# Trophic transfer of microplastics from mysids to fish greatly exceeds direct ingestion from the water column

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**Abstract**

Predators ingest microplastics directly from the environment and indirectly via trophic transfer, yet studies have not investigated the contribution of each pathway to microplastic ingestion in fish. We assessed the relative importance of the two exposure routes using mysids (*Neomysis* spp.) and a benthic fish (*Myoxocephalus brandti*) as a model prey-predator system. We first exposed the mysids to fluorescent polyethylene beads (27–32 μm) at concentrations of 200 and 2,000 μg/L. We then exposed the fish to water containing the same concentrations of polyethylene beads or to nine mysids pre-exposed to polyethylene beads. We quantified the size and overall mass of polyethylene beads in mysids and in fish to assess polyethylene beads fragmentation by the mysids. Mysids ingested 2–3 more polyethylene beads from water containing the higher concentration, and fish ingested 3–11 times more polyethylene beads via trophic transfer than from the water column. The percentage of fragmented particles was higher in mysids and in fish fed bead-exposed mysids, suggesting that the mysids can fragment polyethylene beads. Our experiments demonstrate that trophic transfer is a major route of microplastic ingestion by fish and that prey such as mysids can fragment microplastics. Small particles can translocate from the digestive system into tissues and exert adverse physiological effects. Trophic transfer of microplastics may therefore pose more serious threats to organisms at higher trophic levels.

Keywords: crustacean, trophic transfer, plastic fragmentation, prey-predator interaction, *Neomysis* spp., *Myoxocephalus brandti*

## 1. Introduction

Plastics are ubiquitous in daily life, and global plastic production reached 359 million tons in 2018 (PlasticsEurope, 2019). Jambeck et al. (2015) estimated that 4.8–12.7 tons of plastics are released into the oceans each year. Numerous studies have reported the ingestion of plastics and their potential impacts on marine organisms such as whales, sea turtles, fish, crustaceans, soil fauna and soil microbe (Auta et al., 2017; Cole et al., 2013; Lazar and Gračan, 2011; Rummel et al., 2016; Zhou et al., 2020). Microplastics, defined as plastic pieces smaller than 5 mm in length, are an emerging threat to marine ecosystems (NOAA, 2020) because they are small enough to be ingested by even small organisms (Andrady, 2011; Cole et al., 2011) and difficult to remove from marine environments (Jambeck et al., 2015). Once ingested, microplastics can cause lacerations, inflammation, and starvation (Carbery et al., 2018).

Organisms ingest microplastics directly from their ambient environment or indirectly via trophic transfer (Walkinshaw et al., 2020). Most studies have focused on direct ingestion from the water column, and knowledge of trophic transfer of microplastics is scarce (Au et al., 2017), with the exception of a few reports that did not compare the contributions of direct and indirect ingestion (Farrell and Nelson, 2013; Nelms et al., 2018). Bioaccumulation studies using other environmental contaminants suggested that trophic transfer plays a more important role in the uptake than the waterborne ingestion (Qiao et al., 2000). Thus, trophic transfer may be the major contributor to the ingestion for microplastics too. Understanding the dynamics of microplastics in the marine food chain requires a quantitative comparison between direct ingestion and trophic transfer.

Although microplastics are mostly excreted after passing through the digestive tract, particles smaller than 10 μm can translocate from the gut into other tissues and cause adverse physiological effects, which have been shown in the blue mussel *Mytilus edulis* (Browne et al., 2008; Von Moos et al., 2012). Antarctic krill has been shown to fragment microplastics into particles small enough for tissue translocation to occur, perhaps through its feeding and

digestion processes, which are shared by other small crustaceans such as copepods and mysids (Dawson et al., 2018; Kobusch, 1998; Michels and Gorb, 2015). If fragmentation of microplastics by small crustaceans is common, the particles may pose hazards to organisms at higher trophic levels.

The aim of the present study was to examine the relative ingestion of microplastics by fish from the water column and via trophic transfer from prey. We used a crustacean mysid (*Neomysis* spp.) and a benthic fish (*Myoxocephalus brandti*) as a model prey-predator system. We exposed the fish to fluorescent polyethylene beads (27–32 μm) and to mysids fed the beads to compare direct and indirect ingestion. We also analyzed the size of polyethylene beads ingested by the mysids and fish to assess microplastic fragmentation. We hypothesized the fish ingests more microplastics from the mysids than the water column and that the mysids fragment microplastics into smaller particles.

