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Egestion rates of microplastic fibres in fish scaled to in situ concentration and fish density

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Abstract

1. Microplastics (particles <5 mm) are commonly found in aquatic organisms across taxonomic groups and ecosystems. However, the egestion rate of microplastics from aquatic organisms and how egestion rates compare to other rates of microplastic movement in the environment are sparsely documented.
2. We fed microplastic fibres to round gobies (*Neogobius melanostomus*), an abundant, invasive species in the Laurentian Great Lakes. We conducted two trials where round gobies were fed microplastic-containing food either a single time (1 day) or every day over 7 days.
3. There was no difference in microplastic egestion rates from the 1 day or 7 day feeding trials, suggesting no impact of duration of exposure on egestion (exponential decay rate = −0.055 [±0.016 *SE*] and −0.040 [±0.007 *SE*], respectively). Turnover time of microplastics (i.e., average time from ingestion to egestion) in the gut ranged from 18.2 to 25.0 hr, similar to published values for other freshwater taxa.
4. We also measured microplastics in the digestive tracts of round gobies collected directly from Lake Michigan, U.S.A. Using published values for round goby density and microplastic concentration at the study sites, we calculated areal egestion rate by round gobies (no. particles m^{-2} day^{-1}), and compared it to riverine microplastic export (no. particles m^{-2} day^{-1}). Both area-based rates were of the same order of magnitude, suggesting that round goby egestion could be an important, and potentially overlooked component of microplastic dynamics at the ecosystem scale.
5. Animal egestion is well-known as a major component of nutrient and carbon cycling. However, direct measurements of microplastic fluxes in the environment that include animal egestion rates are uncommon. An ecosystem ecology approach is needed to meet the emerging challenge of generating microplastic budgets for freshwater environments and elsewhere, thereby informing management and mitigation of plastic pollution at a global scale.

KEYWORDS
emerging contaminants, Great Lakes, *Neogobius melanostomus*, plastic pollution, retention time

1 | INTRODUCTION

Global plastic production and the generation of plastic waste has accelerated since the 1950s (Geyer et al., 2017; Rochman & Hoellein, 2020; Worm et al., 2017). Microplastics (i.e., particles <5 mm) are a topic of focus in ecological research because they are pervasive in the environment, interact with a suite of organisms and chemicals, and are consumed by humans (Diaz-Basantes et al., 2020; Hartmann et al., 2019; Lusher et al., 2012; MacIvor & Moore, 2013). Microplastic particles are introduced to aquatic ecosystems through improper waste disposal, wastewater treatment plant effluent, storm-water runoff, tyre wear, biosolids used in agriculture and aerial deposition (Habib et al., 2020; Rillig et al., 2017; Zhang et al., 2019). Microplastics represent a diversity of shapes (i.e., fibres, fragments, pellets) and material types (i.e., individual plastic polymers, as well as mixtures of synthetic, semi-synthetic and processed natural textiles) with an array of chemical additives (Rochman et al., 2019).

Microplastic ingestion has been documented across many taxa (i.e., invertebrates, fish, birds, and mammals) and a quickly growing field of study has emerged to quantify microplastics' physiological impacts (Courtene-Jones et al., 2019; Lusher, McHugh & Thompson, 2013; Senko et al., 2020). Negative effects of microplastic ingestion could include tissue damage and stress responses (Jovanović, 2017). Hydrophobic compounds in the environment such as persistent organic pollutants (POPs) can be sorbed to microplastics (Kim et al., 2015; Rochman et al., 2019), and may be transferred to organisms following ingestion (Critchell & Hoogenboom, 2018; Pedà et al., 2016). Alternatively, microplastics may pass through the digestive tract with minimal interactions of any kind, with impacts variable according to particle properties and organism traits (Earn et al., 2021; Foley et al., 2018; Jovanović, 2017).

Although much recent research examines the consequences of microplastic *ingestion* on fish, the rate at which microplastics leave the digestive tracts (i.e., *egestion* rate) is less well-known (D'Souza et al., 2020; Grigorakis et al., 2017; Roch et al., 2021). Factors which impact microplastic egestion rate in fish include particle characteristics (i.e., size and shape), environmental factors (e.g., temperature), and species- and individual-specific traits such as digestive tract anatomy, body size and trophic level (Hoang & Felix-Kim, 2020; Ory et al., 2018; Parker et al., 2021). Measuring egestion rates is important for understanding the duration of exposure to individual particles, the cumulative microplastic exposure for any individual over a season or a lifetime (Parker et al., 2021; Windsor et al., 2019), and the role of egestion rates from organisms relative to microplastic movement within an ecosystem (D'Souza et al., 2020). For example, if a fish collected from a river has 10 microplastic particles in its digestive tract, without estimates of egestion rate, it is not clear when those particles were ingested or how long they may stay in the digestive tract (McNeish et al., 2018). By combining average egestion rates with measurements of microplastic counts from organisms collected in situ, researchers can better predict when the organisms consumed the microplastics found in the gut at the time of death (Hou et al., 2021). For migratory species, egestion rates are needed to calculate the potential for fish to serve as microplastic vectors across ecosystems (Lusher et al., 2016). Overall, measurements of microplastic egestion rates are needed to understand the role of animals in microplastic dynamics at the ecosystem-scale, but are not commonly measured.

Studies on the ecological dynamics of microplastics benefit from using well-established paradigms and methods for particle and solute transport (Hoellein & Rochman, 2021). For example, microplastic deposition rates in streams can be analysed using particle spiralling metrics, which allow for direct comparison of natural and synthetic particle movement (Hoellein et al., 2019). Likewise, egestion from freshwater animals has been well-studied with regard to the role of waste production on nutrient and carbon cycling at multiple spatial scales, from a benthic patch (e.g., 1 m^2) to entire catchments (Atkinson et al., 2018; Hoellein et al., 2017; Vanni et al., 2013). When egestion, transformation and transport rates of solutes and particles are calculated using the same units at the same site, direct comparisons facilitate insight into the role of animals on ecosystem-scale processes (Atkinson et al., 2016; Capps & Flecker, 2013). No studies have combined microplastic egestion rate with microplastic exposure and animal density in situ, which is needed to situate egestion rates in the broader context of microplastic dynamics within aquatic ecosystems (Krause et al., 2020; Parker et al., 2021).

