Environmental Science & Technology

pubs.acs.org/est

Article

Unpalatable Plastic: Efficient Taste Discrimination of Microplastics in Planktonic Copepods

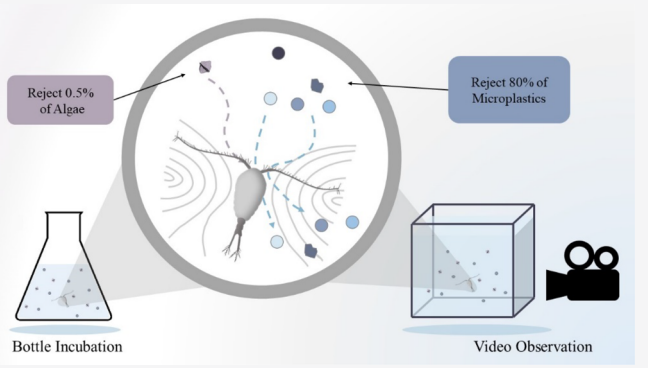
Jiayi Xu,*,# Rocío Rodríguez-Torres,# Sinja Rist, Torkel Gissel Nielsen, Nanna Bloch Hartmann, Philipp Brun, Daoji Li, and Rodrigo Almeda

Cite This: *Environ. Sci. Technol.* 2022, 56, 6455−6465

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Planktonic copepods are the most abundant animals in the ocean and key players in global biochemical processes. Recent modeling suggests that zooplankton ingestion of microplastics (MPs) can disrupt the biological carbon pump and accelerate a global loss of oceanic oxygen. Here we investigate the behavioral responses and ingestion rates of a model feeding-current generating copepod when exposed to microplastics of different characteristics by small-scale video observations and bottle incubations. We found that copepods rejected 80% of the microplastics after touching them with their mouth parts, in essence exhibiting a kind of taste discrimination. High rejection rates of microplastics were independent of polymer type, shape, presence of biofilms, or sorbed pollutant (pyrene), indicating that microplastics are unpalatable for feeding-current feeding copepods and that post-capture taste discrimination is a main sensorial mechanism in the rejection of microplastics. In an ecological context, taking into account the behaviors of planktonic copepods and the concentrations of microplastics found in marine waters, our results suggest a low risk of microplastic ingestion by zooplankton and a low impact of microplastics on the vertical exportation of fecal pellets.



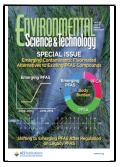
KEYWORDS: *zooplankton, copepods, feeding behavior, microplastic, taste discrimination*

INTRODUCTION

Microplastics are ubiquitous pollutants in the marine environment.[1,2] Understanding the consequences of plastic pollution in marine ecosystems is therefore of major societal and scientific concern. Most biological oceanographic processes are directly linked to the presence and activities of planktonic organisms,[3] which are exposed to microplastics in the water column. This makes zooplankton of particular interest in relation to potential global environmental impacts of microplastic pollution. It has been hypothesized that the ingestion of microplastics instead of organic prey by zooplankton may change the sinking velocity of their fecal pellets,[4] consequently affecting the vertical transportation of carbon and weakening the marine biological carbon pump.[5,6] It is even predicted to accelerate the global loss of ocean oxygen through reduced grazing on primary producers.[7] Among planktonic organisms, copepods are the most abundant animals in the ocean and the dominant zooplankton group.[8,9] Ingestion of microplastics by copepods is potentially the main route by which plankton-sized microplastics enter marine food webs and are transferred to higher trophic levels.[10,11] A better knowledge of the interactions between copepods and microplastics is therefore essential to understand the fate and impacts of marine plastic pollution.

It is well documented that marine macro- and megafauna ingest plastic debris with more than 900 recorded cases of vertebrate species, including sea mammals, sea birds, marine turtles, and fishes, being entangled by or having consumed plastics.[12,13] Additionally, laboratory research has clearly demonstrated that zooplankton, including copepods, ingest plankton-sized microplastics when exposed to high concentrations.[10,11,14−16] However, evidence of microplastic consumption by copepods in the natural environment and its consequences is lacking. Several field surveys have reported the ingestion of microplastics by copepods.[17−21] However, for some of these studies, the reported size of ingested microplastics is outside of the size range of natural prey, and in some cases, it is even larger than the mouth of the copepods, suggesting entanglement or sample contamination rather than actual ingestion. In any case, these field studies indicate a low

Received: January 14, 2022
Revised: April 11, 2022
Accepted: April 13, 2022
Published: April 27, 2022



occurrence of microplastic ingestion in natural zooplankton communities, which contradicts the high probability of microplastic ingestion predicted from laboratory studies.

One reason for this mismatch in the findings of laboratory and field research may be the methods used in the laboratory. Most laboratory research on the ingestion of microplastics by zooplankton has been conducted using virgin spherical microplastics,[10] whereas investigations of zooplankton preferences for different shapes,[4] aging states,[22] and other characteristics of microplastics are still lacking. In addition, most laboratory research involves only bottle incubations that allow little insight into the mechanisms of the interactions between different types of microplastics and copepods. A practical tool to open the "black box" of bottle incubations when investigating the feeding behavior of zooplankton is small-scale video observation. This technique has successfully been applied to study selective feeding behavioral responses of copepods to different species of harmful algae.[23]

In this study, we investigated how different characteristics of microplastics affect the behavioral responses and ingestion rates of the feeding-current feeder *Temora longicornis*. *Temora* species are distributed worldwide from coastal to oceanic waters.[24] Moreover, feeding-current feeding is one of the three dominant feeding modes of planktonic copepods.[25,26] Through modeling, we represent the global distribution of the feeding-current feeding mode with the aim of reflecting the relevance of this feeding mode in the world's oceans.[27,28] Furthermore, we used direct small-scale video observations and parallel bottle incubations to quantify the feeding behavioral responses of copepods to diverse microplastic characteristics that are common in marine environment and biota:[29−31] plastic polymer type (polystyrene (PS) vs polyethylene (PE)), shape (irregular vs sphere), presence of biofilms (bio-fouled vs "clean" microspheres), and sorption of organic pollutants (microspheres with sorbed pyrene vs "clean" microspheres). We hypothesized that feeding-current generating copepods (i) do not discriminate between plastic polymer types; (ii) show a higher rejection of microplastics when plastic particles are irregular (different from normal prey that are typically of regular shape); (iii) have a higher ingestion of bio-fouled microplastics than virgin microplastics; and (iv) show a higher rejection of microplastics with sorbed chemical pollutants. Our results will provide a better understanding of how plastic properties and weathering processes influence the risk of microplastics to enter the marine food webs via zooplankton.

MATERIALS AND METHODS

Spatial Modeling. To represent the global distribution of feeding-current feeders, the global ocean was discretized into roughly 5000 polygons of similar area, and feeding-current feeders were assumed to be represented by the world's most abundant genera: *Paracalanus, Pareucalanus, Parvocalanus, Rhincalanus, Pseudocalanus, Calocalanus, Nannocalanus, Temora, Acartia, Calanus, Centropages, Pleuromamma,* and *Euchaeta*. Observation-based estimates were derived polygon-wise as community-weighted means using abundance observations,[32] body length data,[33] and the procedure described by Brun et al.[34] For model extrapolations, we fitted generalized additive models,[35,36] assuming beta distribution and using the average and range of monthly sea surface temperature (derived from the HadISST1 product[37]) and average chlorophyll a concentration (derived from http://www.globcolour.info/) as predictors.

General Experimental Approach. To test our hypothesis, we conducted four studies:

a) "Polymer type" (PS vs PE), where copepods (*T. longicornis*) were exposed to either (i) algae and virgin, spherical PS microplastics or (ii) algae and virgin, spherical PE microplastics.
b) "Shape" (spheres vs irregular fragments), where copepods were exposed to either (i) algae and virgin, spherical PS microplastics or (ii) algae and virgin, irregular PS fragments.
c) "Biofilms" (bio-fouled vs "clean" microspheres), where copepods were exposed to either (i) algae and spherical PE microplastics with biofilms or (ii) algae and virgin, spherical PE microplastics without biofilms ("clean").
d) "Sorbed pollutants" (microplastics with sorbed pyrene vs microplastics without sorbed pyrene), where copepods were exposed to (i) algae and spherical PE microplastics with sorbed pyrene, (ii) algae and spherical PE microplastics without sorbed pyrene, (iii) algae and spherical PS microplastics with sorbed pyrene, or (iv) algae and spherical PS microplastics without sorbed pyrene.