## 2. Materials & Methods

### 2.1 Animal collection

We collected organisms for the experiment on November 7, 2018 at a seagrass bed in Akkeshi-ko estuary (43°02′ N, 144°52′ E), which is located in eastern Hokkaido, northern Japan. Mysids (*Neomysis* spp., wet weight: 20.9 ± 5.1 mg standard deviation) and juvenile *M. brandti* (wet weight: 6.5 ± 0.8 g standard deviation) were collected using an epibenthic sled. In this area, the mysids are major prey for predatory fish and decapod crustaceans; the fish are among the most dominant predators of mysids, especially at the juvenile stage (Yamada et al., 2010). We acclimated the animals in 30-L aquaria over 5 days with flow-through seawater that were filtered by fine sand to allow them clear possible microplastics from their guts. During the acclimation, we daily fed the mysids microalgae (Shellfish diet 1800; Reed Mariculture) and the fish fresh mysids. Each aquarium contained approximately 100 mysids or 20 fish.

2.2 Microplastic exposure

We used polyethylene microspheres (27–32 μm, 1.025 g/mL; Cospheric LLC, Santa Barbara, CA, USA) that were labeled with green fluorescence (excitation: 414 nm; emission: 515 nm). Global production of polyethylene exceeds production of other plastic polymers, and polyethylene is among the most common types of marine litter identified in the ocean. (Andrady, 2017; Beiras et al., 2018; Burns and Boxall, 2018; Hidalgo-Ruz et al., 2012). We selected this size as it is within the size range of phytoplankton (>10 μm) that mysids feed on (Bowers and Grossnickle, 1978). Fluorescently labeled microbeads were used to distinguish them with any possible contaminated plastics during the experiments so that the results would not be interfered.

A glass beaker was filled with 10 mL of distilled water and boiled in a microwave. Ten microliters of surfactant (Tween 80; polyethylene sorbitol ester, Cospheric LLC) were added and stirred with a glass rod for 30 s. Next, 1 mL of the solution was transferred to a 1.5-mL Eppendorf tube and left at room temperature for 1 h. Following the addition of 50 mg of fluorescent microbeads, the tube was vortexed and the solution was then diluted 100-fold with distilled water. The final concentration of polyethylene beads in the stock suspension was approximately 24 μg or 1,770 particles per 10 μL of suspension stock

To determine that the mysids could ingest the polyethylene beads, we exposed mysids to 2,000–μg/L beads. Fluorescent microscopy showed polyethylene beads in the animals' stomachs, intestines, and fecal pellets (Fig. 1).

2.3 Ingestion experiment for mysids

We conducted a microplastic ingestion experiment on November 12 and 13, 2018. Forty three 1-L plastic bottles were filled with filtered seawater; three bottles contained no microplastics (control), 20 bottles contained 200–μg/L microplastics (low dose), and 20 bottles contained 2,000–μg/L microplastics (high dose). The low dose was chosen as an

environmentally relevant concentration as it is within the same order of magnitude of the microplastic concentration reported in the North Pacific Subtropical Gyre, which is among the most heavily contaminated area around the world (Goldstein et al., 2012). High dose was set as the future concentration in the scenario that microplastic pollution in the North Pacific Ocean keeps growing over the next 50 years (Isobe et al., 2019). We randomly selected adult mysids without juveniles in their brood pouch and placed one in each bottle. We then fed the mysids 2 mg (dry weight) of microalgae. We constantly circulated the seawater by aeration to ensure that the microplastics were evenly distributed in the bottles. After 24 h of microplastic exposure, the mysids were flushed gently with filtered seawater to remove beads from the exoskeleton. Following measurement of their wet weight, the mysids were dissected to remove the stomach and intestine, which were fixed with 70% ethanol and stored in glass vials until analysis.

2.4 Trophic transfer experiment

We conducted a microplastics trophic transfer experiment on November 14–17, 2018. We controlled two factors: the concentration of microplastics (200 or 2,000 μg/L) and the source (water or mysids) (Fig. 2). Thirty-three glass aquaria were prepared, and one fish was assigned to each aquarium and kept without food for 48 h prior to the experiment. In three of the aquaria, each fish was fed only nine plastic-free mysids (control). Five aquaria contained water with a 200-μg/L microplastic suspension and nine plastic-free mysids each, so that the fish would ingest polyethylene beads only from the water column. Five other aquaria contained water with a 2,000-μg/L microplastic suspension and nine plastic-free mysids each. Ten aquaria each contained nine mysids that had been pre-exposed to 200–μg/L microplastics, and 10 aquaria each contained nine mysids that had been pre-exposed to 2,000–μg/L microplastics. We allocated more replicates to the mysids group because the experimental space was limited and the higher variability was expected in this group due to the variation in the amount of microplastics ingested by mysids. The 20 aquaria containing pre-exposed mysids were filled

