

# Bioavailability of Microplastics to Marine Zooplankton: Effect of Shape and Infochemicals

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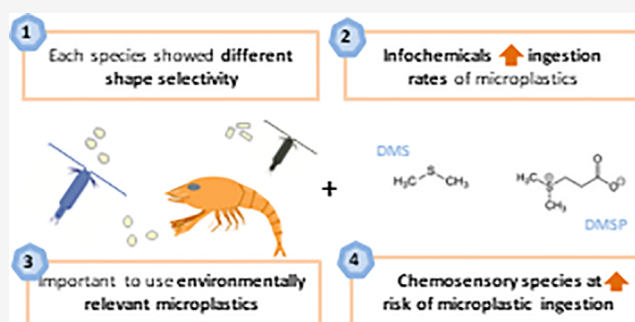


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**ABSTRACT:** The underlying mechanisms that influence microplastic ingestion in marine zooplankton remain poorly understood. Here, we investigate how microplastics of a variety of shapes (bead, fiber, and fragment), in combination with the algal-derived infochemicals dimethyl sulfide (DMS) and dimethylsulfoniopropionate (DMSP), affect the ingestion rate of microplastics in three species of zooplankton, the copepods *Calanus helgolandicus* and *Acartia tonsa* and larvae of the European lobster *Homarus gammarus*. We show that shape affects microplastic bioavailability to different species of zooplankton, with each species ingesting significantly more of a certain shape: *C. helgolandicus*—fragments ( $P < 0.05$ ); *A. tonsa*—fibers ( $P < 0.01$ ); *H. gammarus* larvae—beads ( $P < 0.05$ ). Thus, different feeding strategies between species may affect shape selectivity. Our results also showed significantly increased ingestion rates by *C. helgolandicus* on all microplastics that were infused with DMS ( $P < 0.01$ ) and by *H. gammarus* larvae and *A. tonsa* on DMS-infused fibers and fragments ( $P < 0.05$ ). By using a range of more environmentally relevant microplastics, our findings highlight how the feeding strategies of different zooplankton species may influence their susceptibility to microplastic ingestion. Furthermore, our novel study suggests that species reliant on chemosensory cues to locate their prey may be at an increased risk of ingesting aged microplastics in the marine environment.



## INTRODUCTION

Microplastic (microscopic plastic, 1  $\mu\text{m}$  to 5 mm) is abundant and widespread in the marine environment and has been identified as a contaminant of global environmental and economic concern.<sup>1–3</sup> The risks that larger plastic debris presents to marine organisms have been well documented,<sup>4–7</sup> yet there still remain knowledge gaps regarding the impact of microplastics.<sup>1</sup> Microplastics are not uniform; they are a complex array of different shapes, sizes, and polymers.<sup>8</sup> Microbeads and fibers can enter the environment *via* multiple pathways with direct release from waste water treatment works being a substantial source through the use of plastics in cosmetics and synthetic clothing.<sup>9,10</sup> The degradation of larger plastic debris due to ultraviolet (UV) radiation and wave action can form irregularly shaped microplastic fragments.<sup>10,11</sup> Due to their small size, microplastics are bioavailable *via* ingestion to a wide range of organisms.<sup>12</sup> Ingestion of microplastics has been recorded in many marine species including cetaceans,<sup>13,14</sup> seabirds,<sup>15</sup> molluscs,<sup>16</sup> and zooplankton.<sup>17,18</sup> In species at lower trophic levels, such as zooplankton, ingested microplastics have been shown to cause several detrimental effects, including reduced feeding behavior, growth, and fecundity.<sup>19–21</sup>

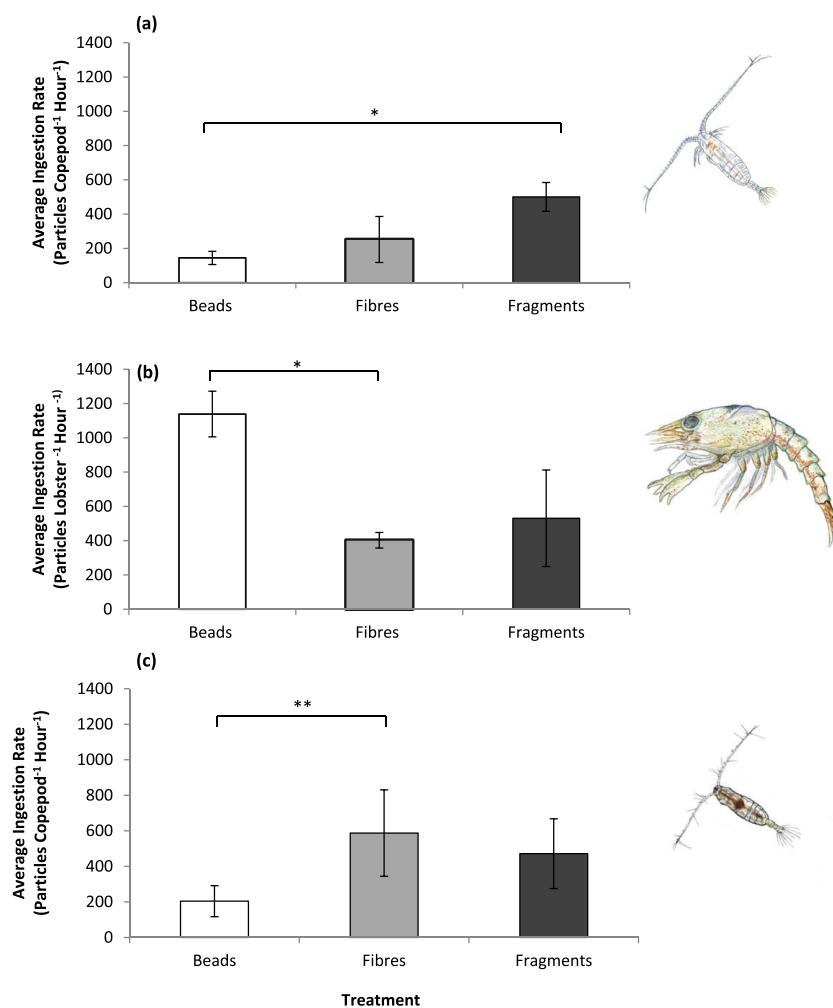
Zooplankton are a crucial food source and provide an important link in the marine food web between phytoplankton and higher trophic levels.<sup>22</sup> Zooplankton comprise many different species of marine invertebrates and some vertebrates (e.g., fish larvae), including those species that spend their entire life cycle (holoplankton), and those with larval stages (meroplankton), in the plankton. Meroplanktonic species develop into adults that are often important constituents of fin-fish and shellfish stocks, which are ecologically and economically important.<sup>23</sup> Previous laboratory studies have shown that zooplankton have the capacity to ingest several different types of microplastics,<sup>17,24</sup> which has also been documented in zooplankton from the wild.<sup>18,25,26</sup> While many studies have investigated the microplastic presence and effect of ingestion, the underlying mechanisms and factors that influence plastic ingestion still remain poorly understood.