In all experiments, copepods were exposed to a nominal prey/microplastic ratio of 1:1 with concentrations of 200 cells mL^{-1} and 200 MPs mL^{-1}. Measured experimental concentrations are shown in Table S1. Feeding behavioral responses of individual copepods, including prey detection, capture, handling, rejection and ingestion of prey and different microplastic types were examined by small-scale video observations, as described in details below. Feeding rates of copepods on the studied prey and microplastics were calculated from both video observations and bottle incubations as described below.

Experimental Organisms. The culture of our model copepod species, *T. longicornis*, originates from samples from the Gullmars fjord (Sweden) and Øresund (Denmark) in 2016. The copepods were subsequently maintained in a continuous laboratory culture at the Technical University of Denmark. They are grown in 30 L tanks with filtered seawater (salinity = 30 psu) at 18 °C in the dark. Copepod cultures were fed a mixed diet consisting of cultured phytoplankton (*Heterocapsa steinii* (formerly known as *H. triquetra*), *Thalassiosira weissflogii*, and *Rhodomonas salina*) and a heterotrophic dinoflagellate (*Oxyrrhis marina*). The phytoplankton and *O. marina* cultures were maintained in the lab.[25]

The day before the experiments, healthy copepod females of similar size (prosome length approximately 740 μm) were sorted under a stereo microscope and kept overnight in glass beakers with 0.2 μm-filtered seawater. From the sorted copepod stock, we picked females for both bottle incubations and video experiments.

The dinoflagellate *H. steinii* was the model prey used in the feeding experiments. The cultures of *H. steinii* were maintained in autoclaved 0.2 μm-filtered seawater with a B1 medium at 16 °C, 150 μmol photons m^{-2} s^{-1}, 12 h light−12 h dark cycle, and salinity of 30 psu. The size distribution and concentration of *H. steinii* were measured with a Beckman Multisizer Coulter Counter before the experiment. Only cultures in the exponential growth phase were used as prey. The equivalent spherical diameter (ESD) of algal cell was approximately 20 μm on average.

Preparation of the Different Types of Microplastics. *Study 1: "Polymer Type".* We used virgin spherical microplastics of the two different plastic polymers: PS and PE. PS

https://doi.org/10.1021/acs.est.2c00322
Environ. Sci. Technol. 2022, 56, 6455−6465

microspheres (20 μm) suspended in water with Tween 80 were purchased from Degradex. PE microspheres (20 μm) were purchased from Cospheric as powder. In both cases, the microplastics were suspended in a 0.01% Tween 80 Milli-Q water solution to prepare the working suspensions.

Study 2: "Shape". To obtain irregular microplastics, PS pellets (500 μm in diameter, purchased from Cospheric) were frozen with liquid nitrogen and subsequently ground using an IKA A11 basic analytical mill. The resulting microplastic fragments were suspended in a 0.01% Tween 80 solution. The suspension of microplastics was then filtered through nylon filters with 30 and 15 μm mesh sizes in sequence to obtain irregular microplastics with an average size of approximately 20 μm. Upon filtration, the fragments were collected and resuspended in a 0.01% Tween 80 solution. PS microspheres (20 μm) were used as spherical microplastics to compare with irregular microplastics of the same size and polymer but with a different shape.

Study 3: "Biofilms". Seawater, containing natural microbial communities, was collected from a Danish estuary, Limfjorden, and filtered through 8 μm polycarbonate filters. To produce bio-fouled microplastics, 20 μm PE microspheres were added to 600 mL Pyrex bottles containing the 8 μm-filtered seawater in a concentration of 50 MPs mL^{-1}. The bottles were placed in a plankton wheel at 1 rpm and incubated with the following conditions: temperature of 18 °C, light intensity of 100 μmol photons m^{-2} s^{-1}, and a photoperiod of 12 h light−12 h dark cycle. The B1 medium (1 mL L^{-1})[38] was added to all the bottles to avoid nutrient depletion. After 4 weeks of incubation, the presence of a biofilm on the MPs was confirmed using DAPI (4′,6-diamidino-2-phenylindole, hydrochloride) staining and examination under an epifluorescence microscope with UV light (Figure 1).

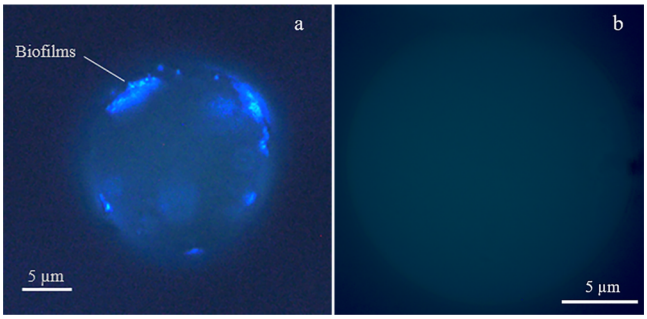


Figure 1. Epifluorescence microscope image of (a) a bio-fouled PE microplastic stained with DAPI under UV illumination and (b) the same microplastic without biofilms. Bright blue fluorescent areas correspond to the DNA of biofouling microorganisms growing on the surface of the microplastic particle.

Study 4: "Sorbed Pollutants". To obtain microplastics with a sorbed hydrophobic pollutant, PE and PS microspheres, respectively, were exposed to a pyrene solution. Pyrene powder was diluted in methanol to prepare a stock pyrene solution of 100 μg mL^{-1}. Ten mg of microplastics was added to acid-washed glass bottles (68 mL) with a pyrene solution of 50 μg L^{-1}. Additionally, 10 mg of microplastics was added to bottles with methanol alone as a control treatment. The bottles were incubated for 72 h in a plankton wheel at 5 rpm at 18 °C in the dark. Upon incubation, we filtered the bottle contents through 5 μm polycarbonate filters, separating the microplastics (residue) from the pyrene solution (filtrate). A subsample of the collected microplastics was resuspended in filtered seawater to be used in the bottle incubations and filming experiments, respectively. Another subsample was stored at −80 °C for later pyrene sorption analysis. The concentration of pyrene sorbed by the microplastics was measured in triplicates of microplastic samples with an Agilent 6890 gas chromatograph. Extraction was performed by adding 4 mL *n*-hexane/acetone (6:4) directly to the vials. The extraction time was 24 h. Chromatographic separation was achieved on an Agilent 6890 gas chromatograph equipped with a 60 m × 0.25 mm inside diameter × 0.25 μm film thickness DB-5 ms column (Agilent Technologies). A 2 μL sample was injected in splitless mode with the sample inlet held at 300 °C. The oven was programmed to 70 °C, then 20 °C/min to 300 °C, and then 50 °C/min to 325 °C and held for 10 min. Helium was used as the carrier gas with a 1 mL/min constant flow. Detection was achieved on an Agilent 5975C triple-axis mass-selective detector operated in SIM mode with the MS source at 230 °C and the quadrupole at 150 °C.

The average concentration of sorbed pyrene in the PE microplastics was 0.059 ± 0.009 μg mg^{-1}. This value is the same order of magnitude as observed by Wang and Wang.[39]

Particle Sizes. The size (equivalent spherical diameter, ESD) and concentration of each type of microplastic in the prepared stock suspensions were measured using a Multisizer Coulter Counter. The average size of PE and PS microspheres was 20.5 ± 0.2 and 20.6 ± 0.5 μm, respectively. The average size of the obtained irregular microplastics was 20.6 ± 1.7 μm. *H. steinii* used for the experiment had an average ESD of 17 ± 0.4 μm.

Video Observation. Before the video recordings, copepods were tethered to a needle from their dorsal surface.[40] Tethering does not affect the feeding selective behavior of *T. longicornis*.[23,41] The video observation was conducted in a thermo-constant filming room (at 16 °C). A 10 × 10 × 10 cm^3 transparent container was placed between an infrared light and a high-speed camera (Phantom V210). In each treatment, 800 mL of the microplastic−alga suspension was added to the container and gently stirred by a magnetic stirrer. Then, a single tethered copepod was attached to a micromanipulator by the other edge of the tether immersed in the mixed particle suspension. Subsequently, the tethered copepod was adjusted to the center of screen field in focus. A 3 h video recording (resolution: 1024 × 512 pixels; frame rate: 100 Hz) was started instantly after preparing the setup. Due to the limited storage space on the camera, each video lasted for a maximum of 100 s. Thus, with 28 recorded videos, a total of 3 h was saved for analysis. All the experimental operations were conducted outside the filming room, and the room was kept in darkness throughout the entire process to minimize any interruption. Three copepod females from each treatment were filmed separately.

