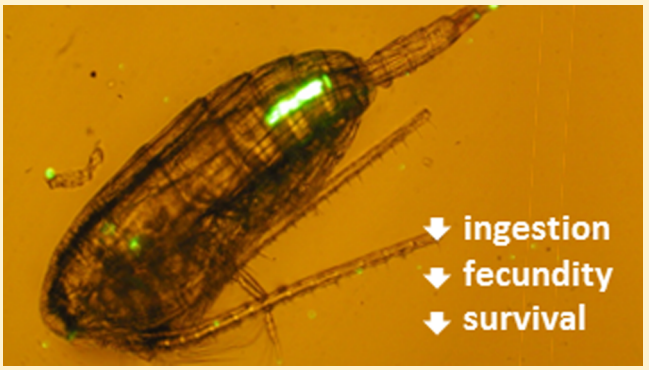
ABSTRACT: Microscopic plastic debris, termed "microplastics", are of increasing environmental concern. Recent studies have demonstrated that a range of zooplankton, including copepods, can ingest microplastics. Copepods are a globally abundant class of zooplankton that form a key trophic link between primary producers and higher trophic marine organisms. Here we demonstrate that ingestion of microplastics can significantly alter the feeding capacity of the pelagic copepod *Calanus helgolandicus*. Exposed to 20 μm polystyrene beads (75 microplastics mL^{-1}) and cultured algae ([250 μg C L^{-1}) for 24 h, *C. helgolandicus* ingested 11% fewer algal cells ($P = 0.33$) and 40% less carbon biomass ($P < 0.01$). There was a net downward shift in the mean size of algal prey consumed ($P < 0.001$), with a 3.6 fold increase in ingestion rate for the smallest size class of algal prey (11.6−12.6 μm), suggestive of postcapture or postingestion rejection. Prolonged exposure to polystyrene microplastics significantly decreased reproductive output, but there were no significant differences in egg production rates, respiration or survival. We constructed a conceptual energetic (carbon) budget showing that microplastic-exposed copepods suffer energetic depletion over time. We conclude that microplastics impede feeding in copepods, which over time could lead to sustained reductions in ingested carbon biomass.



INTRODUCTION

Over the past 50 years plastic litter has become an increasingly conspicuous presence within marine ecosystems.[1] While the risks that larger plastic debris pose to marine life are well documented,[2] we are only just beginning to understand how microscopic plastic debris, termed "microplastics", may be impacting upon aquatic organisms.[3,4] Microplastics describe plastic granules, beads, fragments, and fibers <1 mm in diameter, either manufactured to be microscopic in size or derived from the fragmentation of larger plastic debris following prolonged degradation.[5] Microplastic litter has been identified in aquatic environments across the globe (reviewed by Hidalgo-Ruz et al.[6]) prompting increasing levels of regulation.[7] The abundance of microplastic debris is both temporally and spatially variable, and is subject to the influence of tide, wind and wave action, the effects of upwelling and oceans currents.[8−11] The highest reported waterborne concentrations of microplastics (>80 μm) exceeds 100 000 items m^{-3},[12] however due to the complexities of sampling waterborne microscopic particles, there is no comprehensive data relating to microplastics <333 μm in size. The primary risk associated with microplastics are their bioavailability to marine organisms; a range of marine biota, including fish,[13] seabirds,[14] benthic polychaetes,[15] and zooplankton[16,17] have the capacity to ingest microplastics. Consumption of microplastics can result in adverse health impacts including reduced feeding,[16] loss of energetic reserves,[15] hepatic stress,[18] reduced fecundity and survival,[19] and potentially the transfer of toxic additives and adhered waterborne pollutants to organisms, although this latter process is under some debate.[20−22] Microplastics may have wider ecological impacts, by providing an artificial substrate for microbial colonisation[23,24] and oviposition of pelagic insects,[25] and by altering the properties of zooplankton faecal pellets which have a key role in marine nutrient cycling.[16]

In this study we consider the impact of microplastics on zooplankton feeding, function, and fecundity, using the pelagic copepod *Calanus helgolandicus*. *C. helgolandicus* are a keystone species within marine waters throughout Europe and the northeast Atlantic, where they can constitute up to 90% of mesozooplankton biomass.[26] Their large size, high lipid content and abundance make *C. helgolandicus* a vitally important prey species for the larvae of a number of commercially important fish. *C. helgolandicus* are selective filter-feeders, which use their

