Turning Microplastics into Nanoplastics: Digestive Fragmentation by Antarctic krill

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Abstract

Microplastics (plastics <5mm diameter) are at the forefront of current environmental pollution research. However, little is known about the degradation of microplastics through ingestion. Here, by exposing Antarctic krill (*Euphausia superba*) to microplastics under acute static renewal conditions, we show the first observations of physical size alteration to microplastics ingested by a planktonic crustacean. Ingested microplastics (31.5µm) were fragmented into pieces (<1µm diameter). Previous feeding studies have shown spherical microplastics either; pass unaffected through an organism and are excreted, or are sufficiently small for translocation to occur. We identify a new pathway; microplastics were fragmented into sizes small enough to cross biological barriers, or were egested as a mixture of triturated particles. These findings suggest that current laboratory-based feeding studies may be oversimplifying interactions between zooplankton and microplastics but also introduces a new critical role of this, and potentially other species, in the global biogeochemical cycling and fate of plastic.

Microplastics (plastics <5mm) have been isolated from biota representing the full spectrum of feeding mechanisms, habitats, and trophic levels from zooplankton to megafauna[1]. Marine microplastics are attributed to two main sources; the direct release of micro sized plastic particles into the environment and the *in situ* environmental breakdown of larger plastics. Microplastics are prevalent in the marine environment and degradation occurs continuously on unknown timescales until the polymer is completely mineralized into carbon dioxide, water and biomass[2]. All microplastics are expected to continue fragmenting, thus reaching nano sizes (<1µm). Thus microplastics in the environment are heterogeneous in size and in shape[3], and consequently present a challenge for standardized monitoring[1].

Planktonic suspension and filter feeders may be the most susceptible to microplastic ingestion due to the relatively indiscriminate nature of this feeding strategy[4]. In particular, polyethylene (PE), polypropylene (PP), and expanded polystyrene (PS) are all less dense than seawater, making them buoyant and available to planktonic species[5]. Detrimental health effect have been associated with physical obstruction of the digestive system and associated reduced nutritional condition[6].

Laboratory-based feeding studies are a commonly used approach for the quantification of exposure and associated effects. Often these studies use invertebrate species such as zooplankton, which form the basis of the pelagic food web. Ingestion at this level therefore carries a threat of possible plastic bioaccumulation and biomagnification to higher trophic levels[1]. Organisms are exposed to relatively homogenous, commercially available, plastic beads to replicate environmental condition[3]. Such studies have confirmed numerous planktonic species are capable of ingesting and egesting microplastics[7-11], many of which were associated with toxic and physiological effects[2,12-15]. Despite a growing body of exposure and affect assessments, the ecological consequences of microplastic ingestion by zooplankton remain unclear. Further, the fate and degradation of microplastics, as a consequence of ingestion is rarely considered.

Here we expose Antarctic krill (*Euphausia superba,* hereafter 'krill'), a keystone species in the Antarctic ecosystem, to polyethylene microbeads (27 -32µm diameter) along with an algal food source to determine the fate of microplastics ingested by a planktonic crustacean of high dietary flexibility and ecological importance. Krill predominantly feed on silica diatoms but regularly prey on other zooplankters including salps, copepods and other krill[16]. In terms of biomass, Antarctic krill are extremely abundant, supporting a large number of Southern Ocean predators[17,18] and are a predominant phytoplankton grazer in the Southern Ocean[18,19]. Krill filter feed by forming a feeding basket through which water is passed (Fig. S1A). Food particles are retained on the basket and then transported to the mandibles for mastication[20,21]. The mandible, situated at the base of the oesophagus, is equipped with a cutting and grinding surface[22]. Food is then directed through the short oesophagus into the stomach and gastric mill where it is mixed with digestive enzymes for further mastication[23,24]. Thereafter, particles smaller than the primary filter (0.144 µm) pass through to the digestive gland, and larger particles are

74 directed to the mid and hind gut for egestion[25]. Egested particles are encased in a peritrophic membrane
75 which protects the mid and hind gut from abrasion[26].

76 The digestive gland is the primary site for cellular digestion (Fig S1C)). The gland is made up of groups
77 of blind ending tubules, which are comprised of epithelial cells. Food particles that enter the digestive
78 gland are pumped into the tubules, where digestive enzymes are directly released, thus allowing for
79 nutrient adsorption and intracellular digestion to take place[26-28].

80 Exposed krill and their faecal material were examined microscopically to (1) quantify the size of
81 particles present in the krill digestive system and in egested material, (2) identify where these particles
82 are localised within the digestive system, and (3) examine the effect of particle size on egestion. We
83 find that Antarctic krill are capable of fragmenting pristine PE microbeads into significantly smaller
84 fragments. This is the first report of nanoplastics generated by the ingestion of microplastics in a marine
85 species.

