

## No evidence of microplastic consumption by the copepod, *Temora longicornis* (Müller, 1785) in Chichester Harbour, United Kingdom

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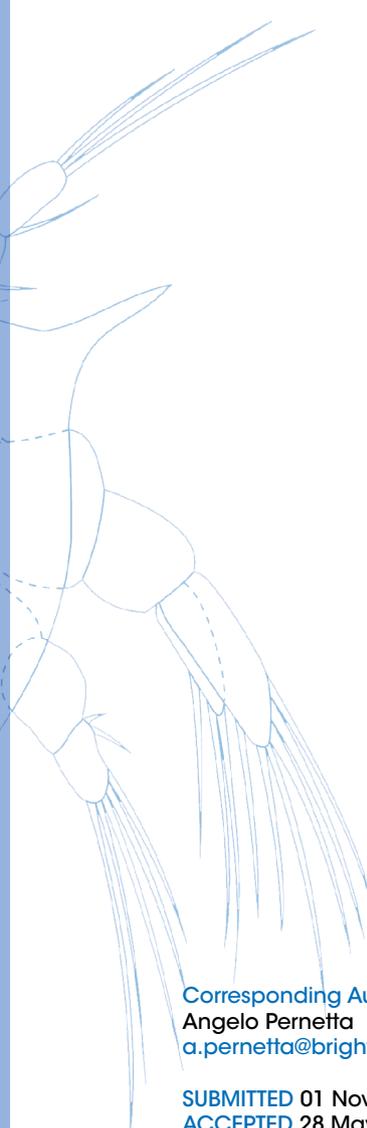
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### ABSTRACT

Increasing global concern with respect to the levels of bioavailable microplastic (<5 mm) contamination in marine environments has led to many studies examining the physiological impacts of microplastic consumption on a range of species. The copepod, *Temora longicornis* (Müller, 1785), is a common inhabitant of the upper epipelagic zone of gulf and estuarine waters of the North Atlantic which we hypothesised would be regularly exposed to microplastic contaminated marine environments. They are therefore at risk of consumption of microplastic pollutants, which could have wider trophic impacts. Microplastic was recorded in all water samples with an average concentration of 8.2 particles/m<sup>3</sup>. However, there was no significant difference in abundance or size of microplastics sampled from three localities within Chichester Harbour, UK. Individual digestion of ninety copepods found no evidence of consumption of any microplastic contaminants above our observable size range of 23 µm. Whilst microplastic pollution remains of wider ecological concern, our results suggest limited support for the potential for this copepod species to transfer these pollutants to higher trophic levels.

### KEYWORDS

Bioaccumulation, microfibers, pollution, seawater, trophic transfer.



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## INTRODUCTION

The ubiquitous nature of plastic contamination in marine environments has resulted in growing global concern (Botterell *et al.*, 2019) due to long persistence times (Welden and Lusher, 2017) and subsequent fragmentation of macroplastics (>5 mm in size) into microplastics. This has led to many studies of this anthropogenic threat documenting a range of adverse effects upon marine ecosystems (Setälä *et al.*, 2014). Microfibers are typically the most commonly identified microplastics within seawater (Beer *et al.*, 2018) and comprise common polymers such as polyester, polypropylene, fluoropolymers, acrylic, polyamide, and polyethylene assumed to derive predominantly from textiles (Courtene-Jones *et al.*, 2017). Recent estimates suggest more than 5 trillion pieces of plastic are present in marine surface waters (Eriksen *et al.*, 2014) and that 1.4 trillion microfibers may be present within the ocean (Mishra *et al.*, 2019). In addition to their inherent threat through consumption they may transfer additives from polymers, such as bisphenol A and phthalates, into both the environment and into organisms upon digestion (Pittura *et al.*, 2018).