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external appendages to create feeding currents that indiscriminately draw waterborne particulates toward the copepod; prey is retained by setae on the maxillae (feeding appendages) and subsequently brought to the mouthparts for ingestion.[27−29] A long-term time-series conducted at L4, a study site in the western English Channel (www.westernchannelobservatory.org.uk), has mapped the seasonal changes in *C. helgolandicus* numbers and egg production rates since 1988: large scale changes are largely indicative of food availability,[30] with peak egg production occurring shortly after the annual spring bloom.[31] A copepod's reproductive output may also change acutely as a result of toxicity or stress, for example following the ingestion of toxic diatoms[32] or metal contaminated algae,[33] or exposure to persistent organic pollutants.[34] The ingestion of plastic or latex beads, used as mimics or tracers of prey, by copepods has been identified in the works of Frost,[27] Paffenhöfer and Van Sant,[35] and Wilson.[36] Cole et al.[16] have recently shown that a range of zooplankton taxa, including *C. helgolandicus*, can ingest microplastics of a similar size to algal prey (7−30 μm; 635−3000 microplastics mL^{-1}), while smaller microplastics (3.8 μm; 40 000 microplastics mL^{-1}) can externally adhere to a copepods' functional appendages. The feeding capacity of the copepod *Centropages typicus* was significantly reduced in the presence of 7.3 μm polystyrene beads (>4000 microplastics mL^{-1}). As feeding is fundamental to the energetic requirements of copepods, there is potential that microplastics may negatively impact upon zooplankton health by reducing feeding in exposed animals.

Here we test the hypothesis that exposure to microplastics will alter the ingestion of algal prey by the copepod *C. helgolandicus*. Our study design incorporated 24 h feeding assays using 20.0 μm polystyrene microplastics at a concentration of 75 particles mL^{-1}. We subsequently conducted a 9 day exposure to determine the impact of microplastics upon reproductive and metabolic function. The results were used together with literature derived data to construct a conceptual model of the energetic costs to the animals based on carbon budgets.

MATERIALS AND METHODS

Copepod Sampling. Zooplankton were sampled from L4 in the western English Channel (50°15′N, 4°13′W) using 200 μm plankton nets in July and August 2013. Samples were transported within insulated boxes, containing 2 L of natural seawater, to Plymouth Marine Laboratory (Plymouth, UK) within 3 h of sampling. Adult female *C. helgolandicus* were identified, through assessment of their shape, size and presence of their genital pore, under a dissecting microscope and transferred to experimental chambers using stork-billed forceps. Experiments were conducted in controlled temperature laboratories matched to the ambient sea surface temperature (SST) of 17.5 ± 0.5 °C.

Algal Prey. *Thalassiosira weissflogii* is a nontoxic, unicellular centric diatom recognized as a prey species for adult *Calanus*.[37−39] Cultures of *T. weissflogii* (CSAR, Swansea University; CCAP 927/1) were maintained on F/2 media with silica, at 15 °C under a 16:8 light dark regimen; media was refreshed weekly to allow for optimal growth conditions. Algal size, cell density and biovolume were quantified daily using a Multisizer 3 coulter counter (Beckman). Carbon biomass of algal prey was estimated using a literature derived conversion factor of 5 nL biovolume ≈1 μg C.[40] During experiments a relatively high concentration of 250 μg C L^{-1} (807 ± 14 cells mL^{-1}) of algae, equivalent to the available carbon present in a spring bloom, was rationed daily to ensure the ingestion rate was saturated throughout the exposure period.

Treatments. For all exposures, *C. helgolandicus* were maintained in 0.2 μm filtered seawater (FSW) containing only *T. weissflogii* (250 μg C L^{-1}) for controls, or *T. weissflogii* (250 μg C L^{-1}) and microplastics (75 beads mL^{-1}) for microplastic treatments. We used 20.0 μm unlabeled, additive free polystyrene (PS) beads (Sigma-Aldrich: 87896 Fluka) as our representative microplastics. While plastics that have suffered environmental degradation are thought to be at risk of leaching toxic monomers and additives,[41] virgin PS (as used here) is considered to have a high stability with negligible styrene migration, hence its widespread use in food and drinks packaging.[42] As styrene rapidly degrades in solution and is only considered toxic in acute doses,[42] we can safely assume any health effects stem from the physical presence of the PS bead, and not monomer leachates. Polystyrene is ubiquitous within sea-surface samples collected from across the globe,[6] and this size of microplastic, ubiquitously used in cosmetics and personal care products,[43] has previously been shown to be readily ingested by *C. helgolandicus*.[16] Environmental sampling for microplastics typically utilizes 333 μm nets,[6] thereby excluding microplastics 20 μm in size. While the environmental concentrations of microplastics of this size are unknown, it is widely postulated that, owing to the perpetual fragmentation and degradation of plastic litter, that as plastics become smaller, the more abundant they will become.[5,44] Our choice of 75 microplastics mL^{-1}, which represents a concentration ∼10% of the available food (particles mL^{-1}), presents the opportunity to explore the fate and impacts of microscopic plastic within the scope of a laboratory based study, without reaching the extreme concentrations used in recent ecotoxicological papers.[19,45] Stock solutions (10 L) were prepared daily, and algal cell density, algal biovolume, and the microplastic concentration of each stock solution were verified using a Multisizer 3 coulter counter (Beckman) prior to experimentation.

