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RESEARCH ARTICLE

A case study investigating temporal factors that influence microplastic concentration in streams under different treatment regimes

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Abstract

Microplastics, particles less than 5 mm in size, are an emerging contaminant in waterways worldwide. Most microplastic studies focus on spatial trends in concentration, but in systems as dynamic as rivers, to draw conclusions from existing spatial studies, we must first examine how microplastic concentrations may change with time and flow conditions. In this study, we investigate how microplastic concentrations change over a 24-h period and between seasonally high and low flows. We do this in two streams, controlling for wastewater treatment strategy: one stream in a watershed where waste is treated with septic systems and the other receiving wastewater treatment plant effluent. We hypothesized that a stream with wastewater treatment plant effluent would exhibit higher and more variable microplastic concentrations than a stream in a watershed with septic systems. Results indicate, however, that there is no significant difference between the two streams despite their differing treatment strategies. Additionally, no significant variation in concentrations was measured over two 24-h sampling campaigns. There was, however, significantly higher concentrations measured in summer low flow conditions relative to spring high flow conditions across both sampled streams (p value <0.001), indicating that increases in stream discharge unrelated to storm events dilute and decrease measured microplastic concentrations. From this, we learn that pairing measured concentrations with a description of flow conditions at sampling time is a requisite for a robust microplastic literature that allows for comparisons between existing spatial studies and extrapolations to global loads.

Keywords Plastic · Wastewater · Septic · Pollution · River · Hydrology · Temporal

Introduction

Pollution by plastics is an emerging presence in waterways across the globe. With a large percentage of marine plastic particles originating from terrestrial sources, commonly estimated at 80% (Andrady 2011), studies have increasingly begun studying plastic concentrations in freshwater systems.

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This freshwater focus coincides with attempts being made to quantify the scale of this global pollution problem and the rate at which it is growing. Of highest concern are plastic particles smaller than 5 mm, known as “microplastics”. Microplastics begin as a variety of products, from small nurdles (plastic pellets) released from manufacturing to larger plastic products that break down over time into easily ingested sizes, making them of concern to the integrity of aquatic ecosystems.

Hydrophobic contaminants such as polychlorinated biphenyls and polycyclic aromatic hydrocarbons have been found to readily adsorb to plastic particles, which also facilitate microbial transport from wastewater treatment plants to receiving waters (Teuten et al. 2009; Rochman et al. 2013; McCormick et al. 2014; Rochman 2015). 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 (Setälä et al. 2014; Vandermeersch et al. 2015; Steer et al. 2017; Critchell and Hoogenboom 2018; Foley et al. 2018). Health risks associated with microplastic ingestion are still unknown (Koelmans et al. 2017; Jovanović et al. 2018).

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 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, which is of particular importance for systems as dynamic and heterogeneous in time and space as rivers. Existing microplastic research focuses predominantly on understanding the effects of the spatial heterogeneity of rivers but leaves questions of how temporal variability affects microplastic concentrations unanswered. Lebreton et al. (2017) estimated that three quarters of the river-sourced annual plastic load enters oceans between May and October, a period of less than half of the year. This discrepancy underscores a need for increased study of long-term temporal variability. Focusing on short timescales, Dris et al. (2018) found that the measured riverine microplastic concentration in quick, 1-min river samples can have a coefficient of variability as high as 45%. Together, these studies support further study on what mechanisms lead to this variation and how concentrations fluctuate at intermediate timescales.

Previous research hints at hydrologic fluxes being a driving mechanism for microplastic origination and transport at the catchment scale. Recent evidence suggests that runoff from storm events is a major source of microplastics to surface water systems. Directly following rainstorms, microplastic concentrations are 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 in watersheds of widely varying size (10^1–10^4 km^2), population density (10^1–10^4 people/km^2), and land-use composition. All found positive correlation, to some extent, between microplastic concentration and degree of urbanization.

Looking at high flows unassociated with rainfall events, 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). In this Seine River study, sampled in absence of surface runoff, microplastic concentrations were negatively correlated with discharge at some sites. This negative correlation points to the presence of some season- and flow-independent sources of microplastics and potentially, a consistent point source emitter.

