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How to get rid of ingested microplastic fibers? A straightforward approach of the Atlantic ditch shrimp *Palaemon varians*☆

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ABSTRACT

Microplastic fibers represent a significant share of the global marine micrcroplastic pollution, particularly in coastal areas. In controlled laboratory experiments, we offered fluorescent microplastic fibers (40–4400 µm lengths, median 150 µm) and spherical microplastic beads (9.9 µm Ø) together with commercial fish food to the Atlantic ditch shrimp *Palaemonetes varians*. The shrimps ingested fibers and beads along with the food. Upon ingestion, the beads and the shortest fibers (up to 100 µm) passed from the stomach into the gut and were egested within the fecal strings. The longer fibers first remained in the stomach but were regurgitated, i.e. extruded through the esophagus, within 12–14 h. Regurgitation is an evolutionary adaptation of particular crustacean species and other invertebrates to remove large and indigestible food particles from the stomach. Accordingly, the process of regurgitation attained a new task nowadays, i.e. the elimination of anthropogenic filamentous microplastic debris from the stomach to avoid harm. This behavioral feature may represent a selective advantage in view of the continuously increasing environmental plastic pollution.

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1. Introduction

An estimated eight million metric tons of plastic waste enter the oceans every year and a further increase by one order of magnitude until 2025 is predicted (Jambeck et al., 2015). In natural environments, plastic items disintegrate due to mechanical action, UV-radiation, or thermo-oxidation, thereby bearing numerous small fragments (Barnes et al., 2009; Andrady, 2011; Cole et al., 2011; ter Halle et al., 2016; Weinstein et al., 2016). Microplastic particles can be ingested by a variety of organisms including small invertebrates. The effects of microplastic ingestion are, however, inconclusive. Various studies showed that particles pass the digestive tract without detectable effects on organisms (e.g. Hämer et al., 2014; Kaposi et al., 2014; Santana et al., 2018; Weber et al., 2018) whereas other studies reported damage to organs, tissues, and cells at experimentally administered particle concentrations ofup to 20 mg L^{-1} (Jeong et al., 2016, 2017) or 2.5 g L^{-1} (von Moos et al., 2012).

Besides irregular fragmented particles, synthetic fibers constitute a significant share of microplastics in the environment, often outnumbering other types of particles, such as fragments and beads. In the brackish waters of the Baltic Sea off Sweden, microfibers accounted for more than 80% of the overall collected microplastics, accounting for about four fibers per cube meter (Gewert et al., 2017). Mathalon and Hill (2014) found 20 to 80 plastic fibers in 10 g of sediment at a beach in the Halifax region (Canada). The fibers probably originated from domestic washing (reviewed by Salvador Cesa et al. (2017)) or from ropes or fishing nets. De Falco et al. (2018) reported up to 6,000,000 fibers with a length of 300–500 µm, released from a typical 5 kg wash load of polyester fabrics. Napper and Thompson (2016) estimated a release of more than 700,000 fibers from a 6 kg wash load of acrylic fabric. Mason et al. (2016) estimated an average daily discharge of 4 million microplastic fibers by each single municipal wastewater treatment plant in the US states of California, New York, Wisconsin, and Ohio. Additionally, fishing gear and ropes, whether in use, lost, or discarded, also significantly contribute to environmental pollution by microplastic fibers (e.g. Barnes et al., 2009; Macfadyen et al., 2009; Cole et al., 2011).

Different to irregular or spherical particles, fibers are widely disregarded in environmental and ecotoxicological studies because it is difficult to differentiate between environmental contaminants and accidently introduced fibers during sample processing in the field and in the laboratory. So far, however, presence of fibers was reported in various species of seafood (Rochman et al., 2015). Blue mussels were examined for their suitability to monitor microplastic pollution in Norwegian coastal waters where mussels contained on average 1.5 microplastics per individual (Bråte et al., 2018). Overall, 83% of the particles investigated in that study were fibers. Synthetic fibers were detected in 63% of assessed brown shrimp from the coastal waters of the Southern North Sea and the Channel (Devriese et al., 2015). Desforges et al. (2015) found that the share of ingested microplastic fibers of the total microplastic particles in *Euphausia pacifica* in the NE Pacific decreased with distance from shore suggesting substantial discharge of synthetic fibers from land-based sources.

Upon ingestion, the fibres may provoke false satiation in animals, either when applied at high concentrations or when they accumulate

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over time in the stomachs as observed in Norway lobster, *Nephrops norvegicus* (Murray and Cowie, 2011; Welden and Cowie, 2016a, 2016b). The water flea, *Daphnia magna*, readily ingested plastic fibers of 300 µm average length but also very long fibers of around 1,400 µm (Jemec et al., 2016). Mortality increased when the animals were exposed to plastic fibers only, but not when they were fed with natural food prior to plastic exposure. The amphipod *Hyalella azteca* was exposed to polyethylene microplastic particles and polypropylene microplastic fibers. Fibers caused significantly higher mortality than particles (Au et al., 2015). The higher toxicity of the fibers corresponded with longer residence time of the fibers in the gut, which, in turn, might have affected the ability to process food and led to severe energetic deficiency (Au et al., 2015).