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Food selectivity has been widely evidenced in copepods, with the capacity to discriminate between algal prey and microplastics.<sup>27,28</sup> There are several abiotic and biotic factors that can affect the biological availability (bioavailability) of microplastics to an organism.<sup>8,29</sup> While the overlap in size of the microplastic and the gape of the individual's mouth is key to ingestion and capture efficiency, other factors such as microplastic shape may affect handling and the capacity for ingestion.<sup>8,28</sup> Many previous laboratory studies have used polystyrene microspheres that have been shown to be readily ingested by a number of species, indicating that this shape is bioavailable to a broad range of taxa.<sup>17,19,30</sup> However, several studies from the field investigating microplastic ingestion in zooplankton found that microfibers were most commonly ingested.<sup>18,25,26</sup> It is unclear whether this shape is more bioavailable or whether it is the most abundant microplastic in the areas sampled. Recent research has shown that differently shaped microplastics can alter the severity of certain biological effects.<sup>31,32</sup> For example, in sheepshead minnow larvae (*Cyprinodon variegatus*), irregularly shaped microplastic fragments negatively affect swimming behavior, decreasing the total distance traveled and their maximum velocity.<sup>32</sup> Additionally Cole et al. showed that the presence of fibrous microplastics can significantly alter prey selectivity in the

copepod *Calanus finmarchicus*.<sup>31</sup> These negative effects could reduce food intake and the available energy for growth, development, and reproductive success.

There is growing evidence that chemosensory cues, not just physical factors, could influence the bioavailability of microplastics.<sup>24,33–35</sup> In the marine environment, plastic can provide a durable substrate for biofouling biota that may produce infochemicals.<sup>36,37</sup> Colonization by infochemical-producing microorganisms, and subsequently the formation and growth of biofilms, could lead to plastic debris acquiring a chemical signal that is attractive to those species that use chemosensory mechanisms when locating, identifying, and ingesting food.<sup>33,38–40</sup> One such chemical is dimethyl sulfide (DMS), a marine trace gas derived from dimethylsulfoniopropionate (DMSP) that is produced by phytoplankton.<sup>39</sup> DMS concentrations typically range from 1 to 7 nM in the surface ocean, with peak concentrations in the North Atlantic Ocean during June–July owing to the annual coccolithophore and dinoflagellate blooms.<sup>41</sup> Recent research has identified that many species demonstrate foraging behavior in the presence of DMS, including loggerhead turtles,<sup>42</sup> seabirds,<sup>33</sup> hard corals,<sup>34</sup> and copepods.<sup>43</sup> The chemical precursor DMSP has also been shown to induce swimming and aggregation behavior in forage fish.<sup>44,45</sup> Breckels et al. demonstrated that DMS plumes

stimulated the grazing behavior response of *Calanus helgolandicus* at concentrations of 1.8–13.1 nM.<sup>46</sup> Recent research by Procter et al. showed that *C. helgolandicus* ingested significantly more DMS-infused microfibers than virgin microfibers.<sup>35</sup> This indicates that complex chemosensory cues may have a role in mediating foraging behavior and therefore consumption of microplastic debris.

Currently, little is known about what factors influence the uptake of microplastics by zooplankton. To better understand the mechanisms behind microplastic ingestion, it is vital to use microplastics that are more representative of those found in the marine environment.<sup>8,47</sup> In the present study, we investigate the effect of microplastic shape and the presence of the infochemicals DMSP and DMS on microplastic ingestion by three species of zooplankton: the widely distributed suspension feeder *C. helgolandicus*, a temperate calanoid copepod and dominant mesozooplankton species in the North Atlantic; the globally distributed calanoid copepod *Acartia tonsa*, an ambush and suspension feeder; and the ambush feeder larvae of *Homarus gammarus* (European lobster)—a species of both economic and social importance in the U.K. We test the hypotheses that (1) species-specific ingestion is significantly different between microplastics of various shapes and (2) infusion of microplastics with DMS or DMSP significantly increases the bioavailability and ingestion of the differently shaped microplastics.

## METHODS

**Zooplankton Sampling and Husbandry.** Zooplankton samples were collected from the Western Channel Observatory station L4, U.K. (50°15'N, 4°13'W) using 200  $\mu\text{m}$  WP2 plankton nets in February 2019. The samples were transported within insulated boxes, containing natural seawater, to Plymouth Marine Laboratory (Plymouth, U.K.) within 3 h of sampling. Adult female *C. helgolandicus* were identified using a dissecting microscope (Wild M5-49361;  $\times 20$  to  $\times 50$  magnification) through assessment of their life stage, size, shape, and distinct genital pore. Individuals were carefully picked out using Storkbill forceps and transferred to 5 L beakers containing filtered seawater. Seawater was filtered *via* a filtration rig through a 0.22  $\mu\text{m}$  nitrocellulose filter (Millipore). European lobster larvae (*H. gammarus*) (stage 1) were obtained in August 2018 from the National Lobster Hatchery, Padstow, U.K. Adult *A. tonsa* were provided from culture by Reefshotz, U.K. in September 2019. Female *A. tonsa* were identified using a dissecting microscope through assessment of their size, shape, and distinct genital pore. All samples were processed and experiments were conducted in controlled-temperature (CT) laboratories matched to the ambient sea surface temperature (SST) of 18 °C (*H. gammarus*, August 2018) and 10 °C (*C. helgolandicus*, February 2019) or culture temperature of 21 °C (*A. tonsa*).