Ingestion Rate. Comprehensive 24h feeding studies were conducted to measure the impact of microplastics on *C. helgolandicus* ingestion rates. Half-liter glass bottles were filled to the brim (total volume: 617 mL) with either control or microplastic enriched stock solution. Adult female *C. helgolandicus* were added to each bottle (five animals per replicate; five replicates per treatment). Further controls (n = 3), without copepods, were set up to measure algal growth without predation. All bottles were secured to a rotating plankton wheel (<5 rpm), and left for 24 h in the dark at ambient SST. Postexposure, 20 mL subsamples were taken from each bottle and immediately analyzed using coulter counter to quantify final algal density, algal biovolume and microplastic concentration. The equation of Frost[46] was applied to calculate *C. helgolandicus* ingestion rates of both *T. weissflogii* (cells copepod^{-1} day^{-1} and μg C copepod^{-1} day^{-1}) and microplastics (beads copepod^{-1} day^{-1}). To reveal size selectivity, ingestion rates were calculated for five 1.1 μm size intervals, encompassing the size range of *T. weissflogii* cells (11.6−17.0 μm).

Extended Exposure. A nine-day exposure was employed to gauge the sublethal impacts of microplastics on *C. helgolandicus* egg production rates, egg size, hatching success, and respiration rates. To ensure only healthy, fertile copepods were used in the exposures, we prescreened the copepods: *C. helgolandicus* (n = 60) were individually placed in 25 mL beakers containing FSW

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with *T. weissflogii* and left overnight; only copepods that survived and produced eggs were selected for subsequent exposures. On initial setup, 1.8 L of control stock solution was poured into 2 L beakers. Egg-production chambers (plexi glass cylinders with a 200 μm mesh base) were inserted into each beaker; these chambers allow eggs and faecal pellets to sink to the bottom of the beaker, but preclude adult copepods, thereby minimizing egg cannibalism. Groups of four healthy, egg-producing *C. helgolandicus* (prosome length: 2.24 ± 0.1 (control); 2.27 ± 0.1 mm (microplastic treatment)) were transferred to each beaker (n = 10), and chambers covered with loosely fitting lids to prevent airborne contamination. Exposures were conducted under a 16:8 light dark regimen at ambient SST. Every 24 h egg-production chambers (containing the copepods) were tapped to displace eggs and then transferred to beakers containing fresh media. On days one−three, all copepods were maintained on *T. weissflogii* (without microplastics) to acclimate copepods to experimental conditions and ascertain baseline egg production, hatching success and egg size. From day four, treatments diverged, with half the copepod groups maintained on only *T. weissflogii* as controls (n = 5), and the other half exposed to *T. weissflogii* and microplastics (n = 5). Microplastic uptake was verified by visually checking the faecal pellets egested by the copepods.

Egg Production Rate, Egg Size, and Hatching Success. The average egg production rate (eggs copepod^{-1} day^{-1}) of the copepods was assessed daily. Eggs and nauplii were collected by pouring the contents of each beaker (after removal of copepods) through a 50 μm mesh. Retained material was carefully washed into gridded Petri dishes, and then eggs and nauplii quantified under a dissection microscope (×120 magnification).

Mean egg size (μm) was determined on days three, five, seven and nine. Eggs were visualized (Olympus IX71; ×400 magnification) and 10 healthy eggs (i.e., circular with no obvious signs of deformation) per replicate selected for assessment. Egg diameter was measured across two planes, using cellSens software (Olympus).

Average hatching success (%) was assessed using eggs collected on days two, four, six, and eight. Following egg counts, Petri dishes were loosely covered to avoid evaporative loss, and then stored at ambient SST under a 16:8 light dark regimen. After 48 h a dissection microscope (×120 magnification) was used to visualize and quantify any unhatched eggs present, and values compared with initial egg and nauplii numbers.