The present study aims at investigating whether microplastic fibers and beads are ingested by the Atlantic ditch shrimp *Palaemon varians* and how they are translocated within the digestive organs of the shrimp. *P. varians* was chosen as test organism because these shrimps are common in coastal regions and estuaries of the Northeast Atlantic where they are particularly exposed to domestic and industrial environmental plastic pollution (Browne, 2015). The animals are relatively small (1–3 cm in length) and widely translucent allowing for direct observation of ingested fluorescent fibers and particles in living animals under a fluorescence stereomicroscope. To clearly observe the fibers and beads, and to track their position in the shrimp, we applied in this study high concentrations well above the environmental levels. We addressed the following questions: 1) do these shrimps ingest microplastic fibers and particles, 2) do the plastic fibers accumulate in the stomach, and 3) and do they cause mortality at the applied experimental conditions?

2. Methods

2.1. Origin of organisms

Atlantic ditch shrimps (*Palaemon varians*, Leach 1813) were either purchased from an aquaristic shop (Futterhaus, Bremerhaven, Germany) or were captured with a hand net in a semi-enclosed former harbor basin in the city of Bremerhaven (Holzhafen; 53° 32.177820′ N, 8° 35.503680’ E). The shrimps were immediately transferred to the laboratories of the Alfred Wegener Institute, where they were maintained in 10-L aquaria in brackish water of 15 PSU, 15 °C, and 16:8 h of indirect illumination and darkness. The shrimps were fed every other day with plant-based fish food (NovoVert, JBL) and, additionally, once a week with freshly hatched *Artemia* sp. nauplii (Great Salt Lake Artemia Cysts, Sanders). The water was exchanged each day after feeding. Animals from both sources were used for preliminary experiments. The feeding experiments reported in this study were solely performed with animals from the urban pond.

2.2. Fluorescent fibers and microbeads

Small plastic fibers were cut from fluorescent polyacrylic wool “Magic Leucht Wolle fluo grün” (Magic Pyramid Bruecher & Partner KG, Frechen, Germany, No. 839512) by hand with scissors under a stereomicroscope. A sample of 0.5 mg contained almost 3000 fibers. The lengths of the fibers ranged between 40 and 4400 µm. The average length was 236 ± 176 (SD) µm, the median length was 189 µm (Fig. 1), and the width of the fibers was 30–35 µm. Due to electrostatic adhesion and their high numbers, fibres could not be counted properly. Instead, the administered quantity in the experiments was expressed by mass. Fluorescent microbeads of polystyrene (PS, Fluoro-Max™ Green Fluorescent Microspheres, 9.9 µm diameter) were purchased from Thermo Fisher Scientific. They were delivered in a suspension of 1% solids in water at a concentration of 20,000 microplastics (MP) μL^{-1}. The fibres and the beads were observed under a fluorescence stereomicroscope (Nikon Instruments, SMZ25) with FITC fluorescence filter (ex: 494 nm; em: 518 nm). Photographs were taken with a Nikon camera DS-Ri1 and attendant software (NIS-Elements Basic Research 4.20.01), which also included features for length measurements that we used for the fibres.

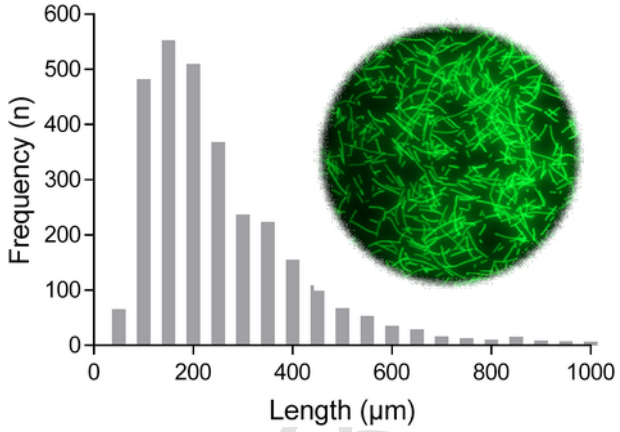

Fig. 1. Length distribution and appearance of fluorescent fibers after production and before administration to the test organisms.

2.3. Experimental procedure

The animals were acclimated to room temperature (22 °C) which was similar to the ambient water temperatures at the time the shrimps were collected (22–24 °C). Prior to the experiments, the test animals were starving for 48 h to void their stomachs from remaining food. The water was exchanged daily to prevent coprophagia. The fullness of the stomachs of the translucent shrimps was visually estimated under the stereo microscope. Only shrimps with empty stomachs were used for the feeding experiments.

2.4. Ingestion of fibres, beads, and food (Exp.1)

Animals received 0.5 mg of plastic fibres and four quantities of flake food (NovoVert, JBL): 0 mg, 2.5 mg, 5 mg, and 10 mg (see also Table S1). The fibres and the food were suspended in 100 ml brackish water (15 PSU) which was previously filtered through a 0.1-µm membrane filter. Animals were kept individually in the experimental units (beakers). Ten replicates were run in each of the four feeding experiments, accounting for 40 animals in total. The shrimps were incubated for 3 h with the food and the fibres. Thereafter, the animals were frozen at −20 °C for 10 min. The wet weights and the lengths from the frontal edge of the eyes to the tip of the uropods were recorded. The stomach, the midgut gland, and the gut of each animal were screened with a fluorescence microscope for microplastic fibres. Subsequently, the organs were dissected and suspended in 100–200 ml of dist. water. The fibres were counted and their lengths were measured under a fluorescence binocular.

