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# Where Is All the Plastic? How Microplastic Partitions across Environmental Compartments within a Large Pelagic In-Lake Mesocosm

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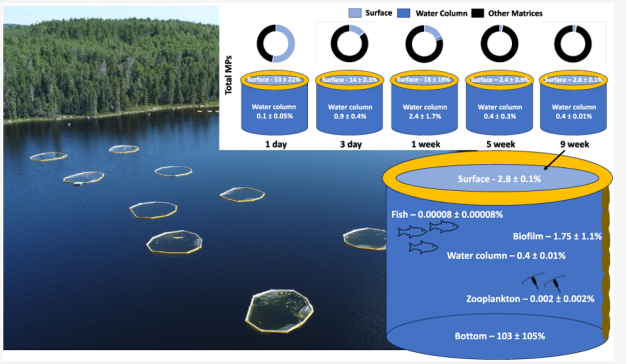
**ABSTRACT:** How microplastics transit within aquatic ecosystems and partition among environmental compartments is not fully understood. To increase understanding, we added microplastic fragments ranging in buoyancy (positive: polyethylene (PE), neutral: polystyrene (PS), negative: polyethylene terephthalate (PET)) and size (~30 to 1400 $\mu$m) to surface waters of closed-bottom, in-lake mesocosms (10 m diameter, 2 m depth). To assess residence time, we measured microplastics in surface waters and the water column over a 9-week period. To measure fate, we measured microplastics in the surface water, water column, bottom detritus, and biota (biofilm on the walls, zooplankton, fish) at 9 weeks. The residence times of microplastics were longer at the surface than in the water column, with less dense and smaller particles having the longest residence times. After 9 weeks, nearly all microplastics were on the bottom, with only 3% on the surface, 0.4% in the water column, 2% in biofilm, and <0.01% in zooplankton and fish. The surface water and biofilm on the walls were larger reservoirs than the water column, suggesting that surface microlayers and biofilm on hard substrates are important, yet overlooked, reservoirs of microplastics in aquatic ecosystems. Results inform future hypotheses relevant to monitoring programs and risk assessments.




**KEYWORDS:** *microplastics, residence time, mass balance, freshwater, aquatic ecosystem, lake*



## INTRODUCTION

Research questions on aquatic microplastics have shifted from whether there are microplastics in the oceans,[1,2] lakes,[3] and rivers[4] to how microplastics reach ecosystems,[2,5] how they move within them,[6] and how they interact with them physically,[7] chemically,[8] and biologically.[9,10] Microplastics are found from benthic sediments to surface waters and everywhere in between (e.g., animals, marine snow, water column[11,12]). Such findings lead to questions about how microplastics reach aquatic ecosystems and how they partition within them, e.g., mechanisms and rate of transport, residence times (or persistence) in each compartment, and the amount of microplastics across compartments over time.

Several studies testing hypotheses about the transport of microplastics have been conducted using a mixture of empirical data and predictive modeling in oceans, and rivers.[13−21] Some studies have also aimed to predict how much plastic resides in different reservoirs (i.e., temporary "resting" places within ecosystems, e.g., surface waters, benthic sediment, beaches) to inform mass balance approaches.[22,23] These studies generally conclude that we do not have enough information to conduct a mass balance. Although models generally suggest most microplastics sink to the bottom of aquatic ecosystems,[6] much remains unknown about the rate at which they sink and the processes driving transport and deposition. Moreover, questions remain about how these unknowns vary by particle size and shape[14,24] and across ecosystems,[25,26] as well as what happens once microplastics enter the sediment (e.g., burial and/or resuspension[27−29]). Understanding the fate and transport of microplastics within ecosystems can improve theoretical models and mass balance predictions. Improving our understanding will also inform environmental manage-

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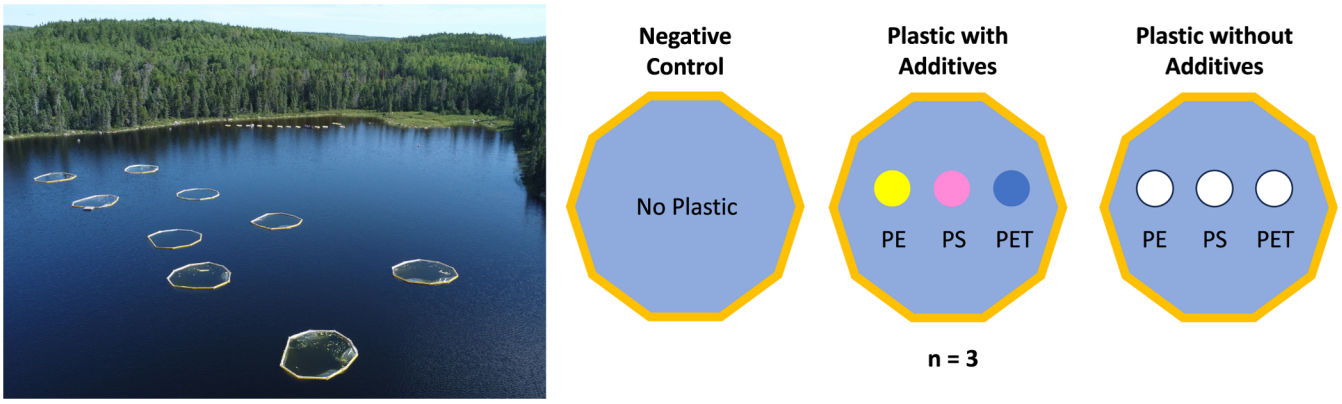


**Figure 1.** Aerial photo of the mesocosm array (left); a diagram of our experimental design (right), consisting of nine experimental units with three treatments: negative control, plastic with additives, and plastic without additives ($n$ = 3 replicate mesocosms per treatment). The treatment with additives had dyes (PE: yellow, PS: pink; PET: blue) and other functional additives (Table 1). The treatment without additives did not include dyes, and the microplastics were colorless.

ment, as the persistence of microplastics within each compartment informs monitoring,[23] risk,[24] and mitigation.[25]

We conducted a large-scale, in-lake mesocosm experiment to measure the fate and transport of microplastics over a 9-week period, calculating residence times and a mass balance. This experiment was conducted at the International Institute for Sustainable Development's Experimental Lakes Area (IISD-ELA), and is a follow-up from an experiment conducted in 2021.[26] Overall, this experiment was designed to investigate how ecological effects of microplastics vary with and without plastic additives as well as to provide a refined description of the exposure landscape (i.e., the amount of microplastics across ecosystem compartments) within a mass balance framework.

