Microplastic Pollution in Relation to Wastewater Treatment Plants, Land Use, Local Physical Conditions, & Biological and Chemical Measures of Water Quality: Results of a Pilot Study on the South Branch Raritan River, New Jersey (USA)

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October 2018

Plastics are ubiquitous in today’s world. Water and beverages come in plastic bottles; a staggering variety of products are packaged in plastic; single-use plastic bags are used by retail stores; plastic fibers are found in many clothes and fabrics.

Plastic pollution is a widespread problem with significant, negative impacts on our rivers and streams. The public is learning that plastic bags, straws and other plastic debris often ends up in our waterways where they continue to accumulate, threatening the ecology of our rivers and ultimately our oceans. Fish and marine animals mistake plastics in the water for food and can ingest them or choke to death.

It is not just freshwater aquatic and marine life at risk. Recent studies have shown that our public drinking water supply is contaminated with “microplastics” – particles of plastic too small to be seen with the naked eye.

In June 2017, Raritan Headwaters Association launched a pilot study to determine potential sources of microplastic pollution on the South Branch Raritan River. We collected and analyzed water samples from 10 sites between Clinton and Branchburg, including four sites upstream of major wastewater treatment plants and four immediately downstream.

Our study found significant amounts of microplastics at every site sampled indicating that even the more rural headwaters streams contain microplastics. We found that some wastewater treatment plants on the river are probably sources of microplastics in the river. The majority of microplastics in the water samples were degraded fragments of bags, wrappers and other plastic objects. We also found that microplastics are associated with other indicators of pollution including specific conductance and phosphates.

The need for action is clear. The existence of microplastics at water treatment plants provides opportunities to target the removal of this pollutant from wastewater discharge. It also provides opportunities to educate families and businesses to decrease plastic waste. In addition, the study highlights the importance of programs like RHA’s annual stream cleanup in which over 1600 volunteers removed 13.3 tons of garbage, including 7,208 plastic bottles and 2,370 plastic bags, from 76 miles of streams in the Upper Raritan, thereby reducing the amount of plastics entering our rivers, drinking water and eventually Raritan Bay.

Microplastics pose a direct threat to the health and safety of our water supplies. Raritan Headwaters is committed to monitoring water quality, informing and engaging the public in watershed protection and advocating for policies that reduce plastic pollution in water.

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Mission of Raritan Headwaters

Raritan Headwaters (RHA) is a 501(c)3 non-profit conservation organization, formed by the 2011 merger of Upper Raritan and South Branch watershed associations (URWA and SBWA), both founded in 1959 to engage New Jersey residents in safeguarding water and natural ecosystems. As one of the largest watershed association in New Jersey, Raritan Headwaters protects, preserves and improves water quality and other natural resources of the Raritan River headwaters region through science, education, advocacy, land preservation and stewardship. Our combined organization is a strong voice in advocating for sound land use policies that protect critical water resources in the region. We are based in Bedminster, with a satellite office in Flemington.

Major RHA programs include water monitoring, ecological research, habitat restoration, land preservation and stewardship, policy and advocacy as well as extensive public education and outreach. Through our long-established Well Testing and Stream Monitoring programs, we have become a trusted source of data on the health of ground and surface water. We work to identify stressors on water quality including pollutants, land use practices, and factors associated with climate change. We monitor the effectiveness of various restoration practices for improving water quality as well as insuring resilience of these systems into the future as the impacts of climate change become more pronounced. We preserve land to protect water quality including properties we own and manage (11 wildlife preserves encompassing 450 acres, plus 32 conservation easements protecting 880 acres). Our stewardship efforts include riparian restoration, invasive plant removal and forest management. Our work engages community residents, including more than 3,200 volunteers and citizen scientists annually, in efforts to protect land, water and natural habitat in our region. www.raritanheadwaters.org

Acknowledgements

We gratefully acknowledge Kate Arnao, RHA Intern, for all her work counting and photographing the microplastics in each sample. We also thank Dr. Nicole Fahrenfeld, professor of engineering and environmental science at Rutgers University, and her students Kathleen Parrish and Sheri Elaker, for providing training in field methods of collection of microplastic samples from streams, separating the size classes in the lab and counting microplastics recovered from the sample. We are grateful for the extensive work they did of removing the organic material from our samples. Kate Arnao and Philip Worster, RHA Summer Research Interns, provided assistance in collecting and help with the first step of cleaning the samples. Melissa Mitchell Thomas, IT Manager and GIS Specialist at RHA, helped identify the location of WWTPs and other dischargers in the study area and produced the map of the study area in figure 1. Toby Davies conducted background research on discharge permits along the river. Dr. Nicole Fahrenfeld and Catie Tobin, Clean Ocean Action, reviewed earlier drafts of this manuscript. This research was funded with support from the environmental non-profit, Raritan Headwaters, and through a grant from the Rutgers Raritan River Consortium.

Cover photo of the South Branch Raritan River in Clinton by Brayden Donnelly.