86 **Results**

87 **Antarctic krill fragment ingested virgin polyethylene**

88 To determine the effect of ingestion on microplastic beads we exposed krill to a 4 day static renewal
89 assay, which incorporated daily feeding on two (low - 20% and high - 80%) PE microplastic and algal
90 diets. Krill were exposed daily for 4 hours to their diet; this was followed by 20 hours in clean seawater.
91 Whole krill were enzyme digested after exposure to isolate the ingested microplastics, as was faecal
92 material collected throughout the experiment. We compared the size distribution of particles from the
93 stock suspension to the distribution of particles within the krill and egested faecal pellets. We found all
94 krill contained a mixture of whole PE microplastic beads and PE fragments that was not consistent with
95 the exposure stock. Beads in the stock suspension had a mean diameter of 31.5µm (± 7.6 Standard
96 Deviation, S.D), whereas the mean particle size isolated from within the krill was, on average, 78%
97 smaller than the original beads (7.1µm ± 6.2 S.D), with some fragments reduced by 94% of their original
98 diameter. Particles isolated from faecal material were also reduced (6.0 µm ± 5.0 S.D). Further, the size
99 distribution of particles within the krill, and excreted particles, were significantly different to beads in
100 the exposure stock (D = 112, $p<0.001$ and D = 113, $p<0.001$ respectively) (Fig. 1A, Fig. S3A). The
101 reduced plastic particle size found in krill and their faecal pellets revealed that Antarctic krill were
102 physically fragmenting beads after ingestion. We found no relationship between krill size and their
103 ability to fragment plastics ($F_{3,15}=2.595$, $p>0.05$, $R^2=0.357$).

104 To ensure that the homogenization process was not responsible for fragmenting the beads we carried
105 out procedural blanks. These consisted of whole krill enzyme digested, beads enzyme digested and
106 beads not subjected to any digestion or homogenization. Beads were unaffected by the sample analysis
107 procedures, neither the homogenisation process nor the digestion enzymes were responsible for
108 fragmenting the beads.

109 Particles from the krill and bead blanks were found to be unaffected by the enzyme digestion protocol.
110 Visually, beads from the stock suspension appeared similar to the bead blanks. As did the whole beads
111 and fragments isolated from the krill and krill blanks. The distributions of particle sizes from
112 experimental and blank samples were very similar, despite unequal sample sizes (Fig. S3). Overall it
113 was determined that krill were responsible for fragmenting the beads.

114 **Repeated exposure decreases fragmentation**

115 Notably, not all ingested beads were fragmented in the current study. To further explore this observation
116 we compared the proportion of fragments to whole beads isolated from whole krill homogenates and
117 faecal pellets exposed to the high and low treatments. The proportion of fragmented beads egested by
118 the krill on days 1 and 4 were compared to assess the effect of repeated exposure. An extra sample point
119 was added on day 4 to assess fine scale temporal variation within a daily cycle after repeated exposure.

120 Whole beads were found in the stomach and midgut content, as well as faecal pellets. Exposure
121 concentration played an important role in the ability of krill to fragment the PE beads; where lower
122 plastic concentration appeared to facilitate the krill's capacity to triturate plastic. Krill contained
123 significantly more whole beads when exposed to a high plastic diet than a low plastic diet (X^2_1 = 323,
124 (N = 67476), p <0.001) (Fig. 1B). Faecal pellets also followed this trend (X^2_1 = 600, (N = 54670), p
125 <0.001). Further examination revealed a significant interaction between time, dose, and the proportion
126 of fragmented plastic ($F_{(1,45778)}$,= 328, p<0.001). Increased dose and repeated exposure appeared to
127 inhibit the ability of krill to triturate plastic. Faecal pellets of high dose krill collected after the first day
128 of exposure, contained a lower proportion of whole beads than faecal pellets collected after the final
129 day of exposure (X^2_1 =384, (N =27317), p <0.001) (Fig. 3A). Whereas, when comparing the first and
130 last day of exposure, krill exposed to low dose plastic appear capable of fragmenting plastics
131 irrespective of repeated expose (X^2_1 = 2, (N = 18465), p >0.05) (Fig. 3A). Faecal pellets of high dose
132 krill collected at 4 and 24 hours on the last day of exposure clearly show an increasing trend of whole
133 beads being egested over the final 24 hours (X^2_1 = 238, (N = 24828), p <0.001) (Fig. 3B). The low and
134 high dose krill both exhibit similar proportions of egested whole beads at 4 hours on the last day of
135 exposure. However, where the high dose krill appear to decrease their ability to fragment plastics over
136 time, the low dose krill exhibited the opposite trend over the final 24 hours. Krill exposed to the low
137 dose egested a higher proportion of fragments suggesting more efficient fragmentation (X^2_1 = 5, (N
138 =17175), p =0.018). Overall it appeared that krill at the beginning of each daily pulse exposure were
139 efficient at fragmentation, as krill ingested more beads the fragmentation decreases.

140 **Tissue localisation of fragments**

141 To further investigate plastic fragment kinetics within the organism, histological cryosections of
142 exposed krill were prepared. We observed microplastics within the esophagus, stomach, digestive gland
143 and midgut of deceased krill (Fig. 2, Fig. S1). Plastic were also visible in the stomach of live krill.

