Measuring the Total Microplastics in Marsh Creek– Upstream and Downstream of the Brentwood Wastewater Treatment Plant

Carrie Thang

ABSTRACT

Microplastic pollution in freshwater ecosystems is a growing concern with detrimental impacts on aquatic ecosystems and human health. There is limited research on microplastics in freshwater ecosystems and challenges to detect and efficiently remove microplastics from wastewater due to lack of standard protocol and removal technologies. This study investigates the presence and distribution of microplastics in Marsh Creek, focusing on the potential contribution of WWTP effluent to microplastic pollution. I took surface water and benthic samples and physical and chemical assessments at 4 sites along Marsh Creek, upstream and downstream of the discharge point on February 11, 2024. To process the samples, I performed filtration, digestion, density separation, quantification, and sorting. Surface water and benthic samples revealed higher total microplastics in the benthic zone (78.02 MP/g) than the surface water (51.90 MP/m^3). The benthic zones contain different substrates with varying capacities to hold microplastics, while surface water microplastics are easily transported with less time to settle. Fibers were the predominant type of microplastic (99.77% of the total microplastics in the surface water, 98.79% in the benthic zone). Upstream sites exhibited higher total microplastic counts than downstream sites, suggesting that the WWTP is not contributing much microplastics. Despite downstream containing half as many microplastics compared to upstream, factors such as flow rate, discharge volume, seasonality, substrate type, and other potential sources of microplastics to the stream should be considered. Nonetheless, downstream sites with WWTP-contributed effluent contained microplastics, indicating the need for improved filtration methods.

KEYWORDS

Freshwater systems, pollution, urban stream, effluent discharge, plastic contamination

INTRODUCTION

Plastic litter is accumulating in ecosystems worldwide and is the dominant type of anthropogenic debris observed in marine environments (Eriksen et al. 2013). This plastic debris can be found in high densities and can have far-ranging impacts on marine ecosystems, such as interacting with marine life and entering the bodies of organisms to cause adverse effects (Yang et al. 2021). When microplastics are ingested by marine organisms, they can negatively affect feeding and digestion (Lusher 2015). Microplastics are small plastic particles less than 5 mm in size (Masura et al. 2015) and can enter the water as manufactured small-sized "primary plastic" objects and "secondary plastics" defined as fragments from the breakdown of larger plastic objects (Drummond et al. 2022). Small microplastics (≤100 um) can be ~6 × 108 times more abundant than larger size classes in freshwater sediment and account for 11.6 to 21.1 million tons of plastic waste suspended on the Atlantic Ocean (Drummond et al. 2022). Despite the ubiquity of plastic pollution in marine ecosystems worldwide, data describing microplastic abundance in freshwater systems is limited.

The San Francisco Bay has a relatively high abundance of microplastics (Sutton et al. 2016), with the majority being fragments and fibers. Microplastics can originate from household, commercial, and industrial wastewater (Montecinos et al. 2022). Namely, fibers from synthetic textiles, fragments from the breakdown of larger plastic items, plastic particles from personal care products, and industrial pellets from commercial manufacturing (Fendall and Sewell 2009, Andrady 2011, Browne et al. 2011). Microplastic concentration in an area depends on specific regional factors, such as population size served and the type of industrial activity carried out (Montecinos et al. 2022). There are likely multiple sources that contribute to microplastic pollution in the San Francisco Bay because of the large population of 7.753 million that is supported by infrastructure around the city, such as roads and freeways, wastewater treatment plants, stormwater catchments, and diverse industries. Pollution pathways and the extensive residence times of microplastics may have contributed to higher levels of microplastic contamination (Sutton et al. 2016).

WWTPs collect and treat wastewater, but are recognized point sources of microplastic pollution in streams (McCormick et al. 2016). Municipal sewage contains high concentrations of microplastics and anthropogenic microliters. Despite the efficiency of WWTPs in removing these pollutants, when considering the large volume of effluent being released by the treatment plant

daily, even a modest amount of microplastics per liter can result in significant amounts of microplastics entering the environment (Conley et al. 2019). Plastic particles are removed in the primary and secondary sedimentation and biological filtration process, however microplastics can pass through the treatment and end up in effluent, entering the receiving water (Talvitie et al. 2015).

Microplastic contamination may be higher in San Francisco Bay compared to other urban areas in North America, including the Great Lakes, the Chesapeake Bay, and the Salish Sea (Sutton et al. 2016). Specifically, 60% of treated wastewater flows into the San Francisco Bay originating from the eight Bay Area WWTPs, with an average total daily discharge of 56 million particles. When comparing the average microplastic concentration in the San Francisco Bay to other urban coastal regions, the average Bay concentration (0.0088 particle/L) is extremely close to the highest concentration obtained from the garbage patch in the North Atlantic Subtropical Gyre (0.0023 particle/L) (Zhu et al. 2021). Although microplastic pollution in the marine environment in Bay Area waters is well-documented, limited data exists describing their specific sources and abundance in freshwater ecosystems. Further, the unknown effectiveness of WWTPs to remove this source of pollution and microplastic discharge via WWTPs raise concerns about this debris in freshwater systems, hindering the development of precise mitigation policies for these point sources.

My study aims to answer the following question: How do WWTPs contribute microplastics in freshwater ecosystems? By asking (1) What are the concentrations of microplastics found in the surface water? (2) What are the concentrations of microplastics found in the benthic zone? (3) How do these concentrations compare upstream and downstream of the WWTP? Based on existing literature, I hypothesize total microplastics will be higher in the benthic zone, total microplastics will be higher downstream from the WWTP, and fibers will be the most abundant type, followed by fragments, pellets, and foam. To evaluate my hypotheses, I will quantify the concentration of microplastics in different zones and compare the microplastic concentration and types between the surface waters and the benthic zone. I will also compare the total microplastics and type between the upstream and downstream sites of the Brentwood WWTP.

METHODS

Study Site

This study was conducted upstream and downstream of the Brentwood Wastewater Treatment Plant. Marsh Creek is a stream located in Contra Costa County in the San Francisco Bay Area in Northern California. Water collected within the Marsh Creek Watershed flows into Marsh Creek Reservoir, through cities (Brentwood, Knightsen, Oakley) downstream, and then into the Sacramento-San Joaquin Delta. These cities primarily consist of suburban development and agricultural land use. As Marsh Creek reaches the NE city bounds of Brentwood, the Brentwood Wastewater Treatment Plant (BWTP) discharges recycled water from the city of Brentwood into the stream channel (Figure 1, Table 1). Through Brentwood, the stream is a straight and engineered channel, with trapezoidal sides four to five meters deep. Stormwater, agricultural, and industrial runoff add to the stream. The effluent discharged from BWTP goes through primary and secondary treatment to remove inorganic matter, heavy material, and biological content. Then, the effluent is tertiary-treated to remove excess contaminants, such as phosphorus and nitrogen.