Respiration Rate. The oxygen consumption rate (μL O_2 copepod^{-1} day^{-1}) of copepods was assessed on day 10 as a proxy for standard metabolic rate. Small glass vials (volume: 2.14 mL) fitted with oxygen-sensitive optical sensor patches were filled with well-aerated control or microplastic-enriched stock solution, and individual *C. helgolandicus* introduced (n = 10 per treatment). Additional vials were set up without copepods (i.e., blanks) to establish the oxygen consumption rates of the algae present within the FSW (n = 5). Small rubber stoppers (bungs) were fitted carefully, ensuring air-bubbles were excluded, and vials transferred to a water-bath maintained at 17.7 ± 0.1 °C. The internal oxygen concentration (μmol O_2 L^{-1}) of each vial was noninvasively measured by scanning the optical sensor patches with an optrode (Fibox 3 LCD trace). Measurements were taken every 30 min until oxygen saturation was <70%. Vials in which *C. helgolandicus* had died (2 copepods in both the control and microplastics treatments) were excluded from further analysis. The oxygen consumption rate of each copepod was calculated for the time-range (≥60 min) in which oxygen depletion was most consistent (i.e., $R^2 \geq 0.99$), taking into account comparative mean oxygen decline measured in blanks.

Survival Rate. The number of live *C. helgolandicus* specimens remaining in each chamber was recorded daily. Dead copepods (typically opaque or cloudy in appearance, and nonmotile) were removed from treatments.

Carbon Budget. Biomass and energetic transfer can be estimated using carbon.[28] Values for ingested carbon biomass were calculated as previously described (Algal Prey). We further applied literature derived conversion factors to our experimental data to estimate the energetic costs (μg C copepod^{-1} day^{-1}) of [A] reproduction, [B] metabolism, and [C] egestion. [A]: The average carbon biomass of the eggs was estimated using their mean equivalent spherical volume (day 7) and a literature derived conversion factor of 0.14 pg C μm^{-3};[47] reproductive costs were calculated by multiplying egg carbon biomass with average egg production rate (day 7). [B]: Metabolic carbon consumption was calculated using average respiration rates (day 9) and established conversion metrics.[48] [C]: Elemental (CHN) analysis of collected faecal pellets was confounded by the presence of the PS microplastics. We therefore estimated losses through egestion as 40% of ingested carbon biomass, based upon a food absorption factor of 0.60 estimated for the copepod *Acartia tonsa* fed upon *T. weissflogii* at concentrations of 250 μg C L^{-1}.[49]

Statistical Analysis. Data is presented as mean ± standard error. Student's t tests were used to compare ingestion rates, reproductive outputs and respiration rates between treatments and dates, with significant difference attributed where $P \leq 0.05$. Regression analysis was used to analyze oxygen consumption rates.

RESULTS

Treatments. For the ingestion experiments, algal prey and microplastic stock concentrations were 234 μg C L^{-1} and 73 beads mL^{-1} respectively. During the 9 day exposure, stock concentrations averaged 245 μg C L^{-1} of *T. weissflogii*, and 65 PS beads mL^{-1} in microplastic-enriched solutions.

Ingestion Rate. In the control group, *C. helgolandicus* ingested 51,500 cells copepod^{-1} day^{-1} on average; comparatively, copepods exposed to microplastics ingested 45,700 cells copepod^{-1} day^{-1} (P = 0.33; Figure 1A). Copepods exposed to the microplastics ingested 3,278 ± 306 PS beads copepod^{-1} day^{-1}. Calculated as carbon biomass, individuals in the control group ingested 16.0 μg C copepod^{-1} day^{-1} (based on five copepods per treatment, this equates to approximately 32% of total carbon available), whereas copepods exposed to microplastics ingested 9.7 μg C copepod^{-1} day^{-1} of prey (P < 0.01; Figure 1B). We identified a shift in the size of prey ingested by copepods in the microplastics treatment. Experimental solutions contained a normal distribution of *T. weissflogii*, ranging from 11.6 to 17.0 μm in diameter (Figure 1C). *C. helgolandicus* exposed to control solution ingested all size classes of *T. weissflogii*, with a preference for the most abundant 13.8−14.8 μm diameter algae (Figure 1D). Copepods exposed to 20.0 μm microplastics, in contrast, consumed only the smallest available prey, with a preference for algae 12.7−13.7 μm in diameter (P < 0.001; Figure 1D).

Egg Production Rates. During the acclimation period (days 1−3), in the absence of microplastics, *C. helgolandicus*

