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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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Title: 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

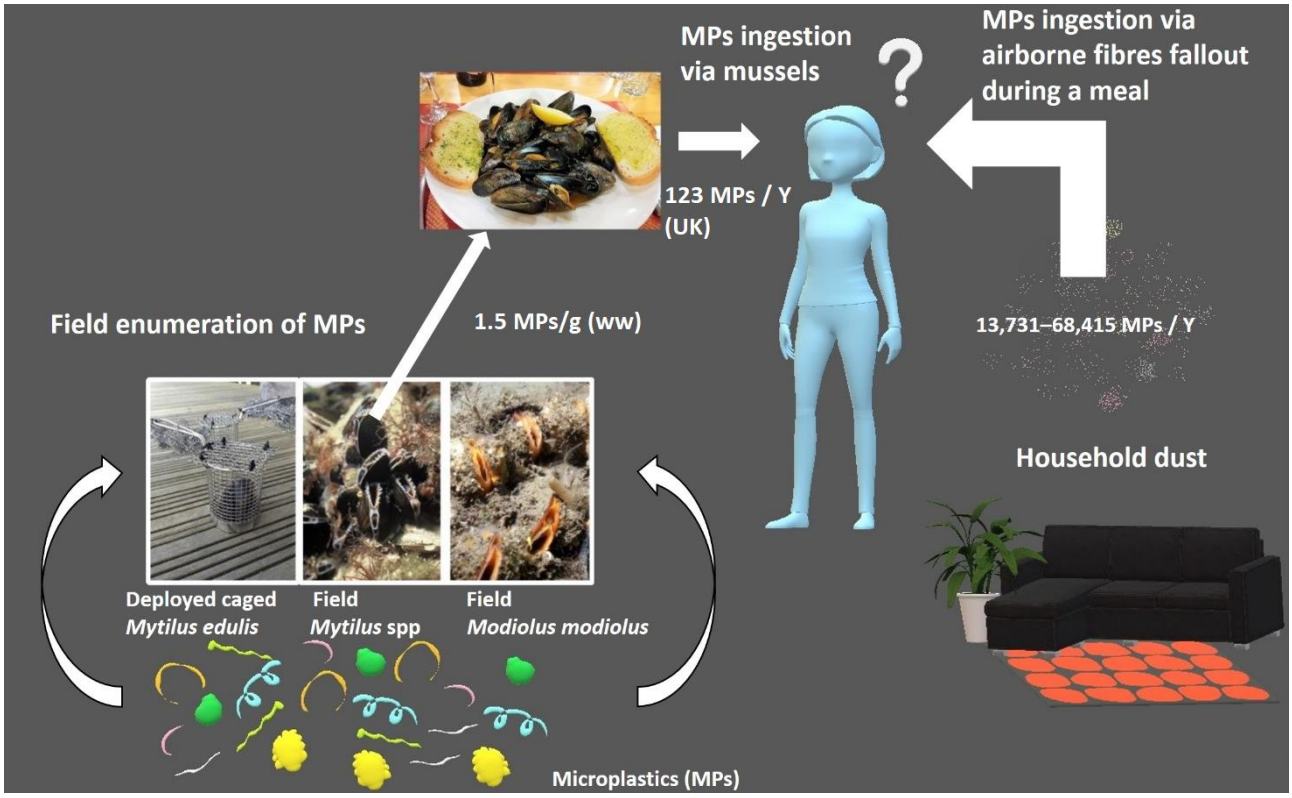
Abstract:

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

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

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

Graphic abstract:



Highlights:

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

1. Introduction

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

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

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

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

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

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

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

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

2. Methods and Materials

2.1 Port Edgar: Caged Deployed Mussels

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

2.2 Field samples collection

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

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

2.3 Sample Processing and MPs Enumeration

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

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

2.4 Validation of Fibre Identification

To verify the accuracy of particle visual observations by the various observers, a re-count of particles was done by one observer on 72 filters. To validate the visual assessment of fibres, a subsample of 30 items were randomly selected and examined with a Perkin-Elmer Spectrum 100 Fourier Transformation Infrared Microscope (FT-IR) equipped with a mercury cadmium telluride (MCT) detector. The spectra were recorded 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 6.3.4.0164, Perkin-Elmer). Spectra obtained were visualised in OMNIC 9.2.106 (Thermo Fisher Scientific Inc.), analysed, and compared against a self-generated library (Blumenröder et al., 2017) and the Hummel Polymer and Additives FT-IR Spectral Library (Thermo Fisher Scientific Inc.). To confirm the proportion of fibres classified as microplastics we used a separate sub-sample of 27 items. These were moved to glass glass staining blocks and further digested using H_2O_2 (50 ºC, 20 - 24 h). Fibres were quantified, moved to a microscope slide, stained with Nile Red in methanol at 1 µg / mL (Erni-Cassola et al., 2017) and covered with a glass cover slip to protect samples from airborne contamination. This methodology has been validated and has a similar accuracy to FT-IR and Raman Spectroscopy (Erni-Cassola et al., 2017; Maes et al., 2017; Shim et al., 2016). Fibres were observed using a Axio Imager M2 fluorescence microscope (10x objective) coupled with an AxioCam MRm camera (ZEISS, Germany) and using the LED Illumination system pE-300 (Cool-LED, USA). For particle visualisation, the filter was set for Fluorescein (FTIC) in green (excitation max at 490 nm and emission max at 525 nm) (Erni-Cassola et al., 2017). Known pristine

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

2.5 Passive Sampling of Airborne Fibres During Meals

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

Two yearly exposure scenarios of human ingestion of particles were calculated: 1) By using the mean number of particles observed in wild caught *Mytillus* spp in this study and the yearly consumption of mussels per capita in the UK and other EU countries and 2) by extrapolating the number of fibres collected 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).

2.6 Data analysis

2.6.1. Field Data and Blanks

The numbers of MPs in each mussel observation were pooled according to site location, season, and 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

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

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

2.6.2 Household Fibres Enumeration

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

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

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

3.3 Field samples

Mytilus spp collected in the field and processed for MPs enumeration (n = 36) were 4.0 ± 0.27 cm (SE) long and their soft tissue mean wet weight (ww) was 4.89 ± 0.694 g (SE). In these mussels the mean load of observed particles was 3 ± 0.9 (SE) particles / g ww, equivalent to 3.2 ± 0.52 (SE) particles per mussel. *Modiolus modiolus* (n = 6) were substantial heavier, 42.91 ± 2.111 (SE) g, and their mean size was 9.2 ± 0.22 (SE) cm. The mean number of MPs observed was 0.086 ± 0.031 (SE) particles / g ww, the equivalent of 3.5 ± 1.29 (SE) particles per mussel.

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

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

3.4 Validation of Fibre Identification

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

3.5 Household Fibres Observation

The mean number of fibres was higher in Household 1 (10 ± 4.2 SE) than in the other two households (Fisher LSD $p \leq 0.0019$). Samples collected during the meal period [1 fibre ± 0.7 (SE)] had a significant lower exposure to airborne fibre contamination (Fisher LSD $p \leq 0.0042$), whereas while cooking the mean fibre fallout was of 5 ± 3.3 (SE) (Fig. 5). If a conservative exposure level is assumed, in bands with a surface of 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 of radius, i.e. an area of 491 cm^2, we could find 114 particles for the same period of time, assuming a constant exposure rate. This is equivalent to a potential exposure to MPs via ingestion of 41,610 particles per year per person, for 20 min during consumption of evening meals. During the cooking period (20 min), if we assume a constant fibre fallout of 5 particles per 4.32 cm^2, the potential human ingestion increases to 207,320 particles per year per person. According to Dris et al. (2017), 33 % of household dust fallout is 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

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

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

The number of particles found in both *Mytilus* spp, 3.0 ± 0.5 (SE), and in the subtidal species *Modiolus modiolus*, 3.5 ± 1.29 (SE) particles per mussel, was similar. However, when these values are converted to number of particles per wet weight of soft tissue, the similarity disappears (3.2 ± 0.52, n = 36, and 0.086 ± 0.031, n = 6, (SE) particles / g ww, respectively). This could be due to *M. modiolus* being larger and having a higher body mass translated into a high capability of their filtration apparatus, capable of clearance rates of up to 37 L / d (Navarro and Thompson, 1996). These results are halved if the accuracy of visual plastic 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 ($p = 0.059$). This was observed, despite the number of MPs / g ww seeming higher in mussels from stations in
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

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

Conclusion:

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

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

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Figures:

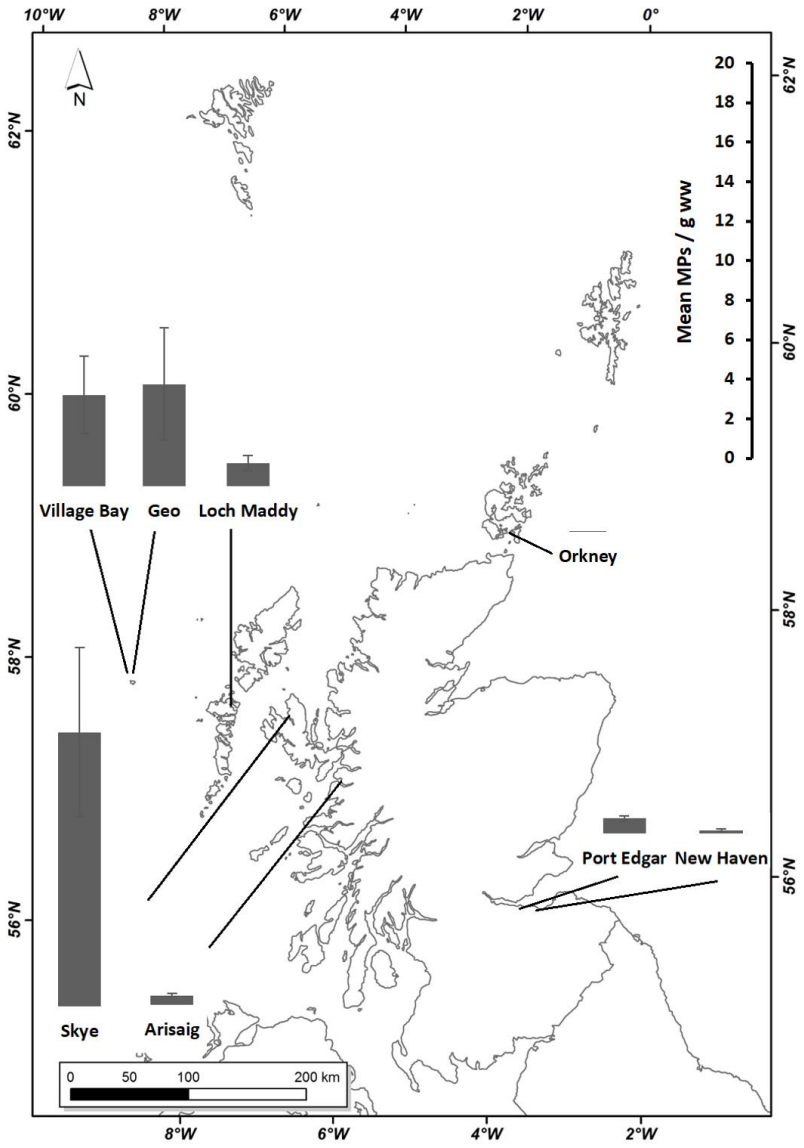

Fig. 1. Map of the Scottish coast indicating sampling stations and mean number (± standard error) of observed fibres and particles per g of mussel soft tissue wet weight. Village Bay and Geo stations are both located in St Kilda island and correspond to the entrance of an intertidal cave and a reef, respectively. Isle of Skye samples were collected in Uig. The Lochmaddy station is located in North Uist. All organisms collected from the wild, with the exception of Newhaven and Port Edgar stations, Edinburgh, where MPs were sampled from deployed caged mussels. All samples were *Mytilus* spp, with the exception of Orkney 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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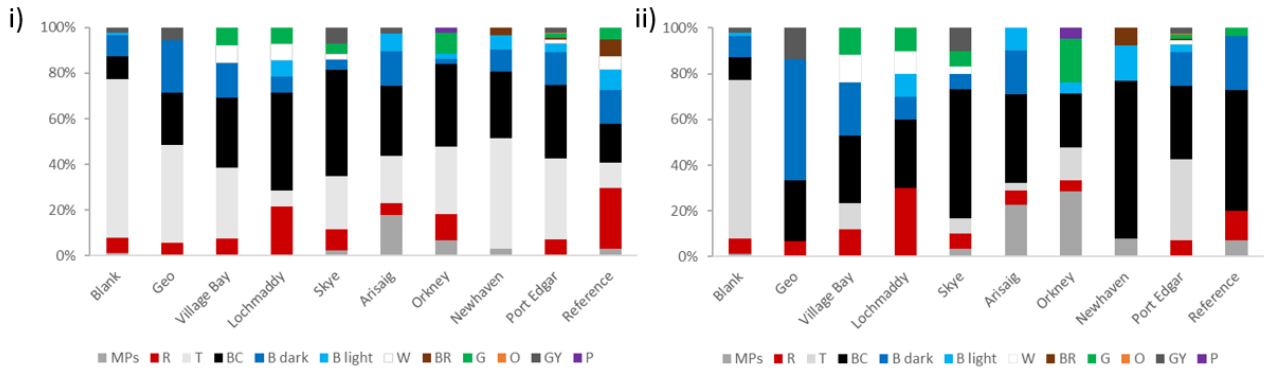

