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The Behavior of Planktonic Copepods Minimizes the Entry of Microplastics in Marine Food Webs

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ABSTRACT: The entry of microplastics (MPs) into marine food webs is a major environmental concern. We investigated how the behavior of planktonic copepods influences the risk of MPs to enter marine food webs by applying a trait-based approach and by combining experiments (bottle incubations and video observations) with biogeographical analyses. We aimed to evaluate which type of feeding behavior is most risky in terms of MP ingestion and which marine geographical areas are more susceptible to MP ingestion by planktonic copepods. We used different species as models of the main foraging behaviors in planktonic copepods: feeding-current, cruising, ambush, and mixed behavior feeding. All behaviors showed a similarly low risk of MP ingestion, up to 1 order of magnitude lower than for similar-sized microalgae. We did not observe any influence of the prey type or MP size (8 and 20 μm) on MP ingestion for any of the behaviors. By mapping the global distribution of feeding behaviors, we showed that feeding-current feeding is the most common behavior, but the risk of MP ingestion remains equally low across the global ocean, independently of the predominant behavior. Overall, our results suggest a low risk of MP ingestion by planktonic copepods and therefore a minimal risk of trophic transfer of MPs via marine pelagic copepods in marine ecosystems.
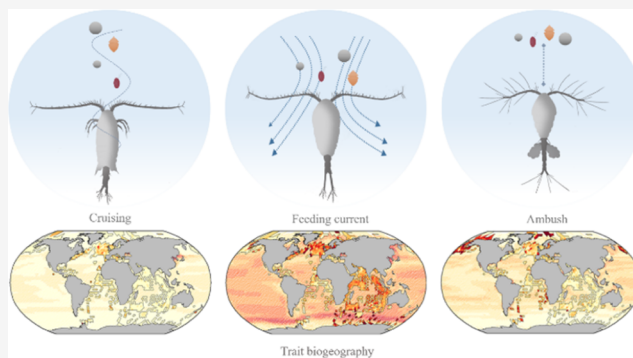
KEYWORDS: microplastics, copepods, trait-based approach, feeding behavior, ingestion

1. INTRODUCTION

Plastic litter is ubiquitous in aquatic ecosystems, with current amounts projected to triple by 2040.[1] As a result, the consequences of plastic pollution in marine ecosystems are of global concern. Part of the plastic litter consists of microscopic plastic particles, microplastics (MPs) with the size fraction <100 μm being the most abundant in marine waters.[2−4] MPs < 100 μm are similar in size to phytoplankton, which makes them potentially available for ingestion by zooplankton.[5−7] Given the key trophic position of zooplankton in marine food webs,[8,9] knowledge of the risk of microplastic ingestion by zooplankton is essential to assess the overall fate of MPs in marine ecosystems.

Planktonic copepods are the dominant zooplankton group and the most abundant animals in the ocean.[10] Copepods are both grazers and prey; therefore, they may influence the entry and biotransfer of MPs in marine food webs.[11] Additionally, copepods can accelerate vertical exportation of MPs via production of fast sinking fecal pellets. Microplastic ingestion by planktonic copepods has been observed in laboratory experiments using high concentrations of MPs.[5−7,12,13] However, field studies show a low occurrence of MP ingestion in natural zooplankton communities.[14−17] Desforges *et al.*

(2015) found 0.026 particles per copepod in the Northeast Pacific Ocean; Sun *et al.* (2018) found 0.13 pieces per individual in copepod species in the East China Sea. These findings contradict the predicted high risk of MP ingestion based on laboratory studies.

A trait-based approach proposes to represent the thousands of planktonic copepod species with few well-chosen model species that reflect the existing diversity in the most important traits. In the context of zooplankton ecology, it is well documented that foraging behavior is a key trait due to its strong influence on feeding rates and predation risk.[18−21] Additionally, functional traits, such as feeding behavior in marine copepods, vary across the global ocean as a function of the environment.[22] A trait-based approach, using feeding behavior as the key trait, is therefore a useful tool to evaluate
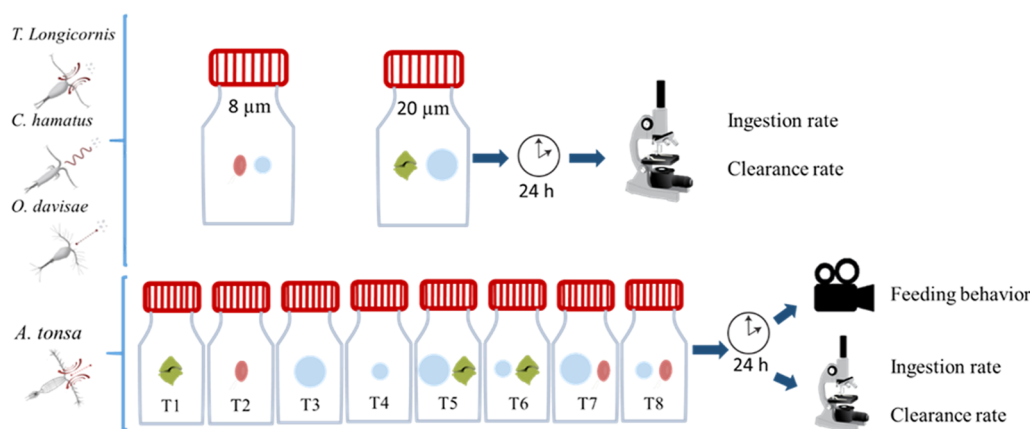
Figure 1. Schematic overview of the experimental studies. The upper bottles represent the bottle incubation experiments of the three main feeding behaviors when exposed to *R. salina* (red alga) and MPs of 8 μm or *H. steinii* (green algae) and MPs of 20 μm. The lower row indicates the incubation and video filming treatments used in the experiment with a mixed behavior feeder, *A. tonsa*, exposed to different combinations of microalgae and MP sizes.

the risk of ingestion and biotransfer of MPs via copepods on a global scale.

