

1 **The behavior of planktonic copepods minimizes the entry of microplastics in marine**  
2 **food webs**

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27 ABSTRACT

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

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44 *Key words:* microplastics, copepods, trait-based approach, feeding behavior, ingestion.

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46 *Synopsis:* We applied a trait-based approach to investigate the relevance of copepod feeding  
47 behavior in the entry of MPs in marine food webs.

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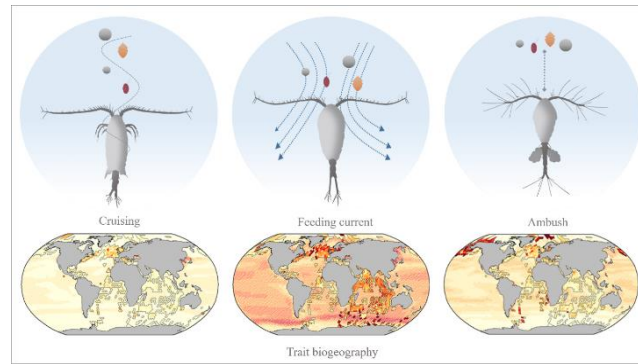
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55 GRAPHICAL ABSTRACT



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73 1. INTRODUCTION

74 Plastic litter is ubiquitous in aquatic ecosystems, with current amounts projected to triple by 2040  
75 <sup>1</sup>. As a result, the consequences of plastic pollution in marine ecosystems are of global concern.  
76 Part of the plastic litter consists of microscopic plastic particles, microplastics (MPs) with the size  
77 fraction < 100  $\mu\text{m}$  being the most abundant in marine waters <sup>2,3,4</sup>. MPs <100  $\mu\text{m}$  are similar in size  
78 to phytoplankton, which makes them potentially available for ingestion by zooplankton <sup>5-7</sup>. Given  
79 the key trophic position of zooplankton in marine food webs <sup>8,9</sup>, knowledge of the risk of  
80 microplastic ingestion by zooplankton is essential to assess the overall fate of MPs in marine  
81 ecosystems.

82 Planktonic copepods are the dominant zooplankton group and the most abundant animals in the  
83 ocean<sup>10</sup>. Copepods are both grazers and prey; therefore, they may influence the entry and  
84 biotransfer of MPs in marine food webs <sup>11</sup>. Additionally, copepods can accelerate vertical  
85 exportation of MPs via production of fast sinking fecal pellets. Microplastic ingestion by  
86 planktonic copepods has been observed in laboratory experiments using high concentrations of  
87 MPs <sup>5-7,12,13</sup>. However, field studies show a low occurrence of MP ingestion in natural zooplankton  
88 communities <sup>14-17</sup>. Desforges et al. (2015), found 0.026 particles per copepod in the Northeast  
89 Pacific Ocean; Sun et al. (2018) found 0.13 pieces per individual in copepod species in the East  
90 China Sea. These findings contradict the predicted high risk of MP ingestion based on laboratory  
91 studies.

92 A trait-based approach proposes to represent the thousands of planktonic copepod species with  
93 few well-chosen model species that reflect the existing diversity in the most important traits. In  
94 the context of zooplankton ecology, it is well documented that foraging behavior is a key trait due  
95 to its strong influence on feeding rates and predation risk <sup>18-21</sup>. Additionally, functional traits, such  
96 as feeding behavior in marine copepods, vary across the global ocean as a function of the  
97 environment <sup>22</sup>. A trait-based approach, using feeding behavior as key trait, is therefore a useful  
98 tool to evaluate the risk of ingestion and biotransfer of MPs via copepods on a global scale.

99 Suspension-feeding planktonic copepods have three main types of feeding behaviors: (1) “feeding-  
100 current feeding”, where they create a current to harvest prey, (2) “cruising feeding”, where they  
101 encounter prey while swimming through the water, and (3) “ambush feeding”, where they wait  
102 motionless for motile prey to enter their sensory reach, or otherwise capture those prey that directly

103 collide with them <sup>18,23,24</sup>. The former two feeding behaviors are categorized as “active feeders”.  
104 Some copepods can be “mixed behavior feeders”, and are able to switch between behaviors  
105 depending on prey type and food availability <sup>23,25,26</sup>. For example, *Acartia tonsa* can either generate  
106 a feeding current to harvest small or non-motile prey or behave as an ambush feeder to capture  
107 large motile prey <sup>26</sup>. Despite the importance of behavior in evaluating the risk of MP ingestion by  
108 zooplankton, behavior is still an understudied variable in MP pollution <sup>27</sup>. For instance, it is  
109 unknown whether *Oithona* (ambush feeding copepods) ingest MPs, which is an abundant and  
110 widely distributed genus across the oceans <sup>28</sup>. Therefore, research about the influence of  
111 zooplankton behavior on MP ingestion is needed to better assess the risk of entrance and transfer  
112 of MPs in marine food webs.

113 The overall aim of this study is to evaluate how the behavior of planktonic copepods influences  
114 the risk of MPs to enter marine planktonic food webs, using bottle incubations and video  
115 observations. We used a trait-based approach and planktonic copepods as model organisms. Our  
116 specific objectives and hypotheses are: (1) to estimate the risk of MP ingestion associated with the  
117 three main feeding behaviors of suspension-feeding copepods (feeding-current feeding, cruising  
118 feeding and ambush feeding) and hypothesize that ambush feeding is the least risky behavior in  
119 terms of MP ingestion since ambush feeders are inefficient in detecting non-motile prey <sup>20</sup>; (2) to  
120 investigate the influence of prey switching behavior on MP ingestion in a mixed behavior feeder.  
121 We hypothesize that presence of a large motile prey will induce ambush feeding, which in turn,  
122 will reduce the ingestion of MPs in a mixed behavior feeder; (3) to evaluate the global risk of  
123 ingestion of MPs in copepods by intersecting the outcome from the experiments with estimates of  
124 the global distribution of the copepods in three feeding behaviors. We hypothesize that areas  
125 dominated by active feeders are more susceptible to the entry of MPs into food webs than areas  
126 dominated by ambush feeders.