In this paper, we introduce the experimental design of the experiment and document the transport and fate of microplastics in the surface water, water column, biofilm on the mesocosm walls, the mesocosm bottom, the zooplankton, and the gastrointestinal tracts (GITs) of fish. Our main objectives were to (1) measure the residence times of microplastics in the surface water and water column, (2) assess how residence time varies by polymer and particle size, and (3) assess the proportions of microplastics across compartments using a mass balance approach. Here, we focused on the plastics with additives, which were easier to characterize due to their color. We hypothesized that over the 9-week period, the majority of the microplastics that we mixed into the surface water would end up at the bottom of the mesocosms, with some sticking to the biofilm on the mesocosm walls. Furthermore, we hypothesized that the residence times would vary based on particle size and density, with smaller and more buoyant particles having longer residence times in water. This experiment is part of the pELAstic project (https://thepelasticproject.com/), a collection of ongoing studies aimed at better understanding the fate and effects of microplastics in aquatic ecosystems. This work is the first of a series for this experiment. Future papers will cover other aspects of microplastics and additive chemical fate, transformation, and effects.

## METHODS

**Experimental Design.** The experiment took place over a 9-week period at the IISD-ELA, near Kenora, Ontario, Canada (49°40′N, 93°44′W). Nine in-lake mesocosms were deployed along the northwestern side of Lake 378 (Figure 1), a typical oligotrophic headwater Canadian Shield boreal lake with a surface area of 251,579 $m^2$, a volume of 1,996,479 $m^3$, and a maximum depth of 16.6 m. Mesocosms were suspended in the pelagic zone where the lake depth ranged from roughly 3 to 6 m. Each mesocosm was a 10-m-diameter, 2-m-deep closed-bottom cylinder (encompassing only the epilimnion). The mesocosms were constructed using a nonpermeable 8-mm-thick, food-grade, low-density polyethylene cylindrical enclosure (Curry Industries, Winnipeg, MN, Canada) attached to a floating vinyl-wrapped Styrofoam decagonal ring (Dow, Midland, MI) held together on the outside by malleable plastic piping and cable ties. No sediment was added to the mesocosms, and thus, the bottom was the same material as the walls. Each mesocosm was held in place by anchoring the floating ring to the lake bottom via concrete weights attached using a black polypropylene rope.

Each mesocosm was filled with roughly 150,000 L of lake water pumped in via a trash pump (Honda Canada) connected with a fire hose. Natural microbial, phytoplankton, and zooplankton communities from L378 were added to the water. To offset mortality from pumping, we added zooplankton from 15 daytime 10 m vertical hauls to each mesocosm using a 0.5 m diameter net with 150 $\mu$m mesh. Before adding microplastics, we allowed the mesocosm communities to acclimate for 1 week. During acclimation, we collected yellow perch (*Perca flavescens*), roughly 70−100 mm in length, from L378 and added 26−28 fish per mesocosm (Table S1), which matched the natural density of this species (approximately 3000 fish per ha) in a nearby reference lake (Lake 239).[27] Fish were collected using a seine net under a collection permit from the Ontario Ministry of Natural Resources (MNR; 1097798) and an animal care permit from the University of Toronto (20012583).

We used an ANOVA design with three treatments (plastic with additives, plastic without additives, and a negative control; $n$ = 3 replicate mesocosms per treatment; Figure 1). The microplastics were composed of an equal mixture of irregular fragments of PE (linear low-density polyethylene (specifically LLDPE)), PS (polystyrene), and PET (polyethylene terephthalate), ranging in density (Table 1). One treatment included plastics with their additives (including colorants), and the other was additive-free (and clear in color; Tables 1 and S2). The sizes of the additive and additive-free plastics were slightly



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Table 1. Details Regarding the Microplastic Fragments Used for Each Treatment, Including Their Chemical Ingredients, Densities, and Size Distributions. See Table S2 for the CAS numbers

| plastic | chemical components (% mass) | density ($g/cm^3$) | size min−max, avg ($\mu m$) |
|---|---|---|---|
| LLDPE additive | 0.25% bismuth vanadate pigment | 0.92−0.95 | 6−939, 209 |
| | 0.05% Chimassorb 944 HALS UV | | |
| | 0.05% Tinuvin 622 HALS UV | | |
| | 0.025% Irganox 1010 Antioxidant | | |
| | 0.025% Irganox 168 Antioxidant | | |
| | 1% Benzotriazole−Acetostab 236 | | |
| | 98.6% LLDPE resin | | |
| LLDPE no additive | LLDPE resin | 0.92−0.95 | 40−1910, 290 |
| PS additive | 0.05% Perylene red | 0.96−1.05 | 5−1960, 197 |
| | 0.15% Titanium dioxide | | |
| | 0.1% Irgafos 126 Antioxidant | | |
| | 0.1% *N,N*-Ethylene bis-stearamide | | |
| | 99.6% PS resin | | |
| PS no additive | PS resin | 0.96−1.05 | 50−1370, 221 |
| PET | 0.25% Ultramarine blue red shade | ∼1.38 | 15−1769, 232 |
| | 0.35% 25u Rutile white | | |
| | 99.4% PET resin | | |
| PET no additive | PET resin | ∼1.38 | 10−640, 104 |

different (Table 1, see Figures S1 and S2 for images of the fragments and particle size distributions), likely related to how differences in their properties with and without additives affected milling. The plastics were produced from TechmerPM (Batavia, IL) and ground into microplastics by Custom Processing Service (Reading, PA).

On June 9, 2022, microplastics were added as a single pulse to six mesocosms at a loading rate of 12.22 kg of microplastics per mesocosm (an estimated 4.4 billion particles). Theoretically, this loading rate would yield a nominal concentration of 29,240 particles/L (matching the highest treatment in our 2021 experiment[26]) if all microplastics were fully mixed homogeneously within the water column—an assumption we did not expect to be true based on previous work.[33] The amount of microplastics added was calculated using mass-to-count relationships for each polymer (SI text, Table S3, and Figures S3−S5). Because the particle sizes were different, and to standardize treatments by particle mass, the number of microplastics in each mesocosm differs slightly. Microplastics were added to the mesocosms by wetting the plastics in mesocosm water within a bucket and then mixing and releasing the mixture just below the surface of the water as we pushed ourselves around the perimeter of the mesocosm in a small boat to distribute them evenly.