2.5. Stomach residence time of microplastic fibers and beads (Exp. 2)

A preliminary feeding experiment showed that the digestive organs (stomach, midgut gland, gut) of *P. varians* were void of microfibers after 24 h. Therefore, we ran an experiment to estimate the

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residence times of food, fibres, and beads. Animals were starved for 48 h prior to the experiment. The maintenance water was exchanged repeatedly and only shrimps with empty stomachs were selected for the experiments. Preliminary observations also showed that *P. varians* ingested fibres more eagerly when they were attached to food items. Therefore, a blend was prepared consisting of 5 mg of flake food, 1 mg of fibres, and 10 µL of 1:10 diluted solution of beads per portion. The control food contained 5 mg of flakes and 10 µl of demineralised water. The food preparations dried overnight at room temperature. Care was taken during the procedure to avoid contamination of the food. The following day, 200-mL petri dishes were filled with 100 ml filtered brackish water (15 PSU) and the prepared food was added with metal forceps. Each of the petri dishes contained about 200,000 beads L^{-1} and 5 mg of fibres as estimated from the amount of food blend added to the water. The animals were placed into the petri dishes and allowed to feed for 20 min. Subsequently, the animals were inspected under the fluorescent microscope for ingested fibres, beads, and food. If the stomachs contained microplastics, the animals were transferred into dishes with 100 ml of fresh and filtered brackish water. Every 2 h, the animals were inspected again and the following parameters were recorded: a) presence of food and microplastics in the digestive organs, b) amount of feces in the petri dish, c) presence of fibres and beads in the feces, and d) presence of mucus plaques at the bottom of the petri dish. After each inspection, the water in the petri dishes was exchanged to enable observation of newly produced feces which was egested during the following 2 h. In total, 27 animals were tested: 9 control animals and 18 fed with beads and fibres. The residence time of fibres was estimated in all 18 animals and the residence time of beads and food in 9 of the 18 animals. See also Table S1.

2.6. Survival

To investigate whether microplastic ingestion had adverse effects on *P. varians*, the survival of the shrimps was recorded for another 48 h. After the experiments were terminated, the animals were continuously fed with flakes (without microplastics) to prevent fatal starvation. Signs of vitality were upright position of the animal, locomotion, and a translucent appearance of the body.

2.7. Stomach movement and regurgitation

Time-lapse recordings were carried out to observe the stomach peristaltic and the egestion of the fibres and beads. Shrimps were fed with food containing fibres and beads as described above. The lateral side of the cephalothorax of the animals was shortly blotted dry with tissue paper and the animals were fixed with a two-component epoxy resin adhesive (UHU, 2-komponenten Epoxidharzkleber) onto the bottom of a petri dish. The dish was filled with brackish water and the animals were observed for 10 h under the fluorescence stereo microscope. Photographs were taken every 10 s with the software program NIS Elements.

2.8. Data analysis

Statistical analysis of data and preparation of graphs was done with the software package GraphPad Prism 7.05 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com). Data sets were analyzed for normal distribution and equal variances. Outliers were identified by the ROUT method. Since data sets were not normally distributed, comparison between groups was performed with sqrt-transformed data, one way ANOVA, and Dunnett's multiple comparison post-hoc test.

The stomach residence times were fitted to the Boltzmann sigmoidal equation:

$$Y = Y_{min} + \frac{(Y_{max} - Y_{min})}{1 + \exp\left[\frac{V_{50} - X}{Slope}\right]}$$

The half-times of the stomach evacuation (V_{50}) and their standard errors (SE) were calculated and compared between animals fed with regular food (control group), microplastic fibers, and microplastic beads, and a mix of microplastic fibres, beads, and food. Treatments with overlapping standard errors ($V_{50} \pm SE$) were considered not significantly different from each other.

3. Data availability

All data supporting the finding of this study are deposited in the Pangea data library . Additionally, information is available from the corresponding author upon reasonable request.

4. Results

4.1. Acceptance of microplastic fibers and beads and their distribution in the digestive organs of shrimps

The length of the shrimps *Palaemon varians* used in this study ranged between 14 and 36 mm and their masses between 88 and 490 mg. Fluorescent acrylic fibers and microspheres were ingested by the shrimps (Fig. 2a). Fibers and microspheres were present in 75% of the stomach from shrimps deployed in Exp. 1. The lengths of the fibers in the stomach ranged from 26 to 2088 µm (236 ± 176 SD), re-