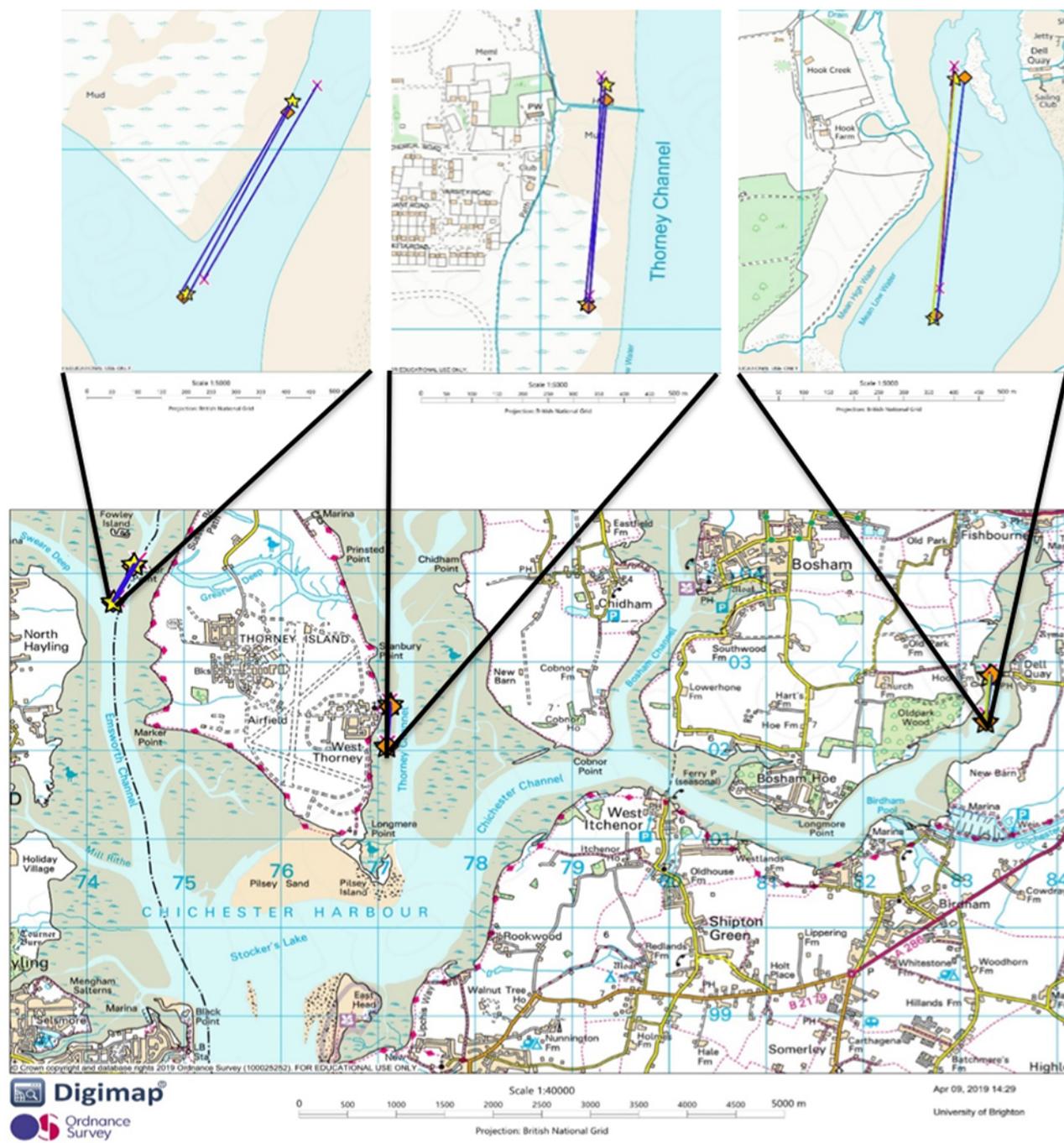
Studies have demonstrated that microplastics have been ingested by a wide variety of marine organisms (reviewed in de Sá *et al.*, 2018), including zooplankton (*e.g.*, Cole *et al.*, 2013; Setälä *et al.*, 2014). Bioaccumulation and retention of microplastics in laboratory trials has been shown to reduce nutritional state, increase mortality and decrease fecundity following experimental exposure (Welden and Cowie, 2016). Contrastingly, field-based studies have suggested ingestion of microplastics in the natural environment, may be transient and have no long-term effect upon organisms (Hämer *et al.*, 2014; Bruck and Ford, 2018). In the case of copepods, experimental exposure to microplastic beads has been shown to result in ingestion via indiscriminate feeding, with *Temora longicornis* (Müller, 1785) showing clumping of ingested microplastic beads in the posterior midgut, as well as adherence to external surfaces (Cole *et al.*, 2013).