Suspension-feeding planktonic copepods have three main types of feeding behaviors: (1) "feeding-current feeding", where they create a current to harvest prey; (2) "cruising feeding", where they encounter prey while swimming through the water; and (3) "ambush feeding", where they wait motionless for motile prey to enter their sensory reach or otherwise capture those prey that directly collide with them.[18,23,24] The former two feeding behaviors are categorized as "active feeders". Some copepods can be "mixed behavior feeders" and are able to switch between behaviors depending on prey type and food availability.[23,25,26] For example, *Acartia tonsa* can either generate a feeding current to harvest small or nonmotile prey or behave as an ambush feeder to capture large motile prey.[26] Despite the importance of behavior in evaluating the risk of MP ingestion by zooplankton, behavior is still an understudied variable in MP pollution.[27] For instance, it is unknown whether *Oithona* (ambush feeding copepods) ingest MPs, which is an abundant and widely distributed genus across the oceans.[28] Therefore, research about the influence of zooplankton behavior on MP ingestion is needed to better assess the risk of entrance and transfer of MPs in marine food webs.

The overall aim of this study is to evaluate how the behavior of planktonic copepods influences the risk of MPs to enter marine planktonic food webs using bottle incubations and video observations. We used a trait-based approach and planktonic copepods as model organisms. Our specific objectives and hypotheses are (1) to estimate the risk of MP ingestion associated with the three main feeding behaviors of suspension-feeding copepods (feeding-current feeding, cruising feeding, and ambush feeding) and hypothesize that ambush feeding is the least risky behavior in terms of MP ingestion since ambush feeders are inefficient in detecting nonmotile prey[20] and (2) to investigate the influence of prey switching behavior on MP ingestion in a mixed behavior feeder. We hypothesize that the presence of a large motile prey will induce ambush feeding, which, in turn, will reduce the ingestion of MPs in a mixed behavior feeder; (3) to evaluate the global risk of ingestion of MPs in copepods by intersecting the outcome from the experiments with estimates of the global distribution of the copepods in three feeding behaviors. We hypothesize

that areas dominated by active feeders are more susceptible to the entry of MPs into food webs than the areas dominated by ambush feeders.

2. MATERIALS AND METHODS

2.1. Experimental Organisms. The trait of relevance in this study is foraging behavior, and thus, the experimental organisms were selected in order to compare among contrasting foraging behaviors. The following species of copepods were used as models of the different foraging behaviors: *Temora longicornis* (feeding-current feeder); *Centropages hamatus* (cruising feeder); *Oithona davisae* (ambush feeder); and *A. tonsa* (mixed behavior feeder). To avoid body size effects on the MP ingestion, we ensured similar body size of the experimental organisms. To this end, we used copepodites I and II of *C. hamatus* and *T. longicornis* and female adults of *O. davisae*, which all have a prosome length of roughly 400 μm. To investigate the prey switching behavior, we used female adults of *A. tonsa*. In all the experiments, specimens were selected from the main cultures the day before the experiment and kept isolated overnight in glass beakers without food to avoid effects from their previous diet.

Copepods were obtained from stock cultures at the Technical University of Denmark (DTU Aqua). Cultures of *T. longicornis* and *C. hamatus* were established from specimens originally collected in Gullmarsfjorden (Sweden) and Øresund (Denmark); *O. davisae* was collected from the Mediterranean Sea (Barcelona harbor, Spain) and *A. tonsa* from the Øresund (Denmark). All copepod species were cultivated at DTU in dim light, at 18 °C and a salinity of 30‰. They were fed *ad libitum* three times per week. *T. longicornis* and *C. hamatus* were fed a mixture of the diatom *Thalassiosira weissfloggi*, the flagellate *Rhodomonas salina,* and two dinoflagellates, *Heterocapsa steinii* and *Oxyrrhis marina*. The copepod *O. davisae* was fed only with the dinoflagellate *O. marina* and *A. tonsa* fed on *R. salina*.

The phytoplankton prey used in this study were *H. steinii* and *R. salina*. We used these two species, with average sizes of 17 and 7 μm, respectively, as they fit the size range of MP beads used in this experiment (20 and 8 μm). Both species were grown in sterilized filtered seawater (FSW) with a salinity of 30‰ with B1 medium[29] at 16 °C in a 12:12 dark/light cycle with a radiation of 150 μmol photons m^{-2} s^{-1}.

2.2. Microplastics. Polyethylene is the polymer type that contributes most to pollution in aquatic environments.[30−32] In this study, transparent polyethylene (PE) spherical beads with a density of 0.96 g cm^{-3} were used as model MPs. The spherical beads were supplied as a dry powder (Cospheric). To prepare the MP suspensions, 1 mg of the powder was suspended in 250 mL glass bottles containing distilled water with 0.01% Tween 80 (Cospheric). We mixed the suspensions through vigorous hand shaking until the particles appeared dispersed and any clusters were broken into individual beads, as verified through microscopy. The size of both algae and MPs was measured using a Beckman Multisizer III Coulter Counter. Two size ranges of PE particles were used, and both follow a normal distribution: the larger particles (Cospheric) had a size range of 13.9−30.3 μm, with a mean equivalent spherical diameter (ESD) of 20.7 μm, and the smaller particles (Cospheric) had a size range of 5−16 μm, with a mean ESD of 7.9 μm. From the stock suspensions, we prepared the following diluted working suspensions for each MP size: 20 μm MPs = 52,080 MPs mL^{-1}, 8 μm MPs = 57,400 MPs mL^{-1}. From the working suspensions, a specific volume is taken to prepare the nominal concentrations (200 or 400 MPs mL^{-1}) in the experimental bottles. The absence of MP aggregates, and the concentration of MPs in the working suspensions, was verified by manual counting under an inverted microscope using Sedgewick−Rafter counting chambers. Microplastic suspensions are kept in the dark in the fridge at 4 °C.

2.3. General Experimental Setup. To estimate the influence of copepod foraging behavior on the ingestion of MPs (objective 1), we conducted bottle incubation experiments with model species of each feeding behavior, simultaneously offering MPs and similarly sized microalgae. We evaluated the ingestion of MPs associated with different behaviors using two different MP and prey sizes: (1) MPs of 20 μm and *H. steinii* (17 μm on average) and (2) MPs of 8 μm and *R. salina* (7 μm on average) (Figure 1).

