

# 1 **Microplastic ingestion by zooplankton**

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13

## 14 **Abstract**

15 Small plastic detritus, termed ‘microplastics’, are a widespread and ubiquitous contaminant of  
16 marine ecosystems across the globe. Ingestion of microplastics by marine biota, including mussels,  
17 worms, fish and seabirds, has been widely reported, but despite their vital ecological role in marine  
18 food-webs, the impact of microplastics on zooplankton remains under-researched. Here, we show  
19 that microplastics are ingested by, and may impact upon, zooplankton. We used bio-imaging  
20 techniques to document ingestion, egestion and adherence of microplastics in a range of  
21 zooplankton common to the northeast Atlantic, and employed feeding rate studies to determine the  
22 impact of plastic detritus on algal ingestion rates in copepods. Using fluorescence and coherent anti-  
23 Stokes Raman scattering (CARS) microscopy we identified that thirteen zooplankton taxa had the

24 capacity to ingest 1.7 – 30.6  $\mu\text{m}$  polystyrene beads, with uptake varying by taxa, life-stage and bead-  
25 size. Post-ingestion, copepods egested faecal pellets laden with microplastics. We further observed  
26 microplastics adhered to the external carapace and appendages of exposed zooplankton. Exposure  
27 of the copepod *Centropages typicus* to natural assemblages of algae with and without microplastics  
28 showed that 7.3  $\mu\text{m}$  microplastics ( $>4000 \text{ ml}^{-1}$ ) significantly decreased algal feeding. Our findings  
29 imply that marine microplastic debris can negatively impact upon zooplankton function and health.

30

### 31 **Keywords**

32 Plastic debris, microplastics, zooplankton, marine pollution, algal ingestion, coherent anti-Stokes  
33 Raman scattering microscopy, CARS

34

### 35 **1. Introduction**

36 It has been estimated that up to 10% of plastics produced globally enters our oceans, so it is of little  
37 surprise that plastic debris is now a pervasive and resilient pollutant of the marine environment [1,  
38 2]. Larger plastic debris, such as monofilament line, plastic strapping and plastic bags, can entangle,  
39 garrotte, drown, or be eaten by an array of marine wildlife [3]. There is compelling evidence that  
40 microplastics – small plastic  $<5 \text{ mm}$  in diameter – also negatively impact upon marine biota [4].  
41 Microplastics consist of synthetic polymer products manufactured to be of a small size, such as  
42 exfoliates in cosmetics [5], and those items derived from the fragmentation of larger plastic debris,  
43 for example polyester fibres from fabrics [6], polyethylene fragments from plastic bags [7] and  
44 polystyrene particles from buoys and floats [8]. Typically, high-density plastics (e.g. polyvinyl  
45 chlorides, polyester) settle out of the water column, whilst low-density plastics (e.g. polyethylene,  
46 polystyrene) remain buoyant, although freshwater inputs, storms and biofilm formation may result

47 in vertical mixing [9, 10]. Floating plastic debris is susceptible to local and ocean currents resulting in  
48 higher-than-average waterborne microplastic concentrations in areas of confluence [11].

49 Microplastics are of environmental concern as their small size makes them available to a wide range  
50 of marine biota [12]. Microplastic ingestion has been demonstrated in marine organisms, including  
51 amphipods, lugworms and barnacles [4], mussels [13], decapod crustaceans [14], seabirds [15], and  
52 fish [16, 17]. Ingested microplastics might obstruct feeding appendages, aggregate and block the  
53 alimentary canal, limit the food intake of an organism or be translocated into the circulatory system  
54 [13, 14]. Further, microplastics may introduce toxicants to the organism: firstly, additives  
55 incorporated into a plastic during manufacture to improve its properties (e.g., phthalates for  
56 malleability and polybrominated diphenyl ethers (PDE) for heat resistance) might leach out of  
57 weathered plastic debris [18, 19]; secondly, the large surface area to volume ratio and hydrophobic  
58 properties of microplastics leave them susceptible to the accumulation of hydrophobic organic  
59 contaminants (HOCs) which could dissociate post-ingestion [20].

60 The extent to which microplastics are ingested and can impact upon zooplankton is uncertain.  
61 Zooplankton have a vital ecological role in marine ecosystems, both as primary consumers in the  
62 marine food web, and in the case of meroplankton, consisting of the juvenile life stage of numerous  
63 commercially important species. The widespread presence of small plastic debris in the water  
64 column makes interactions between zooplankton and microplastics highly likely; indeed, both small  
65 plastic debris and zooplankton >333  $\mu\text{m}$  in diameter have been recurrently sampled together in sea  
66 surface trawls and by continuous plankton recorders [4, 11, 21, 22]. Zooplankton display a range of  
67 feeding modes, which vary by life-stage, species and prey availability [23]. Zooplankton can use a  
68 combination of chemo- and mechano-receptors to select prey, and their ability to preferentially feed  
69 on one species of algae over other algae, plastic beads or detritus has been demonstrated [24-26].  
70 Laboratory experiments, in which latex beads were used to model algal ingestion, have shown that  
71 zooplankton have the potential to ingest small plastics [26-28]. Uptake of these small plastics likely

72 results from indiscriminate feeding modes (e.g. filter-feeding), by which prey with equivalent  
73 spherical diameters (ESD) <100 µm are non-selectively fed upon [23, 29].

74 Due to the complexities of sampling and extracting microplastics from the marine environment,  
75 existing studies have largely focussed on detritus >333 µm [1, 30]. However, there is evidence of  
76 very small microplastics (<100 µm) both in the benthos and water column. Sampling of shoreline,  
77 estuarine and harbour sediments has shown the presence of ~20 µm diameter fibrous polymers [4,  
78 6, 31], and microplastic fibres, granules, films and polystyrene spheres ranging in size from 38 µm – 1  
79 mm [32]. In the water column, sampling with a 80 µm mesh in Swedish coastal waters captured  
80 100,000 times greater concentrations of microplastics than when using a 450 µm mesh, with a  
81 maximal concentration of 102,000 microplastics per m<sup>3</sup> sampled near a polyethylene production  
82 facility [33]. Sampling of microplastics in this size range is exceptional, as such there is currently  
83 insufficient data to determine realistic environmental concentrations of these particles.

84 Here, we investigate the ingestion of minute microplastics, ≤31 µm diameter, by a range of  
85 zooplankton species, and examine their impact on zooplankton function and feeding. To explore the  
86 hypothesis that zooplankton are capable of ingesting microplastics, 15 zooplankton taxa -  
87 representative of abundant mesozooplankton in northeast Atlantic coastal systems - were exposed  
88 to polystyrene spheres in the size range 7.3 – 30.6 µm suspended in natural seawater, then analysed  
89 using fluorescence microscopy. Using the copepod *Temora longicornis*, we explored where 0.4 – 3.8  
90 µm microplastics accumulate, both internally and externally, using a novel bio-imaging technique:  
91 coherent anti-Stokes Raman scattering (CARS) microscopy. Finally, to test the hypothesis that  
92 microplastics negatively impact upon zooplankton feeding, we exposed the copepod *Centropages*  
93 *typicus* to natural assemblages of algae and polystyrene beads, using fluorometry and flow  
94 cytometry to quantify algal ingestion.