The capture, ingestion, and rejection events of *T. longicornis* were counted from the videos. The copepods beat their feeding appendages constantly to maintain the feeding current (percentage of time beating = 99.7 ± 0.1%) and scan the surrounding water. When prey particles were drawn into their detection range, contractions of swimming appendages were observed, in many cases followed by a successful capture of the particle. A behavioral event was defined as "ingestion" when the captured particle was handled, tasted, and finally eaten by the copepod (Movies S1 and S2). On the contrary, a behavioral event was defined as "rejection" when the particle was actively "kicked" away by the copepod after tasting (Movie S3). Although the used prey and microplastics have similar sizes, it

https://doi.org/10.1021/acs.est.2c00322
Environ. Sci. Technol. 2022, 56, 6455−6465

was easy to visually distinguish dinoflagellates from microplastics in the video (Movies S1 and S2) due to their specific morphological characteristics.

Bottle Incubation Experiments. All the glassware used for these experiments was acid-washed with 10% HCl and rinsed three times with Milli-Q water. Experiments were conducted in triplicate in 600 mL Pyrex glass bottles with lids lined with polytetrafluoroethylene (PTFE) protection. Five copepod females were incubated in each bottle. For all the treatments, we prepared three initial bottles (time = 0), three control bottles (without copepods), and three experimental bottles (with copepods). The bottles were first filled with 0.2 μm-filtered seawater (salinity = 30 psu). Aliquots of microplastics and algae working suspensions were added to each bottle to obtain the desired exposure concentrations for each treatment (200 MPs mL^{-1} and 200 cells mL^{-1}). Subsequently, the copepods were added to the experimental bottles. Finally, the bottles were filled up with filtered seawater, closed with a lid, and wrapped in aluminum foil. The bottles were mounted on a plankton wheel (1 rpm) in a temperature-controlled incubation room at 16 °C for 24 h.

At the beginning of the incubation (time = 0), for each treatment, 25 mL samples of the microplastic−alga mixture were collected from the three initial bottles to measure the precise concentration of microplastics and algae added (Table S1). After the 24 h of incubation, 25 mL samples were collected from three experimental and three control bottles, respectively, to measure the final concentration of microplastics and algae. All 25 mL samples were immediately fixed with 1% of Lugol's solution, and subsequently, microplastics and algae were counted under an inverted microscope using Sedgewick−Rafter counting chambers. At the end of the experiment, copepods were examined under a stereomicroscope to verify that there was no mortality during the experiment. We did not observe mortality in any treatment. The ingestion and clearance rates were calculated according to Frost.[42] Selective feeding was evaluated using the electivity index (E).[43] The electivity index of the particle type i (E_i) was calculated as

$$E_i = \frac{Wi - (1/n)}{Wi + (1/n).}$$

with n as the total number of particle types in a given bottle (n = 2) and the coefficient Wi as

$$W_i = \frac{Fi}{\sum Fi}$$

where Fi is the clearance rate of the particle type i and $\sum Fi$ is the sum of clearance rates on all food types. The electivity index (E) ranges between −1 and +1, where 0 indicates no electivity (no selective grazing), negative values correspond to avoidance, and positive values represent selection.

Statistical Analysis. Statistical analysis was performed using IBM-SPSS v25. For each treatment, we statistically analyzed the significant differences between algae and microplastic ingestion and clearance rates. Furthermore, we tested the statistical differences in feeding rates on algae and microplastic types among the treatments. One-way ANOVA was applied followed by pairwise multiple comparisons using the Bonferroni test. In treatment T7 of the incubation experiment, the number of replicates was only two due to the loss of one sample during the analysis; in this case, we used a t test analysis to evaluate the difference between the ingestion of algae and microplastics. Significant difference was determined at $P < 0.05$ (Table S2).

RESULTS

Global Distribution of Feeding-Current Feeding Copepods. Our results show that feeding-current feeding copepods are commonly found across the global oceans, in particular in high and middle latitudes (Figure 2a). In low latitudes, this feeding mode represents approximately 40% of the copepods on average, while in some areas of high latitudes, it reaches 80% (Figure 2b).

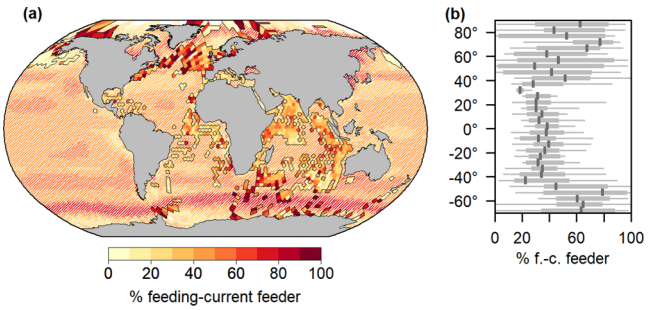


Figure 2. (a) Global and (b) latitudinal distribution of the fraction of feeding-current feeding copepods. Dashed areas represent model extrapolations, and solid colors/latitudinal boxplots are observation-based estimates.

Video Observations: Capture Rates, Ingestion Rates, and Rejection Percentages of Algae and Microplastics. Video observations showed that the copepod *T. longicornis* did not differentiate between algae and microplastics before capture. Within each treatment, the capture rates for algae and microplastics were not significantly different ($P > 0.05$, Figure 3a,b). In addition, there were no significant differences in capture rates on algae between treatments ($P = 0.167$). Capture rates on algae ranged from 2014 to 4423 cells $ind.^{-1}$ d^{-1} (Figure 3a), and capture rates on microplastics ranged from 1445 to 5249 MPs $ind.^{-1}$ d^{-1} (Figure 3b).

Overall, ingestion rates on algae or microplastics did not significantly differ across all treatments (P = 0.166 and 0.184, respectively). The average ingestion rate on algae was 2995 cells $ind.^{-1}$ d^{-1} (Figure 3c), which was 5 times higher than on microplastics (Figure 3d) and similar to the capture rate of algae (Figure 3a).

All examined copepods presented a significantly higher rejection rate of microplastics than algal cells. The average percentage of rejected algae and microplastics, considering all the treatments, was 0.5 ± 0.2 and 78.3 ± 3.2%, respectively (Figure 3e−i). Generally, an algal cell or a microplastic particle was captured and then handled by the copepod for approximately 120 ms before it was tasted. Afterward, most of the algal cells and a few microplastic particles were ingested, while the majority of microplastics were spit out after being tasted for an average of approximately 500 ms.

We did not find significant differences in the rejection percentage of microplastics between polymer types ($P = 0.304$). The average percent of rejection of virgin PS and PE microspheres was 71.9 ± 11.3 and 82.6 ± 4.4%, respectively (Figure 3e). Similarly, the shape of microplastics did not affect the percentage of rejection ($P = 0.964$). Compared to spherical PS, irregular PS was only 0.5% more rejected by *T. longicornis* on average (Figure 3f). The attachment of a biofilm and the

https://doi.org/10.1021/acs.est.2c00322
Environ. Sci. Technol. 2022, 56, 6455−6465

