Microplastics alter feeding selectivity and faecal density in the copepod, *Calanus helgolandicus*

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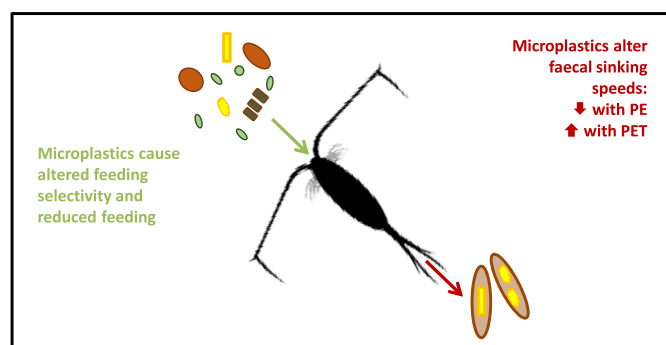
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HIGHLIGHTS

- Copepods are an important link in marine food webs and marine nutrient cycling.
- Investigated effect of microplastics on *Calanus helgolandicus* feeding selectivity
- Assessed sinking rates of faecal pellets contaminated with different microplastics
- *C. helgolandicus* avoided ingesting algae similar in size and/or shape to the microplastic.
- Microplastics with different densities altered the sinking rates of faecal pellets.

GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

Microplastics (1 μm–5 mm) are a ubiquitous marine contaminant of global concern, ingested by a wide range of marine taxa. Copepods are a key component of marine food webs, providing a source of food for higher trophic levels, and playing an important role in marine nutrient cycling. Microplastic ingestion has been documented in copepods, but knowledge gaps remain over how this affects feeding preference and faecal density. Here, we use exposure studies incorporating algal prey and microplastics of varying sizes and shapes at a concentration of 100 microplastics mL^{-1} to show: (1) prey selection by the copepod *Calanus helgolandicus* was affected by the size and shape of microplastics and algae they were exposed to; Exposure to nylon fibres resulted in a 6% decrease in ingestion of similar shaped chain-forming algae, whilst exposure to nylon fragments led to an 8% decrease in ingestion of a unicellular algae that were similar in shape and size. (2) Ingestion of microplastics with different densities altered the sinking rates of faecal pellets. Faeces containing low-density polyethylene sank significantly more slowly than controls, whilst sinking rates increased when faeces contained high-density polyethylene terephthalate. These results suggest that *C. helgolandicus* avoid ingesting algae that are similar in size and/or shape to the microplastic particles they are exposed to, potentially in a bid to avoid consuming the plastic.

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1. Introduction

Microplastics (plastic pieces, 1 μm–5 mm) are pervasive marine pollutants, which have been highlighted as a contaminant of global

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environmental concern (UN Sustainable Development Goal 14 target 14.1.1, GESAMP 2016). Microplastic particles and fibres have been documented ubiquitously throughout the marine realm, including surface waters (Cozar et al., 2014), polar regions (Bergmann et al., 2017; Cincinelli et al., 2017) and deep sea sediments (Woodall et al., 2014). These synthetic particles can be purposefully manufactured, such as cosmetic exfoliates or virgin pre-production pellets, or result from the fragmentation of larger items such as fibres from textiles (Napper and Thompson, 2016), wear of tyres (Boucher and Friot, 2017) and the breakdown of single-use plastics that have degraded over time (Andrady, 2011). Microplastic ingestion has been documented in a wide range of marine organisms including corals, (Hall et al., 2015), fish (Lusher et al., 2013), marine mammals (Nelms et al., 2019), turtles (Duncan et al., 2019), seabirds (Lourenço et al., 2017;) and commercially important shellfish (Murray and Cowie, 2011; Rochman et al., 2015). Exposure to microplastics can result in adverse health effects, including reduced feeding and fecundity in copepods (Cole et al., 2015), reproductive disruption in oysters (Sussarellu et al., 2016), intestinal damage (Lei et al., 2017) and behavioural changes in fish (de Sá et al., 2015).

Zooplankton are an important link between primary producing phytoplankton and higher trophic levels in marine food webs (Kiorboe, 1997; Turner, 2004). Copepods constitute a high proportion of the total zooplankton carbon biomass and Calanus species, which are amongst the largest copepods, may account for >90% of mesozooplankton biomass in regions such as the North and Celtic seas (Bonnet et al., 2005). Experimental studies have demonstrated that zooplankton have the capacity to ingest microplastics (Cole et al., 2013) and field studies have identified that zooplankton, including copepods, euphausiids, jellyfish and fish larvae, consume microplastics in the wild (Desforges et al., 2015; Steer et al., 2017; Sun et al., 2017). Food selectivity has been widely evidenced in copepods, with the capacity to discriminate between algal prey and microplastics (Donaghay and Small, 1979; Huntley et al., 1983). The drivers of this selectivity might include the chemosensory properties of the particles, for example when covered in biofilms (Vroom et al., 2017), the size, which alters capture efficiency, and shape, that may affect handling and capacity for ingestion. This may result in negative effects including, reduced food intake and energy available for growth and reproductive success (Cole et al., 2015).

Copepod faecal material substantially contributes to the flux of carbon and nutrients to deeper waters and to the seabed. Through ingestion of phytoplankton and subsequent repackaging into dense, rapidly sinking faecal pellets, Calanoid copepods play an instrumental role in the biological carbon pump. Their faecal pellets transfer atmospheric carbon dioxide in the form of photosynthetically produced organic matter, or fixed carbon, to the deep ocean, thereby providing food for benthic dwelling organisms and facilitating microbial degradation and remineralisation by microzooplankton (Turner, 2002). A change to the sinking rate of this faecal material has potential ecological consequences affecting a wide range of factors including carbon and nitrogen export out of the euphotic zone, shifting the balance of particulate organic matter (POM) remineralisation and reducing food to the benthos. In a prior study, the sinking rates of copepod faecal pellets contaminated with polystyrene (PS) microspheres were significantly reduced (Cole et al., 2016). If translated to natural systems in highly polluted waters, slower faecal sinking rates may alter POM export, cause faecal pellets to remain in the upper reaches of the ocean for longer and hence increase the likelihood of being consumed by microzooplankton (coprophagy), get fragmented (coprohexy) or degraded by protozoan and microbial communities.

Many previous studies have used PS spheres as representative microplastics, and it has been highlighted that a wider range of plastics, with greater ecological relevance, should be included in exposure studies to better understand the risks microplastics pose to marine life (Botterell et al., 2018; Lenz et al., 2016). Numerous environmental studies report fibres as the predominant particle type (Cole et al., 2011;

Lusher et al., 2016) and 50% of microplastics isolated from copepods in the North Pacific (Desforges et al., 2015) were fibrous. It is currently unclear whether the bioavailability or sinking rates of copepod faecal matter will change with different types of plastic that vary in size, shape and polymeric composition. We predict that the temperate copepod *Calanus helgolandicus* will ingest all types of plastic within their prey size range but that shape and size will influence selection of their algal prey. We also predict buoyant plastic (e.g. polyethylene (PE)) will dramatically reduce sinking rates of contaminated faecal matter, whilst denser plastics (e.g. polyvinyl chloride (PVC), polyethylene terephthalate (PET)) will substantially increase sinking rates.

In this study, we test the hypotheses: (1) that prey selection by the copepod *C. helgolandicus* will be altered depending upon the relationship between prey shape and/or size and that of microplastics available in their surrounding medium; and, (2) that the resulting contamination of copepod faecal pellets with plastics will alter their sinking rates, with buoyancy primarily affected by the density of the polymer. We test these using a mixed-prey exposure containing chain-forming and unicellular algae with copepods over a 24 h period to gain a mechanistic insight into copepod feeding strategies and resultant changes to faecal buoyancy.

2. Methods

Experimental treatments comprised field collected *Calanus helgolandicus* copepods and algal solutions containing assemblages of cultured microalgae, spiked with different types of microplastic at a density of approximately 100 plastics mL^{-1}. Experiments set out to; 1) investigate the effect of microplastic on algal selection and 2) measure the sinking rate of microplastic contaminated faecal pellets.