We fed round gobies (*Neogobius melanostomus*) a diet containing acrylic microplastic fibres for a single feeding or 7 continuous days, and then measured microplastic egestion rate. We expected fish with 1 day of microplastic exposure to show a faster egestion rate compared to fish with 7 days of treatment, as the potential mixing of microplastic fibres within the gut across sequential feeding days could slow egestion (Xiong et al., 2019). We predicted that egestion rates would be similar to previous assessments in similarly sized freshwater species (e.g., goldfish [*Carassius auratus*]; approximately 50% egested in 10 hr; Grigorakis et al., 2017). In addition, we quantified and characterised microplastics and anthropogenic particles in the gastrointestinal tracts of round gobies collected from the environment, and used in situ measurements of their density to calculate microplastic egestion rates for individuals and populations on an area-specific basis.

2 | METHODS

2.1 | Study fish collection

Native to the Black Sea region, round gobies (hereafter 'gobies') became invasive in the Great Lakes of North America ~1990 (Charlebois et al., 1997; Kuhns & Berg, 1999). Gobies are abundant in the littoral areas and some tributaries of the Great Lakes. Gobies are benthic invertivores (Brush et al., 2012; Kornis & Vander Zanden, 2010) that consume microplastics and anthropogenic particles (Hou et al., 2021; McNeish et al., 2018; Munno et al., 2021).

We collected gobies ($N = 68$) using fishing rods and *Lumbricus terrestris* (earthworm) bait along the sea wall at Montrose Harbour

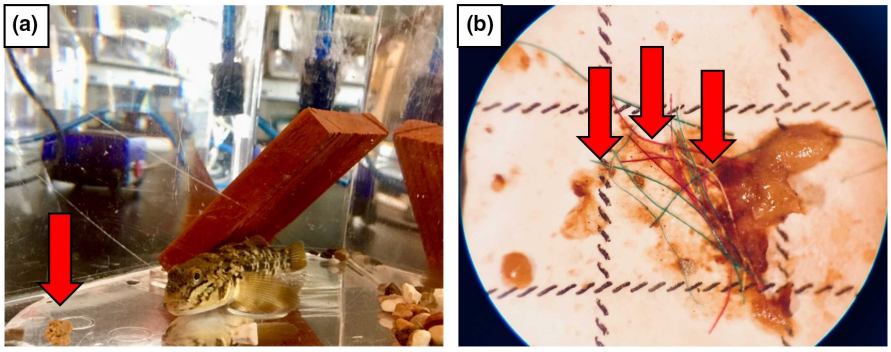

FIGURE 1 (a) Round goby from the 1 day of microplastic feeding showing the food pellet (red arrow) in an experimental aquarium. (b) Filter showing digested remains of a round goby intestinal tract from the 7 days of microplastic diet treatment, with three different coloured fibres from different feeding days indicated by red arrows (dark green, orange and purple)

in Chicago, IL, USA (41°57′44.6″N, 87°38′27.8″W) in summer 2018. Gobies were immediately transferred into buckets with aerated lake water and transported to the laboratory within 2–3 hr of collection. Ten fish were immediately euthanised and preserved. The remaining fish were placed in 50 × 25 × 30 cm acclimation aquaria, with 15 L of water (16°C –23°C) treated with 850 g of API Furan-2 powder to prevent bacterial growth (Mars Fishcare). Aquaria water was de-chlorinated by storing tap water in containers for 24 hr before use. Each aquarium held six to seven fish and was kept aerated with two aquarium air pumps. We placed ceramic tiles in the aquaria as refugia for the territorial gobies. We monitored water temperature daily and changed the water every other day by siphoning out half of the water and replacing it with clean water.

2.2 | Food and microplastic diet preparation

We generated microplastic fibres by cutting acrylic yarn into 1-mm segments in the laboratory. We marked 1-mm lengths on a wooden block, placed a length of yarn on the block, and wearing magnifying glasses (TMANGO, model no. 9892B2) and gloves, cut the yarn with a sterile razor blade into 1-mm sections (Hoellein et al., 2019). The cut yarn was placed in aluminium dishes and covered with foil. We used seven different colours in the experiment (Table S1). Each colour was processed separately to avoid mixing colours. Between cutting different colours, the block was scrubbed, washed with DI water and dried.

We generated "control" food pellets (no microplastics) and microplastic-containing food pellets. Food pellets were made from minced frozen *Glycera dibranchiata* (bloodworms) (OmegaSea) and crushed, unsalted saltine crackers (Nabisco). In a clean aluminium container, we mixed four cubes of bloodworms and two crackers to form a paste, using a pre-cleaned laboratory spatula and forceps to form pellets (diameter 3 mm, N = ~80 per mixture). To make food pellets with microplastic fibres, we wore magnifying glasses to manually count and insert fibres into the wet paste (Table S1). We flattened the paste, manually inserted fibres using forceps, carefully folded over the paste, and rolled it into a pellet (Grigorakis et al., 2017). Pellets were stored in foil-lined plastic trays and covered with paper towels to dry overnight. Control pellets (N = 1,500) and those with microplastic (N = 225) yarn colour were kept separate and stored at room temperature in aluminium dishes covered with foil.

2.3 | Feeding experiments

We conducted two experiments to measure the rate of microplastic fibre egestion. In the first experiment we fed microplastic-containing food pellets to fish one time (hereafter, "1 day"). In the second experiment, fish were fed microplastic-containing food pellets for 7 days. Both experiments had "control" fish (no microplastics) that were fed and sacrificed at the same time points and replication levels.

Before starting both experiments, fish were kept in acclimation aquaria for a week (N = 58 individuals). All fish were fed control food pellets for 5 days, then starved for 2 days before beginning the experimental feeding trial. At the end of the 7 day acclimation period, we euthanised and preserved 10 fish (i.e., five fish per feeding trial; MS-222 Tricaine-S, 0.25 g/L, and 70% ethanol, respectively; Table S2) to examine fish digestive tracts for microplastics (see below). The remaining fish were moved into individual aquaria (25 × 17 × 20 cm) (Grigorakis et al., 2017). Each aquarium had a ceramic tile, 3.3 L of water, and was aerated using an aquarium air pump (Figure 1a). We monitored the water temperature and changed the water as described above. Aquaria were covered throughout the experiments.

