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Determination of the gut retention of plastic microbeads and microfibers in goldfish (*Carassius auratus*)

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HIGHLIGHTS

- Microplastic retention in goldfish was not significantly different from digesta.
- Microbeads and microfibers exhibited similar gut transit and retention times.
- The 90% retention time for microplastics in fish GI-tracts was 33.4 h.

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ABSTRACT

Microplastics are ubiquitous pollutants in aquatic habitats and commonly found in the gut contents of fish yet relatively little is known about the retention of these particles by fish. In this study, goldfish were fed a commercial fish food pellet amended with 50 particles of one of two microplastics types, microbeads and microfibers. Microbeads were obtained from a commercial facial cleanser while microfibers were obtained from washed synthetic textile. Following consumption of the amended pellet, fish were allowed to feed to satiation on non-amended food followed by fasting for periods ranging from 1.5 h to 6 days. Fish sacrificed at different time points were dissected to remove gut contents and the digesta contents retention and microplastic retention was determined. Although a small number of microplastic particles were retained in fish GI-tracts after 6 days (0–3 particles/50), the retention of microplastics was generally similar to the retention of bulk digesta contents. According to a breakpoint regression model fitted to digesta contents and microplastic particles, the 50% and 90% evacuation times were 10 h and 33.4 h, respectively. The results of this study indicate that neither microbeads nor microfibers are likely to accumulate within the gut contents of fish over successive meals.

1. Introduction

Microplastics are a diverse array of synthetic polymer particles that vary in chemical composition, size (from low micrometre scale to an upper size range variously defined between 1 nm and 5 mm), density and shape (Andrady, 2011). They have been observed in most freshwater and marine environments (Eriksen et al., 2014; Corcoran, 2015; Eerkes-Medrano et al., 2015) to such an extent that they were included as sedimentary geochemical markers of the Anthropocene (Waters et al., 2016). Microplastics are often distinguished between those that are synthesized at the defined sizes for an intended application (primary microplastics) relative to particles derived from the breakdown of macroplastics (secondary microplastics). Microbeads are defined as primary microplastics that range in size between 0.1 μm and <5 mm (Environment Canada, 2015) and are used in a wide variety of industrial and consumer applications including personal care products (PCPs). Legislation banning the production of microbeads in PCPs comes into effect in 2017 as passed by the U.S. federal government and similar legislation is under review in Canada. While much of the legislative focus has been on microbeads used in PCPs, other common sources of microplastics to municipal wastewaters include abraded particles from synthetic textiles such as nylon and acrylics, henceforth referred to as microfibers, used in clothing (Browne, 2011).

Concerns have been raised about the ecotoxicology of microplastics in the environment, including their potential to

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bioaccumulate in organisms and subsequent transfer through food webs (Sánchez et al., 2014; Avio et al., 2015a). Zooplankton are capable of ingesting microplastics, potentially mistaking them for food, and can further transfer these to tertiary consumers (Frias et al., 2014; Browne et al., 2013; Setälä et al., 2014; Rehse et al., 2016). Mussels have been shown to accumulate microplastics and transfer them to higher trophic levels (Browne et al., 2013; Von Moos et al., 2012; Collignon et al., 2014). In a study examining 504 fish from the English Channel that included benthic and pelagic species, 36.5% of specimens had microplastics in their gastrointestinal (GI-) tracts (Lusher et al., 2013). Microplastics in the gut contents of field collected fish have subsequently been widely reported in coastal and freshwaters (Sánchez et al., 2014; Neves et al., 2015; Avio et al., 2015b; Phillips and Bonner, 2015; Biginagwa et al., 2016; Bellas et al., 2016). Considering microplastics are being found in fish, there are relatively few studies focussing on the potential of microplastics to bioaccumulate.

Exposure to microplastics in water and food can interfere with normal digestive processes due to intestinal blockage, causing reductions in animal feeding rates and energy assimilation (Besseling et al., 2012), lead to histopathological alteration to intestinal and hepatic tissues of fish (Pedà et al., 2016; Lu et al., 2016) and lower hatching success of fish eggs (Lönnstedt and Eklöv, 2016). Translocation of microplastics from gut to the circulatory system has been demonstrated in mussels (Browne et al., 2008; Von Moos et al., 2012; Avio et al., 2015a) implying that retention of microplastics beyond entrainment in the GI-tract may be possible in some animals. Avio et al. (2015b) and Lu et al. (2016) confirmed microplastics accumulation in hepatic tissues of fish exposed to microplastics at elevated concentrations in water.