**Preparation of Microplastics.** Virgin 20  $\mu\text{m}$  polystyrene beads (Spherotech, Illinois), fresh-cut virgin 20  $\mu\text{m}$  x 10  $\mu\text{m}$  nylon fibers (Goodfellow Cambridge Ltd., prepared following Cole method<sup>48</sup>), and virgin  $\leq 20$   $\mu\text{m}$  nylon fragments (Goodfellow Cambridge Ltd.) were used to represent our three shape classifications (Supporting Information, Figure 1). All microplastics used were yellow in color. The different polymers were used as at the time no nylon microbeads were available. While the size of the microplastics was kept as uniform as possible, due to the different shapes, surface area (beads:  $1.26 \times 10^3 \mu\text{m}^2$ , fibers:  $0.79 \times 10^3 \mu\text{m}^2$ , fragments:

$>1.26 \times 10^3 \mu\text{m}^2$ ) and volume (beads:  $4.19 \times 10^3 \mu\text{m}^3$ , fibers:  $1.57 \times 10^3 \mu\text{m}^3$ , fragments:  $<4.19 \times 10^3 \mu\text{m}^3$ ) varied.

For the experiments with *C. helgolandicus* and *A. tonsa*, glass DURAN experimental bottles (500 mL, total volume 615 mL) were  $\sim 75\%$  filled with 0.22  $\mu\text{m}$  filtered seawater (FSW) and spiked with the 15 mL vials of either beads, fibers, or fragments of virgin (control) or DMSP- or DMS-infused beads, fibers, or fragments. The bottles were then carefully filled to the brim with FSW, which resulted in an overall concentration of 80 microplastics  $\text{mL}^{-1}$  in each of the experimental bottles. All microplastic treatments were incubated in MilliQ water in 15 mL gas-tight vials in a refrigerator at 3 °C for 3 days before use in grazing experiments.

Environmentally relevant concentrations of DMSP and DMS were chosen for our infused treatments.<sup>41,49</sup> DMSP-infused beads/fibers/fragments were prepared by infusion in an aqueous 20 nM DMSP solution (Centrum voor Analyse, Spectroscopie and Synthese, Rijksuniversiteit Groningen, The Netherlands). DMS-infused beads/fibers/fragments were prepared by infusion in a 5 nM aqueous DMS solution (Sigma-Aldrich Company Ltd.). The addition of the infused microplastics to the experimental flasks was performed by directly adding the entire 15 mL solution to the flask at the start of the experiment. This addition to the 600 mL of seawater in the flask resulted in a negligible calculated change in ambient DMSP/DMS concentrations of 0.49 nM (DMSP) and 0.12 nM (DMS).

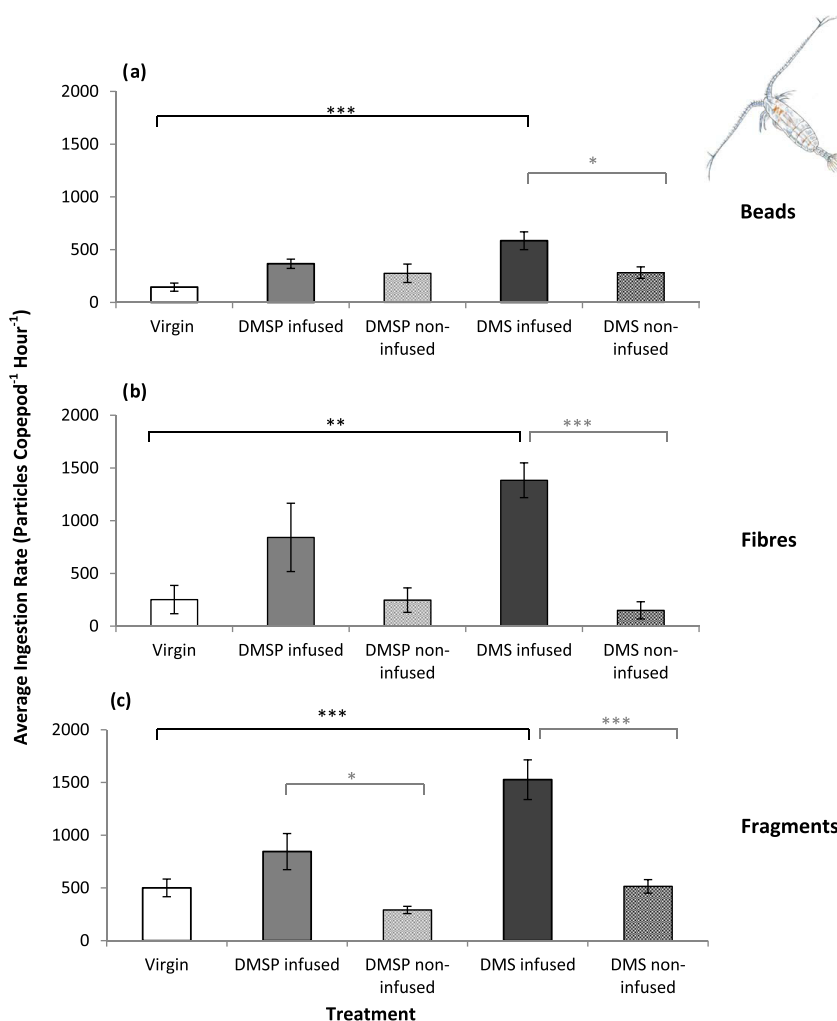
The same process was used for the European lobster larvae. However, due to their cannibalistic nature under limited food availability, larvae were treated individually using smaller experimental bottles (50 mL) and gas-tight vials (1.9 mL). The concentrations of microplastics, DMSP, and DMS remained the same.