BWTP processes four million gallons of water daily from the city of Brentwood's population of 65,000 (City of Brentwood 2022). The discharge into the creek depends on both the volume of influent water entering the treatment plant and the volume of recycled water being used. At the end of the treatment process, effluent from the treatment plant is directed into a three-million-gallon storage tank. When the tank reaches capacity, excess effluent flows into the creek. In the summer when recycled water is in high demand, all of the effluent is directed into the tank, resulting in minimal discharge to the creek for a majority of the day. Conversely, during winter months when demand for recycled water is low, discharge into the creek is continuous (C. Wichert, personal communication).

Table 1. Distance from discharge point and GPS coordinates of sites. The distance of each site upstream or downstream of the discharge point and coordinates are provided.

Site	Meters from Discharge	Coordinates
A	420 meters upstream	37°57’43” N, 121°41’20” W
B	100 meters upstream	37°57’45” N, 121°41’6” W
C	0 – not sampled	37°57’46” N, 121°41’2” W
D	130 meters downstream	37°57’50” N, 121°41’2” W
E	250 meters downstream	37°57’54” N, 121°41’4” W

Figure 1. Map of Marsh Creek and Brentwood WWTP. Points A and B are upstream of the discharge point from the WWTP. Points D and E are downstream of the discharge point from the WWTP. Point C with the yellow arrow is the discharge point, where recycled effluent enters the creek (Moniz 2013). The blue arrow shows the direction of flow.

Data Collection

Study Sites and Characterization

I sampled four extraction points (A, B, D, and E) along Marsh Creek (Table 1, Figure 1), matching four of the five sample sites used by Moniz (2013). Points A and B served as upstream sampling locations, point C was the discharge point that I did not sample, and points D and E were the downstream sampling locations. Each sampling site featured regularly spaced areas with constructed riffles from large-sized bed materials. The sampling sites were interspersed with deeper "pool" or "run" zones, with some "riffle" sites featuring accumulated fine bed material and vegetation encroaching on them. The sampling sites varied in physical characteristics. Site A had vegetation across the stream with no visible rocks. The left bank of Site B and Site D were covered in matted vegetation. Site E was the most clear, with no vegetation in the stream and visible substrate from the pool floor. See Appendix A for photographs of sample sites.

Habitat Assessment and Water Quality Measurements

I used a visual habitat assessment for low-gradient streams. I evaluated and scored each site from 0-20 on ten parameters based on the condition category. For most categories, scores of 0-5 represent poor habitat conditions, 6-10 represent marginal, 11-15 represent suboptimal, and 16-20 represent optimal. Each condition category for scoring included a thorough description of the visual characteristics of the creek required for it to be placed in its appropriate category. I calculated the total scores for each site parameter by summing the scores for the parameters.

I performed a physical and chemical assessment to analyze the stream characteristics at different points of the stream (Barbour et al. 1999). To test the water quality, I measured the pH, salinity, water temperature, conductivity, dissolved oxygen, total dissolved solids, and flow. I used the Extech Instrument DO600 ExStik 2 D.O. meter to measure the dissolved oxygen at Site B, Site D, and Site E before the meter stopped working. I used the Extech Instrument EC500 ExStik 2 pH meter to measure the pH, temperature, total dissolved solids, conductivity, and salinity. Salinity was not measured at Site E. The flow was measured using a flow meter and

wading rod (Gurley Precision Instruments Model 1100) at one site upstream (Site A) and one site downstream (Site E). I measured the width of the creek and collected flow and water depth measurements at intervals of ten units along its width. To measure flow, I counted the number of revolutions of the propeller for 30 seconds. I used the velocity measurements, channel depth, and width intervals to calculate the discharge at each sampling site.

Microplastic Collection

The sampling process for microplastics largely followed Vincent and Hoellein (2021).

Surface water. To sample the surface water, I constructed a homemade drift net (Figure 2). The drift net was made using a reinforced clear plastic tarp, 1.5-inch PVC pipes, rebar stakes, snap fasteners, and a 35 μm Nitex bolt cloth. Using a sewing machine and a pattern designed by the Mendez Lab group at UC Berkeley, I joined the edges of the reinforced plastic tarp together in a cone shape and inserted the 16x9 inch PVC pipe structure into the large opening to provide structural support to the drift net. The team and I then sewed the mesh to the smaller end of the cone. Rebar stakes were affixed to the sides of the drift net to secure it in place during deployment. I set up the drift net at three different locations across the stream at each site. The drift nets were partially submerged approximately 20 cm deep and deployed for ten minutes. The top margin of the net was at the water's surface to collect floating material. Then, I transferred the collected samples from the net to a 16 Oz Wide Mouth Mason Jar by inverting the net and using a squeeze bottle to rinse trapped particles. The samples were stored for laboratory analysis. I took three samples of surface water at Site A and Site E, and two samples of surface water at Site B and D. At Sites B and D, there were fewer samples than expected because of the limited access to the stream. Vegetation and matted roots did not allow for the secure placement of the drift net.

Figure 2. Construction of drift net.

Benthic zone. To sample microplastics from the benthic substrates, I collected substrate samples approximately one foot upstream from the location where the drift net had been positioned earlier. I used a shovel to collect the substrate and transferred the material into a 16 oz. Wide Mouth Mason Jars (Vincent and Hoellein 2021). I took three samples of benthic substrate at Site A and Site E, and two samples of benthic substrate at Site B and D. Similarly to the surface water samples, vegetation and matted roots did not allow access to the substrate at Sites B and D.

Microplastic Processing

These processes with microplastics largely followed Masura (et al. 2015), however we did not use heat or catalyst.

Filtration. To process the microplastics for filtering and counting, I prepared the samples. Firstly, I pre-weighed 2.7 micron sized Whatman filters and foil envelopes that I folded. I vacuum-pumped the surface water samples through the Buchner funnel and used distilled water to thoroughly rinse trapped particles along the sides of the funnel. I placed each filter back into its respective foil envelope and transferred the envelopes into a Quincy Lab Model 10GC Lab

Oven set to 3 (65°C) until the filters were dry. To allow proper airflow, the envelopes were strategically placed in the oven to not overlap. The drying process took three days to completely dry every filter.

To prepare the sediment samples for processing, I individually poured each sample through a stacked arrangement of 4mm (No. 5), 1mm (No. 18), and 0.125mm (No. 120) stainless steel mesh sieves. I thoroughly rinsed the sample with distilled water to wash and transfer all the material through the sieves. I discarded the material from the 4mm (No. 5) and 1mm (No. 18) sieves and only kept material from the 0.125mm (No. 120) sieve (Masura 2015). I checked each emptied sieve for trapped microplastics under a microscope, using fine-tipped forceps to pick them out and transferred them into a vial with ethanol to preserve them. I transferred the material from the 0.125mm (No. 120) sieve back into its original jar and used distilled water to rinse trapped particles. I capped each jar and let the samples settle overnight. Then, I used a pipette to skim off as much top water as possible and vacuumed the skimmed water onto 2.7 micron sized Whatman filters. I transferred each filter into its respective foil envelope and placed them in the oven to completely dry. I loosely covered each jar with aluminum foil and placed the jars with the remaining sediment into a laboratory drying oven with a fan at 65°C for three days.