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## 128 2. MATERIAL AND METHODS

### 129 2.1. *Experimental organisms*

130 The trait of relevance in this study is foraging behavior, and thus the experimental organisms were  
131 selected in order to compare among contrasting foraging behaviors. The following species of

132 copepods were used as models of the different foraging behaviors: *Temora longicornis* (feeding-  
133 current feeder); *Centropages hamatus* (cruising feeder), *Oithona davisae*, (ambush feeder) and  
134 *Acartia tonsa* (mixed behavior feeder). To avoid body size effects on the MP ingestion, we ensured  
135 similar body size of the experimental organisms. To this end, we used copepodites I and II of *C.*  
136 *hamatus* and *T. longicornis* and female adults of *O. davisae*, which all have a prosome length of  
137 roughly 400  $\mu\text{m}$ . To investigate the prey switching behavior, we used female adults of *A. tonsa*. In  
138 all the experiments, specimens were selected from the main cultures the day before the experiment  
139 and kept isolated overnight in glass beakers without food to avoid effects from their previous diet.

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

149 The phytoplankton prey used in this study were *H. steinii* and *R. salina*. We used these two species,  
150 with average sizes of 17  $\mu\text{m}$  and 7  $\mu\text{m}$ , respectively, as they fit the size range of MP beads used in  
151 this experiment (20  $\mu\text{m}$  and 8  $\mu\text{m}$ ). Both species were grown in sterilized filtered seawater (FSW)  
152 with a salinity of 30‰ with B1 medium<sup>29</sup> at 16°C in a 12:12 dark:light cycle with a radiation of  
153 150  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ .

## 154 2.2. Microplastics

155 Polyethylene is the polymer type that contributes most to pollution in aquatic environments<sup>30-32</sup>.  
156 In this study, transparent polyethylene (PE) spherical beads with a density of 0.96  $\text{g cm}^{-3}$  were  
157 used as model MPs. The spherical beads were supplied as a dry powder (Cospheric®). To prepare  
158 the MP suspensions, 1 mg of the powder was suspended in 250 mL glass bottles containing  
159 distilled water with 0.01 % Tween 80 (Cospheric®). We mixed the suspensions through vigorous  
160 hand shaking until the particles appeared dispersed and any clusters were broken into individual  
161 beads, as verified through microscopy. The size of both algae and MPs was measured using a

162 Beckman Multisizer III Coulter Counter. Two size ranges of PE particles were used and both  
163 follow a normal distribution: the larger particles (Cospheric ®) had a size range of 13.9–30.3 µm,  
164 with a mean equivalent spherical diameter (ESD) of 20.7 µm, and the smaller particles (Cospheric  
165 ®) had a size range of 5–16 µm, with a mean ESD of 7.9 µm. From the stock suspensions, we  
166 prepared the following diluted working suspensions for each MP size: 20 µm MPs = 52080 MPs  
167 mL<sup>-1</sup>, 8 µm MPs = 57400 MPs mL<sup>-1</sup>. From the working suspensions a specific volume is taken to  
168 prepare the nominal concentrations (200 or 400 MPs mL<sup>-1</sup>) in the experimental bottles. The  
169 absence of MP aggregates, and the concentration of MPs in the working suspensions, was verified  
170 by manual counting under an inverted microscope using Sedgewick-Rafter counting chambers.  
171 Microplastic suspensions are kept in dark in the fridge at 4°C.

### 172 2.3. General experimental set-up

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

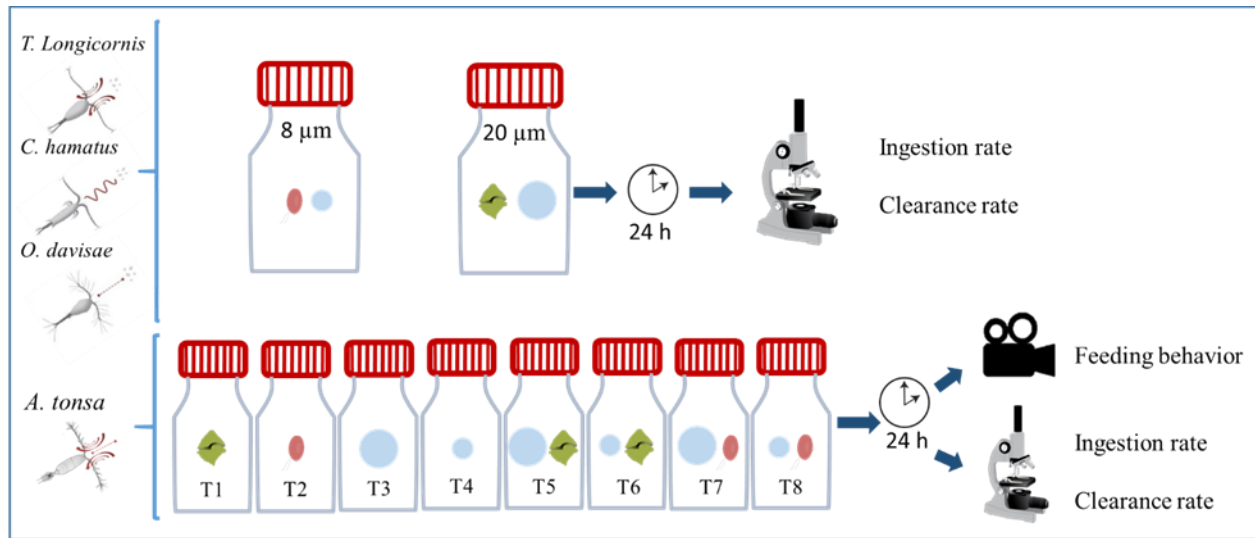
178 To assess the influence of prey switching behavior on MP ingestion in a mixed behavior feeder  
179 (objective 2), we exposed *A. tonsa* to microalgae alone (T1 and T2), MPs alone (T3 and T4), and  
180 MPs in combination with microalgae with different sizes and motility: large MPs and large prey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