Despite the attention being received for microplastic pollution impacts on zooplankton by the scientific community (reviewed in Botterell *et al.*, 2019), there are limited studies of ingestion of

microplastics in natural plankton communities (but see Sun *et al.*, 2018). In particular, copepods, noted as being overwhelmingly abundant and having a pivotal position in marine food webs (Turner, 2004) and highlighted for their susceptibility to microplastic ingestion due to their typical distribution near the surface, are considered an integral link between microplastic transfer and predator and prey interactions (Setälä *et al.*, 2016). Consequently, we conducted field surveys to: 1) determine microplastic concentrations within seawater in Chichester Harbour, UK, and 2) conduct focused sampling of a locally abundant copepod species (*T. longicornis*) to determine the individual uptake of microplastics via ingestion from the environment.

## MATERIAL AND METHODS

Water and plankton samples were collected on the 26<sup>th</sup> of October 2018 from three localities within Chichester Harbour (Fig. 1) on the South East Coast of England (50°79'07"N 000°94'89"W). The harbor has a high level of anthropogenic stressors, lying in close proximity to a major road (A27) and being bordered by the city of Chichester to the East and the town of Emsworth to the West. A large amount of recreational activity takes place throughout the harbor, with a number of slipways, marinas and over 3700 boat moorings. Other water-based activities, including kayaking, windsurfing and recreational fishing take place widely across the harbor. In addition, the harbor has an active commercial oyster fishery and hosts a large bait digging community. Three trawls were undertaken at each of the sampling sites Dell Quay (DQ), Emsworth Channel (EC) and Thorney Channel (TC), which varied in distance from the harbor entrance (7.2 km, 4.5 km and 1.8 km respectively). GPS coordinates, pH, temperature, dissolved oxygen concentration and turbidity were recorded at the beginning and end of each trawl (Tab. 1). A 50 cm diameter plankton net with a mesh size of 20 µm (NHBS, UK) was utilized to collect samples in order to collect any microplastic particles that would have potentially been lost if a standard 250 µm plankton tow was employed. Time and speed of tows were determined during a pilot study carried out



**Figure 1.** Map of Chichester Harbour, East Sussex, U.K. showing the 3 sampling locations. Insets from left to right show locations sampled in Emsworth Channel, Thorney Channel and Dell Quay.

**Table 1.** Water quality parameters collected prior to each trawl at each sampling location in Chichester Harbour showing Mean ± Standard Deviation.

	pH	Temp (°C)	DO (mg/L)	Conductivity (µS/cm)	Turbidity (NTU/FNU)
Dell Quay	7.87 ± 0.32	12.5 ± 0	10.7 ± 0.21	51.77 ± 0.49	5.00 ± 0.56
Thorney Channel	7.67 ± 0.54	13.67 ± 0.05	9.93 ± 0.05	54.33 ± 0.24	2.47 ± 0.40
Emsworth Channel	8.13 ± 0.09	13.4 ± 0.08	10.1 ± 0	54.57 ± 0.05	2.55 ± 1.19

during August 2018. Horizontal tows were carried out at high tide and at slack water and were 10 minutes in duration at 1.5 knots with an average trawl length of 483.58 m. All tows were carried out in triplicate and given the time of year were appropriate for the purposes of this study. The total volume of water which passed through the net was calculated based on the following equation:

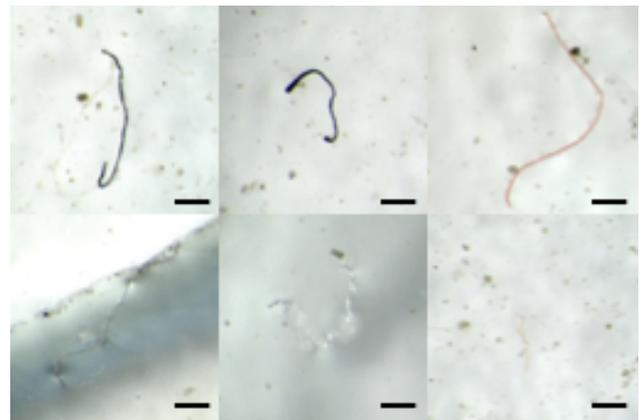
$$V = \pi r^2 \times L$$

Where,  $V$  = volume ( $\text{m}^3$ ),  $r$  = radius of net opening (m) and  $L$  = distance net was towed (m). The plankton tows resulted in an average of  $392.75 \text{ m}^3$  of water being filtered through the net across all sites.

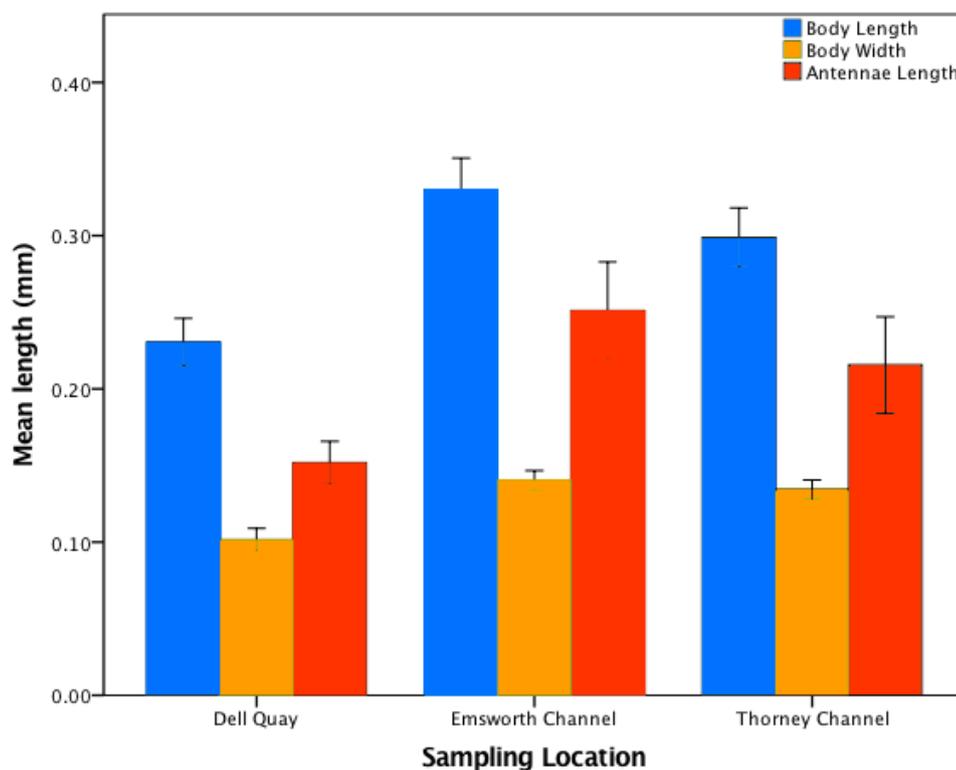
Following the trawl, collected seawater samples were transferred into a 1 L Nalgene bottle and preserved with 4% formalin prior to further analysis. Quantification of microplastic abundance in the water was determined by taking 10 x 1 ml water samples from each of the three tows at each sampling locality. Each 1 ml sample was pipetted on a glass agar plate and observed at 40 x magnification under light microscopy (Leitz Laborlux S). All potential microplastics were photographed, counted and verified using Enders *et al.*'s (2015) established criteria for visual characterization. Lengths of microplastics were measured from photographs using ImageJ (Schneider *et al.*, 2012). Estimates of microplastic concentrations within seawater (microplastics/ $\text{m}^3$ ) were calculated based on the total volume of seawater through the net each trawl and the total number of microplastics recorded in the samples. The surface water samples were dominated by microfibrils and these were identified and documented based on color (Fig. 2).