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Executive Summary

- Microplastics, plastics < 5mm diameter, are an emerging contaminant of concern in aquatic habitats because they are ubiquitous, there are continual inputs of millions of tons, they are associated with direct and indirect ecological effects, and they have been found in drinking water supplies.
- A pilot study was undertaken by Raritan Headwaters (RHA) in June 2017 to determine the potential sources of microplastics from Wastewater treatment plants (WWTPs) and relationship of microplastic concentration to biological, physical habitat, and chemical water quality indicators on the South Branch Raritan River, NJ.
- Microplastics in 2 size classes (363-500 um and 500-2000 um) were sampled at 10 sites, 8 of which were paired as just upstream and just downstream of 4 WWTPs (2 major and 2 minor). In addition, RHA collected biological, physical habitat, chemical,and land use land cover data at 6 of the sites.
- The overwhelming majority of the microplastics in those size classes were secondary in nature (degraded pieces of bags, wraps and other larger objects) as opposed to primary (e.g., microbeads, microfibers, nurdles).
- The greatest concentration of microplastics was from major WWTPs but one of the minor WWTPs had an associated spike in microplastics just downstream.
- Microplastics rose distinctly just downstream of WWTPs as compared to just upstream in three of the four cases.
- Microplastic concentration was strongly positively correlated with specific conductivity, a measure of the dissolved solids and ions in the water, as well as phosphate. No significant correlation was found with biolobical, habitat or land use/land cover variables.
- The highest levels of specific connectivity were found just downstream of the two major WWTPs.
- The existence of point sources of microplastics at WWTPs provides an opportunity to target those places to minimize introduction of microplastics into the environment. It also indicates that water users on the sewer line may take individual measures to decrease introduction of microplastics into wastewater.
- The presence of non-point sources as additional targets along the River is supported by the finding of large amounts of bags and bottles each year during the annual RHA Stream Cleanup. This highlights the importance of these community cleanup programs as well as the role reduction of plastic bags and other single-use plastics bans will play in reducing microplastics.

Introduction

Plastics, synthetic polymers invented in the early part of the 20th century, have become a pervasive part of everyday life. Worldwide, we are now producing nearly **300 million** tons of plastic every year, half of which is for single use. Estimates for the amount of plastic reaching our oceans each year are in the range of more than 8 million tons (from https://plasticoceans.org/the-facts/). Ninety-one percent of plastic is not recycled (Parker 2017). Microplastics, defined as plastic particles <5-mm diameter (Duis and Coors 2016), have become a water contaminant of concern as awareness increases of the uibiquitous and abundant nature of these tiny particles in marine and freshwater ecosystems (Dris et al. 2015; Baldwin et al. 2016; Lechner et al. 2014). Identifying the sources of microplastics and documenting their effects on ecosystem health is important so that targets for decreasing the amount of microplastics entering and accumulating in aquatic ecosystems can be identified.

Some microplastics are intentionally manufactured as micro-structures (eg., microbeads, fibers, nurdles). Others result from the other breakdown of larger macroplastic objects such as plastic bags, bottles, wraps, fishing line and rope when exposed to ultraviolet light, physical weathering, heat, oxygen, and in a few cases microbial activity (review in Duis and Coors 2016). Depending on the polymers in the plastic and the environment where it is deposited, the breakdown to smaller particles and eventually individual polymers may take several years to thousands of years. Because plastics are not naturally occurring polymers they do not naturally decompose; few species of bacteria are equipped to break it down and thus, very few plastics are "biodegradable."

Rivers transport microplastics to lake (Baldwin et al. 2016; Zbyszewski 2011) and marine (Lebreton et al. 2017) environments where they accumulate in the water column and in sediments and eventually may enter the food chain when ingested by zooplankton, bivalves and other organisms that feed on tiny organic particles (review in Duis and Coors 2016). Increasing evidence that microplastics may also affect river and stream ecosystems, coupled with the role they play in transporting microplastics to lakes and the ocean has led to a recent focus on understanding the sources, transport, breakdown, and ecological effects of microplastics in rivers and streams. All are important areas of research necessary in order to identify sources and mitigate potential ecological and health impacts of microplastics.

Sources of microplastics to rivers include point sources such as effluent from wastewater treatment plants (WWTPs) or sewage treatment plants (STPs; Carr et al. 2016; Hoellein et al. 2017; Estahbanati and Fahrenfeld 2016; Ziajahromi et al. 2017; Mason et al. 2016; Talvitie et al. 2015; Kay et al. 2018; McCormick et al. 2016), industrial and combined sewer outflows (CSOs; review in Duis and Coors 2016; Ravit et al. 2017). A variety of non-point sources also exist including discarded plastic litter, plastic that blows from landfills and recycling trucks, and even wear of tires, which eventually enters streams as runoff from stormwater and snowmelt (Baldwin et al. 2016). Following precipitation macro- and microplastics enter waterways at an increased rate (Ravit et al. 2017). Large amounts of plastic debris can enter marine environments from severe or catastrophic weather events like hurricanes, tsunamis, and strong seas (sources in Duis and Coors 2016). More urban areas are expected to have increased inputs of microplastics from non-point sources, resulting from breakdown of discarded plastic waste such as bags, bottles and wraps. Atmospheric deposition of microplastics is also a source to streams and other aquatic ecosystems (sources in Duis and Coors).

There is emerging evidence that microplastics are adversely affecting organisms in rivers and streams including some benthic macroinvertebrates (Redondo-Hasselerharm et al. 2018) and fish (Sanchez et al. 2014; Ravit et al. 2017). One mechanism for these effects may be due to direct physical impairment caused by ingesting plastics (sources in Duis and Coors 2016). Another mechanism may be the toxicity of chemicals in the plastic polymers themselves (eg., polyethylene terephthalate (PET) and polyvinylchloride (PVC; Rochman et al. 2017; Barboza et al. 2018). Hydrophobic organic pollutants, including hexachlorinated hexanes, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), adsorb to the surface of microplastics and may be subsequently ingested at higher concentrations than normally present in the sediment by aquatic organisms resulting in bioaccumulation and biomagnification of these chemical contaminants in the food web (review in Duis and Coors 2016). Another concern is the development of antibiotic resistant bacteria in biofilms on the surface of microplastics (review in Rummel et al. 2017).

