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Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal.

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31 **Title:** Low Levels of Microplastics (MP) in Wild Mussels Indicate that MP Ingestion by Humans is Minimal
32 Compared to Exposure via Household Fibres Fallout During a Meal

33

34 **Abstract:**

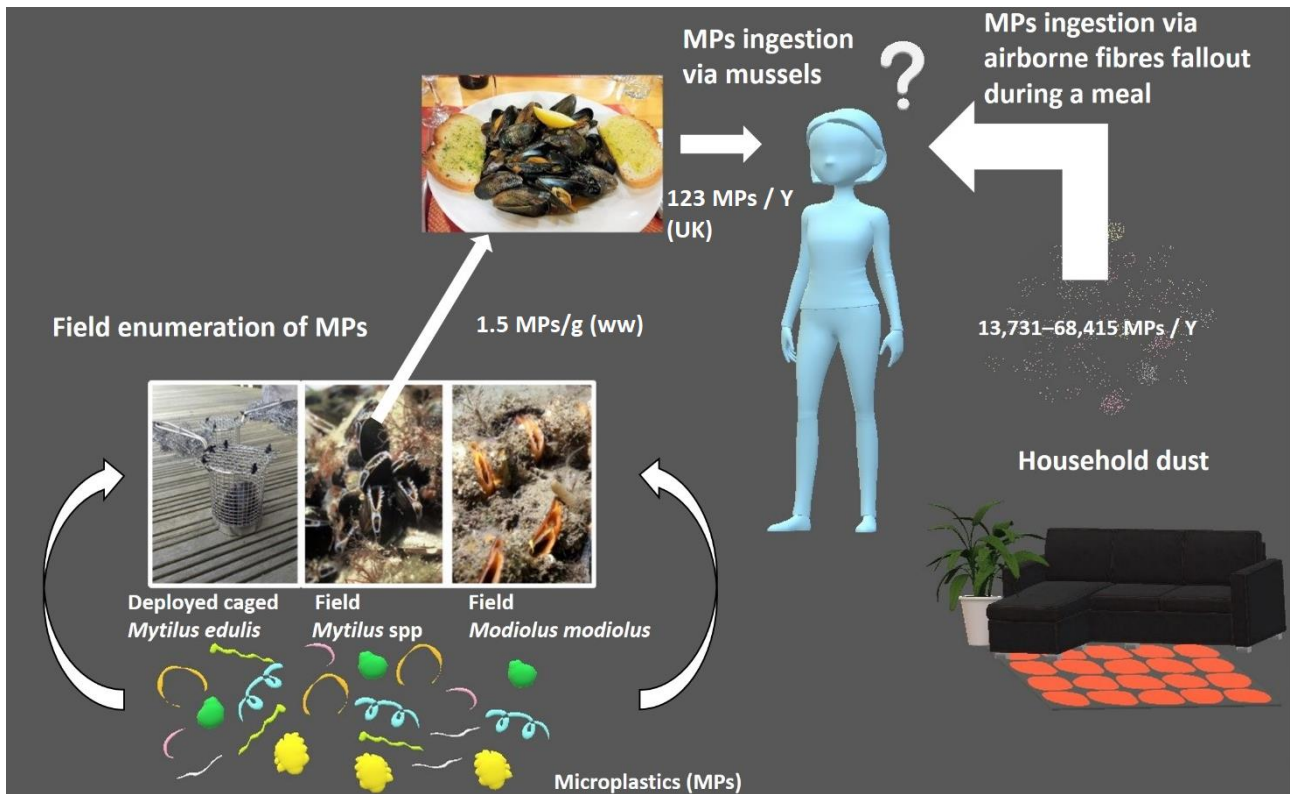
35 Microplastics (MPs) are the most numerous debris reported in marine environments and assessment of
36 the amounts of MPs that accumulate in wild organisms is necessary for risk assessment. Our objective was
37 to assess MP contamination in mussels collected around the coast of Scotland (UK) to identify
38 characteristics of MPs and to evaluate risk of human exposure to MPs via ingestion of mussels. We
39 deployed caged mussels (*Mytilus edulis*) in an urbanised estuary (Edinburgh, UK) to assess seasonal changes
40 in plastic pollution, and collected mussels (*Mytilus* spp and subtidal *Modiolus modiolus*) from eight
41 sampling stations around Scotland to enumerate MP types at different locations. We determined the
42 potential exposure of humans to household dust fibres during a meal to compare with amounts of MPs
43 present in edible mussels. The mean number of MPs in *M. modiolus* was 0.086 ± 0.031 (SE, n=6) / g ww (3.5
44 ± 1.29 (SE) per mussel). In *Mytilus* spp, the mean number of MPs/g ww was 3.0 ± 0.9 (SE, n=36) (3.2 ± 0.52
45 (SE) per mussel), but weight dependent. The visual accuracy of plastic fibres identification was estimated to
46 be between 48 - 50 %, using Nile Red staining and FT-IR methodologies, respectively, halving the observed
47 amounts of MPs in wild mussels. We observed an allometric relationship between the number of MPs and
48 the mussels wet weight. Our predictions of MPs ingestion by humans via consumption of mussels is 123 MP
49 particles/y/capita in the UK and can go up to 4,620 particles/y/capita in countries with a higher shellfish
50 consumption. By comparison, the risk of plastic ingestion via mussel consumption is minimal when
51 compared to fibre exposure during a meal via dust fallout in a household (13,731–68,415
52 particles/Y/capita).

53

54 **Keywords:** Microplastics; Mussels; Fibres, Field Assessment; Airborne Household Dust

55

56 **Summary:** Low levels of microplastics (MP) in wild mussels indicate that ingestion by humans is minimal
57 compared to exposure via household fibres fallout. MP load in wild mussels depends on individual weight.

58 **Graphic abstract:**

59

60

61 **Highlights:**

- 62 1. Report of the first field assessment of microplastics (MPs) using caged deployed mussels (*Mytilus*
- 63 *edulis*)
- 64 2. First report of the presence of MPs in the protected mussel species *Modiolus modiolus*
- 65 3. The number of MPs per mussel wet weight is size-dependent (allometric relationship), and non-
- 66 normalization of the number of MPs per mussel weight change data interpretation
- 67 4. The potential for human ingestion of fibres resulting from household dust is higher than the
- 68 ingestion of fibres via mussel consumption

69 1. Introduction

70

71 Increasing levels of plastic debris are among the most prominent environmental issues faced by
72 government agencies worldwide (e.g. House of Commons, 2016). Small pieces of plastic [1 μm – 5 mm,
73 microplastics (MPs) (Arthur et al., 2009; Browne et al., 2007)] are the most numerous debris reported in
74 marine environments (Eriksen et al., 2013), and contamination by these particulates can present a hazard
75 for aquatic organisms (Cole et al., 2015; Wright et al., 2013). Ingestion of MPs by organisms can facilitate
76 MP exposure across trophic levels (Farrell and Nelson, 2013), including a potential for human exposure via
77 consumption of shellfish (Galloway, 2015). The transfer of small-sized MPs from the lumen of the
78 gastrointestinal tract across epithelial membranes and into internal tissues appears to be minimal (Batel et
79 al., 2016); however, further investigation is necessary, particularly to resolve potential
80 absorption/accumulation of smaller plastic particles (< 1 μm , nanoplastics) in tissues.

81 Despite the numerous concerns regarding the potential negative effects of plastic particles, the
82 establishment of baseline observations and long-term monitoring programmes are still in their early days,
83 especially relating to the use of marine biota, and are highly regional [e.g. San Francisco Bay, USA (Sutton
84 and Sedlak, 2017)]. The determination of the levels of MP contamination in targeted organisms is crucial as
85 it will allow establishment of a temporal and spatial comparison, and enable assessment of real
86 environmental and human health risks.