Timing, with regards not only to flow conditions, but also to diurnal changes, is worth investigating. In this study, we chose to control for wastewater treatment strategy due to suspicions that diurnal patterns in human behavior could affect patterns in river microplastic concentrations in some conditions. While only a few isolated studies provide evidence of microplastic concentrations varying temporally in wastewater (Mason et al. 2016; Warrack et al. 2017), other contaminants sourced from wastewater treatment plants, such as pharmaceuticals, regularly exhibit diurnal signaling, exhibiting twice-per-day peaks 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. Due to the long residence time of sewage treated by septic systems, which rely on long, slow pathways through large volumes of soil for treatment, we expect that diurnal variation in human sewage inputs to septic systems would be intercepted and negated by the drain field. Unlike wastewater treatment plant effluent, no temporal patterns are expected to exist in water or fugitive microplastics treated by septic systems.

For this study, we aimed to determine whether, at sampling time, measured stream microplastic concentrations differ based on the time of day or on flow condition, a term we use to specify seasonally varying discharge. We looked at these two variables while controlling for wastewater treatment strategy, suspecting it to influence the effects of flow and time. To address this goal, we investigated whether microplastic concentrations varied over the course of two 24-h periods, representing high and low flow conditions, on one stream where wastewater is treated by centralized treatment plant and a second where wastewater is treated by septic systems.

We hypothesized that concentrations would be highest in seasonally low flow conditions. Further, we hypothesized that during baseflow conditions, if wastewater treatment plants were a leading source of microplastics to the system, microplastic concentrations in the stream with wastewater treatment plant effluent contributions would peak twice over a 24-h period, reflecting of the diurnal signaling of human use patterns. We suspected that time of sampling would not be an important variable in the stream without a wastewater treatment plant, regardless of flow condition nor would the diurnal signaling be evident in high flows, even in the stream with a wastewater treatment plant.

Methods

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 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 km from one another. We carried out one 24-h sampling event in both streams during August 2016 (low flow period) and a second 24-h sampling event in April 2017 (high flow period).

The primary wastewater treatment plant contributing 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 2500 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). Together, these plants treat waste for approximately 1/5 of the watershed population and discharge it through a single discharge pipe 2.8 km above the sampling site for this study. This second, smaller plant was not included in our temporal analyses 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.

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, including during the August sampling efforts (Scherrieble, K., personal communication, November 10, 2017).

Field data collection

Samples were collected at a designated location within the thalweg of each stream using a Sea-Gear neuston net with 335 μm mesh, as is used in many other surface water

Table 1 Watershed characteristics of the two streams sampled for this study

Watershed characteristics	Six Mile Creek	Fall Creek
Population[a]	4900	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

microplastic studies (e.g., Free et al. 2014; Baldwin et al. 2016; Eriksen et al. 2017), with a 1×0.5-m 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 10 min every 3 h, over two midweek, 24-h 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 km downstream (USGS 2011). 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

Samples were processed following NOAA protocols (Masura et al. 2015), which include a wet sieving size separation step, followed by wet peroxide oxidation and a density separation to digest labile organics and separate dense non-plastic particles from floating plastic particles. Modifications were made to NOAA's wet sieving step: a metal 4.6-mm sieve was used as the upper size filter and a synthetic 0.3-mm 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 0.45-μm 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 (MERI 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.

Understanding the shortcomings of visual counts, for instance that the relationship between visual counts and advanced microscopy measurements is inconsistent between studies (Song et al. 2015; Lenz et al. 2015), this study was designed such that analyses rely on relative differences between sample measurements 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 any count differences were systematic and not a sign of introduced variability. Because visual counts have been cautioned against for particles smaller than 500 μm (e.g., Renner et al. 2018), we also validated a subset of particles using a WITec Alpha300R Confocal Raman Microscope at 20× magnification with a 532-nm laser at 1–2 mW power, with spectra analyzed against Bio-Rad KnowItAll® Informatics System (2018) spectral database. Though only a small number of spectra were identified (n=11), a sensitivity of 100% and precision of 88% indicated to us that our visual identifications matched spectral confirmation closely enough to reasonably rely on our visual findings.

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.

