ABSTRACT

Microplastic Pollution in Surface Waters of Urban Watersheds in Central Texas, USA:
A Comparison Above and Below Treated Wastewater Effluents

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Microplastics are polymer-based particles ranging in size from 50 µm to 5 mm. The behavior of microplastics within freshwater systems remains understudied. The purposes of this study are to assess microplastic levels in spring-fed and runoff-fed freshwater systems in small, urban watersheds above and below local point-source wastewater effluents, to investigate patterns in microplastic spatial distribution and to evaluate the influence that seasonality and land use may have on microplastic frequency and form. A total of 779 surface water samples of 800-mL were collected across five study locales and analyzed via visual inspection. In total, 1,198 microplastics were found, inclusive of fibers (95.0%) and fragments (5.0%). Approximately 57% of all samples were contaminated with microplastics, on average, ranging from 33.3%-80% per study locale. Overall, significant differences between sample site and sampling interval suggest that seasonality and land use influence microplastic frequency, while spatial locale influences particle color and form.

Microplastic Pollution in Surface Waters of Urban Watersheds in Central Texas, USA:
A Comparison Above and Below Treated Wastewater Effluents

by

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A Thesis

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DEDICATION

To God, first and foremost, for blessing me with the opportunity and resources to do the work that I love while pursuing an advanced degree. To my sister, Jamila, for being my sounding board, for serving as a source of encouragement during moments of crisis and for being willing to listen to me talk about all things microplastics no matter the day or the hour. You, Torrence, Samaira and Jori are my tribe and my greatest source of inspiration for why I do what I do, so this degree is as much mine as it is yours. To my mom, Ava, my grandparents, aunts, cousins, friends and loved ones who keep me uplifted in prayer, are my biggest cheerleaders and have provided your unconditional love and support every step of the way. To Darrian, for being my personal life coach and mental health advocate. You came into my life right as this graduate school journey got rough, but you hung in there and have been nothing but supportive and understanding. You keep me grounded and help restore my confidence on those days when I don't believe in myself. I could not have done this without any of you. For that, I am eternally grateful and will do all that I can to continue to serve God by making strides to preserve His creation for future generations and to make you all proud in the process.

We're done, but it's not over.

CHAPTER ONE

Introduction

Plastics are synthetic, manufactured polymers constructed of chains of repeating elemental units, most commonly hydrogen and carbon (American Chemistry Council, 2018). Plastic products play a large role in the everyday lives of humans worldwide as it is versatile, lightweight, cost effective and easily manufactured. As a result of its convenience and durability, plastic production, utilization and subsequently post-consumer plastic waste have largely increased (Dris et al., 2015a). According to the United States Environmental Protection Agency (US EPA), 33.3 million tons of plastic was produced in the United States in 2014, 12.9 percent of which was within the municipal solid waste segment largely consisting of single use containers and packaging products such as bags, sacks, bottles, jars and wraps. Only 9.5 percent (3.2 million tons) of the plastic produced in 2014 was recycled, while 75 percent (25.1 million tons) was deposited into landfills (US EPA, 2018). It is estimated that approximately 10 percent of all plastic waste ends up in the ocean, 80 percent of which originates as land-based trash and the remaining 20 percent a result of "intentional or accidental disposal or loss of goods and waste," (USA EPA, 2017; Greenpeace UK, 2006).

Microplastics are polymer-based particles ranging in size from 50 µm to 5 mm. Microplastics may exist in two forms, primary or secondary. Primary microplastics are manufactured on the microscopic scale and used in everyday consumer products such as facial cleansers and vectors for drugs, while secondary microplastics are formed as a result of the breakdown of primary macroplastics via mechanical, photolytic or chemical degradation processes overtime (Mathalon and Hill, 2014). These degradation processes can result in the further breakdown of microplastics into even smaller fragments, termed nanoplastics (<50 µm). Microplastics can enter into and be transferred within aquatic systems via numerous pathways including, but not limited to improper waste management, road surface, agricultural and storm water runoff following heavy rain events, fishing and other human aquatic recreational activity, industrial waste effluent (Eerkes-Medrano et al., 2015), aerial transport (Dris et al., 2015b), wastewater treatment plant discharge (Gregory, 1996) and residential laundry effluent (i.e. synthetic clothing fibers) (Browne et al., 2011; Dris et al., 2015a). The accumulation of plastic debris deposited into aquatic systems can have physical (i.e. entanglement and ingestion), environmental (i.e. habitat destruction), chemical (i.e. biomagnification, transfer of disease and human pathogens via plastic as vectors, organism exposure to chemical additives and toxins contained in and absorbed by plastics), and ecological (i.e. changes in community composition, plastic particles as habitats for exotic species) adverse effects on aquatic organisms (US EPA, 2017; Ashton et al., 2010; Koelmans et al., 2013; Wagner et al., 2014).