### 186 2.4. Bottle incubation experiments and sample analysis

187 The bottle incubations were carried out in 34 mL Pyrex bottles sealed with a lid protected with  
188 polytetrafluoroethylene (PTFE). The bottles were half filled with 0.2 µm FSW. Aliquots of the MP  
189 working suspensions were added to obtain the desired test concentrations. Afterwards, the  
190 corresponding algae were added. The algae stocks were previously counted under the microscope

191 using a Sedgewick-Rafter counting chamber in order to calculate the stock suspension volume  
192 needed to obtain the desired concentration in the incubation bottles. The nominal concentration of  
193 algae and MPs in the incubations where both types of particles were offered together was 200 MPs  
194 mL<sup>-1</sup> and 200 cells mL<sup>-1</sup>. In the prey switching behavior experiment, where *A. tonsa* was also  
195 exposed to uniform prey (T1-T4), the nominal concentration was 400 MPs or cells mL<sup>-1</sup> to keep  
196 the same amount of total prey (particles and cells) in all treatments. For each treatment, we  
197 prepared triplicates of “Initial bottles” where we determined the concentration of algae and MPs  
198 at time=0, triplicates of “Control bottles” that were incubated with no grazers to follow changes in  
199 prey/MP concentrations during the 24 hours, and triplicates of “Experimental bottles” that were  
200 incubated with the copepods to estimate the ingestion rates after 24 hours. Due to the different  
201 clearance rates among species and between prey/MP types, the number of copepods per bottle was  
202 adjusted, ensuring a recommended decrease in prey concentration of 30%<sup>20</sup>: 12 and 18 ind. bottle<sup>-1</sup>  
203 when *O. davisae* was exposed to 20 µm prey and 8 µm prey respectively, 8 ind. bottle<sup>-1</sup> for *A.*  
204 *tonsa*, and 4 ind. bottle<sup>-1</sup> for *C. hamatus* and *T. longicornis*. Lastly, after adding the copepods to  
205 the bottles, we filled the bottles with the test particle suspensions and closed them. The bottles  
206 were wrapped in aluminum foil to keep them dark and then mounted on a plankton wheel rotating  
207 at 1 rpm in a temperature-controlled room at 16°C for approximately 24 hours. After the  
208 incubation, copepod mortality was checked with a microscope. Mortality was not observed in any  
209 of the experiments. A 25 mL subsample was taken from each bottle, fixed with 1% Lugol’s  
210 solution and stored at 4°C for later analysis. The concentration of MPs and algae in the tested  
211 suspensions were counted under an inverted microscope (x20 magnification) using Sedgewick-  
212 Rafter chambers. We calculated ingestion (I) and clearance rates (F) on MPs and algae according  
213 to Frost (1972)<sup>33</sup>.





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215 Figure 1. Schematic overview of the experimental studies. The upper bottles represent the bottle incubation  
 216 experiments of the three main feeding behaviors when exposed to *R. salina* (red alga) and MPs of 8  $\mu\text{m}$  or  
 217 *H. steinii* (green algae) and MPs of 20  $\mu\text{m}$ . The lower row indicates the incubation and video filming  
 218 treatments used in the experiment with a mixed behavior feeder, *A. tonsa*, exposed to different combinations  
 219 of microalgae and MP sizes.

### 220 2.5. Video observations set-up and behavior analysis

221 The video observations were conducted in a temperature-controlled room at 16°C. The different  
 222 combinations of algae and MPs (Fig. 1) were prepared in 50 mL cell culture flasks with eight  
 223 female adults of *A. tonsa* per flask. The flask was placed between an infrared light and a high-  
 224 speed camera (Phantom V210). The camera was set at a frame rate of 100 fps, a resolution of  
 225 800×600 pixels, and a field of view of 40×30 mm<sup>2</sup>. Each flask was recorded for three hours and  
 226 one 60-second video was saved every hour. The flask was mixed every half an hour to minimize  
 227 particle settlement. Each treatment was conducted in triplicates. The filming was done in a closed  
 228 room in darkness to avoid any light and temperature change or noise that could affect the behavior  
 229 of the copepods.

230 Copepods trajectories were extracted from the resulting videos using a tracking plugin in ImageJ  
 231 (version 1.52n) and behaviors were further analyzed by running an R-script (version 1.3.959). The  
 232 parameters in the script were set based on previous manual frame-by-frame analysis to match the  
 233 threshold of each type of behavior<sup>24</sup>. Between 61 and 119 tracks were analyzed per treatment and  
 234 four different types of behaviors were categorized: swim, sink, jump and hop. “Swim” is the

235 movement made by copepods when actively swimming; “sink” refers to passive sinking due to  
236 negative buoyancy in the absence of active movements; “jump” are active, big and rapid  
237 movements usually to capture prey, and “hop” are active, short movements for the copepod to  
238 relocate back to its original position. The hop-like motion was distinguished from jump by its  
239 slower velocity. Hop is considered a movement that enables copepods to adjust their body  
240 orientation or switch their position in a short range rather than to escape from threats or quickly  
241 scan the water column as is achieved with jumps<sup>34-36</sup>. The time budget (fraction of time of swim,  
242 sink, jump or hop), the duration of each motion bout, the frequency and the velocity of these  
243 motions were calculated for all treatments.