**Water Quality and Chemistry.** We monitored the temperature, dissolved oxygen, turbidity, and photosynthetically active radiation (PAR) weekly from each mesocosm. On four sampling dates, June 8th (pre-addition), June 22nd (week 2), July 12th (week 6), and Aug 10th (week 9), samples were taken to measure particulate and dissolved nutrients (phosphorus, nitrogen, and carbon), soluble reactive silica (SRSi), and chlorophyll-*a*. Water chemistry parameters were analyzed using the methods of Havens et al.[28] Information about the sampling methods, analytical methods, and statistical analyses is in SI Text.

In general, there were no clear patterns among treatments (Tables S4−S6; repeated-measures ANOVAs, $p > 0.05$; Table S6, Figures S6 and S17) for any water quality or chemistry parameter except particulate nitrogen and turbidity. There was a significant treatment effect for particulate nitrogen (repeated-measures ANOVA, $F = 5.561$, $p = 0.043$; Table S6 and Figure S16); however, post hoc tests did not reveal any pairwise differences between treatments ($p > 0.05$) for any study day. For turbidity, there was a significant difference among treatments and days (repeated-measures ANOVA; treatment ($F = 12.9$, df = 2, $p = 0.007$), Day ($F = 5.3$, df = 7, $p < 0.001$; Table S6 and Figure S11)). In general, turbidity (measured from a water sample taken at 1 m depth) was higher and more variable in microplastic treatments. In 2021,[26] we also saw differences among treatments for turbidity, but the patterns were different. In 2021, we observed a significant positive correlation between light penetration and microplastic concentration, which could suggest reduced turbidity due to microplastics. Here, we observed increased turbidity at some time points and no pattern relevant to light penetration. A few studies have looked at the relationships between microplastics and light penetration in aquatic ecosystems. These studies suggest that microplastics can decrease floc size in the water column,[29] which could increase turbidity and reduce light penetration. Additionally, due to the unique radiative properties of microplastics, they may influence light radiation in the water column. Further studies should examine the effects of microplastics and macroplastics, particularly under environmentally relevant concentrations, on turbidity and light availability as these parameters can affect primary productivity.[30]

**Microplastic Sample Collection.** We took samples from only the treatments with plastic additives (including colorants) for easier quantification and characterization. We have no reason to believe that the physical fate would vary between the plastics with and without additives used here. We sampled from two replicate mesocosms to measure the fate and transport of microplastics. We also sampled one control mesocosm to represent field blanks and to measure cross-contamination among mesocosms. We limited our sampling and analysis efforts to these three mesocosms due to the time and resources it takes for laboratory analyses of the samples for microplastics. We sampled the surface water, water column, mesocosm bottom, biofilm attached to the mesocosm walls, zooplankton, and fish and quantified and characterized the amounts, polymers, and sizes of microplastics within each compartment.

The surface water (i.e., at the air−water interface) and water column were sampled at 24 and 72 h, as well as 1, 5, and 9 weeks after the addition of microplastics. We sampled the surface water first so as not to disturb it while sampling the water column. To determine the amount of microplastics on the surface, i.e., within the surface slick (see Figure S18 for examples of what this looked like over time), we used two sampling techniques. We measured the area of the surface slick with a drone (DJI Mini 2). To standardize images for measurements, two pairs of ground control squares, each pair



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with squares situated 2 m apart, were secured to different floating collars of the mesocosms. When flying the drone, images (resolution 12 megapixels) were taken 10 m above the mesocosm. Several images were taken, but the clearest image was used for analysis. The Interactive Perspective plugin in ImageJ was used to line up the images so that the comparative distances among ground control point pairs matched the measured distances in the field (within 0.2 m accuracy). Other than this, there was no image correction. Then, using the polygon tool, we measured the area of the plastic slick. When a plastic slick was mottled, we estimated the proportion of the area covered in plastic and multiplied by the area of the slick. To determine our error, we analyzed the same image from each mesocosm twice and used the average final value. The coefficients of variability for percent cover of microplastics between analyses within one image ranged from 2 to 40%, with an average variability of 17%. To estimate the amount of microplastics per area of surface slick, we sampled one surface core from each mesocosm at each time point from within a representative area of the slick. The surface core was collected by using a glass jar with an 8.6-cm-diameter opening. The jar was held at an angle (roughly 45°) to slice through the surface without disturbing the particles. Once the opening of the jar was underwater and filled to a standard line on the jar, the jar was capped underwater. The sample was then topped with a capful of 99% isopropyl alcohol to prevent biological growth during storage and transport. The slick area was then multiplied by the concentrations of microplastics quantified in the samples taken from the surface slick.

To determine the amount of microplastics in the water column, we sampled 4 L of water from 1 m depth using a peristaltic pump (GeoTech; Denver, CO) with 1/4″ Tygon tubing and inline filtration (with 47 mm diameter nylon filters (Millipore) with 20 $\mu$m pores). To avoid interference from the walls of the mesocosm, the sampling tubes were fed through holes drilled into a cork block that was clipped onto a rope secured across the mesocosm. The cork block was pushed roughly 1 m from the walls of the mesosphere prior to water sampling. To avoid cross-contamination, we used separate tubing for the control and plastic treatment mesocosms. Between samples, the inline filter holder and tubing were rinsed with mesocosm water by running several liters of water through the system. Immediately after sampling, the filters were stored in clean plastic Petri dishes at room temperature until further analysis.

To determine the amount of microplastics on the bottom of the mesocosm, glass Petri dishes were taped to the floor as collection traps. Five glass Petri dishes, 100 mm in diameter, were randomly taped to different areas of the bottom of the mesocosms. At the end of the experiment, a diver located the dishes, capped them immediately, and swam them to the surface. They were transferred immediately with RO water into a clean glass sampling jar and topped with a capful of 99% isopropyl alcohol until further processing. All dishes were recovered from the control, and only four dishes were recovered from the two plastic-treated mesocosms.

To determine the amount of microplastics adhered to the walls, we deployed (with tape) three replicate strips of wall material (2 cm x 6 cm) within each mesocosm along the walls (from top to bottom) from three equidistant points. Each strip had a marking at 1 m depth, and upon sampling, we separated the samples in the water and collected the top 1 m and bottom 1 m of each strip. Strips were collected at the end of the experiment at the same time as the bottom dishes.