144 Mandibles frequently had plastic fragments enmeshed in the grinding surface. The bulk of plastic
145 maceration presumably took place in the stomach and gastric mill, which is responsible for
146 mechanically fragmenting food particles under normal feeding conditions. Due to their predominantly
147 herbivorous diet, Antarctic krill have complex digestive enzymes with high activity[18]. In this study we
148 did not examine the effects of digestive enzymes on microplastics thus cannot rule out the possibly that
149 digestive enzymes did not contribute to the fragmentation displayed in this study. Small food items then
150 pass through a filter then pass through a filter (approximately 0.14µm) into the digestive gland. Thus,
151 large plastic fragments and full sized beads were excluded from the digestive gland and directed to the
152 midgut for excretion.

153 Microscopic limitations precluded a comprehensive investigation into the size and abundance of
154 fragments found in the digestive gland. However, we detected particles in the digestive gland of two
155 out of the five krill examined, within an approximate size range of 150 - 500nm. The digestive gland is
156 responsible for the absorption of digested material into the haemolymph[29]. The presence of PE
157 fragments in the digestive gland revealed krill triturate PE beads to colloidal sizes, which increases the
158 capacity for crossing biological barriers[30].

159 **Size dependent egestion**

160 To examine egestion, we exposed krill to low dose plastic for 10 days, after which their diet was
161 swapped to 100% algae. Faecal pellets were collected for 5 days following the diet change. Small
162 triturated fragments were more persistent and retained within the krill's body for longer than large
163 beads. The proportion of whole beads excreted by krill decreased significantly throughout the egestion
164 period ($X^2_4 = 16$, (N = 21525), p =0.003), with whole beads no longer excreted after three days following
165 the diet change (Fig.4). Fragments were present in faecal material throughout all samples. This finding
166 corresponds well with previous observations of size dependent egestion in marine invertebrates, both
167 in laboratory and wild caught species[31-33].

168 **Discussion**

169 Despite a growing body of research, there are still considerable knowledge gaps regarding spatial
170 patterns and abundance of microplastics in the marine environment. The paucity of studies concerning
171 microplastic ingestion in wild caught zooplankton hampers comparisons to this study. Microplastics
172 isolated from euphausiids and other zooplankton have been found to range in size from 123 µm to
173 ≤2000 µm[7,34], which is more than two orders of magnitude larger than the bead fragments Antarctic
174 krill were found capable of producing in this study.

175 The phenomena of digestive fragmentation has never before been observed in other planktonic
176 crustaceans, such as copepods or isopods, despite the fact that many of which possess similarly
177 developed gastric mills and mouthparts designed for mechanical disruption[29]. However, copepods are
178 theorized to scrape biofilms from the surface of pelagic plastics, inadvertently consuming liberated

plastic fragments[35]. We hypothesise the absence of this observation in other planktonic laboratory crustaceans may be due to the use of different polymers in experiments. Two of the most commonly used laboratory plastics for feeding studies, PE and PS, differ in mechanical properties. The more commonly used PS is a rigid plastic, with a higher capacity to withstand stress than PE[36].

Regardless of their original polymer properties, marine microplastics are largely comprised of secondary plastics, derived from the breakdown of larger plastic items[3,37]. These secondary plastics are subject to weathering and chemical degradation rendering them physically and chemically altered from virgin plastics, such as those used in this study. Weathering serves to reduce the mechanical strength of plastics which leaves them brittle[38,39]. Whilst the capacity of zooplankton to fragment secondary plastics requires further study, we suggest that embrittlement of secondary plastics will facilitate digestive fragmentation. We hypothesize fragmentation of microplastics after ingestion may be more common in the environment than the published literatures currently demonstrate. Previous observations of crabs altering laboratory degraded fibres after ingestion offer weight to this hypothesis[40].

Nonetheless without further testing on other polymers and microplastic particles with varying degrees of degradation, it is difficult to speculate the frequency at which microplastic fragmentation in the environment could be occurring. Polyethylene is one of the most common plastic in the marine environment[41,42], thus even if this phenomenon is restricted wholly to PE; it still could present a significant pathway of microplastic degradation in the marine environment.

In general, polyethylene has a low resistance to UV degradation and recent studies have identified that PE microplastics collected from the North Atlantic subtropical gyre were considerably weathered, with shorter polymer chain lengths, reduced molar mass and were more crystalline than reference PE[39]. Glassy polymers such as PS or Polyethylene terephthalate (PET), however, are stronger and less susceptible to UV degradation [38]. Despite the properties of pristine polymers, all plastics, even those with chemical stabilisers, will eventually degrade in the environment.

The low exposure concentration applied in this study was within the same order of magnitude as microplastic concentrations observed in pelagic systems of the North Pacific Subtropical Gyre[2], which are among the highest concentrations reported globally. Limited pelagic microplastics surveys from the Southern Ocean isolated between 0.0032 and 1.18 particles m^{-3}[43,44], these levels are considerably less than those used in this experiment. In spite of the elevated exposures used in these experiments, considerable bead fragmentation was achieved. These preliminary findings, although limited by scarce environmental data related to plastic <330μm in natural marine systems, suggest that current concentrations may be within the bounds of optimal trituration for krill, but fragmentation efficiency may be affected by chronic exposure. The increased fragmentation of plastic noted at low exposure conditions gives further weight to our hypothesis that digestive fragmentation is more common in the environment than recorded in current literature, which often use similarly high exposure concentrations

214 for exposure experiments[3]. Current contamination levels in the Southern Ocean are theoretically low
215 enough to promote efficient digestive fragmentation by krill species, and in a global context, possibly
216 for other zooplankton with sufficiently developed gastric mills.

