SPATIAL AND TEMPORAL PATTERNS OF MICROPLASTIC CONCENTRATIONS IN ATHENS-CLARKE COUNTY, GEORGIA WASTEWATER TREATMENT SYSTEMS

by

EMILY MADISON MONROE

(Under the Direction of Krista Capps)

ABSTRACT

Microplastics, or plastics that are <5 mm in length or diameter, have been identified as a pollutant of emerging concern in the world's ecosystems. Freshwater systems have been recently recognized as a crucial component of the plastic cycle, transporting plastic pollution from terrestrial to marine systems. Wastewater may contribute a substantial amount of plastic pollution to streams and rivers; yet, limited research has explored the variation in the concentration, volume, and diversity of plastics generated by wastewater. To better understand the spatial and temporal patterns in wastewater-derived microplastic pollution, we quantified and characterized microplastic pollution in effluent from three, distinct sewersheds in Athens-Clarke County, GA. I found that sampling date, particle size, and particle shape were the best predictors of microplastic characteristics in treated wastewater. These findings provide insights as to what types of plastic systems can expect in their wastewater and can aid regulations on reducing plastic pollution.

INDEX WORDS: Plastic Pollution, Microplastics, Freshwater, Wastewater Treatment

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The University of Georgia
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# DEDICATION

I dedicate my work to my loving parents whose words of encouragement and constant support have helped me get through all the challenges of graduate school and life. You have helped shape me into the woman I am today, and for that I am so grateful. I would also like to dedicate this work to the memory of my grandfather, who loved me unconditionally and who always knew I was going to change the world someday. His perseverance and strong work ethic were traits that I hope to emulate.

ACKNOWLEDGEMENTS

First, I would especially like to thank my advisor, Krista Capps, whose unwavering dedication, motivation, and encouragement helped me to complete this project and has helped me become a better scientist. I would like to thank my committee members, Rae McNeish and Amanda Rugenski, for all the support, guidance, and help they have given me throughout this project. I thank my fellow lab mates, especially Emily Martin, for the hours of advice, early morning sampling days, and friendship. I would not have been able to get through this without you all. Finally, I would like to thank my family and my partner, all of whom have believed in me and supported me in more ways than I could have imagined. I love you.

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# CHAPTER 1

# INTRODUCTION

Since the 1950s, the large-scale production and use of plastics has shifted production from reusable materials to single-use products within the global economy (Hou et al., 2021). The total amount of plastic produced annually now exceeds 400 million tons (Geyer et al., 2017), and a large portion of this plastic (31.9 million tons) is discarded, becomes waste, and enters the world's ecosystems (Wong et al., 2020). Plastic waste is now so prevalent in the environment that it has been identified as a useful indicator of the proposed geologic era (Geyer et al., 2017; Simon et al., 2018).

Plastic pollution is persistent, as many of the monomers that are used to produce plastics do not biodegrade (Geyer et al., 2017). Microplastics (plastics <5 mm) have been identified as a contaminant of emerging concern (Hoellein et al., 2019; Li et al., 2020; Rochman et al., 2019). They are considered harmful environmental contaminants because of their ability to leech chemicals, absorb persistent organic pollutants (POPs), and be consumed by animals (Alimi et al., 2018; Carr et al., 2016). Additionally, research has demonstrated that the microbial communities that form on plastic are significantly different than communities that develop on natural substrates (McCormick et al., 2014). Collectively, this work suggests that microplastic pollution may alter the structure and function of affected systems (Green et al., 2017). However, there are other studies that indicate that microplastic concentrations are too low to cause tangible risks to organisms and the environment (Burns & Boxall, 2018; Koelmans et al., 2017; Prata et al., 2019); therefore, more studies are needed to elucidate the toxological risks of microplastics.

For the last decade, the number of articles published on microplastic pollution has increased exponentially. Much of this work has focused on plastic contamination in coastal and marine systems (Zhang et al., 2020). In contrast, comparatively little work has focused on the impact of microplastic pollution on freshwater ecosystems (Hoellein & Rochman, 2021). River networks were previously viewed as conduits for plastic pollution entering marine environments, and researchers assumed that the instream transformation, storage, and export of plastics to surrounding terrestrial ecosystems was negligible (Hoellein & Rochman, 2021). However, emerging research has demonstrated that the flow of plastics is much more complicated. Freshwater systems are a crucial component in the "plastic cycle" (Hoellein & Rochman, 2021), influencing the sources, sinks, and fluxes of plastics entering downstream, terrestrial, and marine environments.

Similar to other contaminants, plastic particles enter the environment in a myriad of ways. One source of microplastic pollution to surface waters is discharge from wastewater treatment plants (WWTP) (Hou et al., 2021; McCormick et al., 2016). Wastewater may contribute a substantial amount of plastic pollution to rivers and streams, as large volumes of microplastics are generated by everyday activities, such as laundering synthetic cloth, and because wastewater treatment facilities are not currently designed to remove plastic waste (Hoellein & Rochman, 2021). This is not to suggest that wastewater treatment cannot be an effective way to remove plastic pollution. For instance, Mason et al. (2016) found that primary treatment (i.e., filtering of solids with screen mesh sizes 6 mm or larger) removed 78% of plastics from effluent, and secondary treatment (i.e., removal of suspended and dissolved organic material and nutrients with microorganisms in large aeration tanks) removed an additional 20% of the plastic from the waste stream. However, even with effective plastic removal, more than 150 billion liters of wastewater is discharged from wastewater treatments each day in the US alone (Mason et al., 2016), suggesting that globally, treated effluent may be a large, but still poorly quantified, contributor of plastic waste to surface waters (Hoellein & Rochman, 2021).

Sewersheds, or the network of stakeholders contributing waste to a wastewater treatment plant, are often heterogeneous in land cover and in the types of waste being produced. Even within jurisdictional and watershed boundaries, sewersheds can have different population sizes, sources of contaminants, and constituents within their wastewater that contribute to the waste stream. Recent work has documented spatial correlations between the types of microplastics found at particular sites and the human activities that are in the surrounding areas (Li et al., 2020). However, only limited research has explored differences in the concentration, volume, and diversity of plastic pollution entering the environment among sewersheds.

The purpose of this study was to address this knowledge gap and explore spatial and temporal patterns in microplastic pollution entering a watershed from three distinct sewersheds. Specifically, I explored how microplastic concentration and composition in treated effluent varied with the sewershed or time of day during four sampling events that occurred monthly between September and December 2020.

# CHAPTER 2

# METHODS

## Study Sites

The fieldwork for this study was conducted in Athens-Clarke County (ACC), Georgia, USA (~306 $km^2$), located approximately 105 kilometers northeast of Atlanta (Figure 1). The county has three water reclamation facilities: North Oconee, Middle Oconee, and the Cedar Creek. North Oconee was built in 1962 and has the capacity to treat 14 million gallons per day. It serves a 119.2 $km^2$ area that includes the downtown business district, the University of Georgia, and most of the major industrial processing facilities in the county. Middle Oconee was built in 1964 and has the capacity to treat 10 million gallons of wastewater per day. The plant primarily serves a 92.24 $km^2$ area with most of the residential and commercial development in the county. Cedar Creek is the newest facility and was built in 1980. It has the capacity to treat 4 million gallons per day; the plant's primary customers are residential homes within a 101.01 $km^2$ area. Cedar Creek also accepts septage collected from onsite wastewater systems from ACC and the counties upstream from ACC on the Oconee River. All three plants discharge treated water into the Oconee River and use the following processes for treatment: screening and grit removal, biological treatment through sludge systems, settling and clarification, and UV disinfection. At the North Oconee and Cedar Creek Water facilities, cascade re-aeration is also completed before the reclaimed water is discharged into the Oconee River to increase oxygen content in the effluent. Because each of the facilities are regulated by the Georgia Department of Natural Resources, Environmental Protection region (GAEPD), the chemical characteristics of the effluent are consistently monitored. Average daily discharge and water quality parameters for the three plants were supplied by water professionals from ACC (Table 2). All the field and lab work for this project was conducted during the COVID-19 Pandemic. All safety guidelines outlined by the United States Centers for Disease Control and the university were followed.