We sampled zooplankton for microplastics from each mesocosm at the end of the experiment using a Wisconsin plankton net with a 0.25 m diameter opening and a 53 $\mu$m mesh. Two 1.5 m vertical net hauls were collected from opposite sides of each mesocosm to ensure a representative sample of the zooplankton community (total sample volume of 147 L) and preserved in 5% sugar-formalin[31] after narcotization in methanol.[32] Counts of zooplankton from these same hauls were used to determine zooplankton densities (numbers per liter) in the mesocosms at the time of sampling. Fish were sampled from each mesocosm at the end of the experiment using a seine net (6 mm mesh, 2 m height, and 30.5 m length). Four fish from each mesocosm were sampled to quantify and characterize the microplastics.

**Microplastic Sample Processing.** In this manuscript, we report on microplastics >53 $\mu$m in size. Microplastics were extracted from water column samples by sonicating the filters in RO water for 10 min and size fractioning the extracted microplastics in RO water through a stainless steel sieve stack with 212 $\mu$m, 106 $\mu$m, and 53 $\mu$m pore sizes. Each size fraction (>212, 106−212, and 53−106 $\mu$m) was rinsed with RO water into a clean glass mason jar for sorting via microscopy.

Surface water samples were size fractioned using the same sieve stack. Each size fraction was subsampled to reduce the number of plastic particles that needed to be counted via microscopy. For subsampling, after transfer with RO to a clean glass jar, RO water with Alcojet detergent (to prevent particle aggregation) was added to achieve exactly 400 mL of a 1% Alcojet solution. Finally, 1 mL of solution (0.25% of the sample) was pipetted from a mixed solution and dried in a clear glass Petri dish for quantification and characterization.

Microplastics were extracted from the bottom samples using a 30% $H_2O_2$ solution. First, a sample was poured over a 53 $\mu$m stainless steel mesh sieve to remove the solution. Then, it was transferred to a clean polypropylene specimen cup with 50 mL of 30% $H_2O_2$. The sample was placed in an oven at 45 °C for 48 h. Once digested, the sample was sieved back through the 53 $\mu$m stainless steel mesh sieve to remove the digestion solution. The sample was then processed in the same fashion as surface water samples.

Biofilm on the wall material was scraped from both sides of the wall strips with a gloved hand into a clean polypropylene specimen cup. A 30% $H_2O_2$ solution was used to rinse the remaining substance off the strip and gloved hand. Samples were then digested, size fractioned, and subsampled as described above, with the exception that 2.5% of the sample was subsampled for quantification and characterization.

Between 11 and 100 individual zooplankton were picked under a microscope using metal forceps for each of the four most common species: *Diaphanosoma birgei, Diaptomus minutus, Eubosmina sp.*, and *Tropocyclops extensus*. To remove microplastics that may have been attached to the outside of the zooplankton, each sample was rinsed before digestion with RO water. Zooplankton were then rinsed into a clean polypropylene jar with 30% $H_2O_2$, digested at 45 °C for 48 h, sieved through a 53 $\mu$m stainless steel mesh, and filtered onto a 20 $\mu$m nylon filter for quantification and characterization. Individual fish were dissected, and GITs were placed in precleaned polypropylene specimen cups. Each GIT was digested in 20% KOH for 48 h at 45 °C, sieved through a


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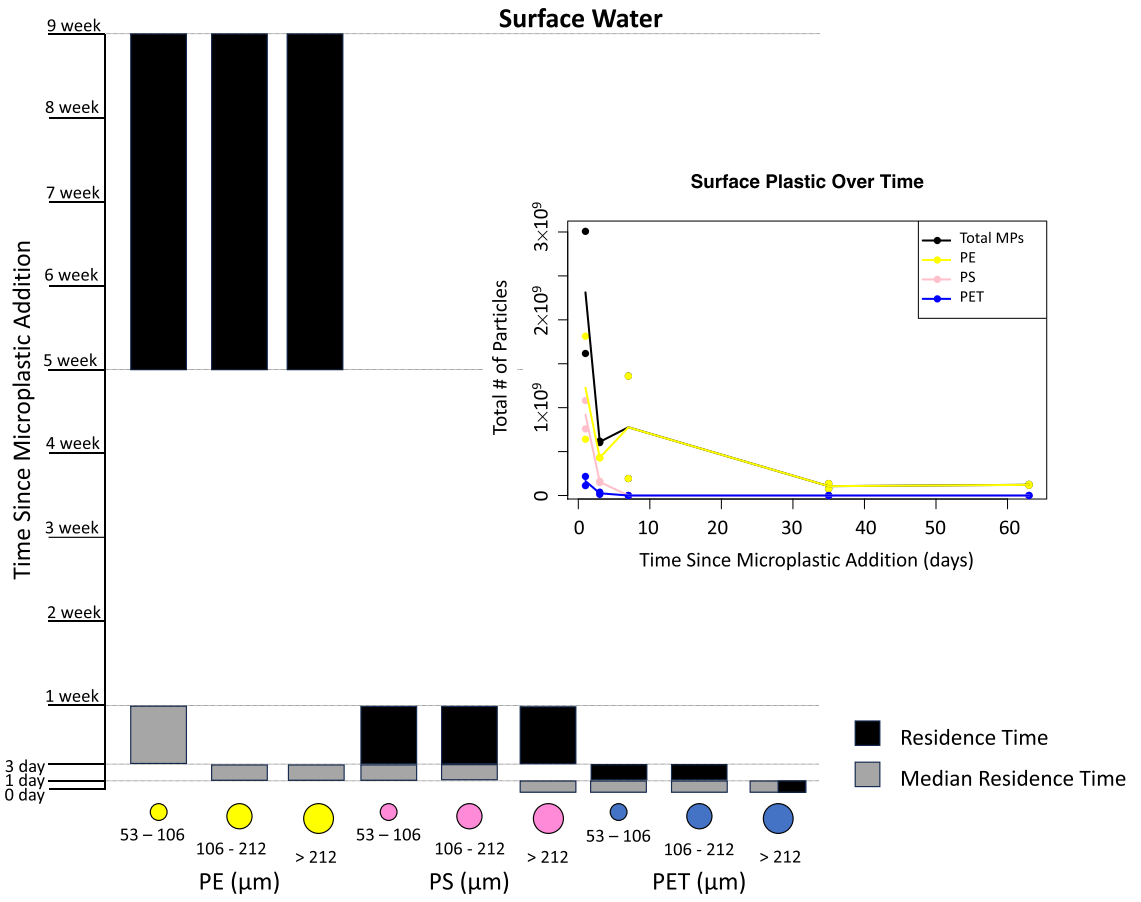


**Figure 2.** Residence times of microplastics in the surface water (shown as time since the addition of microplastics on the *y* axis) are shown in the larger figure categorized by polymer and size fraction (along the *x*-axis). The median residence time (gray boxes) is the delay between when plastic is added and when 50% of the plastic added left the compartment. The residence time (black boxes) is the delay between when plastic is added and when 95% of the plastic added leaves the compartment. The resolution is defined by the sampling events (shown as horizontal lines). The calculated counts of microplastics in the surface water are shown in the inset graph, which has the total number of microplastic particles on the surface of the mesocosm along the *y* axis and the time since the microplastic addition on the *x* axis (in days). The points represent the raw data from each mesocosm, and thus, there are two points per sampling event.