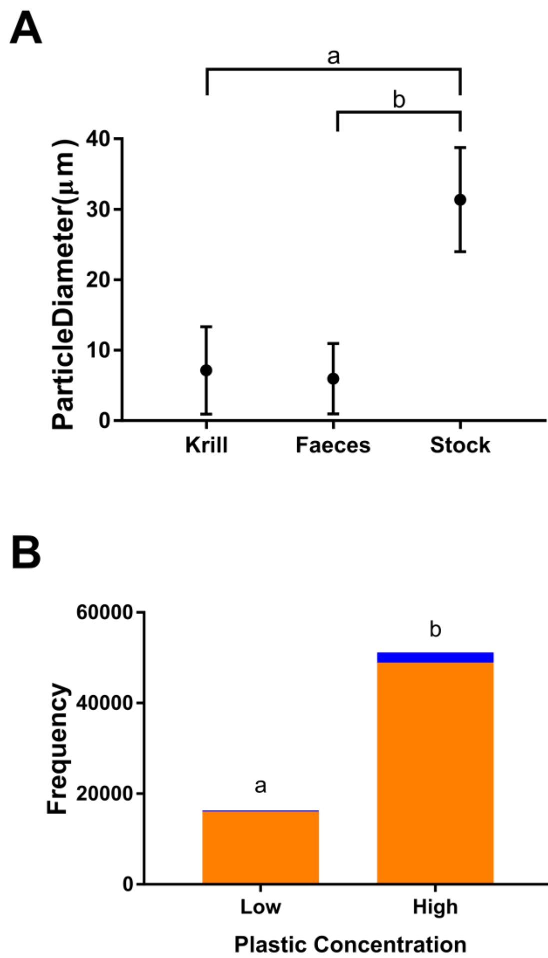
217 We did not examine these fragmented particles for induced toxicological effects. Several laboratory
218 studies have demonstrated the ability of micro and nanoplastics to translocate to the haemolymph[45-47],
219 however in these studies, the exposure particle size was sufficiently small to achieve translocation. We
220 identify the potential for translocation to occur after an organism has physically altered the ingested
221 plastics. This reveals a previously unidentified dynamic in the plastic pollution threat, with the
222 implication that biological fragmentation of microplastics to nanoplastics is likely widespread within
223 most ecosystems. As such, the harmful effects of plastic pollution must take into consideration not only
224 the physical effects to the individual of macro and microplastic ingestion, but also the potential cellular
225 effects of nanoplastics and the ecosystem impacts of biomagnification hereof. The effect of nanoplastics
226 on crustaceans is unknown, although previous studies observed PE microbeads to induce genotoxicity
227 and immunological effects in haemocytes[47].

228 Previous studies have suggested relatively simplistic interactions between microplastics and
229 zooplankton [7,9,10] and biota-facilitated plastic degradation (considered to be predominantly undertaken
230 by microorganisms) is currently considered negligible in the marine environment[2]. However, our results
231 bring into question these previous conclusions. The fate of these altered particles, after egestion, death
232 or predation is completely unknown, and is not necessarily comparable to non-ingested particles.
233 Studies that neglect these interactions may be neglecting a significant pathway of degradation.
234 Interestingly, ter Halle, et al. [48] recently showed that smaller microplastics are fragmented faster than
235 larger particles under environmental conditions. The repercussions of organisms accelerating this
236 process deserve further study.

237 It is also possible that fragmentation resulted from, or was enhanced by, the presence of silica diatoms
238 in the diet. The churning and grinding action of the gastric mill combined with the sharp edges of
239 triturated algae may have fragmented the beads. This could explain the decreased fragmentation in the
240 high exposure treatments, where there was a correspondingly lower algal concentration. However, this
241 mechanism does not explain the temporal variation in fragmentation efficacy with repeated exposure,
242 as krill diet within treatments remained constant over time. Thus fragmentation may have been
243 enhanced by the presence of silica diatoms but it was unlikely to be the sole cause of fragmentation.

244 This study uncovered the ability of an Antarctic keystone species to physically change ingested
245 microplastics in a manner not previously described and in doing so, provides evidence for biologically
246 facilitated production of nanoplastics. We hypothesise fragmented microplastics have increased
247 potential for interaction at the molecular level, as seen in other nanoplastic studies[12], and this warrants
248 significant attention to nanoparticle toxicology in the discussions surrounding global plastic pollution.