Although microplastics are commonly detected in the intestinal tracts of fish, there is limited information characterizing the retention of microplastics by fish. Particle size and shape are likely to influence factors such as GI-retention but limited information is available comparing microplastic types. Neves et al. (2015) observed a higher frequency of fibers in commercial fish gut contents compared to plastic fragments. The above study further reported differences in plastic types in benthic fish, which tended to accumulate a greater proportion of fibers, compared to pelagic fish which contained more fragments. It is not known whether these differences are related to emission patterns and fate of different particle types or whether particle shape might influence the gut retention characteristics of these microplastic types. In this study, the GI-tract retention of two microplastic types, microbeads and microfibers, was determined in goldfish with the objective to determine if i) retention of microplastics by fish exceeds that of food digesta, i.e. exhibits net accumulation in the GI-tract of fish, and ii) to determine if microfibers are retained to a greater or lesser degree than PCP derived microbeads.

2. Methods

2.1. Microplastic source

Microfibers were extracted from clothing (35 cm × 12 cm cut out of a commercial polyester fleece scarf) by mechanical agitation in hot water. Following agitation, the water was sieved through stacked 500 μm, 250 μm and 63 μm sieves. Fibers retained on the 63 μm sieve were removed by tweezer under magnification and size graded to between 50 and 500 μm fiber lengths under a dissecting microscope. Microplastic beads were extracted from a commercial cosmetic product (facial cleanser labelled with polyethylene). The contents of the product was poured onto a 63 sieve and the soluble matrix associated with the product washed with water until only microplastics remained. Microbeads were removed from the sieve under magnification. Fig. 1 provides images of isolated microbeads and microfibers under 5× magnification.

2.2. Experimental

Goldfish were selected as a model fish species because they have been routinely used in many bioaccumulation/toxicokinetic studies owing to their ease of husbandry, tolerance to handling and willingness to accept artificial diets. In their wild state, goldfish are benthic feeders and thus might be expected to accumulate microplastics similar to those reported for other benthic feeders. Fish were exposed to microplastics via food. Commercial fish pellets (0.18–0.21 g, ~3 mm size) were placed in warm water to soften them. Treatment pellets were amended with 50 microbeads or 50 microfibers per pellet by manual insertion of macroplastic particles into each pellet under microscope. Pellets were air dried after manipulation. Control pellets were wetted and dried in an identical manner but not amended with microplastics. The food was prepared in this manner to ensure that every experimental fish consumed exactly 50 microplastic particles to increase precision of gut retention characterization.

Fifty three sexually mature goldfish were fasted for 48 h prior to exposing them to prepared food in order to ensure complete evacuation of gut contents from previous meals and to increase the likelihood that they would accept the microplastic amended pellet provided to them. After fasting, fish were removed from their communal tank and placed in individual fish bowls. Twenty four fish were allocated to the microbead and microfiber treatments, respectively. Five fish were allocated as controls and fed non-amended pellets. Each fish was presented with a single treatment pellet and observed until it was verified that the fish consumed the pellet. After the fish consumed the treatment pellet, non-amended fish pellets were added to the bowl and the fish was allowed to consume to satiation for up to 60 min. Any remaining fish food in