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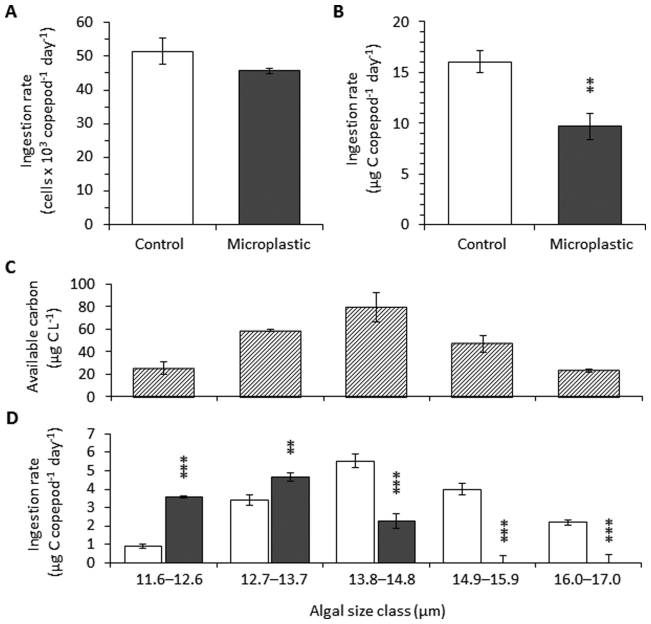

Figure 1. Algal ingestion rates of *T. weissflogii* by *C. helgolandicus* (n = 5), by (A) cell number (cells x 10^3 copepod^{-1} day^{-1}) and (B) biomass (μg C copepod^{-1} day^{-1}). (C) Average algal availability (μg C L^{-1}) in control and microplastic-enriched FSW shows a normal distribution by size. (D) The size of algae preferentially ingested by copepods significantly differs between treatments. Treatments: control (white) and microplastic-enriched (gray). Data expressed as mean ± standard error; asterisks denote significant difference from control (*P < 0.05; ** P < 0.01; *** P < 0.001).

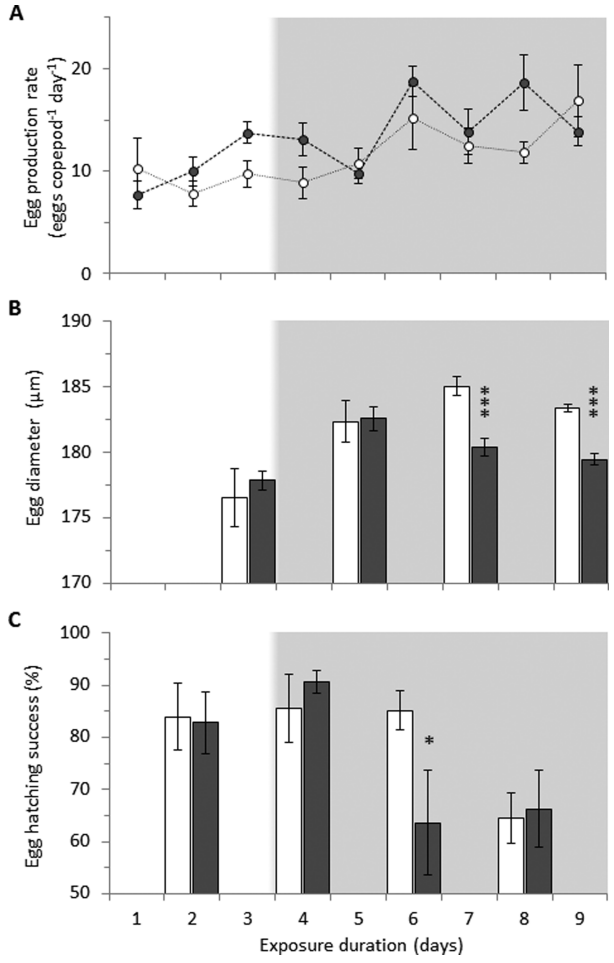

Figure 2. Average daily (A) egg production rates, (B) egg diameter and (C) hatching success of *C. helgolandicus* (n = 5). Treatments: control (white) and microplastic-enriched (gray). Data expressed as mean ± standard error; asterisks denote significant difference from control (* P < 0.05; ** P < 0.01; *** P < 0.001). Light gray background indicates introduction of PS beads in microplastic-enriched treatment (Day 4 onward).

produced 9.3 ± 4.3 (control) and 10.5 ± 1.0 (microplastic) eggs copepod^{-1} day^{-1} (Figure 2A). By the final trimester of the exposure (days 7−9), egg production rates had risen to 13.7 ± 1.4 (control) and 15.5 ± 1.3 (microplastic) eggs copepod^{-1} day^{-1}. This constituted a significant ∼47.5% increase in average egg production rates for copepods in both treatments (P < 0.01). There were no significant differences in egg production rates between controls and microplastic-exposed treatments in any trimester of the exposure (days 1−3, P = 0.21; days 4−6, P = 0.11; days 7−9, P = 0.19).