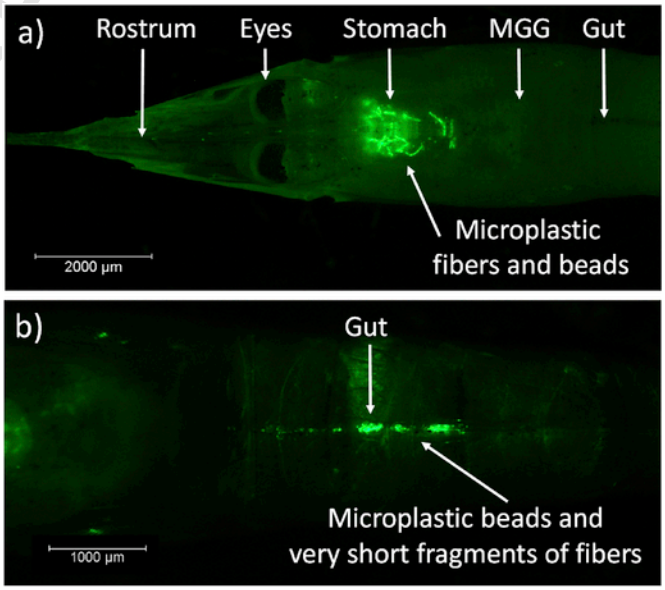

Fig. 2. Dorsal view of a) the cephalothorax and b) the abdomen of the shrimp *P. varians*. The stomach within the cephalothorax is filled with fluorescent fibers and beads. The midgut gland (MGG) posterior to the stomach appears dark and does neither contain fibers nor beads. The gut within the abdomen contains beads and very short fragments (<100 µm) of the microplastic fibers.

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flecting the size range of the administered fibers. No fibers and no microspheres were observed in the midgut glands. Only microspheres and very small fragments of fibers (<100 µm) were present in the gut (Fig. 2b). After 24 h the stomachs were void of fibers and beads. Likewise, no microplastics were present in the guts and in the midgut glands.

4.2. Ingestion of fibers at different food concentrations (Exp. 1)

Animals, which received 0.5 mg of plastic fibers and no food, contained 0 to 8 particles (median 0.5 fibers per animal) in their stomachs after 3 h of exposure (Fig. 3). Ingestion of fibers differed significantly with food concentrations (ANOVA, $F_{3,33}=4.733$, $p=0.0074$). When animals were fed with 2.5 mg of commercial food containing microplastics, the uptake of fibers increased significantly to up to 47 fibers (median: 16.5, $p=0.0027$). Further increase of food to 5 mg reduced the uptake of fibers to maximally 32 items (median: 15) which, however, was still significantly higher than ingestion of fibers of animals without food ($p=0.0395$). At 10 mg of food, up to 17 items were ingested (median: 8) which was not significantly different from the unfed animals ($p=0.1374$). Three individuals ingested exceptional amounts of fibers of 146, 1176, and 1469 items per animal, respectively. These data were identified as outliers and not considered in the statistical comparison nor in the graphical presentation of the results.

4.3. Stomach residence time of microplastic fibers and beads (Exp. 2)

Gut evacuation of the shrimps followed a sigmoidal function. The coefficient of determination varied between $R^2=0.9981$ for food alone (Fig. 4a) and $R^2=9.9883$ for fibers, $R^2=0.9714$ for beads, and $R^2=0.9519$ for the entire mixture of food, fibers, and beads (Fig. 4b). Food remained in all stomachs for the first 4 h but all stomachs were evacuated within 12 h. 50% of stomach evacuation was reached after 8.39 ± 0.13 h (Fig. 4a). Fibers were evacuated from the stomach most rapidly (Fig. 4b). Already after 2 h, the first stomachs were void of fibers. After 6.96 ± 0.55 h, 50% of the animals had no fibers in their stomachs. Thereafter, the animals continuously emptied their stomachs within about 12 h. Beads were evacuated from the stomach at a similar rate as food. Half of the animals showed empty stomachs after

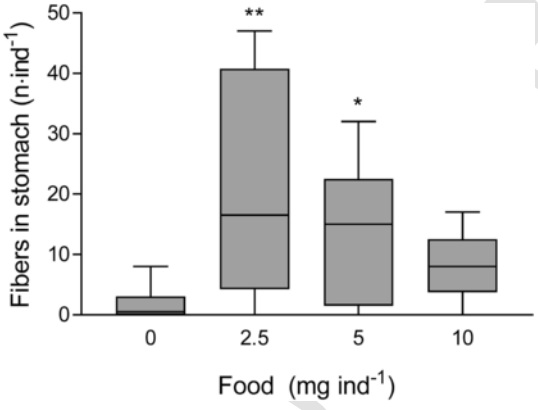

Fig. 3. Numbers of ingested plastic microfibers in stomachs of *Palaemon varians*, which were offered 0.5 mg of microplastic fibres and variable amounts of commercial food (0, 2.5, 5, 10 mg) in 100 ml of water. Box plots show median, 25th and 75th percentile, minimum and maximum values. Asterisks indicate significant differences between animals, which were not fed and those which received 2.5 and 5 mg of food, respectively (ANOVA and Dunnett's multiple comparison on sqrt-transformed data, $p < 0.05$ (*), $p < 0.01$ (**), n = 8–10).