At the microplastic concentrations used in this experiment, we were unable to measure the final concentrations of DMS/DMSP fused to the microplastics as the levels are far below the detection limit of our methods.

**Grazing Experiments.** For all species and for each shape of microplastic, the grazing experiments consisted of (1) a virgin microplastic control group; (2) a DMSP-infused microplastic treatment group; and (3) a DMS-infused microplastics treatment group. Following the initial results with *H. gammarus* larvae, we refined the protocol in the *C. helgolandicus* and *A. tonsa* experiments to include additional controls of (4) DMSP and microplastics added to the experimental bottles separately but concurrently (DMSP noninfused); and (5) DMS and microplastics added to the experimental bottles separately but concurrently (DMS noninfused). Copepods and lobster larvae were starved for a period of 24 h prior to the experiment.

In the *C. helgolandicus* and *A. tonsa* experiment, there were six replicates per treatment for virgin, infused/noninfused DMSP and DMS treatment groups, using copepods that had been acclimatized to the laboratory conditions for 2 days. The algae *Dunaliella tertiolecta*, *Prorocentrum micans*, and *Thalassiosira rotula* were provided as a source of prey during the acclimation period for *C. helgolandicus*. They were cultured on f/2 media, with addition of silica for *T. rotula*, and maintained at 13 °C at a 16:8 light/dark cycle. *A. tonsa* copepods were maintained on their culture media of *Tetraselmis suecica*, *Isochrysis galbana* (T-Iso), and *Chaetoceros muelleri*.

Grazing experiments were carried out in gas-tight 500 mL Pyrex bottles (total volume 615 mL), filled to the brim with



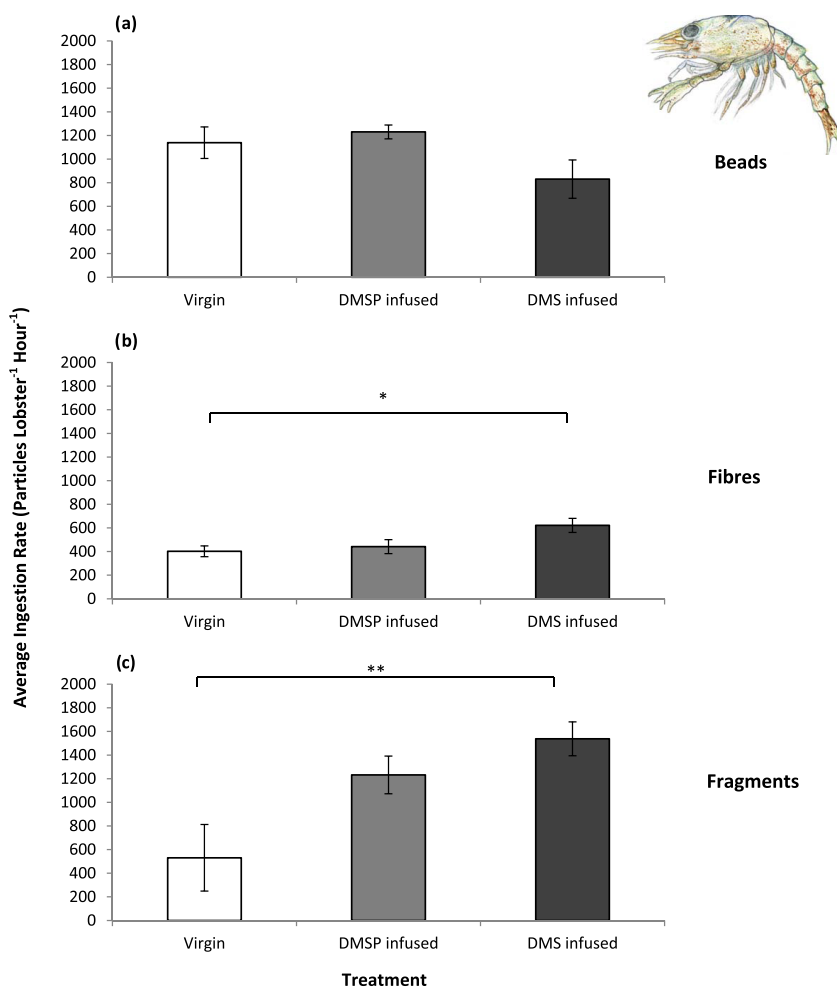
**Figure 2.** *C. helgolandicus* ingestion rates  $\pm$  SE (particles copepod<sup>-1</sup> h<sup>-1</sup>) of differently shaped microplastics: (a) beads, (b) fibers, and (c) fragments, with the different treatments of virgin, DMSP infused, DMS infused, DMSP noninfused, and DMS noninfused. The black significance bars relate to virgin controls and the gray significance bars relate to noninfused controls. The asterisks denote the levels of significant difference in the ingestion rates, \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ . Illustration by Vivienne Botterell.

0.2  $\mu\text{m}$  filtered seawater (FSW). Five healthy adult female *C. helgolandicus* or *A. tonsa* were transferred to each experimental bottle, followed by the addition of microplastics with or without DMSP or DMS. Copepods were not added to the  $T_0$  (time zero, beginning of the experiment) experimental bottles. The experimental bottles were secured to a plankton wheel, rotated at 5 rpm, and left for 6 h in the dark in a CT room at 10 °C (*C. helgolandicus*) or 21 °C (*A. tonsa*). After 6 h, the experiment was stopped and the copepods were removed from each experimental bottle by gently passing the contents of the bottle through a 150  $\mu\text{m}$  mesh into a beaker. The water was returned to the bottle and stored at 3 °C for microplastic enumeration using a FlowCam (Fluid Imaging Technologies Ltd.; see below). The mesh containing the copepods was examined under a dissection microscope and any mortality was recorded. Copepods were then transferred into an Eppendorf tube containing 1 mL of 4% recycled formalin.

In the *H. gammarus* larvae experiment, there were ten replicates per treatment for virgin, DMSP and DMS treatment groups, using lobster larvae that had been acclimatized to the laboratory conditions for 2 days. Frozen plankton (TMC Gamma Blister Red Plankton) was provided as a source of prey during the acclimation period.