with filtered seawater without a microplastic suspension so that uptake of polyethylene beads would be solely from the food source. The number of mysids was determined from a preliminary experiment to ensure that all mysids were consumed by the fish within 24 h. Pre-exposure of the mysids followed the protocol for the first experiment, except that three individuals were placed in each exposure bottle. We constantly circulated the seawater by aeration throughout the experiment to ensure that microplastics were evenly distributed in the aquaria. We did not observe any microplastics on the bottom of the tanks throughout the experiment. We confirmed that all mysids were consumed within 1 h. After 24 h, the fish were gently flushed with filtered seawater to remove beads from the body surface. Following measurement of their wet weight, the fish were dissected to remove the stomach and intestine, which were fixed with 70% ethanol and stored in glass vials until analysis.

2.5 Sample analysis

To extract polyethylene beads ingested by the animals, the stomachs and intestines of mysids or fish were placed in 1.5-mL microtubes with 1 mL of 10% potassium hydroxide solution to dissolve organic matter. We also treated the stock suspension with potassium hydroxide or distilled water as procedure blanks to examine the treatment effects on polyethylene beads. The samples were shaken at 60 rpm at room temperature for more than 1 week.

After all the organic matter was dissolved, the remaining samples were filtered under vacuum through nylon mesh filters (MilliporeSigma, Burlington, MA, USA; nylon membrane hydrophilic filter, pore size: 0.8 μm, filter diameter: 25 mm). The microtubes and funnels were washed three times with 70% ethanol to recover all beads. Next, the filters were fixed between glass slides and the number and size of polyethylene beads were determined under a fluorescent microscope (CKX53; Olympus Corporation, Tokyo, Japan) at 100× magnification. All particles on the filters were individually imaged and analyzed. For fish intestine samples, the images

were taken from 30 randomly selected squares (1.76 × 1.32 mm) on the filter, accounting for approximately 25% of the total filtered area (2.9 cm2), because a substantial number of particles was found. We then estimated the total amount ingested.

The diameter (major axis when a particle was fitted to an ellipse) of each particle within each image was measured using ImageJ software (NIH, Bethesda, MD, USA). We applied thresholds to the fluorescence intensity of each image with the "Intermodes" algorithm, which enabled the exclusion of undigested materials on the filter without interfering with the analysis. Particles larger than 50 μm were excluded from the analysis on the assumption that more than two beads had aggregated. The minimum size threshold was designated as 2.43 μm because the software could not distinguish smaller beads from noise, leading to a final particle diameter range of 2.43–50 μm. Additionally, we determined the number and mass of the ingested particles. Assuming that all particles were ellipsoids, we calculated the mass by the major axis, the minor axis, and the density (1.025 g/mL). We determined the size boundary between whole and fragmented beads as 25 μm (major axis) from the particle size distribution in the stock suspension, and then calculated the fragment frequency by the percentage of fragmented beads against the total number.

## 2.6 Statistical Analysis

No polyethylene beads were found in the control groups, so we excluded them from the statistical analyses. All analyses were conducted in R (R Core Team, 2020). Data are presented as mean ± standard deviation (SD).

To compare the differences in the number and mass of polyethylene beads ingested by mysids, we used generalized linear models (GLMs) with log link functions. For particle numbers, a negative binomial distribution was assumed to account for the overdispersed discrete values using the *glm.nb* function in the "MASS" package (Venables and Ripley, 2002). Overdispersion for the model was checked with the *dispersiontest* function in the "AER"

package (Kleiber and Zeileis, 2008) assuming that the response variable had a Poisson distribution. For particle mass, because the Shapiro-Wilk normality tests showed that the response variable had a non-normal distribution, a gamma distribution was assumed to account for the positive continuous values. We used log-transformed mysid wet weight as an offset in the models. To test the effect of concentration, we performed a likelihood ratio test using the *Anova* function in the "car" package (Fox and Weisberg, 2019).