Ten individual adult *T. longicornis* were isolated from each sample (Total  $n = 30$  per site and 90 in total) using 150 mm glass Pasteur pipettes under a dissection microscope (Leica EZ4). Individual copepods were then imaged using a light microscope (Nikon Eclipse E200) with Moticam software (GX Capture©). Each individual was transferred to glass cavity slides (Agar Scientific), species identity confirmed, and visually assessed under the light microscope for potential attached microplastics. The body length, body width and first antenna length of each were measured

to the nearest  $\mu\text{m}$  using ImageJ (Fig. 3). Antenna length was measured as it has been suggested that the antennae of *T. longicornis* act as mechanoreceptors (Gill, 1986; Yen *et al.*, 1992) and can facilitate determination of the concentration and availability of food items (Yule and Crisp, 1983). Therefore, antenna length may correspond to the number or type of microplastic particles ingested. Copepods were digested individually using a protocol adapted from Enders *et al.* (2017) with a 1:1 ratio of 30% potassium hydroxide (KOH) and sodium hypochlorite (NaClO) (14% active chlorine). In brief,  $60 \mu\text{l}$  of KOH:NaClO was added to an individual copepod in a well of a 96 well plate that had been cleaned with reverse osmosis (RO) water prior to use. The plates were covered using parafilm and kept at room temperature for 12 hours. The protocol of Enders *et al.* (2017) was modified in two ways. After initial digestion trials it was determined that 12 h was the optimal digestion period for *T. longicornis* due to the breakdown of the chitinous carapace. Both KOH (Rochman *et al.*, 2015) and NaClO (Collard *et al.*, 2015) have been shown to be effective over a 12-hour digestion period. Following this step the digested solutions were pipetted into a sterile 1 ml Eppendorf and shaken for 5 minutes on a vortex (IKA 3) to further breakdown any chitinous material. The subsequent solution was then filtered using a vacuum pump (Thermoscientific Nalgene Rapid Flow) with cellulose-acetate membrane filter papers (Satorius Stedim, pore size =  $0.2 \mu\text{m}$ ) and the filter papers were then analysed at 40x magnification



**Figure 2.** The range of color and size of microfibrils detected in surface water samples from the three sites within Chichester Harbour (scale bars =  $100 \mu\text{m}$ ).



**Figure 3.** Mean ( $\pm$  SE) body length, width and antennae length of *Temora longicornis* samples from three sampling localities in Chichester Harbour, UK ( $n = 30$  per locality).

under light microscopy to identify the presence of any microplastics, both fibers and particles.

Contamination risks in such studies are high, therefore in addition to steps to reduce plastic in the environment, control procedural blanks were also used at each stage of the laboratory procedures to account for any airborne contamination (Prata, 2018). Six 1 ml RO water samples were subject to the same conditions as seawater samples, and six blank wells with only the digestion solution were subject to the same procedures as the copepod digestions.

## RESULTS

Microplastic particles were detected in all seawater samples examined from all three sites with an average concentration of 8.2 particles per  $m^3$  of sampled seawater (Tab. 2). Blue microplastics were the most abundant in all three sampling localities, but there was a significant difference in the abundance of colors between the three sampling sites ( $X^2 = 23.891$ ,  $df=10$ ,  $p = 0.008$ ; Tab. 2). However, there was no difference in mean microplastic length between sampling

localities (ANOVA,  $F = 0.92$ ,  $df = 2$ ,  $p = 0.912$ ). The high presence of blue microplastic particles has been commonly documented (Zhao *et al.*, 2016; Jamieson *et al.*, 2019; Wu *et al.*, 2019) although in many instances blue “microplastics” were found to be natural fibres and not synthetic in nature (Zhao *et al.*, 2016; Jamieson *et al.*, 2019).