Streams receive a multitude of inputs of nutrients and contaminants from a variety of point and non-point sources and thereby exert combined stresses on riverine ecosystems. Sewage from WWTPs discharging into surface waters contain high concentrations of nutrients (nitrates and phosphates), organic matter, chloride, emerging contaminants such as pharmaceuticals and personal care products (PPCPs) and other contaminants (Aristi et al. 2015; Baloyi et al. 2014). Bacteria and other microbial pathogens from septic systems, animal waste, and combined sewer overflows (CSOs) may enter streams in stormwater runoff. Microplastics are yet another potential stressor to stream organisms and ecosystem function in streams. Presence of pollution tolerant macroinvertebrates increases downstream of WWTPs (Baloyi et al. 2014). Reliance on healthy stream ecosystems for clean drinking water saves the public billions of dollars every year (source). Many pollutants in streams can make their way into drinking water supplies, including microfibers in 94% of the tap water sampled in the United States averaging 10 microplastic particles per Liter (Orb Media 2017). 93% of bottled water from a variety of popular brands contained much higher concentrations ranging from about 63 microplastic particles per Liter to over 900 particles per Liter (Mason et al. 2018).

Further understanding of the effects and coincidence of microplastics with other water quality parameters as well as the impact and coincidence of microplastics on stream organisms contributes to our understanding of the extent to which this contaminant is affecting the health of stream ecosystems and potentially drinking water quality. Because microplastics are associated with the activities of people, and enter water through a variety of pathways, with WWTPs being a known point source, understanding the association of microplastics with a variety of discharge sources, land use, local physical conditions in the stream, and chemical conditions that might impair water quality and its effects on benthic macroinvertebrate communities to better understand point and non-point sources of microplastics, their impacts on freshwater ecosystems and drinking water quality, and the ways the problem of microplastics might be addressed.

Methods

Study Area and Site Selection:

Study sites were in the lower portion of the South Branch Raritan River before its confluence with the North Branch and Lower Raritan. The South Branch is part of the 470 mi2 North and South Branch Raritan Region, Watershed Management Area 8 (WMA8) in north-central New Jersey. It is the headwaters of a larger Raritan River Basin that covers approximately 2,849 km^2 (1,100 mi^2), making it the largest river basin located entirely within the State of New Jersey. Water from WMA8 flows into the tidal Lower Raritan (WMA9) and then into Raritan Bay and the Atlantic Ocean. The Bay is contiguous with waterways in some of the most heavily populated areas in the country including Newark and Jersey City in Essex and Hudson Counties, respectively, as well as the lower boroughs of New York City including Staten Island, Manhattan and Brooklyn. The streams and rivers of the Raritan Basin eventually supply water to 1.5 million people downstream in urban areas of NJ and potentially affect the water quality in the Bay, New York Harbor, and beaches in New York and New Jersey.

The southern half of WMA8, including most of the study area, is mainly located in the Piedmont physiographic province with its gently rolling terrain and unique soil profile. The Northern half of WMA8 is in the Highlands Physiographic Province. The South Branch Raritan River flows for a total of 82 km (51 miles) from its headwaters in Mount Olive Township. The watershed of the South Branch encompasses a 715 km^2 (276 mi^2) area, consisting of 25 named tributaries, and comprised of all or part of 16 municipalities. The dominant land use-land cover is urban (34.7%) and forest (32.7%) followed by agriculture (18.5%), wetlands (11.1%), and barren land (0.8%; NJDEP land use land cover data 2012). There has been an increase in urbanization over the past 3 decades with suburbs replacing farmland and to a lesser extent forests. Upstream portions of the river are classified as freshwater-2 non-trout as well as freshwater-2 trout maintenance and production (NJDEP). However, in the study area, most of the river is classified as freshwater-2 non-trout stream with a category 2 water designation. Two major and 2 minor WWTPs (Fig 1; Table 1) as well as 2 minor industrial discharges (Fig 1) are located on the South Branch Raritan in the area of study).

Ten sites were selected for microplastic sampling along a 23.07 mile (37.13 km) stretch of the South Branch Raritan River (Fig 1). Seven sites actively monitored as part of Raritan Headwaters stream monitoring program were included and 3 additional sites (sites 4, 6, and 9) that were not part of the current monitoring program but were needed to measure upstream conditions of WWTPs B and C. In addition, a retired site of RHAs, SB08a, was also included. Estahbanati and Fahrenfeld (2016) and Ravit et al. (2017), both of Rutgers University, reported results of studies of microplastics within the study area and there is some site overlap with Estahbanati and Fahrenfeld (2016). Sites in the 2017 study overlapped with a concurrent study of sediments being conducted by Dr. Nicole Fahrenfeld at Rutgers University.

Figure 1. Map of the study area along the lower South Branch Raritan River. Green circles, #1-10, are the stream sampling sites along a 23.07 mile (37.13 km) stretch. Red triangles represent WWTPs from A-D, upstream to downstream. Minor WWTPs are small triangles and major WWTPs are large triangles. The red squares indicate minor industrial discharges. The map does not indicate additional discharges, including other WWTPs, on tributaries to the study site.

Table 1. Characteristics of WWTPs within the study area on the South Branch Raritan River.