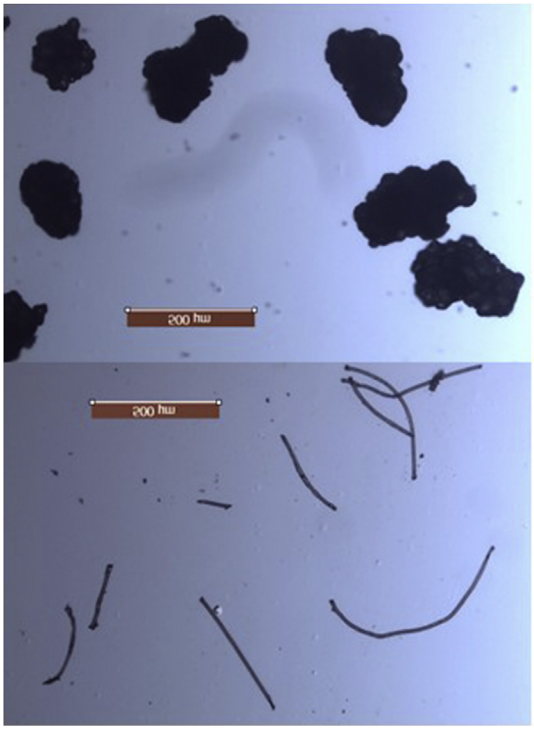

Fig. 1. Image of microbeads (Top) and microfibers (bottom) used for feeding trials (5× magnification).

the bowl was subsequently removed. Fish were fasted for the remainder of the experimental period. Control fish were sacrificed after 1.5 h from feeding the control pellets. Triplicate animals from each treatment were sacrificed after 1.5, 4, 8, 16, 32, 48, 96, and 144 h. The mean ± SE of water temperatures was 14.2 ± 0.21 °C and exhibited no changes over the fasting duration. The mean ± SE body weights of fish from the microbead and microfiber treatments were 24.80 ± 2.77 g and 27.07 ± 3.40 g and were not significantly different from one another ($p > 0.4$; ANOVA). On sacrifice, fish were euthanized by immersion in a solution of MS-222 (100 mg/L) and stored frozen until subsequent analysis. This research was performed under ethics approval from the University of Windsor's Animal Care Committee.

2.3. Microplastic analysis

On analysis, the gut tract of each fish was dissected and removed. The gut contents were pushed through the intestine using tweezers and a probe onto a pre-weighed aluminum weight boat and the gut tract tissues were retained for further analysis. The weigh boat was dried at 110 °C for 1 h and reweighed to determine dry food digesta weight. Subsequently, the dried digesta and gut tissues were re-combined and placed into a 10% KOH solution on a hot plate set at its lowest setting for 1 h. The solution was taken off of the hot plate and after 2 additional hours, 5 mL of 30% H_2O_2 was added to the solution. The solution was poured through a vacuum filtered Buchner funnel using Whatman™ (55 mm) filter papers (1 μm glass fiber filters). Fish carcass samples were also digested in a similar manner. Filter papers from each digestion were analyzed under a stereomicroscope to quantify the number of microplastics remaining in the GI-tract/contents, fish carcass or digested food pellets. Quality control of the method was established by measuring and verifying microbeads and microfibers in 5 amended pellets. The mean ± standard deviation of recoveries of microplastic particles for the digested pellets was 98.8 ± 1.8%.

2.4. Data analysis

Digesta contents weights were standardized to the mean body weight according to:

$$X_{DG(ss)} = X_{DG(s)} \cdot \frac{BW_{(mean)}}{BW_{(s)}} \quad (1)$$

where $X_{DG(ss)}$ is the size standardized digesta weight (g), $X_{DG(s)}$ is the digesta weight measured in an individual fish, $BW_{(mean)}$ is the mean body weight of fish from the treatment and $BW_{(s)}$ is the body weight of the individual fish. The % remaining of digesta contents was calculating by dividing $X_{DG(ss)}$ by the mean $X_{DG(ss)}$ generated for fish sampled at the first time point (1.5 h) and multiplying by 100. For microbeads and microfibers, %remaining was calculated by dividing the number of microplastics measured in a fish's digestive tract by 50 and multiplying by 100.

Statistical analysis was performed using a general linear model (GLM) according to:

$$\text{Model} = \text{Time} + \text{Group} + \text{Time} * \text{Group} + \text{Constant} \quad (2)$$