To measure for contamination from laboratory air, three filter papers were left uncovered for 24 h in areas of the lab where samples were exposed during processing in order to sieve, add reagents, or count. An average of 6.7±2.3 airborne particles were detected in the 24-h deposition blanks. Although in practice, samples were left uncovered for at most 2 h 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.

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, August counts included only eight and seven samples for Fall Creek and Six Mile Creek, respectively.

The experimental variables used in this study were time of day, hydrologic conditions, and 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 α=0.05, first using an analysis of co-variance. For the purpose of presentation, we then ran an analysis of variance treating time of day as a replicate. As a check of whether concentration was affected by different hourly 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, but the interaction term was not found to be significant.

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.

Table 2 Microplastic categories used when describing particles in samples

Category	Description
Fiber	*Thin thread of equal thickness throughout*
Fragment	*Irregularly shaped piece broken off larger debris*
Film	*Thin, flexible, flat sheet*
Foam	*Frothy, sponge-textured particle*
Bead	*Spherical pellet*

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 previous rainfall events in August 2016 and April 2017, respectively, to ensure baseflow conditions. In August, seasonal 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, seasonal high flow conditions were observed: flowrates at the Fall Creek site measured an average of 7.60 m^3/s and at the Six Mile Creek site, 3.79 m^3/s; representing 66% and 117% of the average April monthly discharge, respectively.

Composition of sampled microplastics

Microplastics were found in all samples (Fig. 1). In higher flow conditions, higher average flux is observed (32.6×10^5 particles/s, Fig. 1a), along with lower average concentrations (0.5 particles/m^3, Fig. 1b), as compared to in low flow conditions (5.9×10^5 particles/s; 1.8 particles/m^3). This contradiction between load and concentration suggests that there is a relatively constant presence of microplastics through the year. This baseline indicates that it is the hydrological condition, not other potentially varying factors related to microplastic creation, 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 (Fig. 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, 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 counting. Gewert et al. (2017) found this discrepancy to be 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.

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's t test results suggest that Six Mile Creek's fiber concentration was lower in August low flow samples than in April high flow ones, but this difference was not found to be significant following a Bonferroni correction. The trend in composition may be due to water velocity, which in Fall Creek was 0.06 m/s greater in April than in August, and in Six Mile Creek, was 0.13 m/s higher in April than in August. This difference was not found to be a significant predictor