87 Mussels, already well established biomonitors for environmental contaminants (Andral et al., 2011;
88 Beyer et al., 2017; Kimbrough et al., 2008), are good candidates for assessment of MP exposure in the
89 environment (Beyer et al., 2017). Mussels have the ability to filter large volumes of water [e.g. 30 ml min⁻¹
90 for *Mytilus edulis*, (Clausen and Riisgard, 1996)] and actively filter and trap suspended particulates such as
91 algae and sediments (Bertolini et al., 2017; Engel et al., 2017). In laboratory experiments, the ingestion and
92 retention of MPs within their gut has been observed [72 h (Ward and Kach, 2009) to up to 96h (von Moos
93 et al., 2012)]. The enumeration of particles can thus reflect an integrated exposure over time due to MPs
94 retention either within the lumen of their digestive tract, within internal tissues, or even adherent to tissue
95 surfaces. Because of their wide geographical and spatial distribution, that includes intertidal (e.g. *Mytilus*

96 spp) and subtidal (e.g. *Modiolus modiolus*) environments, mussels can provide information on the MP
97 contamination throughout various locations. However, to our knowledge, there are no long-term
98 monitoring programmes specific for MPs contamination of mussels in place, comparable to other
99 contamination assessment programmes such as the Mussel Watch Program led by The U.S. National
100 Oceanic and Atmospheric Administration (NOAA) and the Mediterranean Science Commission (CIESM)
101 Mussel Watch.

102 The establishment of MPs baseline levels in field mussels is problematic due to the difficulty of inter-
103 studies comparisons. Currently, methods using enzymatic digestion have been developed to assess MP
104 contamination in mussels, enabling a standard quantification of MPs (Catarino et al., 2017; Courtene-Jones
105 et al., 2017). However, early works have used a variety of soft tissue digestions, some of which are
106 aggressive to pH-sensitive polymers resulting in their destruction (Claessens et al., 2013). Recently, less
107 aggressive digestion methods such as with the use of hydrogen peroxide and enzymatic digestion of soft
108 tissue have reported a maximum number of 3 particles / g wet weight (ww) of tissue in farmed (Huahong
109 2017, personal communication) *M. galloprovincialis* sampled in a food market in China (Li et al., 2015), and
110 4.44 particles / g ww of tissue in *M. edulis* from the west coast of Scotland (Courtene-Jones et al., 2017),
111 respectively. However, representative concentration of particles associated with mussels over time and/or
112 over a large geographic area are unknown.

113 Many species of the *Mytilus* genus (e.g. *M. edulis*, *M. galloprovincialis*, *M. californianus*) are of
114 substantial commercial value as seafood items (Food and Agriculture Organization of the United Nations,
115 2017), and there are concerns about the potential for MP transfer and exposure in humans via ingestion
116 (Galloway, 2015; Rochman et al., 2015; Van Cauwenberghe and Janssen, 2014). A potential load of 11,000
117 MPs per year to European shellfish consumers has been hypothesized (Van Cauwenberghe and Janssen,
118 2014), even if so far there is no evidence of the ingestion of MPs by humans through the food chain
119 (CONTAM, 2016; Galloway, 2015). Furthermore, a recent statement issued by the European Food Safety
120 Authority (EFSA) Panel for Contaminants in the Food Chain concludes that occurrence data in shellfish food
121 items is limited (CONTAM, 2016), which implies that exposure levels are largely unknown.

122 Scotland offers a privileged space to assess plastic contamination in mussels, due to the large coastline
123 (11,800 km) facing both the North Atlantic and the North Sea and the wide distribution of various mussels
124 species. Blue mussel (*M. edulis*) farming is a significant economic activity with a registered production of
125 7,732 tonnes in 2016 for the table market (Scottish Government, 2017) and the establishment of baseline
126 data on the current status of MPs contamination in Scotland will have a significant impact in conservational
127 policies. Furthermore, other species, such as the horse mussel (*Modiolus modiolus*), have a special
128 conservational status (Kent et al., 2016) and are protected in all of OSPAR regions (OSPAR Commission,
129 2009). However, there is no information on the relationship of MPs with this species. Preliminary studies
130 have shown that there is potential to use mussels to monitor the presence of MPs in the Scottish coast, and
131 MP contamination has been reported in *M. edulis* specimens from the estuary of the Forth (Edinburgh)
132 (Catarino et al., 2017) and in the west coast of Scotland (Courtene-Jones et al., 2017).

133 The aim of this project was to provide baseline information on the presence of MPs in mussels collected
134 from intertidal and subtidal locations around Scotland, and to assess temporal variation of the MPs
135 associated with *Mytilus edulis* placed in a caged field experiment. In particular, the objectives were: 1) to
136 quantify the presence of MPs in *Mytilus spp* collected at various locations along the Scottish coast, 2) to
137 assess presence of MPs in a subtidal mussel species (*Modiolus modiolus*) and 3) to assess presence of MPs
138 in caged *Mytilus edulis* placed in Edinburgh (a highly populated area) over time (1 year). This work is the
139 first to report on MPs associated with the protected species *M. modiolus* and to use displaced and caged
140 mussels (*M. edulis*) to assess MP contamination. Finally, to clarify the potential human exposure to MPs via
141 mussel consumption, when compared to other sources, we quantified the amount of airborne fibres that
142 food items contaminated within regular household spaces, during the preparation and consumption of a
143 meal. We compared the amount of MPs present within mussels with the amount of MPs that humans
144 potentially consume via airborne fibre contamination of food items within typical households in Edinburgh
145 UK.

146

147 2. Methods and Materials

148

149 2.1 Port Edgar: Caged Deployed Mussels

150

151 Live *Mytilus edulis* obtained from Scottish commercial suppliers, Scottish Shellfish Association, were
152 transferred to Heriot-Watt University (HWU), Edinburgh, UK, and maintained at 10°C in a temperature
153 controlled chamber on 12-12 h light cycle in a static seawater tank (up to 1/3 seawater renewal every
154 week) and fed a diet of live algae (a mixture of *Tetraselmis suecica* and *Tisochrysis lutea*) alternated with
155 commercial Shellfish Diet 1800® (Reed Mariculture, USA). In 2015, during exposure periods, 16-18 mussels
156 were held in the intertidal zone between Spring tides (two weeks) and evenly distributed in cylindrical
157 stainless-steel cages (10 x 8 cm, height and diameter respectively, Fig. S1) in the estuary of the Forth River,
158 Edinburgh, UK, in Port Edgar (N 55°, 59'42", W 3°, 24'30"). A passive sampler (Fig. S1) was attached to each
159 cage, which consisted of a stainless-steel wired scrubber (i.e. pad pot cleaner) of spheroid shape of 5.5 x 2.5
160 cm. Following exposure, mussels and scrubs were collected and frozen until processing for enumeration of
161 MPs. The number of processed samples per campaign was nine mussels, three randomly selected mussels
162 per cage, and two passive samplers, i.e. scrubbers. To check for MPs presence and control the number of
163 particles mussels might already have prior to exposure, reference mussels from the main stock were
164 processed following the same procedure.

165

166 2.2 Field samples collection

167

168 An assessment of field MPs was undertaken on environmental samples of both blue mussels (*Mytilus*
169 *spp*) and horse mussels (*Modiolus modiolus*). It is impossible to visually distinguish between the three
170 known *Mytilus* species occurring in Scotland and/or their hybrids (*M. edulis*, *M. galloprovincialis*, *M.*
171 *trossulus*) (Dias et al., 2009). Therefore, we will refer to blue mussels as any of the collected *Mytilus spp.*
172 Samples were collected throughout 2015 from various locations around Scotland (Fig. 1). Subtidal *Modiolus*
173 *modiolus* were collected by scuba diving, whereas *Mytilus spp* were collected during low tide on intertidal

174 rocky shores. In all cases samples were not given the opportunity to filter feed from the point of collection
175 and frozen immediately after retrieval. A dedicated caged exposure campaign was also undertaken in an
176 urbanised station, Newhaven (Edinburgh, UK), for comparison. *Mytilus edulis* from live stock kept at HWU
177 were placed in cages (above) in the subtidal of the Southeastern part of the Forth Estuary (Fig. 1), for 4
178 weeks during November 2015. After collection, all mussels were transported to HWU facilities, and stored
179 at -20 °C until further processing. The soft tissue of a selected number of mussels (*Mytilus* spp.) was
180 digested (see bellow) for MPs enumeration.