In the first experiment, fish were fed once with one microplastic-containing food pellet (N = 12 fish, microplastic colour = light green). We monitored each fish until they consumed the pellet (range = 0–10 min). Control fish (N = 12) were fed a single non-microplastic-containing food pellet. Fish from the treatment and control groups were euthanised 4, 24 and 96 hr after their exposure (n = 4 fish per time point in control and treatment groups). Individuals in the 96-hr group were fed a single control food pellet each day until euthanasia. Data collection was completed between 24 June and 5 July 2018, and no fish died during the experiment.

The second experiment required feeding fish the microplastic-containing food pellets once per day for 7 days in a row, using a different microplastic colour for each day to track the time elapsed since ingestion. Food consumption was confirmed as described above. Fish were euthanised at 4, 24 and 72 hr after their last exposure (n = 4 fish per time period in control and treatment groups). We set the final time point of 72 hr after last exposure rather than 96 hr

(as for the 1-day exposure), because we found relatively low microplastic at 96 hr in the first experiment, and thus, inferred that the 72-hr sampling would offer greater insight into egestion rates. Fish from the 72-hr group were given one control food pellet every 24 hr after the final microplastic exposure. This experiment was completed from 4 July to 21 July 2018, and no fish died during the experiment. Caretaking and euthanasia followed protocols approved by Loyola University Chicago's Institutional Animal Care and Use Committee.

2.4 | Fish processing and microplastic quantification

All euthanised and preserved fish were processed for microplastics in digestive tissue according to previous research (Hou et al., 2021; McNeish et al., 2018). Firstly, we measured fish total length and recorded the wet weight and sex (McNeish et al., 2018). Fish were dissected on a clean enamel pan, using scalpels and forceps rinsed with filtered DI water (363-μm mesh). The outside of each fish was also rinsed with DI water. We removed the digestive tract by cutting from the urogenital opening to the oesophagus (Hou et al., 2021; Lusher et al., 2013). Digestive tracts were stored in acid-washed glass jars and covered with foil. Using DI water, we rinsed the dissection tools used and the inside of the stomach cavity into the glass jar to avoid sample loss. Between dissections, gloves were changed, and all scalpels, forceps and enamel pans were rinsed with DI water to prevent contamination (Hou et al., 2021; McNeish et al., 2018).

After dissections, fish digestive tracts were dried, digested and filtered. Digestive tracts were dried in individual glass jars at 70°C for 24–48 hr (1,320 Economy Oven, VWR). To break down the organic material, we added 20 mL of iron sulfate catalyst (0.05 M Fe[II]) and 20 mL of 30% hydrogen peroxide (H_2O_2) into each jar and heated the contents (70°C) on a hot plate for 15–20 min. We used a stir-bar to enhance the reaction and added 30% H_2O_2 in increments of 20 mL until the reaction was complete. Wet peroxide oxidation eliminates organic matter without impacting the recovery of the acrylic microplastics (Lusher et al., 2017; Munno et al., 2018). Digested samples were vacuumed through gridded 0.45-μm filters (Whatman™). Filters were transferred into 20-mL aluminium weighing dishes, covered with foil, and dried at 30°C for 4–24 hr (Thermo Fisher Scientific Incubator) (McNeish et al., 2018). Using a dissecting microscope (×25–30 magnification) (model ASZ30L3, Bausch & Lomb), we identified all experimentally added microplastic fibres. The acrylic yarn was uniform in colour and size, so it was easily distinguished from any microplastic fibres already in fish digestive tissues or those that might have been introduced via contamination (Figure 1b) (Hoellein et al., 2019; see controls below).

2.5 | Microplastic abundance and loss in food pellets

We assessed microplastic counts in a subset of food pellets. We first processed prepared food pellets to verify the number of microplastic fibres in the pellet (repeated for each microplastic colour; Table S1) using the same digestion, filtering and quantification procedures described above. In addition, we estimated microplastic leaching from food pellets in the water before goby consumption. To do so, we placed a food pellet in an aquarium with one goby and recorded time to consumption. We immediately removed the fish and filtered the aquarium water onto a gridded filter. We repeated this process four times. Filters ($N = 4$) were processed for microplastics as described above. The mean (±*SE*) time to consumption was 9.25 (±2.1) min, and the mean number of experimental microplastics in the water was 8 (±0.8) particles, or 15% of microplastics in food (Table S3). Thus, we corrected all feeding trials for the initial microplastic abundance of in food pellets by subtracting 15% from the initial concentration. This adds some uncertainty in egestion rate (i.e., a lower starting concentration), but any error is equal across trials, and rates calculated with this method are conservative.

2.6 | Laboratory controls

We performed digestion controls to quantify laboratory contamination ($N = 11$; Table S4). We completed digestions in empty acid-washed glass jars, followed by microplastic processing as described above (McNeish et al., 2018). Controls were used to correct microplastic counts in fish collected from Lake Michigan, and fish acclimated in the laboratory for 7 days before feeding trials (Table S1). Digestion controls also were used to confirm that no experimental microplastics were found in the fish that were not fed microplastics.

2.7 | Polymer identification

We measured the material composition of particles isolated in the digestive tracts of gobies directly captured from Montrose Harbour, Lake Michigan, in gobies that experienced 7 days of acclimation in the laboratory aquaria (just before the start of the feeding experiments), and in laboratory controls. While our intention was to remove and identify all particles during this process, a total of 49% of particles were processed for polymer identification (37 of 97 found) as a result of loss while handling and difficulty in finding all fibres as a consequence of movement of aluminium pans while in storage. We identified 25 particles from fish freshly collected from Montrose Harbour (of 56 found; 45%), seven particles in gobies after 7 days of acclimation (of 21 found; 33%) and five particles from controls (of 20 found; 25%).

We prepared particles for polymer identification as described in Barrows et al. (2018) and Hoellein et al. (2021). We wrapped glass microscope slides in aluminium foil and rinsed with filtered DI water. Using a dissecting microscope, we moved a single particle from the filter to the slide. If the particle colour on the filter did not match the original datasheet it was not removed for polymer ID (i.e., considered contamination). The particle location was noted by gently indenting the foil, and then it was covered by a glass coverslip and taped securely. Later, the glass coverslip was

removed and the slide placed on the stage of a Fourier transform infrared microscope system (μFT-IR; Spotlight 200i equipped with Spectrum Two, Perkin Elmer) in reflectance mode. The spectral acquisition range was 4,000 to 650 cm^{-1} with spectral resolution of 4 cm^{-1}. Spectrum results from 16 scans were compared to a reference library and known standards using SPECTRUM 10 software (Perkin Elmer) (Magni et al., 2019).