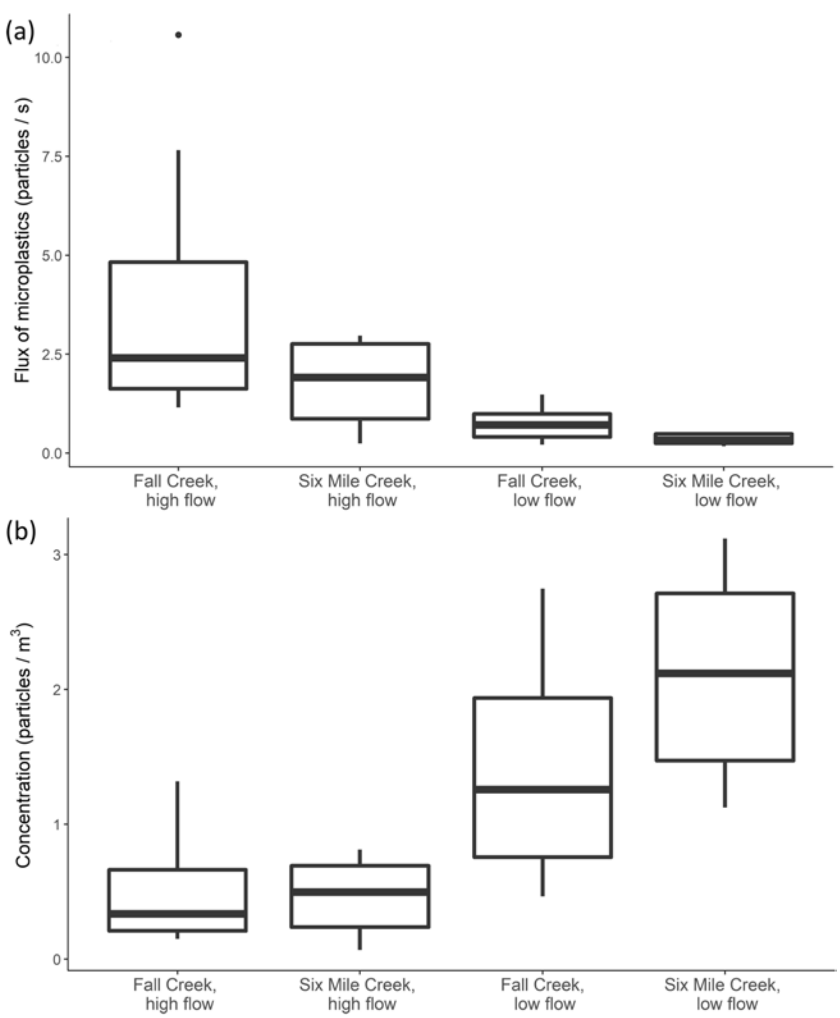

Fig. 1 **a** Flux of microplastics and **b** microplastic concentration measured over 24-h sampling efforts in Fall Creek and Six Mile Creek during August 2016 low flows and April 2017 high flows

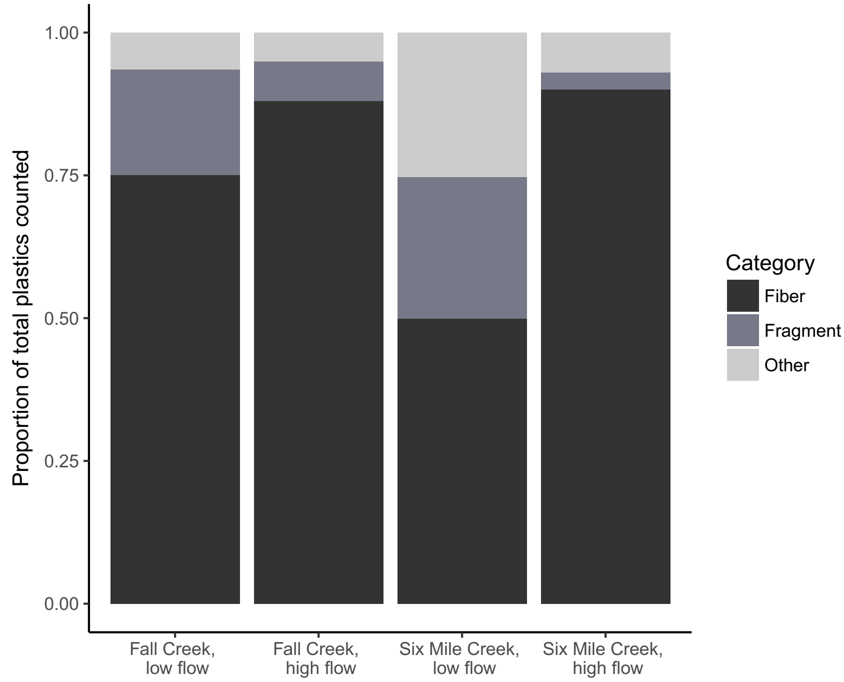

Fig. 2 Microplastic composition, expressed as an average proportion, by count, of all microplastics found on each sample date, divided into categories found: fiber (black), fragment (dark gray), and all other categories, which includes films, beads, and foams (light gray)

when included in the linear model (p=0.81), but may hint at mechanisms for preferential transport or resuspension of certain particles over others during different sampling conditions.

Time of day

Concentration varied with time of day, with the coefficient of variation for given sampling dates ranging from 35% (Six Mile Creek, low flow) to 70% (Fall Creek, high flow). Concentrations at high flows were consistently more variable than those at low flows and Fall Creek samples were more variable than those of Six Mile Creek. The 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 2-h total sampling period, though in that study, length of sampling time was also changed between flow conditions, confounding the interpretation of the results. One possible explanation for our observed variability between flow conditions 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 (p=0.47), as determined using an analysis of covariance.

These two streams were chosen specifically for their differing wastewater management strategies. 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). Other identified point sources are less common, such as regulated discharges from industrial plants (Lechner and Ramler 2015). There are other non-point sources that merit noting here including atmospheric deposition, in-stream resuspension, particle fragmentation, and ground surface runoff. Septic systems, on the other hand, designed to treat wastewater using the soil as a biological and physical filtration system, have not been investigated or suspected as a source of microplastics thus far in the literature, and unlike wastewater treatment plants, are not suspected of discharging water or other constituents cyclically throughout the day.