The study of microplastics in marine environments first began in the early 2000's and the growing mass of supporting literature has since demonstrated the ubiquitous presence of microplastics throughout the world's oceans including but not limited to the deep sea sediments (Woodall et al., 2014; Bergmann et al., 2017; Courtene-Jones, 2017), polar regions (Waller, 2017), intertidal systems (Mathalon and Hill, 2014; Mohamed Nor, 2014; Moreira, 2016), estuarine environments (Gray, 2018), coastal environments (Ng and Obbard, 2006), open water regions (Lusher et al., 2014; Isobe et al., 2017) and throughout the water column as a result of varying densities in microplastic particles (Lusher et al., 2015). Approximately 90% of the plastic debris in the pelagic marine environment are microplastics (EPA, 2017). Due to the widespread, persistent nature of microplastic pollution in marine waters, interactions between microplastics and marine organisms, particularly ingestion, have also been investigated. Microplastic ingestion in fish, plankton, birds, mussels, crustaceans and a host of other marine biota throughout the entirety of the food web has been widely reported in the literature (Peters and Bratton, 2016; Cole et al., 2013; Zhao et al., 2016; Kolandhasamy et al., 2018; Carreras-Colom et al., 2018). Although a wealth of knowledge exists pertaining to microplastic ingestion and uptake in marine biota, very few studies investigate the biological and toxicological effects from plastic ingestion and subsequent exposure to contaminants associated with plastic products such as digestive tract blockage (Wright et la., 2013), hormone regulation (Teuten et al., 2009), endocrine disruption (Fossi et al., 2012) and particle translocation to various organs (Brennecke et al., 2015; Browne, 2008; Hussain et al., 2001) in both marine and freshwater organisms. While the discovery of the ubiquitous presence of microplastic pollution in the world's oceans and their ingestion by marine organisms is anything but novel, microplastics as a persistent pollutant throughout freshwater aquatic ecosystems, has only recently become of increasing concern (Wagner et al., 2014).

The study of microplastic pollution within marine environments highlights the importance of understanding how said pollution is related to the freshwater systems as all water bodies, and the plastic debris it carries with it, lead to the ocean. Studies suggest plastic transport from inland waters as a probable source of marine microplastic pollution (Thiel et al., 2013; Eerkes-Medrano et al., 2015). Marine microplastic pollution and its adverse effects has been the primary focus for the better part of the last five decades and has been thoroughly documented in the literature on a global scale. Microplastic contamination in freshwater systems has recently been reported in lakes (Eriksen et al., 2013; Free et al., 2014), river and lake shore sediments (Klein et al., 2015; Zbyszewski and Corcoran, 2011), as well as urban surface and wastewaters (Wang et al., 2017; Dris et al., 2015b), however, the characterization and abundance of microplastics and their associated sources of origin, fate and transport in freshwater systems remains largely understudied (Eerkes-Medrano et al., 2015). This research is in response to the lack of existing data and understanding pertaining to microplastic pollution in freshwater systems, specifically the surface waters of urban areas and small watersheds within the central Texas region and the Gulf of Mexico.

CHAPTER TWO

Literature Review

Recent studies have pointed to the concept of freshwater systems serving as a source of origin for marine microplastic pollution. As a result, a shift of focus in the literature to microplastic pollution in freshwater systems has recently occurred, however, substantial knowledge gaps still exist. Only a few studies have examined microplastic pollution in lakes and rivers, and even fewer been conducted in streams, urban settings and other aquatic systems on the small watershed scale. The following is a highlight of the some of the most recent assessments of microplastic pollution in freshwater systems reported in the literature.

Microplastic Pollution in Rivers and Lakes

Several studies have reported the presence of microplastics within the surface waters of large lakes and rivers worldwide, which further brings attention to the issue of inland waters serving as transport systems of microplastic to the world's oceans (Klein et al., 2015). Free et al. (2014) examined pelagic microplastics and shoreline anthropogenic debris in Lake Hovsgol, Mongolia, a large, remote mountain lake. An average density of 20,264 particles km^{-2} was found within the lake with decreasing density as distance from the shore increased. The dominant particle forms recovered were fragments and films. Shoreline anthropogenic debris consisted of plastic bottles, fishing gear, and bags. This study is the "first to evaluate abundance, distribution, and composition of pelagic microplastic pollution in a large, remote mountain lake," (Free et al., 2014). Although much lower in abundance in comparison to lakes of similar size, the substantial presence of microplastics in such an isolated area raises the question of aerial transport from surrounding urban areas serving as a source of microplastic input into the remote lake. Microplastic pollution has also been reported in the surface waters of the Laurentian Great Lakes (Lake Huron, Lake Superior and Lake Erie) with an average abundance of 43,000 particles km^{-2}. Sample sites downstream from two major cities contained upwards of 466,000 particles km^{-2}, most of which were multi-colored spheres, suspected to be microbeads from personal care consumer products and thus likely a result of surrounding urban effluent (Eriksen et al., 2013). An additional freshwater study in the United States was conducted on the San Gabriel and Los Angeles Rivers of California, USA. This work specifically measured microplastic abundance and particle composition before and after a rain event. Particle concentrations ranged from 0.01 to 12.9 particles L^{-1} and were mostly comprised of foam. The authors found that smaller plastic particles were sixteen times more abundant in the Los Angeles River and three times more abundant in the San Gabriel River than were larger particles after a rain event, which suggests a local flushing effect during heavy rains, possibly due to increased surface runoff (Moore et al., 2011).

