TEMPORAL AND HYDROLOGIC FACTORS INFLUENCING RIVERINE MICROPLASTIC CONCENTRATIONS

A Thesis

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by

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ABSTRACT

Microplastic pollution is increasingly studied in freshwater systems due to concerns over the ubiquity of the small plastic particles and their potential effects on aquatic organisms. Still unknown is how in-stream characteristics and temporal variabilities may affect observed riverine microplastic concentrations. In this thesis I investigate dams' influence on the spatial heterogeneity of microplastics, the affect that time of sampling (time of day and seasonal flow condition) has on the microplastic concentration measured, and whether upstream wastewater management strategies affect the patterns observed. I find that that dams act as a sink for microplastics, capturing significantly higher concentrations of microplastics in the reservoir behind them than is found in areas of settling upstream or downstream of them. My analysis also demonstrates that flow condition is a significant predictor of microplastic concentration at a given location while other measured parameters, including upstream wastewater management strategies, are not.

By indicating that flow condition is an important control on measured concentration, this research improves future studies' ability to interpret and properly apply the findings of existing riverine microplastic surveys. Additionally, by improving our understanding of how hydrologic conditions and the presence of in-stream barriers affect the transport of microplastics in rivers, this research helps to reduce uncertainty in models that attempt to quantify the magnitude of global microplastic pollution and the rate at which it grows.

BIOGRAPHICAL SKETCH

Lisa grew up in Atlanta, Georgia, where she explored neighborhood creeks at dawn with her best friend and a white dog. By age eight she had spent enough nights camping with her dad on Boy Scout (BSA) trips to earn the official troop t-shirt. Lisa's first experience with research came in the form of a large-scale conservation project during high school where she experimented with best removal techniques for *Ligustrum sinense* (Chinese privet) and went on to lead over forty volunteers in the removal of an acre the invasive, earning her the William T. Hornaday Award from the BSA. After graduating from The Galloway School, this experience leading and educating volunteers in conservation activities took her to Philmont Scout Ranch in New Mexico's Sangre de Cristo mountains where, for a summer, she led novice crews in trail-building and gained valuable experience teaching technical skills, keeping large groups safe in remote settings, and communicating expectations and constructive feedback.

Lisa earned a Bachelor of Science from Clemson University in environmental engineering, and continued on to Cornell University's Soil & Water Lab (SWL) in 2015 with the goal of connecting the traditional engineering solutions learned in undergrad with the natural system responses to similar disturbances and pollutants. To explore the avenues her degrees could lead her, she has spent her summers studying at the University of Cambridge, interning at the U.S. Environmental Protection Agency's Region 8 Headquarters, working on an Extension Service research team studying Hunnicutt Creek in Clemson, South Carolina, and interning with Environmental Defense Fund.

While working toward her master's degree, Lisa trained and mentored 13 undergraduate researchers, several of whom produced work that will be shared in peer-reviewed publications. These students participated in research related to Lisa's involvement in three main projects: the

multiple studies she initiated while spearheading the SWL microplastics team (including the two studies that make up this thesis), a water quality assessment for New York State's Trees for Tribs program, and modeling efforts for culvert flood risk that served county governments in the Hudson River Estuary. Lisa also planned and led a number of water-quality and engineering outreach programs for New York middle and high school students and looks to continue bringing science out of the laboratory in her future professional roles.

TO THE PEOPLE WHO GREW ME IN THE PLACES THAT TAUGHT ME THE MOST

Ten & Plum, Lansbury at Franklin, Shining Rock, the Sunset Meadow

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CHAPTER 1

INTRODUCTION

Background

Pollution by plastics is an emerging problem in waterways across the globe. With an estimated 80% of marine plastic particles originating from terrestrial sources, studies have increasingly begun to study plastic concentrations in freshwater systems, as attempts are made to quantify the scale of this global pollution problem and the rate at which it grows (Andrady, 2011). Of highest concern are particles known as "microplastics", the size fraction of plastics less than five millimeters in diameter. Microplastic particles begin as a variety of products, from small nurdles (small plastic pellets) released from manufacturing to larger plastic items that break down over time to an easily ingested size, making them of concern to the integrity of aquatic ecosystems.

Hydrophobic contaminants such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) have been found to readily adsorb to plastic particles, which also act as transport mechanisms for microbial communities from within wastewater treatment plants to receiving waters (McCormick et al., 2014; Rochman, 2015; Rochman et al., 2013; Teuten et al., 2009). A number of studies have indicated that a wide variety of aquatic organisms from fish to invertebrates ingest microplastics, increasing the risk of physical harm to the organism through false satiation, starvation, or choking, as well as potentially introducing sorbed contaminants into the food chain (Critchell and Hoogenboom, 2018; Foley et al., 2018; Setälä et al., 2014; Steer et al., 2017; Vandermeersch et al., 2015). How great of a health risk the

ingestion of these plastics poses is still unknown (Jovanović et al., 2018; Koelmans et al., 2017).

Motivation

Most research quantifying the amount and the impact of microplastic pollution has focused thus far on marine environments (Cole et al., 2011), where microplastic concentrations amount to 2 to 128 times that of plankton present, indicating ocean plastic concentrations are at levels of particular ecological relevance in these areas (Moore et al., 2002). Much of this marine plastic is assumed to be sourced from rivers, with estimates of annual loads of river-sourced plastics entering the ocean ranging from 0.1 to 2 million tons of plastic per year (Schmidt et al., 2017).

Part of the uncertainty in annual load estimates comes from the large uncertainty associated with freshwater transport of microplastics, of particular importance for systems as dynamic and heterogeneous in time and space as rivers. The models used for these load estimates combine published microplastic concentration values and available hydrologic data, despite the fact that little information exists connecting microplastic transport behaviors with hydrologic condition. Existing microplastics research focuses predominantly on explaining observed spatial heterogeneity of riverine microplastic concentrations using catchment-level characteristics. How microplastic concentrations change across time due to varying inputs and hydrologic conditions, as well as across space due to in-stream disruptions to flow are largely still unknown.

These questions are important to answer for a few reasons. The first is to improve future studies' ability to compare results across existing work. Currently, due to inconsistencies between reported units and methodologies, combining or contrasting

results from various studies is not often possible (Horton et al., 2017b). The majority of existing studies fail to report the flow conditions or time when sampling occurs, but whether the conditions or timing changes the concentrations measured and is important for inclusion in future reports is still unknown.

The second reason is that efforts are being made to quantify the scale and rate of growth of microplastic pollution on a global scale. To do so, concentrations from published studies are combined with hydrologic flow measurements and assumptions are made about how those concentrations from point measurements upstream may be translated into loads entering the oceans. One such assumption is how the presence of dams may affect riverine transport of microplastics (Lebreton et al., 2017). Do microplastics accumulate in the sediment behind dams like many other pollutants including metals, pesticides, and PCBs tend to? Similarly, for these global load estimates, understanding how seasonal difference in flow affect microplastic concentrations enables tighter relationships to be built between hydrologic conditions and microplastic concentrations.

Summary

Through the work shared in this thesis, I attempt to fill two gaps in understanding: 1) how in-stream barriers may be contributing to spatial heterogeneity of riverine microplastic concentrations and 2) how the timing of sample collection, in terms of time of day and flow condition, may affect the concentration measured at a given point in a river, as well as how this relationship may change based on wastewater management strategy being used in upstream.

We find that the presence of dams influences microplastic concentration longitudinally: higher plastic concentrations were found in the sediments and surface water of dams' reservoirs than were found downstream of them. This influence did not extend to influent microplastic concentrations for dams downstream, however, as the lateral contributions of plastics between dams appears to outweigh the local reductions measured.

Additionally, the time of sampling study shows that the flow condition at which samples are taken in a given location does affect the microplastic concentration measured, with highest concentrations measured at lower flow. Factors such as upstream wastewater management strategy and time of day sampled did not significantly influence concentration measured.

Together, these findings aid in the interpretation and informed use of existing research, which often reports riverine microplastic concentration measurements without context of flow condition, river features and flow impediments in the vicinity of sampling, or sample timing.

CHAPTER 2

THE EFFECT OF DAMS ON MICROPLASTIC TRANSPORT IN STREAM ENVIRONMENTS

Introduction

With over 90,500 dams in the United States alone, the impact of these flow impediments is an important consideration for understanding how pollutants are transported in rivers (U.S. Army Corps of Engineers, 2016). Based on the behaviors of pollutants such as metals and PCBs, which are currently known to accumulate with trapped sediments behind dams, it is relevant to consider whether emerging contaminants of concern, namely microplastics, may also accumulate in the sediment of impoundments (Brune, 1953).

An estimated 80% of marine microplastic pollution originates from terrestrial sources (Andrady, 2011). Along with direct inputs from coastal lands, transport along rivers is an important pathway from human-derived plastic pollution sources to the oceans, with an estimated 1.15-2.41 million tonnes of plastic being transported to the ocean by rivers annually (Lebreton et al., 2017). The magnitude of this input is supported by the high concentrations of microplastics found in estuarine environments (Browne et al., 2010; Mathalon and Hill, 2014; Yonkos et al., 2014).