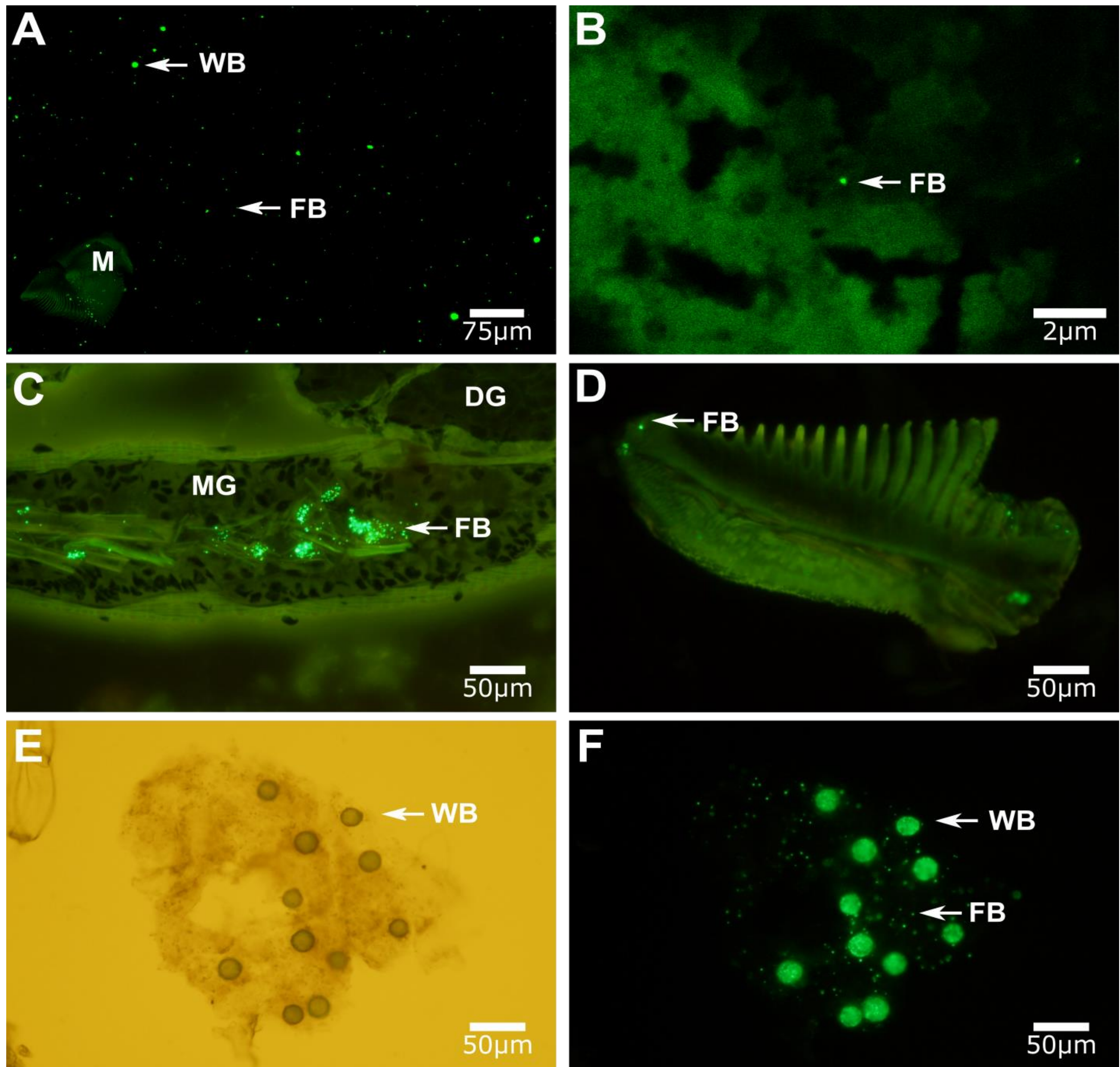
249 Triturated microplastics will likely impact potential particle bioavailability and biomagnification, and
250 likely influence the timescales needed for complete mineralisation.



251

252 **Fig. 1**. Size of polyethylene particles A) Microplastic particle size (mean ± S.D) in all sample
253 types: whole krill homogenates, egested faecal pellets, and in the exposure stock suspension,
254 B) Frequency of whole beads (Blue) and fragments (Orange) isolated from Antarctic krill