Table 1. Sewershed characterization including the land use land cover classifications. Developed HI, MI, LI, and OS indicate that the land use classification for high intensity, medium intensity, low intensity, and open space respectively. Data was selected from land use maps provided by the county.

| | | | Land Cover Classifications (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Water Reclamation Facilities | Sewershed Area (km$^2$) | People Served | Agriculture | Barren | Developed, HI | Developed, MI | Developed, LI | Developed, OS | Forested |
| Cedar Creek | 101.01 | 25,500 | 8.09 | 0.08 | 1.04 | 4.43 | 13.01 | 23.50 | 46.44 |
| Middle Oconee | 92.24 | 49,000 | 4.04 | 0.13 | 3.57 | 8.68 | 16.36 | 23.79 | 36.93 |
| North Oconee | 119.12 | 56,500 | 7.78 | 0.13 | 5.18 | 13.67 | 20.10 | 19.52 | 28.52 |

Table 2. Water chemistry parameters collected by Athens-Clarke County. Data is reported with mean (± standard deviation). The parameters that were monitored included: minimum effluent pH, effluent flow (MGD), effluent temperature (C), effluent biological oxygen demand (%), effluent total suspended solids (TSS; mg/L), minimum effluent dissolved oxygen (mg/L), effluent $NH_3$ (mg/L), and effluent total phosphorus (TP; mg/L). The county maintains daily discharge estimates for each plant, but they are not required to monitor all water parameters daily at each plant. Therefore, I only received daily water chemistry parameters for a subset of the day/plant combinations that were sampled.

| Water Chemistry Parameters | Units | Cedar | Middle | North |
|---|---|---|---|---|
| Minimum effluent pH | -- | 6.6 (± 0.1) | 7.06 (± 0.23) | 7.14 (± 0.23) |
| Effluent flow | MGD | 1.75 (± 0.22) | 3.65 (± 0.25) | 6.64 (± 0.54) |
| Temperature | C | 21.1 (± 4.01) | 19.8 (± 5.91) | 22 (± 5.18) |
| BOD | % | NA | 3.2 (± 0.61) | 3 |
| TSS | mg/L | NA | 3.2 (± 1.69) | 3 |
| DO | mg/L | 8.16 (± 0.21) | 9.01 (± 0.59) | 8.1 (± 0.42) |
| NH3 | mg/L | NA | 0.02 (± 0.59) | 0.01 |
| TP | mg/L | NA | 0.69 (± 0.22) | 0.28 |

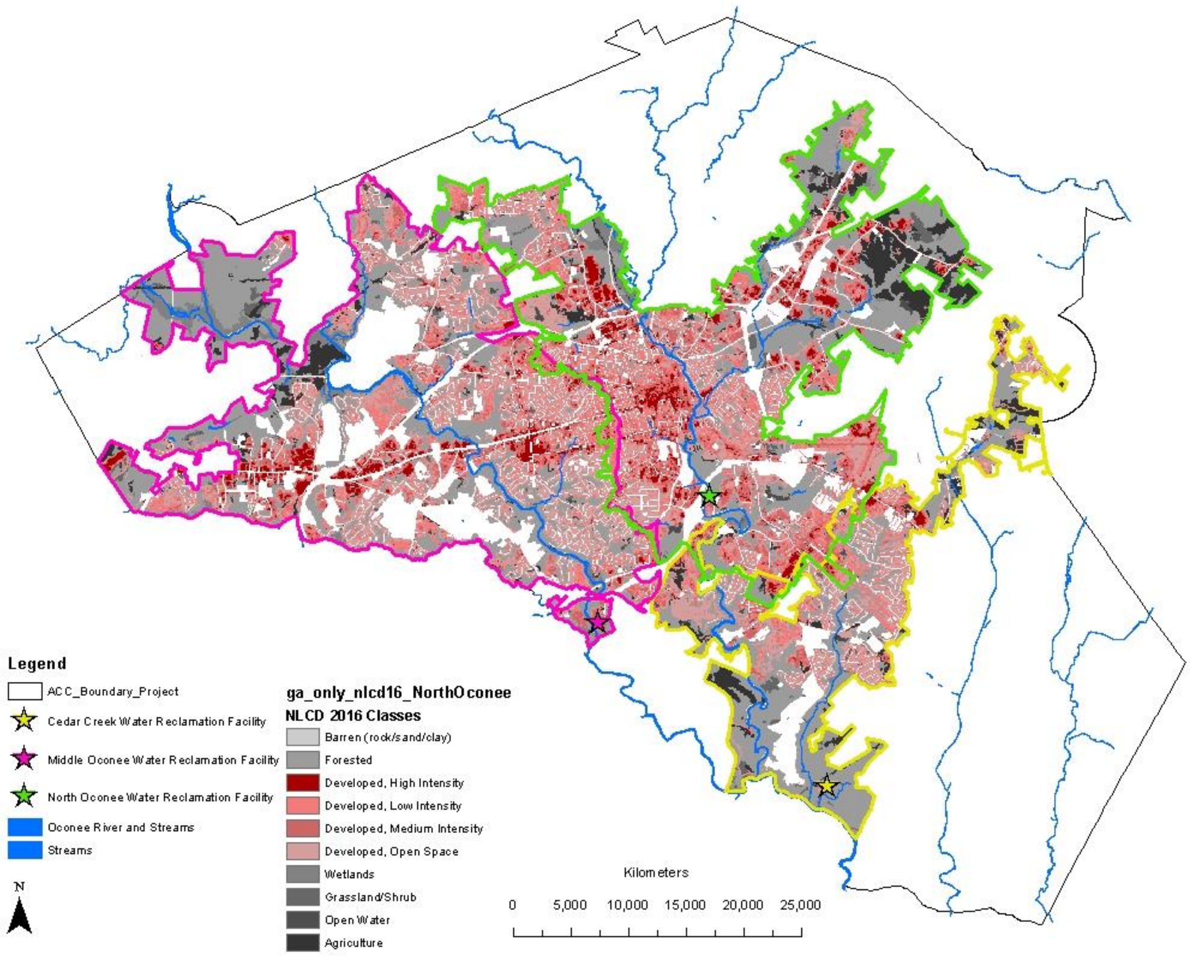


Figure 1. Map of sewersheds and dominant land use in Athens-Clarke County in Georgia, USA. The boundaries of the sewersheds are denoted with color (yellow: Cedar Creek; pink: Middle Oconee; green: North Oconee). All land use data were taken from the Athens-Clarke County GIS Clearing house or were provided by the county. The categories for deciduous forest, mixed forest, and evergreen forest were collapsed into the category "Forested". Shrubs/Scrub and grasslands were condensed into "Grasslands/Shrub". Woody wetlands and emerging wetlands were condensed into "Wetlands". Row crops and pasture/hay was grouped and renamed "Agriculture". The areas without landcover data (white areas) on the map indicate areas in the county that are served by onsite wastewater treatment systems, such as septic systems.