Digestion. The digestion process required 20 mL of 30% H_2O_2 and 20 mL of distilled water. I placed each filter into a 16 Oz. Mason Jar and gathered the jars of sediment samples. Working under the chemical fume hood, I added 20 mL of distilled water and added the 30% H_2O_2 in 10 mL increments to each sample, usually a total of 20 mL of H_2O_2. To speed up the digestion process, I swirled the jar and waited for the reaction to stop bubbling and steaming, which indicated that the reaction had come to completion. To ensure that there was no remaining H_2O_2 and organic material, I added 1 cm sized pieces of meat to each sample. If bubbles appeared, the reaction was not completed. I left all samples for 3-4 days for the reaction to fully come to completion. When the reaction was completed, I used pointed forceps to remove the meat and checked the sample for microplastics under a microscope using magnification up to 60X. I transferred the found microplastics into a small vial with ethanol. For the sediment sample with filters, I used pointed forceps to transfer them into vials. For the smaller sediment samples in the jars, I rinsed each sample with distilled water and poured the sample through a 300-micrometer mesh sieve. I transferred the remaining solids into a vial and used distilled water to rinse the

trapped material from the sieve.

Density separation. To begin the density separation for the bigger sediment samples, I dissolved 337g of NaCl into 1 liter of distilled water to create a solution with 1.2 g/cm3 density. The high density of the solution allowed the microplastics to float to the surface of the solution. I poured the solution into my sediment sample and swirled it to ensure all the stuck material was mobilized. I let the samples settle overnight to prepare for sorting.

I looked through the filters under the microscope and used pointed forceps to individually transfer each microplastic into a small vial with ethanol. Because the filters were sitting in the vial with distilled water used to rinse trapped particles, I poured the liquid through a 300-micrometer mesh sieve and looked at the sieve under the microscope for microplastics. For the sediment samples that were density separated, I used a pipette to transfer the liquid into the small sieve with a 300-micrometer mesh sieve. I looked through the sieve under the microscope and transferred found microplastics into a vial with ethanol. I labeled each vial with its appropriate site and sample number after separating microplastics from each sample.

Imaging and counting microplastics. I poured each ethanol sample containing microplastics for each site into a scanning tray with two silicon rings. Under the scope, I used pointed forceps to move microplastics into the ring to make scanning and counting easier. I placed the top onto the scanning trays and scanned them using an Epson Perfection E600 scanner (Mendez et al. 2018). After that, I classified the microplastics by type (fiber, fragment, line or foam) and counted the microplastics in each sample on ImageJ (Figure 3) (Schneider et al. 2012).

Figure 3. Image of a scan for first benthic sample at Site D.

RESULTS

Visual habitat and physical and chemical assessment

I found that downstream sites, Site D and Site E, had a higher median total habitat assessment score (107.5) compared to the upstream sites (107). The downstream sites had higher median scores on multiple parameters, including pool variability (2), sediment deposition (18), channel flow status (17.5), channel alteration (7.5), channel sinuosity (2.5), and riparian zone width (4) (Table 2).

The flow velocity downstream (1.68 m/s) was 28% greater than the flow rate upstream (1.21 m/s). The discharge upstream was 0.388 m^3/sec at 0.311 meters in depth, while the discharge downstream was greater at 0.667 m^3/sec at 0.427 meters in depth. I calculated the flow rate downstream based on the upstream flow rate (0.388 m^3/s) and the rate of discharge from the WWTP (0.175 m^3/s, an estimated 4 million gallons per day in winter months) and compared it to what I measured with the flow meter (1.21 m^3/s). My results yielded a difference of 0.104 m3/s, which is a close estimate to the calculated flow rate. The conductivity and pH decreased after Site C, which could be explained by the addition of discharge water from the WWTP that may have a different chemical composition from the treatment process (Figure 4).

Table 2. Habitat assessment scores for upstream and downstream sites. The upstream and downstream values are the median of the two sites scored separately.

Habitat Parameter	Marsh Creek, Upstream	Marsh Creek, Downstream
Epifaunal Substrate/Available Cover	15 (11-19)	10.5 (7-14)
Pool Substrate Characterization	20 (20)	13 (13)
Pool Variability	0 (0)	2 (0-4)
Sediment Deposition	14.5 (11-18)	18 (16-20)
Channel Flow Status	16.5 (13-20)	17.5 (15-20)
Channel Alteration	1.5 (0-3)	7.5 (2-13)
Channel Sinuosity	0.5 (0-1)	2.5 (2-3)
Bank Stability	20 (20)	20 (20)
Vegetative Protection	16 (12-20)	12.5 (12-13)
Riparian Vegetation Zone Width	3 (2-4)	4 (4)
Total Score	107 (105-109)	107.5 (107-108)