To assess the influence of prey switching behavior on MP ingestion in a mixed behavior feeder (objective 2), we exposed *A. tonsa* to microalgae alone (T1 and T2), MPs alone (T3 and T4), and MPs in combination with microalgae with different sizes and motility: large MPs and large prey (T5), small MPs and large prey (T6), large MPs and small prey (T7), and small MPs and small prey (T8) (Figure 1). For each treatment, we determined motile behavioral responses using video observations and ingestion rates from bottle incubations. We used *H. steinii* as large motile prey and *R. salina* as small prey of lower motility. The chosen prey concentrations allowed us to film sufficient small-scale interactions between copepods and MPs in a reasonable time frame.

2.4. Bottle Incubation Experiments and Sample Analysis. The bottle incubations were carried out in 34 mL Pyrex bottles sealed with a lid protected with polytetrafluoroethylene (PTFE). The bottles were half filled with 0.2 μm FSW. Aliquots of the MP working suspensions were added to obtain the desired test concentrations. Afterward, the corresponding algae were added. The algae stocks were previously counted under the microscope using a Sedgewick−Rafter counting chamber in order to calculate the stock suspension volume needed to obtain the desired concentration in the incubation bottles. The nominal concentration of algae and MPs in the incubations where both types of particles were offered together was 200 MPs mL^{-1} and 200 cells mL^{-1}. In the prey switching behavior experiment, where *A. tonsa* was also

exposed to uniform prey (T1−T4), the nominal concentration was 400 MPs or cells mL^{-1} to keep the same amount of total prey (particles and cells) in all treatments. For each treatment, we prepared triplicates of "initial bottles" where we determined the concentration of algae and MPs at time = 0, triplicates of "control bottles" that were incubated with no grazers to follow changes in prey/MP concentrations during the 24 h, and triplicates of "experimental bottles" that were incubated with the copepods to estimate the ingestion rates after 24 h. Due to the different clearance rates among species and between prey/ MP types, the number of copepods per bottle was adjusted, ensuring a recommended decrease in a prey concentration of 30%:[20] 12 and 18 ind. bottle^{-1} when *O. davisae* was exposed to 20 μm prey and 8 μm prey, respectively, 8 ind. bottle^{-1} for *A. tonsa*, and 4 ind. bottle^{-1} for *C. hamatus* and *T. longicornis*. Lastly, after adding the copepods to the bottles, we filled the bottles with the test particle suspensions and closed them. The bottles were wrapped in aluminum foil to keep them dark and then mounted on a plankton wheel rotating at 1 rpm in a temperature-controlled room at 16 °C for approximately 24 h. After the incubation, copepod mortality was checked with a microscope. Mortality was not observed in any of the experiments. A 25 mL subsample was taken from each bottle, fixed with 1% Lugol's solution and stored at 4 °C for later analysis. The concentration of MPs and algae in the tested suspensions were counted under an inverted microscope (×20 magnification) using Sedgewick−Rafter chambers. We calculated ingestion (I) and clearance rates (F) on MPs and algae according to Frost (1972).[33]

2.5. Video Observation Setup and Behavior Analysis. The video observations were conducted in a temperature-controlled room at 16 °C. The different combinations of algae and MPs (Figure 1) were prepared in 50 mL cell culture flasks with eight female adults of *A. tonsa* per flask. The flask was placed between an infrared light and a high-speed camera (Phantom V210). The camera was set at a frame rate of 100 fps, a resolution of 800 × 600 pixels, and a field of view of 40 × 30 mm^2. Each flask was recorded for 3 h, and one 60 s video was saved every hour. The flask was mixed every half an hour to minimize particle settlement. Each treatment was conducted in triplicates. The filming was done in a closed room in darkness to avoid any light and temperature change or noise that could affect the behavior of the copepods.