Grazing experiments were carried out in gas-tight 50 mL Pyrex bottles (total volume 65 mL), filled to the brim with 0.2  $\mu\text{m}$  filtered seawater (FSW). One healthy European lobster larva was transferred to each experimental bottle, followed by the addition of microplastics with or without DMSP or DMS. Lobster larvae were not added to the  $T_0$  experimental bottles. The experimental bottles were secured to a plankton wheel, rotated at 5 rpm, and left for 3 h in the dark in a CT room at 18 °C. We then followed the same process as above in the experiments, preserving the individuals in 1 mL of 4% recycled formalin.

A FlowCAM (VS-4 series) was used in autoimage mode to count the number of microplastic particles within a sample and determine the plastic concentration. For the analysis, 40 mL of the sample was pumped at 2 mL min<sup>-1</sup> through the flow chamber fitted with a 100  $\mu\text{m}$  x 2 mm flow cell and a 10x objective lens, which captured the images of the particles at 20 frames s<sup>-1</sup>. FlowCAM uses the software program Visual-Spreadsheet (v 3.4), which can sort the images by selected characteristics such as length. Any images that were not microplastic particles were deleted and the remaining images saved as a new list, which would then be used to generate a count of particles mL<sup>-1</sup>. The FlowCAM was used to determine



**Figure 3.** *H. gammarus* (larvae) ingestion rates  $\pm$  SE (particles lobster<sup>-1</sup> h<sup>-1</sup>) of the virgin, DMSP-infused, and DMS-infused microplastics for all three shapes: (a) beads, (b) fibers, and (c) fragments. The asterisks denote the levels of significant difference in the ingestion rates, \* $P \leq 0.05$ , \*\* $P \leq 0.01$ . Illustration by Vivienne Botterell.

the initial microplastic concentration ( $T_0$ ) and the postexperimental microplastic concentration ( $T_6$ ).

Grazing rates were estimated by comparing changes in the abundance of the microplastics over the experimental period for differently shaped microplastics with or without the addition of DMSP and DMS. Ingestion rates (particles organism<sup>-1</sup> h<sup>-1</sup>) were calculated using an adapted version of the Frost (1972) equation, which accounted for the absence of prey growth during the incubations.<sup>35,50</sup>

The grazing coefficient ( $g$ ) was calculated from eq 1

$$g = 0 - \log \frac{T_6}{T_0} \times \frac{1}{T} \quad (1)$$

where  $T_0$  is the concentration of microplastics mL<sup>-1</sup> at the beginning of the experiment,  $T_6$  ( $T_3$  for *H. gammarus* larvae experiment) is the concentration of microplastics mL<sup>-1</sup> at the end of the experiment (after 3 or 6 h), and  $T$  is the time in hours (Supporting Information, Table 1). The clearance rates ( $F$ , mL organism<sup>-1</sup> h<sup>-1</sup>) were calculated from eq 2

$$F = \frac{V \times g}{n} \quad (2)$$

where  $V$  is the volume of the experimental bottle (mL),  $g$  is the grazing coefficient calculated in eq 1, and  $n$  is the number of

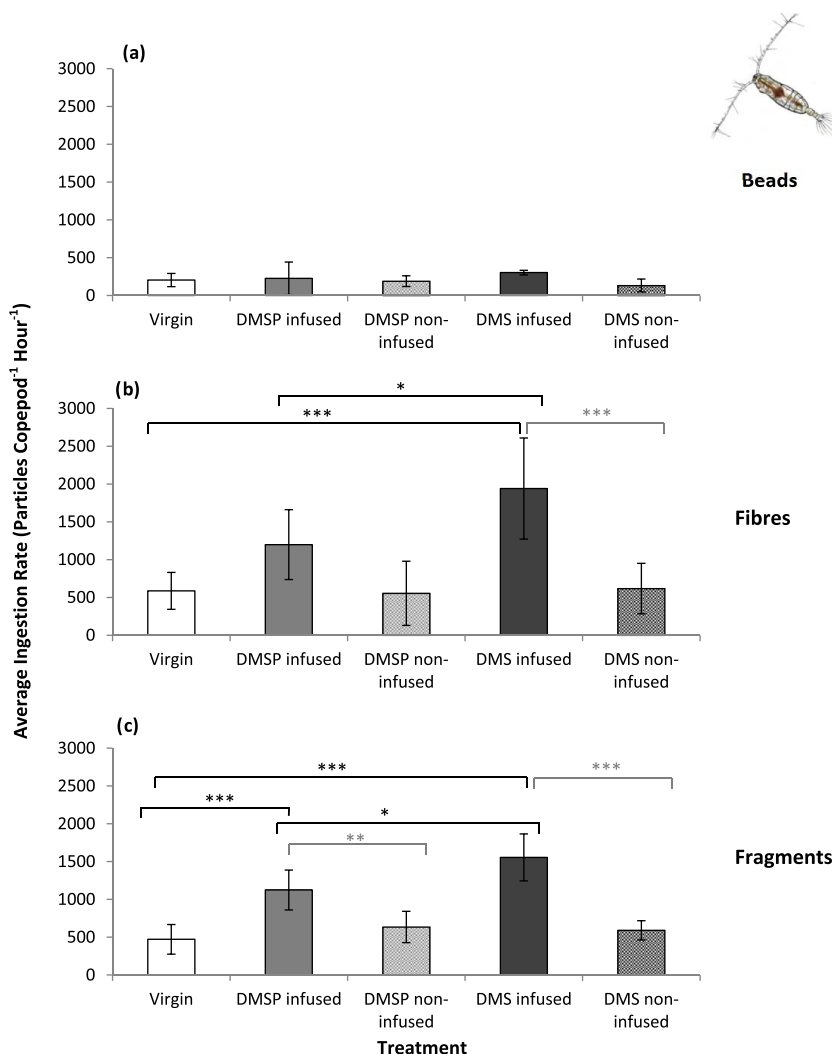
zooplankton per bottle. The ingestion rate ( $I$ ) is then calculated using eq 3

$$I = F \times T_0 \quad (3)$$

**Statistical Analysis.** All data were analyzed using Microsoft Excel (2016) and the statistical software R (version 3.4.1, R Development Core Team 2017). Data were tested for normality using a Shapiro–Wilk test and homogeneity of variance was visually inspected to satisfy parametric requisites. A one-way analysis of variance (one-way ANOVA) and Tukey's post hoc tests were used to compare the ingestion rates from grazing experiments. The significance level was set at  $\alpha = 0.05$ .