2.1. Sample collection

Zooplankton were sampled in January and May 2017 from the Plymouth Marine Laboratory's RV Plymouth Quest from the Western Channel Observatory (station L4; 50°15′N, 4°13′W; https://www.westernchannelobservatory.org.uk/), a site approximately 12 km south-west of Plymouth Sound, UK, which combines coastal influence from the Tamar Estuary and continental shelf conditions (Smyth et al., 2015). Zooplankton were collected via horizontal surface tows using 735 μm mesh plankton nets. Samples were transported in 2 L of seawater, enclosed within a cool box to a temperature controlled laboratory (matched to ambient sea surface temperature at the collection site; SST Jan 10 °C, May 11 °C) at Plymouth Marine Laboratory (Plymouth, UK) within 3 h of collection. On arrival, adult female *Calanus helgolandicus* copepods were carefully, manually selected using a low power microscope (Wild M5-49361; ×20–×50 magnification) and stork billed forceps. They were immediately transferred to a 10 L glass beaker, aerated and maintained in 0.2 μm filtered seawater (FSW; Salinity 34.5–35‰; 24 h darkness; SST) collected from L4, for 72 h during preconditioning to experimental diet treatments (see Algal cultures below).

2.2. Algal cultures

Three algal prey species, the unicellular chlorophyte *Dunaliella tertiolecta* (11 μm), the chain-forming diatom *Thalassiosira rotula* (24 μm) and the dinoflagellate *Prorocentrum micans* (35 μm; Fig. 1), are components of *C. helgolandicus* natural prey and were selected for their size and shape to assess prey selection by the copepods. All prey species were cultured at Plymouth Marine Laboratory after purchase from Swansea University (*P. micans*) and Culture Collection of Algae and Protozoa (*D. tertiolecta* CCAP 19/6B, *T. rotula* CCAP 1085/20) using Guillard's F/2 media for *D. tertiolecta* and *P. micans*, with additional meta-silicates (1 mL L^{-1} of seawater) for *T. rotula* (15 °C; 16:8 light regime; S 34.5–35‰).
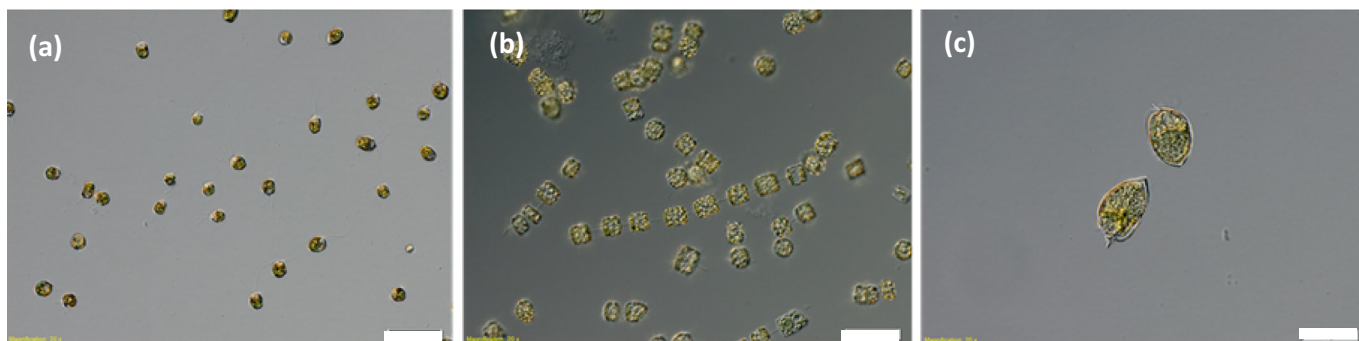
Fig. 1. Cultured single cell algae used in experiments; (a) unicellular chlorophyte, *Dunaliella tertiolecta* (11 μm), (b) chain forming diatom *Thalassiosira rotula* (24 μm) and (c) dinoflagellate, *Prorocentrum micans* (35 μm). Magnification ×20, white scale bars measure 50 μm.

2.3. Microplastic preparation

2.3.1. Dried powder suspension

Fluorescent PE microspheres (0.09 g; Cospheric) were added to 15 mL falcon tubes and 10 mL of 0.01% Tween20 surfactant solution (Thermo Fisher Scientific) was added to aid particle solubilisation. Solutions were thoroughly mixed through vigorous shaking, vortexing and sonicating for 15 min in an ultrasonic bath (Guyson KC3).

2.3.2. Nylon and PET fibres

Nylon 6,6 microfibres were produced using an established 'cryotome' protocol (Cole, 2016). To summarise, nylon 6,6 and PET polyfilaments (Goodfellow) were aligned and embedded in a glycol freezing solution (Neg 50™, Richard-Allan Scientific) and frozen (10 min, −80 °C, New Brunswick U570 ultra low temperature freezer); frozen fibres were sectioned into pre-determined lengths (Table 1.) using a cryogenic microtome (Leica CM1950). Sections were thawed and 'rod' shaped microfibres retrieved via filtration and washed with ultrapure water. For imaging purposes, Nile Red was used to fluorescently dye the nylon microfibers using a solvent-extraction protocol (Cole, 2016). Recovered fibres were suspended in MilliQ water and quantified using Sedgwick Rafter counting cells and stereo microscope (×20 magnification; Wild, M5-49361), where their shape and size were also quantified.

2.3.3. Nylon fragments

Nylon fragments (20 μm) were prepared by size fractionating nylon 6 powder (Goodfellow; AM306010) using 20 μm and 25 μm nylon meshes. Size and shape were visually inspected and quantified using a graticule and stereo microscope (×20 magnification; Wild, M5-49361). The fragments were then fluorescently dyed using Nile Red as per Section 2.3.2.

2.4. Microplastic uptake

Uptake assays were conducted to guide selection of the most appropriate size of each of three common microplastic types that differ in density (Table 1) for use in both the copepod feeding selectivity and sinking rate experiments; low density PE, medium density nylon and high density PET. A single adult female *C. helgolandicus* was transferred to a 50 mL lidded glass bottle ($n = 4$), containing 100 microplastics mL^{-1} and filled with FSW (S 34.5‰; SST; total volume: 74 mL). Controls contained either FSW alone or FSW with equivalent volume of 0.01% Tween20 surfactant solution as used to disperse PE microspheres, and a single *C. helgolandicus*. Lids were securely fastened and bottles installed onto a rotating plankton wheel. After 24 h, the experiment ended and individuals were filtered through a 50 μm mesh, taking care to retain the copepod and any faecal pellets, and preserved in 4% formalin for 48 h before washing thoroughly and storing in 95% ethanol. Microplastic presence and abundance was qualitatively assessed in preserved copepods and faecal pellets under UV light, using an Olympus IMT-2 inverted microscope to guide appropriate size selection for the ingestion studies.

2.5. Ingestion study

To determine the impact of different shaped plastics on algal ingestion rates, we conducted a 24 h feeding study. In brief: 500 mL Duran bottles were filled with 615 mL of FSW, (S 35‰), containing 120 μg C L^{-1} of a mixed, autotrophic algal assemblage (*Prorocentrum micans*; 5 cells mL^{-1} ≈ 25 μg C L^{-1}, *Dunaliella tertiolecta*; 166 cells mL^{-1} ≈ 35 μg C L^{-1} and *Thalassiosira rotula*; 38 cells mL^{-1} ≈ 60 μg C L^{-1}), representing natural carbon availability during spring bloom conditions (Harris et al., 2000; Widdicombe et al., 2010). Abundances were calculated using a Sedgewick Rafter counting chamber and carbon biomass was estimated using a conversion factor of 5 nL biovolume ≈ 1 μg C (Jones et al., 2002). Guillard's F/2 nutrient media was added to algal stocks to ensure algae were nutrient replete prior to study, negating

Table 1
Polymer, shape, density, size and mass concentration of microplastics used to assess uptake in the copepod, *C. helgolandicus* to guide particle selection for ingestion and sinking rate experiments.

Polymer	Shape	Density (g cm^{-3})	Size (μm)	Mass concentration at 100 MP mL^{-1} (g mL^{-1})
Polyethylene	Sphere	0.91–0.96	10–20	2.8×10^{-8}–2.2×10^{-7}
Polyethylene	Sphere	0.91–0.96	20–27	2.2×10^{-7}–5.5×10^{-7}
Polyethylene	Sphere	0.91–0.96	27–32	5.5×10^{-7}–9.2×10^{-7}
Nylon 6,6	Fragment	1.15	20	4.8×10^{-7}
Nylon 6,6	Fibre	1.15	10 × 40	3.6×10^{-7}
Nylon 6,6	Fibre	1.15	23 × 100	4.8×10^{-6}
Polyethylene terephallate	Fibre	1.38	17 × 60	4.0×10^{-6}
Polyethylene terephallate	Fibre	1.38	23 × 70	1.9×10^{-6}

the effects of additional nutrient input from copepod excretions. Treatments were prepared as follows: 1) control without predation; 2) control with predation; 3) nylon fibres (10 × 40 μm; 100 fibres mL^{-1}) and 4) nylon fragments (20 μm; 100 fragments mL^{-1}). Environmental concentrations of microplastics in this size range are not well reported, however there is considerable evidence that concentrations increase with decreasing size (Lenz et al., 2016). Our decision to use 100 microplastics mL^{-1} balanced the desire to achieve near environmental concentrations with the ability to determine any potential effects arising from the microplastic exposures. We therefore used an algae to microplastic ratio of 2:1 to allow a mechanistic insight into prey selection. Five adult female *C. helgolandicus* were added to each bottle (*n* = 5), with the exception of the 'control without predation' treatment, used to ascertain the natural growth of algae over the experimental period. Bottles were rotated on a plankton wheel for 24 h (<5 r.p.m.; 24 h darkness; SST). After 24 h, 200 mL from each bottle was fixed (Lugols 1% final concentration) for algal cell and microplastic quantification using an Olympus IMT2 inverted microscope (×150 magnification: *T. rotula*, *P. micans*, fibres; ×300 magnification: *D. tertiolecta*, fragments) and Utermöhl counting technique (Utermöhl, 1958). Samples were homogenised through inversion before settling 100 mL subsample for treatments 2, 3 and 4 or 50 mL for treatment 1 and leaving to settle for >24 h (50 mL) or >48 h (100 mL). Clearance (mL copepod^{-1} day^{-1}) and ingestion (μg C copepod^{-1} day^{-1}) rates for algal prey and microplastics were calculated using formulae of Frost, 1972.