Copepods trajectories were extracted from the resulting videos using a tracking plugin in ImageJ (version 1.52n), and behaviors were further analyzed by running an R-script (version 1.3.959). The parameters in the script were set based on previous manual frame-by-frame analysis to match the threshold of each type of behavior.[24] Between 61 and 119 tracks were analyzed per treatment, and four different types of behaviors were categorized: swim, sink, jump, and hop. "Swim" is the movement made by copepods when actively swimming; "sink" refers to passive sinking due to negative buoyancy in the absence of active movements; "jump" refers to active, big, and rapid movements usually to capture prey, and "hop" refers to active, short movements for the copepod to relocate back to its original position. The hop-like motion was distinguished from jump by its slower velocity. Hop is considered a movement that enables copepods to adjust their body orientation or switch their position in a short range rather than to escape from threats or quickly scan the water column as is achieved with jumps.[34−36] The time budget (fraction of time of swim, sink, jump, or hop), the duration of each motion bout, and the
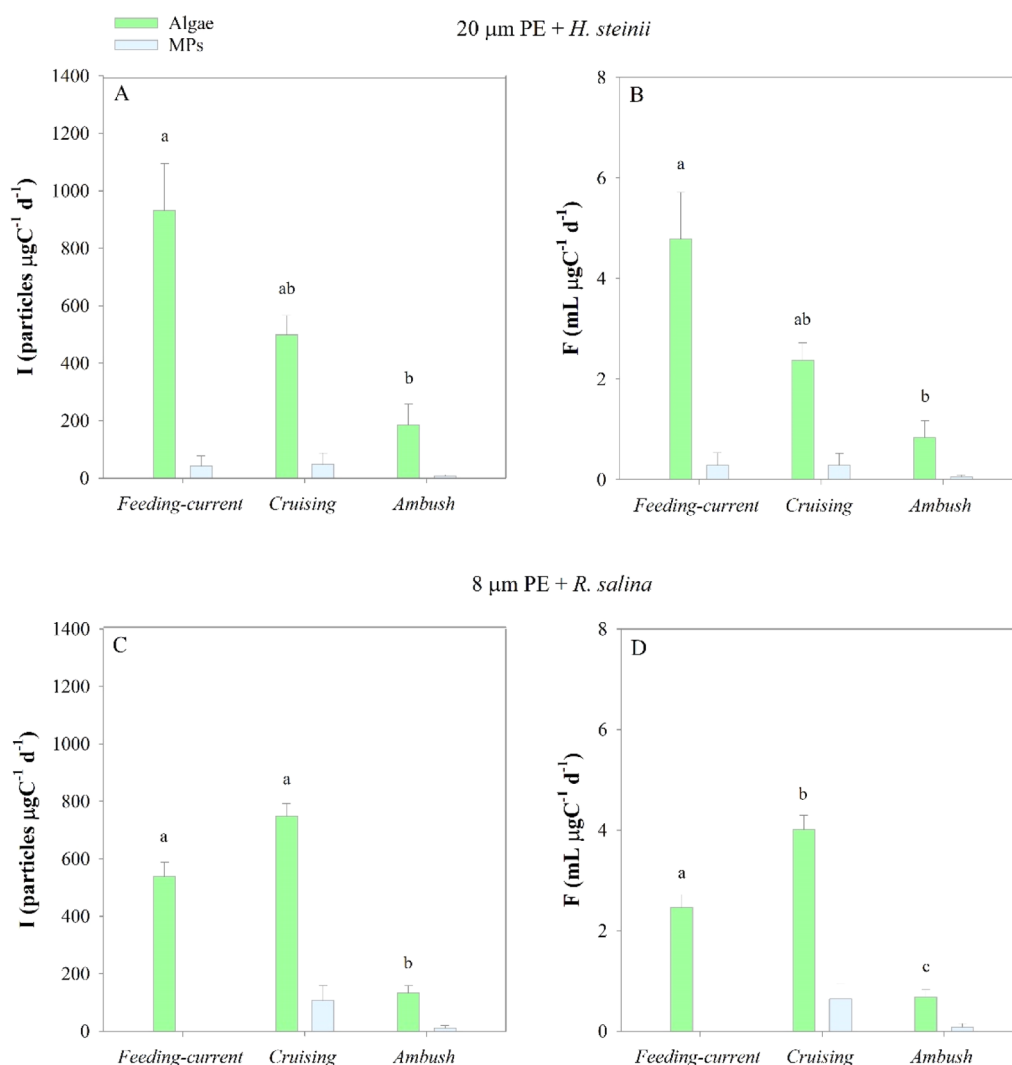
Figure 2. Ingestion (I, particles μg C^{-1} d^{-1}) and clearance rates (F, mL μg C^{-1} d^{-1}) of algae (green bars) and MPs (blue bars) for the three main feeding behaviors of copepods. Top panels (A,B) show the data for MPs and algae of 20 μm in diameter and the bottom panels (C,D) show the data for 8 μm particles and algae. Data are presented as means of triplicates with the standard error. Asterisks (*) represent a statistically significant difference between algae and MP ingestion or clearance rate within each behavior. Letters indicate significance differences among behaviors.

frequency and the velocity of these motions were calculated for all treatments.

2.6. Statistical Analyses of Experimental Data. Statistical analyses were performed using the free statistical computing software R (version 3.6.3) and IBM-SPSS v25SPS. The assumptions of normality of residuals and homogeneity of variances were tested with the Shapiro−Wilks-W-Test and Fligner−Killeen test, respectively. When normality and homogeneity assumptions were valid, differences between three or more groups were analyzed with a one-way analysis of variances (ANOVA, $p < 0.05$), followed by a post hoc Tukey's HSD test. Differences between two groups were analyzed with t-tests. When the normality and homogeneity of variance assumptions were violated, nonparametric Kruskal−Wallis tests were performed.

2.7. Spatial Modeling of the Dominance of the Three Main Feeding Behaviors. We estimated the weight fractions of feeding-current feeders, cruising feeders, and ambush feeders from global compilations of abundance and body length data using data-based estimates where available and model-based interpolations elsewhere. The data were prepared