95

96 **2. Materials and methods**

97 *2.1 Zooplankton sampling*

98 Zooplankton sampling was conducted between November 2011 and October 2012 at Station L4 (50°  
99 15'N, 4° 13'W), a coastal site located in the western English Channel 12 km south of Plymouth, UK  
100 [34, 35]. A 200 µm mesh was used to collect zooplankton via horizontal surface tows and vertical  
101 hauls. Collected zooplankton were held in 2 L of seawater within a coolbox, and transported to  
102 controlled-temperature facilities at Plymouth Marine Laboratory (Plymouth, UK). For all  
103 experimental procedures, we maintained the zooplankton at ambient sea-surface temperatures  
104 (ranging 10-17°C depending on sampling date). Specimens were hand-selected under a dissecting  
105 microscope within two hours of sampling, and then collectively held in 2 L of filtered seawater (0.22  
106 µm Millipore filter) for 24 hours to allow full gut depuration. In all, fourteen mesozooplankton taxa  
107 (size: 0.2-20 mm), representative of the most commonly occurring zooplankton in the western  
108 English Channel and covering a range of life-stages and life-strategies, in addition to cultured  
109 *Oxyrrhis marina*, a heterotrophic dinoflagellate (size: 15-30 µm), were selected for microplastic  
110 ingestion studies (Table 1).

111

112 *2.2 Natural seawater preparation*

113 For the algal ingestion studies, natural seawater (5 L) was collected from the sea surface at station  
114 L4, passed through a 200 µm mesh into a polycarbonate carboy and returned to the laboratory  
115 within 2 hours. The seawater was further screened with a 100 µm mesh to ensure the removal of  
116 any grazing micrometazoans then stored in the dark for 24 hours at ambient sea-surface  
117 temperature to maintain the natural communities of algae at normal concentrations. Prior to  
118 experimental work, the seawater was mixed thoroughly by gentle inversion of the water in the  
119 carboy.

120

121 2.3 *Microplastics*

122 Exposures used commercial polystyrene spheres (SPHERO™ Spherotech). With global production  
123 rates of 10.6 million tons in 2001, polystyrene is the fourth most commonly produced polymer in the  
124 world and its presence as a constituent of marine debris is commonly reported [30, 36]. The bead  
125 sizes used in each experiment (0.4-30.6 µm) were selected to be comparable with the prey size  
126 range of the zooplankton exposed [23, 37].

127

128 2.4 *Microplastic ingestion by zooplankton*

129 To ascertain whether zooplankton ingest microplastics we conducted exposures using fluorescent  
130 polystyrene beads, and used microscopy to assess uptake. Microplastic suspensions were made up  
131 by pipetting 20 µl of 7.3, 20.6 or 30.6 µm diameter fluorescently-labelled (yellow fluorescence: 400-  
132 500 nm excitation, 450-550 nm emission) polystyrene spheres into glass vials containing 20 ml of  
133 filtered seawater (0.1% v/v: 3,000 beads ml<sup>-1</sup> (7.3 µm); 2,240 beads ml<sup>-1</sup> (20.6 µm); 635 beads ml<sup>-1</sup>  
134 (30.6 µm)), then mixed through repeated inversion. With larger zooplankton (e.g. copepods,  
135 decapod larvae, chaetognaths), individual specimens were added directly to the vial ( $n = \geq 6$  per  
136 exposure), and fitted to a rotating plankton wheel (<5 RPM) for 24-hours. For smaller zooplankton or  
137 those with low-survivability in the laboratory (e.g. bivalve larvae, gelatinous holoplankton, *O.*  
138 *marina*), individual specimens were exposed to microplastic suspensions in Petridishes ( $n = \geq 6$  per  
139 exposure) at ambient sea temperature for 1 hour (with the exception of bivalve larvae which were  
140 exposed for 24 hours using this method). Post-exposure, zooplankton were washed with filtered  
141 seawater and transferred to Eppendorf tubes containing 1 ml of 4% formalin . Ingestion was  
142 ascertained by viewing specimens at x40-400 magnification with an Olympus IMT2 inverted light  
143 microscope with fluorescence to determine the presence of polystyrene beads (fluorescing yellow-

144 green) within the alimentary canal or body cavity of the zooplankton. To better understand the  
145 interactions between zooplankton and microplastics, both live and preserved copepods and select  
146 zooplankton specimens were viewed under the microscope for varying lengths of time to observe  
147 the feeding process, ingestion, gut passage and egestion of polystyrene beads.

148

## 149 2.5 *Interactions between microplastics and copepods*

150 To explore the internal distribution and external adherence of microplastics in zooplankton, we  
151 firstly exposed the copepod *Temora longicornis* to polystyrene beads and then employed CARS  
152 microscopy (see below) to visualise their uptake. Microplastic suspensions were formulated by  
153 adding 12  $\mu\text{l}$  of 0.4, 1.7 or 3.8  $\mu\text{m}$  diameter non-labelled polystyrene spheres to 24 ml of filtered  
154 seawater (0.05% v/v:  $1 \times 10^6$  beads  $\text{ml}^{-1}$  (0.4  $\mu\text{m}$ ),  $380 \times 10^3$  beads  $\text{ml}^{-1}$  (1.7  $\mu\text{m}$ ), and  $40 \times 10^3$  beads  
155  $\text{ml}^{-1}$  (3.8  $\mu\text{m}$ )), which were mixed through inversion and sonication. Individual *T. longicornis* ( $n = \geq 6$   
156 per exposure) were added to each vial, rotated at  $<5$  RPM at ambient sea temperature for 24 hours.  
157 Post-exposure, specimens were poured onto a 200  $\mu\text{m}$  mesh suspended in filtered seawater (to  
158 prevent damage to the copepods), washed gently, preserved in 4% formalin and then transferred to  
159 the bio-imaging suite at the University of Exeter (Exeter, UK).

160

## 161 2.6 *Coherent anti-Stokes Raman scattering (CARS) microscopy*

162 CARS microscopy is a novel microscopy technique that provides label-free contrast, based on  
163 vibrational spectroscopy [38] which has exceptional capability for locating polymer particles within  
164 biological tissues with subcellular precision [39, 40]. CARS imaging was performed using a custom-  
165 built microscopy system based on a commercial confocal laser-scanning microscope and a  
166 synchronised dual-wavelength picosecond laser source. Laser excitation was provided by an optical  
167 parametric oscillator (OPO) (Levante Emerald, APE, Berlin) pumped with a frequency doubled

168 Nd:Vandium picosecond oscillator (High-Q Laser Production GmbH). The pump laser generated a 6  
169 ps, 76 MHz pulse train at 532 nm with adjustable output power up to 10 W. The OPO produced  
170 collinear signal and idler beams with perfect temporal overlap and provided continuous tuning over  
171 a range of wavelengths. The signal beam was used as the pump, ranging from 670 to 980 nm and  
172 fundamental of Nd:Vandium (1064 nm) used as the Stokes beam. The maximum combined output  
173 power of the pump and Stokes was approximately 1 W, which was attenuated to reduce the power  
174 at the sample to between 15 - 30 mW. To improve the transmission of the near-IR excitation through  
175 the commercial microscope (IX71 and FV300, Olympus UK) the galvanometer mirrors were replaced  
176 with silver mirrors and the tube lens was replaced with a MgF2 coated lens. The collinear pump and  
177 Stokes beams were directed onto the scanning confocal dichroic which was replaced by a silver  
178 mirror with high reflectivity throughout the visible and NIR (21010, Chroma Technologies, USA). The  
179 forward-CARS signal was collected by the air condenser, transmitted by the dichroic mirror and  
180 directed onto a red-sensitive photomultiplier tube (R3896, Hamamatsu Photonic UK). The epi-CARS  
181 signal was collected using the objective lens and separated from the pump and Stokes beams by a  
182 long-wave pass dichroic mirror (z850rdc-xr, Chroma Technologies, USA) and directed onto a second  
183 R3896 photomultiplier tube at the rear microscope port. The CARS signal was isolated at each  
184 photodetector using a single band-pass filters centred at the anti-Stokes wavelengths. Imaging was  
185 performed using either a 60X water immersion, or 20X air objective (UPlanS Apo, Olympus UK).