53 $\mu$m stainless steel sieve, and filtered onto a 20 $\mu$m nylon filter for quantification and characterization.

**Microplastic Counting and Characterization.** Microplastics were quantified and characterized by a dissecting microscope. Briefly, a wet sample was sorted by putting one spoonful at a time into a clean glass Petri dish and systematically assessing the whole dish. When the full jar was sorted, it was triple-rinsed with RO water, and the rinse water was also assessed for microplastics. For samples that were sorted dry on filters or Petri dishes, the filter or dish was assessed systematically under a microscope. When a suspected microplastic particle was identified, it was recorded by color (blue = PET, pink = PS, and yellow = PE). The first 10 particles of each color within each size fraction were picked and mounted on double-sided tape. The picked particles created a subsample that could be measured and chemically analyzed to confirm the polymer type.

To verify our accuracy in characterizing particles by polymer type, we tested each person who counted microplastics ($n$ = 7) on three samples. For these three samples, all picked particles suspected to be PE, PS, or PET were analyzed via $\mu$-Raman spectroscopy (HORIBA Raman Xplora Plus) in LabSpec6 software using a 785 nm (range 50−2000 cm$^{-1}$) laser. Raman spectra were obtained using a 100× LWD objective (NA= 0.8), resulting in a laser power of 20.2 mW at 100% filter with a spectral resolution of 1.3 cm$^{-1}$ (785 nm excitation laser, 600 grooves/mm). Minimal manual baseline correction was applied to spectra, though the matching software may have applied corrections to the spectra automatically (e.g., baseline, vertical clipping, intensity distortion, horizontal offset, vertical offset, and Raman intensity distortion). Particle identification was based on hit quality index (HQI) determined by matching software (Wiley KnowItAll and ID Expert) and manual inspection of peak alignment and intensity relative to spectral database matches from subscription-based libraries and SLoPP/SLoPP-E.[33] A spectral match generally fell between 80% and 98%, with a few exceptions made based on judgment of spectral features. Average accuracy in characterizing particles for each person ranged from 87 to 100%. Thus, we did not perform Raman spectroscopy on samples other than the three used for testing the accuracy for each counter. However, because there were so few particles overall for zooplankton, we chemically analyzed all particles and spectroscopy-corrected the full data set.

**Quality Assurance/Quality Control (QA/QC).** All sample containers and dishes were triple-rinsed with RO water before use and between samples. Where possible, different equipment was used for controls versus microplastic



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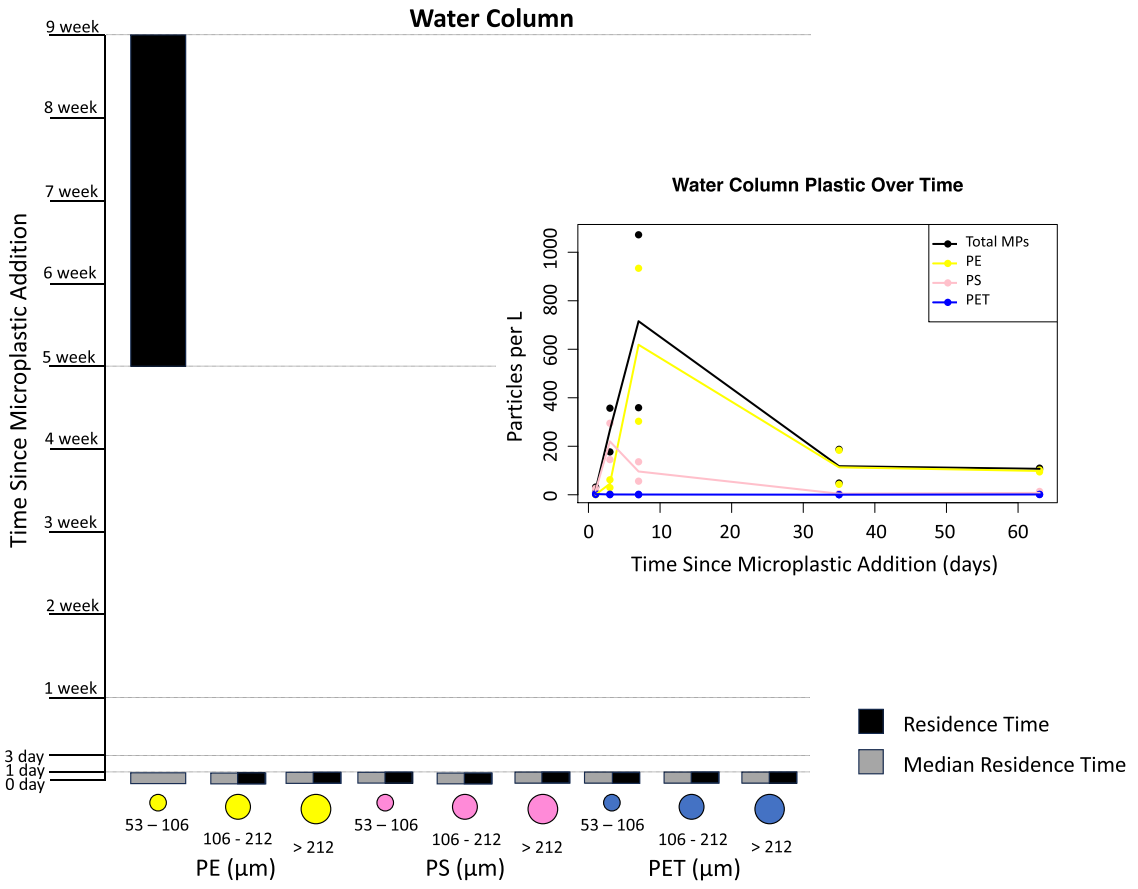