Egg Size. *C. helgolandicus* egg diameters averaged 177.6 ± 2.2 (control) and 177.9 ± 0.8 (microplastic) μm during the acclimation period, (day 3, P = 0.30; Figure 2B). By day 5, average egg size had increased significantly (P < 0.05) to 182.4 ± 3.5 (control) and 182.6 ± 2.1 (microplastic), with no significant differences between treatments (day 5, P = 0.45). In the latter half of the study microplastic exposed copepods produced statistically significant smaller eggs than those laid by control specimens (day 7:185.1 ± 1.7 (control) and 180.4 ± 1.4 (microplastic), P < 0.001; day 9:183.4 ± 0.7 (control) and 179.5 ± 0.9 (microplastic), P < 0.001).

Hatching Success. In the first half of the study, *C. helgolandicus* egg hatching success averaged 82.8−90.7% (Figure 2C), with no significant differences between control and microplastic treatments (day 2, P = 0.45; day 4, P = 0.24). On day 6 the hatching success of microplastic exposed copepods dropped to 63.6 ± 10.1%, a significantly lower egg hatching success than the 85.1 ± 8.4% hatching success observed with control eggs (day 6, P < 0.05). By day 8 the hatching success of control eggs dropped to 64.5 ± 11.0%, closely matching the 66.3 ± 16.3% egg hatching success of microplastic exposed copepods (day 8, P = 0.42).

Respiration. Active metabolic rate for *C. helgolandicus* specimens averaged 0.7 (control) and 0.7 (microplastic) μL O_2 copepod^{-1} day^{-1}, with no significant difference between treatments (P = 0.31; Figure 3A).

Copepod Mortality. Across treatments, three copepods died during the acclimation period (days 1−3; Figure 3B). Two copepods died following the introduction of microplastics on day 4, and a further three copepods died in the microplastic treatment on days 8 and 9. No copepods exposed to control media died during this same time period.

Carbon Budget. A conceptual carbon budget (Figure 4) was constructed using experimental data, collated from the feeding study (ingestion and egestion) and 9 day exposure (reproduction and metabolism), and literature derived conversion factors. These estimated values indicate copepods fed only *T. weissflogii* (controls) could expect energetic losses of −4.4 ± 3.4 μg C copepod^{-1} day^{-1}, while microplastic exposed copepods are predicted to suffer 2-fold greater energetic losses, in the region of −9.1 ± 3.7 μg C copepod^{-1} day^{-1}.

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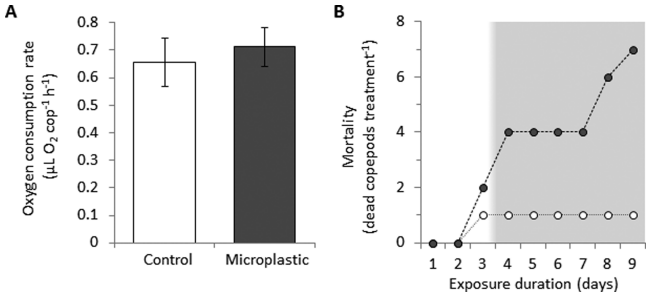

Figure 3. (A) Average oxygen consumption rate of *C. helgolandicus* indicates no significant different in metabolic function of copepods in differing treatments. (B) Cumulative number of dead copepods (mortality) increased in microplastic treatment on day 4 following the addition of microplastics, while there were no deaths during this period in the control treatment. Treatments: control (white) and microplastic-enriched (gray). Data expressed as mean ± standard error; asterisks denote significant difference from control (*$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). Light gray background indicates introduction of PS beads in microplastic-enriched treatment (Day 4 onward).

DISCUSSION

Our results demonstrate that microplastics can have a significant impact on copepod feeding with some notable impacts to the health of the individual. Exposed to 20.0 μm polystyrene beads (75 polystyrene microplastics mL^{-1}), the ingestion rate of *C. helgolandicus* was compromised, with significant reductions in ingested carbon biomass owing to a subtle shift in the size of algal prey consumed from 11.6−17.0 μm to 11.6−14.8 μm. Prolonged exposure to the microplastics resulted in copepods producing smaller eggs with reduced hatching success. No significant changes to egg production rate or oxygen consumption rates were observed. We postulate that microplastics can impede copepod feeding and that sustained reductions in ingested carbon biomass will result in energetic deficiencies and hence reduced growth.