To compare the differences in the number and mass of polyethylene beads ingested by fish, we used GLMs with log link functions. A negative binomial distribution and a gamma distribution were assumed for particle number and mass, respectively. We used log-transformed fish wet weight as an offset in the models. We tested the single and interaction effects in the models by the likelihood ratio test. To address the effect sizes between groups, we report odds ratios with 95% confidence intervals calculated from the estimates and standard errors of the slopes in the models.

To compare the difference in fragment frequency between treatments (stock suspension, potassium hydroxide procedure blank, mysids, fish fed bead-exposed mysids, and fish exposed to waterborne beads), we applied a GLM with a log of the total number of beads as an offset assuming a negative binomial distribution. Following the likelihood ratio test for the treatment effect, we used Tukey's HSD test as post-hoc analysis for pairwise comparisons. Data from the low and high concentration treatments were grouped together in this analysis.

## 3. Results

Ingestion experiment for mysids

Five of the 20 individuals in the low-concentration group and three of the 20 individuals in the high-concentration group died during the experiment. Polyethylene beads ingested by the mysids differed significantly between treatments in both number (likelihood ratio test: $\chi^2(1) = 18.49$, $p < 0.001$) and mass ($\chi^2(1) = 15.61$, $p < 0.001$). Mysids exposed to the

high concentration ingested an average of 266.82 particles per individual (SD: 155.39) for a mean mass of 0.016 ng per mysid (SD: 0.016), a four-fold increase in particle number and six-fold increase in particle mass than mysids exposed to the low concentration, which ingested an average of 65.53 particles per individual (SD: 63.36) for a mean mass of 0.0026 ng per mysid (SD: 0.0028).

Trophic transfer experiment

The number and mass of polyethylene beads ingested by fish varied significantly in concentration and source (Table 1). On average, fish that ingested bead-exposed mysids ingested 8–11 times more particles (Table 1, Fig. 3a) and 3–5 greater mass than fish exposed to polyethylene beads in the water column (Fig. 3b). Fish exposed to the higher concentration of polyethylene beads ingested 2–3 times more particles and 3–6 times greater mass than those exposed to the lower concentration in both groups (fish fed bead-exposed mysids and fish in bead-containing water). There was no significant interaction between bead concentration and bead source (Table 1). Notably, the effect size of the source differed greatly between particle number ($z[1, N = 15] = 0.01$, $p < 0.001$, odds ratio: 13.62, confidence interval: 4.01, 40.15) and particle mass ($t[1] = 0.13$, $p = 0.048$, odds ratio: 4.20, confidence interval: 0.92, 15.53).

Size structure of polyethylene beads ingested by mysid and fish

All mysids exposed to polyethylene beads contained fragmented beads (Fig. 1). Fragment frequency varied significantly between treatments (likelihood ratio test: $\chi^2(4) = 186.88$, $p < 0.001$). Multiple-comparison analysis revealed a significantly higher frequency of fragments in bead-exposed mysids and fish fed bead-exposed mysids than in the stock suspension, potassium hydroxide procedure blanks, or fish exposed from the water column (Fig. 4). The frequency of fragments did not differ between bead-exposed mysids and fish fed bead-exposed mysids. The frequency of fragments was higher in the potassium hydroxide procedure

blanks and in fish exposed from the water column than in the stock suspension. The median particle sizes of bead-exposed mysids and fish fed bead-exposed mysids were 6.41 μm and 6.61 μm, respectively, whereas that of fish exposed from the water column was 32.40 μm.

## 4. Discussion

This study evaluated the pathways of microplastic ingestion by fish and biological fragmentation of microplastics by fish and their prey. We found that trophic transfer from prey to predator contributed more than ingestion of microplastics from the water column, and a higher percentage of fragmented plastics was observed in mysids and in the fish that fed on them. To our knowledge, this study is the first to demonstrate that fish ingest more microplastics through their prey than from the water column and that mysids can fragment microplastics.

Most previous studies of microplastics ingestion or toxicity focused on waterborne exposures at the individual level. While there are some empirical studies on trophic transfer of microplastics in aquatic organisms (Elizalde-Velázquez et al., 2020; Nelms et al., 2018; Setälä et al., 2014), none address their contribution relative to ingestion from the water column. In marine fish, microplastic intake via water is considered a major route of exposure (Roch et al., 2020), but our results showed that it is less important than trophic transfer, likely leading to an underestimation of the effects of microplastics in marine food webs. An increase in microplastic ingestion by predator via trophic transfer raises the concerns on their biomagnification. A recent study, however, showed that biomagnification of microplastics are not likely to occur because they can be excreted from an organism and do not accumulate in the body (Walkinshaw et al., 2020). When ingestion rate exceeds the egestion rate, organisms will accumulate microplastics (Au et al., 2017). As trophic transfer contributes to increase the ingestion rate, continuous exposure from the both pathways needs to be considered in future studies to better explore the potential for biomagnification of microplastics.