181

182 **2.3 Sample Processing and MPs Enumeration**

183

184 The soft tissue of mussels was digested overnight (60 °C) using Corolase® 7089 enzyme mixture, 9.6
185 UHb/mL for *Mytilus* spp and 19.3 UHb/mL for *Modiolus modiolus*, and MPs were extracted and quantified
186 according to Catarino et al. (2017). Mussels length was measured (to 0.1 cm), all soft tissues were removed
187 from the shell, weighed (wet weight to 0.01 g), and placed in a 250-ml glass Erlenmeyer flask for digestion
188 (one mussel per flask). Due to their larger size, *M. modiolus* sub-samples of the entire soft tissue were
189 digested in separate flasks, observed, and particle enumeration pooled per individual. Special care was
190 taken to avoid airborne fibres contamination and samples were covered to avoid air exposure, vials were
191 capped with aluminium foil during digestion, personnel used protective cotton lab coats, equipment was
192 thoroughly rinsed using Milli-Q water, and glassware was acid-washed prior to use. To assess airborne fibre
193 contamination during this procedure, one Milli-Q water control sample (100 ml) was submitted to the same
194 procedure during each digestion event. After digestion, the final product was vacuum filtered [Whatman™
195 filters of cellulose nitrate 0.8 µm]. Filters were observed using a Wild Heerbrugg dissection microscope
196 (Germany, up to x 310 magnification) and particles were classified as fibres, plastic films, spheres and other
197 particles: fibres were elongated and narrow particles, spheres were round shaped, films were thin layers
198 and other particles incorporated all irregular shaped observed MPs. Fibres were further classified by colour.
199 Data was expressed in terms of number of particles per g of mussel wet weight (ww) and number of
200 particles per mussel.

201 For MPs extraction, scrubbers were suspended in a beaker with the aid of a nylon wire, and inserted in a
202 0.5 L super-saturated sodium chloride solution. Corolase® 7089 enzyme mixture was added to a
203 concentration of 0.48 UHb/mL and organic material was digested overnight (50 °C), while stirring. Before
204 collection of the top fraction of the mixture for filtration and MPs separation, the stirring process was
205 ceased and the solution was held static for 3h to allow for separation of particles by density (adapted from
206 Hidalgo-Ruz et al., 2012). Procedural blanks were used in every processing event. Filtration, MPs
207 observation and enumeration performed as described above. Data expressed as the number of observed
208 particles per sample.

209

210 **2.4 Validation of Fibre Identification**

211 To verify the accuracy of particle visual observations by the various observers, a re-count of particles
212 was done by one observer on 72 filters. To validate the visual assessment of fibres, a subsample of 30 items
213 were randomly selected and examined with a Perkin-Elmer Spectrum 100 Fourier Transformation Infrared
214 Microscope (FT-IR) equipped with a mercury cadmium telluride (MCT) detector. The spectra were recorded
215 as the average of 16 scans in the range of 4,000 -600 cm⁻¹ with a resolution of 4 cm⁻¹ (software Spectrum V
216 6.3.4.0164, Perkin-Elmer). Spectra obtained were visualised in OMNIC 9.2.106 (Thermo Fisher Scientific
217 Inc.), analysed, and compared against a self-generated library (Blumenröder et al., 2017) and the Hummel
218 Polymer and Additives FT-IR Spectral Library (Thermo Fisher Scientific Inc.). To confirm the proportion of
219 fibres classified as microplastics we used a separate sub-sample of 27 items. These were moved to glass
220 glass staining blocks and further digested using H₂O₂ (50 °C, 20 - 24 h). Fibres were quantified, moved to a
221 microscope slide, stained with Nile Red in methanol at 1 µg / mL (Erni-Cassola et al., 2017) and covered
222 with a glass cover slip to protect samples from airborne contamination. This methodology has been
223 validated and has a similar accuracy to FT-IR and Raman Spectroscopy (Erni-Cassola et al., 2017; Maes et al.,
224 2017; Shim et al., 2016). Fibres were observed using a Axio Imager M2 fluorescence microscope (10x
225 objective) coupled with an AxioCam MRm camera (ZEISS, Germany) and using the LED Illumination system
226 pE-300 (Cool-LED, USA). For particle visualisation, the filter was set for Fluorescein (FTIC) in green
227 (excitation max at 490 nm and emission max at 525 nm) (Erni-Cassola et al., 2017). Known pristine

228 materials (natural cotton, polyethylene, polypropylene rope fragments, nylon fragments) were subjected to
229 both FT-IR and Nile Red methodologies and used as standards for comparison purposes.

230

231 **2.5 Passive Sampling of Airborne Fibres During Meals**

232

233 To quantify the level of airborne fibres of a food item of similar surface area to a mussel, we used
234 stationary passive samplers adapted from dust collectors described by Adams et al. (2015); Dris et al.
235 (2017). Two rectangular double-sided adhesive white pads ($2 \times 1.8 \text{ mm} \times 1.2 \text{ mm} = 4.32 \text{ cm}^2$) were placed in
236 plastic petri dishes (90 mm diameter) and airborne fibres fallout was collected in April 2017 according to
237 the following treatments: a) control, i.e. petri dish closed, b) petri dish open for 20 min during cooking, c)
238 petri dish open for 20 min during meal consumption; d) petri dish open for 40 (20 + 20 min) min during
239 cooking and food consumption. Petri dishes were transported closed and sealed externally with tape, from
240 the lab to three different households in Edinburgh. They were opened during the evening meal period,
241 closed after exposure according to treatment and sealed until further observation and MPs enumeration.
242 The presence of a sticky-tape surface allowed for a reduced fibre loss and the colour contrast facilitated
243 particle enumeration. Sampling time was shorter than usual household dust passive sampling (Adams et al.,
244 2015; Dris et al., 2017) to account for fibre fallout during the meal period only.

245 Two yearly exposure scenarios of human ingestion of particles were calculated: 1) By using the mean
246 number of particles observed in wild caught *Mytilus* spp in this study and the yearly consumption of
247 mussels per capita in the UK and other EU countries and 2) by extrapolating the number of fibres collected
248 in the passive dust samplers (4.32 cm^2) to that of a regular plate of 12.5 cm of radius (491 cm^2 of area).

249

250 **2.6 Data analysis**

251

252 *2.6.1. Field Data and Blanks*

253 The numbers of MPs in each mussel observation were pooled according to site location, season, and
254 particle type. Particles were enumerated (abundance) per location or sampling season (sample) according

255 to particle type and fibre colour, which resulted in a high proportion of non-detected samples (i.e., no MPs
256 present). Due to the non-parametric nature of these data (high number of zero observations), we used a
257 resemblance permutation-based analysis of similarity (ANOSIM, Primer 5 software) to assess similarities
258 among locations or season based on type of particles present. This analysis method developed by Clarke
259 (1993) and Clarke and Warwick (2001) is widely used in ecology and microbiology for assessing similarities
260 among samples according to species abundance, and a similar approach for field litter data analysis has
261 previously been done by Tekman et al. (2017). The abundance of MPs other than fibres was pooled, as the
262 number of these observed particles was low (total 20 particles in 141 observations). The ANOSIM R statistic
263 obtained at the end of each analysis informs on the (dis)similarity between groups, with a value close to "1"
264 indicating a high dissimilarity of the tested samples (locations) and a R value close to "0" indicating that
265 locations are similar in terms of abundance and diversity of particles (Clarke and Warwick, 2001).

266 a) Blanks: Particles present in procedural blanks were checked verify if they were similar to mussel
267 samples location, and according to the type of fibres/MPs observed. Abundance data was fourth-root
268 transformed (to reduce the influence of large numbers of one fibre type in the final analysis) prior to
269 calculation of the Bray-Curtis similarity matrix, which was followed by a 1-way ANOSIM (factor Location). As
270 the obtained $R = 0.13$ indicated a high similarity between blanks and sample location in terms of the
271 particles present (Fig. 2, i), we concluded that particle counts in observed samples could be strongly
272 influenced by airborne contamination. Therefore, from subsequent data analysis, the type and number of
273 observed fibres in blanks was subtracted from observations corresponding to the same digestion event. A
274 new 1-way ANOSIM was performed and an increased dissimilarity ($R = 0.367$) between blanks and locations
275 confirmed (Fig 2, ii). Scrubs enumeration of MPs equally took into account airborne fibre contamination
276 observed in procedural blank filters and type and number of fibres were subtracted by each processing
277 event.