Although specific sources and sinks of microplastics are largely still unidentified, evidence suggests that microplastic concentrations in rivers vary spatially (A. R. McCormick et al., 2016). Nearby landuse is an oft mentioned source of spatial variability, with higher concentrations of plastics being found in watersheds with high

urban landuse percentage, as well as closer proximity to urban centers being highly correlated with higher surface water microplastic concentrations (Baldwin et al., 2016; Mani et al., 2015). In-stream barriers, however, have not yet been adequately investigated as additional sources of spatial variability, though they have been mentioned as likely sinks for light plastic particles (Lebreton et al., 2017; Mani et al., 2015).

A handful of existing longitudinal surveys published on microplastic concentrations along rivers containing weirs or dams point to the possibility that some microplastic settling may be occurring within impoundments (Zhang et al., 2015, 2017). Settling is likely facilitated by decreased buoyancy due to biofilm accumulation on plastics' surfaces, as evidenced in wastewater treatment plants (Carr et al., 2016) and experimentation (Kaiser et al., 2017; Lobelle and Cunliffe, 2011; Morét-Ferguson et al., 2010). The presence of microplastics in sediments worldwide supports the idea of settling as one fate for microplastics, but the presence of microplastics in sediment found behind dams has not yet been directly investigated (Castañeda et al., 2014; Horton et al., 2017a; Klein et al., 2015; Zhang et al., 2017).

In this study we examine microplastic concentrations in both the surface waters and sediments located upstream of, downstream of, and within dammed impoundments to determine how the presence of dams may influence microplastic concentrations, both locally at individual dams and at a system-scale. We test the hypothesis that dams effectively reduce the microplastic concentrations transported along rivers by trapping plastics in sediments within reservoirs.

Methods

Site Selection

Six dams were chosen for this study: two located in series on Fall Creek and four along Six Mile Creek near Ithaca, New York. The two streams were selected for their similar watershed composition, with particular emphasis on percent urban landuse (Table 2.1) and close proximity, with all dam sites located within a 5 kilometer radius, as shown in Figure 2.1. The dams ranged in height from 1.5 to 18 meters. For each dam, three sampling locations were selected: A) upstream of the reservoir, within the thalweg of the flowing stream; B) centrally within the reservoir, near the downstream end of the impoundment; and C) downstream of the dam, in the thalweg of the stream leaving the plunge pool. Exact location of sampling sites was dictated by accessibility constraints and the presence of sediment deposits.

Table 2.1. Watershed characteristics of the two streams sampled for this study.

Watershed characteristics[a]	Six Mile Creek	Fall Creek
Watershed area (km^2)	127	325
Developed[b] (%)	5	6
Agricultural[b] (%)	22	46
Forested[b] (%)	70	40
Wastewater Treatment Plant	No	Yes

[a]Calculated for most downstream point of sampling on each stream
[b]Calculated from USGS National Land Cover Database, 2011

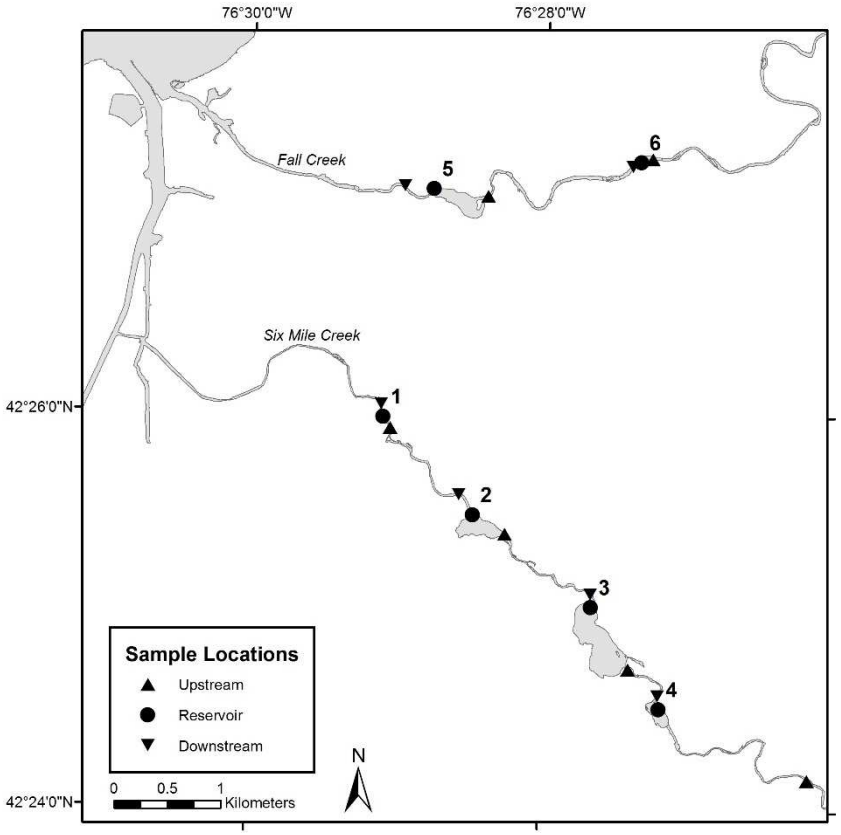

Figure 2.1. Map of sampled locations at each of 6 studied dams. The upstream sampling point at Dam 4 is located a farther distance from the reservoir due to the presence of a police training facility that constrained accessibility.

Sample Collection

A total of 18 paired sediment and surface water grab samples were collected at areas of deposition, one collected at each of the three sampling locations for each dam. Surface water grab samples were collected in two rinsed 1L plastic bottles, which were aggregated during lab processing. Sediment samples were collected with a plastic scoop, with a conduit attached for samples in deeper water (Simmons, 2014). Samples were then transferred into glass jars, where excess water was decanted. Each sediment sample was approximately 400g, wet weight.

Laboratory Processing

Samples were processed in the lab following NOAA wet peroxide oxidation methods for water samples and for bed samples, accordingly (Masura et al., 2015). Water samples were first wet sieved through a #4 sieve with 4.76mm openings and onto 0.335mm mesh to improve comparison ability with the majority of existing literature, which focuses on this larger size fraction. Processed samples were vacuum filtered onto 0.45µm gridded filter paper for safe storage.

Laboratory contamination was minimized by rinsing beakers multiple times with distilled water before use and keeping samples covered. White cotton lab coats and nitrile gloves were worn at all times when handling and transferring samples in an effort to reduce and standardize any contamination coming from processing. To measure any contamination being introduced from laboratory air, three filter papers were left exposed for 24 hours near the locations used for filtration, oxidation processing steps, and counting.

Identification and Counting

Microplastics were visually identified using a dissecting microscope, with standards based on the Marine & Environmental Research Institute's Guide to Microplastic Identification (MERI, 2012) and an additional hardness test performed to exclude any particles brittle under forceps' pressure (Klein et al., 2018). Questionable particles were assumed to be non-plastic. Color and plastic category were noted for each counted particle, as described in Table 2.2. Because studies have indicated that visual microplastic counts may be overestimated by 20-70% due to misidentification, this

study was designed such that findings rely on the relationship between measurements instead of the stand-alone count from individual samples (Ivleva et al., 2017).

Table 2.2 Categories of microplastics used to describe particles identified in samples.

Category	Description
Fiber	*Thin, equal-thickness, thread-like*
Fragment	*Irregular shape, broken-down from larger plastic debris*
Film	*Flat, thin sheet*
Foam	*Sponge-like texture and appearance typical of polystyrene*
Bead	*Round, often colorful pellet*

Statistical Analysis

A total of 18 samples was used for both water and sediment analyses, 6 from Fall Creek, 12 from Six Mile Creek. Location relative to dam position and river were used in a two-way analysis of variance to predict microplastic concentration in sediments first and then a second time for microplastic concentrations in surface water. Dams in each river were treated as replicates. To determine differences in sample composition between water and sediment samples, a student's t-test was run between percent fiber from the total microplastics counted in all water and all sediment samples.

Results and Discussion

Location

Sediment microplastic concentrations varied based on location relative to the dam (Table 2.3). Concentrations measured within the reservoir were significantly greater than those found in sediments either upstream or downstream of the reservoir (Figure 2.2). Although upstream and downstream measurement differences were not statistically significant, for most of the dams, upstream concentrations were higher than downstream concentrations. The exceptions to this were dams 3 and 4 where

downstream concentration was measured to be marginally higher than upstream (Appendix A).

This is a first study to include measurements downstream of an impoundment in comparisons, but similar previous studies do report findings consistent with upstream and reservoir relationships (Mani et al., 2015; Zhang et al., 2015). The downstream measurements included in this analysis provide a clear signal of microplastics accumulating in the sediment behind dams. The mechanism for this accumulation is likely similar to that of sediment, which also tends to settle in the long residence times and slower velocities found in reservoirs.