Microplastics impeded algal ingestion in copepods over 24 h. We observed an 11% reduction in the number of algal cells and 40% reduction in carbon biomass ingested by microplastic exposed copepods. Previously, we identified that >4000 microplastics mL^{-1} could impact the ingestion rate of natural algae by the copepod *Centropages typicus*,[16] and Ayukai[50] found ∼2000 microplastics mL^{-1} could markedly reduce the ingestion rates of 5.6 and 13.4 μm algae by the copepod *Acartia clausi*. Therefore, our results provide the first evidence that copepod feeding can be significantly impacted at concentrations as low as 75 microplastics mL^{-1}.The reduction in ingested carbon biomass can be attributed to a small, albeit significant, shift in the size of algae consumed by copepods exposed to microplastics: offered only algal prey, *C. helgolandicus* ingested all sizes (11.6−17.0 μm) of *T. weissflogii* in proportion to its availability, whereas copepods fed upon algae with 20.0 μm PS microplastics ingested only 11.6−14.8 μm algae. Considering the diverse size of diatoms, phytoplankton, and microzooplankton in the marine environment, periodic shifts in prey size are fundamental for the survival of copepods such as *C. helgolandicus*.[51] However, the change in prey size seen here suggests that the copepods are altering their feeding strategy to avoid ingesting microplastics. Filter-feeding copepods, including *C. helgolandicus*, can demonstrate limited feeding selectivity. For example, *Calanus* can preferentially feed on larger (more nutritious) algae in mixed prey assemblages,[29,52] which Frost[27] hypothesizes stems from the morphology of their maxillae. There is some evidence that copepods can avoid toxic or nonnutritious prey, however in feeding studies neither *Calanus pacificus* nor *A. clausi* were able to differentiate between microplastic beads and algae of a similar size.[27,50] However, with results directly comparable to our own, Donaghay and Small[53] reported that the copepod *Acartia clausi* was able to preferentially feed upon 14 μm algae in the presence of 20 μm latex beads. The authors hypothesized that *A. clausi* were demonstrating postcapture rejection. Microplastics might also be rejected postingestion, as evidenced in the copepod *Eurytemora affinis*.[54] A better understanding of the mechanisms of selectivity against microplastics remains an important research gap for future studies.

Despite the observed shift in prey size selectivity, we found that *C. helgolandicus* readily ingested microplastics. Data from the 24 h feeding study indicates *C. helgolandicus* ingested >3000

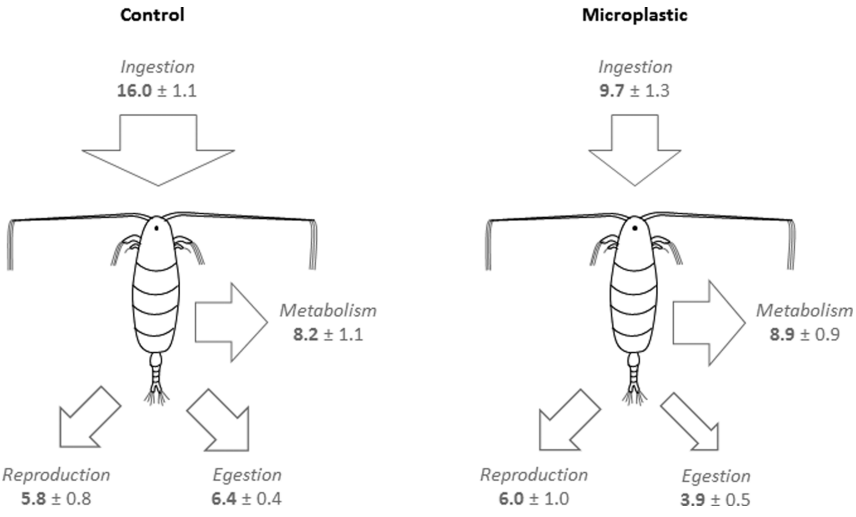

Figure 4. Conceptual carbon budget for *C. helgolandicus* in the absence or presence of microplastics. Arrows indicate energetic inputs and outputs (μg C $copepod^{-1}$ day^{-1}). Values estimated using experimental data and literature derived conversion factors. Data displayed as mean ± standard error. Budget: −4.4 ± 1.8 μg C $copepod^{-1}$ day^{-1} (control); −9.1 ± 1.9 μg C $copepod^{-1}$ day^{-1} (microplastic).

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microplastics copepod^{-1} day^{-1}, which were subsequently visible in their faecal pellets. The 20.0 μm polystyrene beads used here are representative of the small sizes of microplastics used in personal care products[43] and that derive from larger plastic debris such as shopping bags.[55] Our choice of using 75 microplastics mL^{-1} is markedly lower than the exposure concentrations of 4000−25 000,[16] 1000−10 000[17] and 5.25 × 10^5 to 9.1 × 10^{11}[19] microplastics mL^{-1} used in recent studies investigating the impact of plastics upon zooplankton. Within the marine environment microplastic concentrations are spatially and temporally variable, with highest reported densities of ∼18 plastics m^{-3}[11] sampled using relatively coarse (333 μm) nets in the Pacific Ocean, and 102 000 microplastics m^{-3} sampled using finer (80 μm) nets in Swedish coastal waters near an industrial site.[12] This data conforms with the hypothesis that, owing to the perpetual fragmentation of environmental plastics, that as plastics get smaller their abundance will increase.[5] However, the complexities of sampling smaller waterborne particulates means there is currently no direct data on the abundance of ∼20 μm microplastic debris.[6,56] It is important to emphasize that our exposures solely used polystyrene beads as representative microplastics; within the marine environment, microplastic debris encompasses beads, granules, fragments, and fibers, made up of a range of polymers[6] and further work will be required to verify whether these types of plastic also cause adverse health effects to marine copepods.