**Figure 3.** Residence times of microplastics in the water column (shown as time since the addition of microplastics on the *y* axis) are shown in the larger figure categorized by polymer and size fraction (along the *x*-axis). The median residence time (gray boxes) is the delay between when plastic is added and when 50% of the plastic added left the compartment. The residence time (black boxes) is the delay between when plastic is added and when 95% of the plastic added left the compartment. The resolution is defined by the sampling events (shown as horizontal dotted lines). The concentrations of microplastics in the water column are shown in the inset graph, which has the concentrations of microplastic (particles per liter) on the *y* axis and the time since the microplastic addition on the *x* axis (days). The points represent the raw data from each mesocosm, and thus there are two points per sampling event.

treatments in the field. In the laboratory, we wore white cotton laboratory coats, kept samples covered to protect them from dust as much as possible, and worked in a room with a HEPA filter. Samples collected from the control mesocosm helped track field and laboratory procedural contamination and the movement of particles among mesocosms due to wind and waves. Microplastics were present in the control mesocosms, but amounts were much lower than those of the plastic treatments. In general, the counts of microplastics collected from the control mesocosm were 1−3 orders of magnitude lower than from plastic-treated mesocosms. However, for zooplankton, the amounts were low and similar between the control and plastic treatment. This may be due to the relatively low amounts of microplastics in the water column limiting the pelagic zooplankton community's exposure to microplastics. For more details on the amounts and concentrations of microplastics in the control mesocosm, see the Supporting Text and Data. To measure recovery, we conducted spike and recovery tests for all of the methods used across matrices. We spiked water samples with known amounts of each polymer across the size fractions. To measure the recovery of sieving, we did a spike and recovery with one replicate (total recovery = 87%). For $H_2O_2$ digestions, recoveries ranged from 80 to 88% ($n$ = 3). For KOH digestions, recoveries ranged from 70 to 83% ($n$ = 3). See Table S4 for size- and polymer-specific recoveries. To measure the precision of our subsampling procedure, we tested triplicate subsamples and deemed the subsampling method acceptable when the coefficient of variation between triplicates was less than 25%. To measure the accuracy of our size fractionation, we measured 176 of the particles that were picked from three water samples. All researchers were trained via standard training protocols using the particles spiked into the mesocosms. As mentioned above, each researcher could only process samples if the spectroscopy on picked particles showed >70% accuracy. Final data presented here are not recovery-corrected. Only zooplankton samples were blank-corrected.

**Data Analysis.** To estimate the residence times in the surface water and water column, we assessed the rate at which 50% of the particles added to the mesocosms were lost from the compartment (median residence time) and the rate at which 95% of the particles were lost from the compartment (residence time[34]). We calculated residence times for each polymer and size fraction relative to how much was added. For PE, based on our measured size distributions of each polymer, we estimate that 87% of all particles added were >53 $\mu$m (i.e., the size distribution measured here), with 41% > 212 $\mu$m, 32% between 106 and 212 $\mu$m, and 14% between 53 and 106 $\mu$m. For PS, 95% of all particles added were >53 $\mu$m, with 32% > 212 $\mu$m, 38% between 106 and 212 $\mu$m, and 24% between 53


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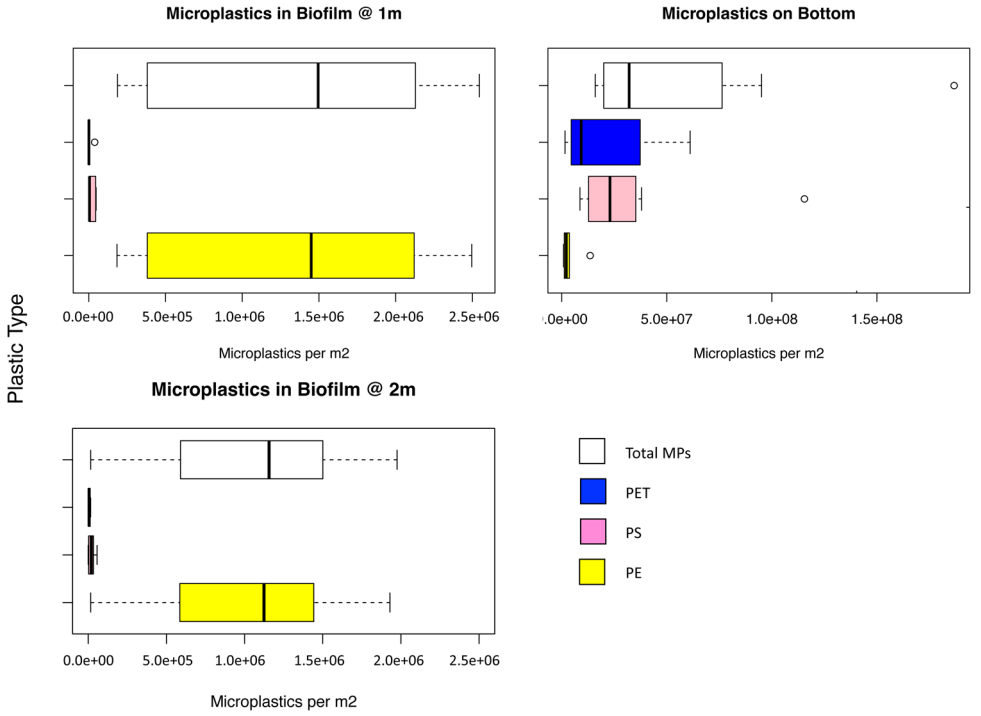


**Figure 4.** Microplastics in the biofilm within the 0−1m strip (top left), in the biofilm within the 1−2 m strip (bottom left) and on the bottom of the mesocosm (top right) in particle per $m^2$. Note the differences in the *x* axis scales between biofilm and bottom samples. Total particle amounts are depicted in white, PE is depicted in yellow, PS in pink, and PET in blue. The box plots show the median concentration as a middle line.

and 106 $\mu$m. For PET, 92% of all particles added were >53 $\mu$m, with 40% > 212 $\mu$m, 28% between 106 and 212 $\mu$m, and 23% between 53 and 106 $\mu$m. Because we sampled only at five time points, we did not fit our data to a model to estimate a more exact residence time. To estimate a mass balance, we scaled up our measured concentrations across the two replicate mesocosms to calculate the predicted total percent of microplastics within each compartment (for further details, see SI Text).