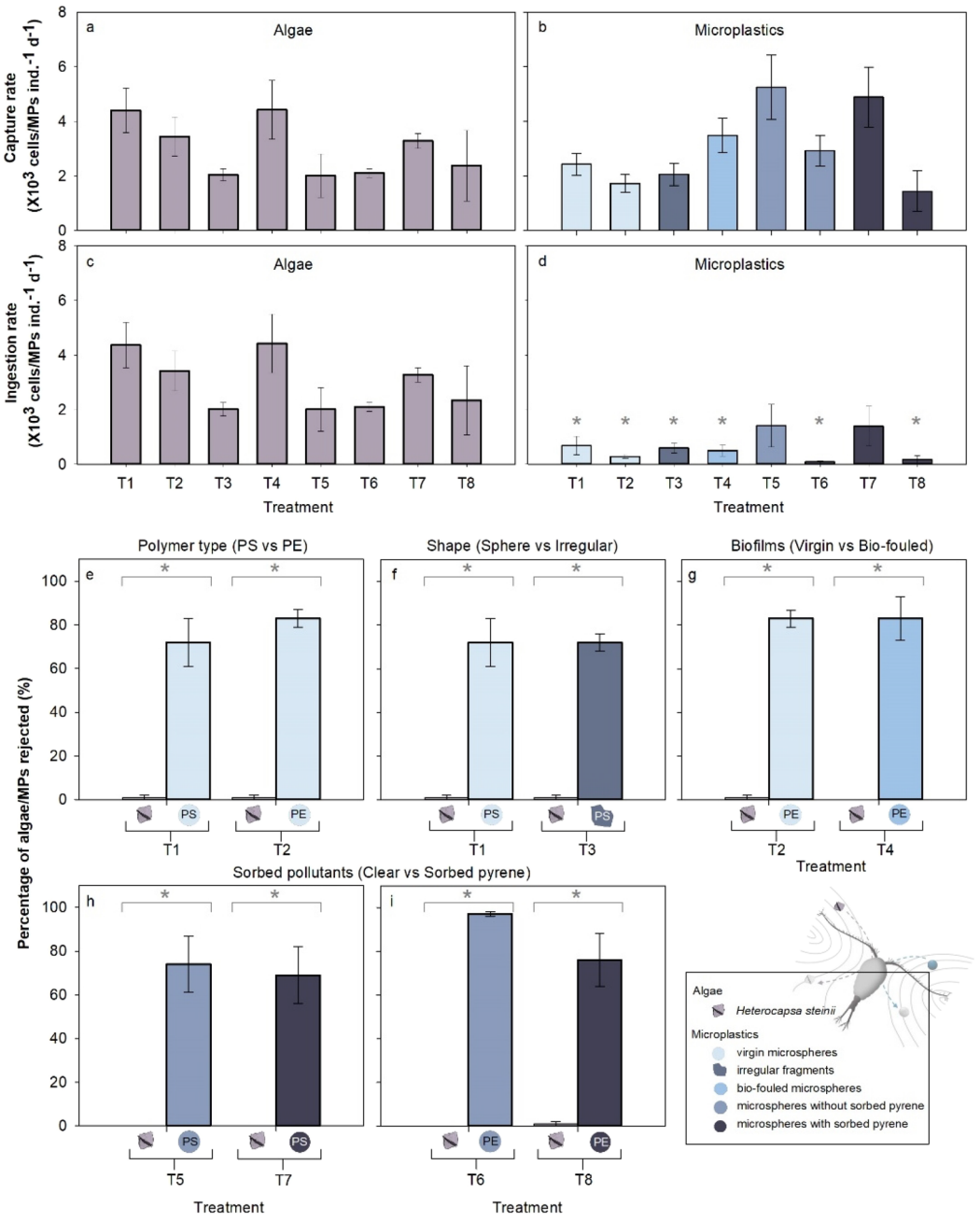


Figure 3. Feeding behaviors of *T. longicornis* on algae and microplastics in the different treatments recorded by a video camera. Capture rates of *T. longicornis* on (a) *H. steinii* and (b) microplastics. Ingestion rates of *T. longicornis* on (c) *H. steinii* and (d) microplastics. (e-i) Percentages of *H. steinii* and microplastics that were rejected by *T. longicornis* when supplied simultaneously. Comparison between treatments added with (e) virgin PS and PE microspheres, (f) spherical and irregular PS, (g) virgin and bio-fouled PE microspheres, and (h–i) clean and pyrene-polluted PS/PE microspheres. Error bars show standard errors ($n = 3$). Note that algae and microplastics were offered together in each treatment. Asterisks (*) represent a statistically significant difference between the algae and microplastic ingestion rate or percentage of rejection within each treatment.

sorption of pyrene did not change the proportion of rejected microplastics either. A total of 82.5 ± 10.2% of bio-fouled PE microspheres were rejected by *T. longicornis*, which was very close to the ratio of clean PE microspheres (Figure 3g). PE microspheres with pyrene, and to a lower degree PS with pyrene, appeared to be rejected less by *T. longicornis* than their control treatments. However, the differences were found not to be statistically significant (Figure 3h,i).

Bottle Incubations: Ingestion and Selection of Algae and Microplastics. The daily ingestion and clearance rates of *T. longicornis* on algae and microplastics were also calculated from 24 h bottle incubations (Figure 4a,b and Table S3). A significant decrease in microplastic concentration only occurred in 7 out of 24 bottles. Besides, in all the treatments, the ingestion of algae was significantly different from the ingestion of microplastic. Ingestion rates of algae (Figure 4a) were 1.4–12.6 times higher than those of microplastics (Figure 4b) and had the same order of magnitude as the rates of algae ingestion measured in the video observation. In general, *T. longicornis* presented a distinct preference for algae and largely avoided eating microplastics when exposed to alga–microplastic mixtures. Overall, no significant differences occurred between

https://doi.org/10.1021/acs.est.2c00322
Environ. Sci. Technol. 2022, 56, 6455–6465

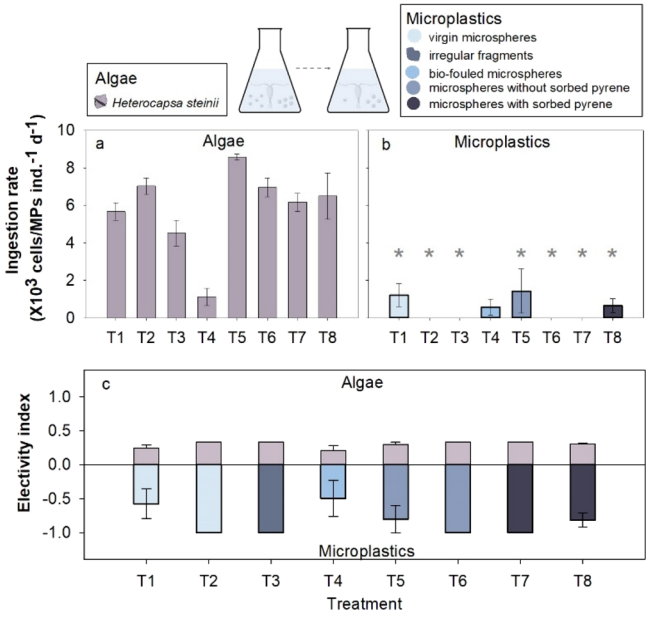


Figure 4. Feeding behaviors of *T. longicornis* on algae and microplastics in the different treatments recorded from bottle incubations. Ingestion rates of *T. longicornis* on (a) *H. steinii* and (b) microplastics. (c) Electivity index of *T. longicornis* among mixtures of algae (top bars) and microplastics (bottom bars). Error bars show standard errors (n = 3). Note that algae and microplastics were offered together in each treatment. Asterisks (*) represent a statistically significant difference between algae and microplastic ingestion rate within each treatment.

algae ingestion rates among all treatments with the exception of the treatment with bio-fouled microplastics (T4). The low ingestion rate on algae in T4 leads to a nonsignificant difference between microplastic and algae ingestion in that treatment. The electivity index (E) of algae varied from 0.21 to 0.33 among treatments, with positive E values indicating selection. By contrast, the electivity index of microplastics varied from −0.49 to −1.00, with negative E values indicating avoidance (Figure 4c).

DISCUSSION

Behavior and Feeding Rates of *T. longicornis* Exposed to Algae and Microplastics. The current knowledge about the ingestion and effects of microplastics on copepods is largely based on bottle incubations.[10,15,16,44,45] Most studies use ingestion rates as the main parameter to describe microplastic consumption and selection by copepods, but this is not sufficient to reveal the underlying mechanisms. On the one hand, copepods could either selectively graze on plastic particles or indiscriminately ingest the particles with the natural prey. On the other hand, copepods could either actively refuse microplastics or passively reduce microplastic intake by physical impacts/interference of microplastics or by chemical toxicity associated with plastics (leachates or sorbed pollutants).[46] During a "black box" bottle incubation, the different mechanisms or processes might lead to similar overall ingestion rates. However, the detection, handling, and rejection rates of microplastics need to be evaluated to understand the impact on the grazer. Here we did not observe any behavioral abnormalities (e.g., stop beating appendages or no grazing movements) when *T. longicornis* was exposed to microplastics with a similar sized microalga. We conclude that the lower ingestion rates of microplastics compared to similar sized natural prey were due to an active selective behavior of the planktonic copepods.

Copepods have been shown to possess diverse sensors on their antennae, feeding appendages, or body surfaces for detecting either hydromechanical or chemical signals created by their prey.[47] The capacity of copepods for remote chemoreception is controversial and discussed in the literature.[48−51] According to the calculation by Tiselius et al.,[51] distant detection was only feasible for prey that is unusually large and leaking chemicals to the environment, while it was more common to observe nearby or touch detection of prey cells within a radius of around 10−50 μm. In the present study, the feeding response (i.e., ingestion or rejection) of *T. longicornis* occurred only after capturing the alga or microplastic particle. This demonstrates a nearby or touch detection, which is similar to observations from previous investigations.[23,27] In addition, the similar encounter and capture rates of algae and microplastics (Figure 3a,b) suggest that *T. longicornis* does not carry out any precapture selection between algae and microplastics. Thus, all 20 μm microplastics and algae were equally perceived and captured when they were very close to the antennae or feeding appendages of *T. longicornis*.