## RESULTS

**Influence of Shape on Microplastic Ingestion.** The copepod *C. helgolandicus* demonstrated a significant variation in the ingestion rates of differently shaped microplastics (Figure 1a, one-way ANOVA ( $F_{(2,15)} = 3.78$ ,  $P = 0.047$ )). A post hoc Tukey test showed that the fragments were ingested significantly more than the beads ( $P = 0.043$ ). The fragments were ingested at a mean rate ( $\pm$ SE) of 500.3 ( $\pm 83.9$ ) fragments copepod<sup>-1</sup> h<sup>-1</sup>, in comparison to the mean number of beads, 144.5 ( $\pm 38.4$ ) beads copepod<sup>-1</sup> h<sup>-1</sup>, and fibers, 251.9 ( $\pm 134.0$ ) fibers copepod<sup>-1</sup> h<sup>-1</sup> (Figure 1a).



**Figure 4.** *A. tonsa* ingestion rates  $\pm$  SE (particles copepod<sup>-1</sup> h<sup>-1</sup>) of differently shaped microplastics: (a) beads, (b) fibers, and (c) fragments, with the different treatments of virgin, DMSP infused, DMS infused, DMSP noninfused, and DMS noninfused. The black significance bars relate to virgin controls and the gray significance bars relate to noninfused controls. The asterisks denote the levels of significant difference in the ingestion rates, \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ . Illustration by Vivienne Botterell.

*H. gammarus* larvae also exhibited a significant variation in the ingestion rates of the different microplastic shapes (Figure 1b, one-way ANOVA ( $F_{(2,26)} = 4.36$ ,  $P = 0.0233$ )). A post hoc Tukey test showed that the beads were ingested significantly more than the fibers ( $P = 0.026$ ) and substantially more than the fragments ( $P = 0.074$ ). The beads were ingested at a mean rate ( $\pm$ SE) of 1138.7 ( $\pm$ 133.4) beads lobster<sup>-1</sup> h<sup>-1</sup>, in comparison to the mean number of fibers, 402.3 ( $\pm$ 45.6) fibers lobster<sup>-1</sup> h<sup>-1</sup>, and fragments, 530.7 ( $\pm$ 281.7) fragments lobster<sup>-1</sup> h<sup>-1</sup> (Figure 1b).

The copepod *A. tonsa* demonstrated a significant variation in the ingestion rates of differently shaped microplastics (Figure 1c, one-way ANOVA ( $F_{(2,15)} = 6.6$ ,  $P = 0.009$ )). A post hoc Tukey test showed that the fibers were ingested significantly more than the beads ( $P = 0.008$ ) and substantially more than the fragments ( $P = 0.06$ ). The fibers were ingested at a mean rate ( $\pm$ SE) of 587.5 ( $\pm$ 243.4) fibers copepod<sup>-1</sup> h<sup>-1</sup>, in comparison to the mean number of beads, 204.1 ( $\pm$ 87.3) beads copepod<sup>-1</sup> h<sup>-1</sup>, and fragments, 471.5 ( $\pm$ 196.4) fragments copepod<sup>-1</sup> h<sup>-1</sup> (Figure 1c).

#### Influence of Infochemicals on Microplastic Ingestion.

The copepod *C. helgolandicus* demonstrated an increase in the

average ingestion rates of the microplastics that had been infused with DMSP and DMS, across all three shapes, in comparison to virgin and also noninfused microplastics (infochemical and microplastics added to the experimental bottles concurrently) (Figure 2). Analysis showed that there were significant differences between the infochemical treatment groups with all three shapes (beads: Figure 2a, one-way ANOVA ( $F_{(4,25)} = 6.235$ ,  $P = 0.0013$ ), fibers: Figure 2b ( $F_{(4,25)} = 8.214$ ,  $P \leq 0.001$ ), and fragments: Figure 2c ( $F_{(4,25)} = 15.21$ ,  $P \leq 0.001$ )). A post hoc Tukey test showed that DMS infusion significantly increased the ingestion rates of the beads (Figure 2a,  $P \leq 0.001$ ), fibers (Figure 2b,  $P \leq 0.01$ ), and fragments (Figure 2c,  $P \leq 0.001$ ) in comparison to the virgin control treatments. In addition, it showed that all microplastics that were infused with DMS were ingested significantly more than those that were not infused with DMS (beads: Figure 2a,  $P \leq 0.05$ , fibers: Figure 2b,  $P \leq 0.001$ , and fragments: Figure 2c,  $P \leq 0.001$ ). This was also found for DMSP-infused and noninfused fragments (Figure 2c,  $P \leq 0.05$ ). There were no significant differences between the virgin control and the noninfused microplastic control for both infochemicals across all microplastic shapes.

In the DMSP- and DMS-infused treatments, the average ingestion rate of the fibers and fragments by *H. gammarus* larvae increased (Figure 3). Analysis showed that there were significant differences between the infochemical treatment groups for both fibers (Figure 3b, one-way ANOVA ( $F_{(2,27)} = 4.481, P = 0.021$ )) and fragments (Figure 3c, one-way ANOVA ( $F_{(2,27)} = 6.372, P = 0.0054$ )). A post hoc Tukey test showed that the presence of DMS significantly increased the ingestion rates of the fibers (Figure 3b,  $P \leq 0.05$ ) and fragments (Figure 3c,  $P \leq 0.01$ ) in comparison to the control virgin treatments. The presence of the infochemicals had no effect on the ingestion rates of the beads (Figure 3a, one-way ANOVA ( $F_{(2,26)} = 2.863, P = 0.075$ )).