Table 2.3. Summary of coefficients for the best fit simple linear regression fit to sediment microplastic concentration. Asterisk (*) indicates statistically significant result. Adjusted R-squared value for this regression model is 0.59.

Parameter	Estimate	Standard Error	t value	p-value
Intercept	89.02	20.96	4.25	0.0008*
Location, reservoir	76.61	22.96	3.34	0.005*
Location, downstream	-24.36	22.96	-1.06	0.31
River, Six Mile Creek	-50.96	19.88	-2.56	0.02*

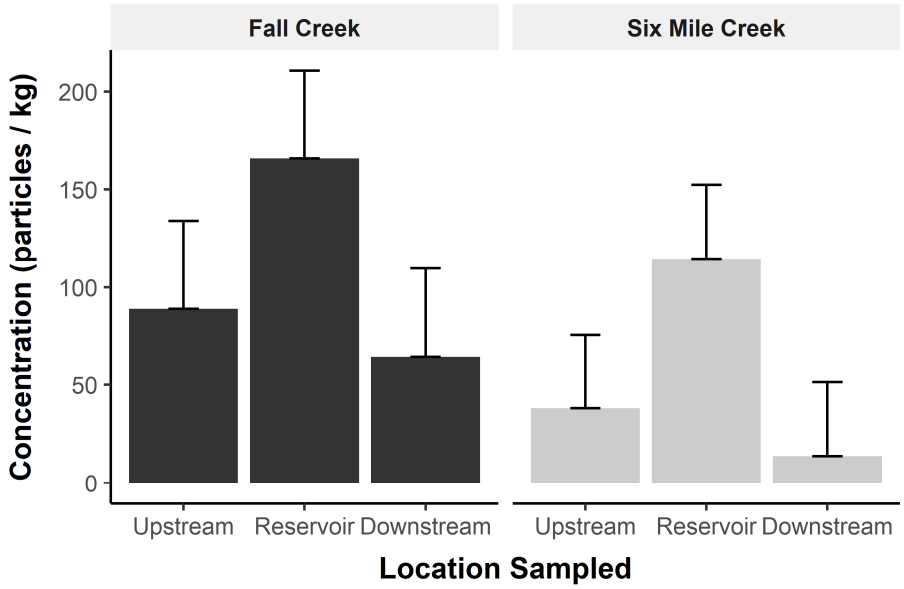

Figure 2.2. Sediment microplastic concentration estimates from a two-way analysis of variance. Fall Creek concentrations represent a mean of two samples, while Six Mile Creek concentrations are a mean of four samples. Error bars represent 95% confidence intervals.

In surface waters, opposite to what is observed in sediments, reservoir concentrations are the lowest of the three locations (Figure 2.3), on average 5.5 particles/L lower than upstream concentrations and 2.25 particles/L lower than downstream samples (Table 2.4). This difference, strengthened further when combined with the high concentration of microplastics in reservoir sediment, is evidence of microplastics settling out of the water column and into the sediment in the slower moving waters of the impoundment.

Table 2.4. Summary of coefficients for the best fit simple linear regression fit to surface water microplastic concentration. Asterisk (*) indicates statistically significant result. Adjusted R-squared value for this regression model is 0.69.

Parameter	Estimate	Standard Error	t value	p-value
Intercept	18.59	1.93	9.62	1.5×10^{-7}*
Location, reservoir	-5.5	2.12	-2.60	0.02*
Location, downstream	-2.75	2.12	-1.30	0.22
River, Six Mile Creek	-10.75	1.83	-5.87	0.000041*

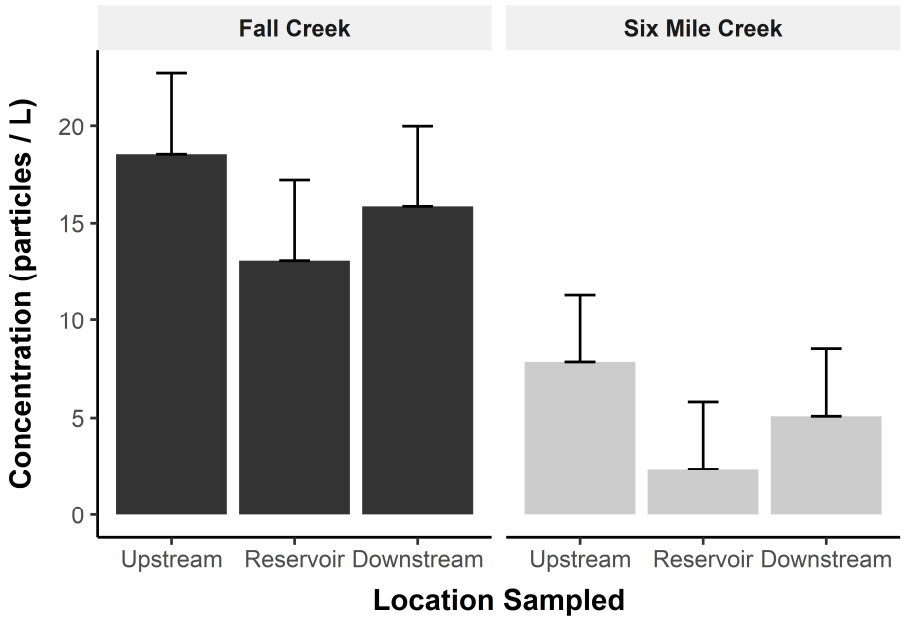

Figure 2.3. Surface water microplastic concentration estimates from a two-way analysis of variance. Fall Creek concentrations represent a mean of two samples, while Six Mile Creek concentrations are a mean of four samples. Error bars represent 95% confidence intervals.

Concentration trends between dams were more variable in water samples than in sediment samples, but a similar pattern still emerged: like sediment, both river and location of sampling were significant predictors of concentration. Some of the variability between dams may be due to the heterogeneity of microplastic concentration along rivers, as observed in a number of different studies which attribute the variability to a variety of hydrologic dynamics as well as human interaction factors (e.g. Dris et al.,

2015; Mani et al., 2015). An initial exploration, however, including dam as a predictor in a three-way analysis of variance along with location and river revealed no additional information over a two-way analysis of variance using only location and river.

With such spatial variability in microplastic concentrations of surface water samples, it is possible that the single time point observation of these dam locations missed longer temporal patterns. Investigating whether repeated sampling over longer timescales may reveal any additional relationships between upstream and downstream concentrations would be an interesting focus for future studies.

Sediment microplastic concentrations were an order of magnitude higher than those found in water samples, which provides evidence of plastic accumulation in the settled fraction. This ratio is about two times greater than differences in concentration reported for paired sediment and water samples from Three Gorges Reservoir (Zhang et al., 2017), but around two orders of magnitude smaller than the difference found in samples from the Ottawa River (Vermaire et al., 2017).

Concentration varied more between rivers than within them, with Fall Creek samples having consistently higher concentrations of microplastics for both sediment and surface water samples. One explanation for the measurable differences in concentration between rivers may be differences in overall microplastic sources to the stream. While sources of microplastics to river systems are still largely unknown, one identified point source is wastewater treatment plant discharge pipes, one of which is located upstream from all dams on Fall Creek, while Six Mile Creek dams are in a watershed void of known point sources of microplastics (Table 2.1). However, we

conducted a more detailed study to test this discrepancy and were not able to discern statistical differences (see: Chapter 3).

One interesting observation from this study is that there is no clear watershed-scale trend in microplastics concentration in either the sediments or water along a series of dams (Appendix A). If we consider upstream concentrations to represent the cumulative impact of the upstream sinks (in this case, dams), they generally increase or remain the same from upstream-to-downstream sites in both the sediments and stream water. This suggests that the longitudinal loading of microplastics overwhelms the potential function of dams as microplastic sinks.

Sample Composition

Separating plastics by morphological category, for both water samples and sediment samples, fibers were the most abundant (Figure 2.5). The majority of fibers were translucent and colorless. Similar to Rodrigues et al. (2018), the other colors most commonly found were black, red, and blue. These fiber colors were consistent between water and sediment samples, as Vermaire et al. (2017) found, suggesting that fibers in the sediment likely settled from the water column. The percentage of fibers, however, in the water sample was significantly higher than that in the sediment samples ($p = 0.02$). This difference was made up for by the higher proportion of fragments found in sediment samples than in water samples.

Existing research points to the possibility that the surface of fragments allows for greater biofilm growth and faster settling. As Kerr and Cowling (2003) found for a number of materials, with increased surface roughness, increased settling speed is observed. Compared to beads or fibers, which have at least one dimension of their

exposed surfaces intentionally manufactured smooth, fragments, having broken down to their current shape, likely have greater surface roughness. This roughness may also improve their ability as a host of microbial biofilms. Much of the literature on settling behaviors focuses on polymer type and density, and how various plastic categories may differentially settle is largely unknown.