2.6. Egestion; Faecal pellet sinking study

To collect faecal pellets for this study, five adult female *C. helgolandicus* were incubated in 500 mL bottles (*n* = 4) containing FSW, (S 35‰) plus 105 μg C L^{-1} of the mixed, autotrophic algal assemblage (*P. micans*; 9 cells mL^{-1} ≈ 30 μg C L^{-1}, *Dunaliella tertiolecta*; 108 cells mL^{-1} ≈ 20 μg C L^{-1} and *Thalassiosira rotula*; 43 cells mL^{-1} ≈ 55 μg C L^{-1}). In addition to the algal mix, treatments were prepared as follows: 1) control with nothing else added; 2) control plus 0.01% Tween20 at volume corresponding to PE prep; 3) high density PET fibres (17 × 60 μm; 100 fibres mL^{-1}); 4) low density PE spheres (10–20 μm; 100 spheres mL^{-1}) and 5) medium density nylon fibres

(10 × 40 μm; 100 fibres mL^{-1}). As per Section 2.5, experimental bottles were rotated on a plankton wheel for 24 h (<5 r.p.m.; 24 h darkness; SST). After 24 h, faecal pellets were collected using a 50 μm mesh sieve and washed into a Petri dish using FSW then stored in the refrigerator at 4 °C for the sinking study, which was completed within 3 days of pellet collection.

Adapting the method of Cole et al., (2016), a clean 2 L measuring cylinder was filled with filtered seawater (34.5‰ S), covered to prevent dust particles entering and placed on a stable workbench at a constant temperature (15 °C). The cylinder was marked at intervals of 40 mm, the first mark occurred 80 mm below the surface to allow for deceleration of the pellets. Using a stereo microscope (Wild M5-49361, ×50 magnification) and eyepiece graticule, faecal pellet length, width and number of encapsulated plastics were recorded. Faecal pellets were then carefully drawn up using a liquid-pipette and gently released once the liquid-pipette tip was submerged just below the water surface; the time taken for the faecal pellet to travel at a constant speed between the two markers was recorded. For analysis, the volume of microplastic in each pellet was determined using the average size of each plastic type used, calculating the volume of the shape (e.g.; cylinder for nylon fibres and sphere for nylon fragments and PE spheres) and multiplied by the number observed.

2.7. Statistical analyses

All data were analysed using R statistical software V 1.0.136 (R Core Team, 2016).

2.7.1. Ingestion rates

All data were tested (Shapiro-Wilk) and visually inspected for distribution and homogeneity of variances and were found to violate *a priori* requisites for linear, parametric tests. Kruskal-Wallis non-parametric tests were therefore performed to assess how each response variable (clearance rate of each algal species) was influenced by the explanatory variable (treatment: control, nylon fibres or fragments) and Dunn's post-hoc pairwise test applied.
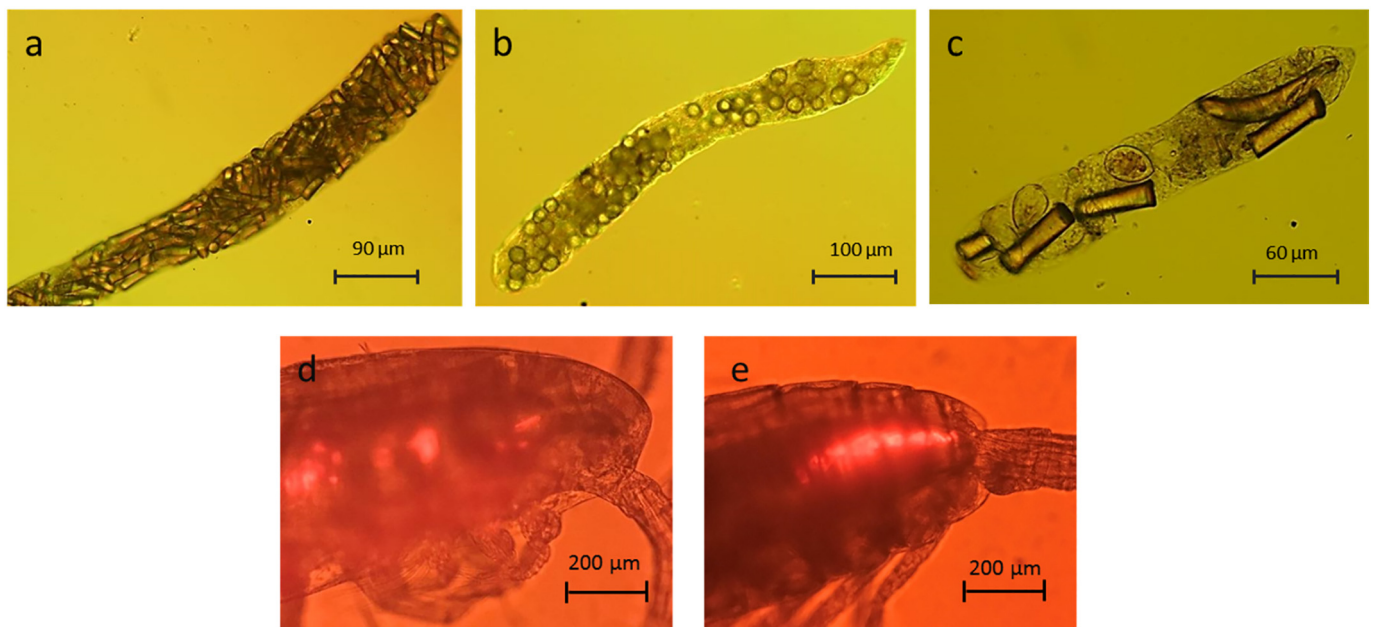