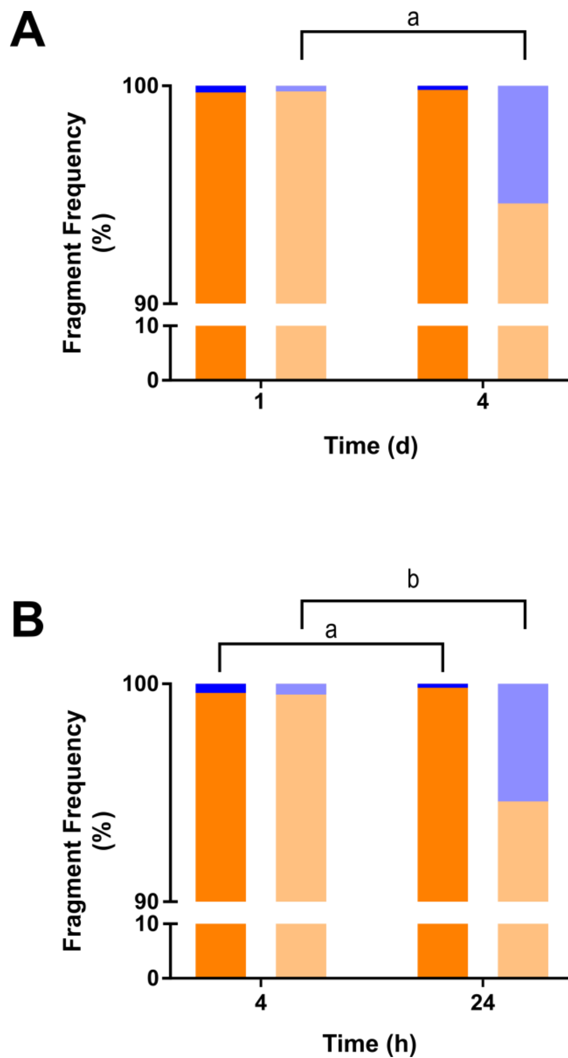
255 (*Euphausia superba*) exposed to a Low (20%) (n= 9) and High (80%) (n=9) plastic
256 concentrations. Letters denote statistically significant differences ($p<0.05$)



257

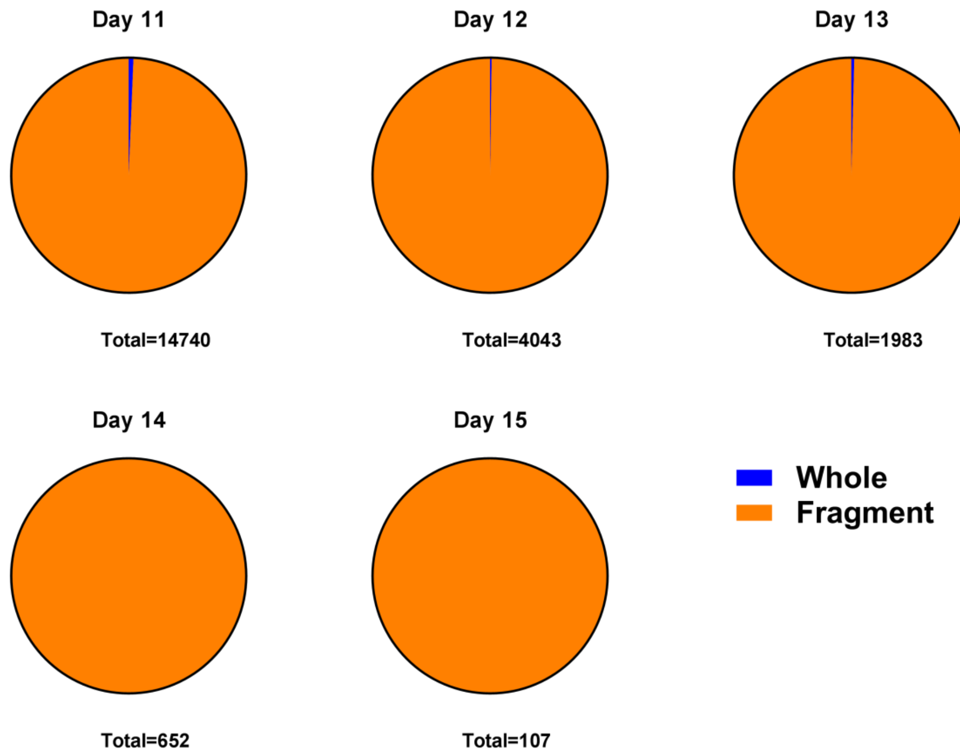
258 **Fig. 2.** Fate of polyethylene beads and fragments after ingestion by Antarctic krill (*Euphausia*
259 *superba*). Krill (n=17) were used for histological analysis A) Beads on a filter paper isolated
260 from digested krill with autofluorescent mandible, B) Digestive gland tissue, C) Midgut and
261 digestive gland tissue, D) Mandible with polyethylene fragments embedded in the surface, E)

262 and F) Faecal pellet with polyethylene beads under bright field and fluorescence microscopy.
263 WB Whole Bead, FB Fragmented Bead, M Mandible, DG Digestive Gland, MG Midgut



264

265 **Fig. 3.** Frequency of whole (Blue) and fragmented (Orange) particles isolated from faecal
266 pellets of Antarctic krill (*Euphausia superba*) exposed to Low (20% - n=3 beakers) (Dark)
267 and High (80% - n=3 beakers) (Light) concentrations at: A) 24h on Day 1 (n=6) and Day 4
268 (n=6), B) 4 (n=6) and 24h (n=6) on Day 4 only. All faecal material per beaker (containing 5
269 krill) was pooled to form a single sample per time point per dose. Letters denote statistically
270 significance differences in the proportion of whole beads excreted over time ($p<0.05$).
271