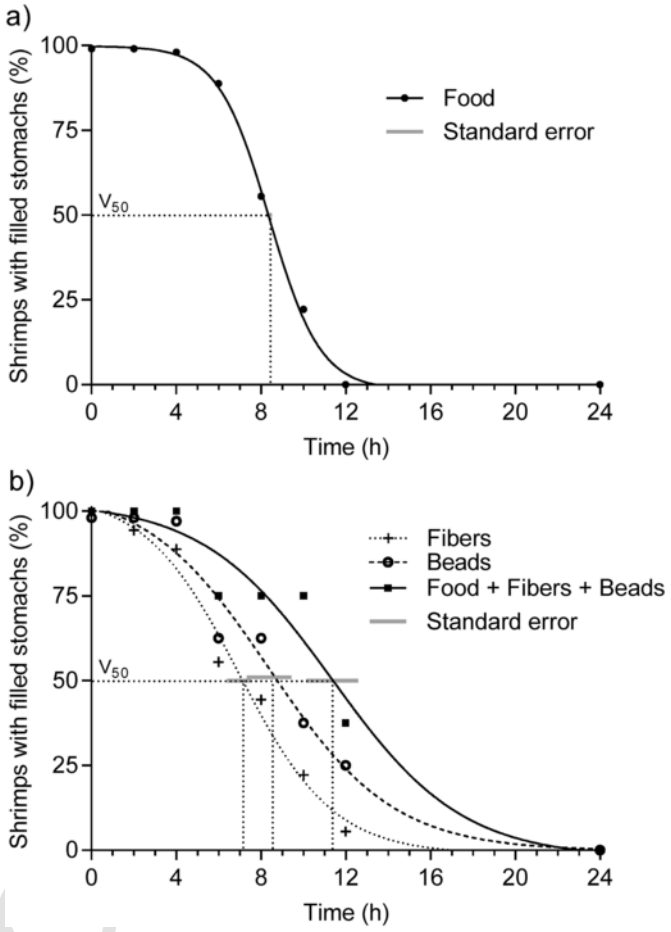

Fig. 4. Evacuation of a) food and b) plastics and food from shrimp stomachs. After feeding, animals were inspected for the presence of food and/or plastics in their stomachs. The share (%) of animals with filled stomachs was plotted against the time. The times at which 50% of the stomachs were empty (V_{50}), and their standard errors, served as measure to compare evacuation times. Overlapping standard error indicate no significant difference between V_{50} of different treatments. The standard error bar in Fig. 4a is smaller than the size of the symbols in the graph. a) 9 shrimps were deployed and analyzed (n = 9). b) 18 shrimps were deployed and n = 18 were analyzed for fibers, n = 9 were analyzed for beads, n = 9 were analyzed for the entire stomach content (fibers, beads, and food).

8.38 ± 1.05 h but complete evacuation took more than 12 h. Total evacuation of food, fibers and beads lasted longest. Half of the animals showed empty stomachs after 11.37 ± 1.22 h (Fig. 4b). Evacuation (V_{50} ± SE) of fibers was significantly faster than evacuation of food. Evacuation of beads did not vary significantly from that of food. Evacuation of the mix of food, fibers, and beads lasted significantly longer than the evacuation of food alone.

4.4. Survival

The ingestion of fibers and microspheres did not seem to enhance mortality of the shrimps. Only one out of 18 animals (i.e. 5.6%) died within 48 h of exposure.

4.5. Egestion and regurgitation

Most of the food remains and the microbeads were transferred from the stomach into the midgut and hindgut of the shrimp. Finally, they were egested as fecal strings, encased by a peritrophic mem-

brane (Fig. 5). Besides microbeads, the fecal strings also contained some small fibers of about 100 µm and less. The large fibers (>100 µm) remained in the stomach but were cleared by regurgitation through the esophagus. Regurgitated material appeared as plaque-like mucous at the bottom of the aquaria (Fig. 6). It contained fluorescent fibers and undefined material, presumably food remains and gastric fluid. An example for a regurgitated plaque with various common air-borne microplastic pollutants is presented in the Supplementary material (Fig. S1).

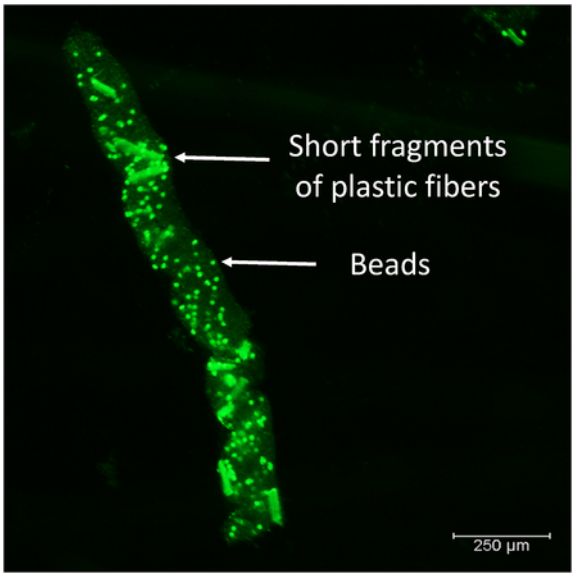

Fig. 5. A fecal string of *Palaemon varians* containing very short fragments of fluorescent microplastic fibers and beads. The fecal string is coated with a chitinous peritrophic membrane.