Fig. 2. Images of contaminated *C. helgolandicus* faecal pellets (a–c) after exposure to solutions containing mixed algal assemblage and a) nylon fibres, b) PE spheres and c) PET fibres and *C. helgolandicus* with fluorescently labelled nylon fibres (d) in digestive tract and (e) being formed into a faecal pellet in the hind gut. All exposures at concentrations of 100 microplastics mL^{-1} with an algae to plastic ratio of 2:1.
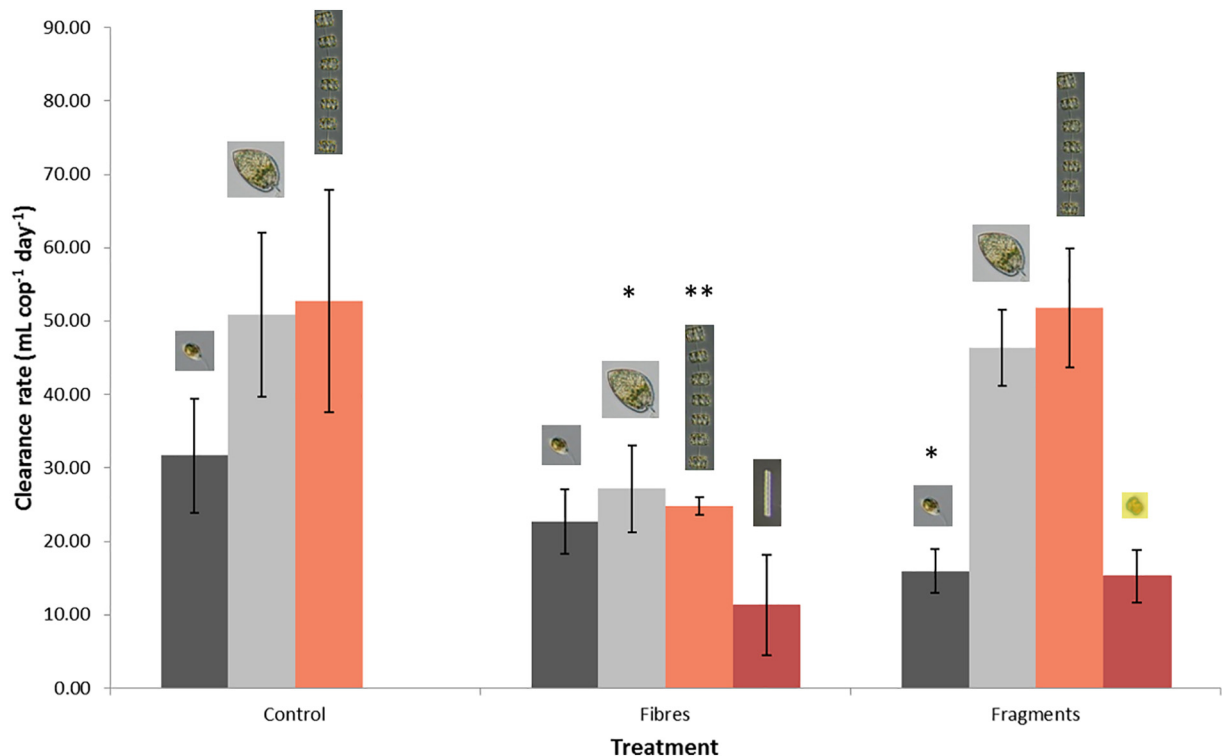
Fig. 3. Mean (±SE) clearance rate (volume of water swept clear of particles) of each algal species (dark grey bars, *D. tertiolecta*; light grey bars, *P. micans*; orange bars, *T. rotula*) and plastic (red bars) cleared per copepod, per day for each treatment. * denotes statistical significance at <0.05, ** at <0.001, Kruskal Wallis (*n* = 5).

2.7.2. Egestion; Faecal pellet sinking rates

Generalised linear modelling (GLM) was conducted to investigate how the explanatory variables (volume of microplastic contained in faecal pellets, faecal pellet volume and polymer type) influenced the response variable (sinking rate). First, a linear regression was conducted to assess the relationship between microplastic volume and faecal pellet volume; collinearity was found to occur therefore microplastic volume was removed from the model, as this variable only applies to plastic treatments and not controls. To achieve model parsimony, a full model was built which included main effects (faecal pellet volume and polymer type) as fixed terms, treatment replicate (*n* = 4) as a random term and main effect interactions. The significance of the random term was tested with GLS and lme functions (nlme package) using REML estimation. The model without a random term returned the lowest AIC value and models which included the random term generated non-significant model coefficients, therefore was excluded from further models. All fixed terms in the model were then tested for significance using GLM. Terms were dropped sequentially and models tested for significance, determined by ANOVA "*F*" test and AIC comparison. Models including interaction terms suggested these resulted in a greater model AIC value and generated non-significant model coefficients, which were also excluded from the final model. Gaussian distribution with 'Identity' link function was used and the model was validated by visually inspecting error distributions and homogeneity of variances relative to linear model assumptions (see Table A.1.).

3. Results

3.1. Ingestion

3.1.1. Microplastic uptake

Adult female *Calanus helgolandicus* readily ingested microplastic fibres, beads and fragments (Table 1; Fig. 2.). The copepods indicated a preference for particles in the size range of 10–20 μm for PE, whilst PET was ingested in greater quantities in the 17 × 60 μm size range. Nylon was readily ingested in both granule and fibre form, the most commonly ingested fibre size being 10 × 40 μm.

3.1.2. Ingestion of algal prey

There was an overall impact to clearance rates of algal prey when exposed to microplastics (H = 45.81, *df* = 2, *p* = 0.05; Fig. 3.). When exposed to nylon fibres, there was an overall reduction in the amount of food ingested by *C. helgolandicus* (H = 5.81, *df* = 2, *p* = 0.05) and a shift in algal preference compared to the control treatment. We observed a reduction in the clearance rates of both *Prorocentrum micans* (H = 3.17, *df* = 2, *p* = 0.04) and a highly significant reduction in clearance rates of *Thalassiosira rotula* (H = 8.97, *df* = 2, *p* = 0.001), which are similar in size and shape (respectively) to the 10 × 40 um fibres (Fig. 4). There was no difference in the clearance of *Dunaliella tertiolecta* (H = 5.49, *df* = 2, *p* = 0.14) compared to controls. When exposed to nylon fragments, total clearance rates were significantly reduced compared to control treatments (H = 5.81, *df* = 2, *p* = 0.01). When assessing clearance rates of individual algal prey, we observed no difference in the clearance rates of *P. micans* (H = 3.17, *df* = 2, *p* = 0.11) or *T. rotula* (H = 8.97, *df* = 2, *p* = 0.16) when compared with control treatments, however there was a significant reduction in the clearance rate of *D. tertiolecta* (H = 5.49, *df* = 2, *p* = 0.01) which is similar in size and shape to the fragments (Fig. 4). When considering the proportions of each algal prey type ingested, the mean proportion of *P. micans* ingested did not vary with treatment (Fig. 5), however exposure to fibres resulted in a 5.7% decrease in ingestion of the similar shaped *T. rotula* and a 5.9% increase in ingestion of *D. tertiolecta*. Conversely, exposure to fragments led to a 7.4% increase in consumption of *T. rotula* but a 7.8% decrease in the similar shaped *D. tertiolecta*.
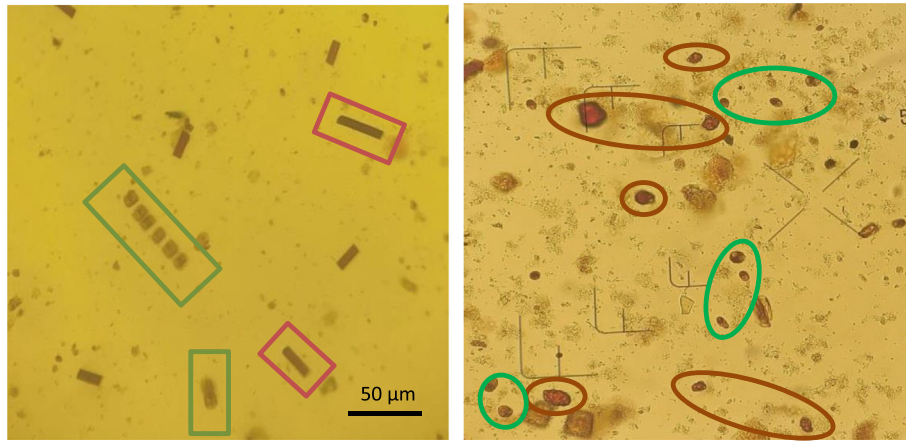
Fig. 4. Images showing similarity between a) nylon fibres (red rectangles) and chain-forming algal prey, *T. rotula* (green rectangles) and b) nylon fragments (circled red) and algal prey species, *D. tertiolecta* (circled green).

3.2. Egestion

Microplastic presence in *C. helgolandicus* faecal pellets altered their sinking rate, but this was dependent on the type of plastic ingested. Treatment (GLM $F_{4,92} = 34.74$, $p \leq 0.001$; Table A.1.) and faecal pellet volume (GLM $F_{1,91} = 29.30$, $p \leq 0.001$) were both significant predictors of faecal pellet sinking rates. Faecal pellets contaminated with low density PE sank significantly slower than the controls ($F_{4,92} = 34.74$, $p \leq 0.001$; Fig. 6), in contrast to the high density PET contaminated pellets which sank significantly faster than controls ($F_{4,92} = 34.74$, $p \leq 0.01$). Neither nylon ($F_{4,92} = 34.74$, $p = 0.25$) or the tween-control ($F_{4,92} = 34.74$, $p = 0.48$) had any significant influence on sinking rates. Faecal pellet volume was positively influenced by microplastic volume when contaminated with all plastic treatments (Fig. 7); PE ($F_{1,16} = 9.32$, $p = 0.006$, $r^2 = 0.29$), PET ($F_{1,18} = 9.32$, $p = 0.007$, $r^2 = 0.34$) and nylon ($F_{1,16} = 6.72$, $p = 0.02$, $r^2 = 0.30$) and is therefore a factor in faecal pellet sinking rates. There was no correlation between the volume of microplastics and sinking rates for PE ($F_{1,23} = 3.14$, $p = 0.09$, adj $R^2 = 0.08$, Fig. 7) or PET contaminated pellets ($F_{1,18} = 2.34$, $p = 0.143$, adj $R^2 = 0.07$) but there was a correlation when contaminated with nylon ($F_{1,23} = 26.6$, $p \leq 0.001$, adj $R^2 = 0.32$). There was no difference in the size of faecal pellets between control and nylon ($F_{4,5.58} = 19.95$, $p = 0.66$), PE ($F_{1,5.58} = 19.952$, $p = 0.98$) or PET ($F_{4,5.58} = 19.95$, $p = $

0.19) treatments but tween-control faecal pellets were smaller than all other treatments ($F_{4,5.58} = 19.95$, $p \leq 0.001$).