## Field collection

Bulk water samples were collected a total of 30 times in 2020 from treated effluent of the three water reclamation facilities in Athens, Georgia, USA (Appendix 1, Table 1). Samples were collected between three time periods each day (6 AM – 8 AM; 10 AM – 12 PM; and 2 PM – 4 PM), on one day each month between September and December (24-Sept-20, 08-Oct-20, 12-Nov-20, 10-Dec-20). In September, samples were only collected between 6 AM-8 AM due to safety concerns associated with the COVID-19 pandemic. During each sampling event, the triplicate water samples were collected using a 2-L stainless steel bucket attached to a 100% cotton rope (Lares et al., 2018; Liu et al., 2019; Magni et al., 2019; Murphy et al., 2016). Samples were stored in amber Nalgene© bottles (Miller et al., 2021). All water samples were collected at sites in the plants after the effluent had been treated by UV. The bucket was rinsed three times with water from the sample site between each sampling event to reduce contamination from site to site. The bottles were uncapped while the samples were poured into the containers and the amount of time the bottles were uncapped was recorded for each of the triplicate samples. The average time the bottles were uncapped (approximately 25 seconds) was used to create the sample used to assess field contamination (i.e., the field blank).

## Lab processing

In the lab, the bulk water samples were homogenized by inverting the sample three times and then poured into a clean graduated cylinder. The total volume for each sample was recorded. After the volume was recorded, the bottle and inner cap were rinsed three times with deionized water to remove the remaining particles. Subsequently, the samples and the rinsed water were poured through a set of 4.75mm, 1 mm, 250 µm, and 25 µm stacked sieves. Sieve sizes were selected to reduce processing time and smaller sieve sizes were used to trap microparticles used in personal care products and fibers from clothing (Miller et al., 2021). The sieves were rinsed with deionized water to improve the fractionating, and the contents from each of the sieves were rinsed into individual 2 oz. containers. All the material on the 4.75 mm sieve was carefully inspected, rinsed with the deionized water, and discarded if it was classified as organic material using methods outlined in De Frond and Munno 2019. After rinsing, each container was covered with aluminum foil and placed in a drying oven at 45°C until the water evaporated. After drying, 30 mL of 15% $H_2O_2$ was added to the sample and digested between 16 and 24 hours in the drying oven at 45°C to digest organic matter (Wiggin & Holland, 2019).

**Lab filtering**

After digesting, the samples were filtered. Eighty-two percent of the samples were filtered on cellulose filers (Whatman™ ME 25/21 ST 0.45 µm gridded filter, 47mm) for primary and secondary particle analysis in the lab. The remaining samples were filtered onto polycarbonate membrane filters (Isopore™ 1.2 µm PC Membrane, 47 mm) for additional analyses. The filtering apparatus was cleaned three times with deionized water and covered with foil to avoid atmospheric contamination (Rochman et al., 2019). The samples were homogenized and poured into the collection cup. All sample containers and lids were rinsed three times to remove all material from the sides of the cups and onto the filters. Quadrant lines were drawn on each filter using a laboratory chemical marker. The cellulose filters were stored in labeled aluminum tins covered in aluminum foil. The polycarbonate filters were placed onto labeled petri slides. The samples were covered and stored at room temperature until they were processed under a microscope.

**Particle quantification and secondary and tertiary confirmation**

Microplastics were initially quantified using light microscopy. Each dry filter was examined at 25-50× magnification under a dissecting microscope and particles were counted at least two times (McNeish et al., 2018; Primpke et al., 2020). Initially, microplastic particles were counted and categorized as fragment, pellet (bead), foam, film, fiber, or fiber bundle (Lusher et al., 2017; Rochman et al., 2019). The sum of the particles was calculated to estimate particle load in the sample. Subsequently, the filters were counted a second time. The secondary counts were either conducted by the same person after a period of at least two weeks since the previous count, or by a different lab member and they were categorized by type (McNeish et al., 2018). During the secondary counts, the color of the particles was also recorded for the sample. As the lab team always wore 100% cotton clothes and facemasks that were pink, any pink fibers were counted and recorded but were not included in the total number of particles for the sample. The total counts for both checks were averaged and reported if the totals were within three particles of one another. If there was greater deviation in the estimates, the filter was counted a third time by the senior lab member. The estimate from the third count was averaged with the previous count that was the most similar to the third estimate and was within three particles of the total count of that estimate. If there was uncertainty in whether a particle was composed of plastic, a needle was heated and placed near the particle (i.e., the hot needle test; (Barrows et al., 2018; De Witte et al., 2014; Devriese et al., 2015)). If the particle shriveled and melted, it was counted as a plastic particle. If the particle shriveled and singed, the particle was classified as a natural particle.

Nile red dye was applied to the remaining polycarbonate filters to quantify microparticles that were too small to see with standard visual identification (Maes et al., 2017; Primpke et al., 2020; Wiggin & Holland, 2019). Filters used for Nile red confirmation were moved from the petri slides to disposable filtration cups using rinsed forceps. Polycarbonate filters (chemically unreactive) were dyed with 5 mL of Nile Red solution (10 ug/mL) made with n-hexane from a stock solution (1 mg/mL) created in acetone. The filtration cups were placed in a box and in a dark cabinet for 30 minutes to incubate at room temperature. The filter was then rinsed three times with 5 mL of n-Hexane, placed back into the petri slide, and allowed to completely dry before examination under the dissecting microscope. The filters were then visually examined using light with a wavelength of 455 nm. The particles that fluoresced under a 455 nm light were counted and classified as fragment, pellet (bead), foam, film, fiber, or fiber bundle (Lusher et al., 2017; Rochman et al., 2019).

**Quality controls during field and laboratory work**

A field blank was taken at each site during each sample period to quantitatively account for contamination from the atmosphere and from clothing. A pre-rinsed, empty Nalgene© bottle was uncapped and left open for the duration of the averaged time that was found from the triplicate samples. This method was designed to mimic the opening and closing of the bottle and the sample exposure to the atmosphere.

Controlling for contamination from atmospheric deposition and clothing was important to account for any particles that may enter the samples that were not from the environmental sample. A field blank was taken at each site to account for the potential contamination (Miller et al., 2021). The field controls were taken at every site during each of the time periods. To control for contamination from clothing, hot pink jackets, hot pink face masks and purple nitrile gloves were worn to prevent fiber shedding into the samples and easy identification of contaminated fibers (Miller et al., 2021). Clothing consisting of 100% cotton was worn during sampling events to minimize microplastic contamination.

Blanks were also created throughout the lab processing. To control for contamination during all lab processes, pink jackets, pink masks, and purple nitrile gloves were worn during every step of the process (Rochman et al., 2019). Bright pink clothing was chosen because that is not a color that is typically found in environmental samples and is unique. Access to the lab was limited and anyone in the space was required to wear the pink clothing. All chemicals were filtered for contaminants and particles before use with the cellulose filters and stored in cleaned glass media bottles.