following the approach described in Brun *et al.* (2016).[22] We first discretized the global ocean into roughly 5000 polygons of similar area. Then, we intersected these polygons with abundance observations from the Coastal and Oceanic Plankton Ecology, Production and Observation Database (COPEPOD).[37] After preprocessing and filtering of the raw data (see Brun *et al.* 2016),[22] we estimated abundance and weight fractions of each taxon in each polygon, multiplying abundance fractions with a cubed body length to obtain weight fractions. The body-length data originated from Brun *et al.* (2017).[38] Then, we identified which taxa in a polygon belonged to each feeding behavior by considering the world's most abundant representative genera as a reference. Feeding-current feeders were represented by *Acartia, Calanus, Calocalanus, Euchaeta, Nannocalanus, Paracalanus, Pareucalanus, Parvocalanus, Pleuromamma, Pseudocalanus, Rhincalanus,* and *Temora*. Cruising feeders were represented by *Centropages, Clausocalanus,* and *Metridia*, and ambush feeders were represented by *Acartia* and *Oithona*. Note that, due to its mixed feeding strategy, the genus *Acartia* was assigned to both feeding-current and ambush feeders. Finally, we calculated
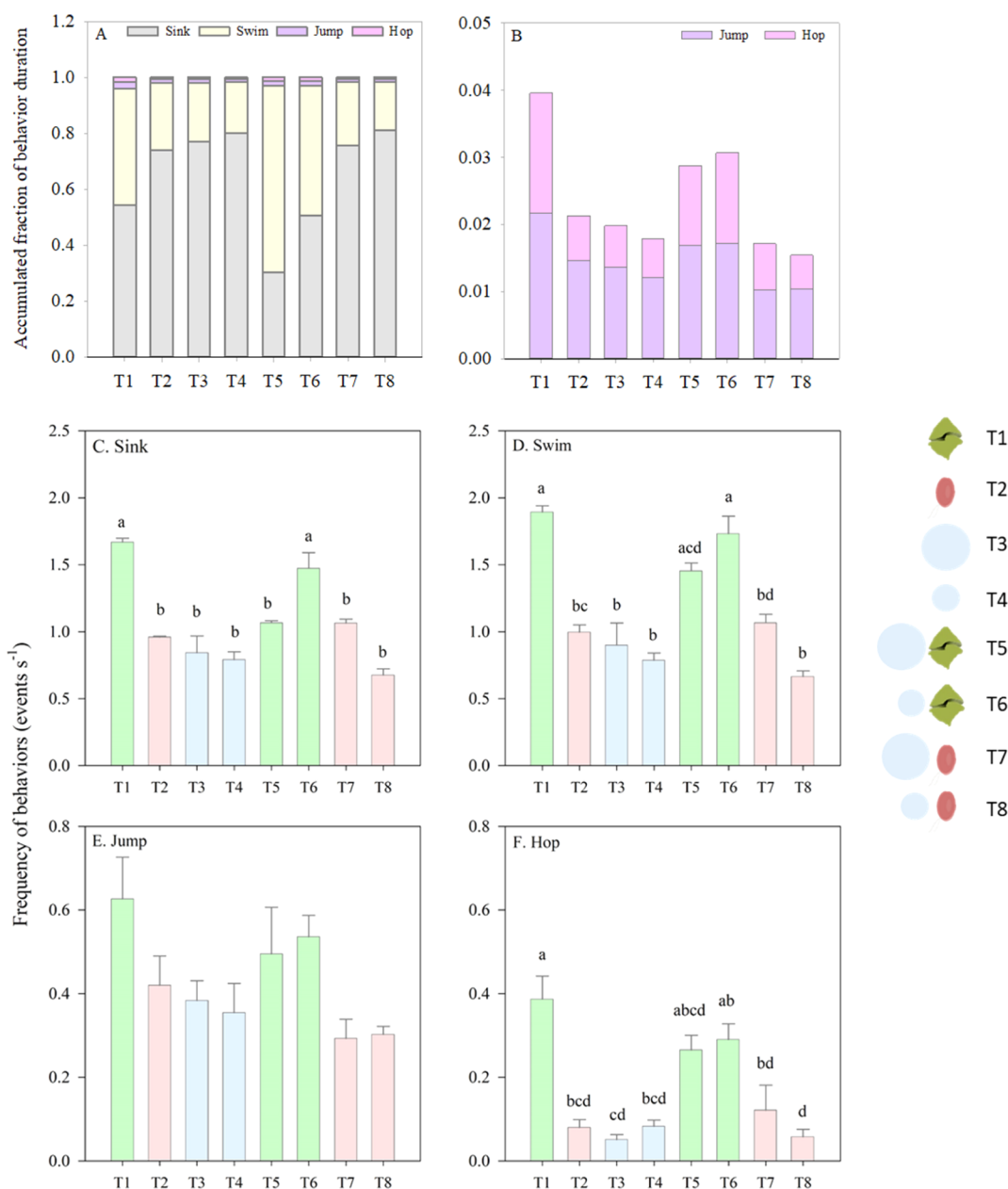
Figure 3. Accumulated fraction (A,B) of the durations of the four behaviors (sink, swim, jump, and hop) and frequency (events s^{-1}) for sink (C), swim (D), jump (E), and hop (F) in the eight different treatments (T1−T8) for the switching behavior copepod *A. tonsa*. Chart A shows the accumulation of the four behaviors, and chart B is a magnification of hop and jump data for a clearer observation. Green bars are for the treatments containing *H. steinii*, red for treatments with *R. salina,* and blue is for treatments with only MPs. Error bars represent the standard error. Letters on top of bars indicate significant differences among treatments based on pairwise comparisons, post hoc analysis.

both the abundance fraction and weight fraction of each feeding behavior relative to all remaining planktonic copepods in each polygon. In total, abundance estimates were available for 584 copepod taxa (species and genera), and 564 of them could be matched with body length information. Feeding-current feeders were represented with 103 taxa, cruising feeders with 42 taxa, and ambush feeders with 32 taxa.

We used statistical modeling to interpolate observation-based estimates to regions of the global ocean that lacked observations. We fitted six generalized additive models,[39] one for each combination of abundance/weight fraction and feeding behavior. As predictors, we considered smooth terms of polygon-wise averages of annual mean and annual range of monthly sea surface temperature (derived from the HadISST1

product),[40] as well as average chlorophyll a concentration (derived from http://www.globcolour.info/). We used the R package mgcv[41] to fit generalized additive models and assumed errors to follow a beta distribution.