#### 244 2. 6. *Statistical analyses of experimental data*

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

#### 252 2.7. *Spatial modelling of the dominance of the three main feeding behaviors.*

253 We estimated the weight fractions of feeding-current feeders, cruising feeders, and ambush feeders  
254 from global compilations of abundance and body length data, using data-based estimates where  
255 available and model-based interpolations elsewhere. The data was prepared following the  
256 approach described in Brun et al. (2016)<sup>22</sup>. We first discretized the global ocean into roughly 5000  
257 polygons of similar area. Then, we intersected these polygons with abundance observations from  
258 the Coastal and Oceanic Plankton Ecology, Production and Observation Database (COPEPOD)<sup>37</sup>.  
259 After preprocessing and filtering of the raw data (see Brun et al. 2016)<sup>22</sup>, we estimated abundance  
260 and weight fractions of each taxon in each polygon, multiplying abundance fractions with cubed  
261 body length to obtain weight fractions. Body-length data originated from Brun et al. (2017)<sup>38</sup>.  
262 Then, we identified which taxa in a polygon belonged to each feeding behavior by considering the  
263 world’s most abundant representative genera as a reference. Feeding-current feeders were

264 represented by: *Acartia*, *Calanus*, *Calocalanus*, *Euchaeta*, *Nannocalanus*, *Paracalanus*,  
265 *Pareucalanus*, *Parvocalanus*, *Pleuromamma*, *Pseudocalanus*, *Rhincalanus*, and *Temora*. Cruising  
266 feeders were represented by *Centropages*, *Clausocalanus*, and *Metridia*, and ambush feeders were  
267 represented by *Acartia* and *Oithona*. Note that, due to its mixed feeding strategy, the genus *Acartia*  
268 was assigned to both feeding-current and ambush feeders. Finally, we calculated both abundance  
269 fraction and weight fraction of each feeding behavior relative to all remaining planktonic copepods  
270 in each polygon. In total, abundance estimates were available for 584 copepod taxa (species and  
271 genera), and 564 of them could be matched with body length information. Feeding-current feeders  
272 were represented with 103 taxa, cruising feeders with 42 taxa, and ambush feeders with 32 taxa.

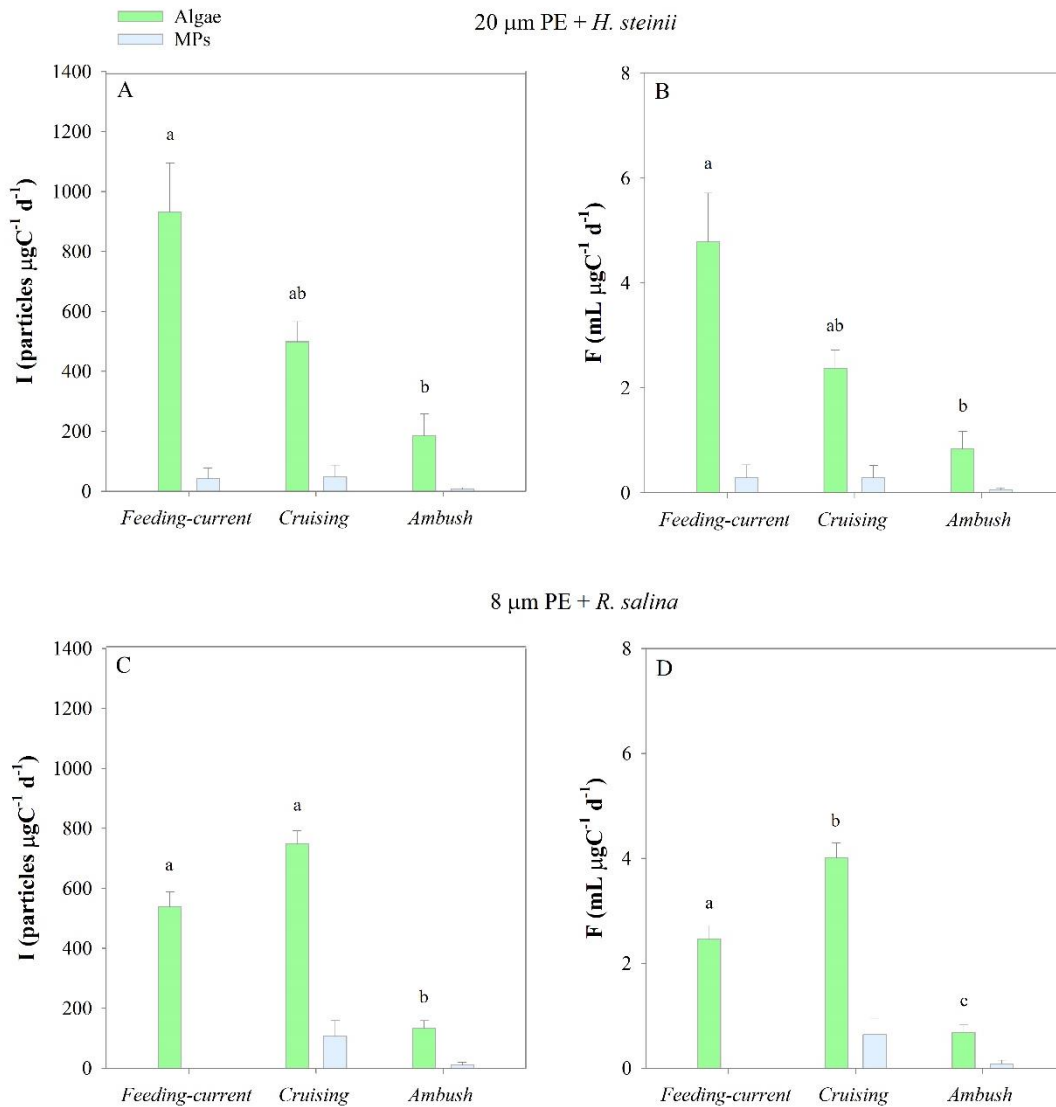
273 We used statistical modelling to interpolate observation-based estimates to regions of the global  
274 ocean that lacked observations. We fitted six generalized additive models <sup>39</sup>, one for each  
275 combination of abundance/weight fraction and feeding behavior. As predictors we considered  
276 smooth terms of polygon-wise averages of annual mean and annual range of monthly sea surface  
277 temperature (derived from the HadISST1 product) <sup>40</sup>, as well as average chlorophyll *a*  
278 concentration (derived from <http://www.globcolour.info/>). We used the R package *mgcv* <sup>41</sup> to fit  
279 generalized additive models and assumed errors to follow a beta distribution.