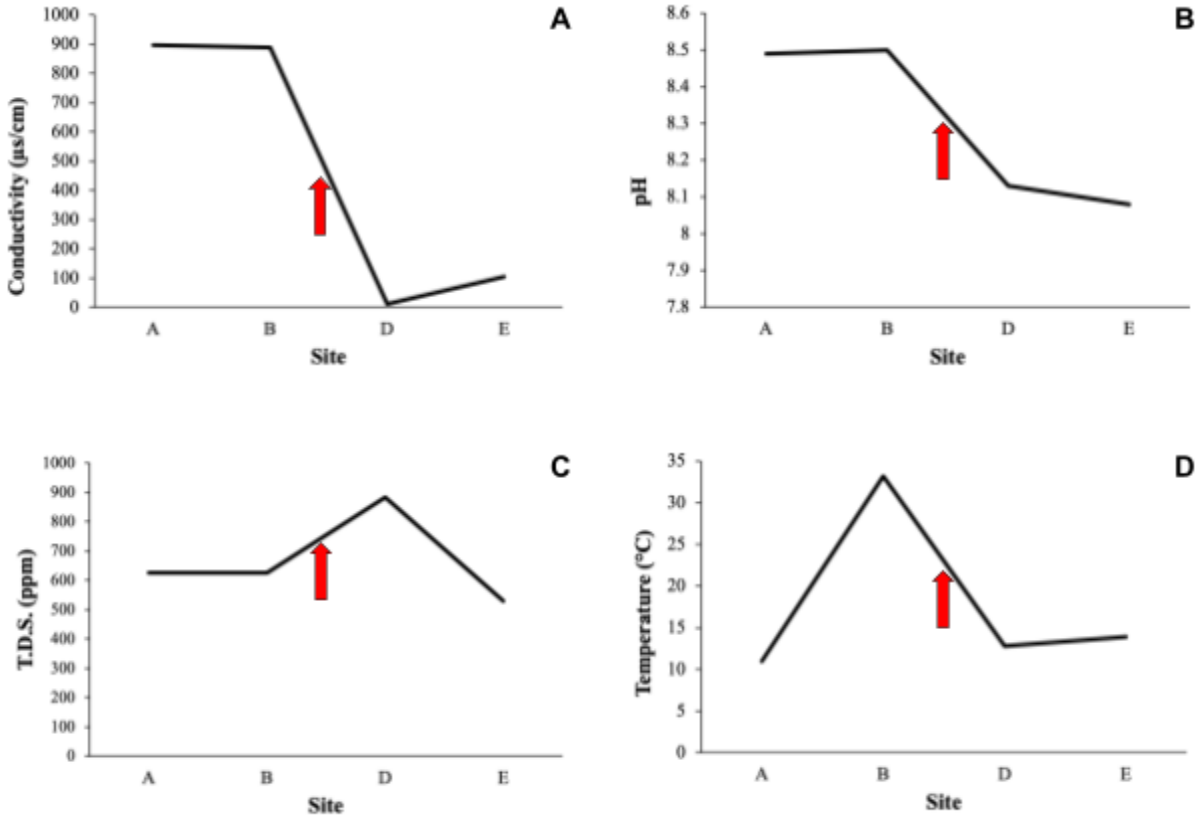

Figure 4. Water quality measurements from each site. (A) Conductivity, (B) pH, (C) T.D.S., (D) Temperature. The arrows indicate the discharge point, Site C. That dissolved oxygen was not due to an issue with the meter.

Surface water microplastics

I analyzed the total microplastics in the surface water individually for each site. I recovered a total of 436 microplastics, with microplastic types including fibers, fragments, and lines. Site B (125 microplastics, 16.79 MP/m^3) and Site E (153 microplastics, 16.33 MP/m^3) yielded the highest total of microplastics in the surface water (Figure 5B). Of the total microplastics found, microfibers accounted for 99.77% and lines accounted for 0.23% (Figure 5A). I did not observe fragments, pellets, or foam in the surface water.

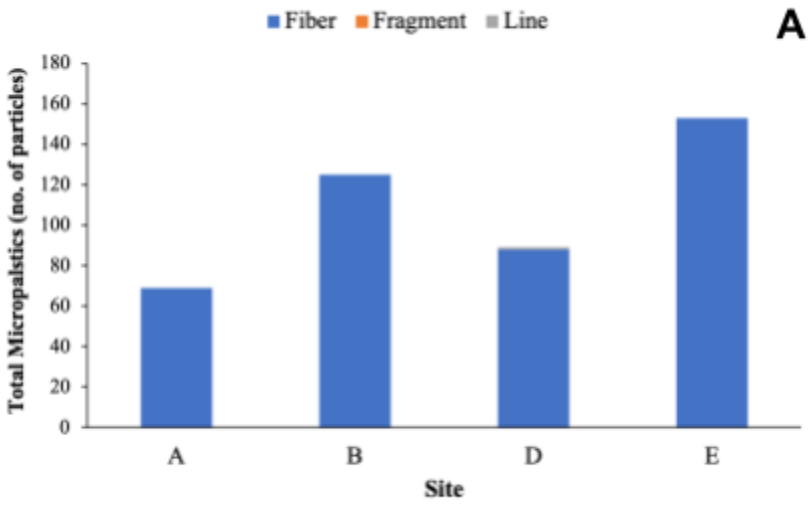

Figure 5A. Total microplastics from the surface water (no. of particles) sorted by types from each site. Three surface water samples were taken at Site A. Three surface water samples were taken at Site B. Two surface water samples were taken at Site D. Two surface water samples were taken at Site E. Microplastic types accounted for include fibers, fragments, and lines.

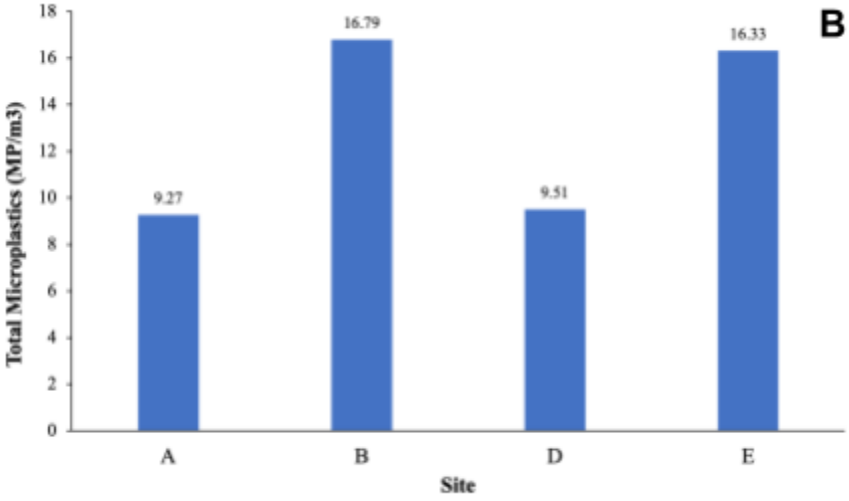

Figure 5B. Total microplastics from the surface water from each site (MP/m^3). Three surface water samples were taken at Site A. Three surface water samples were taken at Site B. Two surface water samples were taken at Site D. Two surface water samples were taken at Site E.

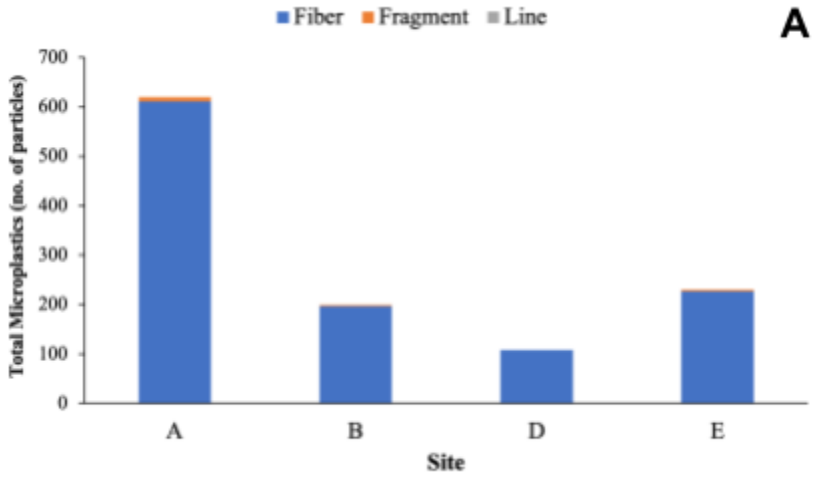

Figure 6A. Total microplastics from the benthic zone sorted by types across sites. Microplastic types accounted for include fibers, fragments, and lines.