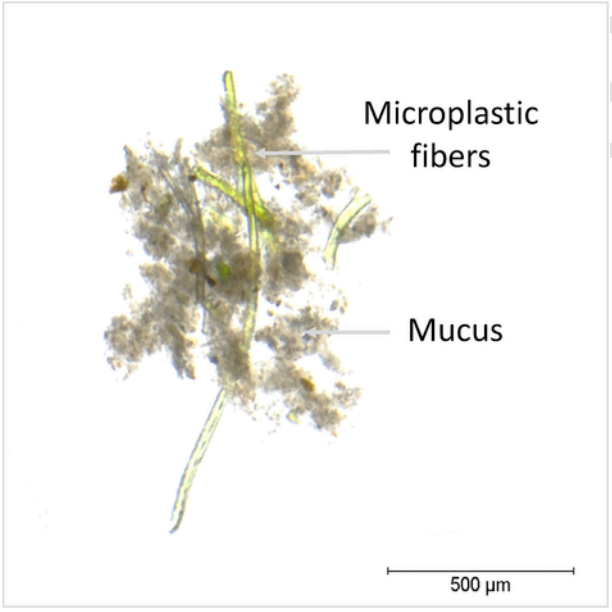

Fig. 6. Mucus-like plaque of regurgitated material at the bottom of the aquarium of animals fed with experimentally administered green microplastic fibers (MPFs) and food. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

The process of regurgitation is shown in a time-lapse movie (Supplementary material). The fluorescent fibers were visible in the stomach (Fig. 7a). After contraction of the stomach, the fibers appeared in the esophagus (Fig. 7b). Fig. 7c and d show the release of the stomach content including fibers through the esophagus. Thereafter, the regurgitated material dispersed (Fig. 7e). Finally, the stomach was empty and few fluorescent particles remained attached to the setae of the mouthparts (Fig. 7f). The process of stomach evacuation lasted less than 3 min.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.envpol.2019.113068.

5. Discussion

Fluorescent microplastic fibers and microspheres were ingested by the shrimps, *Palaemon varians*, preferably together with the offered food. The lengths of the fibers resemble those of fibers collected in the habitat of the shrimps, the brackish Baltic Sea waters off Sweden (290–2700 µm, Gewert et al., 2017). The fibers were retained in the stomach while the microbeads were passed further into the gut. The latter were egested together with undigested food remains within fecal strings. After few hours, the fibers disappeared from the stomach as well and mucous plaques containing fluorescent fibers appeared at the bottoms of the aquaria in which the animals were maintained. The fibers were not egested through the gut, but left the stomach through the esophagus. This process has been reported previously as regurgitation.

Regurgitation is a common process in various invertebrates (Table 1). It is associated with e.g. defense behavior, food transfer among individuals, extracellular digestion, or defense suppression. In crustaceans, it has been described as a mechanism to remove large and hardly digestible food particles from the stomach. Already Forster and Gabbott (1971) reported that regurgitation of parts of the indigestible food remains after the meal complicated the accurate determination of assimilation efficiencies in the prawns *Palaemon serratus* and *Pandalus platyceros*. In the paddle crab *Ovalipes catharus* (Brachyura) shell fragments were cleared from the foregut by regurgitation after virtually all soft parts had been digested (Haddon and Wear, 1987). The speed of food procession and onset of regurgitation depended on temperature but not on animal size. At 20 °C, regurgitation happened within 3–6 h, at 11 °C within 11 h, and at 9 °C within 18 h.

Even the pelagic pluteus larvae of the Pacific Sand Dollar, *Dendraster excentricus*, are able to regurgitate too large or offending particles from their esophagus by a reverse wave of peristaltic contractions (Burke, 1981). Kaposi et al. (2014) observed that the number of plastic particles ingested by the pluteus larvae of the sea urchin *Tripneustes gratilla* decreased with time. The authors suggested that the larvae recognized the low nutritional value of the plastic particles and rejected their ingestion or egested them over time. Another mechanism to select between digestible and indigestible food items is the formation of pseudofeces by certain bivalve species, such as the Blue mussel *Mytilus edulis* (Beninger and St-Jean, 1997; Beninger et al., 1999).

Apart from our laboratory experiments under controlled and clean conditions (Wesch et al., 2017) a few animals were left in an open uncontrolled area where they were exposed to the common airborne microplastics. These animals regurgitated plaque with various dark and blue-colored fibers (Fig. S1), which are most likely contaminants originating from clothes or other air-borne discharge. They resemble those fibers collected from the atmospheric fallout of urban environments or indoor facilities (Dris et al., 2016, 2017). This example,