## RESULTS AND DISCUSSION

**Microplastic Fate and Transport in Water.** The concentration of microplastics added to the surface water of the mesocosms decreased over time (Figure 2). The residence time at the surface (i.e., delay between when microplastics were added and when 95% left the surface[34]) was between 5 and 9 weeks following the plastic addition (Figure 2). The visible surface slick decreased over time from an average of 38.4 $m^2$ (±0.7 StDev; 48% of the surface area of the mesocosm) 24 h after addition to an average of 1.6 $m^2$ (±0.8; 3% of the surface area of the mesocosm) 9 weeks after addition. The residence times on the surface varied by polymer density and particle size, decreasing with increasing density and size (Figures 2 and S19; Supporting Data). For example, the surface residence times were between 2 and 3 days for PET and 36 and 63 days for PE. For PE, the median residence times (i.e., delay between when microplastics were added and when 50% had left the surface[34]) were between 2 and 3 days for the larger size fractions (>106 $\mu$m) and between 4 and 7 days for the smaller (53−106 $\mu$m). Further work should assess how this varies with wind conditions, e.g., if the concentrations on the surface and just below become similar under windier conditions due to mixing.[35]

Microplastic concentrations in the water column increased over time during the first week, as plastics from the surface sank and then decreased to relatively stable concentrations by week 5 (Figure 3). At 24 h after addition, average concentrations in the water column were 21 (±16) particles/L. This increased to 267 (±128) particles/L at 72 h and 716 (±504) particles/L at 1 week. At 5 weeks and 9 weeks, concentrations were relatively similar at 118 (±98) particles/L and 108 (±4) particles/L, respectively. This pattern was generally driven by the smallest size fraction of PE as it was transported from the surface water over time (Figure 3). The median residence times of all polymers and size fractions in the water column were between 0 and 1 days. The resident time was also between 0 and 1 days, except for the 53−106 $\mu$m size fraction of PE, which was between 36 and 63 days. The persistence of microplastics in the water column varied with particle density and size, with the lighter and smallest particles staying in the water column for the longest period (Figure S20; Supporting Data), as predicted by various models and observations.[34,36]

The relatively large proportion of microplastics and relatively long residence times in the surface slick, compared to the water column, confirm our predictions[26] that sampling water from just below the surface (10 cm) is not always representative of the surface, where microplastics form a slick in the surface microlayer (i.e., air−water interface). This is consistent with other studies that have observed differences between quantities and characteristics of microplastics between surface trawls and near-surface water column samples.[37,38] This is an important consideration for monitoring programs, as the water column (including the water just below the surface) should be considered a different compartment than the surface, both of which are relevant to exposure in risk assessment.



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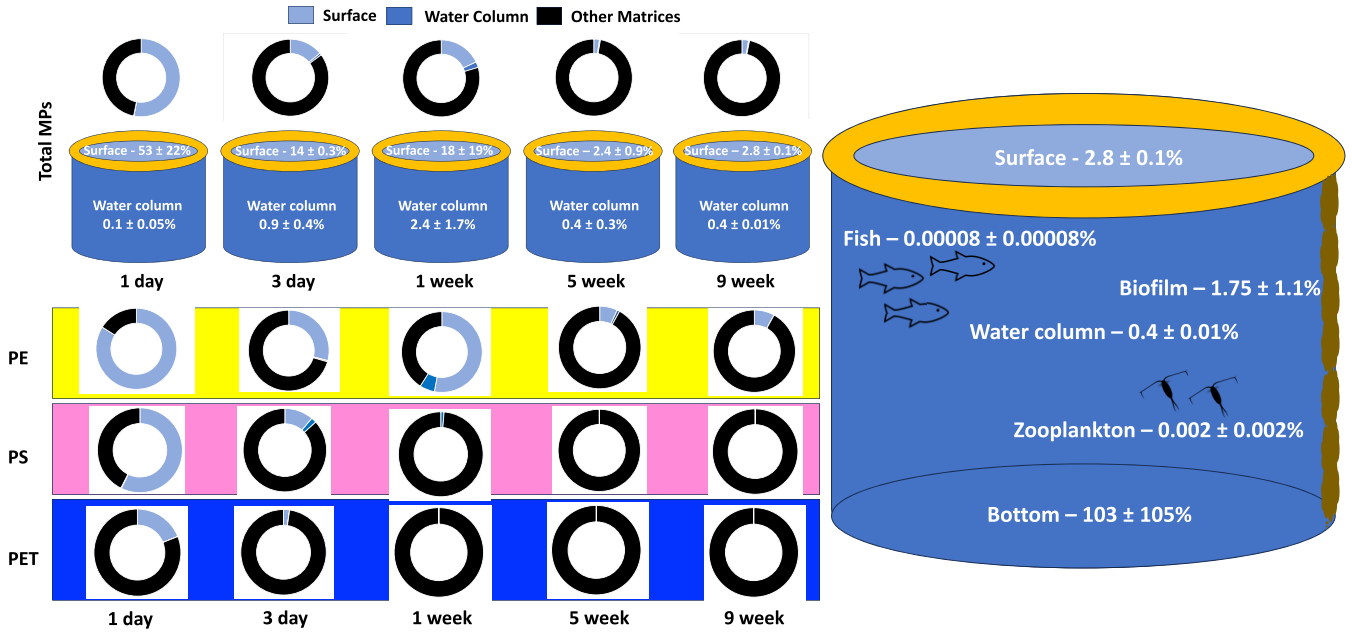


**Figure 5.** Left: (Top) Proportion of microplastics in the surface water and water column over time. The rings on the top show the proportion of all of the plastic added to the mesocosms within the surface waters (light blue), water column (dark blue), and other compartments (black). The depictions of the mesocosms show the percentages of the total amount of microplastics added in the surface waters and water column at each time point sampled. (Bottom) Proportion of each polymer in the surface water and water column over time. The rings show the proportion of each polymer added to the mesocosms within the surface waters (light blue), water column (dark blue), and other matrices (black). Right: Overall exposure landscape of total particles across the mesocosms measured after 9 weeks. Each percent is the average percent of microplastics in that compartment relative to the total amount added to the mesocosms. Numbers are average ± standard error across the two sampled mesocosms.