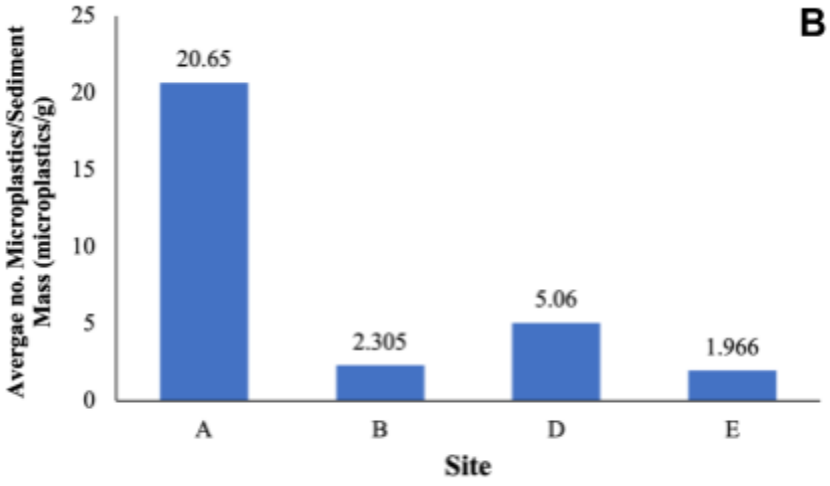

Figure 6B. Average number of microplastics per sediment mass from the benthic zone at each site. I calculated these values by dividing the total microplastics by the sediment mass and took the average individually for each site.

Benthic Zone Microplastics

I analyzed the total microplastics in the benthic zone individually for each site and observed a total of 1157 microplastics. The total microplastics and average number of microplastics per gram of sediment mass were highest at Site A (620 microplastics, 20.65 MP/g) and Site D (230 microplastics, 5.06 MP/g) (Figure 6B). Microfibers made up 98.79% of the total

microplastics found, and fragments made up 1.21% of the total (Figure 6A). Across all sites, more microplastics were found in the benthic zone compared to the surface water.

Total Microplastics

Upstream vs. downstream

Total microplastics in the surface water, measured in MP/m^3, and benthic zone, measured in MP/g, were higher upstream versus downstream of the effluent discharge point (Table 3A, Table 3B). In the surface water, Site B had the highest total of the upstream sites while Site E had the highest total of the downstream sites (Table 3A). In the benthic zone, Site A had the highest total of the upstream sites and Site E likewise for downstream sites (Table 3B). Both the surface water and benthic zone contained more microplastics upstream (Figure 7).

Table 3. Comparison of total microplastics. (A) Surface water (MP/m^3), (B) Benthic Zone (MP/g) to the distance of the site from effluent discharge.

A

Site	Distance from Effluent (m)	Total Microplastics (MP/m^3)
A	420 meters upstream	9.27
B	100 meters upstream	16.79
D	130 meters downstream	9.51
E	250 meters downstream	16.33
Upstream		26.06
Downstream		25.84

B

Site	Distance from Effluent (m)	Total Microplastics (MP/g)
A	420 meters upstream	61.69
B	100 meters upstream	4.61
D	130 meters downstream	5.82
E	250 meters downstream	5.90
Upstream		66.3
Downstream		11.72

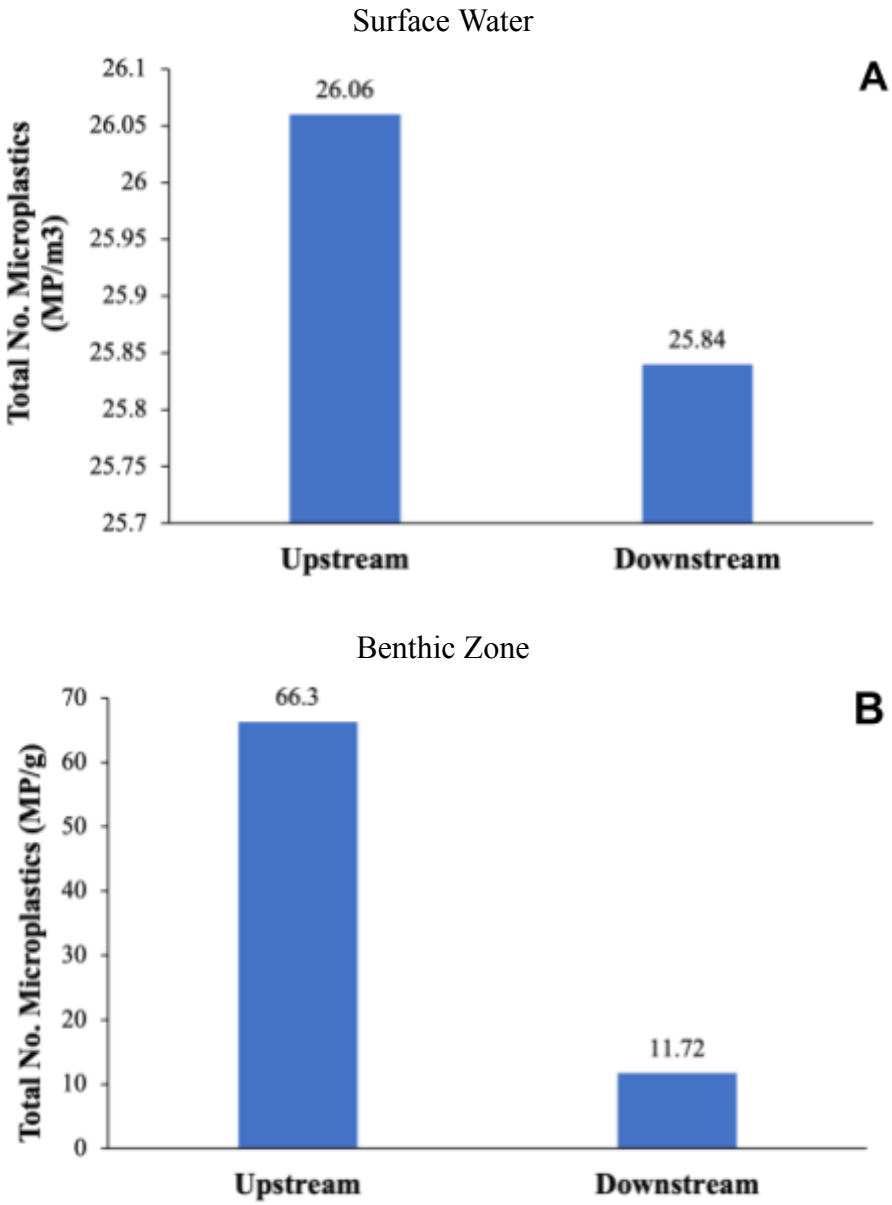

Figure 7. Total microplastics upstream and downstream. (A) Surface Water, (B) Benthic Zone. I calculated the total number of microplastics in the upstream and downstream in benthic zone by summing up the microplastics/g of sediment for the upstream or downstream sites. I calculated the total number of microplastics in the upstream and downstream in surface water by summing up the MP/m^3 for the upstream or downstream sites.

Over Distance

Total microplastics did not decrease with distance downstream from the effluent discharge point. In the downstream surface water samples, Site D, which is 130 meters downstream from the effluent, had the lowest total microplastics at 9.51 MP/m^3 (Table 3).