4. Discussion

4.1. Ingestion

Our results reveal that exposure to microplastics at concentrations of ~100 plastics mL^{-1} not only caused an overall reduction in *Calanus helgolandicus* feeding, but also influenced prey selection. Nylon fibres impeded ingestion of algae of a similar size or shape and caused a shift in the preference of consumed prey. The copepods *C. helgolandicus* reduced their intake of the similarly shaped chain forming diatom *Thalassiosira rotula* and the similar sized dinoflagellate *Prorocentrum micans*, but ingestion of the small flagellate *Dunaliella tertiolecta* remained unchanged. Exposure to nylon fragments did not alter the total consumption of algal prey, however there was a significant reduction in the ingestion of *D. tertiolecta*, which is similar in size and shape to the fragments. These results suggest that *C. helgolandicus* avoided ingesting algae that were similar in size and/or shape to the microplastic particles they were exposed to, potentially in a bid to avoid consuming the plastic.
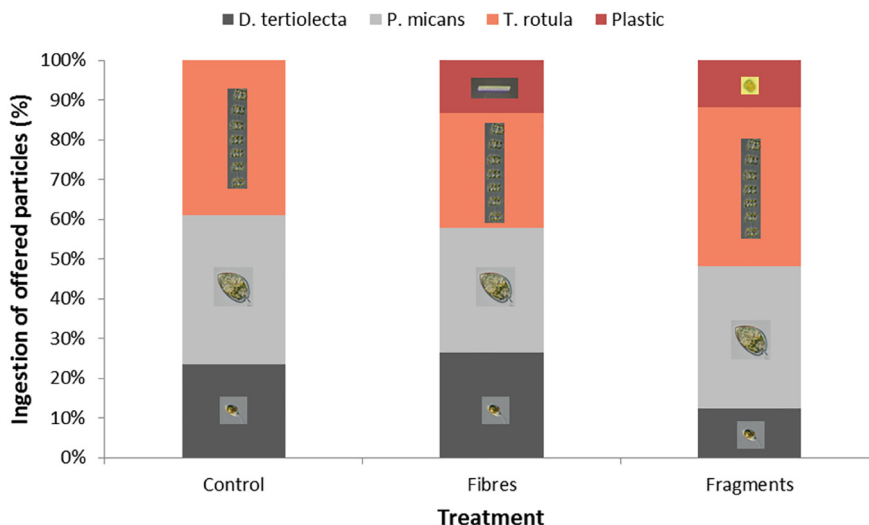

Fig. 5. Proportion of each offered particle type ingested for each treatment (*n* = 5). Dark grey blocks = *D. tertiolecta*, light grey = *P. micans*, peach = *T. rotula* and coral = plastic.
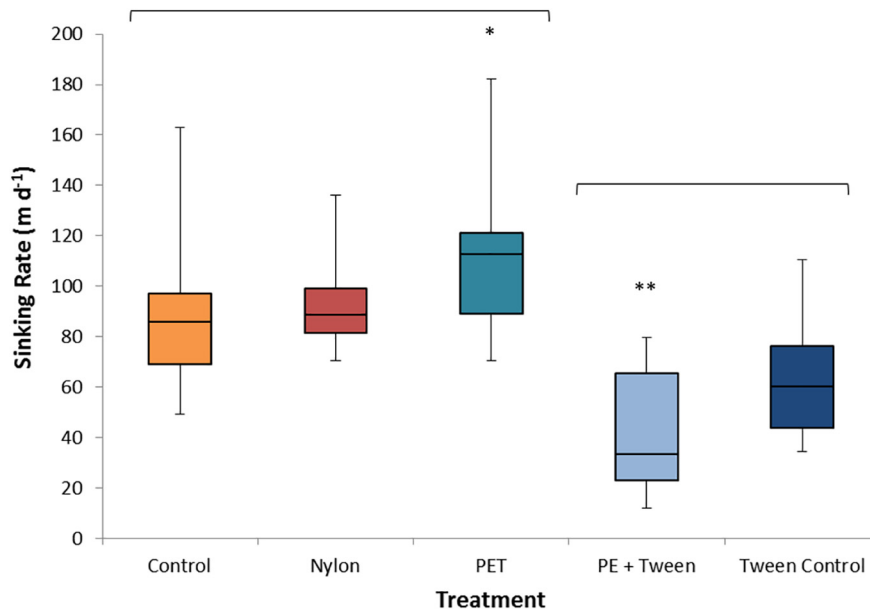
Fig. 6. Box and whisker plots of sinking rates (m d^{-1}) of control and microplastic contaminated faecal pellets. * denotes statistical significance at <0.01, ** at <0.001, GLM ($n = 4$).

Calanus sp. copepods primarily feed by generating a feeding current using appendages around their mouth (Cannon, 1928). Copepods have demonstrated complex selective capabilities when it comes to particle ingestion. A previous study observed a 40% reduction in the total carbon biomass ingested by *C. helgolandicus* when exposed to microplastic and this was due to a subtle shift in algal cell size preference away from the PS microplastics that were present (Cole et al., 2015). Some studies suggest selectivity is a function of size (Harvey and Sc, 1937; Meyer et al.,

2002), others have reported selection based on nutritional value; i.e. phytoplankton cells versus PS beads (Fernández, 1979) or that live food is preferable to detritus determined by chemo and mechanoreceptors in the zooplankton (Paffenhöfer and Sant, 1985). How and why zooplankton select one particle over another has been widely debated, with unselective feeding also reported (Djeghri et al., 2018; Leiknes et al., 2014); often highly variable feeding rates are seen and interpretation of copepod feeding strategies is notoriously difficult. Differences in
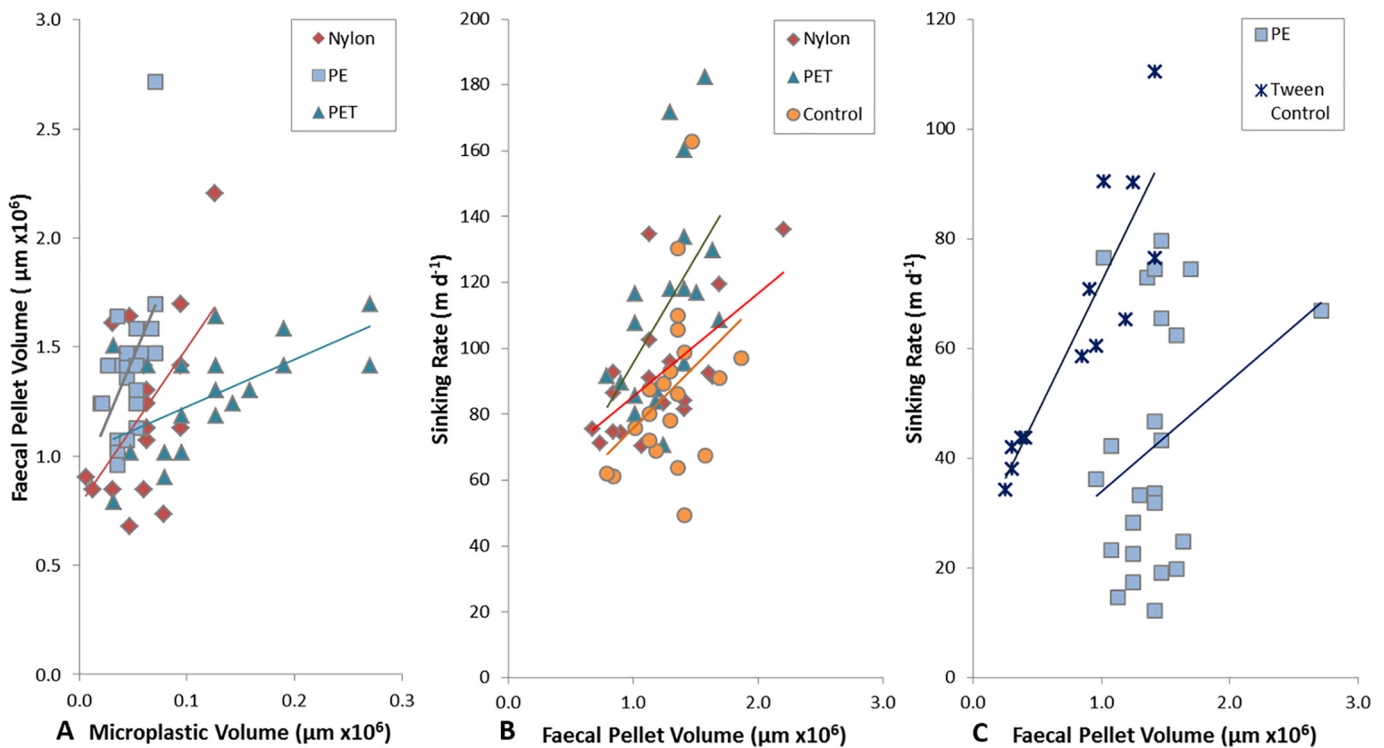