Wastewater Treatment Plant (WWTP) Discharging to South Branch Raritan River	Microplastic Sampling Site No. just downstream from facility	Description of facility
A	2	2.3 mgd facility; major
B	5	minor
C	7	3.8 mgd conventional activated sludge; major
D	9	minor

Data Collection:

Microplastics

Microplastic samples were collected in mid-stream at all 10 sampling sites (2 duplicates) using a 5" diameter, 15" long Student Plankton Net, 363 µ mesh made of nylon (Fieldmaster, Lenexa, Kansas). Collection of samples in the field and extraction of microplastics in the lab followed the methods described in Estahbanati and Fahrenfeld 2016. At the 10 study sites, nets were submerged in the water perpendicular to flow with a portion of the net opening above the water to collect floating particles. Nets remained in the water for one hour per sampling event. River velocity was determined at the time of sampling by measuring the time for a ping pong ball to travel a set distance averaged over three measurements. Nets were transferred to the lab for analysis.

The contents of each net were rinsed with distilled water into a series of sieves (2000, 500 and 250 um aperture size). The contents of the 2000 um sieve was discarded and the remaining contents of each sieve containing particles that passed through the 500-2000 were transferred to individual 200 ml beakers and the contents dried overnight at 70 degrees Celsius. The organic content of each sample was oxidized by hydrogen peroxide catalyzed by iron (II) (Baker et al. 2015; Estahbanati and Fahrenfeld 2016). The solutions were transferred to a funnel to facilitate density separation, covered with foil and left overnight for settling. In the density separation step dense particles settled and buoyant particles, including microplastics, floated on the surface. Settled particles were discarded and floating particles were rinsed with DI water and transferred to a glass petri dish (Estahbanati and Fahrenfeld 2016).

Recovered particles were visualized under a reflected microscope by a single obersver (Kate Arnao) for all samples. For both size classes of particles (363-500 um and 500-2000 um), all suspected microplastic particles were counted and photographed using a camera mounted on the microscope. Guidelines on identifying microplastics versus non-plastic particles were provided by a variety of unpublished resources as well as Hidalgo-Ruz et al. (2012). Though most of the organic material in samples was removed during the peroxide reaction, almost all the samples still contained a varying amount of organic material resistant to dissolving in peroxide including high lignin and cellulose materials (wood, plant fibers), chitin (insect parts), and possibly wool fibers. Particles were also categorized as to whether they were primary or secondary microplastics based on visual inspection of morphology including shape and surface texture. The concentration of microplastics in field samples was determined by dividing the number of microplastics counted by the volume of the sample collected (the product of the cross sectional area of the submerged net opening, river velocity, and length of time of sample collection). Best efforts were made to distinguish microplstics from non-plastic debris using available guidance. All samples have been saved for future analysis for plastic polymer composition using FTIR, which was not available at the time of this analysis.

Benthic Macroinvertebrate, Physical Habitat, Chemical and Bacterial Sampling

Seven of the 10 sites were also monitored in 2017 for water quality using biological, chemical and physical habitat assessments. The monitoring is part of the Raritan Headwaters (RHA) annual stream monitoring program for WMA8. RHA has annually collected data on benthic macroinvertebrates and habitat, and chemical data at 63-68 fixed monitoring sites since 1992 using the protocols developed by the USEPA (1999) and later refined by NJ Department of Environmental Protection (NJDEP; Poretti et al. 2007). In addition, beginning in 2017, Raritan Headwaters began collecting baseline chemical data on water quality parameters at all of its stream monitoring sites. In addition, data on macroplastics (plastic bottles and bags) has been collected by Raritan Headwaters along the river as part of the watershed-wide annual stream cleanup.

Visual and biological stream assessments are performed annually between June 15 and 30. By using the same sampling window each year the information gathered can be easily compared across years. Stream monitoring sites have been chosen so that they are suitable to use with the U.S. Environmental Protection Agency's Rapid Bioassessment protocol (Barbour et al. 1999). The protocol RHA uses is briefly described below.

The suitability of the riparian habitat is complete by completing a visual site assessment and calculating stream flow. A total habitat score is calculated (Table 2; Barbour 1999; NJDEP 2015). To collect a biological sample, a net is used perform a "kick" in the stream, disturbing the benthic habitat in a particular riffle so that all cobble, sand, and debris flow into the net. This is completed at 10 riffles or other suitable habitats in the stream. Once completed, samples are sent to Normandeau Labs in Stowe, Pa, an EPA certified laboratory, where an expert taxonomist identifies all macroinvertebrates collected down to the genus level and if possible species level. Using the relative abundances and pollution tolerances at the family level (Table 2; NJ Impairment Score and underlying metrics; Poretti et al. 2007) and genus level (Table 2; High Gradient Macroinvertebrate Index and underlying metrics; Jessup 2007), the macroinvertebrate community can be characterized and compared across sites and years.

Land Use Land Cover

Data on Land use land cover were obtained from NJDEP and analyzed by catchment area for each site ArcGIS(ESRI). Calculations included total area of the catchement, percent land use land cover type (forest, wetland, agriculture, urban and barren), and percentage change for each land use land cover between 1986 and 2012.

Table 2. Water quality parameters and brief description or source for methods used for collection.