280

## 281 3. RESULTS

### 282 3.1. *Ingestion of MPs in relation to feeding behavior*

283 The incubation experiments, that compare the three main feeding behaviors, showed that ingestion  
284 (I, particles cop.<sup>-1</sup> d<sup>-1</sup>) and clearance rates (F, mL cop.<sup>-1</sup> d<sup>-1</sup>) of algae were at least one order of  
285 magnitude higher than of MPs in all treatments, independently of the particle size (Fig. 2). Active  
286 feeders (cruising and feeding-current feeders) had higher ingestion and clearance rates of algae  
287 than ambush feeders. However, there was no difference among the three species regarding the  
288 ingestion of MPs. All showed low ingestion and clearance rates of PE MP microsphehres (on  
289 average, 8 µm: 32.4 MPs cop.<sup>-1</sup> d<sup>-1</sup> and 20 µm: 39.2 MPs cop.<sup>-1</sup> d<sup>-1</sup>) compared to similar sized  
290 microalgae (S.I. Table 1).



291

292 Figure 2. Ingestion (I, particles  $\mu\text{gC}^{-1} \text{d}^{-1}$ ) and clearance rates (F, mL  $\mu\text{gC}^{-1} \text{d}^{-1}$ ) of algae (green bars) and  
 293 MPs (blue bars) for the three main feeding behaviors of copepods. Top panels (A, B) show the data for MPs  
 294 and algae of 20  $\mu\text{m}$  in diameter and the bottom panels (C, D) show the data for 8  $\mu\text{m}$  particles and algae.  
 295 Data is presented as means of triplicates with the standard error. Asterisks (\*) represent a statistically  
 296 significant difference between algae and MP ingestion or clearance rate within each behavior. Letters  
 297 indicate significance differences among behaviors.

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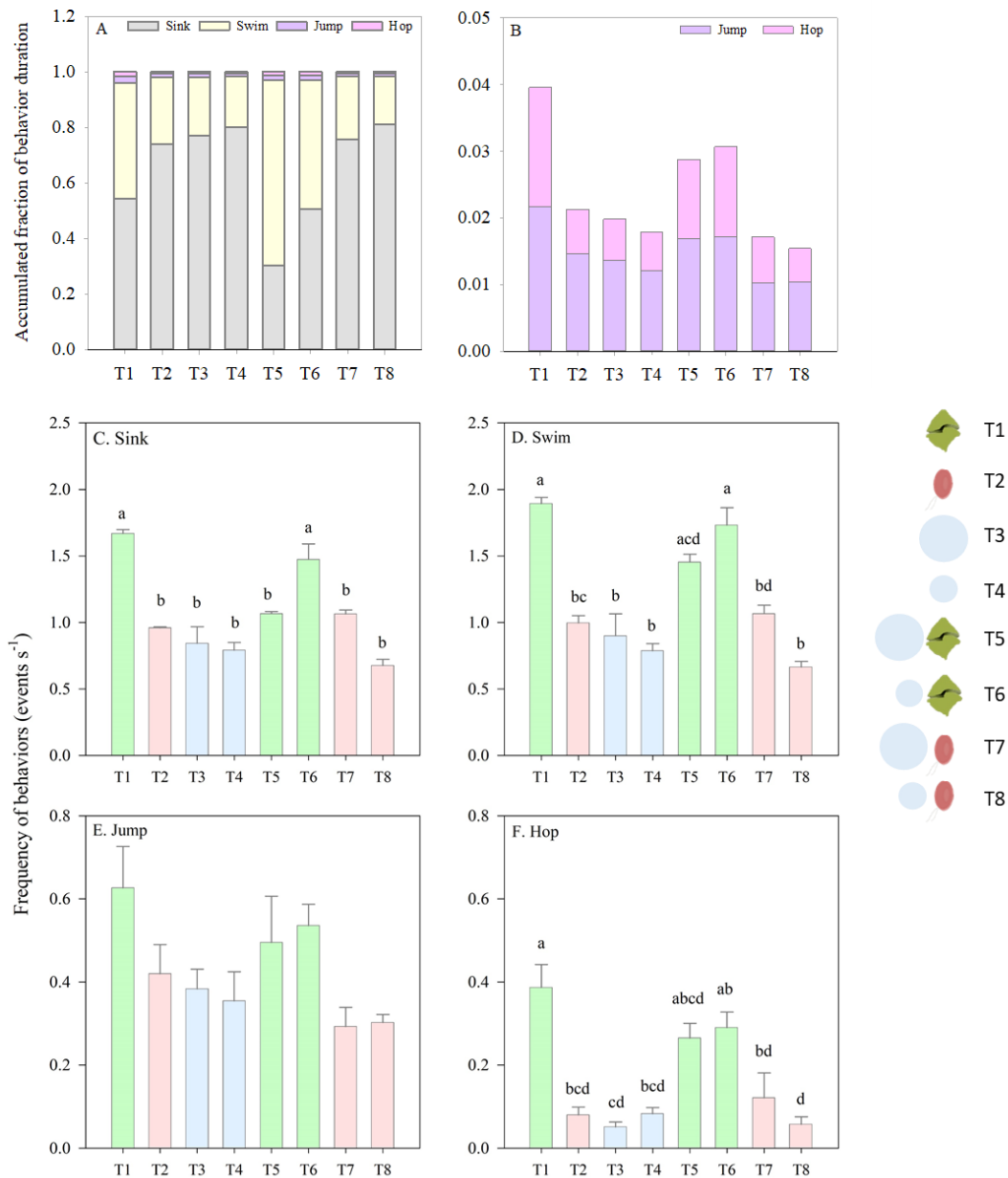
301 3.2. Influence of prey type on behavior and MP ingestion in a mixed behavior feeder

302 Behavioral responses from video observations

303 The accumulated fraction (0-1) of the duration of the four swimming behavior responses clearly  
304 reflects the dominance of sinking or swimming in all treatments (Fig. 3 A and B). The fraction of  
305 swimming was the highest in treatments 1, 5 and 6. Those are the treatments with *H. steinii* and  
306 correspond to the treatments with the highest ingestion and clearance rates (Fig. 4). The  
307 accumulated duration of the jumping was equally distributed in all treatments without significant  
308 differences.