278 b) Port Edgar: To understand if sampling seasons were similar in terms of abundance and diversity of
279 fibres and MPs, a 1-way ANOSIM was performed using the Bray-Curtis similarity matrix obtained after a
280 fourth-root transformation. To compare sampling seasons in terms of total load of particles in the exposed
281 mussels (particles / g ww), a 1-way ANOVA was performed after \log_{10} transformation of data. ANOVA was

282 preceded by homogeneity of variance (Levene's) and normality (Shapiro–Wilk) tests. A probability level of P
283 < 0.05 was used to determine if differences were statistically significant. The analysis was followed by
284 Fisher's LSD post hoc test for multiple comparisons. Total number of particles per passive sampler (scrub)
285 present in each season was tested for significant differences using a 1-way ANOVA ($p < 0.05$).

286 c) Observations on Wild Mussels: The number of MPs per g of soft tissue was dependent on the total
287 mass of the mussels tissue following an allometric equation ($y = -1 + 10^{0.53 - 0.37 \times \log_{10} x}$, $p = 5 \times$
288 10^{-6}). Using the mean weight specific pumping rate (L / h / g) of *M. edulis* according to wet weight (g)
289 ($y = 0.001 \times 10^{2.65 - 0.29 \times \log_{10} x}$, $p < 0.01$, Jones et al., 1992), we computed a model able to predicted
290 number of plastics per g of wet weight of mussel tissue according to mussel wet weight specific pumping
291 rate. The number of MPs observed was \log_{10} transformed and analysed using a co-variance analysis
292 (ANCOVA) to check for significant differences among locations, using total soft tissue wet weight as a
293 continuous independent variant. To understand if sampling between locations were similar in terms of
294 observed types of fibres and other MPs a 1-way ANOSIM was performed using the Bray-Curtis similarity
295 matrix obtained after a fourth-root transformation.

296

297 2.6.2 Household Fibres Enumeration

298 The mean number of observed fibres in procedural blanks (1 ± 0.33 SE) was subtracted from observed
299 samples in each sample (similar type of observed fibre). A two-way ANOVA after square-root
300 transformation was used to check for differences between treatments and households (two independent
301 variables). Data was previously checked for homogeneity of variance (Levene's) and normality (Shapiro–
302 Wilk). In case probability P was lower than 0.05, analysis was followed by Fisher's LSD post hoc test for
303 multiple comparisons.

304

305

306 3. Results

307

308 3.1 Procedural blanks

309

310 The majority (98.6%) of particles observed in blanks were fibres of which 70 % were transparent (Fig. 2).
311 The mean number of particles observed per procedural blank processed during digestion of soft tissue of
312 mussels (n = 22) was 6.5 ± 0.95 (SE), while in procedural blanks from processing of scrubbers (n = 3) was 3.5
313 ± 2.04 (SE). When the number and type of particles observed in blanks per digestion event is subtracted
314 from the observed particles in each sample, the relative abundance of each particle type changes in
315 sampling stations (Fig. 2).

316

317 3.2 Port Edgar

318

319 Exposed *Mytilus edulis* mussels (n = 62) were 5.2 ± 0.08 cm (SE) long and their soft tissue mean wet
320 weight (ww) was 5.63 ± 0.284 g (SE). Mussels exposed in Port Edgar (n = 62) had a mean load of $0.74 \pm$
321 0.125 (SE) particles / g ww, equivalent to 3.4 ± 0.48 (SE) particles per mussel. Of the observed particles, 99
322 % were fibres. The mean number of particles observed per passive sampler (i.e. scrubber, n = 14) was $2.1 \pm$
323 0.69 (SE), with a maximum number of 9 particles per scrubber. With the exception of one sphere observed
324 in a sampler exposed in Spring 2015, all other observed particles were fibres. In reference mussels (n = 15),
325 from the main stock kept in the lab, a mean value of 0.6 ± 0.12 (SE) particles / g ww of soft tissue of mussels
326 was observed, corresponding to a mean value of 4 ± 0.6 (SE) particles per mussel. Reference mussels were
327 within the same size range of exposed mussels: 5.0 ± 0.25 cm (SE) long and their soft tissue mean wet
328 weight was 6.74 ± 0.943 g.

329

330 The MPs in mussels collected in different seasons were similar in terms of present types of fibres and
331 MPs (ANOSIM R = 0.048). However, in terms of numbers of MPs in mussels, significantly more particles
were observed in mussels during the first winter sampling campaign (Winter 1) in Port Edgar (Fisher LSD $p \leq$

332 0.0001) (Fig. 3, i). Number of particles per passive sampler did not differ significantly through season (Fig. 3,
333 ii).

334

335 **3.3 Field samples**

336

337 *Mytilus* spp collected in the field and processed for MPs enumeration (n = 36) were 4.0 ± 0.27 cm (SE)
338 long and their soft tissue mean wet weight (ww) was 4.89 ± 0.694 g (SE). In these mussels the mean load of
339 observed particles was 3 ± 0.9 (SE) particles / g ww, equivalent to 3.2 ± 0.52 (SE) particles per mussel.

340 *Modiolus modiolus* (n = 6) were substantial heavier, 42.91 ± 2.111 (SE) g, and their mean size was 9.2 ± 0.22
341 (SE) cm. The mean number of MPs observed was 0.086 ± 0.031 (SE) particles / g ww, the equivalent of $3.5 \pm$
342 1.29 (SE) particles per mussel.

343 The number of particles per wet weight (g) of soft tissue was dependent on soft tissue mass ($y = -1 +$
344 $10^{0.53 - 0.37 \times \log_{10} x}$, $p = 5 \times 10^{-6}$) (Fig. 4, i), but did not differ according to location ($p = 0.059$,
345 ANCOVA), when total weight used as a co-variant in the statistical analysis. Using the mean ww-pumping
346 rate presented by Jones et al. (1992), was possible to compute a model of the predicted number of plastics
347 per g of wet weight of mussel tissue according to mussel wet weight specific pumping rate ($x =$
348 $0.001 \times 10^{2.23 + 0.78 \times \log_{10}(y+1)}$, where x is the mussel pumping rate (L / h / g ww) and y is the number of
349 MPs per g of mussel ww) (Fig. 4, ii). Locations were very similar between themselves in terms of observed
350 types of fibres and MPs (ANOSIM R = -0.011) observed in samples.

351 Based on an assumption that humans ingest MPs via the consumption of mussels, we can predict the
352 number of particles to which they are exposed. According to SEAFISH (2016) (Seafood Authority for the UK)
353 there is a mean annual consumption of 8.2 Kg of seafood per capita in the UK [2014 data], of which c.a. 1 %
354 is of mussels (82 g). Assuming each wild mussel would have a load of 3 particles per g, of which only about
355 50 % would be microplastics (see section 3.4), we can conclude that an individual could be exposed up to
356 123 particles per year, via ingestion of mussels in the UK. In countries with a higher consumption rate of
357 mussels (3.08 kg / y / capita) such as Spain, France or Belgium (Food and Agriculture Organization of the
358 United Nations, 2017), a consumer would be exposed up to 4,620 particles / y via mussel ingestion.

359

360 **3.4 Validation of Fibre Identification**

361

362 In the 72 independent re-count of observed particles from field samples, 10 differed from the original
363 number of fibres reported. The mean deviation ($|\text{observed value} - \text{expected value}|$) of the re-counts was
364 0.4 ± 0.08 (SE) fibres and the mean error ($|\text{observed value} - \text{expected value}| / \text{expected value} \times 100$) was
365 $5.4 \% \pm 1.13$ (SE). Fifty percent of the 30 observed fibres using FT-IR were identified and the two most
366 common types of observed polymers were Polyethylene terephthalate (Polyester or PET, 40 %) and
367 Poly(ether-urethane) (20%). Using the Nile Red staining method (Fig. S2), we were able to confirm that 48
368 % of the observed 27 fibres were microplastics. Plastic fibres were easily distinguished from “natural” ones
369 as latter would not show fluorescence, or from cotton, which although stained, are flattened (in opposition to
370 cylindric polymer fibres) and presented a very characteristic twisted ribbon-like shape (Fig. S2). Stained
371 plastic fibres measured between 0.2 and > 2 mm length, and 0.01 – 0.05 mm thickness.