The energetic costs of producing healthy eggs means the reproductive success of copepods is intrinsically linked with their feeding.[30,57] In adult *Acartia*, up to 85% of carbon biomass attained from food is used toward growth (i.e., egg production in adult females).[58] Across numerous copepod species, provision of higher prey concentrations or prey of greater nutritional value is associated with higher egg production rates and better rates of hatching success.[28] The sensitivity of copepod egg production and viability in response to food and environmental conditions, and their importance for secondary production, make these variables apt biomarkers of health.[59] We found that when exposed to polystyrene microplastics copepods produced significantly smaller eggs with reduced hatching success at different points of the extended exposure. These effects were most noticeable 3−4 days after the introduction of microplastics to the treatment; this lag can be attributed to the rate of oogenesis (egg production), which typically occurs over a matter of days in calanoid copepods.[59−61] As the size of an egg is proportional to its carbon biomass, we conclude that the significant reduction in egg volume on days 7 and 9 resulted from reduced ingested carbon biomass (owing to microplastic exposure) of the adult copepods. A drop in hatching success on day 6 likely results from this reduction in egg carbon biomass, however, maternal stress may also influence egg viability, and we propose this might be explored using molecular analysis in future work. Lee et al.[19] found that when exposed to 0.5 and 6 μm microplastics, the number of nauplii which hatched from eggs produced by the benthic copepod *Tigriopus japonicus* was significantly reduced. Further, Pacific oysters exposed to microplastics during oogenesis have been shown to produce significantly fewer and smaller oocytes than observed in controls.[62] In both treatments, egg production rates increased during the exposure. We believe this stems from the provision of algae at 250 μg C L^{-1}, exceeding the energetic requirements necessary to support maximal growth rates in *C. helgolandicus*.[26] Furthermore, we associate reduced hatching success on the final 2 days of the exposure with the use of a monoalgal diet; using a single algal species was necessitated by the experimental design, however, over prolonged periods monoalgal feeding can result in a shortfall of polyunsaturated fatty acids and amino acids required for sustained copepod egg viability.[30,52]

Energy assimilated from food is required for growth (reproduction in adults), maintenance, metabolic processes, and laying down energetic reserves (lipids). In the marine environment, prey concentrations are both spatially and temporally variable. When faced with starvation, zooplankton can adapt by decreasing metabolic rate or traveling further to increase prey encounter rates.[58,63] However, our data showed no significant differences in the metabolic rate of copepods in differing treatments.

We calculated a carbon budget using measures of metabolism, reproduction, and ingestion. Values were converted into carbon production (μg C copepod^{-1} day^{-1}) using literature derived conversion factors; while widely applicable, these conversion figures stem from studies with different experimental set-ups, and therefore resultant data must be considered as estimated. Nonetheless, the budget helps identify that microplastic exposed copepods will have much higher energetic deficiencies than controls, predominantly owing to the 40% reduction in ingested carbon biomass. When food is sparse, the lipid reserves of a copepod may be engaged to makeup the shortfall in energy.[64,65] However, these reserves are limited, and wax esters and triglycerides contained within the lipids of *C. helgolandicus* may be depleted within 3 days of starvation. Wright et al.[15] identified prolonged microplastic exposure could result in energetic depletion in marine worms, with concurrent increases in phagocytic activity indicative of inflammation. Presuming microplastics are resulting in energetic deficiencies in copepods, we could expect their lipid reserves to be rapidly consumed, with repercussions for the health of the individual. Copepod deaths witnessed on days 8 and 9 in the microplastic treatment may well be the result of such energetic deficiencies. Recent research also indicates zooplankton survival may be significantly impacted when exposed to high microplastic concentrations.[19,66] Energetic deficiencies and reduced survival in microplastic exposed copepods may also impact upon higher trophic organisms which rely on the high lipid content of copepods for their own sustenance. As such, we believe it is now increasingly important to better understand the density of bioavailable microplastics in biota-rich waters, and test whether environmentally relevant concentrations of plastic litter can impact keystone species, such as *Calanus*, including the consequences for commercially important predators.

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Notes

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