186

## 187 2.7 *Impact of microplastics on copepod feeding*

188 To determine whether microplastics negatively impact upon a copepod's ability to ingest natural  
189 prey, we exposed the copepod *Centropages typicus* to natural assemblages of algae with and  
190 without microplastics, and compared algal ingestion rates between treatments. In our initial  
191 experiment, designed to identify the size of microplastic that would have the greatest impact on *C.*  
192 *typicus* feeding, we exposed individual *C. typicus* specimens ( $n = \geq 6$  per exposure) to 23 ml of natural

193 seawater containing 0 or 23  $\mu\text{l}$  of 7.3 or 20.6  $\mu\text{m}$  fluorescent polystyrene beads (0.1% v/v), rotated at  
194 <5RPM for 24 hours. To quantify algal concentrations within the natural seawater pre- and post-  
195 exposure, we vacuum filtered the exposure media through a glass fibre filter, and then transferred  
196 the filter to 7 ml of acetone, held at 4°C in the dark for 24 hours. The chlorophyll levels within the  
197 acetone solution were measured using a Turner fluorometer. Since 7.3  $\mu\text{m}$  microplastics had the  
198 most notable impact on *C. typicus* feeding, we conducted a further experiment to establish a dose-  
199 response relationship between microplastic concentration and food uptake. Microplastic  
200 suspensions consisted of 0, 2.5, 5, 10 or 20  $\mu\text{l}$  additions of 7.3  $\mu\text{m}$  fluorescent polystyrene beads in  
201 23 ml of natural seawater. A 1.8 ml aliquot of natural seawater was taken from all vials at  $T_0$  and  
202 fixed with 40  $\mu\text{l}$  of 50% glutaraldehyde (4% final concentration), inverted for 2 minutes, refrigerated  
203 at 4°C for 30 minutes and subsequently snap-frozen in liquid nitrogen and stored in a -80°C freezer  
204 prior to analysis using analytical flow cytometry. Individual *C. typicus* ( $n = \geq 6$  per exposure) were  
205 added to experimental vials, while controls (with no copepod) were set up to determine natural  
206 growth or decline of algae over the exposure period. The vials were incubated on a rotating plankton  
207 wheel (5RPM) for 24 hours in the dark. Post-exposure ( $T_{24}$ ), a further 1.8 ml aliquot was fixed (as  
208 with  $T_0$ ). Flow cytometric analysis was carried out on thawed natural seawater samples using a BD  
209 Accuri C6 flow cytometer [41]. Particle abundance data was subsequently used to calculate the  
210 ingestion rates of algae by *C. typicus* [42].

211

## 212 2.8 *Statistical analysis*

213 Data was analysed using Microsoft Excel. Student's T-tests were used to compare experimental data  
214 with controls, with significant difference attributed where  $P \leq 0.05$ . Regression analysis was used to  
215 analyse the correlation between algal ingestion rates and microplastic concentration.

216

217 **3. Results**

218 **3.1 Microplastic ingestion by zooplankton**

219 The majority of zooplankton (13 of 15) exposed to polystyrene beads (7.3 – 30.6 µm) demonstrated  
220 the capacity to ingest microplastics (Table 1). Organisms exhibiting uptake included copepods (Fig. 1i  
221 + Fig. 1ii), bivalve larvae (Fig. 1iii) and decapod larvae (Fig. 1iv + Fig. 1v). Only two specimens –  
222 chaetognaths (*Parasagitta* sp.) and siphonophorae (Cnidaria) – showed no evidence of ingestion. All  
223 four species of copepods examined demonstrated some affinity for ingesting microplastics, with  
224 *Centropages typicus* and *Temora longicornis* able to consume 7.3, 20.6 and 30.6 µm polystyrene  
225 beads (Fig. 1ix). The other copepods showed evidence of size-based selectivity: *Acartia clausi*  
226 ingested 7.3 µm beads but ingested significantly less 20.6 and 30.6 µm beads, and *Calanus*  
227 *helgolandicus* showed significantly less affinity for 30.6 µm beads than for 7.3 µm beads. The  
228 decapod Brachyurans demonstrated variability in microplastic ingestion depending upon life-stage:  
229 brachyuran zoea showed no affinity for 20.6 µm beads, while the more-developed brachyuran  
230 megalopa readily ingested such beads. *Obelia* sp., Paguridae larvae and Porcellinidae (zoea)  
231 exhibited individual variability in their ability to ingest polystyrene beads, with less than half the  
232 exposed specimens in a cohort showing evidence of microplastic uptake.

233 Live observations of copepods, euphausiids and doliolids found microplastics were ingested via filter-  
234 feeding. In copepods and euphausiids, this process relied upon the rapid movement of the swimming  
235 legs and external appendages, which generated a feeding current that indiscriminately drew  
236 surrounding beads towards the organism. With doliolids, we observed the microplastics being drawn  
237 through the anterior siphon into their body cavity, where the polystyrene beads were entrapped and  
238 drawn towards the gut. *Oxyrrhis marina*, a single celled heterotrophic dinoflagellate, demonstrated a  
239 more direct method of ingestion, locating particles with their flagella and then engulfing the  
240 polystyrene beads. Post- ingestion, copepods typically aggregated beads within the anterior mid-gut,  
241 shifted them to the posterior mid-gut via peristaltic action (Fig. 1i + Fig. 1ii) and egested them within

242 densely-packed faecal pellets (Fig. 1vi + Fig. 1viii). Typically, microplastic-laden faecal pellets were  
243 egested within hours. In the absence of food, individual microplastic beads could remain in the  
244 intestinal tract of *C. helgolandicus* for up to 7 days (data not shown). During observations of both live  
245 and preserved zooplankton specimens, including copepods, decapod larvae and euphausiids,  
246 microplastics often adhered to the specimens' external surfaces. In copepods that died during the  
247 exposure period, polystyrene beads would coat the carapace in vast numbers; similarly, beads were  
248 observed to cling to the shed carapace of a moulting *C. helgolandicus* copepodite. In live specimens,  
249 microplastics were found to concentrate between the external appendages of copepods, including  
250 the swimming legs, feeding apparatus, antennae and furca (Fig. 1vii).