372

373 **3.5 Household Fibres Observation**

374

375 The mean number of fibres was higher in Household 1 (10 ± 4.2 SE) than in the other two households
376 (Fisher LSD $p \leq 0.0019$). Samples collected during the meal period [$1 \text{ fibre} \pm 0.7$ (SE)] had a significant lower
377 exposure to airborne fibre contamination (Fisher LSD $p \leq 0.0042$), whereas while cooking the mean fibre
378 fallout was of 5 ± 3.3 (SE) (Fig. 5). If a conservative exposure level is assumed, in bands with a surface of
379 4.32 cm^2 we expect the arrival of 1 particle in 20 min. We can then extrapolate that in a plate with 12.5 cm
380 of radius, i.e. an area of 491 cm^2 , we could find 114 particles for the same period of time, assuming a
381 constant exposure rate. This is equivalent to a potential exposure to MPs via ingestion of 41,610 particles
382 per year per person, for 20 min during consumption of evening meals. During the cooking period (20 min),
383 if we assume a constant fibre fallout of 5 particles per 4.32 cm^2 , the potential human ingestion increases to
384 207,320 particles per year per person. According to Dris et al. (2017), 33 % of household dust fallout is
385 microplastics, whereas other fibres can be of natural origin, such as cellulose. We can then extrapolate that

386 human ingestion of microplastics during evening meals could be in a range of 13,731 – 68,415 particles per
387 year.

388

389 **4. Discussion**

390 We observed that airborne fibres contamination of processed soft tissue, during microplastics (MPs)
391 assessment in field mussels, can alter the relative proportion of the type of fibres observed in samples.
392 Quantification of MPs in field organisms will need to take contamination into account and by subtracting in
393 the data not only the number, but also the type of particles observed in blanks, leading to a more reliable
394 data analysis and report. Airborne fibres contamination of field samples is a challenge in MP studies and
395 this can take place any time during collection, processing and/or microscopic observation, with sample
396 dissection and digestion of mussels being the most vulnerable steps to contamination (Catarino et al.,
397 2017). Contamination is extremely hard to eliminate, even in highly controlled and/or forensic conditions
398 (Torre et al., 2016; Woodall et al., 2015). So it is essential to systematically use procedural blanks to
399 quantify it (Catarino et al., 2017), to apply good laboratorial practices (GLP) for quality assurance (Torre et
400 al., 2016) and to report openly real levels of contamination, so that appropriate data analysis,
401 interpretation and inter-studies comparisons can be done. Even though we applied GLP (*see* Catarino et al.,
402 2017), we observed contamination in our procedural blanks, which, if not taken into consideration in
403 particle counts, influenced data analysis results and therefore interpretation (Fig. 2). So, in our data
404 analysis, we subtracted the type and number of observed fibres in blanks from observations corresponding
405 to the same digestion event, aiming at obtaining a more representative sampling from what can be found
406 in the field.

407 We have shown that the use of cage deployed mussels in the field can be an effective method to
408 quantify and assess plastic pollution in the field. This assessment is particularly useful in areas where
409 mussel beds are not necessarily present, such as an urban port (Forth Estuary, Port Edgar, Edinburgh, UK).
410 We were also able to detect seasonal changes in the particle load, even if the proportion of each type of
411 fibre was similar throughout the sampling seasons. The same seasonal change was not detected when

412 using passive samplers (scrubs). We recommend the use of mussels instead of this specific type of
413 samplers, due to a reduced number of particles captured compared to mussels, inability to detect season
414 changes and to the more complex/time consuming extraction procedure. Samples indicated that mussels
415 had a higher number of particles in the first 2015 winter campaign, corresponding to a high water flow
416 period in the River Forth (Fig. S3., National River Flow Archive). Rivers are a major source of microplastics in
417 marine coastal environments (Lebreton et al., 2017), and increased land runoff during winter/early Spring
418 in Europe increases the number of microplastic particles in the water(Lebreton et al., 2017), as detected in
419 our samples. The use of caged mussels to monitor microplastics has clear advantages that are very similar
420 to other biomonitoring purposes. These include improved experimental control over sampling period and
421 location, and use of mussels as bioindicators in areas where mussels may not be present (Beyer et al.,
422 2017). We highly recommend the progression in this area and the development of standardized procedures
423 for future field studies using caged organisms.

424 Mussels kept in the lab and used as reference also presented particles. These were kept in a
425 temperature-controlled chamber, where open tanks were subject to a strong air-conditioned ventilation
426 and in a space available to several other department users. Airborne fibres can be present in this
427 environment and so exposure to atmospheric contamination was likely the major source of these particles
428 in mussel tanks. However, deployed mussels were kept in the field for periods of over two weeks and as
429 mussels are capable of particle depuration within 72 h after exposure (Ward and Kach, 2009), we believe
430 that the number of particles observed reflects local and punctual field pollution and not lab contamination.

431 The number of particles found in both *Mytilus* spp, 3.0 ± 0.5 (SE), and in the subtidal species *Modiolus*
432 *modiolus*, 3.5 ± 1.29 (SE) particles per mussel, was similar. However, when these values are converted to
433 number of particles per wet weight of soft tissue, the similarity disappears (3.2 ± 0.52 , $n = 36$, and $0.086 \pm$
434 0.031 , $n = 6$, (SE) particles / g ww, respectively). This could be due to *M. modiolus* being larger and having a
435 higher body mass translated into a high capability of their filtration apparatus, capable of clearance rates of
436 up to 37 L / d (Navarro and Thompson, 1996). These results are halved if the accuracy of visual plastic
437 particle identification (FT-IR and Nile Red staining techniques) is accounted for, an estimation within the

438 range of recent reports on freshwater clams (81 %) (Su et al., 2018), fish gut contents (22 %) (Wagner et al.,
439 2017) and coastal bivalves (6 %) (Phuong et al., 2017). If we now consider a value of 1.5 MPs / g ww *Mytilus*
440 spp, the amount of particles observed in our study shows lower levels of plastic contamination in wild
441 caught mussels, such as for *M. galloprovincialis* (3 particles / g ww) (Li et al., 2015), and for Scottish *M.*
442 *edulis* (4.44 particles / g ww) (Courtene-Jones et al., 2017). This can be further due to
443 sampling/methodological and/or regional contamination differences or due to seasonal variability.

444 This was the first time that the presence of microplastics was reported from subtidal *M. modiolus*, a
445 species with a protected status in OSPAR areas. *Modiolus modiolus* is well known to actively trap
446 suspended particulates such as algae and sediments (Lindenbaum et al., 2008), and can double sediment
447 deposition rates (Kent et al., 2017); engineering large reefs of substantial biodiversity value (Sanderson et
448 al., 2008). The role of mussels in the deposition rates of MPs in biogenic reefs and on the possibility of
449 trapping particles together with sediments is unknown. The observed microplastics in field samples from
450 both *Mytilus* spp and *M. modiolus* indicates the need to assess the real risk to other species that might be
451 present in these biogenic reefs.