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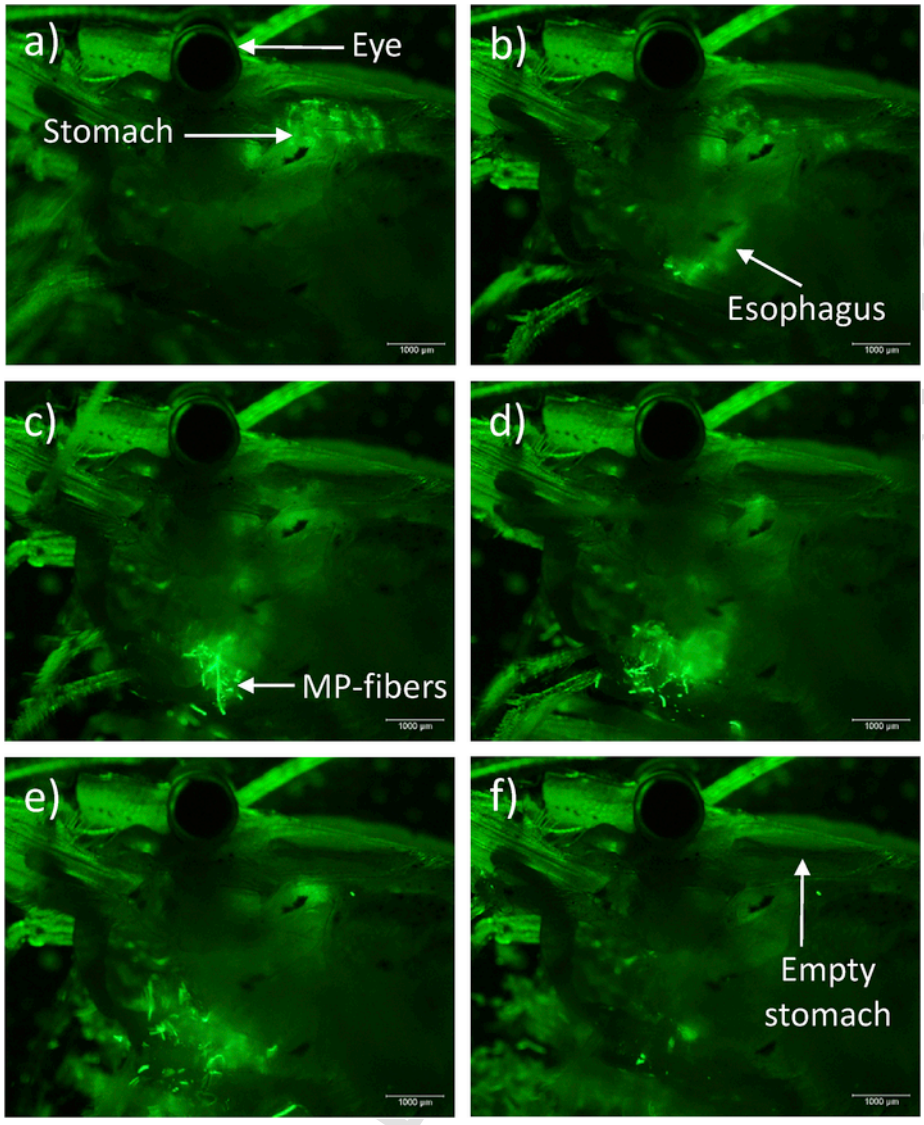

Fig. 7. Sequence of time-lapse recordings showing regurgitation of fluorescent fibres and beads from a lateral view of *P. varians* cephalothorax (a–d). The whole process of regurgitation lasted 160 s. Thereafter, the stomach of the shrimp was void of fibres and beads (e, f). MP-fibers = microplastic fibres.

however, clearly demonstrates that the shrimps are also capable of regurgitating the meanwhile ubiquitously occurring microplastic contaminants.

The microplastic fibres used in our experiments had a similar shape as e.g. cellulose or lignin fibres. They were likely too large to be passed into the gut but were, instead, retained in the stomach and then regurgitated. However, when the animals received only food without microplastics, regurgitation also took place suggesting this behavior being no exclusive reaction to plastic contamination. An explanation for this behavior could be an adaptation of *P. varians* to the omnivorous, especially the herbivorous and detrivorous life style in particle-rich estuarine waters. In this way, the numerous indigestible organic and inorganic particles could be eliminated before injuring or clogging the digestive tract of the organism. Accordingly, the mortality of *P. varians* was low in our experiments. Only one animal died for unknown reason.

Different to *P. varians*, the closely related species *Palaemonetes pugio*, was not able to clear its digestive tract from polypropylene fibres after a 3 h exposure to 34 and 96 µm long fibres (Gray and Weinstein, 2017). As a result, 100% (34 µm) and 43% (93 µm) of the shrimps died within 96 h. *P. pugio* lives in salt marshes at the Atlantic US coast of North America and, thus, encounters similar environmental and trophic conditions as *P. varians*. However, the ability to regurgitate fibres seems not to have evolved in this species. Similarly, the Norway lobster *Nephrops norvegicus* was shown to be unable to regurgitate indigestible items. Fibers accumulated in the stomach, formed small balls, and clogged the stomach. These balls could only be removed when the ectodermal structures of the stomach were shed off during moulting (Welden and Cowie, 2016a,b). Intermoult periods, however, lasted for several months during which animals showed signs of starvation due to the accumulation of the indigestible material in their stomachs. Besides filling the stomach with indigestible material, the microplastic particles may cause inflammation reactions and oxidative stress as shown in mollusks, rotifers, and copepods (von Moos et al., 2012; Jeong et al., 2016, 2017).

Although regurgitation has been described for several crustacean species, the anatomical requirements, the physiological control mechanisms, and the triggers for the regurgitation process are still un-

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Table 1
Crustaceans and other invertebrate species showing regurgitation.