251

### 252 3.2 *Interactions between microplastics and copepods*

253 CARS microscopy used a blend of transmitted light to capture the structure of the copepod, and  
254 Raman shifts of  $2845\text{ cm}^{-1}$  (C-H) and  $3050\text{ cm}^{-1}$  (aromatic C-H) to visualise the polystyrene (Fig. 2i).  
255 *Temora longicornis* ingested both 1.7 and 3.8  $\mu\text{m}$  polystyrene beads; use of Z-stacking – in which 2D  
256 images at incremental focal plains are layered together to form a 3D image – confirmed that  
257 microplastics clumping in the posterior mid-gut were, indeed, internalised (Fig. 2ii; yellow dots), but  
258 sufficient resolution to identify microplastic translocation was not possible. CARS imaging confirmed  
259 that microplastics adhere to the external appendages of the zooplankton: polystyrene beads (0.4 –  
260 3.8  $\mu\text{m}$ ) accumulated between the filamental hairs on appendages, including the furca (Fig. 2iii; blue  
261 dots), rear swimming legs (Fig. 2iii; red dots) and antennules, and between the segments of the  
262 carapace, particularly around the urosome and swimming legs.

263

### 264 3.3 *Impact of microplastics on copepod feeding*

265 Using chlorophyll concentration as a proxy for algal abundance, we identified that 7.3  $\mu\text{m}$   
266 microplastics had a significant impact on algal ingestion by the copepod *Centropages typicus* (data  
267 not shown) and identified a significant dose-response relationship between ingestion rates and the  
268 concentration of 7.3  $\mu\text{m}$  polystyrene beads. Exposed to seawater – containing natural assemblages  
269 of algae – *C. typicus* ingested  $\sim 12$  *Synechococcus* sp.  $\text{ind}^{-1} \text{h}^{-1}$  (Fig. 3i) and  $\sim 24$  picoeukaryotes  $\text{ind}^{-1} \text{h}^{-1}$   
270 (Fig. 3ii). These ingestion rates decreased when additionally exposed to  $\sim 4,000$  microplastics  $\text{ml}^{-1}$ ;  
271 this decrease was statistically significant at concentrations of  $\geq 7,000$  microplastics  $\text{ml}^{-1}$  (t-test:  $P$   
272  $\leq 0.05$ ). When considering all of the  $< 20 \mu\text{m}$  ESD algal groups identified using flow cytometry –  
273 *Synechococcus* sp., picoeukaryotes, nanoeukaryotes and cryptophytes – in combination (hereafter  
274 referred to as “total algae”), *C. typicus* presented total algal ingestion rates of  $\sim 34$  algae  $\text{ind}^{-1} \text{h}^{-1}$  in  
275 the absence of microplastics. Total algal ingestion rates for *C. typicus* were significantly reduced with  
276 the addition of  $\geq 4,000$  microplastics  $\text{ml}^{-1}$  (t-test:  $P \leq 0.05$ ; Fig. 3iii). Furthermore, we identified a  
277 strong, logarithmic relationship ( $R^2 = 0.70$ ,  $P \leq 0.05$ ) between the ingestion rate of total algae and  
278 microplastic concentration (Fig. 3iv).

279

#### 280 **4. Discussion**

281 Our results show that a range of zooplankton common to the northeast Atlantic can ingest  
282 microplastics (1.4 – 30.6  $\mu\text{m}$  diameter), with capacity for uptake varying between species, life-stage  
283 and microplastic size. Microplastics were indiscriminately ingested via filter-feeding and later  
284 egested in faecal pellets, typically within a matter of hours. Microplastics accumulated on the  
285 external surface of dead zooplankton, and were found trapped between the external appendages of  
286 live copepods. We visualised 1.7 and 3.8  $\mu\text{m}$  polystyrene beads clustered within the alimentary canal  
287 and aggregated between the setae and joints of external appendages. Lastly, we demonstrated that  
288 the presence of 7.3  $\mu\text{m}$  polystyrene beads could significantly reduce the algal ingestion rate of the  
289 copepod *Centropages typicus*, in a dose-response relationship.

290 We demonstrated that thirteen zooplankton taxa – including holoplankton, meroplankton and  
291 microzooplankton – have the capacity to ingest polystyrene beads in the absence of natural food. All  
292 four copepod species showed uptake of microplastics, with varying degrees of selectivity: *T.*  
293 *longicornis* and *C. typicus* ingested 7.3, 20.6 and 30.6  $\mu\text{m}$  beads, while *A. clausi* and *C. helgolandicus*  
294 fed on 7.3  $\mu\text{m}$  beads but less frequently ingested larger beads. Using CARS microscopy, we further  
295 identified that *T. longicornis* could ingest 1.7 and 3.8  $\mu\text{m}$  microplastics, however, we found no  
296 evidence of 0.4  $\mu\text{m}$  beads being ingested. Brachyuran larvae only ingested 20.6  $\mu\text{m}$  polystyrene  
297 beads as megalopa (post-zoea larvae), with no uptake observed when in the earlier zoea stage.  
298 Microplastics were also ingested by the filter-feeding euphausiids and doliolids, and *Oxyrrhis marina*,  
299 a heterotrophic dinoflagellate that ingests motile or immotile prey through engulfment via a non-  
300 permanent cytosome [43]. These findings corroborate the results of several previous studies, which  
301 documented the uptake of  $<100 \mu\text{m}$  microplastics by *Acartia tonsa* [28], *Calanus pacificus* adults,  
302 copepodites and nauplii [26, 44, 45], *Oxyrrhis marina* [46], ciliates [47, 48], echinoderm larvae [27]  
303 and salps [49].

304 We did not observe microplastic uptake in *Parasagitta* sp. (chaetognaths) following 1- or 24- hour  
305 exposures to 30.6  $\mu\text{m}$  beads, or siphonophorae (Cnidaria) exposed to 20.6  $\mu\text{m}$  plastics, possibly as a  
306 result of handling stress, or more likely because these zooplankton are raptorial predators and feed  
307 actively, so were not enticed to capture the immotile microplastics [37]. Furthermore, only 10-50%  
308 of *Obelia* sp., Paguridae larvae and Porcellinidae (zoea) specimens presented with polystyrene beads  
309 in their intestinal tracts post-exposure. As we also observed size-selective ingestion in *A. clausi* and  
310 *C. helgolandicus*, it is important to consider how microplastics may impact on different zooplankton  
311 feeding strategies. Zooplankton use both mechanoreception (i.e. detection of pressure disturbances  
312 within the water) and chemoreception (i.e. detection of infochemicals emitted by algal cells) to  
313 sense prey [29, 37]. As such, the clean immotile beads used in our algal-free experiments are less  
314 likely to be detected by exposed zooplankton, although it is possible that aged microplastics, that  
315 have developed bio-films during their residence within the marine environment [10], may generate a

316 chemosensory response; this effect was observed in the copepod *Eurytemora affinis* which more  
317 readily ingested beads spiked with bacteria than when offered beads alone [50]. While some  
318 copepods will continuously filter-feed regardless of prey availability, others (e.g. *C. pacificus*, *A.*  
319 *tonsa*) can limit their movement and filter-feed at reduced rates to conserve energy when faced with  
320 low food-concentrations [51, 52]. The presence of algae promotes greater uptake of microplastics in  
321 the filter-feeding copepods *Calanus pacificus* [26] and *Eucalanus pileatus* CV copepodites [53];  
322 notably, *A. clausi* only ingests 16  $\mu\text{m}$  polystyrene beads in the presence of algae [24]. Some  
323 zooplankton can ingest or reject prey upon capture, depending on surface characteristics and charge  
324 of the particle, both echinoderm larvae and the copepods *A. clausi* and *E. pileatus* can reject plastic  
325 beads that coalesced within their mouthparts [27, 53, 54]. The presence of microplastics may also  
326 alter the behaviour of zooplankton, limiting their capacity to feed: in *Acartia tonsa* copepodites,  
327 contact with 45  $\mu\text{m}$  plastic beads caused the organisms to “jump”, limiting time dedicated to feeding  
328 bouts and reducing their clearance rates by 60% [55].