3. RESULTS

3.1. Ingestion of MPs in Relation to Feeding Behavior. The incubation experiments that compare the three main feeding behaviors showed that ingestion (I, particles cop.$^{-1}$ d^{-1}) and clearance rates (F, mL cop.$^{-1}$ d^{-1}) of algae were at least 1 order of magnitude higher than of MPs in all treatments, independently of the particle size (Figure 2). Active feeders (cruising and feeding-current feeders) had higher ingestion and clearance rates of algae than ambush
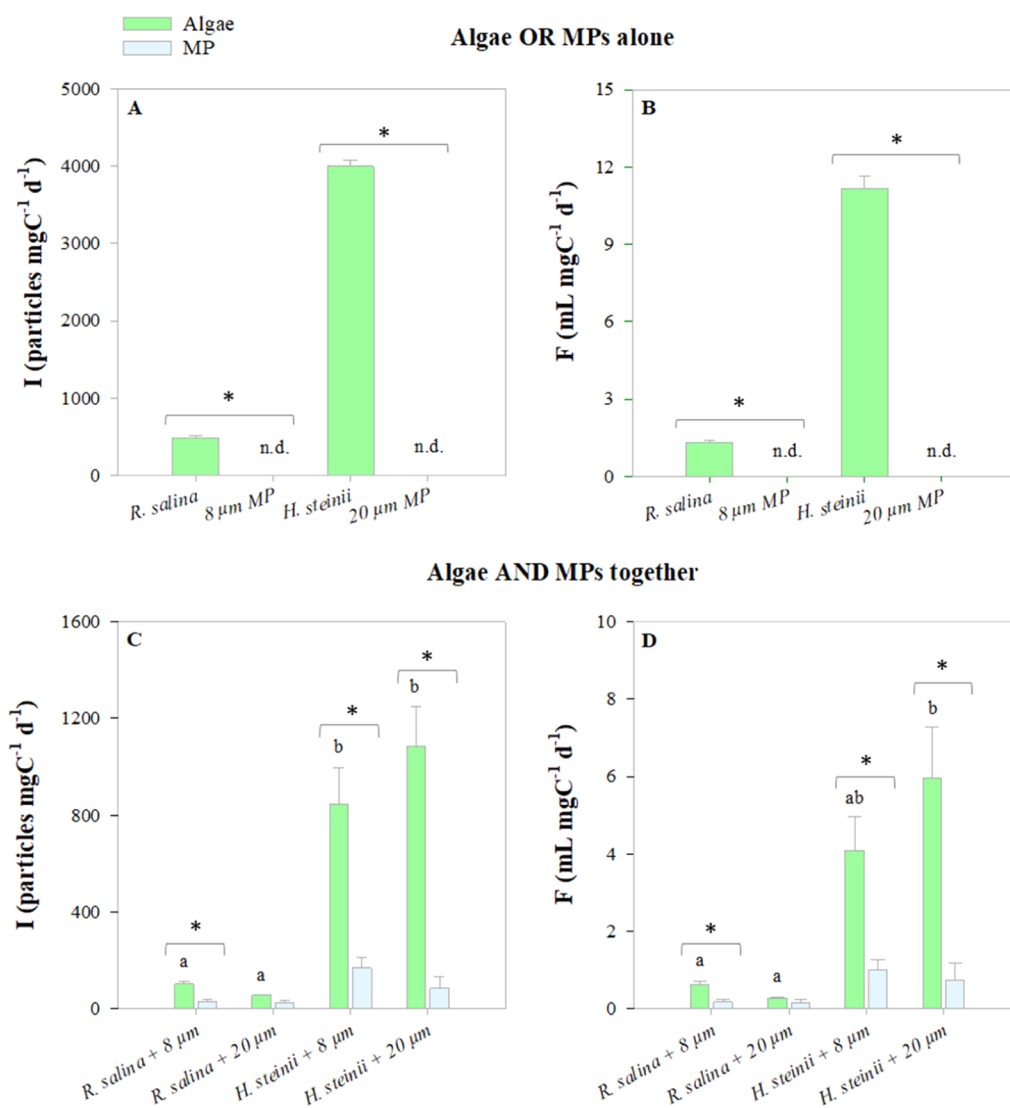
Figure 4. Ingestion (I, particles μg C^{-1} d^{-1}) and clearance rates (F, mL μg C^{-1} d^{-1}) of *A. tonsa* when they were fed with single particle (A,B) or two particles simultaneously (C,D): algae (*R. salina* or *H. steinii*) in green and MPs (8 or 20 μm PE beads) in blue. Data are presented as means of triplicates with the standard error. Letters indicate significant difference of algae ingestion or clearance rates between treatments. Asterisks (*) represent a statistically significant difference between algae and MP ingestion or clearance rate within each treatment.

feeders. However, there was no difference among the three species regarding the ingestion of MPs. All showed low ingestion and clearance rates of PE MP microspheres (on average, 8 μm: 32.4 MPs cop.$^{-1}$ d^{-1} and 20 μm: 39.2 MPs cop.$^{-1}$ d^{-1}) compared to similar-sized microalgae (Supporting Information Table S1).

3.2. Influence of Prey Type on Behavior and MP Ingestion in a Mixed Behavior Feeder. *3.2.1. Behavioral Responses from Video Observations: A. tonsa.* The accumulated fraction (0−1) of the duration of the four swimming behavior responses clearly reflects the dominance of sinking or swimming in all treatments (Figure 3A,B). The fraction of swimming was the highest in treatments 1, 5, and 6. Those are the treatments with *H. steinii* and correspond to the treatments with the highest ingestion and clearance rates (Figure 4). The accumulated duration of the jumping was equally distributed in all treatments without significant differences.

Sink and swim were the most frequent movements (Figure 3C−F). There were no significant differences among treat-

ments for jump frequency. However, for sink, swim, and hop, we found significant differences between several treatments (Supporting Information Table S2). The frequency of sink, swim, and hop was always higher for the treatments with *H. steinii*. Sink, swim, and hop frequencies never differed significantly in treatments with *R. salina* or MPs alone.

Regarding the duration of the sinking bouts, they were shorter in the treatments with *H. steinii* (Supporting Information Figure S2A). For swimming bouts, only treatment 5 showed statistically significant differences with T1, T3, T4, and T7 (Supporting Information Figure S2B). Jumps were by far the fastest movement (Supporting Information Figure S3), as expected. However, the duration of the individual bouts of jumps and hops did not vary significantly among treatments (Supporting Information Figure 2C,D).