Similarly in the downstream benthic zone samples, Site D had the lowest total microplastics at 5.82 MP/g (Table 3).

DISCUSSION

Discharged effluent from WWTPs is likely contributing microplastics into streams, as benthic substrates influenced by WWTP effluent had the highest mean concentration of microplastics compared to other sources of microplastics (Wang et al. 2022). Microplastics are an overlooked water contaminant due to the expectation that WWTPs are 96-99.9% effective in filtering out microplastics (Drummond et al. 2022) and their small size of 5mm (Masura et al. 2015). I determined the total microplastics and microplastic types found in the surface water and benthic zone at four different sites along the creek, finding that the benthic zone contained more microplastics than surface water. Fibers were the most prominent microplastic type, and total microplastics were highest upstream. The total of microplastics upstream was 1.74 times greater than the total of microplastics found downstream.

Physical and Chemical Assessment

Marsh Creek is a heavily urbanized and engineered stream channel, channelized to allow stormwater to move quickly through a channel, thus reducing the risk of flooding (Moniz 2013). When comparing the habitat scores of all the sites, downstream sites had a higher average habitat score compared to upstream sites. This is because downstream sites receive a higher volume of water, which has beneficial effects on these regions of the stream (Moniz 2013). Moniz (2013) received the same results when performing a habitat assessment at the same sites at Marsh Creek. Variations in our scores existed among the study sites, but differences in values between upstream and downstream sites demonstrate the influence of effluent on water chemistry (Table 4).

Using the net size, the average velocity from the flow measurements, and the number of microplastics found at each site, I calculated the number of microplastics found per cubic meter of water for all the surface water samples. Site A contained 9.27 MP/m3, Site B contained 16.8 MP/m3, Site D contained 9.51 MP/m3, and Site E contained 24.57 MP/m3. Although there is no

trend between these values, Site E may have the highest concentration of microplastics because of the higher velocity downstream, which may give the microplastics less time to settle in the sediment or may prevent storage of microplastics in sediments at Site D.

Total Microplastics: Surface Water and Benthic Zone

The surface water and benthic zone both contained microplastics, suggesting that different ecological zones of the creek will all hold microplastics. I found that total microplastics in the surface water and benthic zone differed significantly, with 72.63% of microplastics residing in the benthic zone and 27.36% residing in the surface water. Because the stream has an artificial channel with constructed riffles, these are considered to be built up areas where sediment can collect. Sediment samples were limited due to the depth of the channel that did not allow us to look at the deeper depositional zones. Upstream sites, Sites A and B, featured heavy vegetation and matted roots, and had sand and smaller rocks distributed with relatively bigger rocks. The downstream sites had visibly more fine silt and clay in the benthic zone.

Microplastics tend to have long residence times in the benthic zone because they become trapped between substrates and end up stuck in the algae and fungus (Drummond et al. 2022). The benthic zone is made up of different microhabitats that vary in their ability to retain microplastics (Vincent and Hoellein 202). Vincent and Hoellein (2021) found similar results in the benthic zone, with depositional zones (e.g., FBOM) containing higher concentrations of microplastics. Corroborating this, Wang et al. (2022) found that benthic substrates influenced by WWTP effluent had the highest mean concentration of microplastics compared to stormwater, industrial runoff, and agricultural runoff. Therefore, urban streams that collectively receive stormwater, industrial runoff, agricultural runoff, and WWTP effluent will experience significantly higher concentrations of microplastics than normal streams. The predominant surrounding landscape at the four study sites at Marsh Creek was agricultural and industrial, which can also contribute microplastics into the stream via runoff.

The total microplastics found were limited to what I was able to collect at each site, with some sites having easier access to the substrate and some being more difficult. To standardize my results, I calculated the number of microplastics per sediment mass in grams. When considering this, Site A had the highest average number of microplastics per sediment mass (20.65 MP/g),

and Site E had the lowest average (1.96 MP/g). I calculated the average microplastics from the upstream sites (506.5 microplastics) to serve as my baseline. Therefore, my downstream values serve as the "background level." After subtracting the baseline value from each downstream site individually, I observed that Site D yielded -309.5 microplastics and Site E yielded -123.5 microplastics. Using these values to evaluate the contribution from the WWTP to the channel, my results suggest that the WWTP is not contributing a positive amount of microplastics to the stream.

Microplastic Types: Surface Water and Benthic Zone

Fibers and fragments were found in the surface water and benthic zone with varying abundances. However, fibers dominated the other microplastic types. Montecinos et al. (2022) and McCormick et al. (2016) observed similar trends, with fibers being the most abundant type of microplastic. Of the total concentration of microplastics found after the WWTP, their study observed that 70% were microfibers (Montecino et al. 2022). A significant portion of microplastics consisted of fibers in both upstream and downstream sites, with higher fiber concentrations observed downstream across all sites except two (McCormick et al. 2016). I observed a similar trend, with a significant amount of fibers in both upstream and downstream sites. Fibers made up 99.05% of the total microplastics found, and fragments and lines made up less than 1% combined. The primary source of microfibers come from domestic and commercial laundering, and as many as 700,000 microfibers can be released into the wastewater each cycle (Liu et al. 2022), which explains why we saw a majority of fibers. Filtering at the household laundry level could be an impactful way to mitigate this point source.

On my sampling day, there was a noticeable amount of litter and potential sources of microplastics in and around the stream. This includes clothing, plastic bags, and plastic bottles, all of which can break down to release fragments and fibers. Surface water samples are limited to the volume of water captured by the drift net at the exact section of the water. Therefore, total microplastics from the surface water were likely underestimated because of the small net opening and mesh size. The amount of microplastics found in the benthic zone is also dependent on the type of substrate present on the floor of the creek, as different substrates have varying abilities to retain microplastics. The number of surface water and benthic samples taken at each

site was not consistent due to vegetation growth at certain sites. Three surface water and benthic zone samples were taken at Site A. Three surface water and two benthic samples were taken at Site B. Two surface water and benthic samples were taken at Site D. Two surface water and three benthic samples were taken at Site E. Common substrates in the stream were rocks, sand, mud, root mats, and bigger rocks. Sites A and B featured matted roots and heavy vegetation, and had significantly more microplastics than other benthic habitats. However, calculating per m^3 of water, or per gram of sediment allows for elimination of this issue with uneven sampling.

Total Microplastics: Upstream vs. Downstream

Overall total microplastics were higher upstream of the WWTP compared to downstream. Site D, the downstream site closest to the discharge point, contained significant less microplastics. The WWTP collects water from the city of Brentwood and is treated, so the water from the discharge point is not from Marsh Creek. However, the WWTP can add water that contains less microplastics and dilute the amount of microplastics per cubic meter of water. At the Langueyú stream basin, the total microplastic concentration at the point after discharge from two WWTPs was 72,000 ± 19,000 MP/L. The main source of microplastics was the discharge from the WWTPs, making up 97% of the total concentration (Montecinos et al. 2022). My results differed from Montecinos et al. (2022), with significant disparities in the total microplastics found. The maximum total microplastics found was 53 million MPs/s, with a flow rate of 0.7 m^3/s, while my maximum total microplastics found was 0.383 MPs/s at Site E. My findings do not support the trend of increased microplastic concentration downstream.