A similar mesocosm experiment, also conducted in a lake, used particles similar in size and density to our smallest size fraction of PS.[34] They found residence times in the water column of just under 1 day, nearly identical to what we observed here. If these patterns are general, concentrations in the water column would be expected to persist only above 0 with frequent inputs of microplastics to the system. As such, concentrations in the water may be pseudopersistent; i.e., the consistent presence reported in the water column worldwide is due to consistent inputs of microplastics to aquatic ecosystems. Perhaps most interestingly, for particles within this size range, if we turned off the tap (i.e., prevented further input), the amount in the water column would likely fall to near-zero rapidly. However, these patterns may vary for different morphologies (e.g., fibers, foam, film), polymer types, and particle sizes. Moreover, they may vary under different environmental conditions.

**Microplastic Fate in Other Compartments.** Almost all of the microplastics quantified within the biofilm on the walls were PE (98% on average; Figures 4 and S21), consistent with the surface water and water column. The concentrations of microplastics were greater in the top meter of the walls (average 1,372,000 per $m^2$ ± 200,818) than in the bottom meter (average 1,065,778 per $m^2$ ± 624,768). Most microplastic particles observed in the wall biofilm were within the >212 $\mu$m size fraction, and the least were in the 53−106 $\mu$m size fraction. If the biofilm on the walls is indicative of beaching (i.e., plastic washing up on shorelines), this suggests that larger buoyant particles are more likely to be deposited on beaches and smaller particles are more likely to be in the water column.[39]

In general, most microplastics sank to the bottom by the end of the experiment (Figure 4). This is consistent with global models suggesting microplastics eventually sink regardless of polymer density.[6] Visibly, we could see the PET particles sink immediately upon addition (see our previous study[26] for underwater video), followed by the PS and the PE, following a pattern with polymer density. Within the bottom samples collected, the majority of particles observed in our samples were PS and PET (58% and 36% on average, respectively) and average total concentrations ranged from 83,202,532 (±76,552,958) particles per $m^2$ to 31,670,886 (±18,776,665) particles per $m^2$. There was no clear pattern with particle size, at least not that could be observed at this final sampling point (Figure S22). Among samples within a mesocosm, we observed a coefficient of variation of 75%. This suggests that we may have some sampling error. This could be due to not having a large enough sample size (i.e., not sampling enough of the area of the mesocosm floor) to capture the variability (or heterogeneity of microplastics across the bottom) or due to our sampling methods themselves. Based on our visual observations during additions, the denser microplastics, i.e., PET, sank immediately, and thus the distribution on the bottom was not homogeneous. Moreover, according to Bloesch and Burns,[40] our "sediment traps" were too shallow to accurately capture sedimentation.

Microplastics were ingested by zooplankton and fish. Here, we report the average concentrations of microplastics per individual zooplankton and fish (within the GITs). More detailed assessments will follow in subsequent manuscripts. Zooplankton contained, on average, 0.04 particles (±0.04) per individual. All of these particles were PE, which was the most abundant polymer in the water column at the time of sampling. Fish contained, on average, 137 particles (±150) per individual. The majority of these particles were PE (59%),



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followed by PET (25%) and PS (16%), suggesting that fish were fed from all compartments of the mesocosm.

**The Exposure Landscape: Where Is All the Plastic?** At the end of the 9-week experiment, across the two replicate mesocosms, 2.9−3.2% of the plastics added remained at the surface, 0.39−0.41% were in the water column, 1.5−2.4% were in the biofilm on the walls, and 62−163% were on the bottom. The large range for the bottom compartment reflects the large variability of our bottom samples (as mentioned above). Better sampling methods are needed to better predict this compartment. Overall, <0.01% of the microplastics were in the fish and zooplankton. Here, we were better able to close a mass balance for the >53 $\mu$m particles (Figure 5) than in Rochman et al. (2024) where we did not sample from the surface water or bottom of the mesocosms.

Over time, the amount on the surface rapidly decreased as the amount in the water column increased until about the 5-week sampling point (Figure 5). One day after the additions, an average of 53 (±22)% of the microplastics were at the surface and an average of 0.1 (±0.05)% were in the water column—leaving just under half estimated to be in other matrices. At the 3- and 7-day sampling points, 14 (±0.3) and 18 (±19)% were estimated to be at the surface and an average of 0.9 (±0.4)% and 2.4 (1.7)% in the water column. By the 5-week sampling point, 2.4 (±0.9)% were estimated to be at the surface and 0.4 (±0.3)% in the water column (similar to our observation at 9 weeks).

Models predict that most microplastics sink to the bottom[6] and that the next largest reservoir may be the coastlines,[41] which may be represented by the biofilm on the walls. The greater residence time of microplastics on the surface water of our experimental unit compared to the water column suggests that particles are retained in the surface microlayer at relatively high concentrations. This pattern has been shown before in the ocean for microplastics[42] and is similar to other organic contaminants.[43] When monitoring microplastics, we should recognize the surface as a separate compartment from the water column. This compartment, also known as the neuston, is the surface layer of oceans, lakes, and rivers that concentrates nutrients and is frequented by many aquatic invertebrates that specialize in feeding from it.[44] We should also recognize the importance of sediment for quantifying the amount of microplastics in a system and especially for looking at trends over time (particularly in deposition zones). For risk assessment, all compartments are important, as organisms interact with them uniquely based on their life history strategies. With respect to toxicity testing, these dynamics are also important as microplastics are not expected to homogeneously mix in solution, and this will affect the actual exposure experienced by test organisms. In the environment, these dynamics are important to inform the design of monitoring and risk assessment programs.

However, our observations should be taken at face value as patterns relevant to transport and fate vary for different morphologies, types, and sizes of microplastics. Moreover, the fate likely varies when a whole water column is included, adding complexity via thermal layers and currents, instead of just the top 2 m. In addition, the surface slick of microplastics may not be the same when water is not encompassed in a mesocosm that shields some of the wind and may change the physical dynamics of the surface. Finally, our sampling methods varied across compartments, varying in the collection volume and time period. The different methods may have led to bias in our data and thus our interpretations. As such, future studies should experiment with different types of microplastics and under different-sized mesocosms and conduct observational experiments in nature to understand how the dynamics vary with different microplastic characteristics and under different environmental conditions. Prior to sampling, more effort should be made to determine the sampling volume needed to obtain an accurate representation of the fate of microplastics across compartments.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.5c01441.

Supporting text, tables, and figures with more details about methods, microplastics in the control mesocosm, water quality, microplastic characteristics, and polymer- and size-specific fate (PDF)

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### Notes

The authors declare no competing financial interest.

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