329 Post-ingestion, polystyrene beads were observed to coalesce within the mid-gut of copepods prior  
330 to egestion. While gut-retention times of these microplastics were typically similar to natural food  
331 items (i.e. egestion occurred within hours), a follow-up experiment found some *Calanus*  
332 *helgolandicus* individuals retained microplastics for up to 7 days. Microplastics found in the marine  
333 environment include fibres, granules and fragments manufactured from a range of polymers [30]; if  
334 such irregularly-shaped and fibrous microplastics were ingested, they may become entangled within  
335 the intestinal tract, potentially resulting in a non-biodegradable gut-blockage and greater gut-  
336 retention times. Plastic fibres entangle within the intestinal tracts of Nephrops in this manner [14],  
337 while fish [16, 17] and seabird dissections [15] have demonstrated that marine wildlife can retain a  
338 range of plastic detritus within their stomachs near-indefinitely. Prolonged gut-retention times of  
339 plastics and gut-blockages in zooplankton may limit the ability of these organisms to ingest and  
340 digest food, and may pose a toxic risk. During manufacture, a suite of additives (e.g. plasticisers,  
341 flame-retardants, anti-microbials) are added to plastics, and large surface area to volume ratio and

342 hydrophobic properties of microplastics make them particularly susceptible to the adherence of  
343 waterborne contaminants (e.g. PCBs, DDT and PAHs) [19]. The leaching of additives and  
344 disassociation of toxic contaminants post-ingestion has been modelled in polychaete worms [56] and  
345 demonstrated in streaked shearwaters [57]. In zooplankton, as with other marine biota, these  
346 contaminants might be considered endocrine-disruptors, carcinogenic or toxic, with repercussions  
347 for growth, sexual development, fecundity, morbidity and mortality [58, 59]. Of further concern is  
348 trophic-transfer: microplastics (and contaminants released from microplastics) within lower-trophic,  
349 keystone organisms such as zooplankton may result in the trophic-transfer of these contaminants up  
350 the food-chain, with the potential for bio-accumulation and therefore adverse health consequences  
351 in higher trophic organisms.

352 Copepods that died during exposures, and shed moults of copepodites, were coated in microplastics  
353 – presumably because of hydrophobic- or static-attractions between the negatively-charged  
354 polystyrene (average zeta potential: -41.8 mV) and organic material – a process that acts to  
355 concentrate microplastics from the surrounding seawater. Our observations of microplastic laden  
356 faecal pellets egested by copepods provided no indication that passage through the alimentary canal  
357 had any discernible impact on the microplastics. However, plastics may alter the density and  
358 structural integrity of faecal pellets with potential repercussions on vertical carbon flux [60]. During  
359 our studies, we also found microplastics were becoming trapped between the external appendages  
360 and carapace segments of live copepods. We found that very small microplastics (0.4 – 3.8  $\mu\text{m}$ )  
361 became lodged between the filamental hairs and setae of the antennules, furca and the swimming  
362 legs [29, 61]. As these appendages have key roles in copepod function and behaviour, this may have  
363 repercussions for locomotion, ingestion, mating and mechanoreception, that may limit their ability  
364 to detect prey, feed, reproduce and evade predators.

365 We found that the presence of 7.3  $\mu\text{m}$  beads significantly reduced the amount of algae eaten by the  
366 copepod *Centropages typicus*, whereas 20.6  $\mu\text{m}$  beads showed no discernible impact on algal

367 consumption. This suggests *C. typicus* can preferentially feed upon algae over 20.6  $\mu\text{m}$  beads (but  
368 could not differentiate between the algae and 7.3  $\mu\text{m}$  beads), or, that only the smaller beads impact  
369 on copepod feeding (i.e. 7.3  $\mu\text{m}$  beads are small enough to become entrapped between external  
370 appendages or be recurrently ingested). A similar finding has been observed with *Acartia clausi* and  
371 *Calanus pacificus* nauplii, which selectively fed upon small algae while avoiding larger beads, but  
372 could not discriminate between algae and beads of a similar size [24, 45, 54]. We found that a  
373 concentration of 4,000 beads  $\text{ml}^{-1}$  was enough to result in significantly reduced algal ingestion rates.  
374 This relationship reached saturation at concentrations of  $>5000$  beads  $\text{ml}^{-1}$ . Two previous studies  
375 have found similar results, where the ingestion rates of the copepod *A. clausi* [24] and *C. pacificus*  
376 [45] were significantly reduced by the presence of beads of a similar size to the algae. A reduction in  
377 algal feeding may have severe consequences for copepods, as limited energy intake, in particular  
378 with species that have minimal lipid reserves (e.g. *Centropages*, *Acartia*), could result in decreased  
379 fecundity and growth, or increased mortality [24, 62]. We do not yet know whether 5000 particles  
380  $\text{ml}^{-1}$  can be considered an environmentally relevant concentration for microplastics  $<10$   $\mu\text{m}$  in size.  
381 Perpetual fragmentation of plastic litter, coupled with the increasing popularity of household  
382 products containing microscopic plastic exfoliates [5], suggests marine plastic debris is becoming, on  
383 average, smaller over time [63]. However, due to the complexities of sampling and extraction, and in  
384 the absence of unified sampling methodologies, microplastics are still considered to be an under-  
385 researched fraction of marine litter, with no consistent data relating to plastic detritus  $<333$   $\mu\text{m}$  in  
386 diameter [1, 30, 64]. Further, we must consider that microplastics made of polymers other than  
387 polystyrene, potentially laden with chemical additives or adhered contaminants, could result in  
388 different interactions with zooplankton with variable impacts on function.

389 Our findings confirm that ingestion of marine microplastic debris by zooplankton in the ocean is  
390 feasible. Potential impacts include reduced function and health of the individual, trophic-transfer of  
391 contaminants to predators, and the egestion of faecal pellets containing microplastics. Better

392 knowledge of the extent of microplastic contamination of oceans waters is now a research  
393 imperative.

394

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400

### 401 **Figure Legends**

402 Figure 1: Microplastics of different sizes can be ingested, egested and adhere to a range of  
403 zooplankton, as visualised using fluorescence microscopy: (i) the copepod *Centropages typicus*  
404 containing 7.3 µm polystyrene (PS) beads (dorsal view); (ii) the copepod *Calanus helgolandicus*  
405 containing 20.6 µm PS beads (lateral view); (iii) a D-stage bivalve larvae containing 7.3 µm PS beads  
406 (dorsal view); (iv) a Brachyuran (decapod) larvae (zoea stage) containing 20.6 µm PS beads (lateral  
407 view); (v) a Porcellanid (decapod) larvae, containing 30.6 µm PS beads (lateral view); (vi) 30.6 µm PS  
408 beads in the posterior-gut of the copepod *Temora longicornis* during egestion, (vii) 1.4 µm PS beads  
409 trapped between the filamental hairs of the furca of *C. typicus*; (viii) a *T. longicornis* faecal pellet  
410 containing 30.6 µm PS beads; (ix) proportion of copepods (*Acartia clausi*, *Calanus helgolandicus*,  
411 *Centropages typicus* and *Temora longicornis*) with microplastics in their guts following 24 hours of  
412 exposure to 7.4, 20.6 and 30.6 µm polystyrene beads. \* denotes statistically significant ( $P \leq 0.05$ )  
413 lower consumption of larger beads compared with that of 7.3 µm beads. Scale bar (grey line): 100  
414 µm.