Filter controls were created using deionized water by placing a cellulose fiber into the filtering apparatus and processed using the same methods as the field samples. Three filter controls were created for every six samples processed. Digestion controls were also run by adding 30 mL of the 15% $H_2O_2$ to a clean sample cup. Samples were then filtered and processed using the same methods as the environmental samples. Digestion controls were created at the start of every new filtering day. These samples were placed in the aluminum tins and stayed covered until quantification under the microscope.

To assess atmospheric contamination within the lab before beginning sample, a blank cellulose filter was placed in a weigh boat and left exposed to the lab room for 24 hours. This was repeated over three consecutive days. The filters were then covered in aluminum foil and remained covered until quantification under a microscope.

**Data Analysis**

To examine the patterns in microplastic abundance, concentration, and morphologies, I built generalized linear models (GLMs) to test the effects of sampling time, sampling date, sampling location, size fractionations, and microplastic morphology on the abundance counts and concentrations after methods described in Hou et al. (2021), Nix et al. (2018), and Hall et al.

(2018). I identified the appropriate statistical distribution (e.g., Poisson, Gaussian, Negative Binomial [NB], Zero-Inflated Poisson [ZIP], or Zero-Inflated Negative Binomial [ZINB]) for the microplastic data. The best statistical distribution for the data set was the negative binomial [NB]. The appropriate distribution was identified through model selection (*model.sel (),* MuMIn Package; Barton 2020) and Akaike's information criterion corrected for sample size ($AIC_c$) for each of the possible distributions (Appendix 1, Table 2).

Completed models were ranked based on the $AIC_C$ and model weights ($W_i$) to determine the best model. 95% confidence intervals (*confint(),* stats package; R Core Team 2019) were calculated for the best fitting model. Other models within the selection were considered competitive and were reported if they had an $AIC_c$ difference within 2 of the top-ranking models (Hou et al. 2020). For all models, I assessed whether the datasets met the assumptions of homoscedasticity using K-S Lilliefor's test and Levene's test and I reviewed the residuals of the model to assess appropriateness of fit. A Wald Chi-Squared test was used to assess the significance of explanatory variables in the best-fitting model. Post-hoc Tukey contrasts for multiple comparison of means were also conducted on each of the significant explanatory variables in the model. I also compared the concentrations of microplastics among sites using a GLMM assess whether sampling location was a significant factor in the different concentrations of microplastics. All statistical analysis was conducted using the R statistical software with R version 4.1.1.

Estimates of daily and annual flux of microplastic pollution from wastewater treatment plants in ACC into the Oconee River were estimated by multiplying the mean microplastic concentration from each plant by the average daily discharge for each plant. This value was then multiplied by 365 for each plant to obtain an annual flux estimate. I reported the sum of the estimated totals from each plant to estimate the annual discharge of microplastic particles into the Oconee River from the three water reclamation facilities.

# CHAPTER 3

# RESULTS

I processed 90 samples (approx. 95 L of wastewater effluent) from the three water reclamation facilities during this study. I identified a total of 3,496 microplastic particles in the samples. Three size fractions (4.75 mm – 1 mm, 1 mm – 250 µm, 250 µm - 25 µm) and six microplastic morphology categories (fiber, fiber bundles, films, fragments, foams, and beads) were isolated from the effluent. The average concentration (No. Particles/L) of microplastics was 38.03 ± 9.58, 36.63 ± 9.22, and 35.55 ± 9.93 (mean ± SD) for Cedar Creek, Middle Oconee, and North Oconee, respectively.

Among all samples collected, the majority of the microplastics were fibers (89.99% of all particles), followed by fragments (7.21%), films (2.12%), foam (0.34%), fiber bundles (0.2%), and beads (0.14%), respectively (Figures 2, 3). On average, smaller microplastics were more common in the effluent of all plants. Size class 250 µm - 25 µm was the most common (46.22% of all particles) followed by size class 1mm - 250 µm (28.46%) and size class 4.75mm-1mm (25.31%). Average concentration (No. Particles/L) of microplastics were 29.11 ± 8.22, 43.15 ± 9.82, 32.96 ± 8.06, and 36.93 ± 7.23 (mean ± SD) for dates in September, October, November, and December, respectively (Figure 4). The average concentrations of microplastics from samples collected between 6 AM – 8 AM, 10 AM – 12 PM, or 2 PM – 4 PM were 34.86 ± 10.25, 38.85 ± 7.65, and 37.26 ± 9.97 (mean ± SD), respectively.

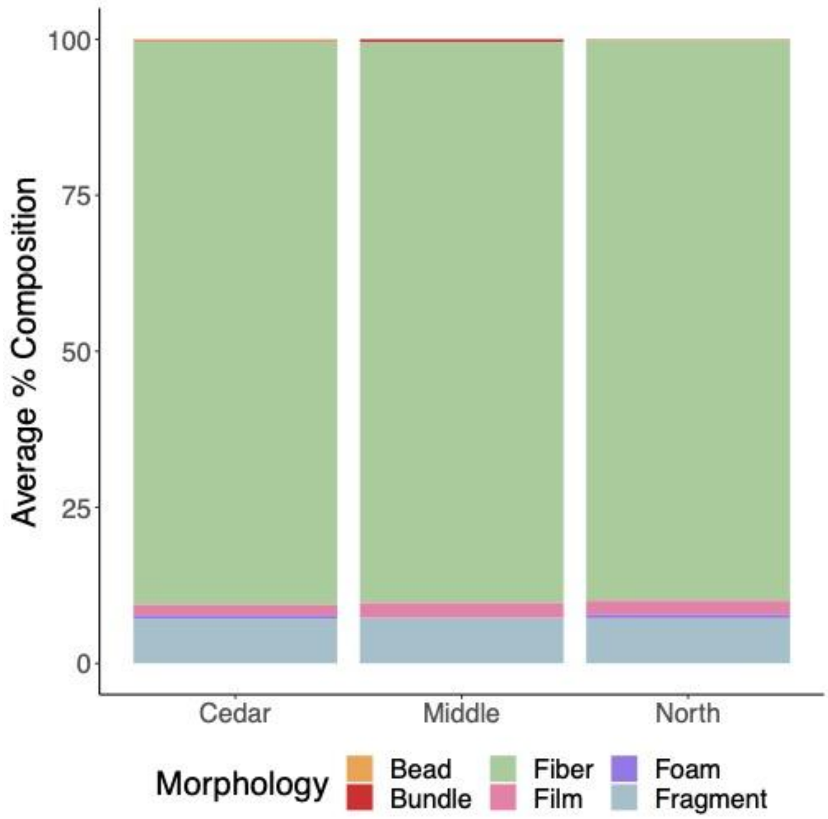


Figure 2. The average morphological composition of all microplastics that were identified in all the samples collected from the Cedar Creek, Middle Oconee, and North Oconee water reclamation facilities during this study.

Nile red was used to increase accuracy when counting fragment particles that were too small to see when using visual classifications, as the fluorescing dye allows smaller particles to be seen more easily. With the Nile Red analysis, I found that the average abundance of all microplastic particles was 108.5 (± 95.46),175.25 (± 20.9), and 200 (± 59.14) for Cedar Creek, Middle Oconee, and North Oconee, respectively (Appendix 1, Table 5). The concentration of microplastics with the Nile red analysis was 193.18 (± 88.2), 168.45 (±11.78), 188.33 (± 53) for

Cedar Creek, Middle Oconee, and North Oconee, respectively. All data were presented as mean (± standard deviation).