The percentage of smaller plastics recovered from the mysids and fish fed on bead-

exposed mysids was significantly higher than in the stock suspension, potassium hydroxide procedure blanks, and fish exposed from the water column. This finding supports our hypothesis that mysids fragment plastics. Dawson et al. (2018) reported that Antarctic krill can fragment microplastics into nanoplastics. An another recent study, by Mateos-Cárdenas et al. (2020), showed that the freshwater amphipod *Gammarus duebeni* can also fragment microplastics. Many small crustaceans that feed on phytoplankton or detritus, such as krill, amphipods, and mysids, have similar digestive mechanisms, including developed mandibles and chitinous and thick barbed spines in their stomach (Dawson et al., 2018; Friesen et al., 1986; Mateos-Cárdenas et al., 2020). These specific features and mastication in the feeding process explains the capacity for fragmenting plastic particles (Dawson et al., 2018; Mateos-Cárdenas et al., 2020). Although the Antarctic krill used by Dawson and colleagues fragmented over 90% of the plastics ingested, our mysids fragmented only 34% (after subtracting the value in the potassium hydroxide procedure blank), perhaps because of differences in diet. Krill is known to feed mostly on phytoplankton while mysids are omnivorous (Siegfried and Kopache 1980; Nakamura et al., 2020), so krill may have a more developed physical digestive system to grind harder cell structure of phytoplankton. We cannot fully exclude the possibility that the mysids fed selectively on smaller particles, but it is unlikely because mysids preferentially consume phytoplankton exceeding 10 μm in size (Bowers and Grossnickle, 1978; Friesen et al., 1986). Potassium hydroxide fragmented some polyethylene beads, but at a lower frequency than the mysids.

After ingestion by organisms, microplastics can be retained in the digestive tract and cause lacerations, inflammation, and starvation (Carbery et al., 2018). As our study showed, trophic transfer is a significant route of microplastic ingestion and may increase the chance to have such negative impacts on the fish. Particularly, fish exposed to the lower concentration only through the mysids ingested more polyethylene beads than fish exposed to the higher concentration only through the water column. This suggests that trophic transfer of even low

concentrations of microplastics have greater impacts on the fish than waterborne ingestion of high concentrations. Yin et al. (2019) demonstrated that waterborne exposure of Korean rockfish (*Sebastes schlegelii*) to 15-μm polystyrene microplastics altered both energy reserves and behavior. Although larger particles (27–32 μm) were used in our study, the fish fed with bead-exposed mysids contained the more comparable particle size of microplastics (Median: 6.61 μm) to those used in Yin et al. (2019). The concentration used by Yin et al. (2019) was similar to the high-exposure treatment (2,000 μg/L, $1.5 \times 10^6$ particles) in the current study. As the low-exposure treatment (200 μg/L, $1.5 \times 10^5$ particles) is within environmentally relevant concentrations (Goldstein et al., 2012), the actual exposure of predatory fish may be underestimated when it does not take trophic transfer into account.

Our findings also suggest that feeding on small crustaceans such as mysids increases the ingestion of fragmented particles that are small enough to translocate from the digestive tract into other tissues and cause physiological effects (Lu et al., 2016; Von Moos et al., 2012). Although we did not examine the translocation of the fragmented particles into fish tissues, they are theoretically small enough for the translocation to occur. Lu et al. (2016) exposed zebrafish to 5-μm polystyrene particles and observed accumulation in the liver. The smallest particles observed in our study were 2.43 μm in length, and could potentially translocate to tissues. As we found, feeding on plastic-containing prey increases the number of particles ingested by fish, and the prey can fragment the particles into smaller sizes, so trophic transfer may cause more adverse biological effects in predators.