3.2.2. Ingestion and Clearance Rates from Bottle Incubation: A. tonsa. *A. tonsa* ingested both algae species when offered alone, albeit eight times more of *H. steinii* than *R. salina* (Figure 4A,B). In contrast, the ingestion and clearance rates of both MP sizes were zero when exposed to MPs alone
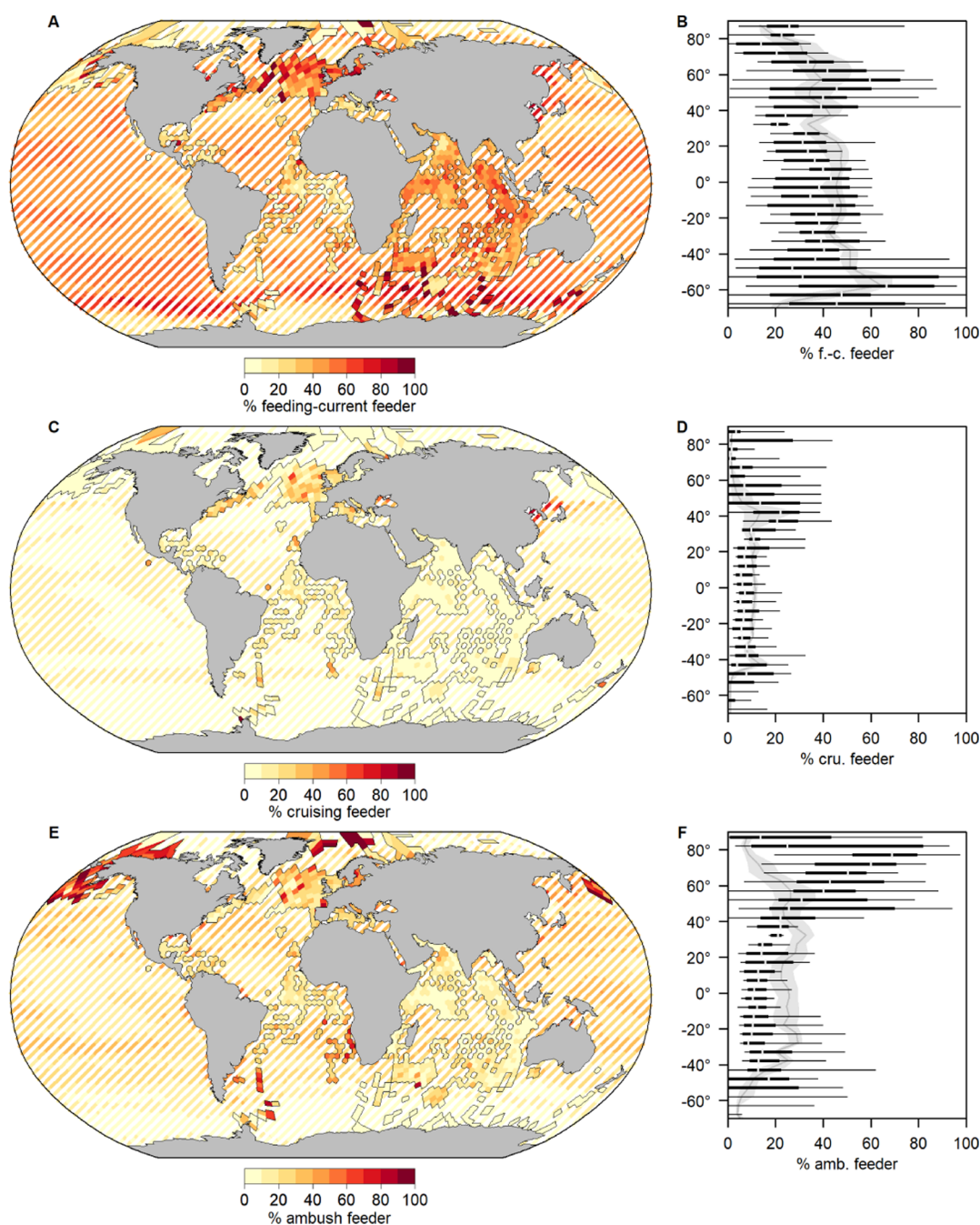
Figure 5. Global (left) and latitudinal (right) distribution of the abundance fraction of each feeding behavior: feeding-current feeders (A,B); cruising feeders (C,D), and ambush feeders (E,F). Dashed areas in maps represent model extrapolations, and solid colors represent observation-based estimates. Latitudinal boxplots are observation-based estimates, where central lines represent medians, boxes represent interquartile ranges, and whiskers represent 95% confidence intervals. Underlying polygons represent interquartile ranges of model-based estimates and underlying gray line represents median of model-based estimates. The map has some limitations due to the lack of available field data for copepods smaller than 200 μm.

(Figure 4A,B). When *A. tonsa* was simultaneously exposed to MPs and algae, the copepods ingested both types of particles (Figure 4C,D). However, the ingestion and clearance rates of MPs were 2−10 times lower than those of algae (Figure 4C,D).

Ingestion and clearance rates of *R. salina* were also significantly lower than those of *H. steinii* when offered together with MPs (Figure 4C,D). The ingestion (0−274 particles μg C^{-1} d^{-1}) and clearance (0−1.8 mL μg C^{-1} d^{-1}) rates of MPs were generally low and did not differ significantly

between treatments with different MP and algae combinations (Figure 4C,D).

3.3. Global Distribution of Copepods in the Three Main Feeding Behaviors. The three feeding behaviors (feeding-current, cruising, and ambush feeders) are present all around the globe. However, feeding-current feeders are the most abundant (Figure 5), followed by ambush feeders. Feeding-current feeders are particularly abundant above 40° latitude in both hemispheres (Figure 5A,B). Cruising feeders show highest relative abundances in temperate latitudes, but

stable fractions of around 10% also occur in subtropical and tropical areas. Above 60° latitude, cruising feeders become rare (Figure 5C,D). Model-based estimates of the relative abundance of ambush feeders peak between 60 °N and 30 °S at around 25% of individuals and declines toward higher latitudes. However, in the northern hemisphere, higher relative abundances of ambush feeders are repeatedly observed at higher latitudes (Figure 5E,F). When weight fractions instead of abundance fractions are compared, the patterns remain similar, but the fractions of feeding-current feeders generally increase, while the fractions of ambush feeders decrease (Supporting Information Figure S1).

4. DISCUSSION

4.1. Influence of the Feeding Behavior on MP Ingestion.
Our first hypothesis (ambush feeding is the least risky behavior in terms of MP ingestion) was not verified since all three behaviors showed a similarly low ingestion of MPs compared to natural prey (algae). As expected, ingestion rates of PE MP beads were very low in strict ambush feeders, which rely on hydromechanical signals to detect their prey[42] and have low clearance rates on nonmotile prey.[20,43] The nonmotile nature of MPs makes them nondetectable to ambush feeders. They can however still perceive MPs if they directly intercept/collide with the feeding/sensorial structures of the copepod, as observed for other nonmotile particles.[20,42] This mechanism may account for the nonzero ingestion of MPs by ambush feeders observed in our experiments.

For feeding-current feeders, where both motile and non-motile prey enter their feeding current, the ingestion of MPs was lower than expected. In this case, a postcapture discrimination of MPs can explain the low ingestion of MPs, as recently demonstrated for different MP types by Xu *et al.* (2022).[44] The feeding currents, created by copepods, act as "scanning currents", where prey is drawn into and, subsequently, perceived and handled individually.[18] The prey can be perceived only when it is touched, or nearly touched, by the setae on the feeding appendages.[45] Chemical discrimination occurs after tasting as observed for toxic algae[46] and MPs.[44] Therefore, an efficient active rejection of MPs by tasting (chemical discrimination) can explain the low ingestion of MPs in feeding-current feeders observed here. Taste-discrimination in the feeding-current feeder *T. longicornis* occurs even when MPs are covered with biofilms, suggesting that it is the synthetic plastic polymer "core" making the MP unpalatable to these planktonic copepods.[44] Fibers are not used in this study. However, due to the nonmotile nature of these particles and the observed ability of copepods to discriminate MPs,[41] low ingestion of fibers is expected for the studied copepods. At low rates, feeding-current feeders accidently ingested some MPs (Figure 2A,B), which can be due to (1) a relatively high concentration of MPs used in these experiments and (2) an optimization of food intake by reducing the handling time.