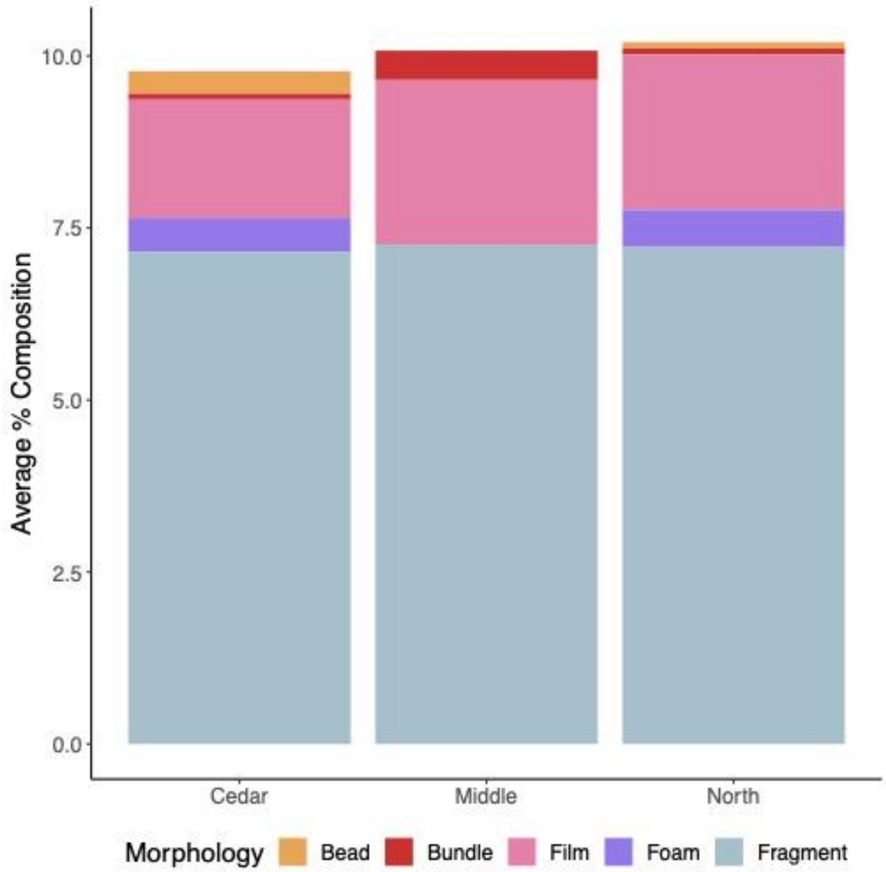


Figure 3. The average morphological composition of all microplastic morphologies measured in this study except fibers, which were identified in all the samples collected from the Cedar Creek, Middle Oconee, and North Oconee water reclamation facilities during this study.

Microplastic abundance patterns were best explained by the date sampled, the morphologies that were present within the sample, and the size classes in which the microplastics were analyzed (Table 3). Models that included these variables with water treatment plant included as a random factor explained 62.4 % of the variation (Table 3). There was no significant effect of the water reclamation facility on microplastic concentrations in the water samples (Figure 4). There were no significant differences in plastic abundance in samples collected in September, November, and December. However, there were significantly more microplastics detected in samples collected in October relative to the other dates other dates (Figure 5, Table 4). Post hot tests (Appendix 1, Table 5) indicated that the smallest particle sizes I accounted for through our sampling process were most common in our samples (Figure 6) and that fibers were significantly more common among all our samples than any other particle morphology (Figure 7).

Table 3. Results from Wald Chi-Squared test of best-fitting model

| | Chisq | Df | Pr(>Chisq) |
|---|---|---|---|
| Sample Date | 29.864 | 3 | 1.47E-06 |
| Fraction | 116.355 | 2 | <2.2e-16 |
| Morphology | 1265.509 | 5 | <2.2e-16 |

Table 4. Model selection results evaluating the best model for microplastic abundance for models with sample location (site) as the random effect. Null models were included as reference regardless of if the null was a competing model or not. Models with AICs greater than 2 were not considered competitive and we not reported.

| Model | df | LL | AICc | ΔAICc | wi |
|---|---|---|---|---|---|
| Date + Fraction + Morphology | 13 | -1074.81 | 2176.37 | 0 | 0.624 |
| Morphology + Fraction * Date | 19 | -1071.15 | 2181.88 | 5.51 | 0.0397 |
| Morphology | 8 | -1135.58 | 2287.44 | 111.07 | <0.001 |
| Null | 3 | -1505.15 | 3016.35 | 839.98 | <0.0001 |

Notes: LL is long-link ratio; $AIC_c$ is Akaike's information criterion corrected for sample sizes; $\Delta AIC_c$ is the difference from the best model; $w_i$ is the $AIC_c$ weight.

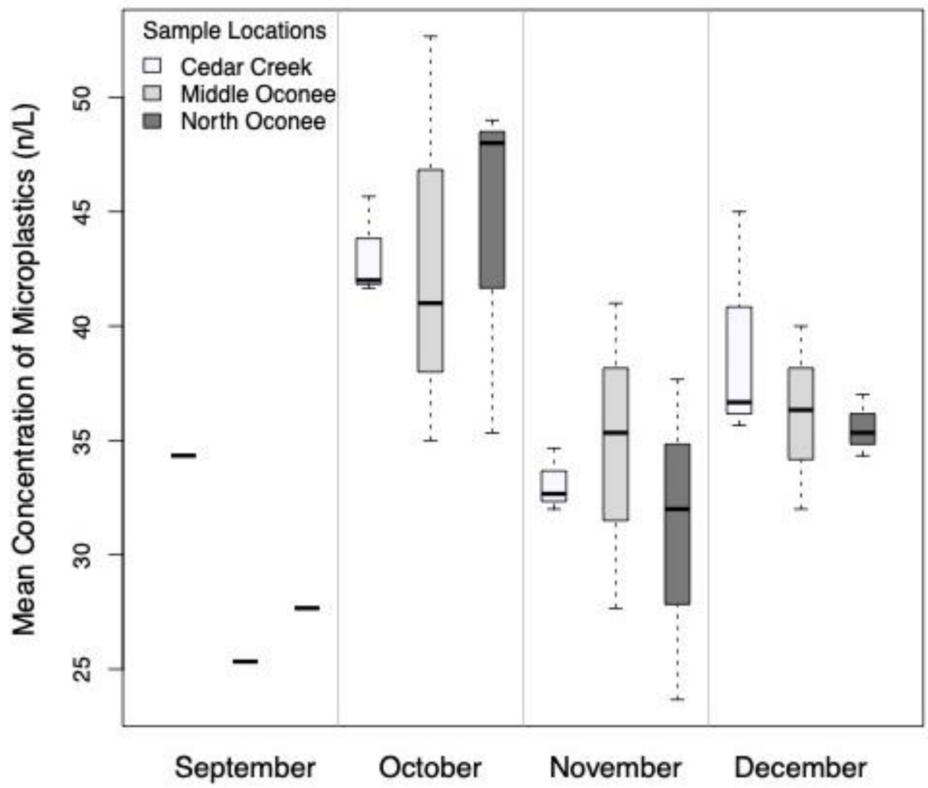


Figure 4. Mean microplastic concentration for each water reclamation facility by date. In September, we only collected samples during the early morning sampling period (6-8AM).