It is important to consider the size difference of the city and its population when comparing the total microplastics found. Brentwood, CA is 30.4 km^2 with a population of 67,000, while the city of Tandil where the Langueyú stream basin is, is 50 km^2 with a population of 111,483. The larger city and population size of Tandil may influence the total microplastics found. Furthermore, the small net size limited the amount of water and material I collected. There was effluent actively being discharged from the WWTP when I was sampling, which could influence my results. Corroborating that, because I sampled in the winter, there is low water demand and high flows of continuous discharge into the stream that could have also affected my results.

Microplastics in Marsh Creek

Although I observed less microplastics downstream of the WWTP compared to upstream, there were still microplastics recovered in the downstream sites because of the addition of treated water from the city of Brentwood that discharges into Marsh Creek. My results for the total microplastics found in the surface waters and benthic zones at each site suggest that WWTP effluent does contribute microplastics to Marsh Creek. Marsh Creek is a highly urbanized and channelized stream, with deeper areas that are straight and more shallow and slow areas with constructed riffles. The deep and straight areas may contain depositional zones that store more microplastics.

The Brentwood WWTP uses TetraTech deep bed sand filters, which is a downflow sand filter for the filtration of effluent from municipal WWTPs. The water is filtered through a 6-foot deep layer of sand. The sand is highly engineered, with a 2-3 mm effective size, 0.8-0.9 sphericity factor, 2.65 specific gravity, and a 1.3 uniform coefficient (C. Wichert, personal communication). The 2-3 mm effective size refers to the range of diameters of the sand particles, the sphericity factor refers to the relative spherical shape of the sand particles, the specific gravity is the ratio of the density of the sand particles to the density of the water, and the uniformity coefficient is a measure of the sand particle size distribution (Maiyo et al. 2023, Cescon and Jiang 2020). The 0.8-0.9 sphericity factor means that the sand particles are relatively in spherical shape and can pack together more efficiently to improve filtration performance, and the specific gravity of 2.56 indicates that the sand particles are more dense than water, so the sand settles to prevent it from floating away. Finally, the uniformity coefficient of 1.3 suggests that most sand particles fall within a similar size range, which prevents uneven flow through the filter. It is crucial to consider these factors in the sand particles because they play a pivotal role in ensuring efficient filtration. Although I did not measure all my microplastic particles, I measured 20 microplastics as a representative sample of their sizes to compare them with the filter size of the Brentwood WWTP. The microplastics had an average of 1.65 mm, a maximum of 5.15 mm, and a minimum of 0.37 mm. The small size and thinness of the microplastics may allow it to bypass the deep bed sand filters.

Because I sampled in the winter, there was continuous flow into the creek. However some of the water is recycled, so capturing microplastics downstream of the discharge point is not entirely representative of the amount of microplastics in the water. I observed a majority of fibers that are likely coming from laundering activities from the city, but other sources of microplastics into the stream may impact these results. Clearly, even without inputs from the WWTP, Marsh Creek already has a high level of existing microplastic fibers from surface water inputs stored in the sediment and mobilized in surface water flows.

Limitations

Although total microplastics visibly decreased downstream after the WWTP, concrete conclusions about other streams can not be drawn from these results. The total amount of microplastics present during the day and time of collection is a snapshot of the creek's characteristics. The volume of discharge received by the creek is dependent on seasonality and water demand. Because I had limited samples, I recommend that more sampling is done along Marsh Creek to have a better understanding on microplastics upstream and downstream and to perform statistical tests to observe differences. Therefore, a direct comparison of my findings with those from different dates or different creeks is unfeasible, due to variations in seasonality, site conditions, and behavior of WWTPs. However, microplastics are certainly present.

Future Directions

To gain a deeper understanding of the role of WWTPs as a potential source of microplastics requires research on other urban streams with similar and differing conditions as Marsh Creek. Factors such as rates of microplastic deposition, depositional zones, flow rate, disturbances, and microplastic pathways into freshwater streams are necessary for further study. More work on Marsh Creek should be done at different times of the year to observe peak water processing and when microplastic contribution is highest.

Broader Implications

Although microplastics are small in size, they pose a significant threat to marine organisms (Vincent and Hoellein 2021) and human health (Wright and Kelly 2017). Microplastic pollution is a prominent pollution source that requires more research to mitigate it precisely and properly. The challenge to precisely detect and efficiently remove microplastics from wastewater are due to the lack of standard protocol and removal technologies (Tang and Hadibarata 2021). Furthermore, there are no treatment methods specifically designed to remove microplastics and limited research on removal efficiency of microplastics at different stages of the WWTPs (Sun et al. 2019). Conventional WWTPs employ three steps, pre-treatment, primary treatment, and secondary treatment (Sadia et al. 2022), however effluent quality can be improved with the addition of advanced oxidation, tertiary treatment with sand filtration, and membrane filtration (Raju et al. 2018). Currently, there are studies exploring different techniques to removing microplastics from wastewater: physical/chemical removal technologies, biological removal techniques, and sustainable removal techniques. However, there are associated drawbacks such as secondary contamination, cost effectiveness, and addition of metal ions. There are new and sustainable strategies that are highly efficient and low cost for efficient microplastic removal powered by solar energy, such as 3D solar evaporator and photocatalysis, but have not been implemented in treatment plants (Sadia et al. 2022).

This work aimed to study the effectiveness of WWTPs to filter out microplastics before discharging their effluent into streams. I discovered that the effectiveness of the Brentwood WWTP in removing microplastics is not ideal when considering the large volume of effluent that is discharged daily from the treatment plant. WWTPs play an important role in cleaning our water and must be able to keep up with the increasing demand for water. Creating a guide for MP sampling in the future would be valuable, as it would standardize the sampling process, improve data accuracy, and reduce sampling errors (Sun et al. 2019). Another approach would be developing technology at the household scale to prevent microplastic contamination at the source (Schmaltz et al. 2020). Additional studies on microplastic pathways and WWTP effectiveness can allow scientists to better understand plastic pollution dynamics in urban streams. Thereby, more effective policies for monitoring and mitigation can be developed to keep our water clean.

ACKNOWLEDGEMENTS

I thank Patina K. Mendez for giving me the opportunity to join Team Caddis and properly training me to be successful in my project. Over the last year, Patina K. Mendez provided exceptional support with establishing procedures for microplastic sampling and processing, developing my study design, and being there every step of the way in writing and analysis. I appreciate the entirety of Team Caddis for the unforgettable lab memories, and especially Jacquie Ramos and James Anthony Campbell, for assisting me on my sampling day. I am extremely thankful to Casey Wichert from the Brentwood Wastewater Treatment Plant for sending me gauge data, details on the filters used at the WWTP, and information on the frequency of discharge by the WWTP. Finally, I thank the Environmental Sciences Class of 2024, particularly Jerry Sun, Elijah Macalinao, and Dhruthi Sri Mandavilli for their support, company, and feedback, as both my friends and classmates.