Current scientific literature abounds with microplastic fish ingestion studies, primarily of marine species, but are particularly sparse within the North America region, specifically of freshwater species within Central Texas, as well as the greater Gulf of Mexico area. In response to this shortage of data, Peters and Bratton (2016) examined the affects that urbanization have on microplastic ingestion by two species of freshwater sunfish in the Brazos River Basin of Central Texas. The authors reported that 45% of the fish collected contained microplastics within their guts, inclusive of microplastic fibers as well as large amounts of macroplastic, metal, Styrofoam and fishing material. This study also showed that fish from collected from urban sites had the highest mean number of microplastics ingested in comparison to that of upstream and downstream sites along the river channel, suggesting that “human development and local urbanization are two possible factors influencing the occurrence of microplastic ingestion by sunfish” (Peters and Bratton, 2016). A similar study assessed the occurrence of microplastic ingestion by fish in various Texas watersheds of the Gulf of Mexico, inclusive of freshwater drainages and one estuary. Of the fish collected, 8% of the freshwater species and 10% of the marine species contained microplastics in their gut tract. Agreeing with the findings of Peters and Bratton, this study also demonstrated a higher percentage of occurrence for ingestion in urbanized streams (29%) versus non-urbanized streams (5%) (Phillips and Bonner, 2015).

In addition to microplastic pollution studies in the surface waters and fish of lakes and rivers, several studies have also investigated the presence of microplastics in sediments and on the shorelines of river and lake systems. River shore sediments of the large Rhine and highly industrialized Main Rivers in Germany contained upwards of 4,000 particles kg^{-1}, with polyethylene, polypropylene and polystyrene of the most abundant polymer types (Klein et al., 2015). In another publication, Zbyszewski and Corcoran (2011) conducted shoreline surveys at Lake Huron, Canada to assess the distribution and degradation of plastic particles along the beaches. They found that of the plastic debris recovered, 94% was comprised of plastic pellets at multiple sample sites, the majority of which were found near the industrial sector of the lake and decreased in density with increasing distance from said area. Particle analysis utilizing Fourier transform infrared spectroscopy (FTIR) indicated that the predominant type of plastic present was polyethylene (Zbyszewski and Corcoran, 2011). A second study in Canada, conducted by Castañeda et al. (2014), quantified microplastic pollution in St. Lawrence River sediments. In this work, microplastics, particularly polyethylene microbeads, were recovered within the sediments of ten freshwater sites along a 320 km section. Microbead abundance varied widely across sites, however, the mean density was reported as 13,832 microbeads m^{-2}. Particle size also varied with location where smaller microbeads were associated with locations receiving municipal or industrial effluent in comparison with those sites not receiving effluent (Castañeda et al., 2014).

Microplastic Pollution in Urban Surface Waters

Dris et al. (2015b) conducted a microplastic pollution study in urban areas of Greater Paris and confirmed microplastic presence in wastewater, surface water and atmospheric fallout. High levels of fibers were reported in wastewater (260-320 x 10^3 particles m^{-3}), while overall contamination significantly decreased in the treated effluent to 15-50 x 10^3 particles m^{-3}. A unique case study conducted in Ljubljana, Slovenia investigated the emission, fate and transport of microbeads within wastewater treatment plants (WWTP), their interactions and subsequent release into surface waters. The purpose of this study was to estimate "daily emission of microbeads from consumers to the sewerage system, their fate in biological WWTPs and finally their release into surface waters". Laboratory simulation experiments indicated that approximately 52% of microbeads are captured in sludge during the treatment process, particularly particles ≤ 70 µm in size, while larger particles are released in the effluent. The researchers estimated approximately 112,500,000 particles being discharged daily into the surface waters of Ljubljana with an average emission rate into the sewer system of 15.2 mg per person per day, equating to a microbead concentration of 21 particles m^{-3} (Kalčíková et al., 2017).