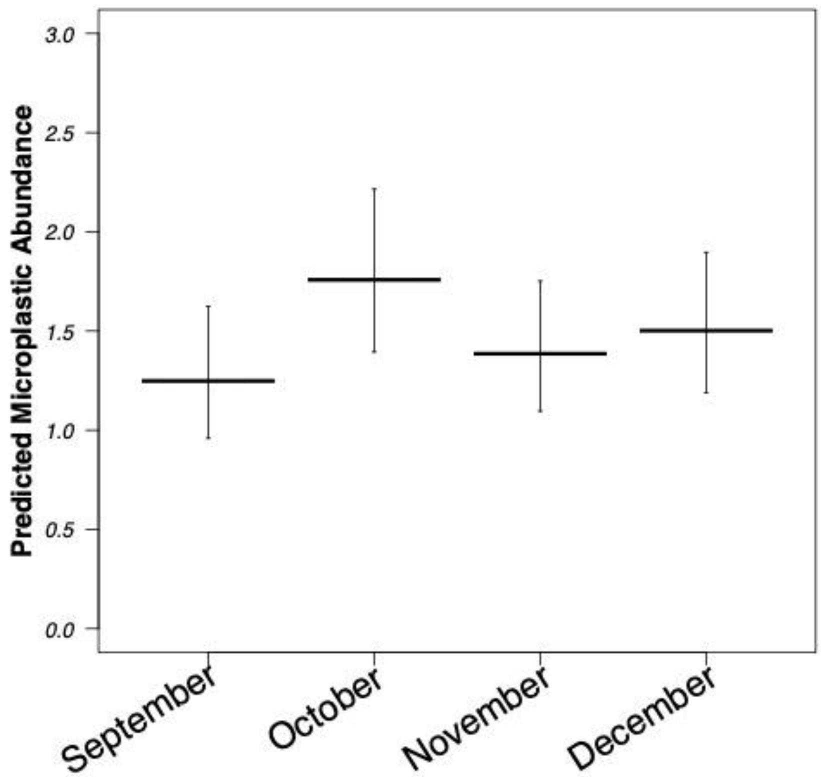


Figure 5. GLMM results for the date as a significant predictor of microplastic abundance in the samples. Error bars denote the upper and lower 95% confidence intervals.

Table 5. Statistical analysis using the Tukey contrasts for multiple comparison of means for plastic abundance for the dates sampled

| Linear Hypotheses | Estimate | Standard Error | Z Value | P Value |
|---|---|---|---|---|
| September - October | -0.34 | 0.08 | -4.29 | <0.001 |
| November - October | -0.24 | 0.05 | -4.49 | <0.001 |
| December - October | -0.16 | 0.05 | -3.04 | 0.012 |
| September - November | -0.1 | 0.08 | -1.28 | 0.57 |
| September - December | -0.18 | 0.08 | -2.3 | 0.1 |
| December - November | 0.08 | 0.05 | 1.48 | 0.44 |

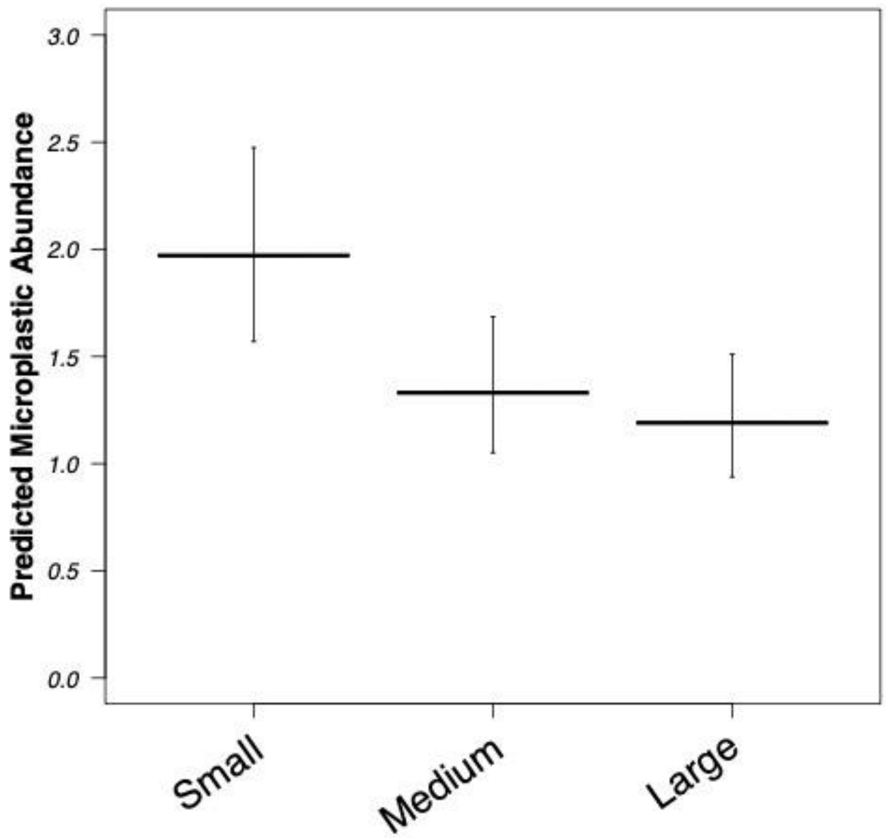


Figure 6. GLMM results for the sample fractionation as a significant predictor of microplastic abundance in the samples (small: size fraction 250 µm - 25 µm; medium: size fraction 1 mm - 250 µm; large: size fraction 4.75 mm – 1mm). Error bars denote the upper and lower 95% confidence intervals.

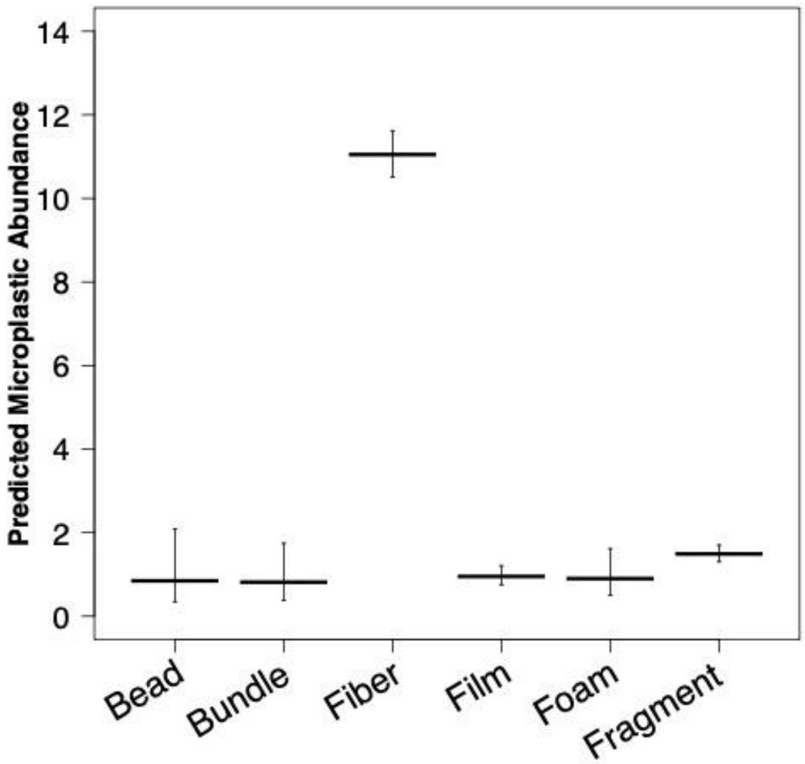


Figure 7. GLMM results for microplastic morphology as a significant predictor of microplastic abundance in the samples. Error bars denote the upper and lower 95% confidence intervals.