The evaluation and selection of prey by the copepods occurred post-capture when prey touched the setae on the feeding appendages and in the mouth. The duration of the subsequent handling time is mainly caused by the position of the prey particle when it is first captured.[52] In our study, the duration was variable, but it did not show any statistically significant difference between prey types. The handled particle was pushed into the mouth, tasted, and either ingested immediately by *T. longicornis* or spat out. In many cases, microplastics were handled, tasted, and spat out several times until finally being pushed away by *T. longicornis*. Tasting was therefore the main mechanism used by *T. longicornis* to discriminate microplastics from normal prey.

Results from both video observation and bottle incubation further showed that algal ingestion by the studied copepod was not impeded by the presence of microplastics at the studied concentrations (≈200 MPs mL^{-1}). To evaluate if the used microplastic concentration can affect the ingestion rates of the studied copepod, we compared our results with previous studies with the same copepod species and type of alga but in the absence of microplastics.[27,53] According to those data, when *T. longicornis* was given dinoflagellate *H. steinii* as the sole food, the ingestion rate increased linearly with the algal concentration. If we consider the initial algal concentration (200 cells mL^{-1}) used here, the ingestion rates are estimated to be around 4500 cells ind.$^{-1}$ d^{-1}, which are very close to the ingestion rates measured in this study (Figures 3c and 4a). Early studies have similarly demonstrated that algal ingestion by other copepods, for example, *Acartia clausi* and *Calanus pacificus*, was not affected by the presence of virgin plastic microspheres.[54,55] Similarly, fecal pellet production rates of arctic copepods, which are directly related to ingestion rates, were not affected by the presence of virgin microplastics at a concentration of 20 MPs mL^{-1}.[16]

Effects of Shape and Polymer Type on Microplastic Ingestion. The shape of microplastics is one of the characteristics that may regulate copepods' selective ingestion of microplastics. Botterell et al.[11] further hypothesized that different feeding strategies of copepods might lead to different preferences for microplastic shapes. According to their experiments, feeding-current feeders ingested more fragments than

https://doi.org/10.1021/acs.est.2c00322
Environ. Sci. Technol. 2022, 56, 6455−6465

fibers, suggesting differences in selectivity depending on the microplastic shape. In the present study, we hypothesized that copepods will reject microspheres to a smaller extent than irregular fragments due to their similar shape to the natural prey. However, the ingestion rates of the feeding-current feeder *T. longicornis* on different shapes of microplastics showed no significant difference: upon capture, spherical and irregular PS particles were rejected in the same proportion (≈70%). Meanwhile, the algae offered together with microplastics were rarely rejected (≈1%).

The plastic polymer type is another factor that may affect the ingestion of microplastics by copepods.[18] Polymers differ in several physical and chemical characteristics, like hardness and density.[56] We measured the selection of two polymer types (PS vs PE) in this study and found no significant difference between ingestion rates on virgin PS and PE microspheres. The similar high percentage of rejected microplastics by copepods (Figure 3e) suggests that copepods select similarly strictly against the two polymer types, corroborating our hypothesis.

The high rejection rate of all types of microplastics tested in our experiments indicates that an irregular shape and PE/PS polymers may not be crucial factors for *T. longicornis* to selectively reject a specific microplastic particle. The reasons for the high rejections may be that copepods dislike the chemical composition of virgin plastic or that virgin microplastics lack the organic signals, which help the copepods to recognize the particles as food. However, more studies on other microplastic physical characteristics (colors, additional shapes, other polymer types, etc.) could be very relevant to give a better overview of the effects of different microplastic types on zooplankton.

Effects of Weathering on Microplastic Ingestion. Weathered microplastics are more bioavailable for marine organisms and potentially harmful for aquatic ecosystems due to their biofilm or absorbed pollutants.[22,57−59] When a primary microplastic enters the aquatic environment, bacteria quickly colonize the surface, and within the subsequent weeks, the dominant bacterial species could entirely change and create a new biofilm community depending on environmental conditions.[60] The organisms growing on the plastic surface release metabolic products that can make microplastics smell and taste more like food particles.[61] For example, it was observed that microplastics with biofilm were preferred by some copepod species over virgin microplastics.[22] Another study showed that microplastics infused with dimethyl sulfide (DMS) or dimethylsulfoniopropionate (DMSP), compounds that are naturally synthesized by marine phytoplankton, were ingested to a larger extent by *Calanus helgolandicus* and *Acartia tonsa* compared to clean microplastics.[11] Therefore, we hypothesized that bio-fouled microplastics would be ingested to a higher degree than clean ("virgin") plastic particles in our experiment. We observed, however, that only a few bio-fouled microplastics were ingested by *T. longicornis*. Based on both video observation and bottle incubation, ingestion rates of bio-fouled microplastics were similar to "virgin" microplastics (Figures 3 and 4). The bio-fouled microplastics were rejected at the same rates (≈80%) than virgin microplastics and other types of microplastics (Figure 3e−i). This indicates that the presence of biofilms did not promote the ingestion of microplastics in our study. Possibly, the biofilms on our microplastics had an organic signal that copepods cannot detect or the biofilms were not thick enough to completely inhibit the chemical signals of the synthetic polymers, which make microplastics unpalatable to copepods. The ingestion and impact of bio-fouled microplastics are of high interest for example due to their role as available surface for invasive species or for antibiotic resistant bacteria.[62,63] Therefore, further studies are needed to evaluate the impact of these biofilm-coated microplastics.

Microplastics are potential vectors of harmful chemicals sorbed from the environment.[64] Since plastics were reported to absorb high concentrations of PAHs (polycyclic aromatic hydrocarbons) like pyrene[39,64] and copepods have the ability to avoid diesel oil in water,[65] we hypothesized that *T. longicornis* also has the ability to avoid plastic particles contaminated with pyrene. However, no significant difference was observed between capture rates of pyrene-contaminated microplastics and virgin microplastics. This indicates that either the concentration of signals from pyrene-contaminated microplastics was not sufficient to stimulate the remote chemosensitivity of copepods or the compounds associated with our pyrene-contaminated microplastics are not perceivable to *T. longicornis*.

Theoretically, the aging of microplastics (biofilm formation and sorption of chemicals) could either promote or impede microplastic ingestion by copepods. However, based on the high rejection rates of all types of microplastics observed in this study, the influence of microplastic aging appears limited for planktonic copepods with an efficient tasting-discrimination technique, like *T. longicornis*.

Ecological Implications. *Feeding Behavior Is a Key Trait To Understand the Entry of Microplastics into Marine Food Webs.* Zooplankton, having an important trophic role in connecting primary producers and higher trophic levels, are considered one of the main vectors for small microplastics into marine food webs.[66] However, there is so far little evidence to support this hypothesis.[67,68] Approximately 11,500 copepod species are known worldwide,[69] and they can be grouped into three main feeding modes: ambush feeders, cruising feeders, and feeding-current feeders. Ambush feeders need a physical disturbance in the surrounding water to detect their prey. Cruising feeders swim and feed on the particles they encounter on their way. Feeding-current feeders, like *T. longicornis,* create a feeding current to draw and scan prey within their current. Active feeders (feeding-current feeders and cruising feeders) are one order of magnitude more efficient than ambush feeders at getting nonmotile prey (e.g., diatoms).[25,26] Since microplastics are nonmotile and captured in the feeding current at the same rate as motile prey, feeding-current generating copepods are more susceptible to encounter and ingest microplastics than ambush feeding copepods. Therefore, feeding-current feeders may play a particularly important role in enabling microplastics to enter marine food webs. More importantly, feeding-current feeding copepods are the dominant zooplankton group in many oceanic areas, especially in the coastal and higher-latitude areas of the northern hemisphere that normally contain high densities of microplastics and other pollutants simultaneously (Figure 2). Hence, the foraging behavior of zooplankton is the key trait to understand the entry of microplastics into marine food webs. However, although filter feeding was hypothesized to be the riskier foraging behavior in terms of microplastic ingestion, our results indicate that feeding-current feeding copepods are very efficiently discriminating microplastics, reducing the risk of ingestion and the entry of microplastics into marine food webs. Recent field studies support the low risk of ingestion of microplastics by planktonic copepods.[21,70,71]

Ingestion of Microplastics by Planktonic Copepods in the Natural Environment Is Expected To Be Much Lower than

https://doi.org/10.1021/acs.est.2c00322
Environ. Sci. Technol. 2022, 56, 6455−6465