Variable (Abbreviation)	**Brief Description/Source**
Biological (Benthic Macroinvertebrate)	Barbour et al. 1999; Poretti et al. 2007; https://www.state.nj.us/dep/wms/bfbm/amnet.html
High Gradient Macroinvertebrate Index (HGMI)	Jessup 2007
New Jersey Impairment Score (NJIS)	Poretti et al. 2007
Family Richness (F_Rich)	No. of Families
Genus_Richness (G_Rich)	No. of Genera
Family-Level Biotic Index (FBI)	An index of pollution tolerant families; Poretti et al. 2007?
Hilsenhoff Biotic Index (HBI), Genus level	An index of pollution tolerant genera; Hilsenfoff 1987
Percent Non-Insect Genera (Non_Insect)	Percentage of the Genera that are non-insect indicating increasing pollution tolerance
Percent Ephemeroptera, Plecopter, Trichoptera (EPT)	Percentage of pollution intolerant Genera
H2 Genera (H2)	Pollution Sensitive Uncommon Genera
H3 Genera (H3)	Pollution Sensitive Common Genera
Bacteria	
Escheria coli count	EPA Method Method 1603
Chemical	
Temperature (Temp)	Degrees Celsius: YSI Probe
Turbidity (Turb)	LaMotte Kit
pH	YSI Probe
Dissolved Oxygen (DO)	YSI Probe
Phosphate (P)	LaMotte Kit
Nitrate (N)	LaMotte Kit
Specific Conductance (SPC)	YSI Probe
Habitat Quality	
Total Habitat Score (HAB)	Habitat Score: Ratings of embeddedness, bank structure, stream bottom, woody debris, periphyton, and vegetated buffer are combined into a habitat score (Barbour et al. 1999; NJDEP 2015)
Land Use Land Cover, Catchement (2012)	NJDEP 2012 Land Use Land Cover GIS data used to calculate the percentage of major habitat categories standardized per unit area (acres)
Total Catchement Area	
% Forest (FOR)	
% Wetland (WET)	
% Agriculture (AGR)	
% Urban (URB)	
% Water (WAT)	
% Barren (BAR)	

Statistical Analysis:

Number of microplastics in each of the 2 size classes and overall were explored for trends in concentration going from upstream to downstream along the South Branch Raritan River. Mean microplastic concentration in 2 size classes and overall, respectively, were compared between 8 sites upstream (n=4) and downstream (n=4) of WWTPs using student's paired sample t-tests. In addition, mean microplastic concentration was compared between upstream sites (n=6) and sites downstream of WWTPs (n=4) using independent sample t-tests to allow for inclusion of all microplastic sampling sites in the analysis. The reason for the two tests is that not all the "upstream" sites were paired directly with WWTPs and what is far downstream of one site is upstream of another. All test were considered significant at $p<0.05$.

In addition, a subset of 7 sites that were monitored by RHA for biological, chemical, habitat, and bacterial parameters were used to relate microplastic concentrations to other water quality parameters using Spearman rank correlation. Because water quality parameters were collected at the 7 current RHA monitoring sites, these sites were used in these analyses to explore relationships between microplastic concentration and biological, chemical, habitat and bacterial data for the site. All tests were considered significant at $p<0.05$.

Results

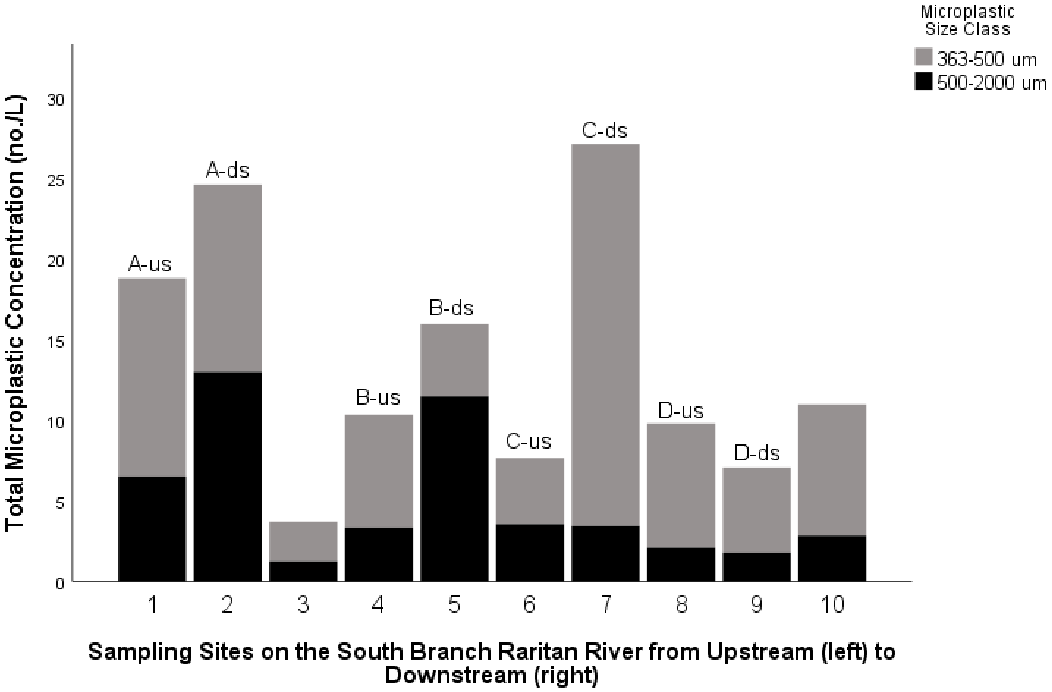
Microplastic particles in both size classes were found in samples collected at all 10 sites along the South Branch Raritan River. A monotonous trend from upstream to downstream was not detected but instead a fluctuation in microplastics with increases at both major WWTPs and one of two minor WWTPs relative to just upstream apparent (Fig. 2). Mean and standard deviation of microplastic concentration upstream (n=6, including 4 sites just upstream and 2 additional not associated with WWTPs) and downstream (n=4) of WWTPs for both size classes and overall total are provided in Table 3.

Table 3. Mean and standard deviation of microplastics in 2 size classes (363-500 um and 500-2000 um) and total upstream (US) and downstream (ds) of WWTPs.

Size Class	Location Relative to WWTP	Concentration (no./L)	
		Mean	Standard Deviation
363-500 um	**US**	6.602	3.379
	DS	11.266	8.877
500-2000 um	**US**	3.312	2.102
	DS	7.428	5.639
Total	**US**	9.914	5.148
	DS	18.693	9.112

Sites 4 and 6 had duplicate samples taken from the site. Mean values were used for both size classes of microplastics for the two sites.