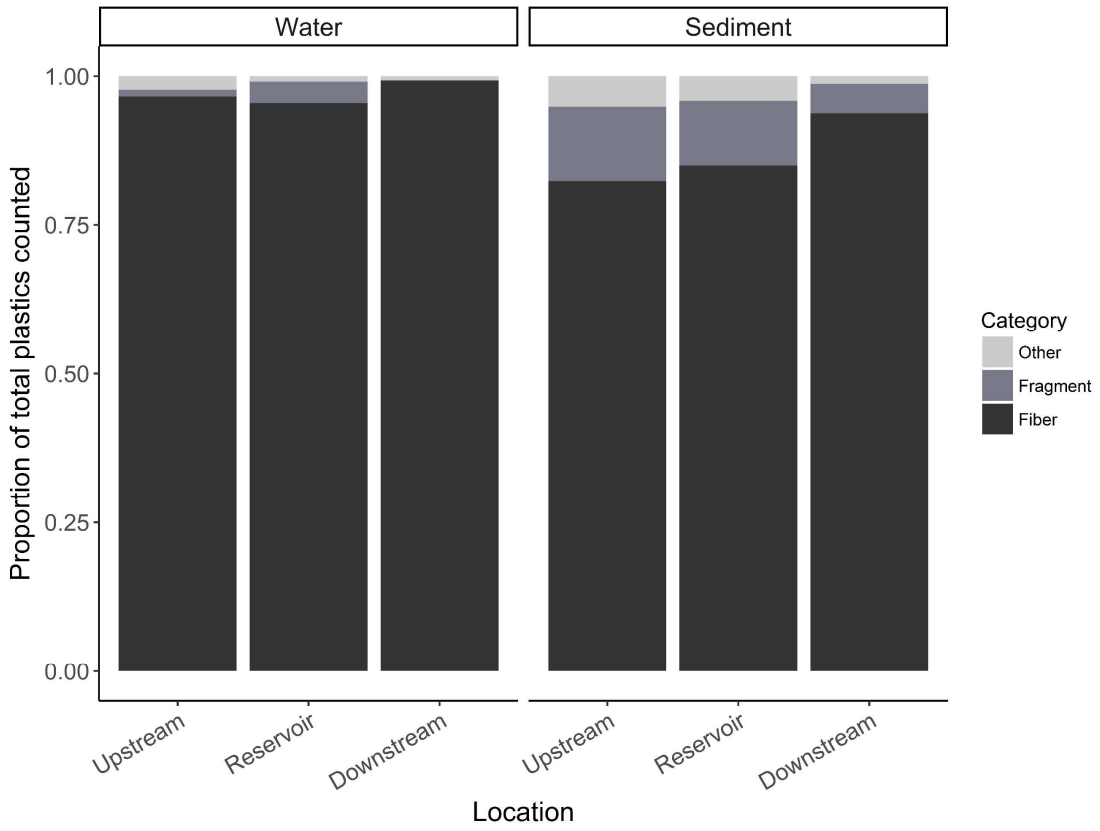

Figure 2.5. Categories of plastics found, plotted as proportions of the total plastics counted in water and sediment samples collected at upstream, reservoir and downstream sampling locations. Colors represent categories found: fiber (black), fragment (dark grey), and all other particles, which includes films, foams, and beads (light grey).

Interestingly, there is not only a difference in the average proportion of each plastic type, but in sediment samples there is also a noticeably greater variation in the proportion of plastic categories than there is in water samples (Figure 2.5). Upstream

and reservoir sediment samples, in particular, have a greater share of non-fiber plastics than any of the water samples. The diversity of plastics in sediment as compared to water may point to the length of time that it takes particles to accumulate in the sediment. With such a small fraction of the surface water sample being fragments, it likely takes long periods of time for fragments to accumulate in the large quantities measured in sediment samples. This indicates that plastics measured in sediments represent a greater span of time than do the surface grab samples of the water column. Downstream sediment composition appears most similar to reservoir water plastic composition.

Contamination

Laboratory blanks collected an average of 6.7 +/- 2.3 microplastics. In practice, no sample was left uncovered for more than 1-2 hours total during processing and counting, but as a conservative measure, this average value was subtracted from all sample counts for all analysis and reporting.

Conclusions

This study provides evidence that microplastics accumulate in the sediment behind dams. For both surface water and sediment samples, samples in the reservoir were significantly different than those in moving water. For water samples, microplastic reservoir concentrations were lower. For sediment samples reservoir concentrations were higher. This connection indicates that settling of microplastics from the surface waters is happening within the slower water of the reservoir. Additionally, the distribution of plastic categories did vary based on sampling location, all sediment and water samples were dominated by fiber particles.

As more effort is made to quantify annual loads to lakes and oceans by river transport, this study underscores the importance of including the presence of dams in quantification models and hints at the influence of dams and weirs as mechanisms for plastic retention in the watershed.

CHAPTER 3

IS IT TREATMENT, TIME, OR FLOW? INVESTIGATING THE FACTORS THAT INFLUENCE MICROPLASTIC CONCENTRATION IN FRESHWATER SYSTEMS

Introduction

Existing microplastics research focuses predominantly on understanding the effects of the spatial heterogeneity of rivers but leaves questions of how temporal variability affects microplastics concentrations unanswered. Lebreton et al. (2017) found that three quarters of the global river-sourced annual microplastic load enters oceans between May and October, a period of less than half of the year, indicating a need for increased study of this seasonal-scale temporal variability. Focusing on short timescales, Dris et al. (2018) found that the measured river microplastic concentration in quick, one-minute river samples can have a coefficient of variability as high as 45%. Together these studies support further investigation on how concentrations fluctuate over different timescales and what mechanisms lead to this variation.

Previous research hints at hydrologic fluxes being a driving mechanism for microplastic origination and transport at the catchment scale. Recent work supports a conceptual model for in-stream microplastic concentrations to be introduced to surface water systems with runoff. Directly following rainstorms, microplastic concentrations were found to be positively correlated with increased stream discharge (Moore et al., 2011; Yonkos et al., 2014; Faure et al., 2015; Baldwin et al., 2016). Research supporting this finding includes studies of watersheds of widely varying size (10^1 - 10^4 km^2),

human population density (10^1 - 10^4 people/km^2) and landuse composition. All found positive correlation, to some extent, between microplastic concentration and degree of urbanization. Dris et al. (2015) considered the relatively large Seine River watershed above Paris, France (area approx. 32,000 km^2, population density ~230 people/km^2; Garcia-Armisen and Servais, 2007), and found that in the absence of surface runoff, microplastic concentrations were negatively correlated with discharge at some sites (and at another, not statistically significant). This negative correlation points to the presence of some season- and flow-independent sources of microplastics and potentially, a consistent point source emitter.

Commonly, wastewater treatment plants are identified as a leading point source of microplastics and the default explanation for localized increases in microplastic concentrations (e.g. Mani et al., 2015; Ziajahromi et al., 2016). Few other points sources have been identified and those that have are less abundant in the landscape, such as regulated discharges from industrial plants (Lechner and Ramler, 2015). While only a few isolated studies provide evidence of microplastic concentrations varying temporally in wastewater (Mason et al., 2016; Warrack et al., 2018), other contaminants sourced from wastewater treatment plants, such as pharmaceuticals, regularly exhibit diurnal signaling, following behavior patterns of human consumption (Browne et al., 2011; Nelson et al., 2011). These human use and treatment plant discharge patterns support the idea that treatment plants may also be relevant to a study of microplastic temporal trends.

A number of studies have shown that wastewater treatment plants are effective at removing large percentages of microplastics from wastewater, often reported above

90% removal, but indicate that the amount remaining in the effluent still amounts to a large number of microplastic particles (Dris et al., 2015; Talvitie et al., 2015; Murphy et al., 2016). As an example, Murphy et al. (2016) report that a wastewater treatment plant located in Glasgow, Scotland successfully removed 98.4% of influent microplastics, but the small fraction remaining in the effluent amounted to considerable daily load after accounting for the large volumes of water being processed by the plant. In some cases, this large output appears to be substantial even compared to background conditions for many rivers, with increased microplastic concentrations commonly being detected downstream of wastewater treatment plants as compared to upstream measurements (McCormick et al., 2014; Estahbanati and Fahrenfeld, 2016).

For this study, the main objective was to determine whether certain times of sampling are more representative in terms of stream microplastic concentrations than others and how daily-temporal trends compare between streams with different wastewater treatment strategies. To address this goal, we investigated whether microplastic concentrations varied over the course of two 24-hour periods, representing high and low flow conditions, on streams where wastewater is treated primarily with a wastewater treatment plant and where it is treated solely with septic systems. We hypothesized that concentrations would vary over the sampling time period and between high and low flows. Further, we hypothesized that during baseflow conditions, if wastewater treatment plants were a leading source of microplastics to the system, a diurnal signal would be most evident in a stream with wastewater treatment plant effluent contributions, but would not be detectable in a stream with only septic systems in its watershed.

Methods

We broadly aimed to determine if microplastic concentrations vary temporally within a diurnal cycle and across two flow regimes. Second, we wanted to determine the effect of wastewater treatment strategies (centralized wastewater treatment vs decentralized treatment via septic systems) on the magnitude and temporal variability of in-stream microplastic concentrations through field observations.