Predicted from Laboratory Experiments. Although data from laboratory experiments have shown a high degree of microplastic ingestion by copepods,[14,67] the concentrations used are higher (10−1000MPs mL^{-1}) than what are currently observed in marine surface waters (<0.0001−0.01 MPs mL^{-1}).[72,73] Consequently, the chance of encountering and capturing a microplastic particle by a copepod in the natural environment is much lower. As discussed above, we showed that copepods like *T. longicornis* can detect the plastics, evaluate their edibility, and actively reject 80% of all captured microplastics. We observed that copepods can make mistakes in the selection of prey (20% in this study). The high ingestion of microplastics by copepods observed in laboratory experiments is likely an artifact due to the unrealistically high concentrations of microplastics used in the bioassays. Therefore, especially when exposed to low concentrations, the risk of microplastic ingestion by feeding-current feeding copepods appears to be minor. The selective behavior of feeding-current feeders minimizes the ingestion of microplastics and entry of microplastics in planktonic food webs. However, it is important to note that the entrance of microplastics into marine food webs can still happen through organisms that do not have the ability to discriminate between natural prey and microplastics or that use other mechanisms, e.g., visual detection, to select their prey (e.g., fish larvae).[13,74]

Can Zooplankton Ingestion of Microplastics Disrupt the Biological Carbon Pump? The biological carbon (C) pump is the mechanism by which inorganic carbon fixed through photosynthesis is exported out of the surface layer via biological processes. The biological C pump is crucial for the sequestration of CO_2 and climate regulation.[75] Planktonic copepods are keystone components of the biological C pump by ingestion of primary production, export of particulate organic matter via fecal pellet and carcass production, vertical migrations, and respiration during hibernation[76,77] (Figure 5). The adverse biological effects of microplastics, shown in laboratory studies, have raised concerns about the impact of microplastic pollution on the C cycle.[78]

Kvale et al.[7] predicted that a physical effect of microplastic pollution via zooplankton negatively affects the biological C pump and consequently the global ocean oxygenation. Kvale's model assumes that the ingestion and selection of microplastics by zooplankton are only driven by the ratio of microplastics to natural food. However, this is not the case for planktonic copepods, where the foraging behavior and prey selection capability of copepods are key aspects that determine the ingestion of microplastics as demonstrated here. Due to the capture mechanisms (ambush feeder) and taste discrimination (feeding-current feeders) of copepods, the ingestion of microplastics is expected to be low and therefore also their impacts on planktonic copepods. The grazing of zooplankton is not negatively affected by the ingestion of virgin microplastics at *in situ* concentrations of microplastics (0.0001−0.01 MPs mL^{-1}).[30,73] In our studies, the ingestion rates of copepods on algae were not affected by the presence of MPs and were similar to those in the absence of MPs.[79] Production and sinking rates of fecal pellets are also key processes in the biological C pump. Assuming that the presence of microplastics inside the fecal pellets increases their buoyancy, the pellets would be recycled in the water column, reducing the C sequestration in the bottom waters. However, similar to grazing rates, fecal pellet production and sinking rates are not expected to be affected by the ingestion of microplastics under natural conditions.[16] Therefore, it is unlikely that physical impacts of microplastics can disrupt the role planktonic copepods play in the global biological carbon cycle. Overall, our results indicate that, while there is a risk of entry of microplastics in the marine food webs, planktonic copepods are not expected to be a major entry route.

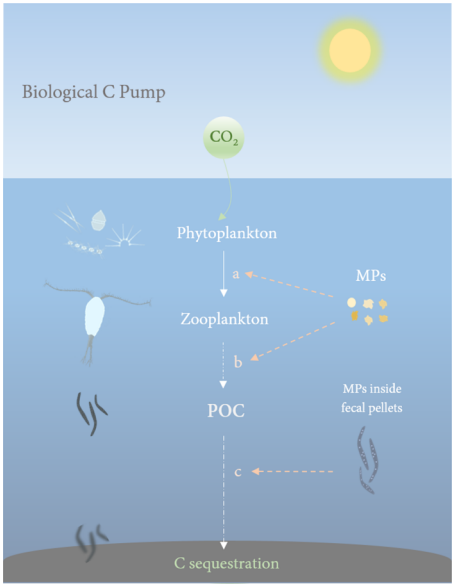


Figure 5. Simplified scheme of the key role of zooplankton in the "biological carbon (C) pump" and the potential impacts of microplastics (MPs) on the transfer and exportation of carbon: (a) decreased zooplankton grazing, (b) reduced fecal pellet production, and (c) lower sinking velocity of fecal pellets containing ingested microplastics. POC, particulate organic carbon.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c00322.

List of actual microplastic and algae concentrations used in both incubation and filming experiments (Table S1); analysis of feeding on algae and microplastics (Table S2); and bottle incubation data (Table S3) (PDF)
Temora longicornis ingesting an algal cell (AVI)
Temora longicornis ingesting a plastic microsphere (AVI)
Temora longicornis rejecting a plastic microsphere (AVI)

■ AUTHOR INFORMATION

Corresponding Author

Jiayi Xu − *State Key Laboratory of Estuarine and Coastal Research, East China Normal University, 200241 Shanghai, China;* orcid.org/0000-0003-3885-7774; Email: jyxu@sklec.ecnu.edu.cn

Authors

Rocío Rodríguez-Torres − *National Institute of Aquatic Resource, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark;* orcid.org/0000-0003-0288-4949

Sinja Rist − *National Institute of Aquatic Resource, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark; Department of Environmental Engineering, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark*

Torkel Gissel Nielsen − *National Institute of Aquatic Resource, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark;* orcid.org/0000-0003-1057-158X

https://doi.org/10.1021/acs.est.2c00322
Environ. Sci. Technol. 2022, 56, 6455−6465

Nanna Bloch Hartmann − *Department of Environmental Engineering, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark*
Philipp Brun − *Swiss Federal Institute for Forest, Snow and Landscape Research. WSL, CH-8903 Birmensdorf, Switzerland*
Daoji Li − *State Key Laboratory of Estuarine and Coastal Research, East China Normal University, 200241 Shanghai, China;* orcid.org/0000-0002-3447-3485
Rodrigo Almeda − *National Institute of Aquatic Resource, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark; EOMAR, IU-ECOAQUA, Biology Department, University of Las Palmas de Gran Canaria, 35017 Tafira Baja, Las Palmas, Spain*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.est.2c00322

Author Contributions

#J.X. and R.R.-T. contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was made possible by the support from the National Natural Science Foundation of China (42006124), the Shanghai Sailing Program (19YF1413900), the Danish center for research into marine plastic pollution ("MarinePlastic") supported by the Velux Foundation (Project no. 25084), the JPI Oceans Project RESPONSE (Towards a risk-based assessment of microplastic pollution in marine ecosystems) supported by the Innovation Fund Denmark (IFD, 9087-00006B), a research grant from the 2019 Maritime DTU-Orients Fund to R.A., and a grant from the Spanish Ministry of Science and Innovation through the Ramón y Cajal Program to R.A. (RYC2018-025770-I). This study was furthermore supported by the H2020 CLAIM project (Cleaning Litter by developing and Applying Innovative Methods in European seas; Grant Agreement No. 774586) to T.G.N. Thanks to Mikael Emil Olsson for the chemical analysis and to Hans Van Someren Gréve for the copepod vector graphic.