where time is the time since feeding (h), group represents a categorical variable specified as digesta retention treatment 1, digesta retention treatment 2, microfibers and microbeads. Under cases where the interaction term (Time * Group) was non-significant, analysis of covariance (ANCOVA) was performed to adjust for time as a covariate and increase the statistical power of the group comparison test. Where the interaction term was found to be significant, GLMs were performed on subsets of the data to test for differences between selected group comparisons. GLM(1) tested for differences in digesta retention time between treatment 1 and treatment 2. GLM(2) tested for differences in digesta retention time and microfiber retention from measurements taken in treatment 1. GLM(3) tested for differences in digesta retention time and microbead retention from measurements taken in treatment 2. Finally, GLM(4) tested for differences in microfiber and microbead retention. Data transformation was necessary owing to failure of normality of the % retention data on the combined data (digesta, microbeads and microfibers). However, when the fist time point (1.5 h) was removed, transformation of % retention data by natural log transformation yielded a normal data set ($p > 0.05$; Lillefor's test). Thus, statistical comparisons by GLM were performed with the 1.5 h time point removed and applying a ln transformation. Non-transformed digesta retention data (inclusive of the 1.5 h time point) for individual fish were subsequently fit to an exponential model using non-linear least squares regression according to:

$$\%Retained = 100 \cdot e^{-B \cdot time} \quad (3)$$

where 100 is constant forcing 100% of gut contents retention at time 0, b is the fitted coefficient and time is time since feeding (h). The ability of Eq. (3) calibrated independently to gut contents to predict microplastic retention was evaluated using goodness of fit tests by performing a linear regression on observed (microplastic) vs model (Eq. (3)) predicted digesta retention. The goodness of fit result was evaluated by determining if the slope was significantly different from 1, the constant was significantly different from 0 and by evaluating the magnitude of the coefficient of determination. All statistics were performed using Systat 13 statistical software. Except where otherwise noted, measures of central tendency and variation are expressed as mean and standard error (SE).

3. Results and discussion

3.1. Digesta retention

During experimental trials, all fish were observed to consume the microplastic amended treatment pellet. No fish mortalities occurred nor were there apparent signs of distress following exposure to the amended food pellet. Fish sacrificed at the 1.5 h time point had a mean $X_{DG(ss)}$ weight of 0.60 ± 0.04 g. This corresponds to a food consumption of 2.32% body weight across the treatments and is consistent with expected food consumption rates in fasted fish.

A general linear model (GLM(1) as described in methods) was performed to compare % retention of digesta between the two treatments. The GLM and ANCOVA revealed a non-significant ($F_{1,39} = 0.92$; $p > 0.3$; ANCOVA) difference in digesta retention between the treatments after adjusting for time as a covariate. Given that digesta retention did not significantly differ between the two treatments, the data were combined and fit to the exponential model yielding the following solution:

$$\%Retained = 100 \cdot e^{-0.069 \cdot time}; \ R^2 = 0.69 \quad (4)$$

Based on Eq. (4), the time to evacuate 50% and 90% of digesta was 10.0 and 33.4 h, respectively. Overall, the exponential model fit described the temporal trends of digesta contents well during the first 24 h but tended to underestimate observed digesta contents at longer time points (Fig. 2). This may be related to the method of separating gut contents from the intestinal tissues which could have included residual gut secretions and/or sloughed cells/tissues generated from the GI-tract processing method itself. However, the

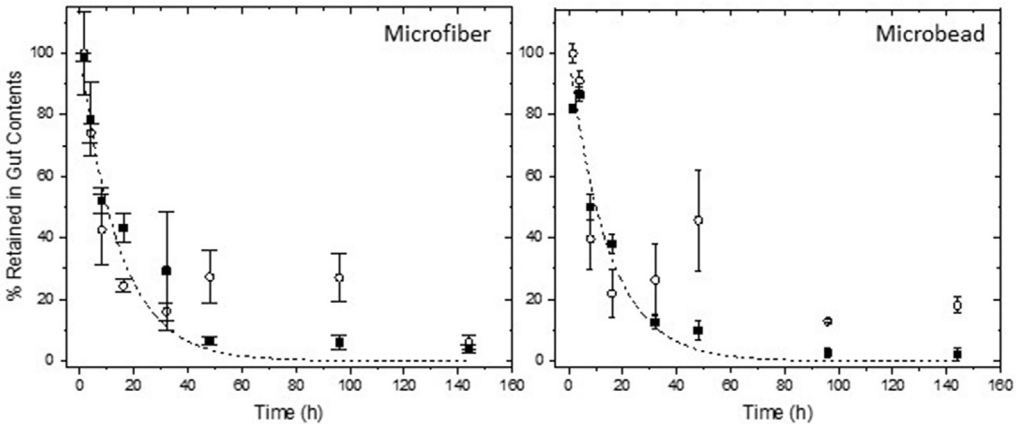

Fig. 2. Gut retention of digesta and microplastics in gold fish post feeding. Left graphic presents mean microfiber (■) retention compared to digesta (O). Right graphic presents mean microbeads (■) retention compared to digesta (O). Dashed line is the exponential fit to the combined digesta retention data (Eq. (4)). Error bars are standard error.