Site Description

Microplastics were sampled on two tributaries to Cayuga Lake: Six Mile Creek and Fall Creek (NY USA). The sampling locations were selected to have similar watershed contributing areas, land use, topography, vegetation, soils, population densities, and contrasting wastewater management strategies (Table 3.1). Six Mile Creek watershed is characterized by septic system waste management, while Fall Creek is served primarily by a centralized wastewater treatment plant. Sampling locations in Fall Creek and Six Mile Creek were located 11 kilometers from one another. Twenty-four-hour sampling was carried out in both streams during August 2016 (low flow period) and April 2017 (high flow period).

The primarily wastewater treatment plant discharging 2.8 kilometers upstream of the Fall Creek site operates as a sequencing batch reactor, with a standard on-off discharge cycle year-round, averaging 0.5 million gallons per day and serving around ~2,500 people. A second, smaller wastewater treatment plant (0.1 million gallons per day, ~700 people), consisting of two aeration lagoons, also discharges through the same effluent pipe (Rahm et al., 2016). Time-specific data for the discharge cycle of the sequencing batch reactor was made available from the plant only for the corresponding

sampling event in April but was reported anecdotally to run at similar time intervals year-round and therefore was assumed to be relatively similar in timing during August sampling efforts.

Table 3.1. Watershed characteristics of the two streams sampled for this study.

Watershed characteristics	Six Mile Creek	Fall Creek
Population[a]	4,900	14,650
Watershed area (km^2)	105	280
Population density (people/km^2)	47	52
Urban[b] (%)	3	5
Agricultural[b] (%)	24	49
Forested[b] (%)	69	38
Wastewater management strategy	Septic systems	Treatment plant

[a]Calculated by census block from the 2010 US Census
[b]Calculated from USGS National Land Cover Database 2011

Field Data Collection

Samples were collected at a designated location within the thalweg of each stream using a Sea-Gear neuston net with 335 micron mesh, as is used in many other surface water microplastic studies (e.g. Free et al., 2014; Baldwin et al., 2016; Eriksen et al., 2017), with a 1 x 0.5 meter rectangular opening (Sea-Gear Corp., Miami, FL, USA). A single location within each stream was marked with rebar to maintain a consistent sampling reference location. The net was deployed at the designated location for ten minutes every three hours, over two mid-week, 24-hour periods: August 24-25, 2016 and April 26-27, 2017.

The net opening was never fully submerged to ensure floating plastics were collected, and in order to avoid including bedload in the samples, space was left between the bottom of the net and the stream bed. To calculate volume of water sampled, the depth of water entering the net was multiplied by the average velocity at the mouth of

the net, as measured at the beginning and end of each sample collection. Stream discharge throughout the sampling period was recorded by the USGS gauge located at the Six Mile Creek site and measured by the velocity-area wading method (Herschy, 1985) at the Fall Creek site, which was then correlated to a USGS gauge located 12 kilometers downstream (USGS). For transport back to the laboratory, samples were rinsed from the net into the cod end in the field using a pressurized sprayer and stored in glass jars.

Laboratory Processing

NOAA wet peroxide oxidation with density separation methods were used to digest labile organics and separate dense non-plastic particles from floating plastic particles (Masura, et al. 2015). Modifications were made to the wet sieving process, with a metal 4.6mm sieve as the upper size filter and a synthetic 0.3mm mesh section of the netting material used as the lower size fraction to more accurately match the lower size bound collected during field sampling. Following density separation, samples were filtered onto gridded 0.45µm filters, which were placed in small petri dishes for easier visual inspection and safe storage.

The entire contents of each digested-separated sample were counted visually with a dissecting microscope. To standardize particle identification between counts, the Marine & Environmental Research Institute's visual "Guide to Microplastic Identification" was used (2015), with an additional hardness test performed on questionable samples to check for particles' ability to withstand forceps' pressure (Klein et al., 2018). The color and particle category were noted for each identified microplastic, as described in Table 2.2.

Due to a tendency for visual inspection to systematically overestimate microplastic counts, this study was designed such that analyses rely on relative values and not the absolute concentrations (Hidalgo-Ruz et al., 2012; Eriksen et al., 2013). For better consistency between samples, all samples were counted by a single researcher. A second researcher also performed counts to ensure differences were systematic and not a sign of introduced variability.

Contamination Reduction

To reduce and standardize any introduced microplastic contamination, blue nitrile gloves and white cotton lab coats were worn at all times when handling samples. Samples were kept covered with aluminum tins while in the lab. Deionized water was used to clean all sieves and containers before introducing the sample.

A series of five blanks were run through the lab to measure contamination that may have been introduced during processing. To measure for contamination from laboratory air, three filter papers were left uncovered for 24 hours in areas of the lab where samples were exposed during processing in order to sieve, add reagents, or count. Procedural contamination was tested by processing three deionized water blanks through all materials and methods of the laboratory protocol. All blanks were run after all samples had been processed.

Statistical Methods

For April counts, nine samples from Fall Creek and nine from Six Mile Creek were included in the analysis, but due to sample losses in the lab for August counts only

eight and seven samples were included from Fall Creek and Six Mile Creek, respectively.

The experimental variables used in this study were time of day, hydrologic conditions, and stream. The following linear regression was fit to the data, with stream correlating to wastewater management strategy:

$$Concentration = \beta_0 + \beta_1 TimeOfDay + \beta_2 FlowCondition + \beta_3 Stream$$

To better match the prediction that time of day may influence concentration in a sinusoidal fashion, time of day was coded as a normalized, cyclical value ranging from zero (at 0:00 and 24:00) to one (at noon).

Statistical significance of trends was determined against a null hypothesis where neither stream, hydrologic condition, nor time of day influenced concentration, with $\alpha = 0.05$. As a check of whether concentration was affected by different timing of high and low points for each stream, a second model was run that included an interaction term between stream and time of day.

As a secondary check on the influence of wastewater management strategy on microplastic concentrations, wastewater treatment plant discharge records from sequencing batch reactor effluent discharge pumps in April 2017 were compared to Fall Creek microplastic concentrations. To do so, curve fitting was performed on microplastic concentration and time since last batch reactor discharge, anticipating that a clear peak in microplastic concentration would occur at a given lag time if there was indeed a clear signal of wastewater treatment plant microplastic inputs to the system.

As a general indication of factors influencing sample composition in terms of microplastic category, a linear model was fit to the percentage of fibers, with stream and

flow condition used as predictors. Student's t-tests were used as a finer scale indication of relationships between the stream and flow condition combinations for microplastic category composition.

Results and Discussion

Hydrologic Conditions

Over the course of both August and April sampling, the stream flowrate remained relatively constant. Sampling was performed 3 and 4 days after the previous rainfall events in August 2016 and April 2017, respectively, to ensure baseflow conditions. In August, low flow conditions were observed: Fall Creek flowrate averaged 0.52 m^3/s, and Six Mile Creek flowrate remained around 0.17 m^3/s. These flowrates represent 23% and 29% of the average August monthly discharge in Fall Creek and Six Mile Creek, respectively. In April, high flow conditions were observed: flowrates at the Fall Creek site measured an average of 7.60 m^3/s and 3.79 m^3/s at the Six Mile Creek site; representing 66% and 117% of the average April monthly discharge, respectively.

Composition of Sampled Microplastics

Microplastics were found in every sample collected across both streams, months, and sub-daily sampling (Figure 3.1). We qualitatively review the effect of watershed population on microplastic discharge (Figure 3.1b) and demonstrate that microplastics load per capita compared well across the two streams, suggesting similar behavior with respect to influence of population density on microplastic contaminant generation.