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728 Fig. 2. Relative abundance of the observed types of particles in blank samples, sampling stations and
729 reference mussels (stock): i) before subtraction of number and type of fibres in blank treatments and ii)
730 after subtraction of number 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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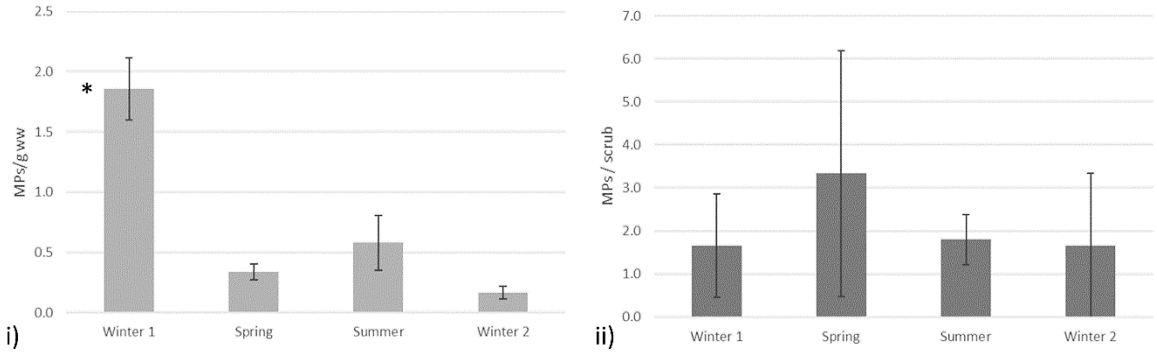

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737 Fig. 3. i) Mean number (± SE, n = 9 - 21) of particles per g ww of mussels soft tissue and ii) mean number

738 (± 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).

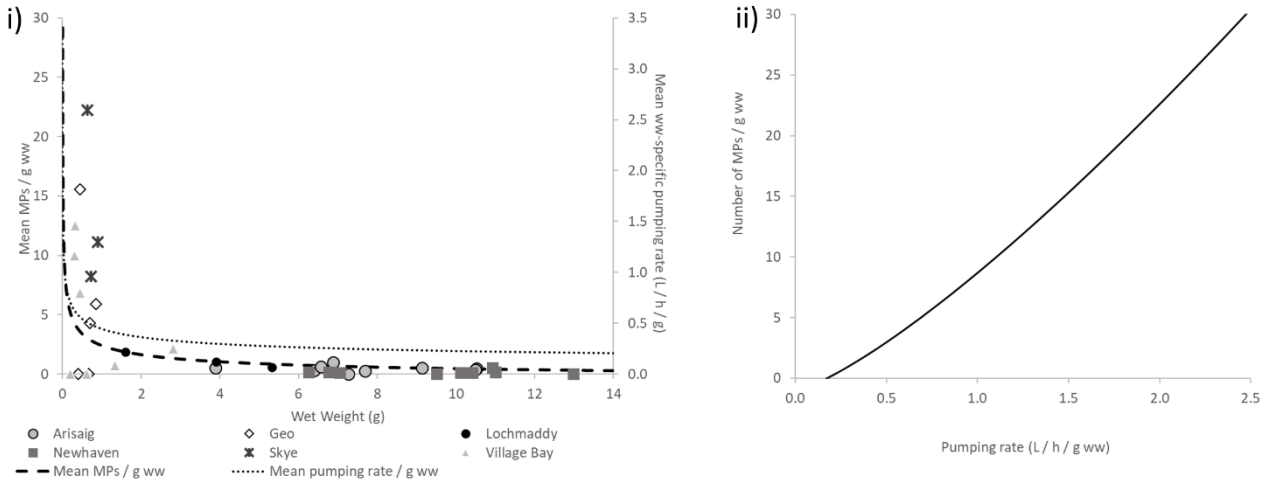

Fig. 4. i) Number of microplastics (MPs) per g ww of *Mytilus* spp plotted according to total soft tissue wet weight ($y = -1 + 10^{0.53 - 0.37 \times \log_{10} x}$, $p = 5 \times 10^{-6}$) and mean weight specific pumping rate (L / h / g) of *M. edulis* according to wet weight (g) ($y = 0.001 \times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