Species	Taxon	Regurgitated material	Reference
Cancer pagurus	Crustacea – Brachyura	Exoskeletal fragments	Lawton (1989)
Cancer gracilis	Crustacea – Brachyura	Food at low salinity	McGaw (2006)
Cancer productus	Crustacea - Brachyura	Not specified stomach content	McGaw (2007)
Ovalipes catharus	Crustacea – Brachyura	Shell fragments	Haddon & Wear (1987)
Scylla serrata	Crustacea – Brachyura	Shell fragments	Hill (1976)
Palaemon serratus	Crustacea - Caridea	Cellulose fibers	Fair et al. (1980)
Palaemon serratus	Crustacea - Caridea	Indigestible material	Forster and Gabbott (1971)
Pandalus platyceros	Crustacea - Caridea	Cellulose, lignin, fragments of fish scales and bones	Forster and Gabbott (1971)
Formica sanguinea	Insecta – Formicidae	Food exchange	Wallis (1961)
Lasius flavus	Insecta – Formicidae	Extracellular digestion, defense	Cammaerts (1995)
Malacosoma americanum	Insecta – Lepidoptera	Enteric fluid for defense	Peterson et al (1987)
Spodoptera littoralis	Insecta – Lepidoptera	Suppression of plant defense	Vadassery et al. (2012)
Schistocera emarginata	Insecta – Caelifera	Gastric fluid for defense	Sword (2001)
Locusta migratoria	Insecta – Caelifera	Crop fluid for defense	Freeman (1968)
Various species	Instecta	Suppression of plant defense	Timisena and Mikó (2017)
Dendraster excentricus	Echinodermata, Echinoidea	Offending particles	Burke (1981)
Pleurobranchaea californica	Mollusca – Gastropoda	Experimentally administered rotten squid or soap solution	McClellan (1982)

known. Sousa and Petriella (2006) suggested, that the lack of an oesophagial valve at the entrance of the pyloric stomach of the shrimp *Palaemonetes argentinus* may enable regurgitation. However, the mud crab, *Scylla serrata* has a tri-lobed valve between the short esophagus and the cardiac stomach (Barker and Gibson, 1978) but is able to regurgitate shell fragments (Hill, 1976). The regurgitation process starts with rapid waves of contraction in the posterior region of the cardiac stomach forcing food upward, while the simultaneous opening of the esophagus allows expulsion of the food (McGaw and Curtis, 2013). To facilitate food expulsion, it appears necessary to tighten the passage between stomach and midgut to avoid pressing the stomach content into the gut. Many crustaceans, like the brachyuran crabs *Scylla serrata* and *Menippe rumphii,* possess a cardio-pyloric valve separating the cardiac and pyloric regions of the stomach allowing the passage of only very small particles (Barker and Gibson, 1978; Erri Babu et al., 1982). Additional comparative studies on crustaceans, which are capable of regurgitation (Table 1) and those which are not, like the Norway lobster, *N. norvegicus*, are required to understand the behavior and physiological mechanism of regurgitation.

Our experiments also indicate that shrimps are able to empty their stomach contents selectively. Indigestible plastic fibers were evacuated faster than food. Apparently, the animals recognized that the plastic fibers did not provide nutrients and, thus, released the synthetic stomach content on average 1.4 h earlier than the food content. A mixture of food and fibers, however, increased the stomach evacuation time by 3 h. We suggest, that the presence of plastic fibers mimics additional food, which, apparently, needs more time to be processed and digested. Consequently, the process of regurgitation seems to be linked to the sensory recognition for mechanical and chemical stimuli within the digestive organs as reported for e.g. insects (Park and Kwon, 2011).

The shrimps ingested most of the fibers along with food but only few when fibers were offered alone. This resembles natural feeding behavior where shrimps would avoid ingestion of indigestible material. However, indigestible or hardly digestible compounds of organisms such as e.g. silica shells of diatoms, chitin bristles of annelids, or calcareous shells of bivalves are inevitably ingested as part of the prey and need to be eliminated from the digestive tract. In addition to chemical and mechanical selection of food items and gastric filter-separation of chyme and solids (Saborowski, 2015), regurgitation represents another sorting mechanism to efficiently separate assimilable material from indigestible remains.

6. Conclusions

Regurgitation reflects an evolutionary adaptation of crustaceans to remove large indigestible natural food items from the stomach. Nowadays it may also serve as an efficient mechanism to get rid of ingested particulate anthropogenic pollutants, such as microplastic fibers. Species, which are able to extricate themselves from such a burden may have better risk scores and will be less affected by microplastic pollution than others which are not able to do so. In turn, knowledge about biological features of species, such as regurgitation or other sorting mechanisms in the digestive tract, can help to estimate the vulnerability of species to ecotoxicological threats. This issue might be of interest for various stakeholders, including aquaculture farmers and environmental policy makes and should be addressed in future environmental risk assessment strategies.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.113068.

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E T O C B L U R B

The evolutionary developed process of regurgitation in shrimps provides nowadays a suitable mechanism to eliminate ingested anthropogenic microplastic fibers from the stomach, avoiding accumulation and, thus, health impairment.

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