Comparisons of *T. longicornis* showed a significant difference between sampling localities in body length (ANOVA,  $F = 7.523$ ,  $df = 2$ ,  $p = 0.001$ ), body width (ANOVA,  $F = 10.144$ ,  $df = 2$ ,  $p < 0.001$ ), and antennae length (ANOVA,  $F = 10.144$ ,  $df = 2$ ,  $p = 0.036$ ). Tukey’s post-hoc analyses showed copepods sampled from Dell Quay to be significantly smaller in all measurements than those from both Emsworth and Thorney channels (Fig. 3). This is likely due to Dell Quay being furthest from the harbor mouth, with less water exchange and increased boating activity, giving the channel higher turbidity than either Emsworth or Thorney Channels, resulting in a reduction of prey items. Decreased phytoplankton concentrations have been shown to reduce *T. longicornis* body size (Breteler and Gonzalez, 1988), therefore the reduced

**Table 2.** Microplastic counts, sizes and concentrations recorded in each sampling location in Chichester Harbour

Microplastic counts by colour	Dell Quay	Emsworth Channel	Thorney Channel	Total
Black	75	68	34	177
Blue	108	79	72	259
Red	31	39	44	114
Yellow	6	10	3	19
Grey	22	16	7	45
Transparent	43	44	35	122
Total number of recorded microplastic particles	285	256	195	736
Mean $\pm$ SE microplastic size (mm)	0.250 ( $\pm 0.017$ )	0.241 ( $\pm 0.225$ )	0.238 ( $\pm 0.017$ )	0.243 ( $\pm 0.109$ )
Mean concentration (particles/m <sup>3</sup> )	9.5	8.5	6.5	8.2

body size observed in this study may be a result of the increased turbidity impacting phytoplankton densities in Dell Quay. There was little variation between other recorded water quality parameters (Tab. 1). Following the digestion and filtration procedures, no microplastic fragments or fibres were detected in any of the *T. longicornis* samples from any of the sites ( $n = 90$ ). In contrast, 15 microplastic fibres were recorded in the six contamination controls (mean = 2.5 fibres per replicate), suggesting a true absence of microplastics in the *T. longicornis* samples. FTIR analysis was not utilised for this study as no consumption of microplastics by *T. longicornis* was observed and no correlation between microplastic color and consumption could be made.

## DISCUSSION

Coastal areas are hotspots of microplastic accumulation, with estuaries and rivers being significant input pathways for plastics into oceans (Lebreton *et al.*, 2017). Whilst our results confirmed the ubiquitous presence of microplastic contamination in the water sampled within Chichester Harbour at comparable levels to sites in the eastern Mediterranean (van der Hal *et al.*, 2017) and northern Adriatic Sea (Gajšt *et al.*, 2016), our results showed no evidence of microplastic consumption by *T. longicornis*. This lack of consumption may be a result of dietary selectivity by *T. longicornis*. Previous feeding studies having shown this species to alter behavioral responses when exposed to toxic dinoflagellates, whereby continued exposure to

toxic *Alexandrium* spp. resulted in individual copepods showing high rates of regurgitation (Xu *et al.*, 2017). It may be that this species exhibits a similar response when exposed to microplastic particles during feeding. Other field-based studies have shown very low levels of microplastic consumption by individual *Neocalanus cristatus* Krøyer, 1848 (0.026 particles/individual, Desforges *et al.*, 2015), and Copepoda more broadly (0.33 particles/individual, Kosore *et al.*, 2018).

Given the observed microplastic sizes in our study relative to the mean body lengths of *T. longicornis* sampled, our results suggest that the potential for trophic transfer of microplastic pollution is likely to be low for this particular species. It is possible that accumulation of microplastic in higher species, such as mesopelagic fish, may be more likely a result of direct consumption of microplastic pollutants that are mixed with zooplankton in the surface waters (Lusher *et al.*, 2016). Furthermore, nano-sized plastics, beyond the limit of detection in this study, may be present and may be transferred to higher trophic levels. It is acknowledged that detecting particles below 100  $\mu\text{m}$ , especially those found in the environment, is difficult to undertake (Huvet *et al.*, 2016) which may explain why, to date, we are not aware of any literature that has recorded nano-sized particles in wild copepods. However, given the relatively small sample size and single focal species employed in this study, further research is needed to determine whether incidences of microplastic consumption occur at significant levels in other zooplankton species, which may impact on higher trophic levels in estuarine environments.

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