Strict cruising feeding is a less common behavior in zooplankton that is advantageous when passive interception is inefficient in the aquatic environment. Cruising feeders detect prey remotely depending on hydromechanical and chemical cues.[47] Since virgin MPs do not generate any of these cues, the detection and capture of MPs are expected to be low, which could explain the low ingestion of MPs observed in the studied cruising feeder. Our results agree with findings from

field studies, which indicate a low occurrence of MP ingestion in natural zooplankton communities.[14−17,48]

4.2. Influence of Prey Type on Behavior and MP Ingestion in the Mixed Behavior Feeder.
Algae species influenced the feeding behavior of *A. tonsa*. The time fraction allocated to swim, which relates to active feeding, were significantly higher in treatments with *H. steinii* than in other treatments (Figure 3A). In contrast, the time fraction allocated to sink, which relates to ambush feeding, were low in that treatments (Figure 3A). Correlating to the results from incubation experiments, the ingestion rates and clearance rates of *H. steinii* were significantly higher than the rates of *R. salina* as well (Figure 4). In addition, all the other observed behaviors (Figures 3C−F, Supporting Information S2 and S3) also indicated that when large-sized *H. steinii* existed, *A. tonsa* was livelier and more like an active feeder. In this study, *A. tonsa* never presented as a strict ambush feeder that almost sink all of the time, which was observed by Kiørboe (1996)[26] when *A. tonsa* was offered a pure ciliate suspension as food. It is because neither *H. steinii* nor *R. salina* used in this study is as large or motile as the ciliate used in that study. The active feeding behavior of *A. tonsa* in this study is more practical in nature.

The ingestion of MPs, however, was not correlated with the ingestion of algae. PE MP beads were not ingested by the mixed behavior feeder *A. tonsa* when they were offered alone, without prey. Even in combination with prey, a significantly low ingestion of MPs was observed independently of the increasing ingestion of algae. This indicates that chemical and hydromechanical cues of algae induce algal ingestion by *A. tonsa*, but the ingestion of MPs occurs accidently in the presence of prey. Therefore, our second hypothesis (presence of large motile prey induces ambushing, which, in turn, will reduce MP ingestion) should be rejected. The mechanisms behind the low ingestion of MPs are the same as described above for the different behaviors of copepods: low detection when ambushing and postcapture chemical discrimination when behaving as a feeding-current feeder.

4.3. Global Distribution of Planktonic Copepod Feeding Behaviors and the Risk of Entrance of MPs in Marine Food Webs.
The three main feeding behaviors of planktonic copepods are present throughout the world's oceans, with specific latitudinal distributions. Using the currently available data, we estimated the spatial distribution of the three main behaviors in order to identify areas with a high risk of entrance of MPs into marine food webs. However, given the low ingestion of MPs we found for all three analyzed feeding behaviors, we could not define such areas.

In this study, feeding-current feeders were found to be the most abundant planktonic copepods. However, in the observational database used, the abundance of ambush feeders is likely underestimated due to the lack of data for the size fraction smaller than 200 μm.[37] The availability of more accurate field data would allow us to improve the estimates of observed trait distribution, resulting in an improved accuracy of the spatial distributions. Furthermore, including other abundant and relevant copepods taxa in a similar study, such as *cyclopoida Oncaea* and *harpacticoid Microsetella*, would enrich the knowledge about the role of copepods in the entry of MPs in marine food webs.

4.4. Ecological Implications.
Copepods are a key link between primary producers and higher trophic levels in marine food webs. Based on this study and Xu *et al.* (2022) results, the

risk of MP ingestion in copepods is low due to their feeding behavior and postcapture discrimination of MPs.[44] Therefore, significant biotransfer of MPs via copepods appears unlikely, particularly if we consider the concentration of MPs commonly found in marine surface waters.[2,49−51] The low MP ingestion of the studied particles by copepods also implies a low vertical exportation of MPs via fecal pellets and minor consequences on the biological carbon pump.

Since we did not find differences in MP ingestion among foraging behaviors, we cannot pinpoint any marine biogeographic areas with a higher risk of MP ingestion by planktonic copepods based on their behavior. Thus, the risk of MP ingestion in planktonic copepods globally is expected to be low. Our study includes the main behaviors of suspension feeding copepods, which dominate zooplankton communities. However, we know little about the risk of MP ingestion by aggregate colonizing copepods (e.g., *Microsetella* and *Oncaea*), which feed on marine snow. MPs (e.g., fibers and plastic fragments) are known to aggregate and concentrate in marine snow.[52] Therefore, copepods with this understudied behavior can potentially be at a higher risk in terms of MP ingestion.

Marine vertebrates have a higher risk of MP ingestion than planktonic copepods. In contrast to planktonic copepods, where there is little evidence of MP ingestion in the field, ingestion of MPs has been frequently observed in marine mammals, sea birds, marine turtles, and fishes.[6,53] The risk seems higher for visual predators, which can mistake MPs for food items, particularly if they have the same color.[54−56] Therefore, there is a risk of entry of MPs in the marine food webs, but planktonic copepods are not expected to be a major entry route. A display of expected results with other MP types and concentrations were presented along this discussion. However, future experiments using different conditions, such as the presence of leachates or detailed evaluation of fibers, would provide valuable knowledge to this understudied topic. Overall, our results and discussion indicate a low global risk of MP entry in marine food webs via planktonic copepods— especially when considering the current concentration of MPs found in surface waters combined with the behavioral responses of copepods to MPs.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c04660.

> Map of global copepod's behavior distribution in biomass; duration of behavioral responses for *A. tonsa*; velocity of behavioral responses for *A. tonsa*; ingestion and clearance rates for all studied copepods; statistical output from the general analysis of each behavior in the *A. tonsa in* video observations; and statistical output from the analysis of the interactions among treatments for *A. tonsa* in video observations (PDF)

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