272

273 **Fig. 4.** The proportion of PE plastic fragments (Orange) to whole beads (Blue) isolated from
274 Antarctic krill (*Euphausia superba*) (n=15 krill) faecal material collected over 5 days, after
275 switching from 10 days of low dose microplastic exposure, with daily static renewal, to a
276 clean algae diet. Total refers to the total number of particles measured in each 24 hour period
277 of faecal material.
278

279 # Methods

280 A microplastic feeding stock suspension was made from commercially available (Cospheric LLC CA,
281 USA - UVPMS-BG-1.025) fluorescent green polyethylene microbeads (27-32µm diameter, 1.030 or
282 1.026 g cm^{-3}). The beads were confirmed to be polyethylene by FTIR spectroscopy using a PerkinElmer
283 FTIR spectrometer (Fig. S4). The bead size range was selected to closely conformed to the size range
284 of the algal food, simultaneously offered to the krill (see below). Density was selected to be close to
285 neutrally buoyant in 0°C seawater. The physical properties of the microbeads were characterised using
286 images of beads subsampled from the feeding stock (see Sample Analysis section below).

287

288 ## Exposure Design

289 Mixed sex Antarctic krill were collected from the Southern Ocean (66.33 S, 59.34 E) in the Austral
290 summer of 2014/2015. Krill were maintained in the Marine Research Facilities at the Australian
291 Antarctic Division, Tasmania according to previously established methods until use in experiments [49].
292 Adult krill (n=65, wet weight: 0.556 ± 0.117mg, length: 41.1 ± 3.7mm; were acclimatised for 24hrs
293 prior to the start of experiments in 5L glass beakers. Krill were randomly selected for use in the
294 experiment from apparently healthy free swimming schooling adults. Krill were collected into buckets
295 by repeatedly dipping a small net into the same region of the tank as the krill schooled anticlockwise.
296 Buckets contained 15 krill; these were randomly distributed amongst beakers, so each beaker contained
297 five adult krill in 4 L seawater. Block randomisation was applied to distribute krill amongst treatments.
298 The sex of individuals was not determined in the experiment. Seawater temperatures were maintained
299 at 0°C (± 0.5) and beakers were kept in total darkness throughout the experiment. Exposure seawater
300 was collected from Bruny Island, Tasmania, and filtered to 0.2 µm. Filtered seawater was pre chilled to
301 0°C (±0.5) before krill were added. The dietary exposure suspension was prepared daily from stock
302 using fluorescent plastic microbeads with concentrated instant non-viable algae *Thalassiosira*
303 *weissflogii* (Reed Mariculture Inc, CA, USA). The size range for *T. weissflogii* cells was 5-20 µm
304 according to the manufacturer. Although this is slightly smaller than the microplastics beads, Antarctic
305 krill can feed efficiently on particles >2 µm up to whole zooplankton (~3 mm). Dietary exposure
306 suspensions were made up as a portion of the krill's dietary requirements under laboratory conditions,
307 100% algae equates to 0.00798 mg *T. weissflogii* (dry weight) per beaker. Harvested krill were
308 euthanized in liquid nitrogen or formalin. The seawater physiochemical parameters for the two
309 experiments are outlined in Tables S1 and S2.

310

311 ## Particle Size Experiment

312 Four day feeding and egestion experiments were carried out on 45 Antarctic krill. Nominal daily
313 exposure suspensions were made up to 20% or 80% microplastics by weight, which equated to
314 approximately 29 or 116 beads mL^{-1} (402 or 1606µg L^{-1}). Krill were transferred daily to exposure

315 suspension and allowed to feed for 4 hours, before being transferred with a stainless steel dip net to a
316 clean beaker for 20 hours. Before transfer, krill were flushed with cold fresh filtered seawater to remove
317 plastics that may adhere to the exoskeleton. Upon transfer to the exposure suspension, krill were
318 observed to be feeding almost immediately. Control krill were fed 100% algae. Faecal pellets were
319 collected after 24 hours exposure on days 1 and 4. An extra sample point was added on day 4 to assess
320 fine scale temporal variation after repeated exposure, thus faecal pellets were collected at 4 and 24 hours
321 on day 4 (refer to SI Figure 2). All beakers of krill were harvested for particle size and tissue localisation
322 analysis after 96hrs. Three krill from each beaker were randomly selected for particle size analysis
323 (n=18 krill). As the beads were fragmented after ingestion, the total bead ingestion rates of could not
324 be calculated from stomach content or egested material.

325 **Tissue Localisation Experiment**

326 To investigate tissue localisation of ingested plastic, two krill from each beaker were randomly selected,
327 fixed in formalin, and used for histological cryo-section (20µm) analysis. Slides were stained with H&E
328 or remained unstained**.** Slides were examined using an Olympus BX60 fluorescence microscope or
329 Zeiss-780 Laser Scanning Confocal microscope with a fluorescent filter of 488 nm excitation and 526
330 nm emission.

331 In addition, to investigate if krill could fragment plastics <1µm and the possibility of fragments entering
332 the digestive gland, five krill were exposed to 100% plastic diet (approx. 2063µg L^{-1} or 149 beads mL^{-}
333 ^{1}) for 24hrs, with no water changes. These 5 krill were all used for tissue localisation analysis.