415

416 Figure 2: Coherent anti-Stokes Raman scattering (CARS) microscopy: (i) Spontaneous [•] and  
417 stimulated [•] peaks for polystyrene beads, Raman shifts of  $2845\text{ cm}^{-1}$  (C-H) and  $3050\text{ cm}^{-1}$  (aromatic  
418 C-H) were used to visualise the polystyrene; (ii)  $3.4\text{ }\mu\text{m}$  microplastics accumulated in the alimentary  
419 canal [ac] of the copepod *Temora longicornis* (yellow dots); beads further adhered to the exterior of  
420 the copepod's urosome [u], furca [f] and posterior swimming legs [sl] (blue dots); (ii)  $3.4\text{ }\mu\text{m}$   
421 microplastics (red dots) adhered to the external surface of the posterior swimming legs of *T.*  
422 *longicornis*. Scale bar [grey line]:  $50\text{ }\mu\text{m}$ .

423

424 Figure 3: Exposure to increasing concentrations of microplastics in the copepod *Centropages typicus*  
425 ( $n = \geq 5$ ). Treatments comprise seawater containing natural assemblages of algae [A] with 4,000 [B],  
426 7,000 [C], 11,000 [D] and 25,000 [E]  $7.3\text{ }\mu\text{m}$  polystyrene beads per ml. \* denotes statistically  
427 significant ( $P \leq 0.05$ ) lower ingestion rates (cells individual<sup>-1</sup> hour<sup>-1</sup>) than in controls. Graphs show  
428 ingestion rates of: (i) *Synechococcus* sp.; (ii) Picoeukaryotes; (iii) all algae present; (iv) plot comparing  
429 positive *C. typicus* algal ingestion rates at differing microplastics concentrations - logarithmic  
430 regression:  $R^2 = 0.70$  ( $P \leq 0.05$ ).

431

**Table 1: The capacity for a range of zooplankton to ingest microplastics, demonstrated using fluorescent microscopy.**

Organism	Taxonomy	Microplastic	Exposure	Ingestion
<b>Holoplankton (Copepods)</b>				
<i>Acartia clausi</i>	Copepoda (Calanoida)	7.3	24	Yes
<i>Acartia clausi</i>	Copepoda (Calanoida)	20.6	24	No
<i>Acartia clausi</i>	Copepoda (Calanoida)	30.6	24	Partial
<i>Calanus helgolandicus</i>	Copepoda (Calanoida)	7.3	24	Yes
<i>Calanus helgolandicus</i>	Copepoda (Calanoida)	20.6	24	Yes
<i>Calanus helgolandicus</i> (juv.)	Copepoda (Calanoida)	20.6	24	Yes
<i>Calanus helgolandicus</i>	Copepoda (Calanoida)	30.6	24	Partial
<i>Centropages typicus</i>	Copepoda (Calanoida)	7.3	24	Yes
<i>Centropages typicus</i>	Copepoda (Calanoida)	20.6	24	Yes
<i>Centropages typicus</i>	Copepoda (Calanoida)	30.6	24	Yes
<i>Temora longicornis</i>	Copepoda (Calanoida)	7.3	24	Yes
<i>Temora longicornis</i>	Copepoda (Calanoida)	20.6	24	Yes
<i>Temora longicornis</i>	Copepoda (Calanoida)	30.6	24	Yes
<b>Holoplankton (Other)</b>				
Doliolidae	Tunicata	7.3	1	Yes
Euphausiidae	Euphausiacea	20.6	24	Yes
<i>Parasagitta</i> sp.	Chaetognatha	20.6	1	No
<i>Parasagitta</i> sp.	Chaetognatha	30.6	24	No
<i>Obelia</i> sp.	Cnidaria (Hydrozoa)	20.6	1	Partial
Siphonophorae	Cnidaria (Hydrozoa)	20.6	1	No
<b>Meroplankton</b>				
Bivalvia (larvae)	Mollusca	7.3	24	Yes
Brachyura (megalopa)	Decapoda	20.6	24	Yes
Brachyura (zoea)	Decapoda	20.6	24	No
Caridea (larvae)	Decapoda	20.6	24	Yes
Paguridae (larvae)	Decapoda	20.6	24	Partial
Porcellanidae (zoea)	Decapoda	30.6	24	Partial
<b>Microzooplankton</b>				
<i>Oxyrrhis marina</i>	Dinoflagellata	7.3	1	Yes

Microplastic uptake is based upon the number of individuals in a treatment ( $n \geq 6$ ) that contained beads in their alimentary canals or body cavity following 1 or 24 hour exposures to either 7.3, 20.6 or 30.6  $\mu\text{m}$  fluorescent polystyrene beads. ESD = Equivalent Spherical Diameter. Scoring system: Yes (>50%); Partial (<50%); No (0%).