Fig. 7. Relationship between (A) volume of microplastic per faecal pellet, (μm ×10^6; nylon, red diamonds; PE, blue squares; PET, green triangles) and faecal pellet volume (μm ×10^6) and sinking rates (m d^{-1}) and faecal pellet volume (μm ×10^6) for (B) nylon, red diamonds; PET, green triangles; control, yellow circles; and (C) PE, blue squares; tween-control, blue stars. Slopes represent linear relationship (see Section 3.2 for r^2 values), lm ($n = 4$).

these rates may be explained by a wide variety of factors, including light conditions, temperature, food quality, size and abundance and pre-exposure to the experimental diet (Huntley, 1988). The copepod *Acartia clausi* has demonstrated complex grazing behaviour which includes the ability to optimise capturing food particles whilst avoiding non-food particles and to reject food post-capture (Donaghay and Small, 1979). Similarly, when offered mixtures of phytoplankton cells and PS beads, *Calanus pacificus* were able to discriminate between particles of different types, although they were not wholly efficient at rejecting the non-food PS beads (Huntley et al., 1983). It is possible that as the copepods are unable to digest the plastics, they display a learned behavioural response by attempting to avoid food of a similar size or shape which may explain the results seen in our study. It has not been possible to differentiate from our results, or predict, whether it is size or shape that is more important in the particle selection seen here, however size was determined more influential than shape in experimental studies investigating microplastic ingestion and entanglement in mysid shrimp (*Praunus* sp.) and three-spined sticklebacks (*Gasterosteus aculeatus*) (Lehtiniemi et al., 2018), prompting further investigations to quantify.

Mechanoreception, used in the handling of individual particles, is a recognised mechanism for prey detection in many Calanoid copepods. Legier-Visser et al. (Legier-Visser et al., 1986) suggested that copepods could detect and work out the size and location of a particle based on the pressure disturbance created in the feeding current. This mechanism would give credence to our suggestion here that *C. helgolandicus* may be rejecting food particles that mimic the size and shape of the microplastic. It has been suggested however, that mechanoreception can only be triggered when chemoreceptors are activated (Paffenhöfer and Jiang, 2016), based on historical studies using PS spheres as non-food particles when conducting mechanistic feeding trials. Adult female *Eucalanus pileatus* rejected PS spheres once three or more had been passed to the mouth, only ingesting the plastic once phytoplankton cells were also offered and detected in the feeding current (Paffenhöfer and Sant, 1985). More recently however, microplastic nylon fibres infused with dimethyl sulfide (DMS), an infochemical produced by many phytoplankton species, were ingested by *C. helgolandicus* up to three times more readily than non-infused nylon fibres (Procter et al., 2019), but the copepods did still ingest the non-DMS infused fibres despite no phytoplankton being offered. Behavioural studies are recommended to investigate this matter further.

Recorded microplastic abundance in marine surface waters is highly variable, both spatially and temporally, ranging from zero in some studies to >100,000 microplastic particles m^{-3} in a Swedish industrial harbour (Noren, 2007) (also see (Shim et al., 2018) and references therein). Due to methodological constraints, environmental concentrations of microplastics in the size range of those used in this study are not well known, however there is evidence to suggest microplastic concentrations increase with decreasing size (Lenz et al., 2016). The vast majority of waterborne microplastic concentration data has been obtained using a 333 μm net, therefore current reported environmental concentrations typically refer to microplastic particles larger than those used in this study. Whilst enhanced concentrations were used in our study compared to those reported for larger microplastics in the environment ($\times 10^3$ to 10^6), fragmentation of plastic (Andrady, 2011) will likely increase the number of plastics in the small size fractions; a scenario where the concentrations used in our study may potentially represent future microplastic hotspots or accumulation zones. Due to high biological productivity and the close proximity to land-based pollution sources, coastal areas are predicted convergence hotspots of zooplankton and microplastic accumulation (Clark et al., 2016). In coastal waters off California, USA, the ratio of microplastics to zooplankton was reported as 1:3 (Lattin et al., 2004) and near Plymouth, UK, microplastics outnumbered fish larvae by 27:1 (Steer et al., 2017). By altering their prey selection, copepods may shift the balance of phytoplankton community composition and such shifts have been known to lead to the development of harmful algal blooms (Hallegraeff, 2010). However, given

current concentrations and the wide range of shapes and sizes of microplastics sampled from the marine environment, such a shift would seem unlikely. A bigger concern may be for the health of the copepod themselves, where chronic exposure to plastic leads to nutrient deficiency, reduced feeding and impeded reproductive output (Cole et al., 2015). The increased handling times involved in the copepod selecting the food items may also lead to carbon deficits which in turn would have consequences for the health of the individual.

4.2. Egestion

Our results confirm that microplastic contamination of copepod faecal pellets alter their sinking rates, and those rates are primarily affected by the density of the polymer. These results compliment a previous experiment that demonstrated *C. helgolandicus* faecal pellets contaminated with low density PS, sank more slowly than uncontaminated pellets (Cole et al., 2016).

Faecal matter produced by zooplankton play a significant role in the ocean's biological carbon pump, the transport of photosynthetically-produced organic matter, or fixed carbon, away from surface waters to deeper water and sediments, and the remineralisation through grazing by zooplankton and microbial degradation (Turner, 2002). Plastic contaminated faecal pellets may alter this flux of carbon to the seabed, extending or decreasing transport times depending on the type and potentially, quantity, of plastic ingested. Our results support the idea that zooplankton faecal pellets contaminated with low density plastics such as PE may remain in surface waters for longer than uncontaminated pellets. Slowly sinking faeces are less likely to reach the sea floor (Turner, 2015), which increases the potential for repackaging of microplastics through coprophagy, the ingestion of faecal pellets (Cole et al., 2016; Iversen and Poulsen, 2007), or degradation by the microbial community (De La Rocha and Passow, 2007). Slower sinking rates may also increase the propensity for fragmentation by other zooplankters, breaking the pellets into smaller pieces and thus reducing sinking even further. Whilst not quantified in our study, Cole et al. (2016) observed increased fragmentation of faecal pellets when contaminated with PS beads, potentially increasing retention in the photic zone further. Reduced sinking rates may also allow for the degradation of the organic matter contained in the pellet to be taken up by microorganisms in the surface waters, shifting the balance of nutrient recycling from the water column to the surface, and affecting the flow of carbon to the seabed; thus alternatively fuelling faster mineralisation near the warmer water surface, than in the deeper ocean. This biogeochemical cascade may have potentially significant implications for the ocean carbon cycle and the ability of the seafloor to accumulate organic carbon fixed in photic waters, requiring future research. Furthermore, faecal pellets containing low density polymers may remain within the upper surface waters and undergo predominantly lateral advection rather than vertical flux, potentially altering also the geographical location of carbon stores due to extended buoyancy.

In contrast to low density polymers, faeces contaminated with high density polymers such as PET may increase the rate at which the carbon-rich pellets are conveyed away from surface waters. Total carbon flux varies both spatially and temporally, alongside phytoplankton, zooplankton and microbial abundance and species composition (Wilson et al., 2013), potentially also influencing microplastic dispersal. For example, krill faecal pellets were highly abundant in sediment traps deployed along the Western Antarctic Peninsula during January 2009, but were completely absent at the same location the following month (McDonnell and Buesseler, 2010). Similarly, faecal pellets contributed up to 48% of the total particulate carbon flux during a 15 year time-series study in the northeast Pacific deep sea (Wilson et al., 2013). Diel vertical migration, the synchronous daily migration of many zooplankton species and a wide range of other taxa, may also present a potential route for microplastic transport from surface to deeper waters (De La Rocha and Passow, 2013). Whilst our study did not extend to

fish faecal pellets, microplastics have been identified in the gastrointestinal tracts of adult (Lusher et al., 2013) and juvenile (Steer et al., 2017) fish and it is plausible to suggest that pellet density of small, herbivorous fish may also be influenced by ingested microplastics and contribute to altering carbon transport. Microplastic contamination of faecal pellets may therefore directly influence the lateral and vertical distribution of microplastics at locations where high densities of zooplankton or shoaling fish co-occur with microplastic hotspots and result in a significant shift in carbon export from surface waters.