452 In the MPs enumerated in *Mytilus* spp, there is mass dependent relationship when particle numbers are
453 reported per wet weight of mussel (Fig. 4, i). When using this relationship and normalizing our data with
454 mussel weight (co-variant), our (ANCOVA) analyses showed no significant difference between locations ($p =$
455 0.059). This was observed, despite the number of MPs / g ww seeming higher in mussels from stations in
456 Skye, the largest island in the Inner Hebrides archipelago, and St Kilda (Village Bay and Geo), an isolated
457 archipelago in the North Atlantic Ocean, containing the westernmost islands of the Outer Hebrides of
458 Scotland (Fig 1). This discrepancy can be explained by the fact that in *Mytilus edulis*, and other *Mytilus*
459 species, both pumping rates (water transport) and filtration rates (particle clearance) decrease with higher
460 soft tissue mass (ww), among other parameters (Jacobs et al., 2015; Riisgard et al., 2014; Tsuchiya, 1980).
461 Gill area also scales with weight and size of the organism (Jones et al., 1992; Riisgard et al., 2014), a key
462 feature in controlling the individuals pumping, filtration and biodeposit rates (Jones et al., 1992; Tsuchiya,
463 1980). According to our model (Fig 4, ii), a higher number of particles per g ww will accumulate in mussels

464 with higher pumping rates per g ww, i.e. in smaller individuals. Future studies will need to take a size effect
465 into consideration, and will need to clearly establish if within same sampling stations, larger mussels will
466 trap lower number of fibers due to size dependent filtration rates. Other factors such as soft tissue mass
467 and sexual maturation (i.e. gonadal development, which accounts heavily into weight) will play an
468 important role when reporting data according to soft tissue wet weight. When establishing a monitoring
469 programme of microplastic contamination using *Mytilus* spp., we recommend the use of individuals with a
470 wet weight over 6 g. To our knowledge, this is the first time the relationship of mass of soft tissue to
471 number of reported particles was observed in a microplastics report and we expect it may be applicable to
472 other species of filter-feeders.

473 The most frequently observed particles in our samples were fibres (99 %) and of which a high proportion
474 is estimated to be Polyester (Polyethylene Terephthalate), in accordance to observations in other bivalves
475 (Browne et al., 2011; Rochman et al., 2015; Su et al., 2018). It is believed that a large number of fibres
476 present in marine environments are of textile origin, for instance released during washing cycles (Napper
477 and Thompson, 2016; Sillanpää and Sainio, 2017) and not trapped in waste water plants (Browne et al.,
478 2011). Textile fibres are associated with dyes resistant to washing detergents and bleach agents which stay
479 sorbed to fibers as a result of the presence of binders and mordants (*reviewed in* Drumond Chequer et al.,
480 2013; Qian et al., 2017). Toxicity of these agents and dyes (*see* Drumond Chequer et al., 2013; Klemola,
481 2015), and their subproducts due to photodegradation (de Luna et al., 2014), via textile fibres ingestion is
482 virtually unknown for aquatic organisms, and requires further investigation. There is also a lack of
483 information on retention times by *Mytilus* spp of ingested fibres, as these can differ of those of microbeads,
484 and that could influence the level of exposure of mussels to co-contaminants during particle passage
485 through their gut.

486 Using a conservative approach, we calculated that the incidental human ingestion of airborne fibers
487 during a meal can lead to an exposure between 13,731 – 68,415 particles / y / person. This is a much higher
488 figure when compared with the potential ingestion of particles via mussels consumption: 123 particles per
489 year in the UK or up to 4,620 particles / y in countries such as France, Belgium or Spain. The latter figure is

490 also lower than the previously reported 11,000 / y / European consumer, calculated by Van Cauwenberghe
491 and Janssen (2014). In Europe and around the world, various species of mussels, namely from the *Mytilus*
492 genus, have a highly commercial value and they are highly appreciated shellfish items (Food and Agriculture
493 Organization of the United Nations, 2017). Urban dust fallout can be composed of 33 % of microplastics
494 (Dris et al., 2017) and has been pointed as a potential source of MPs for human exposure, including through
495 ingestion (Dehghani et al., 2017; Dris et al., 2017). Younger children are in particular risk of ingesting of
496 microplastics via dust and airborne fibres, with estimated rates of indoor dust ingestion ranging between
497 2.2 mg / d for teenagers and 41 mg / d for toddlers (Wilson et al., 2013). The concerns to human health by
498 ingestion of MPs via shellfish is minimal compared to much higher levels of exposure via household dust
499 ingestion. Besides particle load, exposure to associated co-contaminants (toxicants) in both cases can be of
500 concern, and so we suggest a strong emphasis on the investigation of environmental relevant levels of
501 exposure via marine microplastics and other major sources.

502

503 Conclusion:

504

505 Quantification of MPs using field mussels is a feasible procedure to assess field contamination and the
506 use of deployed specimens is an effective advantageous method in the establishment of monitoring
507 programmes, in particular in locations where mussel beds are not present. Our data shows that even in
508 remote locations such as St Kilda, Scotland, mussels can indicate the presence of MPs. A better knowledge
509 of baseline levels of contamination is necessary, to allow for recommendations to policymakers, such as on
510 better waste treatment, and to establish real levels of environmental contamination of microplastics in
511 mussels and other species. The size of studied mussels and their soft tissue mass will need to be taken into
512 consideration when particle load is being reported, as this seems weight/size dependent, and data will
513 require standardization to be comparable between studies. Good laboratory practices are not sufficient to
514 completely eliminate airborne fibre contamination of samples, so the use procedural blanks is of high
515 importance, as well as the honest report of observed particles in blanks, so to establish comparable
516 reports. Quantification of the proportion of plastic fibres from field observations (using FT-IR and/or Nile

517 Red) is critical to establish realistic concentrations of synthetic particles in the environment. Finally,
 518 concerns of human exposure to MPs via shellfish ingestion need to be placed into context, since their
 519 potential for ingestion is minimal when compared to exposure to MPs via household dust fallout.

520

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532

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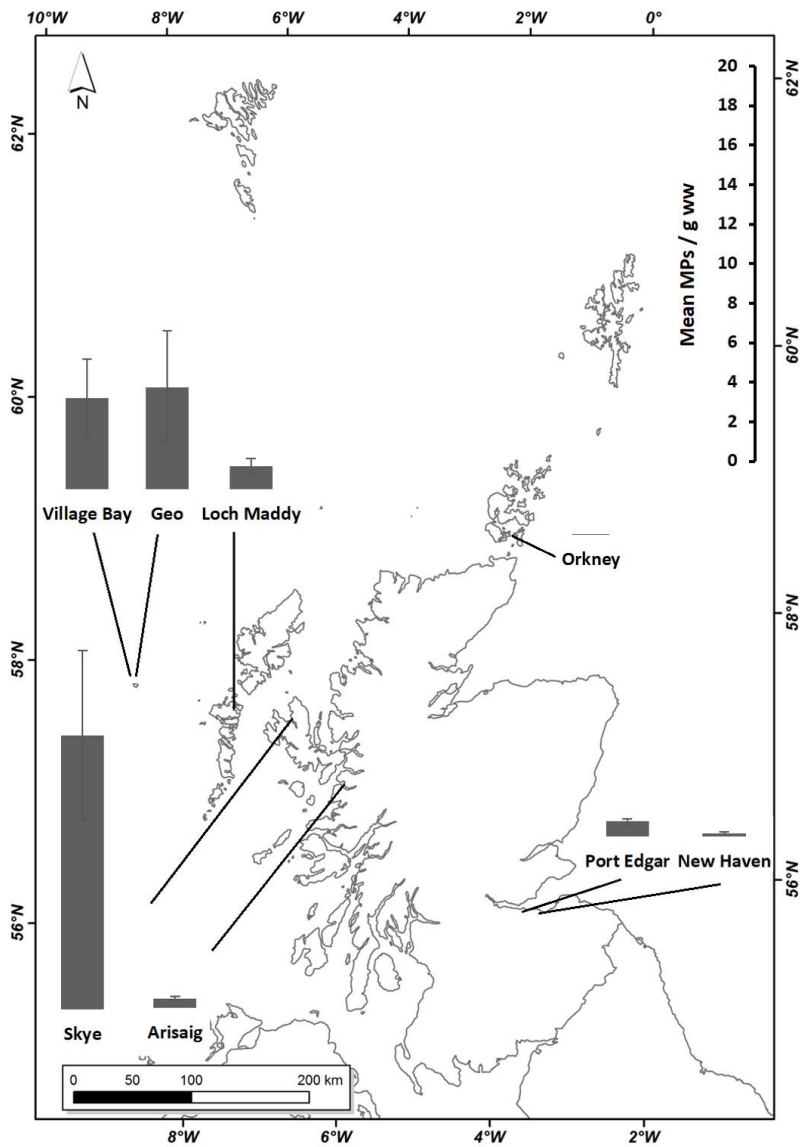
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712 **Figures:**