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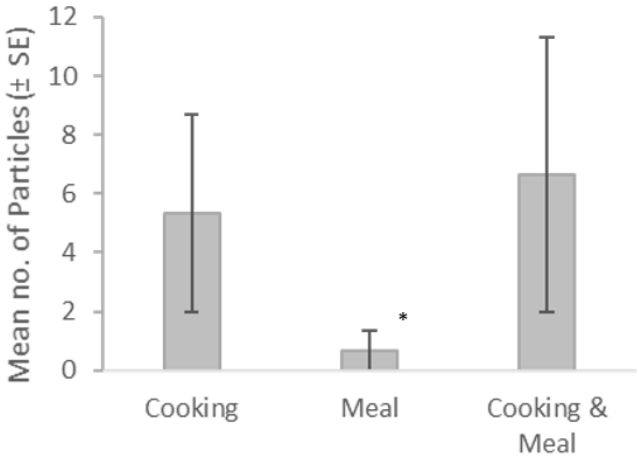

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755 Fig. 5. Mean number of particles (± SE, n = 3) observed at different sampling times in households in

756 Edinburgh, UK. The symbol * indicates statistically significant differences ($p \leq 0.003$).

Supplement Information

Environmental Pollution, Series A, Ecological and Biological

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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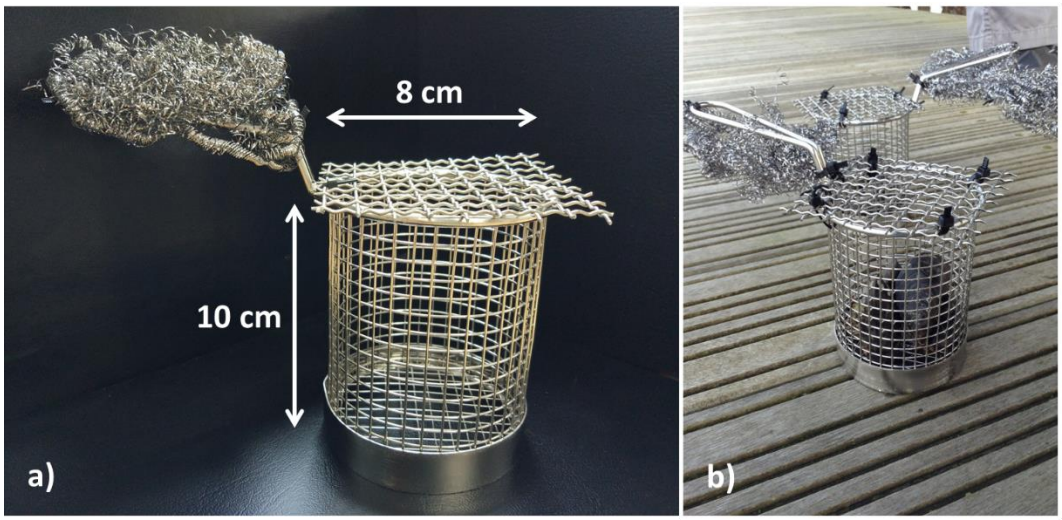

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788 Fig. S1. a) Inox cages and passive sampler (scrubs) used in the field to deploy mussels for microplastics
789 capture and enumeration; b) *Mytilus edulis* mussels in cage immediately before a deployment experiment
790 in Port Edgar, Edinburgh,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