In the DMSP- and DMS-infused treatments, the average ingestion rate of the fibers and fragments by *A. tonsa* increased (Figure 4). Analysis showed that there were significant differences between the infochemical treatment groups for both fibers (Figure 4b, one-way ANOVA ( $F_{(2,25)} = 11.6, P \leq 0.001$ )) and fragments (Figure 4c, one-way ANOVA ( $F_{(2,25)} = 21.2, P \leq 0.001$ )). A post hoc Tukey test showed that the presence of DMS significantly increased the ingestion rates of the fibers (Figure 4b,  $P \leq 0.05$ ) and fragments (Figure 4c,  $P \leq 0.01$ ) in comparison to the control virgin treatments. The presence of the infochemicals had no effect on the ingestion rates of the beads (Figure 4a, one-way ANOVA ( $F_{(2,25)} = 1.7, P = 0.18$ )). In addition, it showed that the fibers and fragments that were infused with DMS were ingested significantly more than those that were not infused with DMS (Figure 4b,  $P \leq 0.001$ , and fragments: Figure 4c,  $P \leq 0.001$ ). This was also found for the DMSP-infused and noninfused fragments (Figure 4c,  $P \leq 0.01$ ). There were no significant differences between the virgin control and the noninfused microplastic control for both infochemicals across all microplastic shapes.

## DISCUSSION

Our study reveals that both shape and the infusion of infochemicals can affect the ingestion rate of microplastics in *C. helgolandicus*, *A. tonsa*, and *H. gammarus* larvae. Each species selectively ingested significantly more of a certain microplastic shape (Figure 1), indicating that shape is an important factor that influences microplastic bioavailability. *C. helgolandicus* ingested more fragments; *H. gammarus* ingested more beads; and *A. tonsa* ingested the most fibers. We further observed that infusion with the infochemical DMS significantly increased the ingestion rates of microplastics in all three species (Figures 2–4). This highlights that chemosensory species utilizing DMS as an infochemical may be at an increased risk of microplastic debris ingestion. These findings add to the growing evidence of the importance of testing environmentally relevant microplastics in zooplankton grazing studies in contrast to the predominately used virgin beads, to fully elucidate the mechanisms behind microplastic ingestion.<sup>8</sup>

**Effect of Microplastic Shape on Its Bioavailability to Zooplankton.** Previous laboratory research has shown that many species of zooplankton will readily ingest microplastic beads including *C. helgolandicus* and *A. tonsa*.<sup>17,51</sup> However, microplastic ingestion has not previously been observed in *H. gammarus* larvae. The fragments and fibers have also been shown to be ingested by *C. helgolandicus*.<sup>28,31</sup> Furthermore, in wild copepods sampled in the natural environment, irregularly shaped and fibrous microplastics have been identified.<sup>18,25</sup> However, it is unclear whether these microplastics from the wild samples are due to prey selectivity of the species,

differences in gut retention time, or simply a representation of the most prevalent microplastic in the environment under investigation.

Our results show that while all microplastic shapes were ingested, each of the species selectively ingested one shape preferentially over the others (Figure 1). The selectivity of the species could be explained by the different feeding strategies, with particular shapes being easier to handle, or species-specific capacities to ingest. *C. helgolandicus* ingested the most fragments, *H. gammarus* the most beads, and *A. tonsa* the most fibers. *C. helgolandicus* is a suspension feeder, using appendages around their mouths to generate a feeding current.<sup>52,53</sup> Whereas *A. tonsa* is an ambush feeder, a complex grazing behavior requiring a stimulus to optimize capturing prey items yet avoiding nonfood items, but can also switch to suspension feeding when consuming small phytoplankton by generating a feeding current.<sup>54,55</sup> It is the smallest of the three species used in these experiments and therefore may have found the smaller diameter of the fibers (10  $\mu\text{m}$ ) easier to ingest. Additionally, it is possible that differently shaped microplastics may generate different eddies, through disturbances in the feeding current or water flow, which may be more or less attractive to species with different feeding strategies. Similarly to *A. tonsa*, *H. gammarus* larvae are also thought to be ambush feeders consuming both phytoplankton and zooplankton.<sup>56</sup> Unlike copepods, which have a singular naupliar eye that is light sensitive, lobster larvae have two compound eyes, which are not only light sensitive but also have a large view angle and are able to detect fast movement.<sup>57,58</sup> This more developed spatial vision may aid the lobster in prey selection and subsequently may have played a role in the selectivity of the microplastic beads over the other shapes. The microplastic shape may also resemble the species' natural prey source. The microplastics used in this current study overlapped in size with the species prey. The beads and fragments may resemble spherical algae and the fibers could resemble chain-forming diatoms.<sup>28</sup> Shape selectivity could also be explained by species shifting their prey preference.<sup>31</sup> Recent research by Cole et al. and Coppock et al. suggest that *Calanus* species may shift prey selectivity to avoid ingesting microalgae that are of a similar size and shape to the microplastics that they were exposed to, potentially to avoid consuming the plastic particles.<sup>28,31</sup> However, future behavioral experiments are recommended to further understand this mechanism of microplastic shape selectivity.

Certain microplastic shapes have been shown to have more adverse effects than others in species of zooplankton, with previous research highlighting negative effects on swimming behavior by the fragments<sup>32</sup> and feeding behavior by the fibers.<sup>31</sup> It is crucial to understand which shapes have the highest bioavailability in a species to understand the effect commonly ingested microplastic shapes could have on the health of the individual as any negative effects could reduce food intake and available energy for growth, development, and reproductive success. Future experiments should consider differences in gut retention time between differently shaped microplastics as certain shapes may be retained longer than others. This is imperative to understanding whether the microplastics we find in zooplankton sampled in the natural environment were due to selectivity of the species, representative of the microplastics present in the environment, or are retained for longer in the gut. The length of time the microplastics are retained for has implications not only for the

health of the individual but also for the transport of the microplastics through the water column by diel vertical migration of zooplankton and also through the food web when zooplankton is consumed by predators.<sup>28,59</sup>

**Role of Infochemicals in Increasing the Bioavailability of the Microplastics.** To investigate whether these chemicals would stimulate grazing, *C. helgolandicus*, *A. tonsa*, and *H. gammarus* larvae were exposed to microplastics (beads, fibers, and fragments) that had been infused in artificial DMS and DMSP solutions of environmentally relevant concentrations. Our results show that the presence of the infochemical DMS can lead to significant increases in the ingestion rate of microplastics in three species of zooplankton. This indicates that chemosensory species utilizing DMS as an infochemical may be at an increased risk of microplastic debris ingestion.