■ REFERENCES

(1) Cózar, 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. Plastic debris in the open ocean. *Proc. Natl. Acad. Sci.* **2014**, *111*, 10239−10244.
(2) Lim, X. Microplastics are everywhere — but are they harmful? *Nature* **2021**, *593*, 22−25.
(3) Parsons, T. R.; Takahashi, M. *Biological oceanographic processes*; Pergamon Press: Oxford. UK. (1973).
(4) Coppock, R. L.; Galloway, T. S.; Cole, M.; Fileman, E. S.; Queirós, A. M.; Lindeque, P. K. Microplastics alter feeding selectivity and faecal density in the copepod. *Sci. Total Environ.* **2019**, *687*, 780−789.
(5) Wieczorek, A. M.; Croot, P. L.; Lombard, F.; Sheahan, J. N.; Doyle, T. K. Microplastic ingestion by gelatinous zooplankton may lower efficiency of the biological pump. *Environ. Sci. Technol.* **2019**, *53*, 5387−5395.
(6) Shen, M.; Ye, S.; Zeng, G.; Zhang, Y.; Xing, L.; Tang, W.; Wen, X.; Liu, S. Can microplastics pose a threat to ocean carbon sequestration? *Mar. Pollut. Bull.* **2020**, *150*, No. 110712.
(7) Kvale, K.; Prowe, A. E. F.; Chien, C.-T.; Landolfi, A.; Oschlies, A. Zooplankton grazing of microplastic can accelerate global loss of ocean oxygen. *Nat. Commun.* **2021**, *12*, 2358.
(8) Humes, A. G. How many copepods? *Hydrobiologia* **1994**, *292-293*, 1−7.
(9) Cornils, A.; Sieger, R.; Mizdalski, E.; Schumacher, S.; Grobe, H.; Schnack-Schiel, S. B. Copepod species abundance from the Southern Ocean and other regions (1980−2005) − a legacy. *Earth Syst. Sci. Data* **2018**, *10*, 1457−1471.
(10) Botterell, Z. L. R.; Beaumont, N.; Dorrington, T.; Steinke, M.; Thompson, R. C.; Lindeque, P. K. Bioavailability and effects of microplastics on marine zooplankton: A review. *Environ. Pollut.* **2019**, *245*, 98−110.
(11) Botterell, Z. L. R.; Beaumont, N.; Cole, M.; Hopkins, F. E.; Steinke, M.; Thompson, R. C.; Lindeque, P. K. Bioavailability of Microplastics to Marine Zooplankton: Effect of Shape and Infochemicals. *Environ. Sci. Technol.* **2020**, *54*, 12024−12033.
(12) Kühn, S.; van Franeker, J. A. Quantitative overview of marine debris ingested by marine megafauna. *Mar. Pollut. Bull.* **2020**, *151*, No. 110858.
(13) Ugwu, K.; Herrera, A.; Gómez, M. Microplastics in marine biota: A review. *Mar. Pollut. Bull.* **2021**, *169*, No. 112540.
(14) Cole, M.; Lindeque, P.; Fileman, E.; Halsband, C.; Goodhead, R.; Moger, J.; Galloway, T. S. Microplastic ingestion by zooplankton. *Environ. Sci. Technol.* **2013**, *47*, 6646−6655.
(15) Almeda, R.; Rodriguez-Torres, R.; Rist, S.; Winding, M. H. S.; Stief, P.; Hansen, B. H.; Nielsen, T. G. Microplastics do not increase bioaccumulation of petroleum hydrocarbons in Arctic zooplankton but trigger feeding suppression under co-exposure conditions. *Sci. Total Environ.* **2021**, *751*, No. 141264.
(16) Rodríguez-Torres, R.; Almeda, R.; Kristiansen, M.; Rist, S.; Winding, M. S.; Nielsen, T. G. Ingestion and impact of microplastics on arctic Calanus copepods. *Aquat. Toxicol.* **2020**, *228*, No. 105631.
(17) Desforges, J. P. W.; Galbraith, M.; Ross, P. S. Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. *Arch. Environ. Contam. Toxicol.* **2015**, *69*, 320−330.
(18) Sun, X.; Liu, T.; Zhu, M.; Liang, J.; Zhao, Y.; Zhang, B. Retention and characteristics of microplastics in natural zooplankton taxa from the East China Sea. *Sci. Total Environ.* **2018**, *640-641*, 232−242.
(19) Kosore, C.; Ojwang, L.; Maghanga, J.; Kamau, J.; Kimeli, A.; Omukoto, J.; Ngisiag'e, N.; Mwaluma, J.; Ong'ada, H.; Magori, C.; Ndirui, E. Occurrence and ingestion of microplastics by zooplankton in Kenya's marine environment: first documented evidence. *African J. Mar. Sci.* **2018**, *40*, 225−234.
(20) Md Amin, R.; Sohaimi, E. S.; Anuar, S. T.; Bachok, Z. Microplastic ingestion by zooplankton in Terengganu coastal waters, southern South China Sea. *Mar. Pollut. Bull.* **2020**, *150*, No. 110616.
(21) Zheng, S.; Zhao, Y.; Liangwei, W.; Liang, J.; Liu, T.; Zhu, M.; Li, Q.; Sun, X. Characteristics of microplastics ingested by zooplankton from the Bohai Sea. *China. Sci. Total Environ.* **2020**, *713*, No. 136357.
(22) Vroom, R. J. E.; Koelmans, A. A.; Besseling, E.; Halsband, C. Aging of microplastics promotes their ingestion by marine zooplankton. *Environ. Pollut.* **2017**, *231*, 987−996.
(23) Xu, J.; Hansen, P. J.; Nielsen, L. T.; Krock, B.; Tillmann, U.; Kiørboe, T. Distinctly different behavioral responses of a copepod, Temora longicornis, to different strains of toxic dinoflagellates, Alexandrium spp. *Harmful Algae* **2017**, *62*, 1−9.
(24) Di, C.; Temora Baird, I. 1850. ICES Identif. *Leafl. Plankt.* **2021**, *195*, 17.
(25) Almeda, R.; Someren Gréve, H.; Kiørboe, T. Prey perception mechanism determines maximum clearance rates of planktonic copepods. *Limnol. Oceanogr.* **2018**, *63*, 2695−2707.
(26) Kiørboe, T. How zooplankton feed: Mechanisms, traits and trade-offs. *Biol. Rev.* **2011**, *86*, 311−339.
(27) Gonçalves, R. J.; Van Someren Gréve, H.; Couespel, D.; Kiørboe, T. Mechanisms of prey size selection in a suspension-feeding copepod, Temora longicornis. *Mar. Ecol.: Prog. Ser.* **2014**, *517*, 61−74.
(28) Kiørboe, T.; Visser, A.; Andersen, K. H. A trait-based approach to ocean ecology. *ICES J. Mar. Sci.* **2018**, *75*, 1849−1863.
(29) Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T. S. Microplastics as contaminants in the marine environment: A review. *Mar. Pollut. Bull.* **2011**, *62*, 2588−2597.