309 Sink and swim were the most frequent movements (Fig. 3 C-F). There were no significant  
310 differences among treatments for jump frequency. However, for sink, swim and hop we found  
311 significant differences between several treatments (S.I. Table 2). The frequency of sink, swim and  
312 hop were always higher for the treatments with *H. steinii*. Sink, swim and hop frequencies never  
313 differed significantly in treatments with *R. salina* or MPs alone.

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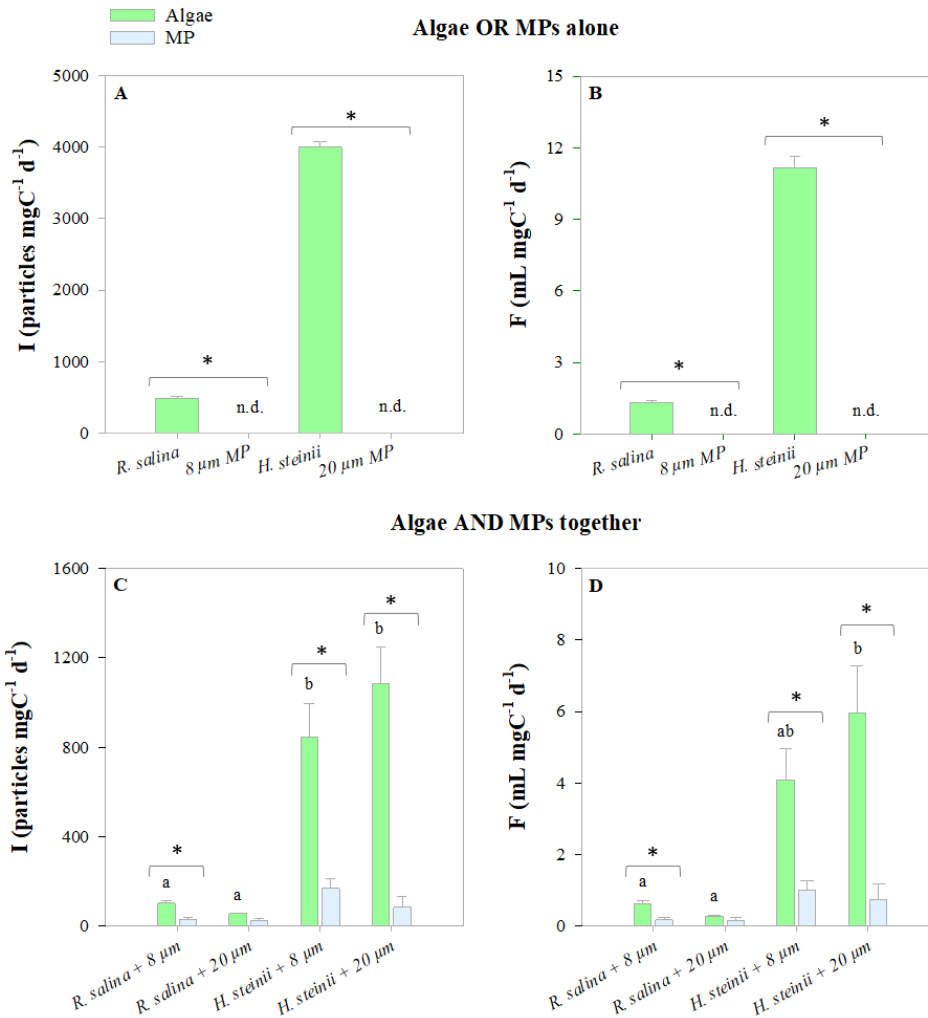
316 Figure 3. Accumulated fraction (A-B) of the durations of the four behaviors (sink, swim, jump and hop)  
 317 and frequency (events s<sup>-1</sup>) for sink (C), swim (D), jump (E) and hop (F) in the eight different treatments  
 318 (T1-T8) for the switching behavior copepod *A. tonsa*. Chart A shows the accumulation of the four behaviors  
 319 and chart B is a magnification of hop and jump data for a clearer observation. Green bars are for the  
 320 treatments containing *H. steinii*, red for treatments with *R. salina* and blue is for treatments with only MPs.  
 321 Error bars represent the standard error. Letters on top of bars indicate significant differences among  
 322 treatments based on pair-wise comparisons, post- hoc analysis.

323 Regarding the duration of the sinking bouts, they were shorter in the treatments with *H. steinii* (S.I.  
324 Fig. 2A). For swimming bouts, only treatment 5 showed statistically significant differences with  
325 T1, T3, T4 and T7 (S.I. Fig. 2B). Jumps were by far the fastest movement (S.I. Fig. 3), as expected.  
326 But the duration of the individual bouts of jumps and hops did not vary significantly among  
327 treatments (S.I. Fig. 2C and D).

328 *Ingestion and clearance rates from bottle incubation: A. tonsa*

329 *A. tonsa* ingested both algae species when offered alone, albeit eight times more of *H. steinii* than  
330 *R. salina* (Fig. 4A and B). In contrast, the ingestion and clearance rates of both MP sizes were zero  
331 when exposed to MPs alone (Fig. 4A and B). When *A. tonsa* was simultaneously exposed to MPs  
332 and algae, the copepods ingested both types of particles (Fig. 4C and D). However, the ingestion  
333 and clearance rates of MPs were 2-10 times lower than of algae (Fig. 4 C and D).