Plastics present in the oceans contain a variety of toxic chemicals which are either absorbed to the polymer surface from the surrounding water, in particular persistent organic pollutants (POPs; Andrady, 2011) or incorporated during the manufacture process, called plastic additives (Cole et al., 2011). If plastics are ingested by organisms, those chemicals can transfer from the plastics to their tissues and cause adverse health effects (Hermabessiere et al., 2017; Tanaka et al., 2015). Our findings imply that trophic transfer increases microplastic

exposure to predators and may accelerate the bioaccumulation of toxic chemicals derived from microplastics. Also, fragmentation of microplastics by small crustaceans could facilitate the release of chemical substances from microplastics due to the migration of the chemicals from the core to the surface of the particle (Wright and Kelly, 2017). Although the biomagnification of microplastics may not occur (Burns and Boxall, 2018; Walkinshaw et al., 2020), plastic-derived chemicals could biomagnify because they can accumulate in organisms' tissues. More advance research is needed on the effects of trophic transfer and fragmentation of microplastics on the behavior of plastic-derived chemicals.

Particle number has been commonly used as a unit of measurement in studies of microplastic ingestion and toxicity, but the lack of standardization precludes direct comparisons between studies. We therefore used particle mass to quantify ingestion and support particle counts. We found that the effect sizes for the difference between ingestion from the water column and ingestion by trophic transfer varied considerably between the number and mass of polyethylene beads. This difference can be attributed to the fragmentation by mysids. As fish ingested fragmented particles through mysids, quantification by number caused an overestimation in the actual quantity of polyethylene beads ingested by the fish, and analysis by mass was more accurate (small: $OR < 1.5$; medium: $1.5 < OR < 5$: large: $OR > 5$; Chen et al., 2010). Other organisms could also have the capacity to alter the size structure of plastics, then whether showing ingestion values just in the number or mass makes a huge difference in the interpretation of those results. Other organisms may also fragment plastic microspheres, and we therefore strongly recommend that results be analyzed by mass and not by particle number for more accurate quantification.

## 5. Conclusion

We demonstrated that trophic transfer is a major contributor to microplastic ingestion by fish and that prey such as mysids can fragment microplastics. Smaller particles can

translocate from the digestive tract into tissues and exert adverse physiological effects. Increased amount and smaller sizes of microplastics via trophic transfer could possibly amplify plastic-mediated adverse effects on fish. Concerns have been voiced about the impacts of plastic-derived chemicals on marine ecosystems. Trophic transfer and fragmentation of microplastics may also facilitate bioaccumulation and biomagnification of such chemicals. More research on trophic transfer of microplastics and their fragmentation by organisms is warranted.

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**Table 1 Results of likelihood ratio test on the generalized linear model for the number and mass of polyethylene beads ingested by fish as functions of concentration and source**

| Response variable | Fixed factors | LR Chisq | Df | *P*-value |
|---|---|---|---|---|
| Number | Concentration | 2.955 | 1 | 0.086 |
| | Source | 23.395 | 1 | < 0.001 |
| | Concentration*Source | 0.3571 | 1 | 0.550 |
| Mass | Concentration | 5.7254 | 1 | 0.017 |
| | Source | 8.1623 | 1 | 0.004 |
| | Concentration*Source | 0.0875 | 1 | 0.767 |

**Figure captions**

**Fig. 1.** Fluorescent stereomicroscope images of polyethylene (PE) beads ingested by *Neomysis* spp. in (a) stomach, (b) intestine, and (c) fecal pellet. (d) Beads isolated from a mysid. FB, fragmented beads; WB, whole beads.

**Fig. 2.** Experimental design of trophic transfer (Experiment 2). Control fish were not exposed to microplastics through the water column or food. Fish_Water denotes fish fed plastic-free mysids and exposed to polyethylene (PE) beads suspended in the water column for 24 h. Fish_Mysid denotes fish fed mysids that had been pre-exposed to PE beads but the aquarium water did not contain a microplastic suspension.

**Fig. 3** Average number (a) and mass (b) of polyethylene beads ingested by fish (*Myoxocephalus brandti*) from the water column or through ingestion of bead-exposed mysids. Bead concentration was 200 µg/L (low) or 2,000 µg/L (high). Error bars represent the standard deviation.

**Fig. 4.** Frequency of polyethylene bead fragmentation in a stock suspension (SS), potassium hydroxide procedure blank (KOH), mysids exposed to polyethylene beads (Mysid), fish fed bead-exposed mysid (Fish_Mysid), and fish exposed to polyethylene beads in the water column (Fish_Water). Results are shown as box and whisker plots with median (solid horizontal line), interquartile range (25th and 75th percentiles; box), and the 10th and 90th percentiles (whiskers). Different letters denote significant differences by post-hoc comparison ($p < 0.001$, GLM with post-hoc Tukey's HSD).