334 **Egestion Experiment**

335 To examine particle sizes egested over an extended period, 15 krill divided into 3 beakers were exposed
336 for 10 days to a 20% diet (approx. 401µg L^{-1}) of plastic following the same basic design as the Particle
337 Size Experiment. After 10 days, the diet was switch to 100% algae for five days. Faecal pellets were
338 collected at 4 and 24 hours every day of the five day egestion period. Faecal material was pooled per
339 beaker per 24 hours resulting in 15 samples.

340 **Sample Analysis**

341 Body burden analysis was carried out using an enzyme digestion followed by visual identification of
342 ingested microplastics under a fluorescent microscope. Krill were flushed with milli-q water, blotted
343 dry, weighed (to 3 d.p), and heated to 65°C in a water bath, after which the exoskeleton was removed.
344 Krill were then homogenised using a glass rod, and digested using proteinase K *adapted from* [50], which
345 was previously shown to have negligible effects on PS bead integrity. Digestion efficacy was not
346 optimal as hard chitinous structures often remained after digestion. Digested krill were filtered under
347 vacuum onto Millipore gridded 0.45µm filters and air dried overnight. Filters were fixed between glass
348 coverslips and analysed for microplastics using a Zeiss-780 Laser Scanning Confocal microscope with
349 a fluorescent filter with a Plan-Apochromat 10X/0.45 M27 lens, with a numerical aperture of 0.45.
350 Microplastic fragments were imaged in five randomly selected squares (6.97 × 6.98 mm; total area of

351 2.4 cm^2) on the filter paper, which accounted for 25% of the total filtered area. Images were verified by
352 eye, and compared to controls to examine for undigested chitinous material with autofluorescence. Of
353 the 165 images taken, 2 images were excluded on the basis of chitinous material with autofluorescence
354 (See Fig 2. panel A for example of excluded image). These were too large to be mistaken as a
355 microplastic beads and were clearly distinguishable as mandibles. The diameter (major axis when
356 particles were fitted to an ellipse) of each particle within each image was measured using imaging
357 software (FIJI GPL v2)[51]. A minimum threshold was applied to the fluorescence intensity of each image
358 to ensure only beads were counted by the imaging software. Thresholds were set to a minimum of 65
359 and maximum of 255 which allowed background material, including undigested exoskeleton (except
360 for mandibles), algal cells and the filter paper, to be excluded without interference to the analysis. Size
361 exclusions were applied to particles which had a diameter >50µm, on the basis these were 2 or more
362 beads too close together for the imaging software to distinguish individual beads and accurately measure
363 size.

364 **Bead fragmentation**

365 To test that the sample analysis procedures were not responsible for fragmenting the beads, procedural
366 blanks were carried out in a pilot study and throughout the experiment. Procedural blanks consisted of
367 krill and beads or just beads. Krill blanks consisted of 7 krill taken from a pilot study. The krill were
368 digested as per the method described in the Sample Analysis section, except the krill were not
369 homogenised. After digestion, exoskeleton remained intact but the tissue was completely digested, krill
370 were vortexed and the stomach was opened to liberate any remaining beads. The sample was then
371 filtered and imaged as per the method described in the Sample Analysis section. Bead blanks consisted
372 of beads in the absence of krill and were not homogenised. Beads were added to buffer and enzyme,
373 then digested, filtered and imaged as per methods outlined in the Sample Analysis section. Bead blanks
374 were examined after enzyme digestion with FTIR spectroscopy (Fig. S5), but ingested beads and
375 fragments were unable to be detected on the cellulose filters with FTIR due to the low concentration
376 and/or small size of the particle.

377 **Statistical Analysis**

378 Two-sample Kolmogorov-Smirnov Tests (two tailed, $\alpha \leq 0.05$) were used to compare the
379 particle size distribution from the stock microbeads to the size distribution of plastics isolated
380 from the digested krill, and from the particles isolated from the faecal pellets. The proportion
381 of whole beads compared to fragments in digested krill and in faecal pellets was compared
382 between doses using Chi squared analysis (two tailed, $\alpha \leq 0.05$). For all proportion tests (Chi
383 squared and linear regression), beads with a diameter ≥25µm were classified as whole beads,
384 beads <25µm were considered fragments. This cut off was selected by eye using the standard
385 distribution of the stock beads. Kolmogorov-Smirnov Tests (two tailed, $\alpha \leq 0.05$) were used to
386 test for normality. The data was $\log_{10}$ transformed and comparison between fragment size,

387 sample time and plastic dose in the faecal pellets was determined with a two-way ANOVA
388 (two tailed, $\alpha \leq 0.05$). Multiple linear regression was used to examine relationships between
389 the length and weight of the krill and their ability to fragment plastics. Means are expressed as
390 mean ± standard deviation (S.D) unless otherwise stated.

391 **Data Availability**

392

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400 means. A.D and C.K preformed laboratory experiments. R.K and A.D interpreted histological data.
401 W.H and A.D designed image analysis methods. A.D analysed the data. A.D prepared the manuscript,
402 all authors contributed substantially to revisions.

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