The sequencing batch reactor treatment plant upstream of the Fall Creek sampling site discharged at 2-h intervals during the sampling period, which were finer time scales than the 3-h 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. Though not an often used statistic for differentiating wastewater treatment plant influence in the literature, we note that flow contribution percentages are much lower than any of wastewater treatment plant contributions in streams presented by McCormick et al. (2016).

Had the wastewater treatment plant been a significant contributor of microplastics for the overall Fall Creek system, a time lag of peak influence on concentration would be expected at the sampling site. Based on the average stream velocity at sampling and the distance between the wastewater treatment plant outfall and sampling site (2.85 km) with no dispersion and assuming a constant microplastic concentration in the effluent with time, the peak would be expected in just over 50 min. No such trend was observed (Supplementary Materials, Fig. S1).

The results' consistency between streams with varied wastewater management strategies suggests that (1) for these systems, the presence of a treatment plant instead of septic systems as a waste management strategy does not, as a default, lead to greater microplastic concentrations or differentiated signals, despite evidence from studies such as McCormick et al. (2014, 2016) which indicate 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 any wastewater treatment plant along a sampled river will be of importance in microplastic concentration patterns. It also indicates that additional research should be done to uncover whether there is a threshold of treatment plant size, septic system 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, at least of a particular scale in relation to the receiving body, are equal performers in terms of microplastic inputs to the stream. Indications of microplastic movement through agricultural soils indicate that there may be a possibility that some microplastics from septic leach fields, particularly from failed, short-circuiting systems, could eventually enter local streams (Zubris and Richards 2005; Rillig et al. 2017), but this is unproven, as lateral transport of these particles has not yet been observed. Our 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 Estahbanati and Fahrenfeld (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 (Yonkos et al. 2014; Baldwin et al. 2016), 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.

Stream discharge and seasonality

Flow condition was a significant predictor of microplastic concentration ($p<0.001$), as determined through an analysis of variance for flow condition and stream, which treated time of day as a replicate (Table 3). 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 (Fig. 1b). 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) used 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, wastewater treatment plants being one commonly mentioned candidate. Since the same relationship was observed in a stream without wastewater treatment plant inputs, there is an apparent need for further research into the mechanisms and sources of microplastic introduction during baseflow conditions that goes beyond wastewater management strategies.

Although we designed our study to account for different baseflow conditions, we recognize that there may be other contributing factors between April and August sampling dates. For example, there may be more people using streams for recreation in the summer and, thus, potentially

Table 3 Summary of coefficients for the analysis of variance. Asterisk (*) indicates statistically significant result. Adjusted R^2 value for this regression model is 0.5, with an F-statistic of 16.23 on 2 and 30 degrees of freedom and a p value <0.001

Parameter	Estimate	Standard error	t value	p value
Intercept	1.63	0.195	8.34	<0.001*
Stream (Six Mile)	0.27	0.223	1.21	0.24t
Flow condition (High)	−1.25	0.224	−5.60	<0.001*

adding microplastics as part of this activity. However, our results showed a smaller flux of microplastics in the streams during the summer (Fig. 1a), so we conclude that this is an unlikely explanatory factor. We also considered that people may travel during the summer and that students largely leave the area. However, we chose our sampling sites to be well upstream of the two major colleges/universities in the area, Cornell University and Ithaca College, in order to minimize the student impact.

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.

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 microplastic 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 that 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.

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 (Enders et al. 2015; Dris et al. 2018). 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 >335 μm 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.

Conclusions

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

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 fluxes were highest in high flows. This study was unable to detect differences in concentration based on time of day, nor was it able to find any relationship between wastewater treatment plant discharge timing and concentration peaks for microplastics. Our results suggest that a simplistic conceptual model describing wastewater treatment plants as the primary and most significant point sources of microplastic contamination in a freshwater system may be an oversimplification. We found no significant difference in concentration or flow-related patterns of microplastics in a stream with wastewater treatment plant inputs and another with septic systems.

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 that 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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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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