334 Ingestion and clearance rates of *R. salina* were also significantly lower than of *H. steinii* when  
335 offered together with MPs (Fig. 4C and D). The ingestion (0-274 particles  $\mu\text{gC}^{-1} \text{d}^{-1}$ ) and clearance  
336 (0-1.8 mL  $\mu\text{gC}^{-1} \text{d}^{-1}$ ) rates of MPs were generally low and did not differ significantly between  
337 treatments with different MP and algae combinations (Fig. 4C and D).



338

339 Figure 4. Ingestion (I, particles  $\mu\text{gC}^{-1} \text{d}^{-1}$ ) and clearance rates (F,  $\text{mL } \mu\text{gC}^{-1} \text{d}^{-1}$ ) of *A. tonsa* when they  
 340 were fed with single particle (A and B) or two particles simultaneously (C and D): algae (*R. salina* or *H.*  
 341 *steinii*) in green and MPs (8  $\mu\text{m}$  or 20  $\mu\text{m}$  PE beads) in blue. Data is presented as means of triplicates with  
 342 the standard error. Letters indicate significant difference of algae ingestion or clearance rates between  
 343 treatments. Asterisks (\*) represent a statistically significant difference between algae and MP ingestion or  
 344 clearance rate within each treatment.

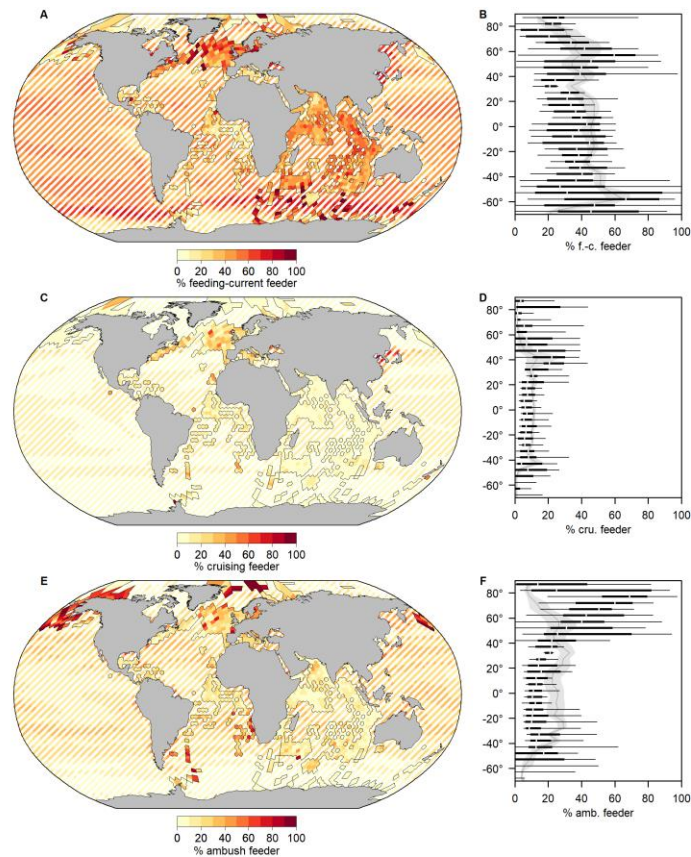
### 345 3.3 Global distribution of copepods in the three main feeding behaviors

346 The three feeding behaviors (feeding-current, cruising and ambush feeders) are present all around  
 347 the globe. However, feeding-current feeders are the most abundant (Fig. 5), followed by ambush  
 348 feeders. Feeding-current feeders are particularly abundant above 40° latitude in both hemispheres  
 349 (Fig. 5A and B). Cruising feeders show highest relative abundances in temperate latitudes but



350 stable fractions of around 10% also occur in subtropical and tropical areas. Above 60° latitude,  
 351 cruising feeders become rare (Fig. 5C, D). Model-based estimates of the relative abundance of  
 352 ambush feeders peak between 60°N and 30°S at around 25% of individuals and declines towards  
 353 higher latitudes. However, in the northern hemisphere higher relative abundances of ambush  
 354 feeders are repeatedly observed at higher latitudes (Fig. 5E, F). When weight fractions instead of  
 355 abundance fractions are compared, the patterns remain similar but the fractions of feeding-current  
 356 feeders generally increase, while the fractions of ambush feeders decrease (S.I. Fig. 1).

357



358

359 Figure 5. Global (left) and latitudinal (right) distribution of the abundance fraction of each feeding behavior:  
 360 feeding-current feeders (A, B); cruising feeders (C, D) and ambush feeders (E, F). Dashed areas in maps  
 361 represent model extrapolations and solid colors represent observation-based estimates. Latitudinal boxplots  
 362 are observation-based estimates, where central lines represent medians, boxes represent interquartile ranges  
 363 and whiskers represent 95% confidence intervals. Underlying polygons represent interquartile ranges of  
 364 model-based estimates and underlying grey line represents median of model-based estimates. The map has  
 365 some limitations due to the lack of available field data for copepods smaller than 200  $\mu\text{m}$ .

366 4. DISCUSSION

367 *Influence of the feeding behavior on MP ingestion*

368 Our first hypothesis (ambush feeding is the least risky behavior in terms of MP ingestion) was not  
369 verified since all three behaviors showed a similarly low ingestion of MPs compared to natural  
370 prey (algae). As expected, ingestion rates of PE MPs beads were very low in strict ambush feeders,  
371 which rely on hydromechanical signals to detect their prey<sup>42</sup> and have low clearance rates on non-  
372 motile prey<sup>43, 20</sup>. The non-motile nature of MPs makes them non-detectable to ambush feeders.  
373 They can however still perceive MPs if they directly intercept/collide with the feeding/sensorial  
374 structures of the copepod, as observed for other non-motile particles<sup>42, 20</sup>. This mechanism may  
375 account for the non-zero ingestion of MPs by ambush feeders observed in our experiments.