2.8 | Data analysis: Egestion rate and scaling to in situ conditions

We calculated the microplastic egestion rate as the proportion of microplastics remaining per fish over time for the 1- and 7-day microplastic feeding trials. Firstly, we calculated the proportion of microplastic remaining relative to the amount ingested: [no. fibres/fish]/[no. fibres/pellet] * 100 for each individual (where the number of fibres per pellet was corrected to account for loss of microplastics from pellets as described above). We used an exponential decay model to estimate egestion rate, with the equation $y = 100e^{(-kt)}$, with the y-intercept set at 100%, where $|k|$ is the decay rate constant (units: proportion/hr) and t is time (hr). We also calculated the half-life (T_{50}; time to egest 50% of microplastics) with the equation $T_{50} = \ln(2)/k$, and the turnover time (i.e., mean time spent by a particle in transit) as $(1/-k)$. We used exponential decay rather than other models (i.e., linear) as it provided the best fit, and its use in previous research allowed for direct comparison of egestion parameters with the literature (Grigorakis et al., 2017; Hoang & Felix-Kim, 2020; Roch et al., 2021).

Generalised linear models (GLM) were used to determine if microplastic abundance (no./fish) patterns were explained by time, feeding trial (1- or 7-day), and fish body length, wet mass and sex, similar to methods from Hou et al. (2021), Hall et al. (2018) and Nix et al. (2018). The best statistical distribution (Gaussian, Poisson, Zero-inflated negative binomial [ZINB], Zero-inflated Poisson [ZIP] or Negative binomial [NB]) for this pooled dataset was identified as NB with model selection (*model.sel()* [MuMIn package]; Barton, 2020) and Akaike's information criterion corrected for sample size (AIC_c; Table S5). A series of NB GLM analyses (*glmmTMB()*, [glmmTMB package]; Brooks et al., 2017) were constructed with all variables as fixed effects in models. Continuous variables were checked for autocorrelation (*cor()* [stats package]; R Core Team, 2019). No models were constructed with fish mass and body length due to autocorrelation ($r \geq |0.3|$). All univariate and additive multivariate model possible combinations were explored (14 models total + Null model). The overall best model and competing models were determined by ranking models based on model weights (w_i) and AIC_c (Table S6). Model residuals were extracted (*simulateResiduals()* [DHARMa package]; Hartig, 2021) from the best-fitting model and were found to have no significant outliers or dispersion, and did not significantly deviate from uniformity and had homogenous variances. The 95% confidence interval was calculated for model variables for the best-fitting model (*confint()* [stats package]; R Core Team, 2019). Models competing with the best-fitting model were identified if within an AICc difference (ΔAICc) of 2 from the top performing model. The best-fitting model was compared to the Null model and competing models via loglink ratio to determine if there was a significant difference between models (*ANOVA()* [stats package] and *lrtest()* [lmtest package]) to increase confidence in the best-fitting model. The best-fitting model was significantly different than the Null model, yet not significantly different compared to competing models. An ANOVA Type II was used to discern if the GLM main effects were significant (*ANOVA()* [car package]; Fox et al., 2022). Pairwise comparisons between feeding treatments were conducted with Tukey honestly significant difference tests by treatment estimated marginal means to determine if egestion patterns were different between treatments (*pairs()* [emmeans package]; Lenth, 2022). The best-fitting model was checked for collinearity and all variables had an variation inflation factor <2 and were considered to not be collinear (*check_collinearity()* [performance package]; (Lüdecke et al., 2021).

We combined measurements of microplastic egestion rates with in situ measurements of anthropogenic particles in round gobies from our study and data from the literature (Hou et al., 2021; McNeish et al., 2018). We conducted a literature search for measurements of microplastics measured in round gobies using Google Scholar (date 15 July 2021). We divided particle concentration in fish at each site by turnover time (i.e., average time for microplastic egestion) to obtain daily egestion rate for gobies at each site:

$$\text{Egestion rate}\left(\text{no. fish}^{-1}\text{day}^{-1}\right) = \text{particle concentration(No. / fish)} / \text{turnover time(days)} \quad (1)$$

We used the two turnover times measured in this study (i.e., 18.2 and 25.0 hr) as well as a longer time for a conservative estimate (36.0 hr). We also searched literature for measurements of round goby density in shallow Great Lakes habitats (Google Scholar; date 15 July 2021), and used published values representing a range of measurements for in situ density of round gobies (no. fish/m^2) in coastal habitats of southern Lake Michigan (Chotkowski & Marsden, 1999; Marsden et al., 1996). We then multiplied daily egestion rate for individual fish at each study site (using the 25.0 hr turnover time) by a range of in situ density estimates to obtain a rate of particle egestion for gobies per unit area (no. m^{-2} day^{-1}).

$$\text{Areal egestion rate}\left(\text{no. m}^{-2}\text{day}^{-1}\right) = \text{Individual egestion rate}\left(\text{no. fish}^{-1}\text{day}^{-1}\right) \times \text{density}\left(\text{fish / m}^2\right) \quad (2)$$

3 | RESULTS

3.1 | Microplastic retention

Our first experiment documented microplastic egestion in round gobies following a single exposure. Four hours after the single microplastic diet exposure, the fish contained a mean (±*SE*) of 97 (±7.2)% of

the microplastics in the food pellet. This was reduced to 17.6 (±5.7)% after 24 hr and to 0.6 (±0.6)% after 96 hr (Figure 2a; Table S7). We did not find any of the experimental microplastic fibres in our control fish. The exponential decay model showed a decay rate constant of 0.055 hr^{-1} (adjusted $R^2 = 0.874$, $p < 0.001$; Figure 2a).

We repeated the analysis for fish which were fed microplastics for 7 sequential days. We only found fibres from the final 3 days of feeding: Day (D)5 (dark green), D6 (orange) and D7 (purple; Figure 2b). We found no experimental microplastic fibres in our control fish. The exponential decay model showed a decay rate constant of 0.040 hr^{-1} (adjusted $R^2 = 0.506$, $p < 0.001$; Figure 2b; Table S8).