435 **References**

- 436 1. Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T. S., Microplastics as contaminants in the  
 437 marine environment: A review. *Marine Pollution Bulletin* **2011**, *62*, 2588-2597.
- 438 2. Thompson, R. C., Plastic debris in the marine environment: consequences and solutions.  
 439 *Marine Nature Conservation in Europe* **2006**, *193*, 107-115.
- 440 3. Derraik, J. G. B., The pollution of the marine environment by plastic debris: a review. *Marine*  
 441 *Pollution Bulletin* **2002**, *44*, (9), 842-852.
- 442 4. Thompson, R. C.; Olsen, Y.; Mitchell, R. P.; Davis, A.; Rowland, S. J.; John, A. W. G.;  
 443 McGonigle, D.; Russell, A. E., Lost at Sea: Where Is All the Plastic? *Science* **2004**, *304*, (5672), 838.
- 444 5. Fendall, L. S.; Sewell, M. A., Contributing to marine pollution by washing your face:  
 445 Microplastics in facial cleansers. *Marine Pollution Bulletin* **2009**, *58*, (8), 1225-1228.
- 446 6. Browne, M. A.; Crump, P.; Niven, S. J.; Teuten, E.; Tonkin, A.; Galloway, T.; Thompson, R.,  
 447 Accumulation of microplastics on shorelines worldwide: Sources and sinks. *Environmental Science &*  
 448 *Technology* **2011**, *45*, (21), 9175-9179.
- 449 7. O'Brine, T.; Thompson, R. C., Degradation of plastic carrier bags in the marine environment.  
 450 *Marine Pollution Bulletin* **2010**, *60*, (12), 2279-2283.
- 451 8. Davidson, T. M., Boring crustaceans damage polystyrene floats under docks polluting marine  
 452 waters with microplastic. *Marine Pollution Bulletin* **2012**, *64*, (9), 1821-1828.
- 453 9. Lattin, G. L.; Moore, C. J.; Zellers, A. F.; Moore, S. L.; Weisberg, S. B., A comparison of  
 454 neustonic plastic and zooplankton at different depths near the southern California shore. *Marine*  
 455 *Pollution Bulletin* **2004**, *49*, (4), 291-294.
- 456 10. Lobelle, D.; Cunliffe, M., Early microbial biofilm formation on marine plastic debris. *Marine*  
 457 *Pollution Bulletin* **2011**, *62*, (1), 197-200.
- 458 11. Collignon, A.; Hecq, J.-H.; Galgani, F.; Voisin, P.; Collard, F.; Goffart, A., Neustonic  
 459 microplastic and zooplankton in the North Western Mediterranean Sea. *Marine Pollution Bulletin*  
 460 **2012**, *64*, (4), 861-864.
- 461 12. Barnes, D. K. A.; Galgani, F.; Thompson, R. C.; Barlaz, M., Accumulation and fragmentation of  
 462 plastic debris in global environments. *Philosophical Transactions of the Royal Society B: Biological*  
 463 *Sciences* **2009**, *364*, (1526), 1985-1998.
- 464 13. Browne, M. A.; Dissanayake, A.; Galloway, T. S.; Lowe, D. M.; Thompson, R. C., Ingested  
 465 Microscopic Plastic Translocates to the Circulatory System of the Mussel, *Mytilus edulis* (L.).  
 466 *Environmental Science & Technology* **2008**, *42*, (13), 5026-5031.
- 467 14. Murray, F.; Cowie, P. R., Plastic contamination in the decapod crustacean *Nephrops*  
 468 *norvegicus* (Linnaeus, 1758). *Marine Pollution Bulletin* **2011**, *62*, (6), 1207-1217.
- 469 15. van Franeker, J. A.; Blaize, C.; Danielsen, J.; Fairclough, K.; Gollan, J.; Guse, N.; Hansen, P.-L.;  
 470 Heubeck, M.; Jensen, J.-K.; Le Guillou, G.; Olsen, B.; Olsen, K.-O.; Pedersen, J.; Stienen, E. W. M.;  
 471 Turner, D. M., Monitoring plastic ingestion by the northern fulmar *Fulmarus glacialis* in the North  
 472 Sea. *Environmental Pollution* **2011**, *159*, (10), 2609-2615.
- 473 16. Boerger, C. M.; Lattin, G. L.; Moore, S. L.; Moore, C. J., Plastic ingestion by planktivorous  
 474 fishes in the North Pacific Central Gyre. *Marine Pollution Bulletin* **2010**, *60*, (12), 2275-2278.
- 475 17. Davison, P.; Asch, R. G., Plastic ingestion by mesopelagic fishes in the North Pacific  
 476 Subtropical Gyre. *Marine Ecology Progress Series* **2011**, *432*, 173-180.
- 477 18. Talsness, C. E.; Andrade, A. J. M.; Kuriyama, S. N.; Taylor, J. A.; vom Saal, F. S., Components  
 478 of plastic: experimental studies in animals and relevance for human health. *Philosophical*  
 479 *Transactions of the Royal Society B: Biological Sciences* **2009**, *364*, (1526), 2079-2096.
- 480 19. Teuten, E. L.; Saquing, J. M.; Knappe, D. R. U.; Barlaz, M. A.; Jonsson, S.; Björn, A.; Rowland,  
 481 S. J.; Thompson, R. C.; Galloway, T. S.; Yamashita, R.; Ochi, D.; Watanuki, Y.; Moore, C.; Viet, P. H.;  
 482 Tana, T. S.; Prudente, M.; Boonyatumanond, R.; Zakaria, M. P.; Akkhavong, K.; Ogata, Y.; Hirai, H.;  
 483 Iwasa, S.; Mizukawa, K.; Hagino, Y.; Imamura, A.; Saha, M.; Takada, H., Transport and release of

484 chemicals from plastics to the environment and to wildlife. *Philosophical Transactions of the Royal*  
485 *Society B: Biological Sciences* **2009**, *364*, (1526), 2027-2045.

486 20. Mato, Y.; Isobe, T.; Takada, H.; Kanehiro, H.; Ohtake, C.; Kaminuma, T., Plastic Resin Pellets  
487 as a Transport Medium for Toxic Chemicals in the Marine Environment. *Environmental Science &*  
488 *Technology* **2001**, *35*, (2), 318-324.

489 21. Moore, C. J.; Moore, S. L.; Leecaster, M. K.; Weisberg, S. B., A Comparison of Plastic and  
490 Plankton in the North Pacific Central Gyre. *Marine Pollution Bulletin* **2001**, *42*, (12), 1297-1300.

491 22. Moore, C. J.; Moore, S. L.; Weisberg, S. B.; Lattin, G. L.; Zellers, A. F., A comparison of  
492 neustonic plastic and zooplankton abundance in southern California's coastal waters. *Marine*  
493 *Pollution Bulletin* **2002**, *44*, (10), 1035-1038.

494 23. Wirtz, K. W., Who is eating whom? Morphology and feeding type determine the size relation  
495 between planktonic predators and their ideal prey. In 2012; Vol. 445, pp 1-12.

496 24. Ayukai, T., Discriminate feeding of the calanoid copepod *Acartia clausi* in mixtures of  
497 phytoplankton and inert particles. *Marine Biology* **1987**, *94*, (4), 579-587.

498 25. DeMott, W. R., Discrimination Between Algae and Detritus by Freshwater and Marine  
499 Zooplankton. *Bulletin of Marine Science* **1988**, *43*, (3), 486-499.

500 26. Frost, B. W., Feeding Behavior of *Calanus pacificus* in Mixtures of Food Particles. *Limnology*  
501 *and Oceanography* **1977**, *22*, (3), 472-491.

502 27. Hart, M. W., Particle Captures and the Method of Suspension Feeding by Echinoderm Larvae.  
503 *The Biological Bulletin* **1991**, *180*, (1), 12-27.

504 28. Wilson, D. S., Food Size Selection Among Copepods. *Ecology* **1973**, *54*, (4), 909-914.

505 29. Mauchline, J., *The Biology of Calanoid Copepods*. Academic Press: London, 1998.

506 30. Hidalgo-Ruz, V.; Gutow, L.; Thompson, R. C.; Thiel, M., Microplastics in the marine  
507 environment: A review of the methods used for identification and quantification. *Environmental*  
508 *Science & Technology* **2012**, *46*, (6), 3060-3075.

509 31. Browne, M. A.; Galloway, T. S.; Thompson, R. C., Spatial Patterns of Plastic Debris along  
510 Estuarine Shorelines. *Environmental Science & Technology* **2010**, *44*, (9), 3404-3409.

511 32. Claessens, M.; Meester, S. D.; Landuyt, L. V.; Clerck, K. D.; Janssen, C. R., Occurrence and  
512 distribution of microplastics in marine sediments along the Belgian coast. *Marine Pollution Bulletin*  
513 **2011**, *62*, (10), 2199-2204.

514 33. Lozano, R. L.; Mouat, J. *Marine litter in the North-East Atlantic Region: Assessment and*  
515 *priorities for response.*; KIMO International: 2009.

516 34. Harris, R., The L4 time-series: the first 20 years. *Journal of Plankton Research* **2010**, *32*, (5),  
517 577-583.

518 35. Smyth, T. J.; Fishwick, J. R.; Al-Moosawi, L.; Cummings, D. G.; Harris, C.; Kitidis, V.; Rees, A.;  
519 Martinez-Vicente, V.; Woodward, E. M. S., A broad spatio-temporal view of the Western English  
520 Channel observatory. *Journal of Plankton Research* **2010**, *32*, (5), 585-601.