Figure 2. Concentration of microplastics in two size classes (363-500 um and 500-2000 um) and total for 10 sampling sites numbered 1-10 going from upstream to downstream on the South Branch Raritan River. WWTPs are identified by letter (A-D). A and C are major WWTPs and B and D are minor WWTPs. Upstream (us) and Downstream (ds) indicates microplastic sampling site location relative to the wastewater treatment plant.



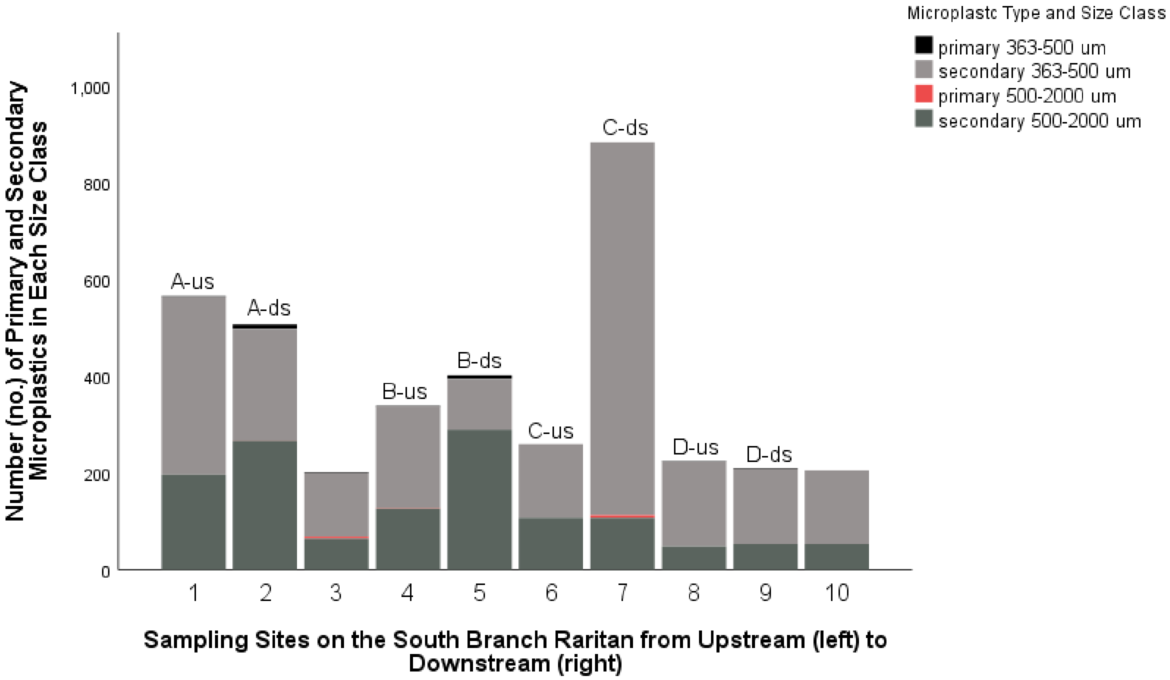
Microplastics Upstream and Downstream of Wastewater Treatment Plants

Paired upstream (n=4) and downstream (n=4) did not show a statistical difference for any size class (363-500 um and 500-2000 um) or combined (Fig. 2.; p=0.206, p=0.088, p=0.055, respectively). When means of upstream and downstream were compared for all parameters (microplastic, biological, chemical, bacterial, habitat) available for a particular site, none differed (p>0.05).

Primary vs. Secondary Microplastics

Microplastics in both size classes were predominantly secondary (Fig. 3), and included a variety of fragments and flakes from larger plastics. The few primary plastics detected included beads and fibers.

Figure 3. Absolute number (no.) of primary and secondary microplastics counted in each size class from samples taken along the South Branch Raritan River.



Relationship of microplastic concentration to other water quality parameters

Of the 6 chemical parameters included in the analysis, 2 showed a significant correlation with microplastic concentration; concentration of microplastics in the 363-500 um size class was strongly positively correlated with SPC (0.943, $p<0.01$, Table 4; $R^2=0.637$, $p=0.057$, Figure 4a) and microplastic concentration in the 500-2000 um size class was strongly positively correlated with phosphate (0.845, $p<0.05$, Table 4; $R^2=0.566$, $p=0.084$, Figure 4b).

None of the biological, habitat, bacterial or land use/land cover variables were correlated with microplastic concentration.

Table 4. Summary of significant ($p<0.05$* and $p<0.01$**) Spearman Rank Correlations of microplastics with other Water Quality Parameters

Microplastic Concentration Category	**Biological**	**Chemical**	**Habitat**	**Bacterial**	**Land Use/Land Cover**
363-500 um	--	SPC (0.943**)	--	--	--
500-2000 um	--	Phosphate (0.845*)	--	--	--
Total Combined	--	--	--	--	--

Significant correlations were further explored through graphing and regression analysis (Figure 3 a-e) to look for trends relating to WWTPs and other factors that might explain the observed relationships.

Figure 4a. Relationship, regression line and R^2, between specific conductance and microplastic concentration in the 363-500 um size class. Large red triangles indicates major WWTPs and the small triangle indicates a minor WWTP.