Generalised linear models revealed that time and feeding trial were consistent explanatory variables across the best-fitted and competing models (Table S6). Models that included time and/or feeding trial as one or both explanatory variables had c. 100% and 73.5% of the model weights, respectively (Table S6). The best-fitted model included time and feeding trial as explanatory variables (Table S6), with time as a significant predictor of microplastic abundance in fish (Tables 1, S9). Feeding trial was not a significant predictor of the model, suggesting that egestion rates were similar between the 1- and 7-day feeding experiments (Tables 1, S9).

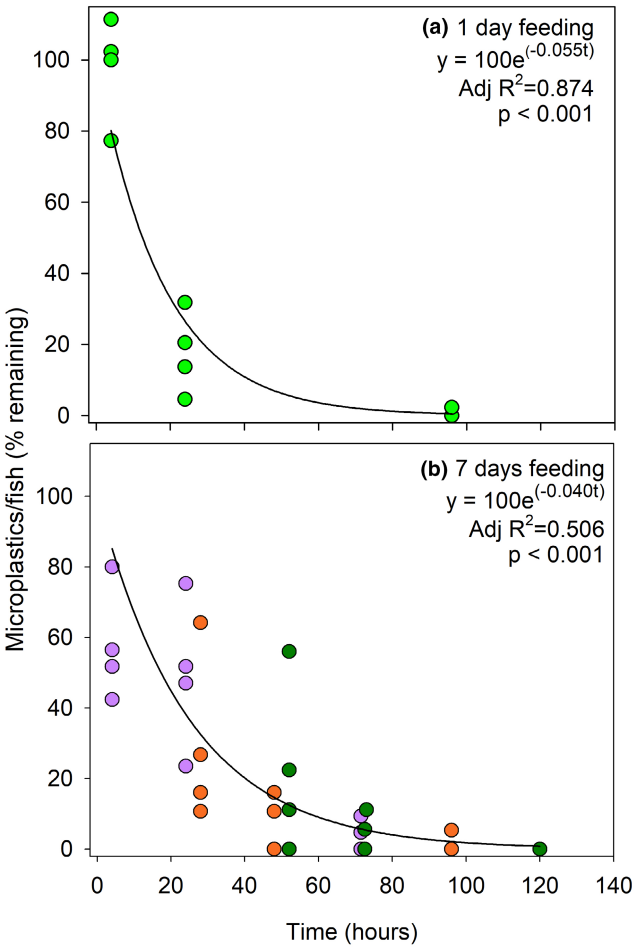

FIGURE 2 Relative abundance of microplastics remaining in fish digestive tracts after time since ingestion following (a) a single microplastic feeding, and (b) 7 days of microplastic feeding. In the 7 days exposure, purple fibres were fed on Day (D)7 (the final day), orange fibres on D6, and dark green fibres on D5. Regression results to fit the data using an exponential decay model (forced to y-intercept of 100%) are included in each panel.

3.2 | Microplastic abundance and polymer identification in gobies

Freshly collected fish from Lake Michigan showed mean (±*SE*) of 3.7 (±0.7) microplastics/fish ($N = 10$). After the 7-day acclimation period in laboratory aquaria, the gobies showed a mean (±*SE*) of 0.4 (±0.7) microplastics/fish ($N = 10$; Table S2). Polymer identification showed a mixture of natural, semi-synthetic and synthetic material types. For the freshly collected fish, 32% of particles were cellulose, 56% were processed cellulose (e.g., semi-synthetic rayon) and 12% were synthetic (e.g., polyester and polypropylene; Table S10). After 7 days of acclimation in the aquaria, two of the five identified particles were semi-synthetic (i.e., rayon) and three were cellulose. In the laboratory controls, we identified five particles: two were semi-synthetic, two were polyester and one was acrylic (Table S11).

3.3 | Scaling up egestion rates over time and by area

Our literature search showed that microplastics in round gobies have been measured in North America and Europe, with variation in concentration (Table 2). Similar values were found for gobies in this study and from nearby site on Chicago's Lake Michigan coast (mean [±*SE*] of 3.70 [±0.70] particles/fish and 2.1 [±0.6] particles/fish, respectively). Higher values were reported elsewhere in the Great Lakes including Milwaukee Harbour (22.9 [±6.2] particles/fish) and Hamilton Harbour (31 [±3.4] particles/fish) (McNeish et al., 2018; Munno et al., 2021). In the Rhine River (Switzerland), Roch and Brinker (2017) found a mean (±*SE*) of 1.25 (±0.05) particles/fish (Table 1). Also in the Rhine River, Bosshart et al. (2020) found one microplastic particle in 417 round gobies examined, although non-synthetic microfibers also were found (range = 0–4 fibres/fish, found in 12.7% of fish collected).

Our literature search for measurements of round goby density in shallow Great Lakes habitats revealed a range of values (Table 3). Chotkowski and Marsden (1999) reported juvenile density on sand in southern Lake Michigan as high as 133/m^2, and densities of adults on cobbles were 3.35–19/m^2. Marsden et al. (1996) reported goby densities that exceeded 40/m^2 in Grand Calumet Harbour (southern Lake Michigan). Goby density on various habitats (i.e, mud, sand, cobble, boulder) in Hamilton Harbour, Lake Ontario, Canada ranged from 2.2 to 34.9 individuals/m^2 (Vélez-Espino et al., 2010). Finally, in waterways near Detroit, MI, round gobies on rocks and sand ranged from 0.3 to 9 individuals/m^2 (Ray & Corkum, 2001; Table 3).

TABLE 1 Model coefficients, statistical results, and 95% confidence intervals from the top model and competing models evaluating the effects of time, feeding trial (1- or 7-day feeding experiment), and fish sex and length on microplastic abundance in fish.

					95% CI	
Coefficient	**Estimate**	***SE***	**Z**	***p***	**Lower**	**Upper**
Time + Trial						
Intercept	3.6394	0.2116	17.197	**<0.0001**	3.256	4.056
Trial: 7 Days	−0.4590	0.2490	−1.843	0.0653	−0.962	0.051
Time	−0.0470	0.0049	−9.646	**<0.0001**	−0.057	−0.038
Time						
Intercept	3.4680	0.1894	18.310	**<0.0001**	3.113	3.851
Time	−0.0511	0.0051	−10.000	**<0.0001**	−0.061	−0.042
Time + Trial + Sex						
Intercept	3.7295	0.2258	16.518	**<0.0001**	3.321	4.181
Time	−0.0451	0.0049	−9.228	**<0.0001**	−0.056	−0.036
Trial: 7 Days	−0.6031	0.2712	−2.224	**0.0261**	−1.150	−0.050
Sex: Male	−0.3970	0.3731	−1.064	0.2873	−1.124	0.341

Note: Significant values ($p \leq 0.05$) are in bold.