fitted model produced retention estimates that were generally consistent with other studies on digesta retention in fish of similar size and temperature. Stehlik et al. (2015) reported full clearance of gut contents from clearnose skate (*Raja eglanteria*) by 48 h when held at 15 °C. Yellow perch held at 17.1 °C exhibited a gut evacuation coefficient of 0.035·time (h^{-1}) based on a log linear model which implies a 50% digest retention of 19.8 h and 90% retention of 65 h (Gingras and Boisclair, 2000).

3.2. Microplastic retention in GI-tracts

Control fish sacrificed after 1.5 h were examined for evidence of microplastics in gut contents and carcass samples. No microplastics were found in control fish or within their gut contents. In addition, 10 control fish pellets were examined for presence of microplastics. Similar to control fish, microplastics were not observed in non-ammended food pellets.

During the first sampling point (1.5 h), there was good recovery of microplastics within the gut contents of treatment fish. For microfibers, 2 fish had 50 microfibers recovered (100% recovery) and the third fish had 48 fibers recovered in the GI-tract. For the microbeads, 40 to 44 particles (80–84% recovery) were recovered from fish during the first time point. Small numbers of microplastics were recovered at the 144 h time point (1–3 microfibers in replicate 144 h sampled fish and 0 to 3 microbeads in triplicate fish).

A general linear model (GLM) was applied to test percent retention of all treatments (digesta from each treatment, microbeads and microfibers) within the study. Both Time ($F_{1,76} = 88.1$; $p < 0.001$) and the Group x Time ($F_{3,76} = 3.09$; $p < 0.05$) interaction terms were significant but group was not significant ($F_{3,76} = 0.212$; $p > 0.8$) in the overall GLM. Due to the significant interaction terms, additional GLMs were applied to subsets of the data to evaluate for differences in retention on selected measurements. GLM(2) and the ANCOVA revealed no significant differences ($F_{1,39} = 0.959$; $p > 0.5$; ANCOVA) in microfiber and digesta retention. Similarly, GLM(3) and ANCOVA revealed non-significant ($F_{1,39} = 4.00$; $p > 0.05$; ANCOVA) differences in microbead retention from gut digesta retention. Finally, a comparison of microfiber and microbead retention yielded non-significant differences ($F_{1,39} = 0.678$; $p > 0.4$; ANCOVA) from one another. Microplastic and microfiber retention with time along with digesta contents trends are presented in Fig. 2.

For microfibers, the linear regression between %microfiber retention and gut digesta model (Eq. (4)) prediction yielded a slope of 0.96 ± 0.09, constant of (7.33 ± 4.07) and coefficient of determination (R^2) of 0.85. The above slope was not significantly different from unity ($t_{1,22} = 0.042$; $p > 0.5$; *t*-test) and the constant was not different from zero ($t_{1,22} = 1.80$; $p > 0.05$; *t*-test). For microbeads, the goodness of fit test produced a similar slope (0.94 ± 0.04) that was not significantly different from unity ($t_{1,22} = 1.44$; $p > 0.1$; *t*-test) and constant (3.67 ± 2.06) not significantly different from zero ($t_{1,22} = 1.79$; $p > 0.05$; *t*-test) with an R^2 of 0.95. It is perhaps notable that the digesta retention model (Eq. (4)) which was calibrated only to digesta retention data explained even more variation in microplastic retention than digesta contents itself. This was mainly related to the better fit of model predictions to microplastic retention at the later time points (Fig. 2). The reason for the differences in model fit across measurements is attributed to the fact that microplastic exposure was controlled with a high degree of precision compared to gut contents. Although each fish was given exactly 50 microplastic particles, they were provided with food ad libitum after verifying their consumption of the microplastic amended pellet. Thus, digesta contents would have varied to a greater extent between fish compared to microplastic exposures. Overall the goodness of fit tests indicates that the gut digesta retention model adequately described the retention of both microplastic types.