REFERENCES

Andrady, A. L. 2011. Microplastics in the marine environment. Marine Pollution Bulletin 62:1596–1605.

Barbour, M. T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: Periphton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S.

Browne, M. A., P. Crump, S. J. Niven, E. Teuten, A. Tonkin, T. Galloway, and R. Thompson. 2011. Accumulation of Microplastic on Shorelines Worldwide: Sources and Sinks. Environmental Science & Technology 45:9175–9179.

Cescon, A., and J.-Q. Jiang. 2020. Filtration Process and Alternative Filter Media Material in Water Treatment. Water 12:3377.

Conley, K., A. Clum, J. Deepe, H. Lane, and B. Beckingham. 2019. Wastewater treatment plants as a source of microplastics to an urban estuary: Removal efficiencies and loading per capita over one year. Water Research X 3:100030.

Drummond, J. D., U. Schneidewind, A. Li, T. J. Hoellein, S. Krause, and A. I. Packman. 2022. Microplastic accumulation in riverbed sediment via hyporheic exchange from headwaters to mainstems. Science Advances 8:eabi9305.

Eriksen, M., S. Mason, S. Wilson, C. Box, A. Zellers, W. Edwards, H. Farley, and S. Amato. 2013. Microplastic pollution in the surface waters of the Laurentian Great Lakes. Marine Pollution Bulletin 77:177–182.

Fendall, L. S., and M. A. Sewell. 2009. Contributing to marine pollution by washing your face: Microplastics in facial cleansers. Marine Pollution Bulletin 58:1225–1228.

Liu, J., Q. Liu, L. An, M. Wang, Q. Yang, B. Zhu, J. Ding, C. Ye, and Y. Xu. 2022. Microfiber Pollution in the Earth System. Reviews of Environmental Contamination and Toxicology 260:13.

Lusher, A. 2015. Microplastics in the Marine Environment: Distribution, Interactions and Effects. Pages 245–307 *in* M. Bergmann, L. Gutow, and M. Klages, editors. Marine Anthropogenic Litter. Springer International Publishing, Cham.

Maiyo, J. K., S. Dasika, and C. T. Jafvert. 2023. Slow Sand Filters for the 21st Century: A Review. International Journal of Environmental Research and Public Health 20:1019.

Masura, J., J. Baker, G. Foster, C. Arthur, and C. E. Herring. 2015, July. Laboratory Methods for the Analysis of Microplastics in the Marine Environment : Recommendations for Quantifying Synthetic Particles in Waters and Sediments.

McCormick, A. R., T. J. Hoellein, M. G. London, J. Hittie, J. W. Scott, and J. J. Kelly. 2016. Microplastic in surface waters of urban rivers: concentration, sources, and associated bacterial assemblages. Ecosphere 7:e01556.

Mendez, P. K., S. Lee, and C. E. Venter. 2018. Imaging natural history museum collections from the bottom up: 3D print technology facilitates imaging of fluid-stored arthropods with flatbed scanners. ZooKeys 795:49–65.

Moniz, P. J. 2013. The Ecological Benefits of using Recycled Water for Streamflow Augmentation during the Dry Season in Marsh Creek, Brentwood, California.

Montecinos, S., M. Gil, S. Tognana, W. Salgueiro, and J. Amalvy. 2022. Distribution of microplastics present in a stream that receives discharge from wastewater treatment plants. Environmental Pollution 314:120299.

Raju, S., M. Carbery, A. Kuttykattil, K. Senathirajah, S. R. Subashchandrabose, G. Evans, and P. Thavamani. 2018. Transport and fate of microplastics in wastewater treatment plants: implications to environmental health. Reviews in Environmental Science and Bio/Technology 17:637–653.

Sadia, M., A. Mahmood, M. Ibrahim, M. K. Irshad, A. H. A. Quddusi, A. Bokhari, M. Mubashir, L. F. Chuah, and P. L. Show. 2022. Microplastics pollution from wastewater treatment plants: A critical review on challenges, detection, sustainable removal techniques and circular economy. Environmental Technology & Innovation 28:102946.

Schmaltz, E., E. C. Melvin, Z. Diana, E. F. Gunady, D. Rittschof, J. A. Somarelli, J. Virdin, and M. M. Dunphy-Daly. 2020. Plastic pollution solutions: emerging technologies to prevent and collect marine plastic pollution. Environment International 144:106067.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671–675. doi:10.1038/nmeth.2089

Sun, J., X. Dai, Q. Wang, M. C. M. Van Loosdrecht, and B.-J. Ni. 2019. Microplastics in wastewater treatment plants: Detection, occurrence and removal. Water Research 152:21–37.

Sutton, R., S. A. Mason, S. K. Stanek, E. Willis-Norton, I. F. Wren, and C. Box. 2016. Microplastic contamination in the San Francisco Bay, California, USA. Marine Pollution Bulletin 109:230–235.

Yang, H., G. Chen, and J. Wang. 2021. Microplastics in the Marine Environment: Sources, Fates, Impacts and Microbial Degradation. Toxics 9:41.

Talvitie, J., M. Heinonen, J.-P. Pääkkönen, E. Vahtera, A. Mikola, O. Setälä, and R. Vahala. 2015. Do wastewater treatment plants act as a potential point source of microplastics? Preliminary study in the coastal Gulf of Finland, Baltic Sea. Water Science and Technology 72:1495–1504.

Tang, K. H. D., and T. Hadibarata. 2021. Microplastics removal through water treatment plants: Its feasibility, efficiency, future prospects and enhancement by proper waste management. Environmental Challenges 5:100264.

Vincent, A. E. S., and T. J. Hoellein. 2021. Distribution and transport of microplastic and fine particulate organic matter in urban streams. Ecological Applications 31:e02429.

Wang, J., K. Bucci, P. A. Helm, T. Hoellein, M. J. Hoffman, R. Rooney, and C. M. Rochman. 2022. Runoff and discharge pathways of microplastics into freshwater ecosystems: A systematic review and meta-analysis. FACETS 7:1473–1492.

Wright, S. L., and F. J. Kelly. 2017. Threat to human health from environmental plastics. BMJ:j4334.

Zhu, X., K. Munno, J. Grbic, L. M. Werbowski, J. Bikker, A. Ho, E. Guo, M. Sedlak, R. Sutton, C. Box, D. Lin, A. Gilbreath, R. C. Holleman, M.-J. Fortin, and C. Rochman. 2021. Holistic Assessment of Microplastics and Other Anthropogenic Microdebris in an Urban Bay Sheds Light on Their Sources and Fate. ACS ES&T Water 1:1401–1410.

APPENDIX A: Physical Site Characterization

Site A

Figure A1. The channel width was 9-10 feet.

Site B

Figure A2.

Site C

Figure A3.

Site D

Figure A4.

Site E

Figure 5A.