No distinct relationship was observed between faecal pellet volume and the sinking rates in either PET or PE treatments. Whilst this was unexpected and contrary to many studies (Turner, 2002), it is in agreement with previous observations made between *Calanus* faecal pellet sinking rates and volumes when offered different diets (Bienfang, 1980). One explanation for our results may be due to potential variation in the size of each of the plastics ingested. The size of the plastics used were variable, however microplastic size in each pellet was not calculated and only mean size was used to calculate plastic volume.

Here, we have highlighted that animals respond very differently to microplastics of differing size, shape and polymer, and would advocate that it is important to move away from using solely PS beads as a representative plastic if we are to gain a fuller understanding of the threat microplastics pose to marine life. Our results suggest that microplastic fibres will have a more pronounced effect on copepod feeding than fragments, leading to subsequent health implications. Fibres are by far the largest reported fraction of microplastic in the marine environment and therefore pose a significant threat to copepod health and ecosystem functioning. With increasing amounts of plastic entering the oceans each year; an estimated input of up to 24 million tonnes annually by 2025 (Jambeck et al., 2014), whilst it is unlikely that current estimated microplastic levels in the ocean will significantly alter the biological pump balance, it is important to investigate and consider future scenarios based on plastics continuing to enter the oceans at predicted rates. We have demonstrated that pelagic biota can play an instrumental role in altering the properties and redistribution of plastic in the marine environment and it is now prudent to uncover the role benthic biota may impart on plastic burial in marine sediments.

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Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgements

References

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030.

Bergmann, M., Wirzberger, V., Krumpen, T., Lorenz, C., Primpke, S., Tekman, M.B., Gerdts, G., 2017. High quantities of microplastic in Arctic deep-sea sediments from the HAUSGARTEN observatory. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.7b03331 acs.est.7b03331.

Bienfang, P.K., 1980. Herbivore diet affects fecal pellet settling. Can. J. Fish. Aquat. Sci. 37, 1352–1357. https://doi.org/10.1139/f80-173.

Bonnet, D., Richardson, A., Harris, R., Hirst, A., Beaugrand, G., Edwards, M., Ceballos, S., Diekman, R., López-Urrutia, A., Valdes, L., Carlotti, F., Molinero, J.C., Weikert, H.,

Greve, W., Lucic, D., Albaina, A., Yahia, N.D., Umani, S.F., Miranda, A., Dos Santos, A., Cook, K., Robinson, S., Fernandez De Puelles, M.L., 2005. An overview of *Calanus helgolandicus* ecology in European waters. Prog. Oceanogr. 65, 1–53. https://doi.org/10.1016/j.pocean.2005.02.002.

Botterell, Z.L.R., Beaumont, N., Dorrington, T., Steinke, M., Thompson, R.C., Lindeque, P.K., 2018. Bioavailability and effects of microplastics on marine zooplankton: a review. Environ. Pollut. 245, 98–110. https://doi.org/10.1016/j.envpol.2018.10.065.

Boucher, J., Friot, D., 2017. Primary Microplastics in the Oceans: A Global Evaluation of Sources. IUCN, Gland, Switzerland.

Cannon, H.G., 1928. On the feeding mechanism of the copepods, *Calanus finmarchicus* and *Diaptomus gracilis*. J. Exp. Biol. 6, 131–144.

Cincinelli, A., Scopetani, C., Chelazzi, D., Lombardini, E., Martellini, T., Katsoyiannis, A., Fossi, M.C., Corsolini, S., 2017. Microplastic in the surface waters of the Ross Sea (Antarctica): occurrence, distribution and characterization by FTIR. Chemosphere 175, 391–400. https://doi.org/10.1016/j.chemosphere.2017.02.024.

Clark, J., Cole, M., Lindeque, P.K., Fileman, E., Blackford, J., Lewis, C., Lenton, T.M., Galloway, T.S., 2016. Marine microplastic debris: a targeted plan for understanding and quantifying interactions with marine life. Front. Ecol. Environ. 14, 317–324. https://doi.org/10.1002/fee.1297.

Cole, M., 2016. A novel method for preparing microplastic fibers. Sci. Rep., 1–9 https://doi.org/10.17795/ajehe-4541.Research.

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62, 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T.S., 2013. Microplastic ingestion by zooplankton. Environ. Sci. Technol. 47, 6646–6655. https://doi.org/10.1021/es400663f.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. Environ. Sci. Technol. 49, 1130–1137. https://doi.org/10.1021/es504525u.

Cole, M., Lindeque, P.K., Fileman, E., Clark, J., Lewis, C., Halsband, C., Galloway, T.S., 2016. Microplastics alter the properties and sinking rates of zooplankton faecal pellets. Environ. Sci. Technol. 50, 3239–3246. https://doi.org/10.1021/acs.est.5b05905.

Cozar, A., Echevarria, F., Gonzalez-Gordillo, J.I., Irigoien, X., Ubeda, B., Hernandez-Leon, S., Palma, A.T., Navarro, S., Garcia-de-Lomas, J., Ruiz, A., Fernandez-de-Puelles, M.L., Duarte, C.M., 2014. Plastic debris in the open ocean. Proc. Natl. Acad. Sci. 111, 10239–10244. https://doi.org/10.1073/pnas.1314705111.

De La Rocha, C.L., Passow, U., 2007. Factors influencing the sinking of POC and the efficiency of the biological carbon pump. Deep. Res. Part II Top. Stud. Oceanogr. 54, 639–658. https://doi.org/10.1016/j.dsr2.2007.01.004.

De La Rocha, C.L., Passow, U., 2013. The biological pump. Treatise on Geochemistry: Second Edition, 2nd ed Elsevier Ltd https://doi.org/10.1016/B978-0-08-095975-7.00604-5.

Desforges, J.P., Galbraith, M., Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. Arch Env. Contam Toxicol 69, 320–330. https://doi.org/10.1007/s00244-015-0172-5\r10.1007/s00244-015-0172-5 (pii).

Djeghri, N., Atkinson, A., Fileman, E.S., Harmer, R.A., Widdicombe, C.E., McEvoy, A.J., Cornwell, L., Mayor, D.J., 2018. High prey-predator size ratios and unselective feeding in copepods: a seasonal comparison of five species with contrasting feeding modes. Prog. Oceanogr. 165, 63–74. https://doi.org/10.1016/j.pocean.2018.04.013.

Donaghay, P.L., Small, L.F., 1979. Food selection capabilities of the estuarine copepod *Acartia clausi*. Mar. Biol. 52, 137–146. https://doi.org/10.1007/BF00390421.

Duncan, E., Broderick, A., Fuller, W., Galloway, T., Godfrey, M., Hamann, M., Limpus, C., Lindeque, P., Mayes, A., Omeyer, L., Santillo, D., Snape, R., Godley, B., 2019. Microplastic ingestion ubiquitous in marine turtles. Glob. Chang. Biol., 744–752 https://doi.org/10.1111/gcb.14519.

Fernández, F., 1979. Particle selection in the nauplius of *Calanus pacificus*. J. Plankton Res. 1, 313–328. https://doi.org/10.1093/plankt/1.4.313.

Frost, B.W., 1972. Effect of size and concentration of food particles on the feeding behaviour of the marine planktonic copepod *Calanus pacificus*. Limnol. Oceanogr. 805–815.

Hall, N.M., Berry, K.L.E., Rintoul, L., Hoogenboom, M.O., 2015. Microplastic ingestion by scleractinian corals. Mar. Biol. 162, 725–732. https://doi.org/10.1007/s00227-015-2619-7.

Hallegraeff, G.M., 2010. Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. J. Phycol. 46, 220–235. https://doi.org/10.1111/j.1529-8817.2010.00815.x.

Harris, R.P., Irigoien, X., Head, R.N., Rey, C., Hygum, B.H., Hansen, B.W., Niehoff, B., Meyer-Harms, B., Carlotti, F., 2000. Feeding, growth, and reproduction in the genus Calanus. ICES J. Mar. Sci. 57, 1708–1726. https://doi.org/10.1006/jmsc.2000.0959.

Harvey, B.H.W., Sc, D., 1937. Note on Selective Feeding XXI. pp. 97–100.

Huntley, M., 1988. Feeding biology of *Calanus*: a new perspective. Hydrobiologia 167/168, 83–99.