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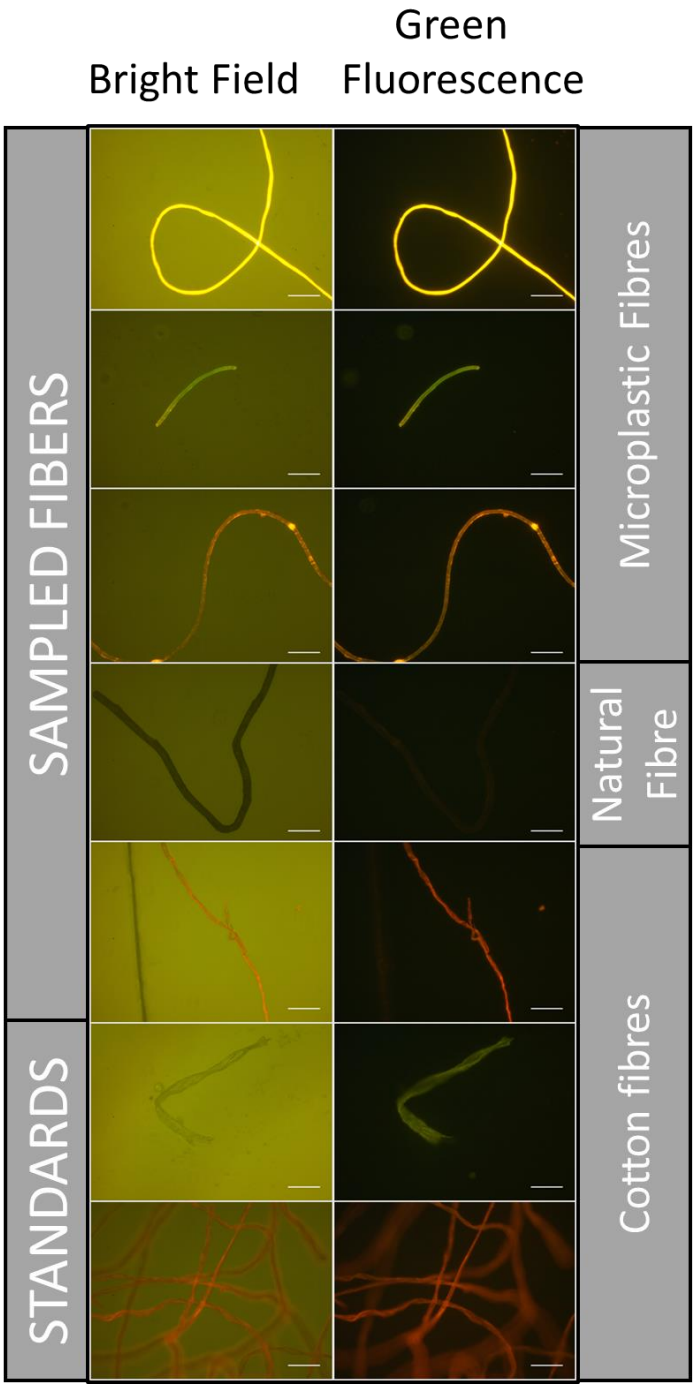

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793 Fig. S2. Microscope image of sampled and standard fibres (cotton) stained with Nile Red (1 µg / mL).

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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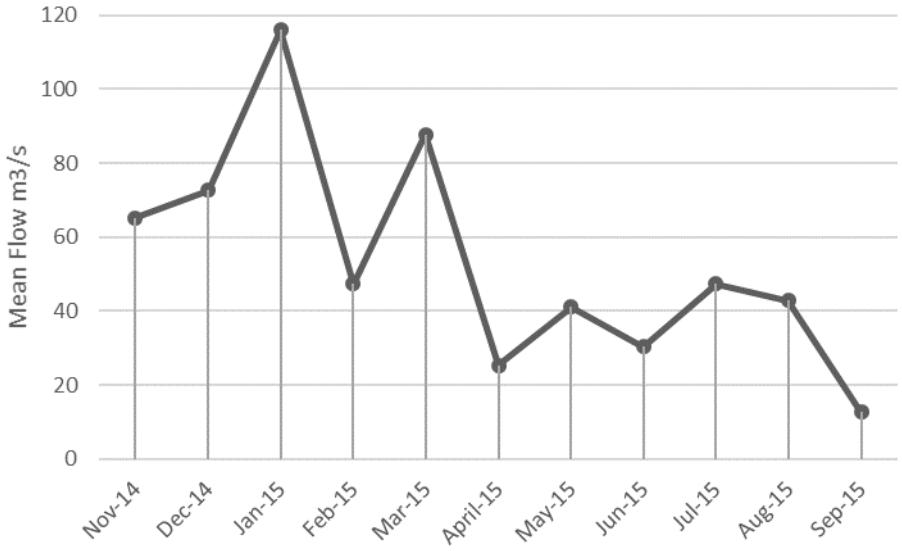

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800 Fig. S3. Mean daily flow (m^3 / s) at the station #18011, Forth at Craigforth, River Forth (Scotland, UK).

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

802 23/10/2017).