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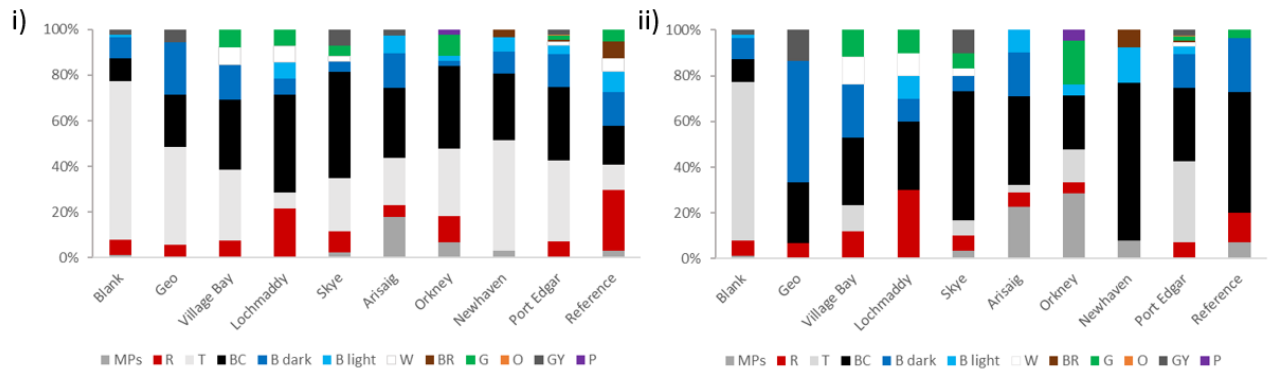
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715 Fig. 1. Map of the Scottish coast indicating sampling stations and mean number (\pm standard error) of
 716 observed fibres and particles per g of mussel soft tissue wet weight. Village Bay and Geo stations are both
 717 located in St Kilda island and correspond to the entrance of an intertidal cave and a reef, respectively. Isle
 718 of Skye samples were collected in Uig. The Lochmaddy station is located in North Uist. All organisms
 719 collected from the wild, with the exception of Newhaven and Port Edgar stations, Edinburgh, where MPs
 720 were sampled from deployed caged mussels. All samples were *Mytilus* spp, with the exception of Orkney
 721 where only *Modiolus modiolus* were collected. Using FT-IR analysis and Nile Red staining, we estimate that

722 only half of the observed fibres are plastic polymers. For the allometric relationship of MPs / g ww and
 723 mussels soft tissue ww, see Fig. 4.

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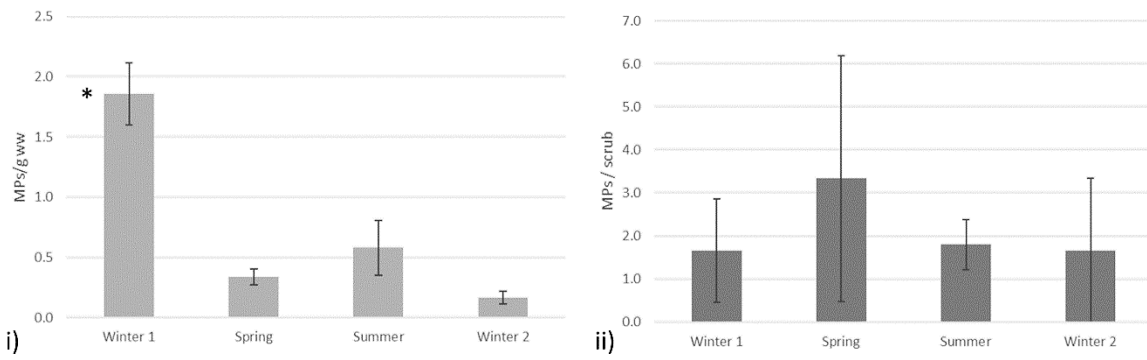
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728 Fig. 2. Relative abundance of the observed types of particles in blank samples, sampling stations and
 729 reference mussels (stock): i) before subtraction of number and type of fibres in blank treatments and ii)
 730 after subtraction of number and type of fibres in blank treatments. Abbreviations: MPs: other observed
 731 microplastics besides fibres, R: Red fibres, T: Transparent fibres, BC: Black fibres, B: Blue fibres, W: White
 732 fibres, BR: Brown fibres, G: Green fibres, O: Orange fibres, GY: Grey fibres, P: Purple fibres.

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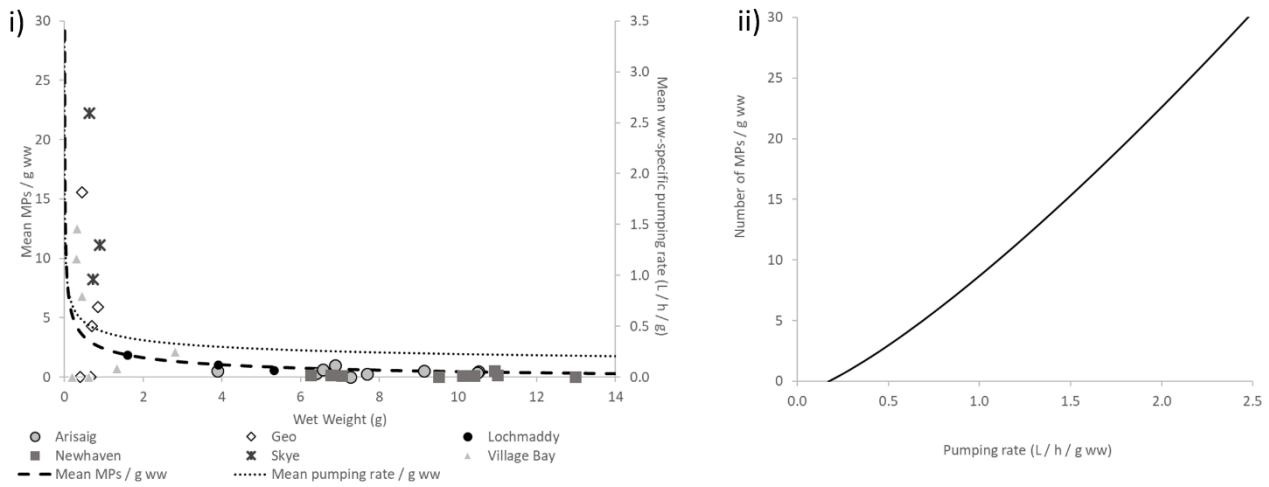
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737 Fig. 3. i) Mean number (\pm SE, $n = 9 - 21$) of particles per g ww of mussels soft tissue and ii) mean number
 738 (\pm SE, $n = 3 - 5$) of particles per passive sampler (scrub), observed at different sampling seasons, 2015, in
 739 Port Edgar, Edinburgh, UK. Symbol * (i) indicates statistically significant differences ($p < 0.0001$, Fisher LSD).

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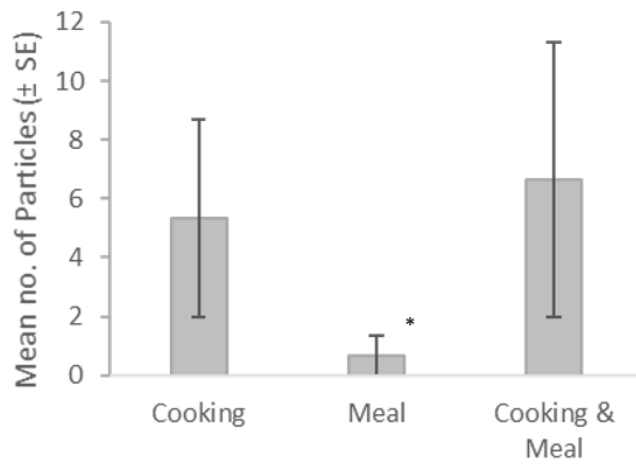
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744 Fig. 4. i) Number of microplastics (MPs) per g ww of *Mytilus* spp plotted according to total soft tissue
 745 wet weight ($y = -1 + 10^{0.53 - 0.37 \times \log_{10} x}$, $p = 5 \times 10^{-6}$) and mean weight specific pumping rate (L / h
 746 / g) of *M. edulis* according to wet weight (g) ($y = 0.001 \times 10^{2.65 - 0.29 \times \log_{10} x}$, $p < 0.01$) as per Jones et
 747 al 1992. ii) Model, computed using previous equations, of the predicted number of plastics per g of wet
 748 weight of mussel tissue according to mussel wet weight specific pumping rate ($x = 0.001 \times$
 749 $10^{2.23 + 0.78 \times \log_{10}(y+1)}$, where x is the mussel pumping rate (L / h / g ww) and y is the number of MPs per g
 750 of mussel ww).