TABLE 2 Literature values for microplastics in round gobies

			Microplastic	
Study	**Location and water body**	**No. fish**	**Mean (no./fish)**	***SE***
This study	Montrose Harbour, Lake Michigan	10	3.7	0.7
Hou et al. (2021)	Calumet Harbour, Lake Michigan	17	2.1	0.6
McNeish et al. (2018)	Milwaukee Harbour, Lake Michigan	9	22.9	6.2
Munno et al. (2021)	Hamilton Harbour, Lake Ontario	84	31	3.4
Roch and Brinker (2017)	Rhine River, Switzerland	15	1.25	0.05

TABLE 3 Literature values for area-specific density of round gobies in Great Lakes

Study	**Location and water body**	**Habitat**	**Density (no./m^2)**
Chotkowski and Marsden (1999)	Calumet Harbour, Lake Michigan	Cobble	3.4–28
Marsden et al. (1996)	Calumet Harbour, Lake Michigan	nr	40
Ray and Corkum (2001)	Detroit River, St. Claire River, Lake St. Clair	Rocks, sand	0.3–9
Vélez-Espino et al. (2010)	Hamilton Harbour, Lake Ontario	mud, sand cobble, boulder	2.2–34.9

Abbreviation: nr, not reported.

We scaled-up egestion rates from data collected in this study literature values. Mean daily egestion rate per individual (no. particles fish^{-1} day^{-1}), ranged from a low of 1.8 particles fish^{-1} day^{-1} at Calumet Park (with 36 hr turnover time), to a high of 30.2 particles fish^{-1} day^{-1} at Milwaukee Harbour (with an 18.2 hr turnover time; Figure 3a). Using goby densities that represented a range of literature values (3, 28 and 40 individuals/m^2; Table 3), areal egestion rates ranged from a low of 6 particles fish^{-1} day^{-1} at Calumet Park (using 3 individuals/m^2 density) to a high of 879 particles fish^{-1} day^{-1} at Milwaukee Harbour (using 40 individuals/m^2 density; Figure 3b).

3.4 | Laboratory controls

We processed 11 laboratory digestion controls to account for contamination. We found a mean (±*SE*) of 1.82 (±0.48) non-experimental microplastic fibres per filter in the controls. We used a correction factor of 2 particles/sample for microplastic counts in fish collected from Lake Michigan, and after the 7-day acclimation period in the laboratory that occurred before the feeding trials (Table S2). The size and colour of the microplastic fibres in the laboratory controls were different from our experimentally added, acrylic microplastic fibres. We found one acrylic fibre in

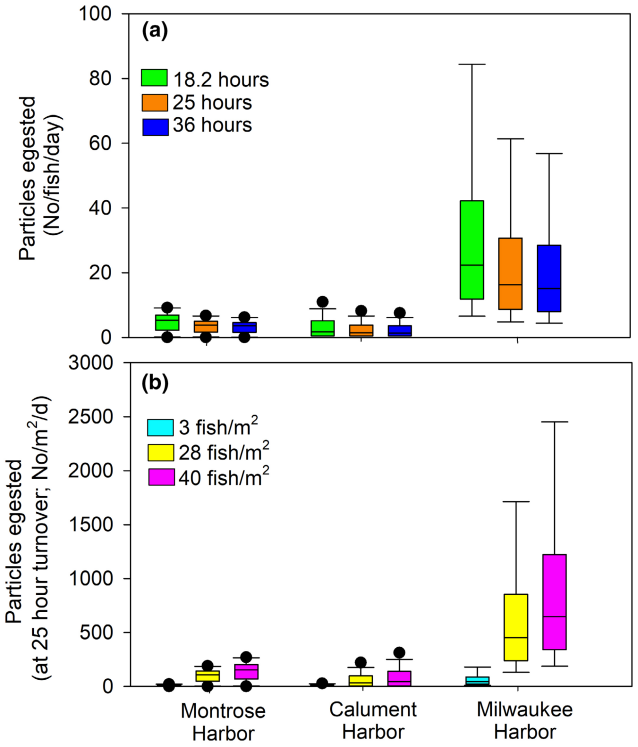

FIGURE 3 Microplastic egestion scaled to in situ conditions for microplastic in round gobies and round goby density at Montrose, Calumet and Milwaukee harbours in southern Lake Michigan, USA. (a) Particle egestion rates per fish, scaled according to three turnover time estimates. (b) Areal particle egestion rates scaled to a range of fish density. The centre line indicates the median, the box edges indicate the 25–75 percentiles, the brackets indicate the 10–90 percentiles, and any individual points indicate outliers from that range.

our laboratory controls; however, it was dark blue, a colour not used in the feeding trials (Table S10).

4 | DISCUSSION

Microplastics are commonly found within digestive tracts of aquatic organisms across ecosystem types and taxa (Li et al., 2019; Lusher et al., 2016; Rochman et al., 2015), but rates of microplastic egestion are less commonly measured. Our analyses of egestion rates demonstrated relatively swift egestion of microplastic fibres from a common fish (i.e., 18–25 hr), and combined with in situ measurements, showed high potential cumulative exposure rates for individuals, and for rapid cycling of microplastics from goby habitats on an areal basis. Examining rates of microplastic egestion is crucial to quantifying the role of fish on microplastic movement at the ecosystem scale.

4.1 | Microplastic egestion in round goby

We found no difference in microplastic egestion rate between fish which were fed microplastics once, relative to those fed microplastics for 7 consecutive days, suggesting minimal cumulative impact of microplastic ingestion on egestion rate. We expected slower egestion with 7 days of exposure, as microplastic fibres ingested in sequential meals could mix and aggregate, becoming more difficult to transport and slow egestion. The data showed no evidence of such an effect, so we concluded that for microplastic fibres of this size class, there is no impact of repeated exposures on microplastic transit within the digestive tract.