I estimated the daily and annual contribution of microplastic pollution entering the Oconee River through each of the water reclamation facilities by multiplying the average concentration of microplastics in samples from each plant during the study by the average daily discharge. My estimates suggest that an estimated 1.6 billion microplastics each day or 601 billion microplastic particles each year could be entering the Oconee River through wastewater effluent from Athens-Clarke County (Table 5).

Table 6. Estimated microplastic flux from effluent flows. MP represents the number of microplastic particles. MP/L indicates the concentration of microplastics in No. Particles/L. MGD indicates million gallons per day while MLD indicates million liters per day.

| Sample Location | MP/L | Average Discharge rate MGD | Average Discharge Rate MLD | million MP/day | billion MP/year |
|---|---|---|---|---|---|
| Cedar Creek | 38.03 (± 9.58) | 1.75 | 6.62 | 252 (± 63) | 92 (± 23) |
| Middle Oconee | 36.36 (± 9.22) | 3.65 | 13.82 | 502 (± 127) | 183 (± 47) |
| North Oconee | 35.55 (± 9.93) | 6.64 | 25.14 | 894 (± 250) | 326 (± 91) |

Analyses of microplastic controls and blanks can help determine where microplastic contamination occurs. Filter controls contained contamination of 4±3 particles per filter, while digestion controls contained 5±2 particles per filter (mean ± SD). Fibers were the dominant microplastic morphology found in both the digestion and filter controls. Atmospheric and sample collection contamination were collected as blank samples alongside the bulk water sample collection. The sampling contamination was 7± 4 particles per filter, with fibers also being the dominant morphology (mean ± SD).

# CHAPTER 4

# DISCUSSION

Examining the spatial and temporal drivers of microplastics from wastewater treatment plants is critical to understanding the diverse ways in which wastewater production and treatment is contributing to surface water pollution and the plastic cycle. The results from this study help fill an important gap in our understanding of the variability of wastewater-derived microplastic pollution. By generating this kind of information, we can make informed decisions about patterns in microplastic pollution and how best to design infrastructure and to create local policies to effectively manage plastic waste.

Effectively predicting spatial and temporal variation in microplastics entering watersheds through wastewater effluent is essential if we hope to reduce plastic pollution. My results indicated that there were no significant differences in microplastic concentrations among the three water reclamation facilities included in this study during the study period. Though the concentrations I report here are higher than some studies, (Raju et al., 2020), they align with values reported by other work. (Jiang et al., 2020; Leslie et al., 2013). I did not document differences in microplastic concentrations among the sampling periods each day, indicating that the treatment process was consistent within each plant through the day (Bayo et al., 2020).

In contrast, I did document a significant effect of sample date; there were significantly more microplastics collected in samples collected in October than any of the other dates. Greater plastic abundance did not seem to be associated with a weather event that may have increased runoff (Appendix 1, Table 3). However, the October date was the only sample date that occurred immediately after a home game for the University of Georgia Football team, when local population numbers most likely increased. Due to restrictions associated with the pandemic, the number of people in town to tailgate and attend the game was much lower than usual. For instance, only 20,524 fans were reported to have attended the game, which means the stadium was only operating at 22-25% capacity. If this increase in population was the driver of changes in plastic abundance, it is very likely under non-pandemic conditions, wastewater-derived microplastic pollution is exceptionally variable through time in ACC, and changes as students and fans move in and out of town throughout the year. Future work should focus on estimating temporal variation in plastic pollution in communities that experience frequent shifts in population size.

Smaller microplastics are commonly found in wastewater treatment effluent. Recently published work examining microplastic size structure in effluent document between 64% and 83% of all the plastics sampled were smaller than 1 mm (Bayo et al., 2020; Lares et al., 2018). In my study, ~75% of the plastics found in our samples were within this range. Murphy et al. (2016) suggested that larger particles may settle out into the sludge during the treatment process, but smaller particles may remain suspended and move through the entire process. Larger plastic particles could also be broken down into smaller particles during the treatment process due to physical abrasion when moving through the grit chambers and via exposure to UV light (Jiang et al., 2020). It is important to note, I only collected samples out of the water column of the effluent, and this may have impacted the size composition of the samples.

Microplastic fibers were the most common morphology of plastics I documented, comprising almost 90% of all the microplastics in the samples. This finding was not particularly surprising as the sewersheds were dominated by residential, rather than industrial, clients. In many higher income countries, the wastewater treatment plants receive wastewater from residential washing machines (Napper & Thompson, 2016), and a single piece of clothing can emit more than 1,900 fibers during each wash (Browne et al., 2011). Fibers may have been more common in my samples because they may be able to pass through the treatment process more easily that plastics with other morphologies. Jiang et al. (2020), documented a 100% removal rate of foam and tubular microplastics, but only ~66% of fibers were removed during wastewater treatment. Microfiber retention may be minimal due to their shape; they can fit through filters longitudinally (Raju et al. 2020). Furthermore, there is substantial evidence supporting the claim that microplastic fibers can move through almost all current membrane technologies (Murphy et al. 2016; Raju et al., 2020).

Sampling microplastics and sampling in wastewater effluent present unique challenges (Miller et al. 2021), and this study was not an exception. Though field blanks, filter controls, and digestion controls were accounted for, samples were not blank corrected (Miller et al., 2021), and I may have overestimated the number of particles entering the river. Additionally, I was unable to access samples from the untreated waste entering the plant. Therefore, my estimates of plastic pollution entering the watershed through wastewater treatment do not account for all the plastics exported from the plant in the sewage sludge. This waste may stay in the Oconee River watershed as landfill material or fertilizer or may be exported to other watersheds for disposal or reuse.

Increasing numbers of studies are highlighting the potential influence of wastewater effluent on microplastic pollution in aquatic systems (McCormick et al., 2016; McNeish et al. 2018). Alimi et al. (2018) estimated that eight trillion pieces of microplastics enter aquatic environments through wastewater treatment plants each day in the US. Here, I estimated the potential flux of microplastics entering the Oconee River daily through the three water reclamation facilities is approximately 1.6 billion particles. Research also demonstrates that the distribution of wastewater-derived plastic pollution is heterogeneous, even at smaller spatial scales. For example, research in the Chicago River documented a 10-fold increase in microplastic particles downstream of wastewater treatment facilities relative to upstream sites (Alimi et al., 2018). Most likely, the distribution and flow of the plastic pollution through the Oconee River also depends on the proximity to each of the reclamation facility discharge points.

The wastewater treatment process can be an effective way to reduce microplastic pollution entering rivers through effluent. Studies have estimated that between approximately 76-99% of microplastics can be removed from wastewater as it is treated (Lares et al., 2018; Liu et al., 2019; Long et al., 2019; Murphy et al., 2016). This indicates that many more plastics are being introduced to the system than I reported in effluent concentrations. Most likely, these plastics are removed as part of solid waste and are brought to a landfill or land application facility or are converted into fertilizer (Golwala et al., 2021). In either case, plastics trapped in solid waste can be re-introduced to a watershed through erosion and runoff and may still contribute to watershed-level plastic pollution (Golwala et al., 2021).