376 For feeding-current feeders, where both motile and non-motile prey enter their feeding current, the  
377 ingestion of MPs was lower than expected. In this case, a post-capture discrimination of MPs can  
378 explain the low ingestion of MPs, as recently demonstrated for different MP types by Xu et al.  
379 (2022)<sup>44</sup>. The feeding currents, created by copepods, act as “scanning currents”, where prey is  
380 drawn into and, subsequently, perceived and handled individually<sup>18</sup>. The prey can be perceived  
381 only when it is touched, or nearly touched, by the setae on the feeding appendages<sup>45</sup>. Chemical  
382 discrimination occurs after tasting as observed for toxic algae<sup>46</sup> and MPs<sup>44</sup>. Therefore, an efficient  
383 active rejection of MPs by tasting (chemical discrimination) can explain the low ingestion of MPs  
384 in feeding-current feeders observed here. Taste-discrimination in the feeding-current feeder *T.*  
385 *longicornis* occurs even when MPs are covered with biofilms, suggesting that it is the synthetic  
386 plastic polymer ‘core’ making the MP unpalatable to these planktonic copepods<sup>44</sup>. Fibers are not  
387 used in this study. However, due to the non-motile nature of these particles and the observed ability  
388 of copepods to discriminate MPs<sup>41</sup>, low ingestion of fibers is expected for the studied copepods.  
389 At low rates, feeding-current feeders accidentally ingested some MPs (Fig 2A and B), which can  
390 be due to 1) a relatively high concentration of MPs used in these experiments and 2) an  
391 optimization of food intake by reducing handling time.

392 Strict cruising feeding is a less common behavior in zooplankton that is advantageous when  
393 passive interception is inefficient in the aquatic environment. Cruising feeders detect prey remotely  
394 depending on hydromechanical and chemical cues<sup>47</sup>. Since virgin MPs do not generate any of  
395 these cues, the detection and capture of MPs is expected to be low, which could explain the low

396 ingestion of MPs observed in the studied cruising feeder. Our results agree with findings from field  
397 studies, which indicate a low occurrence of MP ingestion in natural zooplankton communities<sup>14-</sup>  
398 <sup>17, 48</sup>.

#### 399 *Influence of prey type on behavior and MP ingestion in the mixed behavior feeder*

400 Algae species influenced the feeding behavior of *A. tonsa*. The time fraction allocated to swim,  
401 which relates to active feeding, were significantly higher in treatments with *H. steinii* than in other  
402 treatments (Fig 3A). In contrast, the time fraction allocated to sink, which relates to ambush  
403 feeding, were low in that treatments (Fig 3A). Correlating to the results from incubation  
404 experiments, the ingestion rates and clearance rates of *H. steinii* were significantly higher than the  
405 rates of *R. salina* as well (Fig. 4). In addition, all the other observed behaviors (Fig. 3C-F, S.I. Fig.  
406 2 and 3) also indicated that when large sized *H. steinii* existed, the *A. tonsa* was livelier and more  
407 like an active feeder. In this study, *A. tonsa* never presented as a strict ambush feeder that almost  
408 sink all of the time, which was observed by Kiørboe (1996)<sup>26</sup> when *A. tonsa* was offered a pure  
409 ciliate suspension as food. It because neither *H. steinii* nor *R. salina* used in this study is as large  
410 or motile as the ciliate used in that study. The active feeding behavior of *A. tonsa* in this study is  
411 more practical in nature.

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

#### 422 *Global distribution of planktonic copepod feeding behaviors and the risk of entrance of MPs in* 423 *marine food webs*

424 The three main feeding behaviors of planktonic copepods are present throughout the world's  
425 oceans, with specific latitudinal distributions. Using the currently available data, we estimated the  
426 spatial distribution of the three main behaviors in order to identify areas with high risk of entrance  
427 of MPs into marine food webs. However, given the low ingestion of MPs we found for all three  
428 analyzed feeding behaviors, we could not define such areas.

429 In this study, feeding-current feeders were found to be the most abundant planktonic copepods.  
430 However, in the observational database used the abundance of ambush feeders is likely  
431 underestimated due to the lack of data for the size fraction smaller than 200  $\mu\text{m}$ <sup>37</sup>. Availability of  
432 more accurate field data would allow us to improve the estimates of observed trait distribution,  
433 resulting in an improved accuracy of the spatial distributions. Furthermore, including other  
434 abundant and relevant copepods taxa in a similar study, such as cyclopoida *Oncaea* and  
435 harpacticoid *Microsetella*, would enrich the knowledge about the role of copepods in the entry of  
436 MPs in marine food webs.

#### 437 *Ecological implications*

438 Copepods are a key link between primary producers and higher trophic levels in marine food webs.  
439 Based on this study and Xu et al., (2022) results the risk of MP ingestion in copepods is low, due  
440 to their feeding behavior and post-capture discrimination of MPs<sup>44</sup>. Therefore, significant  
441 biotransfer of MPs via copepods appears unlikely, particularly if we consider the concentration of  
442 MPs commonly found in marine surface waters<sup>2, 49-51</sup>. The low MP ingestion of the studied  
443 particles by copepods also implies a low vertical exportation of MPs via fecal pellets and minor  
444 consequences on the biological carbon pump.

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

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

466

#### 467 **Supporting information:**

468 Map of global copepod's behavior distribution in biomass (S.I. Figure 1)

469 Duration of behavioral responses for *Acartia tonsa* (S.I. Figure 2)

470 Velocity of behavioral responses for *Acartia tonsa* (S.I. Figure 3)

471 Ingestion and clearance rates for all studied copepods (S.I. Table 1)

472 Statistical output from the general analysis of each behavior in the *A. tonsa in video* observations  
473 (S.I. Table 2)

474 Statistical output from the analysis of the interactions among treatments for *A. tonsa in video*  
475 observations (S.I. Table 3)

476

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#### 717 **CRedit authorship contribution statement**

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#### 727 **Declaration of competing interest**

728 The authors declare that they have no known competing financial interests or personal relationships  
729 that could have appeared to influence the work reported in this paper