We suggest two reasons for the lack of difference in microplastic egestion between the 1- and 7-day microplastic feeding trials: digestive tract anatomy and particle characteristics. Firstly, the morphology of the fish gastrointestinal tract could influence the likelihood of interactions among ingested microplastics, and it varies by species and development stage. Predatory fish may have more complex anatomy (e.g., stomach) relative to herbivores which have less anatomical differentiation (Roch et al., 2021; Wilson & Castro, 2010). For example, Roch et al. (2021) documented active transport of microplastics in a predatory fish with a stomach (e.g., *Oncorhynchus mykiss*; rainbow trout) and passive transport in a stomach-less common carp (*Cyprinus carpio*). The digestive anatomy of round goby, considered a generalised zoobenthivore, is not entirely resolved in the literature (Kobegenova & Dzhumaliev, 1991; Wilson & Castro, 2010). Overall, further study of the role of digestive tract anatomy on patterns of microplastic transport in gobies and other species is warranted.

In addition to anatomical considerations, we note that the microplastic fibres in the feeding trials were homogenous in size and material type, which along with their relative flexibility and the lack of weathering (e.g., particles can be more rigid and/or brittle via UV light exposure), could impact our results relative to in situ conditions, where fish are exposed to a diversity of particles with highly variable physical properties. For example, Grigorakis et al. (2017) found that the egestion rate of microplastic fibres was slightly faster than the rate for beads (although not significantly different), and the authors speculated that different microplastic shapes may be retained in the digestive system at different rates. To the best of our knowledge, however, no previous experiments have quantified egestion rate of microplastic shapes for individual and mixed particle treatments. Future studies which examine the mixture of materials, shapes and sizes that occur in situ are needed to measure potential interactions among materials as they move within organisms' digestive systems (Xiong et al., 2019).

Particle size interacts with digestion processes to determine microplastic egestion rates in fishes. Roch et al. (2021) fed rainbow trout and common carp a gradient of microplastic sizes (c. 0.02–1 mm polymethylmethacrylate fragments), and showed that trout actively egested large particles relative to smaller ones, whereas carp egestion of microplastics across size classes was passive. Results suggested that some sorting via unknown physiological processes facilitated preferential excretion of large microplastics by trout (Roch et al., 2021). To date, detection of microplastics within fish digestive tracts has been biased towards larger particles, as the evolution of methods to detect smaller particles (e.g., <15 µm) is newly emerging (Brander et al., 2020; Lusher et al., 2017). As the

field matures, quantifying egestion rates across a wide gradient of particle sizes will be critical for understanding total microplastic exposures for fish.

4.2 | Ecological implications of microplastic egestion

Our egestion rate results are similar to other studies which quantified microplastic egestion rate using feeding trials and exponential decay coefficients (Table 4). Results from other studies show generally faster T_{50} than our results (12.6–17.3 hr), including 10 hr for goldfish (*Carassius auratus*; Grigorakis et al. (2017), 4–12.1 hr for rainbow trout and carp (*O. mykiss* and *C. carpio*, respectively; Roch et al., 2021), and 3.8–9.5 hr for larval fathead minnows (*Pimephales promelas*; Hoang & Felix-Kim, 2020). Across the nine published measurements of T_{50} for microplastic egestion, the scale of variability was relatively narrow, with an average 9.0 hr (range 3.8–17.3 hr; Table 4). Clearly there is variability among study species and particles (i.e., polymer type, size and shape) that merit additional study for understanding the physiological and ecological drivers of microplastic egestion (Roch et al., 2021; Xiong et al., 2019). However, the composite data represent a critical starting point by providing a time-constrained range of values that can be used for estimates of microplastic egestion by fish when building new models of microplastic dynamics at the ecosystem scale.

Two of the key questions that arise from measurements of microplastics in fish specimens are: (1) When were the microplastics ingested? and (2) Does microplastic abundance in fish reflect environmental concentrations? That is, microplastics may be on a relatively brief trip through organisms, or may accumulate over a longer period of time. The answers to those questions are critical for broader ecological conclusions including the potential for microplastic bioaccumulation, and the use of fish as biomonitors for plastic pollution. Drawing inferences from egestion rates measured in this study and in the literature, we suggest limited potential for microplastic bioaccumulation, and the use of fish as biomonitors for plastic pollution.

Bioaccumulation is observed for some chemical pollutants in aquatic ecosystems, which increase within organisms during their lifetime, but it is not clear if that occurs for microplastics (Krause et al., 2020). Our egestion results do not suggest that long-term retention or bioaccumulation of microplastic fibres is possible, at least for the shape, size and polymer type studied. Analyses of microplastic egestion within similar particle size ranges from other fish also do not suggest that bioaccumulation occurs for individuals (Grigorakis et al., 2017; Hoang & Felix-Kim, 2020; Roch et al., 2021). McNeish et al. (2018) found that microplastic abundance in round gobies was positively related to body size. In that case, the data may initially appear to suggest bioaccumulation, however, the higher abundance was attributed to a larger gut, rather than increased retention. When microplastic abundance in gobies was expressed in units of body mass (number/g

TABLE 4 Summary of egestion rates from freshwater fish measured via exponential decay rates following microplastic feeding from this study and published values

Study	Taxon	Micro-plastic feeding	Stage	Mean Length (cm)	Mean wet weight (g)	Type	Shape	Size	Exp. decay (k/hr)	T_{50} (hr)	Turn-over (hr)
This study	Neogobius	1 time	Adult	9.6	12.4	Acrylonitrile	Fibre	0.04 × 1 mm	−0.055	12.6	18.2
	Melanostomus	Daily, 7 days	Adult	9.7	11.4	Acrylonitrile	Fibre	0.04 × 1 mm	−0.040	17.3	25.0
Roch et al. (2020)	Oncorhynchus	1 time	Adult	11.6	18.4	PMMA	Fragment	0.0427 mm	−0.057	12.1	17.5
	Mykiss	1 time	Adult	11.6	18.4	PMMA	Fragment	1.086 mm	−0.173	4.0	5.8
	Cyprinus	1 time	Adult	8.5	7.9	PMMA	Fragment	0.0427 mm	−0.095	7.3	10.5
	carpio	1 time	Adult	8.5	7.9	PMMA	Fragment	1.086 mm	−0.151	4.6	6.6
Grigorakis et al. (2017)	Carassius	1 time	Adult	nr	24.8	Polyester	Fibre	0.05–0.5 mm	−0.069	10.0	14.5
	Auratus	1 time	Adult	nr	27.1	Polyethylene	Bead	~0.2 mm			
Hoang and Felix-King (2020)	Pimephales	1 time	Larva	nr	nr	Polyethylene	Bead	63–75 µm	−0.073	9.5	13.7
	Promelas	1 time	Larva	nr	nr	Polyethylene	Bead	125–150 µm	−0.182	3.8	5.5