(30) Rist, S.; Vianello, A.; Winding, M. H. S.; Nielsen, T. G.; Almeda, R.; Rodriguez-Torres, R.; Vollertsen, J. Quantification of plankton-sized microplastics in a productive coastal Arctic marine ecosystem. *Environ. Pollut.* **2020**, *266*, No. 115248.
(31) Cverenkárová, K.; Valachovičová, M.; Mackul'ak, T.; Žemlička, L.; Bírošová, L. Microplastics in the food chain. *Life* **2021**, *11*, 1−18.
(32) O'Brien, T. D. COPEPOD: The Global Plankton Database. *An overview of the 2010 database contents, processing methods, and access interface*. U.S. Dep. Commer. NOAA Tech. Memo. NMFS-F/ST-, 28 pp (2010).
(33) Brun, P.; Payne, M. R.; Kiørboe, T. A trait database for marine copepods. *Earth Syst. Sci. Data* **2017**, *9*, 99−113.
(34) Brun, P.; Payne, M. R.; Kiørboe, T. Trait biogeography of marine copepods - an analysis across scales. *Ecol. Lett.* **2016**, *19*, 1403−1413.
(35) Hastie, T.; Tibshirani, R. *Geberalized additive models*. (London: Chapman & Hall/CRC 1990).
(36) Wood, S. N. *Generalized additive models: An introduction with R*, Carlin, B. P.; Chatfiled, C.; Tanner, M.; Zidek, J. Eds. (CRC Press, 2006).
(37) Rayner, N. A.; Parker, D. E.; Horton, E. B.; Folland, C. K.; Alexander, L. V.; Rowell, D. P.; Kent, E. C.; Kaplan, A. Global analyses of sea surface temperature, sea ice, and night marine air temperature since the late nineteenth century. *J. Geophys. Res.* **2003**, *108*, 4407.
(38) Hansen, P. The red tide dinoflagellate Alexandrmm tamarense: effects on behaviour and growth of a tintinnid ciliate. *Mar. Ecol.: Prog. Ser.* **1989**, *53*, 105−116.
(39) Wang, W.; Wang, J. Comparative evaluation of sorption kinetics and isotherms of pyrene onto microplastics. *Chemosphere* **2018**, *193*, 567−573.
(40) Cowles, T. J.; Strickier, J. R. Characterization of feeding activity patterns in the planktonic copepod Centropages typicus Kroyer under various food conditions. *Limnol. Oceanogr.* **1983**, *28*, 106−115.
(41) Xu, J.; Kiørboe, T. Toxic dinoflagellates produce true grazer deterrents. *Ecology* **2018**, *99*, 2240−2249.
(42) Frost, B. W. Effect of size and concentration of food particles on the feeding behaviour of the marine planktonic copepod Calanus pacificus. *Limnol. Oceanogr.* **1972**, *17*, 805−815.
(43) Vanderploeg, H. A.; Scavia, D. Two electivity indices for feeding with special reference to zooplankton grazing. *J. Fish. Res. Board Canada* **1979**, *36*, 362−365.
(44) Bellas, J.; Gil, I. Polyethylene microplastics increase the toxicity of chlorpyrifos to the marine copepod Acartia tonsa. *Environ. Pollut.* **2020**, *260*, 114059.
(45) Koski, M.; Søndergaard, J.; Christensen, A. M.; Nielsen, T. G. Effect of environmentally relevant concentrations of potentially toxic microplastic on coastal copepods. *Aquat. Toxicol.* **2021**, *230*, No. 105713.
(46) Yu, J.; Tian, J.; Xu, R.; Zhang, Z.; Yang, G.; Wang, X.; Lai, J.; Chen, R. Effects of microplastics exposure on ingestion, fecundity, development, and dimethylsulfide production in Tigriopus japonicus (Harpacticoida, copepod). *Environ. Pollut.* **2020**, *267*, No. 115429.
(47) Heuschele, J.; Selander, E. The chemical ecology of copepods. *J. Plankton Res.* **2014**, *36*, 895−913.
(48) Strickler, J. R. Calanoid Copepods, Feeding Currents, and the Role of Gravity. *Science* **1982**, *218*, 158−160.
(49) Moore, P. A.; Fields, D. M.; Yen, J. Physical constraints of chemoreception in foraging copepods. *Limnol. Oceanogr.* **1999**, *44*, 166−177.
(50) Jiang, H. H.; Osborn, T. R.; Meneveau, C. Chemoreception and the deformation of the active space in freely swimming copepods: a numerical study. *J. Plankton Res.* **2002**, *24*, 495−510.
(51) Tiselius, P.; Saiz, E.; Kiørboe, T. Sensory capabilities and food capture of two small copepods, Paracalanus parvus and Pseudocalanus sp. Limnol. *Oceanogr.* **2013**, *58*, 1657−1666.
(52) Paffenhöfer, G. A.; Strickler, J. R.; Alcaraz, M. Suspension-feeding by herbivorous calanoid copepods: A cinematographic study. *Mar. Biol.* **1982**, *67*, 193−199.
(53) Dam, H. G.; Lopes, R. M. Omnivory in the calanoid copepod Temora longicornis: Feeding, egg production and egg hatching rates. *J. Exp. Mar. Bio. Ecol.* **2003**, *292*, 119−137.
(54) Fernández, F. Particle selection in the nauplius of Calanus pacificus. *J. Plankton Res.* **1979**, *1*, 313−328.
(55) Ayukai, T. Discriminate feeding of the calanoid copepod Acartia clausi in mixtures of phytoplankton and inert particles. *Mar. Biol.* **1987**, *94*, 579−587.
(56) Rodriguez, F. Plastic. *Encyclopaedia Britannica*, at <https://www.britannica.com/science/plastic> (2020)
(57) Andrady, A. L. The plastic in microplastics: A review. *Mar. Pollut. Bull.* **2017**, *119*, 12−22.
(58) Capolupo, M.; Sørensen, L.; Jayasena, K. D. R.; Booth, A. M.; Fabbri, E. Chemical composition and ecotoxicity of plastic and car tire rubber leachates to aquatic organisms. *Water Res.* **2020**, *169*, No. 115270.
(59) Kettner, M. T.; Rojas-Jimenez, K.; Oberbeckmann, S.; Labrenz, M.; Grossart, H.-P. Microplastics alter composition of fungal communities in aquatic ecosystems. *Environ. Microbiol.* **2017**, *19*, 4447−4459.
(60) Oberbeckmann, S.; Osborn, A. M.; Duhaime, M. B. Microbes on a bottle: substrate, season and geography influence community composition of microbes colonizing marine plastic debris. *PLoS One* **2016**, *11*, No. e0159289.
(61) Procter, J.; Hopkins, F. E.; Fileman, E. S.; Lindeque, P. K. Smells good enough to eat: Dimethyl sulfide (DMS) enhances copepod ingestion of microplastics. *Mar. Pollut. Bull.* **2019**, *138*, 1−6.
(62) Reisser, J.; Shaw, J.; Hallegraeff, G.; Proietti, M.; Barnes, D. K. A.; Thums, M.; Wilcox, C.; Hardesty, B. D.; Pattiaratchi, C. Millimeter-sized marine plastics: A new pelagic habitat for microorganisms and invertebrates. *PLoS One* **2014**, *9*, No. e100289.
(63) Zhang, Y.; Lu, J.; Wu, J.; Wang, J.; Luo, Y. Potential risks of microplastics combined with superbugs: Enrichment of antibiotic resistant bacteria on the surface of microplastics in mariculture system. *Ecotoxicol. Environ. Saf.* **2020**, *187*, No. 109852.
(64) Rochman, C. M.; Manzano, C.; Hentschel, B. T.; Simonich, S. L. M.; Hoh, E. Polystyrene plastic: A source and sink for polycyclic aromatic hydrocarbons in the marine environment. *Environ. Sci. Technol.* **2013**, *47*, 13976−13984.
(65) Seuront, L. Zooplankton avoidance behaviour as a response to point sources of hydrocarbon-contaminated water. *Mar. Freshw. Res.* **2010**, *61*, 263−270.
(66) Griffin, R. L.; Green, I.; Stafford, R. Accumulation of marine microplastics along a trophic gradient as determined by an agent-based model. *Ecol. Inform.* **2018**, *45*, 81−84.
(67) Setälä, O.; Fleming-Lehtinen, V.; Lehtiniemi, M. Ingestion and transfer of microplastics in the planktonic food web. *Environ. Pollut.* **2014**, *185*, 77−83.
(68) Costa, E.; Piazza, V.; Lavorano, S.; Faimali, M.; Garaventa, F.; Gambardella, C. Trophic transfer of microplastics from copepods to jellyfish in the marine environment. *Front. Environ. Sci.* **2020**, *8*, 1−7.
(69) Walter, T. C., Boxshall, G. *World of Copepods Database*. Accessed at http://www.marinespecies.org/copepoda on 2021-11-18. (2021).
(70) Outram, L., Hurley, J., Rott, A., Crooks, N., Pernetta, A. P. *No evidence of microplastic consumption by the copepod, Temora longicornis (Müller, 1785) in Chichester Harbour, United Kingdom. Nauplius* 28, (2020), DOI: 10.1590/2358-2936e2020033.
(71) Kuddithamby, G.; Almeda, R.; Nielsen, T. G.; Lorenz, C.; Vianello, A.; Rodriguez-Torres, R.; Iordachescu, L.; Papacharalampos, K.; Vollertsen, J. Where does the marine plastic go? Abundance and distribution of MPs in the Kattegat Sea. *SETAC Europe 32st Annual Meeting Abstract Book* (2022).
(72) Lenz, R.; Enders, K.; Nielsen, T. G. Microplastic exposure studies should be environmentally realistic. *Proc. Natl. Acad. Sci.* **2016**, *113*, E4121−E4122.
(73) Beiras, R.; Schönemann, A. M. Currently monitored microplastics pose negligible ecological risk to the global ocean. *Sci. Rep.* **2020**, *10*, 22281.

(74) Ory, N. C.; Sobral, P.; Ferreira, J. L.; Thiel, M. Amberstripe scad Decapterus muroadsi (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. *Sci. Total Environ.* **2017**, *586*, 430−437.
(75) Henson, S.; Le Moigne, F.; Giering, S. Drivers of Carbon Export Efficiency in the Global Ocean. *Global Biogeochem. Cycles* **2019**, *33*, 891−903.
(76) Turner, J. T. Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. *Prog. Oceanogr.* **2015**, *130*, 205−248.
(77) Jónasdóttir, S. H.; Visser, A. W.; Richardson, K.; Heath, M. R. Seasonal copepod lipid pump promotes carbon sequestration in the deep North Atlantic. *Proc. Natl. Acad. Sci.* **2015**, *112*, 12122−12126.
(78) Shore, E. A.; deMayo, J. A.; Pespeni, M. H. Microplastics reduce net population growth and fecal pellet sinking rates for the marine copepod, Acartia tonsa. *Environ. Pollut.* **2021**, *284*, No. 117379.
(79) van Someren Gréve, H. Zooplankton motile behavior: traits and trade-offs in planktonic copepods. PhD thesis, Technical University of Denmark. (2017).

https://doi.org/10.1021/acs.est.2c00322
Environ. Sci. Technol. 2022, 56, 6455−6465