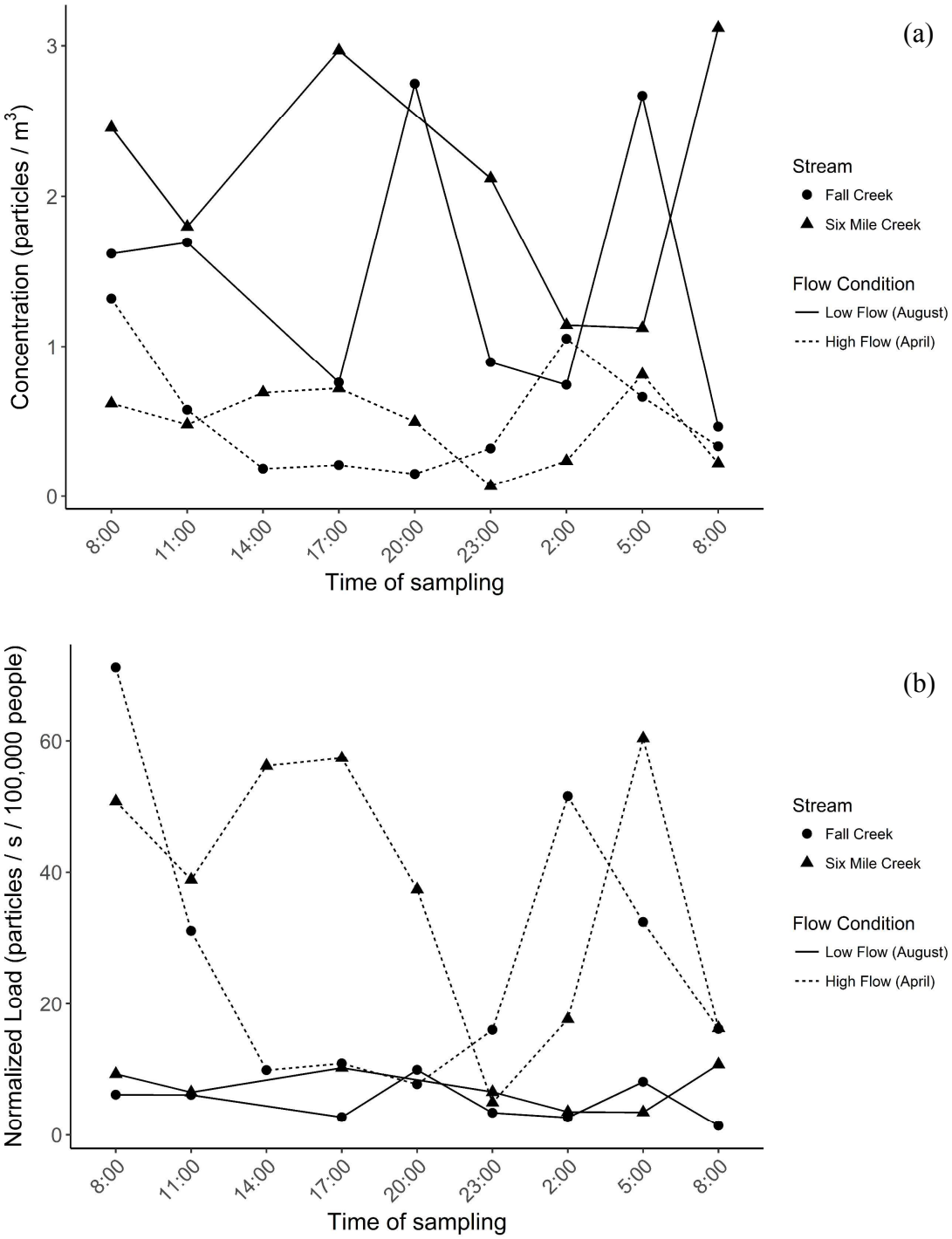

Figure 3.1. a) Microplastic concentration measured over 24-hour sampling efforts in Fall Creek (circles) and Six Mile Creek (triangles) during August 2016 low flows (solid line) and April 2017 high flows (dashed line). b) Microplastic loads normalized by upstream watershed population.

In higher flow conditions, higher loads are observed, along with lower concentration. This contradiction suggests that it is the hydrological condition, not other potentially varying factors such as increased fragmentation due to increasing UV exposure or microbial activity, that is controlling the microplastic concentrations observed in this system.

Regardless of flow condition or wastewater management strategy, fibers made up the majority of all collected microplastics, averaging 87% of the microplastics found per sample, by count (Figure 3.2). This is a common trend in river samples and ocean samples alike (Desforges et al., 2014; Baldwin et al., 2016; Dris et al., 2015; Kanhai et al., 2017). Particles were predominantly red, black, and blue in color, a trend also found in the Vandermeersch et al. (2015) study of microplastics in mussels. Due to their visual contrast in the sample, colored particle counts may be included with higher consistency than more neutral colored particles, which are more likely to be missed during counts. Gewert et al. (2017) found this discrepancy to by the case during quality assurance experiments in which 60% of intentionally introduced transparent fibers were missed during counting, while all other microplastic particles and colored fibers that had been added to artificial samples were recovered.

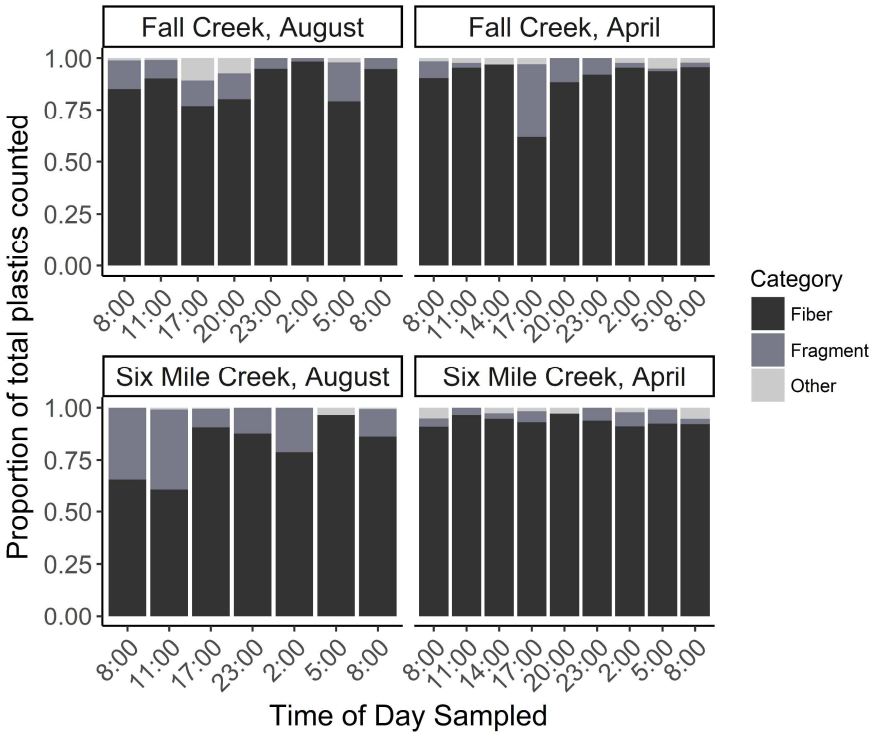

Figure 3.2. Microplastic composition of all samples, expressed as a proportion, by count, of total microplastics counted by sample, divided into categories found: fiber (black), fragment (dark grey), and all other categories, which includes films, beads and foams (light grey).

The percentage of fragments tended to be higher in August low flow samples than in April high flow ones. This is supported by a linear model which indicates that flow condition ($p = 0.03$), but not stream ($p = 0.81$), was a significant predictor for the percentage fibers present in a sample. Student t-test results suggest that Six Mile Creek's fiber concentration was lower in August low flow samples than in April high flow ones ($p = 0.04$), while Fall Creek showed no significant differences between composition percentages ($p = 0.60$). When applying a Bonferroni correction to account for the multiple tests being run for the analysis, however, this finding was not significant ($p = 0.125$). One factor that may support this trend in composition percentages between sampling efforts, though, may be that average velocities in Fall Creek were a mere

0.02 m^3/s greater in April sampling than in August sampling while in Six Mile Creek average stream velocity during sampling was 0.04 m^3/s higher during April sampling. This difference was not found to be a significant predictor when included in the linear model ($p = 0.81$), but may hint at mechanisms for preferential transport of certain particles over others during different sampling conditions.

Time of Day

Concentration varied a great deal with time of day, with the coefficient of variation ranging from 35% (Six Mile Creek, low flow) to 70% (Fall Creek, high flow). Higher variability was measured consistently in high flows and in Fall Creek samples. This positive relationship between higher flows and higher coefficients of variation was also seen by Dris et al. (2018), who investigated minute-scale samples over a two-hour total sampling period, though in that study, length of sampling time was also changed between flow conditions, confounding the results. One possible explanation for our observed variability may be that in higher flows, greater turbidity allows for less evenly distributed microplastic particles and perhaps uneven resuspension of particles from the bed into the sample. To better uncover the source of this variability, future studies should incorporate both finer timescales and better understanding of the influence of methodological uncertainties on overall variability observed.

Despite the observed variability, time of day was not a significant predictor for microplastic concentration for both Six Mile Creek and Fall Creek (Table 3.2). This was the case even when accounting for potential lag between concentration peaks due to stream site. The sequencing batch reactor treatment plant upstream of the Fall Creek sampling site discharged at two-hour intervals during the sampling period, which were

finer time scales than the three-hour sampling intervals used at Fall Creek. Even during discharge intervals, the wastewater treatment plants contribute at most 0.5% of the April Fall Creek flow and no more than 3% of the total Fall Creek flow in August, which supports evidence in the sampling data that indicates the wastewater treatment plants' microplastic contributions during the two sampling efforts were minimal. These percent flow contributions are much lower than any of wastewater treatment plant contributions in streams presented by McCormick et al. (2016).

Table 3.2. Summary of coefficients for the simple linear regression fit to the data. Asterisk (*) indicates statistically significant result. Adjusted R-squared value for this regression model is 0.5.