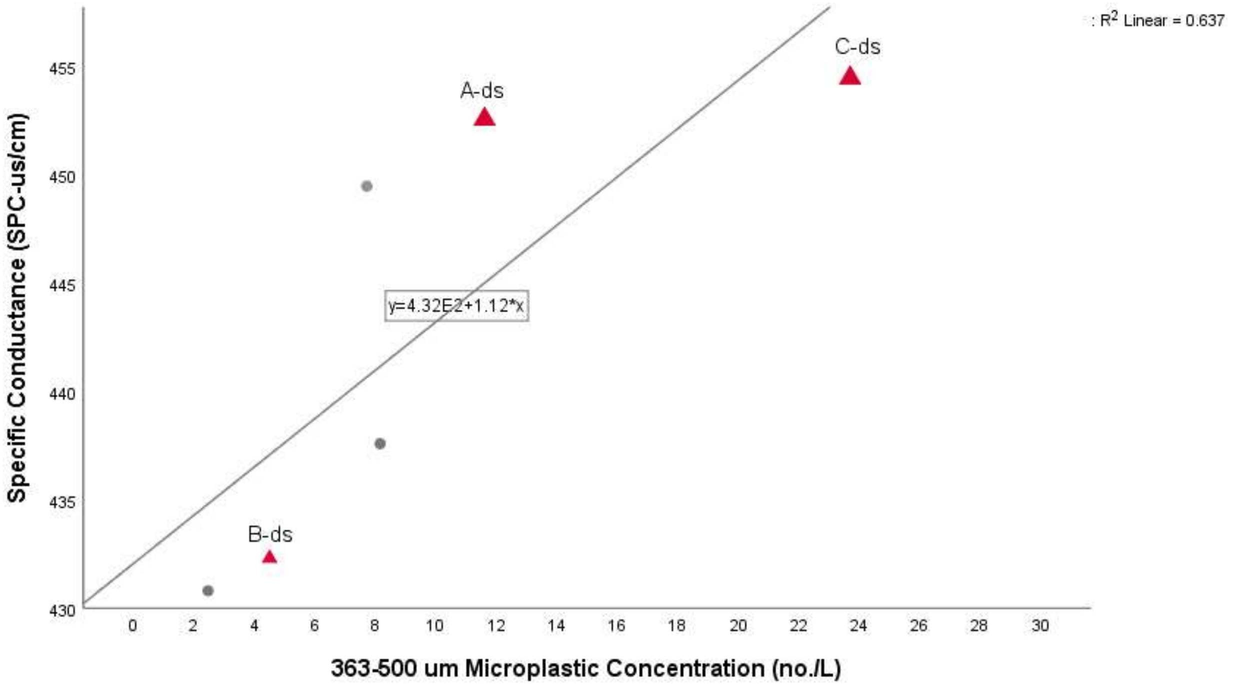
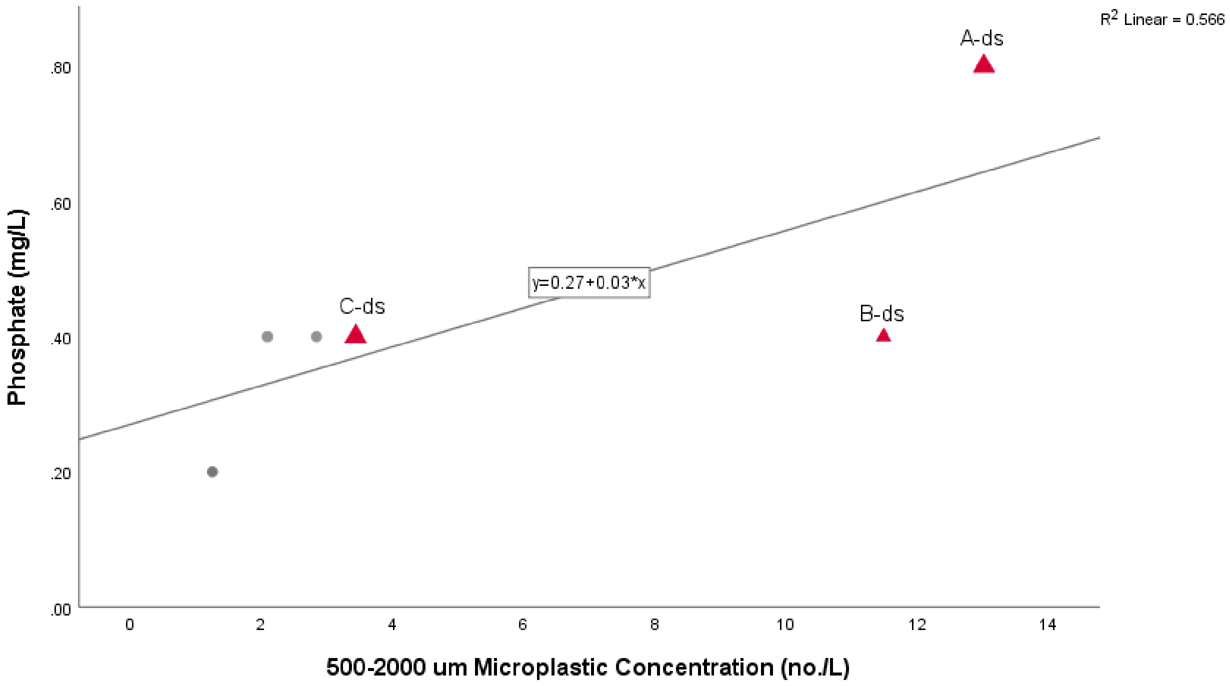


Figure 4b. Relationship, regression line and R^2, between phosphate and microplastic concentration in the 500-2000 um size class. Large red triangles indicates major WWTPs and the small triangle indicates a minor WWTP.



Other positive correlations include SPC is positively correlated with phosphate (0.808**) and pH (0.829*).