Similar observations were generated for the marine isopod *Idotea emerginata* fed a diet spiked with microplastic particles and fibers (Hämer et al., 2014). In the study by Hämer et al. (2014), microplastic particles appeared in the stomach and gut contents of isopods but were also readily egested with the feces. Mazurais et al. (2015) examined microplastic retention in European sea bass (*Dicentrarchus labrax*) larvae when exposed to microplastics added to food. The above authors observed a correlation between microbeads in the gut of larvae with concentration of microbeads added to the diet. However, the authors noted that microbeads were fully cleared from the gut of larvae after 2 days post exposure and could be identified in feces suggesting passive retention in the gut contents of fish.

Microplastics were also examined in carcass samples of treatment fish but were not observed apart from the gut tissue and gut contents analyzed separately and discussed above. This differs from the results of Avio et al. (2015b) who observed translocation of polyethylene and polystyrene microplastics to liver of laboratory held mullet (*Mugil cephalus*) exposed to microplastics in water (nominal microplastic dose was 2.5×10^3 particles/L of polyethylene or polystyrene particles sized from 100 to 1000 μm) for 7 days. Between 1 and 2 microplastic particles per individual were

detected in liver of exposed fish, although the presence of microplastics in liver was two orders of magnitude lower than what was observed in gut contents of fish. Similarly, Lu et al. (2016) exposed zebrafish (*Danio rerio*) to solutions containing 5 or 20 μm diameter polystyrene microplastics at concentrations of between 4.5×10^6 to 2.9×10^8 particles/L for 7 days. The above authors reported that 5 μm microplastics accumulated in fish gills, liver and gut, whereas larger microplastics (20 μm in diameter) accumulated only in fish gills and gut but not in liver. Time to steady state of microplastics in zebra fish was reported to be 48 h, implying rapid clearance from animals consistent with the gut retention data presented here (Lu et al., 2016). While the present study failed to identify microplastic translocation in fish tissues apart from their detection in the GI tract, this could be a function of exposures to different microplastic types, different dosing strategies, levels of exposures and differences in the method of detection of microplastics in exposed animals. The lack of translocation of larger (20 μm plus) sized microbeads to liver in zebra fish as reported by Lu et al. (2016) is consistent with the present work given that particles greater than 63 μm were utilized but is not consistent with Avio et al. (2015b) who exposed fish to microplastics of comparable size to this research. Avio et al. (2015b) and Lu et al. (2016) provided continuous exposures of fish to microplastic contaminated water for up to 7 d days compared to a single dose from a microplastic amended meal applied in the present study. The above authors also used nominal microplastic concentrations in water that were considerably higher than what is present in natural waters. Avio et al. (2015b) used a more sensitive microplastic extraction/detection technique that employed a combination of density gradient separation and oxidant treatment which was shown to yield higher recoveries of microplastics from animal tissues then the oxidation treatment alone. Lu et al. (2016) utilized microplastic particles with encapsulated fluorescent dies to facilitate their detection in tissues which potentially yielded much lower detection limits then the visual method employed here. Thus, even though microplastics had very good recovery in pellets and gut contents of early time point sacrificed fish from the present work, translocation of smaller microplastic particles when exposed at higher concentrations or under long term exposures cannot be ruled out based on the results of this study.

4. Conclusions

Microplastics of two distinct particle shapes (microbeads and microfibers) exhibited similar retention in the GI-tract of goldfish compared to bulk food and digesta. Although a small number of particles were retained in fish after 6 days of fasting, there was no evidence for net bioaccumulation of microplastics in the GI-tract or internal translocation to tissues of fish post exposure. This implies that the potential for long term entrainment and retention of textile derived microfibers or PCP-derived microbeads in fish is relatively low and the detection of microplastics in fish gut contents in the environment most likely represents recent exposures to microplastics in the diet as opposed to cumulative retention across multiple meals. However, this study was limited to evaluation of only two microplastic types and one species of fish. As such, further research to characterize microplastic retention by fish species over different plastic types, shapes and dietary concentrations may be warranted.

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