Huntley, M.E., Barthel, K.G., Star, J.L., 1983. Particle rejection by *Calanus pacificus*: discrimination between similarly sized particles. Mar. Biol. 74, 151–160. https://doi.org/10.1007/BF00413918.

Iversen, M.H., Poulsen, L.K., 2007. Coprorhexy, coprophagy, and coprochaly in the copepods *Calanus helgolandicus*, *Pseudocalanus elongatus*, and *Oithona similis*. Mar. Ecol. Prog. Ser. 350, 79–89. https://doi.org/10.3354/meps07095.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2014. Plastic waste inputs from land into the ocean. Science (80-.) 347.

Jones, R.H., Flynn, K.J., Anderson, T.R., 2002. Effect of food quality on carbon and nitrogen growth efficiency in the copepod *Acartia tonsa*. Mar. Ecol. Ser. 235, 147–156. https://doi.org/10.3354/meps235147.

Kiorboe, T., 1997. Population regulation and role of mesozooplankton in shaping marine pelagic food webs. Hydrobiologia 363, 13–27. https://doi.org/10.1023/A:1003173721751.

Lattin, G.L., Moore, C.J., Zellers, A.F., Moore, S.L., Weisberg, S.B., 2004. A comparison of neustonic plastic and zooplankton at different depths near the southern California shore. Mar. Pollut. Bull. 49, 291–294. https://doi.org/10.1016/j.marpolbul.2004.01.020.

Legier-Visser, M.F., Mitchell, J.G., Okubo, A., Fuhrman, J.a., 1986. Mechanoreception in calanoid copepods. Mar. Biol. 90, 529–535. https://doi.org/10.1007/BF00409273.

Lehtiniemi, M., Hartikainen, S., Näkki, P., Engström-Öst, J., Koistinen, A., Setälä, O., 2018. Size matters more than shape: ingestion of primary and secondary microplastics by small predators. Food Webs 17, e00097. https://doi.org/10.1016/j.fooweb.2018.e00097.

Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., Raley-Susman, K.M., He, D., 2017. Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*. Sci. Total Environ. 619–620, 1–8. https://doi.org/10.1016/j.scitotenv.2017.11.103.

Leiknes, Ø., Striberny, A., Tokle, N.E., Olsen, Y., Vadstein, O., Sommer, U., 2014. Feeding selectivity of *Calanus finmarchicus* in the Trondheimsfjord. J. Sea Res. 85, 292–299. https://doi.org/10.1016/j.seares.2013.05.012.

Lenz, R., Enders, K., Nielsen, T.G., 2016. Microplastic exposure studies should be environmentally realistic. Proc. Natl. Acad. Sci. 113, E4121–E4122. https://doi.org/10.1073/pnas.1606615113.

Lourenço, P.M., Serra-Gonçalves, C., Ferreira, J.L., Catry, T., Granadeiro, J.P., 2017. Plastic and other microfibers in sediments, macroinvertebrates and shorebirds from three intertidal wetlands of southern Europe and west Africa. Environ. Pollut. 231, 123–133. https://doi.org/10.1016/j.envpol.2017.07.103.

Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar. Pollut. Bull. 67, 94–99. https://doi.org/10.1016/j.marpolbul.2012.11.028.

Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2016. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal. Methods 9, 1346–1360. https://doi.org/10.1039/C6AY02415G.

McDonnell, A.M.P., Buesseler, K.O., 2010. Variability in the average sinking velocity of marine particles. Limnol. Oceanogr. 55, 2085–2096. https://doi.org/10.4319/lo.2010.55.5.2085.

Meyer, B., Irigoien, X., Graeve, M., Head, R.N., Harris, R.P., 2002. Feeding rates and selectivity among nauplii, copepodites and adult females of *Calanus finmarchicus* and *Calanus helgolandicus*. Helgol. Mar. Res. 56, 169–176. https://doi.org/10.1007/s10152-002-0105-3.

Murray, F., Cowie, P.R., 2011. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). Mar. Pollut. Bull. 62, 1207–1217. https://doi.org/10.1016/j.marpolbul.2011.03.032.

Napper, I.E., Thompson, R.C., 2016. Release of synthetic microplastic plastic fibres from domestic washing machines: effects of fabric type and washing conditions. Mar. Pollut. Bull. 112, 39–45. https://doi.org/10.1016/j.marpolbul.2016.09.025.

Nelms, S.E., Barnett, J., Brownlow, A., Davison, N., Deaville, R., Galloway, T., Lindeque, P., Santillo, D., Godley, B., 2019. Microplastics in marine mammals stranded around the British coast: ubiquitous but transitory? Sci. Rep., 1–8 https://doi.org/10.1038/s41598-018-37428-3.

Noren, F., 2007. Small plastic particles. Coastal Swedish Waters. KIMO Rep, pp. 1–11.

Paffenhöfer, G.A., Jiang, H., 2016. Comment: on phytoplankton perception by calanoid copepods. Limnol. Oceanogr. 61, 1163–1168. https://doi.org/10.1002/lno.10294.

Paffenhöfer, G.-A., Sant, K.B. Van, 1985. The feeding response of a marine planktonic copepod to quantity and quality of particles. Mar. Ecol. Prog. Ser. 27, 55–65. https://doi.org/10.3354/meps027055.

Procter, J., Hopkins, F.E., Fileman, E.S., Lindeque, P.K., 2019. Smells good enough to eat: dimethyl sulfide (DMS) enhances copepod ingestion of microplastics. Mar. Pollut. Bull. 138, 1–6. https://doi.org/10.1016/j.marpolbul.2018.11.014.

R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria URL. https://www.R-project.org/.

Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., Teh, F.-C., Werorilangi, S., Teh, S.J., 2015. Anthropogenic debris in seafood: plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci. Rep. 5, 14340. https://doi.org/10.1038/srep14340.

de Sá, L.C., Luís, L.G., Guilhermino, L., 2015. Effects of microplastics on juveniles of the common goby (*Pomatoschistus microps*): confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ. Pollut. 196, 359–362. https://doi.org/10.1016/j.envpol.2014.10.026.

Shim, W.J., Hong, S.H., Eo, S., 2018. Marine microplastics: abundance, distribution, and composition. In: Zeng, E. (Ed.), Microplastic Contamination in Aquatic Environments: An Emerging Matter of Environmental Urgency. Elsevier, pp. 1–26 https://doi.org/10.1016/B978-0-12-813747-5.

Smyth, T., Atkinson, A., Widdicombe, S., Frost, M., Allen, I., Fishwick, J., Queiros, A., Sims, D., Barange, M., 2015. The western channel observatory. Prog. Oceanogr. 137, 335–341. https://doi.org/10.1016/j.pocean.2015.05.020.

Steer, M., Cole, M., Thompson, R.C., Lindeque, P.K., 2017. Microplastic ingestion in fish larvae in the western English channel. Environ. Pollut., 1–10 https://doi.org/10.1016/j.envpol.2017.03.062.

Sun, X., Li, Q., Zhu, M., Liang, J., Zheng, S., Zhao, Y., 2017. Ingestion of microplastics by natural zooplankton groups in the northern South China Sea. Mar. Pollut. Bull. 115, 217–224. https://doi.org/10.1016/j.marpolbul.2016.12.004.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci., 201519019 https://doi.org/10.1073/pnas.1519019113.

Turner, J.T., 2002. Zooplankton fecal pellets, marine snow and sinking phytoplankton blooms. Aquat. Microb. Ecol. 27, 57–102. https://doi.org/10.3354/Ame027057.

Turner, J.T., 2004. The importance of small pelagic planktonic copepods and their role in pelagic marine food webs. Zool. Stud. 43, 255–266.

Turner, J.T., 2015. Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. Prog. Oceanogr. 130, 205–248. https://doi.org/10.1016/j.pocean.2014.08.005.

Vroom, R.J.E., Koelmans, A.A., Besseling, E., Halsband, C., 2017. Aging of microplastics promotes their ingestion by marine zooplankton. Environ. Pollut. 231, 987–996. https://doi.org/10.1016/j.envpol.2017.08.088.

Widdicombe, C.E., Eloire, D., Harbour, D., Harris, R.P., Somerfield, P.J., 2010. Long-term phytoplankton community dynamics in the Western English Channel. J. Plankton Res. 32, 643–655. https://doi.org/10.1093/plankt/fbp127.

Wilson, S.E., Ruhl, H.A., Smith, K.L., 2013. Zooplankton fecal pellet flux in the abyssal northeast Pacific: a 15 year time-series study. Limnol. Oceanogr. 58, 881–892. https://doi.org/10.4319/lo.2013.58.3.0881.

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1, 140317. https://doi.org/10.1098/rsos.140317.