The implications of this study suggest that there may be large amounts of wastewater-derived microplastics entering the Oconee River from Athens-Clarke County. My work suggests that fibers are the dominant source of the microplastic pollution entering systems which is not surprising because this region is dominated by residential systems. To address this type of pollution, local governments may need to develop education campaigns and subsidized plastic waste mitigation programs that target household plastic generation produced through washing machines. Technologies such as the Cora Ball and Lint LUV-R have been shown to significantly reduce the number of microfibers in washing machine waste (McIlwraith et al., 2019). The results of this study add to the body of work documenting the contribution of wastewater derived microplastic to plastic pollution globally. Future work is needed to examine wastewater-derived microplastic pollution from a larger river network perspective to estimate the contribution of river networks to the global plastic cycle.

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APPENDIX 1

Appendix S1: Table 1. Sampling dates and times analyzed for the three Wastewater Treatment Plants in Athens-Clarke County.

| Sample Location | Dates Sampled | Times Sampled |
|---|---|---|
| North Oconee | 9/24/2020 | 6 - 8 AM |
| | 10/8/2020 | 6 - 8 AM, 10 AM - 12 PM, 2 - 4PM |
| | 11/12/2020 | 6 - 8 AM, 10 AM - 12 PM, 2 - 4PM |
| | 12/20/2020 | 6 - 8 AM, 10 AM - 12 PM, 2 - 4PM |
| Middle Oconee | 9/24/2020 | 6 - 8 AM |
| | 10/8/2020 | 6 - 8 AM, 10 AM - 12 PM, 2 - 4PM |
| | 11/12/2020 | 6 - 8 AM, 10 AM - 12 PM, 2 - 4PM |
| | 12/20/2020 | 6 - 8 AM, 10 AM - 12 PM, 2 - 4PM |
| Cedar Creek | 9/24/2020 | 6 - 8 AM |
| | 10/8/2020 | 6 - 8 AM, 10 AM - 12 PM, 2 - 4PM |
| | 11/12/2020 | 6 - 8 AM, 10 AM - 12 PM, 2 - 4PM |
| | 12/20/2020 | 6 - 8 AM, 10 AM - 12 PM, 2 - 4PM |

Appendix S1: Table 2. Akaike's information criterion corrected for sample counts (AICc) values for the statistical distribution of microplastic particles for the dataset with all of the abundances together (pooled). Abbreviations: NB = negative binomial; ZINB = zero-inflated negative binomial, ZIP = zero-inflated Poisson; ΔAICc = AICc difference from the best model; wi = AICc weight.

| Dataset | Distribution | AICc | ΔAICc | $w_i$ |
|---|---|---|---|---|
| Pooled | NB | 3014.33 | 0 | 0.7335 |
| | ZINB | 3016.35 | 2.03 | 0.2665 |
| | Gaussian | 3308.95 | 294.62 | <0.0001 |
| | ZIP | 4642.09 | 1627.76 | 0.00 |
| | Poisson | 4642.86 | 1628.53 | 0.00 |

Appendix S1: Table 3. Precipitation and weather data collected from the National Climatic Data Center for the dates sampled and the 7 days proceeding.

| Month | Day | Precipitation (inches) | Word Weather |
|---|---|---|---|
| September | 17 | 3.6 | 1 |
| | 18 | 0 | |
| | 19 | 0 | |
| | 20 | 0 | |
| | 21 | 0 | |
| | 22 | 0 | |
| | 23 | Trace | |
| | 24 | 0.5 | 1 |
| October | 1 | 0 | |
| | 2 | 0 | |
| | 3 | 0 | |
| | 4 | 0 | |
| | 5 | 0 | 1 |
| | 6 | 0 | 1 |
| | 7 | 0 | |
| | 8 | 0 | 1 |
| November | 5 | 0 | |
| | 6 | 0 | |
| | 7 | 0 | |
| | 8 | Trace | |
| | 9 | 0.2 | 1 |
| | 10 | 0.3 | 18 |
| | 11 | 0.47 | 128 |
| | 12 | 0.49 | 1 |
| December | 3 | 0 | |
| | 4 | 0 | 13 |
| | 5 | 0 | |
| | 6 | 0 | 1 |
| | 7 | M | 1 |
| | 8 | 0 | |
| | 9 | 0 | |
| | *10* | *0* | |

*Note: word weather symbols represent qualitative weather information. 1 is fog or mist; 2 is fog reducing visibility to ¼ mile ot less; 3 is thunder; 8 is smoke or haze.*

Appendix S1: Table 4. Summary table of the Nile red counts with abundance, concentration, and estimated plastic loads coming out of the three wastewater treatment plants. Results are reported with mean (± standard deviation).

| Sample Location | Average Abundance | MP/L | Average Discharge Rate MLD | Billion MP/day | Trillion MP/year |
|---|---|---|---|---|---|
| Cedar | 208.5 (± 95.46) | 193.18 (± 88.22) | 6.62 | 13 (± 0.58) | 4.67(± 0.21) |
| Middle | 175.25 (± 20.9) | 168.45 (± 11.78) | 13.82 | 23(± 0.16) | 8.49 (± 0.06) |
| North | 200 (± 59.14) | 188.33 (±53) | 25.14 | 47 (± 1.33) | 17.28 (± 0.49) |

Appendix S1: Table 5. Statistical analysis using the Tukey Contrasts for Multiple Comparison of abundance means for the size fraction and microplastic morphology categories.

| Linear Hypotheses | Estimate | Standard Error | Z Value | P Value |
|---|---|---|---|---|
| Medium - Large | 0.11 | 0.05 | 2.03 | 0.105 |
| Small - Large | 0.5 | 0.05 | 9.89 | <1e-04 |
| Small - Medium | 0.39 | 0.05 | 7.92 | <1e-04 |
| | | | | |
| Bundle - Bead | -0.04 | 0.6 | -0.06 | 1 |
| Fiber - Bead | 2.57 | 0.46 | 5.6 | <0.001 |
| Film - Bead | 0.12 | 0.47 | 0.25 | 1 |
| Foam - Bead | 0.06 | 0.56 | 0.11 | 1 |
| Fragment - Bead | 0.56 | 0.46 | 1.22 | 0.79 |
| Fiber - Bundle | 2.61 | 0.39 | 6.72 | <0.001 |
| Film - Bundle | 0.15 | 0.4 | 0.38 | 1 |
| Foam - Bundle | 0.1 | 0.49 | 0.2 | 1 |
| Fragment - Bundle | 0.6 | 0.39 | 1.53 | 0.58 |
| Film - Fiber | -2.45 | 0.12 | -20.13 | <0.001 |
| Foam - Fiber | -2.51 | 0.3 | -8.45 | <0.001 |
| Fragment - Fiber | -2 | 0.07 | -28.68 | <0.001 |
| Foam - Film | -0.05 | 0.32 | -0.17 | 1 |
| Fragment - Film | 0.45 | 0.14 | 3.29 | 0.01 |
| Fragment - Foam | 0.5 | 0.3 | 1.66 | 0.5 |