Parameter	Estimate	Standard Error	t value	p-value
Intercept	0.05	0.01	6.09	9.7×10^{-13}*
Time of day	0.01	0.01	0.70	0.49
Stream (Six Mile)	0.01	0.01	1.25	0.22
Flow condition (High)	-0.04	0.01	-5.84	2.48×10^{-6}*

Stream Discharge and Seasonality

Flow condition was a significant predictor of microplastic concentration (Table 3.2). Though in neither August nor April conditions the two streams were significantly different from each other, microplastic concentrations for both stream were significantly higher during August's low flows than in April's high flows (Figure 3.1a). This correlation is opposite of what Moore et al. (2011) reported when high flow samples were collected immediately following runoff events. In contrast, for this study, seasonally high baseflow conditions were used for high flow samples. Dris et al. (2015) use similar high flow conditions to this study, where runoff is not a precondition for high flow measurements, and found the similar high flow-low concentration correlation

reported in this study. This discrepancy in what constitutes "high flows" underscores the importance of runoff as one mechanism for introducing plastics to the system, particularly from urban areas currently recognized as a leading nonpoint source of microplastics, attributable to materials and behaviors as far ranging as dry cleaner exhaust and macroplastic litter (Eriksen et al., 2018). It also points toward the existence of another source of microplastics independent of flow condition or time of year, which highlights the need for further research into the mechanisms and sources of microplastic introduction during baseflow conditions, wastewater treatment plants being one commonly mentioned candidate.

Wastewater Management Strategy

In both high and low flow conditions, wastewater management strategy was not a significant driver of microplastic concentration (Table 3.2). Unlike effluent concentration and upstream-downstream comparison studies that have shown wastewater treatment plants to be significant contributors to microplastic contamination of some rivers (McCormick et al., 2014; A. McCormick et al., 2016), this study found no difference between microplastic concentrations downstream from a wastewater treatment plant effluent pipe and in a watershed exclusively using septic systems. The results' consistency between streams with varied wastewater management strategies suggests that (1) wastewater treatment plants are no more or less a source of microplastics than septic systems, assuming wastewater is a primary microplastics source, or (2) there are other sources that are abundant enough to mask potential wastewater signals at watershed scales. This finding potentially contradicts the existing research that assumes a primary significance of wastewater treatment plant presence

along a sampled river, and it indicates that additional research should be done to uncover whether there is a threshold of treatment plant size, septic system field density, or low-background microplastic concentration at which wastewater treatment plant inputs do begin to dominate stream microplastic loads.

Since similar concentrations and categories of microplastics were collected across both streams, our results could indicate that septic systems and wastewater treatment plants are equal performers in terms of microplastic inputs to the stream. Indications of microplastic movement through agricultural soils indicates that there may be a possibility that some microplastics from septic fields, particularly from failed, short circuiting systems, could eventually enter local streams (Rillig et al., 2017; Zubris and Richards, 2005), but this is unproven, as lateral transport of these particles has not yet been observed. The results could alternatively be an indication that the main driver of microplastic levels in both of these streams is a different input unrelated to wastewater treatment. This is similar to Estibahni et al. (2016) who found background concentrations at their control location, which lacked wastewater treatment plant inputs, to be higher than those near plant effluent inputs. Previous studies have indicated that the presence of urban areas correlate to increased microplastic concentrations, but with no runoff events occurring in the days before each sampling event, a baseflow mechanism for introduction of human-activity sourced microplastics to the streams in this study remains unclear.

Contamination

An average of 6.7 +/- 2.3 airborne particles were detected in the 24-hour deposition blanks. Although in practice, samples were left uncovered for at most two

hours at a time while counting and processing, as a conservative measure this average value has been subtracted from all reported concentrations. For comparison, stream sample counts contained an average of 91 particles. Additional discussion of procedural contamination results is included in Appendix B.

Methodology Extensions

Although our study offers new insight into microplastic behavior in riverine systems and has been designed in ways to minimize the shortcomings—including limited sizes captured in neuston nets and inability to quantify uncertainty—of existing methods, it is still to some extent limited by those shortcomings and provides opportunities for improvement by future studies.

a. Sampling Design

The purpose of this study was to study temporal differences in microplastic concentrations and we did so by focusing on two streams with differing primary wastewater treatment strategies. Due to our study design, conclusions about whether the wastewater treatment plant serves as a source cannot explicitly be answered. What we can say is that the stream with wastewater treatment plant inputs does not behave significantly differently from one without them and also that for this system, the microplastics signal originating from the wastewater treatment plant effluent is not detectable above background levels. Existing literature indicates that this trend may not hold in differently sized rivers or for treatment plants with different unit operations or treatment volumes. To make conclusions centered on the wastewater treatment plant microplastic inputs themselves, measurements would need to be taken above, below and

potentially from the outfall pipe itself, but this lies outside the scope of this study focused on system-scale patterns.

Our assessment of microplastic concentrations with respect to wastewater treatment techniques used a small-scale batch reactor wastewater treatment plant and a much smaller aeration lagoon treatment plant. The addition of these small plants is of value in the existing literature in that it adds complexity to our understanding of what the presence of a wastewater treatment plant may mean for microplastic concentrations, while underscoring the diversity of affects that treatment plants may have based on their unit operations, scale and discharge patterns (Mason et al., 2016).

Future studies may find it beneficial to have samples from upstream and downstream of plants as well as across streams with different treatment streams to dig deeper into the topic introduced here. It should also be noted, as Magnusson and Norén (2014) point out, that the distance between wastewater treatment plant outfall and stream sampling point matters in terms of observed concentration. This suggests upstream-downstream samples should include multiple distances downstream of discharge pipes in order to make conclusions about observed patterns. In our study, we also compare treatment plant regimes without including a baseline of an uninhabited, wastewater-free watershed in our assessment. Understanding how our measured inhabited systems compare against an uninhabited control would improve our ability to make hypotheses about sources of baseflow microplastic loads.

b. Size Fractions Analyzed

Other studies have highlighted the wide range of microplastic sizes not being captured in net studies due to mesh sizes that impose a lower bound on the size range

captured (Dris et al., 2018; Enders et al., 2015). Neuston nets are beneficial in stream samples in that they allow large volumes of water to be captured while also providing ample mesh to prevent clogging and flow reduction from high organic loads during sampling, particularly in late summer and fall sampling of smaller order streams. Along with this benefit, however, comes the risk of losing sampled microplastics in the large amount of mesh fabric, despite thorough rinse efforts. Grab samples may provide a mechanism for capturing smaller size fractions and have for that reason been found to measure higher concentrations of total microplastics in a system (Barrows et al., 2017), but in doing so they may increase error measurements on concentrations due to a reduced volume of stream water being captured per sample.

With this study, we have only captured and analyzed the larger >300um fraction of microplastics. As Conkle et al. (2018) note, many primary microplastics are manufactured to be of the size fraction much smaller than this. This adds an obstacle to making general claims about temporal patterns of all microplastics along rivers, particularly sourced from wastewater treatment plants, and additional, differing stories could emerge from similar, future studies that look at a smaller or wider size range of microplastic particles.

c. Enumeration Techniques

This study includes visual counts, which have been exclusively used in a large percentage of studies. Since the work of this study was completed, a number of more advanced enumeration methods have been described, tested, and recommended including FT-IR microscopy and fluorescent techniques, e.g. the use of Nile Red, and visual counts have been cautioned against for particles smaller than 500um (e.g. Renner

et al., 2018). With new enumeration techniques now more widely accepted, future efforts to expand on this study must include validation of counts using these advanced methods. Understanding the shortcomings of visual counts, for instance that relationships between visual counts and advanced microscopy measurements are inconsistent between studies (Lenz et al., 2015; Song et al., 2015), this study was designed intentionally such that conclusions could be based on the relative difference between our counts instead of individual count totals, with the understanding that visual count uncertainty cannot be resolved.

Conclusions

This goal of this study was to identify whether factors such as time of sampling, in terms of both time of day and flow condition, as well as primary wastewater management strategy (centralized wastewater treatment plant versus decentralized septic systems) affect the microplastic concentration measured at a given location.

Unlike previous studies which show localized increased concentrations corresponding to wastewater treatment plant effluent, this study finds no significant difference between a site downstream of a wastewater treatment plant outfall and one in a watershed relying on septic systems for wastewater treatment. Our results suggest that a simplistic conceptual model describing wastewater treatment plants as lone and significant point sources of microplastic contamination in a freshwater system may be an oversimplification.

This study does show that concentrations measured at the same location but in different flow conditions are significantly different. Microplastic concentrations in high flows were significantly lower than concentrations in low flows, while measured

microplastic loads were highest in high flows. Time of day when sampling occurs did not affect measured concentration.

These results indicate that in the dynamic systems of rivers, reporting flow condition during spatial studies is important, particularly to aid future attempts to compare results between studies or sampling efforts. This study also indicates the continued investigation into terrestrial sources of microplastics is crucial to constructing informed microplastic budgets and quantifying uncertainty associated with them.