In *C. helgolandicus*, the ingestion rates of all three microplastic shapes were significantly increased when infused with DMS and while not significant, DMSP infusion also substantially increased microplastics uptake. *H. gammarus* larvae exhibited a similar pattern to *A. tonsa*, with significantly more microplastic fibers and fragments ingested when infused with DMS. However, DMSP infusion only substantially increased microplastic fragment ingestion in *H. gammarus* and neither infochemical affected the ingestion rate of the microplastic beads. However, DMSP infusion did significantly increase the ingestion rate of both fibers and fragments in *A. tonsa*, but like DMS had no effect on bead ingestion. While the majority of the microplastics infused with DMS were ingested at a higher rate in all species, DMS infusion had no effect on the ingestion of the microplastic beads by the lobster larvae. However, the microplastic beads in the virgin treatment group were ingested at the highest rate in comparison to the fibers and fragments, which implies that the microplastic shape had a higher bioavailability and an overriding effect on the chemoattractive potential of DMS. It is possible that the more developed vision of the larvae likely aided in prey detection and selection, and that the larvae used a range of chemo- and mechanoreceptors in combination with visual cues. This highlights that in some species, microplastic shape, based on the particles that are easier to handle or mimic the preferred prey items, may present a greater bioavailability than other factors including attraction by infochemicals.

Following the initial results with *H. gammarus* larvae, we refined the protocol in the *C. helgolandicus* and *A. tonsa* experiments, to include additional controls in order to investigate whether simply the presence of infochemicals in the surrounding seawater induced an increase in the ingestion rates. Here, the addition of virgin, noninfused microplastics to the experimental flasks was performed separately but concurrently to the addition of 15 mL of DMS or DMSP solution (see [Methods, Preparation of Microplastics](#)). However, there was no significant difference in the ingestion rates between the virgin controls and the noninfused controls across all microplastic shapes. Furthermore, our results ([Figures 2 and 4](#)) show that there was a significant difference between the noninfused DMS controls and the infused DMS treatments for almost all three different shapes of the microplastics. This therefore suggests that it is not just the presence of DMS in the seawater, but the presence of DMS on the plastic itself that stimulates increased ingestion.

In the marine environment, the infochemicals DMSP and DMS could be present on the plastic surface due to biofilm

formation by DMSP/DMS-producing microorganisms, which form part of the diverse microbial community of the plastsphere.<sup>38</sup> Many of these organisms within the plastsphere are important prey items readily consumed by several species of zooplankton.<sup>60</sup> This could increase the ability of zooplankton to locate the plastic particles if they mimic the scent of natural prey and in turn could increase consumption of the microplastics. It is important to note that these experiments are a simplification of the natural environment separating visual biofilm effects from chemical cues associated with DMSP/DMS. There still remains a significant knowledge gap in assessing the ability of the microplastics to gain an infochemical signature through the formation of a biofilm. This work seeks to further understand the interspecies response to DMSP/DMS, yet future investigation is still required to understand the response to additional infochemicals and importantly the detection thresholds of the chemosensory organisms.

**Environmental Relevance and Risk Assessment.** In this study, we demonstrate that both the shape and the presence of infochemicals can affect the ingestion rate of the microplastics in three species of zooplankton. This research highlights the importance of using a greater diversity of the environmentally relevant microplastics in laboratory experiments. For our infochemical infusion experiments, we used environmentally relevant concentrations of DMSP and DMS. While this triggered increased uptake of the microplastics in all three of the species, currently there is very little understanding of the interspecies response to other infochemicals, limited knowledge on the detection threshold of chemosensory species, and a poor understanding of the chemical gradients emanating from the microscopic particles. The microplastics used in this study were chosen to be as similar as possible (*i.e.*, size and color); however, due to the use of different shapes, they did vary slightly in volume and surface area, which could affect the ingestion rates in some species. It is possible that those microplastics with a greater surface area, such as fragments, may gain a greater infochemical signature. Similarly, polymer type could also affect the ingestion rates. Different polymers have been shown to develop different microbial biofilm communities, which in turn could produce different infochemicals.<sup>61</sup> While we used nylon fibers and fragments we also used polystyrene beads as we were unable to find a nylon equivalent. The concentration of the microplastics used in this study exceeds those currently observed in the marine environment; however, there is very little environmental information relating to microplastics in the size range of 10–30  $\mu\text{m}$  due to technical difficulties in sampling, extracting, and identifying such particles.<sup>31</sup> However, where data is available, they suggest an inverse relationship between particle size and abundance, and hence, the smaller the microplastics, the larger their concentration.<sup>62</sup> Recent research by Lindeque *et al.* has demonstrated that the 333  $\mu\text{m}$  nets commonly used for microplastic sampling underestimate microplastic abundance, particularly for <333  $\mu\text{m}$  microplastics that are within the optimal prey size range of numerous marine organisms.<sup>63</sup> While it is important to investigate the risk that environmentally relevant microplastic concentrations pose to marine organisms, it is essential to understand the mechanisms by which microplastics become bioavailable to a species, can cause harm, identify end points, and understand the sensitivity of different species at every life stage, and it is also crucial to conduct these studies at elevated/future scenario concen-



trations.<sup>8,31</sup> Such research is key to establishing no-effect thresholds for the development of effective risk assessments for species, populations, and the ecosystem.

## ■ ASSOCIATED CONTENT

### SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.0c02715>.

Images of the microplastics and microplastic concentration data at  $T_0$ ,  $T_3$ , and  $T_6$  (PDF)

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### Notes

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