Discussion

Microplastic concentrations and the dominance of secondary plastics in the South Branch Raritan River were similar to findings from other studies along the river (Estahbanati and Fahrenfeld 2016, Ravit et al. 2017) and in other regions. It is likely that primary plastics and fibers were either not captured because they are smaller in size than the mesh used for sampling or they are being removed at the WWTP. Furthermore, there is not a continuous pattern of increasing or decreasing microplastics from upstream to downstream but rather microplastics increased at WWTPs, with the exception of one minor plant, and then gradually decreases likely due to settling out of plastics and dilution from water inputs. Obvious spikes in microplastics at three of the four WWTPs was not supported statistically, likely due to high variance in number of microplastics among paired sites for total microplastics and in both size classes. For example, some sites directly downstream of WWTPs appeared to have higher concentrations of the larger microplastics (WWTPs A and B) whereas another appeared to have higher concentrations of the smaller size class downstream (WWTP C). This in turn is likely due to differences in 1) the volume of effluent treated and discharged into the river, supported by smaller spikes at minor WWTPs, 2) potential inputs from industrial sources, 3) differences in the filtering capabilities and thus concentration of microplastics in effluent from each of the WWTPs, 5) sampling of sites on different days under different flow regimes, and 4) additional discharges from WWTPs and industrial sources along tributaries of the South Branch Raritan River. The latter included one discharge operated by Exxon Corp. on Beaver Brook upstream of sampling site 2, with an extremely high SPC, and thus, site 2 had an already high input of microplastics and SPC. In addition, a major WWTP operated by Flemington Borough, located on Bushkill Brook upstream of its confluence with the South Branch near/upstream of sampling site 6, could have potentially contributed to observed microplastics and other effects on water quality at that site.

It is suprising the benthic macroinvertebrate community was not detectably negatively impacted in areas downstream of WWTPs where microplastic concentrations were also highest. The HGMI, which is influenced by the pollution-sensitivity of benthic macroinvertebrate taxa in a community, did not show a significant correlation with microplastic concentration. Pollution greatly influences sensitive macroinvertebrates especially those in the families Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) while favoring some non-insect organisms. However, sensitive organisms are also influenced by local habitat conditions, which varied greatly among sites downstream of WWTPs and may explain why a direct correlation was not detected.

Chemical variables associated with discharge from WWTPs exhibited surprisingly few positive correlations with microplastic pollution. We would have expected nitrates, phosphates, temperature and pH to increase with microplastics because it was assumed they are at higher concentrations just downstream of WWTPs. Only SPC and phosphate, both as predicted, showed positive correlations with microplastics. We would have also expected temperature, pH, and nitrate to increase downstream to have an association with microplastics. Variability among WWTPs and other factors are likely explanations.

The positive relationship of microplastic concentration with specific conductance warrants further exploration. It is not known whether microplastics are impacting SPC or whether ions and Total Dissolved Solids (TDS), strong correlates of SPC, are coincident with high microplastic concentration. The correlation of phosphate (in the form of orthophosphate) with microplastic concentration supports this. In addition, charged microplastic particles may be contributing to increased SPC.

The predominance of secondary microplastics in the samples is an interesting finding and indicates the likelihood of multiple sources of microplastics. It appeared that secondary microplasics are entering the river from WWTPs and the origin and nature of those plastics needs to be investigated. In addition to the point sources (WWTPs) investigated in this study, the presence of non-point sources should be acknowledged as additional contributors of observed microplastic pollution. A significant amount of discarded plastic is picked up each year as part of an annual stream cleanup organized by Raritan Headwaters thoughout North and South Branch Raritan Region (WMA8; https://www.raritanheadwaters.org/get-involved-2/stream-cleanup-page/). In April 2018, 13.3 tons of trash was collected from 56 sites in Hunterdon, Somerset and Morris counties along 76 miles of stream in WMA8. In 2018, trash included 7,208 plastic bottles and 2,370 plastic shopping bags, which were quantified, but also included a variety of other plastics including mylar balloons and pool liners. The recovered plastic would eventually degrade into smaller plastics and potentially make their way into rivers and streams, especially as runoff and during flooding in severe storms as has been demonstrated in other studies (eg., Ravit et al. 2017). This adds further value to such annual clean-up programs. Beyond the important role of decreasing macroplastics such as bags and bottles, beautifying the community, and bringing public attention to the health of streams and rivers, these programs will also decrease the amount of microplastics that would eventually arise if plastic garbage were left in place to degrade.

Conclusions

The presence of point sources such as WWTPs allows for targeted programs to reduce microplastics from entering our aquatic ecosystems. Further investigations into ways of reducing the use of microplastics and technologies for collecting microplastics in homes and businesses before they enter the wastewater treatment systems as well as new technologies for removing microplastics at WWTPs, would help to reduce the volume of microplastics in aquatic ecosystems. There are also non-point sources of microplastics including discarded plastic trash and influx of microplastics in stormwater runoff that needs to be addressed. In general, programs to reduce the amount of plastics in use such as regulations eliminating microbeads in pharmaceutical and personal care products (PPCPs), bans on plastic bags and straws, encouraging reusable/refillable bags and containers as well as package-free products, and wide-spread recycling will aid in reduction of microplastics as the secondary plastics from the breakdown of bags and wraps. Presently, New Jersey is set to make great strides in limiting single-use plastics by enacting a plastic bag ban. Several municipalities in the state are enacting their own ordinances to address plastic pollution. In all respects, individual consumer behaviors and choices as well as support of local, state and federal limits on plastic use can have a big influence on the amount of microplastics entering our rivers and streams and eventually our drinking water.

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