521 36. PlasticsEurope Plastics - the facts 2010.  
522 [http://www.plasticseurope.org/documents/document/20101028135906-](http://www.plasticseurope.org/documents/document/20101028135906-final_plasticsthefacts_26102010_lr.pdf)  
523 [final\\_plasticsthefacts\\_26102010\\_lr.pdf](http://www.plasticseurope.org/documents/document/20101028135906-final_plasticsthefacts_26102010_lr.pdf)

524 37. Kiørboe, T., How zooplankton feed: mechanisms, traits and trade-offs. *Biological Reviews*  
525 **2011**, *86*, 311-339.

526 38. Moger, J.; Johnston, B. D.; Tyler, C. R., Imaging metal oxide nanoparticles in biological  
527 structures with CARS microscopy. *Opt. Express* **2008**, *16*, (5), 3408-3419.

528 39. Garrett, N. L.; Lalatsa, A.; Begley, D.; Mihoreanu, L.; Uchegbu, I. F.; Schätzlein, A. G.; Moger,  
529 J., Label-free imaging of polymeric nanomedicines using coherent anti-stokes Raman scattering  
530 microscopy. *Journal of Raman Spectroscopy* **2012**, *43*, (5), 681-688.

531 40. Garrett, N. L.; Lalatsa, A.; Uchegbu, I.; Schätzlein, A.; Moger, J., Exploring uptake mechanisms  
532 of oral nanomedicines using multimodal nonlinear optical microscopy. *Journal of Biophotonics* **2012**,  
533 *5*, (5-6), 458-468.

- 534 41. Tarran, G. A.; Heywood, J. L.; Zubkov, M. V., Latitudinal changes in the standing stocks of  
535 nano- and picoeukaryotic phytoplankton in the Atlantic Ocean. *Deep-Sea Research II* **2006**, 1516-  
536 1529.
- 537 42. Frost, B. W., Effect of size and concentration of food particles on the feeding behaviour of  
538 the marine planktonic copepod *Calanus pacificus*. *Limnology and Oceanography* **1972**, *17*, 805-815.
- 539 43. Roberts, E. C.; Wootton, E. C.; Davidson, K.; Jeong, H. J.; Lowe, C. D.; Montagnes, D. J. S.,  
540 Feeding in the dinoflagellate *Oxyrrhis marina*: linking behaviour with mechanisms. **2011**, *33*, (4), 603-  
541 614.
- 542 44. Huntley, M. E.; Barthel, K. G.; Star, J. L., Particle rejection by *Calanus pacificus*: discrimination  
543 between similarly sized particles. *Marine Biology* **1983**, *74*, 151-160.
- 544 45. Fernandez, F., Particle selection in the nauplius of *Calanus pacificus*. *Journal of Plankton*  
545 *Research* **1979**, *1*, (4), 313-327.
- 546 46. Hammer, A.; Grüttner, C.; Schumann, R., The Effect of Electrostatic Charge of Food Particles  
547 on Capture Efficiency by *Oxyrrhis marina* Dujardin (Dinoflagellate). *Protist* **1999**, *150*, (4), 375-382.
- 548 47. Christaki, U.; Dolan, J. R.; Pelegri, S.; Rassoulzadegan, F., Consumption of Picoplankton-Size  
549 Particles by Marine Ciliates: Effects of Physiological State of the Ciliate and Particle Quality.  
550 *Limnology and Oceanography* **1998**, *43*, (3), 458-464.
- 551 48. Juchelka, C. M.; Snell, T. W., Rapid toxicity assessment using ingestion rate of cladocerans  
552 and ciliates. *Arch. Environ. Contam. Toxicol.* **1995**, *28*, (4), 508-512.
- 553 49. Chan, W. Y.; Witting, J., The impact of microplastics on salp feeding in the tropical Pacific.  
554 *The ANU Undergraduate Research Journal* **2012**, *4*.
- 555 50. Powell, M. D.; Berry, A., Ingestion and regurgitation of living and inert materials by the  
556 estuarine copepod *Eurytemora affinis* (Poppe) and the influence of salinity. *Estuarine, Coastal and*  
557 *Shelf Science* **1990**, *31*, (6), 763-773.
- 558 51. Lam, R. K.; Frost, B. W., Model of Copepod Filtering Response to Changes in Size and  
559 Concentration of Food. *Limnology and Oceanography* **1976**, *21*, (4), 490-500.
- 560 52. Tiselius, P., Behavior of *Acartia tonsa* in Patchy Food Environments. *Limnology and*  
561 *Oceanography* **1992**, *37*, (8), 1640-1651.
- 562 53. Paffenhöfer, G.; Van Sant, K. B., The feeding response of a marine planktonic copepod to  
563 quantity and quality of particles. *Mar Ecol Prog Ser* **1985**, *27*, 55-65.
- 564 54. Donaghay, P.; Small, L., Food selection capabilities of the estuarine copepod *Acartia clausi*.  
565 *Marine Biology* **1979**, *52*, (2), 137-146.
- 566 55. Hansen, B.; Hansen, P. J.; Nielsen, T. G., Effects of large nongrazable particles on clearance  
567 and swimming behaviour of zooplankton. *Journal of Experimental Marine Biology and Ecology* **1991**,  
568 *152*, (2), 257-269.
- 569 56. Teuten, E. L.; Rowland, S. J.; Galloway, T. S.; Thompson, R. C., Potential for Plastics to  
570 Transport Hydrophobic Contaminants. *Environmental Science & Technology* **2007**, *41*, (22), 7759-  
571 7764.
- 572 57. Betts, K., Why small plastic particles may pose a big problem in the oceans. *Environmental*  
573 *Science & Technology* **2008**, *42*, (24), 8995-8995.
- 574 58. Lithner, D.; Larsson, Å.; Dave, G., Environmental and health hazard ranking and assessment  
575 of plastic polymers based on chemical composition. *Science of The Total Environment* **2011**, *409*,  
576 (18), 3309-3324.
- 577 59. Oehlmann, J. r.; Schulte-Oehlmann, U.; Kloas, W.; Jagnytsch, O.; Lutz, I.; Kusk, K. O.;  
578 Wollenberger, L.; Santos, E. M.; Paull, G. C.; Van Look, K. J. W.; Tyler, C. R., A critical analysis of the  
579 biological impacts of plasticizers on wildlife. *Philosophical Transactions of the Royal Society B:*  
580 *Biological Sciences* **2009**, *364*, (1526), 2047-2062.
- 581 60. Urrère, M. A.; Knauer, G. A., Zooplankton fecal pellet fluxes and vertical transport of  
582 particulate organic material in the pelagic environment. *Journal of Plankton Research* **1981**, *3*, (3),  
583 369-387.

- 584 61. DeMott, W. R.; Watson, M. D., Remote detection of algae by copepods: responses to algal  
585 size, odors and motility. *Journal of Plankton Research* **1991**, *13*, (6), 1203-1222.
- 586 62. Dagg, M., Some effects of patchy food environments on copepods. *Limnology and*  
587 *Oceanography* **1977**, 99-107.
- 588 63. Andrady, A. L., Microplastics in the marine environment. *Marine Pollution Bulletin* **2011**, *62*,  
589 (8), 1596-1605.
- 590 64. Doyle, M. J.; Watson, W.; Bowlin, N. M.; Sheavly, S. B., Plastic particles in coastal pelagic  
591 ecosystems of the Northeast Pacific ocean. *Marine Environmental Research* **2011**, *71*, (1), 41-52.

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