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755 Fig. 5. Mean number of particles (\pm SE, $n = 3$) observed at different sampling times in households in756 Edinburgh, UK. The symbol * indicates statistically significant differences ($p \leq 0.003$).

Supplement Information

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759 Environmental Pollution, Series A, Ecological and Biological

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761 Low Levels of Microplastics (MP) in Wild Mussels Indicate that MP Ingestion by Humans is Minimal

762 Compared to Exposure via Household Fibres Fallout During a Meal

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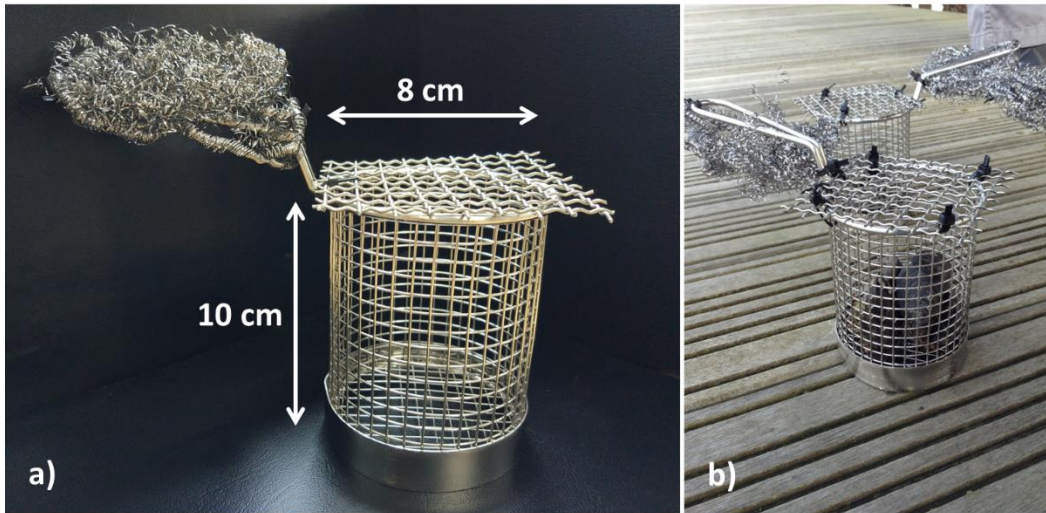
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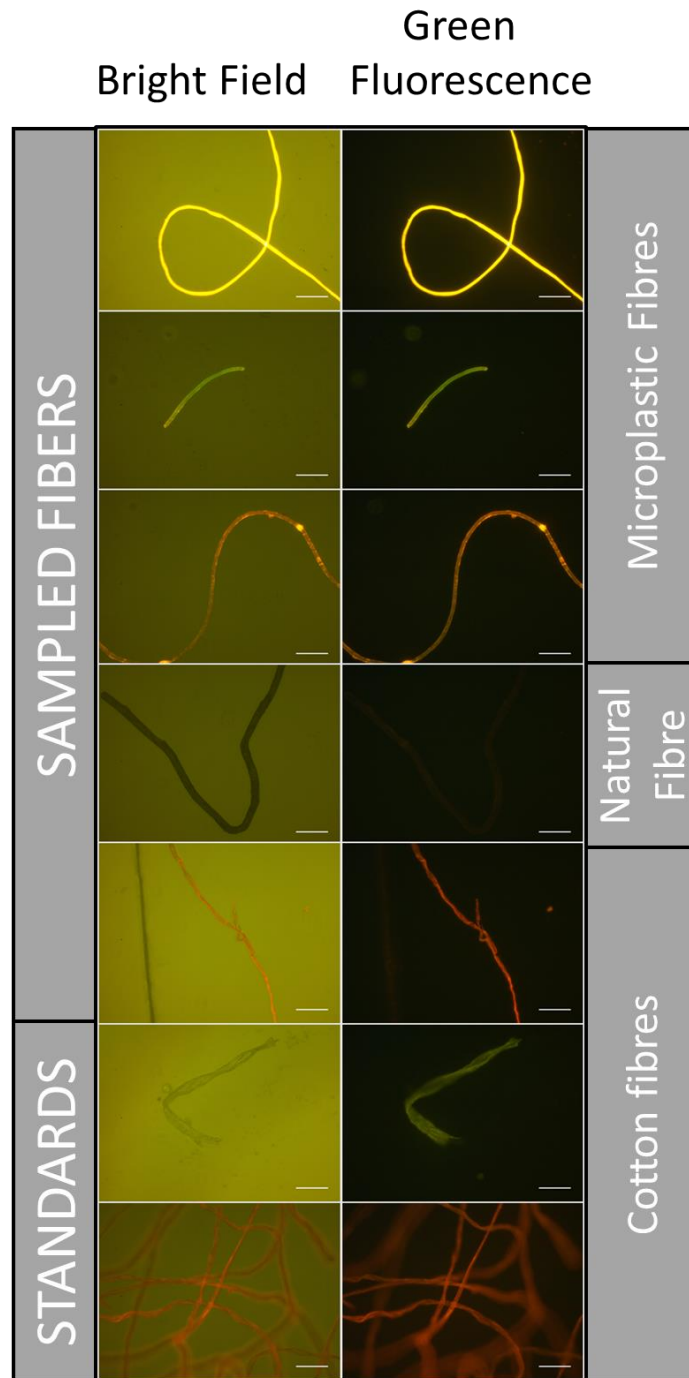


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788 Fig. S1. a) Inox cages and passive sampler (scrubs) used in the field to deploy mussels for microplastics
789 capture and enumeration; b) *Mytilus edulis* mussels in cage immediately before a deployment experiment
790 in Port Edgar, Edinburgh, UK.

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793 Fig. S2. Microscope image of sampled and standard fibres (cotton) stained with Nile Red (1 μg / mL).

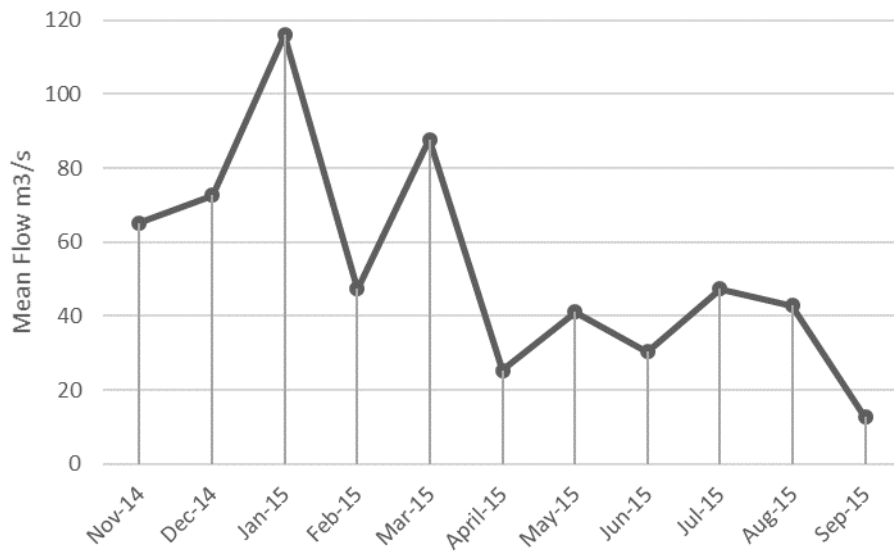
794 Right column shows fibres under the bright field, and in the left under a green fluorescent FITC filter

795 (excitation max at 490 nm and emission max at 525 nm). Scale bar set at 0.1 mm.

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800 Fig. S3. Mean daily flow (m^3 / s) at the station #18011, Forth at Craigforth, River Forth (Scotland, UK).

801 Data available at the National River Flow Archive (nrfa.ceh.ac.uk) until 30/09/2017 (accessed on

802 23/10/2017).