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APPENDIX A

Influence of individual dams on study findings

To illustrate how similar the relationships are between upstream, reservoir, and downstream samples for each dam, the individual measurements for each sample are included as Figure A1 (surface water concentrations) and Figure A2 (sediment concentrations). In these figures, each bar represents a single measurement and for analysis (see: Chapter 2), all dams along a given river were treated as replicates. Dams are plotted from upstream to downstream moving left to right, by river. For the location of each dam number along Fall and Six Mile Creeks, refer to Figure 1.

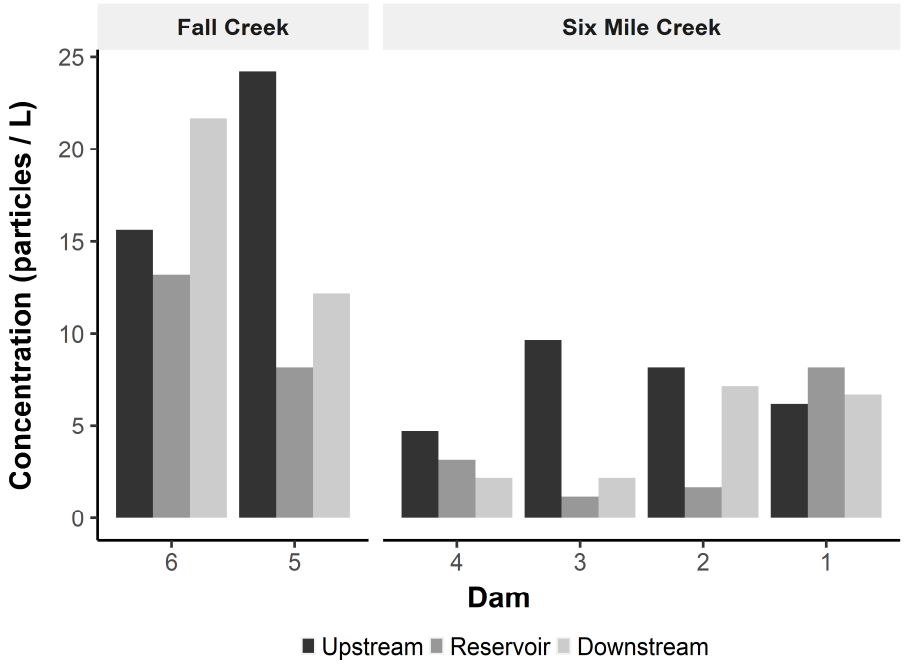

Figure A1. Measured surface water microplastic concentrations at each dam, with color indicating the relative location at each dam each sample was collected: in the thalweg upstream of the reservoir (black), in the reservoir (dark grey), in the thalweg downstream of the dam (light grey).

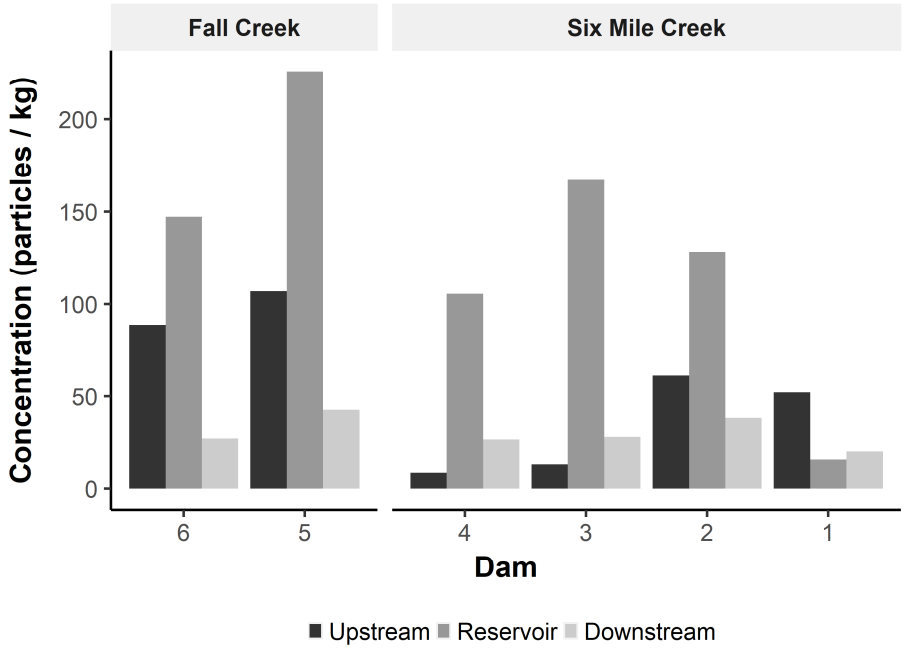

Figure A2. Measured sediment microplastic concentrations at each dam, with color indicating the relative location at each dam each sample was collected: in the thalweg upstream of the reservoir (black), in the reservoir (dark grey), in the thalweg downstream of the dam (light grey).

For surface water samples, we see greater variability between the sampling locations by dam, but in all cases, except at Dam 1, upstream concentrations are higher than reservoir concentrations, supporting the overall assertion that plastics settle out of suspension and enter the sediment in the slower velocities of reservoirs.

Dam 1 is not only the anomaly in terms of concentration for water samples, but also for sediment samples. Reservoir concentrations of microplastic at Dam 1 are lower than upstream and downstream measurements. Reservoir surface area was tested as one possible predictor of concentration, but it was found to be not significant. Other factors affecting these concentration patterns may include residence time, water depth, age or remaining capacity of reservoir, or other hydraulic parameters.

APPENDIX B

Additional commentary on factors influencing concentration

Secondary analysis on Fall Creek's microplastic concentrations in relation to the lag since last sequencing batch reactor discharge showed no clear relationship (Figure B1). The second, smaller plant was not included in further analysis as it was assumed to be contributing constant microplastic concentrations throughout the entire sampling period due to the long residence times and constant mixing of aeration lagoon systems.

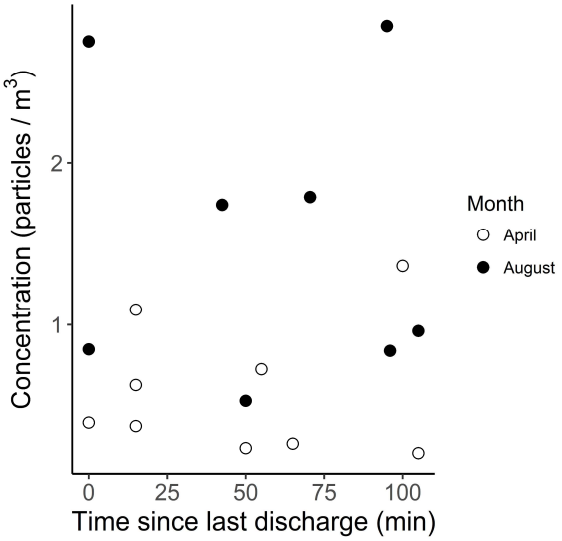

Figure B1. The difference between time of sampling and time of the most recent sequencing batch reactor discharge related to concentration of microplastic measured during high-flow April (empty circle) and low-flow August (filled circle) sampling. August wastewater treatment plant discharge timing is approximate.

Had the sequencing batch reactor been a significant contributor of microplastics, a time lag of peak influence on concentration would be expected. Based on the average velocity at sampling and the distance between the wastewater treatment plant outfall and sampling site (2.85 kilometers) with no dispersion and assuming a

constant microplastic concentration in the effluent with time, the peak would be expected in just over 50 minutes. No such trend was observed.

Contamination measured in procedural blanks

Five procedural blanks run through the entirety of laboratory processing steps following the completion of this study collected an average of 60 microplastic particles, 94% of which were fibers, by count. This number likely stems from an accumulation of fibers in the 0.335mm synthetic mesh used during wet sieving resulting in cross-contamination between samples. Due to equipment being shared among several concurrent studies, it is difficult to tell when and from where the contamination began accumulating in equipment. Sample counts do not show any systematic trends based on time of processing. In comparing counts and categories to that measured on the blanks, as well as to other studies, it is reasonable to assume that the contamination measured at this later date in the lab is higher than the amount introduced to samples included in this study; therefore, procedural blank counts have not been removed from sample concentrations. In future studies, procedural blanks should be run between each sample.

One of the shortcomings of much of the previous literature on microplastics is the lack of quantification work done to uncover levels of contamination and uncertainty. To best contextualize the numbers we report for this study, we have made attempts to include all information related to our contamination prevention strategies (e.g. rinsing dishware, keeping samples covered, wearing cotton lab coats and gloves when working with samples, avoiding synthetic clothing during sample collection whenever possible, running procedural blanks to the best of our abilities). Though our attempts have not entirely eliminated introduced contamination from handling or successfully quantified

all contamination resulting from the laboratory environment and procedures, our hope is that this will continue to inspire others to fully describe their procedures and methods and include the findings of efforts to quantify contamination. By sharing this information, better standards can be developed and shared in the field for both detecting contamination and reporting findings. Through this standardization, findings across studies can be better compared, in order to better leverage results worldwide and in so doing, develop a better understanding of the scale and behavior of this global pollution problem.