Note: Egestion rates from Grigorakis et al. (2017) are for fibres and beads combined, rates from Hoang and Felix-King 2020 are from the trials with no re-consumption.
Abbreviations: nr, not reported; PMMA, polymethylmethacrylate; exp., exponential; T_{50}, time to 50% egestion; turnover, mean time for particle egestion.

wet weight), there was no difference among individuals (McNeish et al., 2018). Finally, although bioaccumulation may not occur for particles in the size range for the current study and those listed in Table 3 (0.04–1 mm), it may occur for other particle sizes. In particular, very small particles (i.e., <5 μm) could be assimilated across the gut lining, become redistributed to other tissues and permanently retained (Zeytin et al., 2020). In addition, large particles could become permanently stuck, and therefore represent long-term accumulation within the digestive tract (Puig-Lozano et al., 2018).

Microplastic turnover times of ≤24 hr in this study and others (Table 3), suggest the microplastics found within the study organisms were recently ingested, and thus the amount in the fish might be reflective of the amount in the environment at the time of collection; thereby fish may be a bioindicator of pollution. However, attempts to compare microplastic in fish to environmental concentrations are limited and show contrasting results (McNeish et al., 2018; O'Connor et al., 2020; Perry et al., 2020). In addition, there are several considerations to be assessed before any taxon can be considered for this role. Firstly, the microplastics found within the digestive tracts in a single fish likely represent a subset of the total microplastics in the environment. Some fish taxa show avoidance of some colours and sizes of microplastics (Xiong et al., 2019), and smaller particles may be assimilated (Zeytin et al., 2020). Secondly, the amount of microplastics within a fish may reflect only a brief window of time near its death, and likely indicate conditions at a specific habitat or recent prey item. A single fish species may have limited applicability to represent the microplastic pollution status of an ecosystem. Species that are generalist in their habitat use and feeding, and show little discrimination in particle selection would be best suited as potential bioindicators.

Egestion rates of microplastics by fish documented in this study and others offer an important framework for estimation of long-term exposure to microplastics and plastic-associated chemicals for individuals and populations. Round gobies retain microplastic fibres for about 1 day on average, so it is most likely that new microplastics are *continuously* ingested and egested. An individual organism's exposure to microplastics over a period of weeks or months is much higher than is reflected in the amount of microplastic in their gut on any one date (D'Souza et al., 2020). Also, microplastic particles may have sorbed POPs (e.g., polycyclic aromatic hydrocarbons), which can be desorbed in the gut (Rochman et al., 2019). Microplastic egested from the digestive tract will re-enter the environment and may adsorb new chemicals, which may be re-ingested. This perspective that many microplastic particles are passing through individual fish over short time scales (days to weeks) is not in harmony with many laboratory-based assessments of microplastic ingestion, which focus on single exposures (Hoang & Felix-Kim, 2020).

Combining the egestion rate, estimates of microplastic abundance within organisms and organism density in situ, is needed to place laboratory-based analysis in an ecological context. We compared areal egestion rate for microplastics in round gobies in the Milwaukee River to microplastic export from the river measured in a previous analysis using a "back of the envelope" approach. Riverine export is measured as the number of particles (i.e., mass of solutes) that leave a river over a given unit of time, relative to the watershed area (e.g., no. particles/watershed area/time). McNeish et al. (2018) used grab samples to measure microplastics concentrations of 30 particles/L in the Milwaukee River in summer 2016. With discharge of 7.8 m^3/s on the date of collection, and a watershed area of 1,803 km^2, this equates to watershed-scale microplastic export of 11.2 particles m^{-2} day^{-1}. Watershed export shares identical units as the areal egestion rates from round gobies. We note the watershed export estimate of 11.2 particles m^{-2} day^{-1} is the same order of magnitude as the areal-specific microplastic egestion rate for gobies, which ranged from 6 to 879 particles m^{-2} day^{-1}. This preliminary comparison is intriguing, and suggests that goby egestion may be an important component of microplastic dynamics at the mouth of the Milwaukee River. However, we note a few key caveats. These calculations are based on a modest amount of data collected at different times, and error surrounding the estimates may be large. In addition, the total areal coverage of goby habitat for the region is not known, but if documented, would add additional context into the relative magnitude of the area-based rates of river export and goby egestion. In any case, the framework here places microplastic egestion rates from animals within the context of ecosystem-scale processes, a fundamental principle of ecosystem ecology for the study of elemental cycles (Atkinson et al., 2016). This approach is a roadmap for generating data needed to fill in microplastic budgets in aquatic ecosystems.

5 | CONCLUSIONS

Studies on the rate of microplastic egestion within fish and other aquatic organisms are relatively limited, and require unification with in situ assessments of microplastic dynamics. Our results suggest that microplastic fibres are passed through the digestive system of a common freshwater fish species with an average turnover time of 18.2–25 hr, despite single or sequential microplastic ingestion. Because the study species is so abundant and well-studied, combining egestion rates with microplastic measurements within digestive tissues of gobies, and published values for in situ goby density, we compared daily export of microplastics from the Milwaukee River to areal-egestion rate by gobies, which were in the same order of magnitude. More research is needed to investigate microplastic retention by fish across a range of environmentally relevant microplastic characteristics, including concentration, polymer types, sizes and shapes. Future studies also should consider the egestion rate dynamics to estimate an individual's total exposure to microplastics over the course of a season or lifetime, and compare egestion rates to other rates of input, retention and movement of microplastics in the environment.

AUTHOR CONTRIBUTIONS

Conceptualisation: TH, RM, LH, Developing methods: TH, RM, LH. Data analysis: TH, RM, LH. Preparation of figures and tables: LH, TH, RM. Conducting the research: LH, TH, data interpretation: LH, TH, RM, writing: LH, TH, RM.

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DATA AVAILABILITY STATEMENT

All data are shown in the supplemental materials, including all measurements from the feeding trials, microplastic abundance from gobies collected in situ and after laboratory acclimation periods, laboratory control results, and analyses for polymer identification.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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