Researchers in the United States have also investigated the role of wastewater treatment plants (WWTP) as a point source of microplastics in rivers by quantifying and comparing microplastic levels upstream and downstream of a WWTP effluent site in the North Shore Channel in Chicago, Illinois (IL), USA. Microplastic concentrations measured in this study met or exceeded levels reported in oceans and the Great Lakes. In contrast with the findings of Dris et al. (2015b), the authors reported substantially higher mean concentrations of microplastics downstream from the WWTP (17.93 particles m^{-3}) versus upstream (1.94 particles m^{-3}), confirming WWTP effluent as a point source of microplastics (McCormick et al. 2014). Two years later, McCormick et al. published a second WWTP comparison study that was conducted at nine highly urbanized rivers in IL, USA. The findings were parallel to that of McCormick et al.'s (2014) study, in that higher microplastic concentrations downstream of the WWTP effluent was observed in seven of the nine rivers with a mean influx rate of 1,338,757 particles per day. Dominant particle forms extracted included pellets, fibers and fragments and analyzed polymers were identified as polypropylene, polyethylene and polystyrene (McCormick et al., 2016).

Another case study involving microplastic pollution in urban surface waters was conducted by Wang et al. (2017) in the largest city in central China, in which twenty urban lakes and rivers were assessed for the presence of microplastics. Concentrations ranged from 1660.0 ± 639.1 to 8925 ± 1591 particles m-^{3}, with the most common particle form being colored fibers. Other particle forms present in samples included granules, films and pellets. FTIR analysis indicated that polyethylene and polypropylene were the most abundant polymer type, while Nylon and polystyrene were also detected. Microplastic levels varied with location and decreased as distance from the city increased, which demonstrates the affects that urbanization and anthropogenic inputs may have on the microplastic levels within the aquatic systems in closer proximity to the center of the city (Wang et al., 2017).

Inconsistencies in the definition of 'microplastic', variations in field and laboratory protocols (i.e. mesh size, type of media sampled, overall sample size, volume of sample) and differences in reporting units make comparison between studies almost impossible, however, the aforementioned studies all serve as evidence to support the idea that microplastics are present in both the sediments and surface waters of various types of freshwater systems subjected to varying degrees of urbanization. This brief literature review also highlights the increasing popularity of this emerging field internationally. One consistency that is important to note is the widespread repeated indication of three dominant polymer types across studies: polyethylene, polypropylene and polystyrene. These three polymers are among the most versatile and commonly used plastics in the United States in everyday products, with polypropylene being the most widely produced polymer around the world (Wang et al., 2017; US EPA, 2018). Multiple studies also mention spatial/geographical variation of microplastic concentrations, namely decreasing levels of microplastics with increased distance from potential sources (major cities, industrial plants, etc.) which addresses overarching research questions regarding distribution patterns and transport of microplastics once they enter an aquatic system.

However, apart from McCormick et al. (2015 and 2016)'s studies of the Illinois River and Chicago North Shore Channel, there are no studies that compare microplastic levels above and below sewage effluent, nor are there studies examining microplastic pollution in the headwaters of small watersheds, specifically not in Texas as both Peters and Bratton (2016) and Phillips and Bronner (2015) were below sewage outfall.

Purpose of the Study

The purpose of this study is to assess and compare microplastic pollution levels in spring-fed and runoff-fed freshwater systems, from springs to creeks and rivers, in highly urbanized small watersheds above and below local point-source wastewater effluents. Additionally, to investigate patterns in microplastic spatial distribution relative to the stream gradient and cross-sectional profile and to evaluate the influence that factors such as seasonality, urbanization, land use type and the associated human activities may have on the presence, origin, fate and transport of microplastics within small watersheds. The complex hydrology of the systems chosen as study locales are largely beyond the scope of this project. Thus, with relatively basic field collection methods, this work is to serve as a foundational study and first look at the presence and behavior of microplastics in the upper portions of small urban watersheds. For the purposes of this research, microplastics will be defined as "artificial polymers (e.g. polyester or nylon), and manufactured products (i.e. manufactured natural and non-natural material), that range in size from 50 to 5000 μm" (Masura et al., 2015; Peters and Bratton, 2016).

Research Questions

1. Do microplastic pollution levels differ significantly above and below sewage outfalls?

2. Does the degree of urbanization or human use of the landscape influence the level of microplastic pollution present in streams?

3. Do microplastic pollution levels and/or particle type differ significantly within and between an urban watershed fed by a high discharge spring and lower volume streams fed by run-off, groundwater and a low discharge spring?

4. Are there any apparent patterns in the spatial and/or temporal distribution of plastics along the stream gradient and/or across the stream profile?

Hypotheses

1. Run-off fed sample sites above local point source sewage effluent will have lower microplastic pollution levels than sample sites below local point source sewage effluent.

2. Sample sites that are subjected to frequent occurrences of local direct human contact via recreational land use (i.e. swimmers, boaters and tubers) will have higher microplastic pollution levels than those sample sites that are geographically isolated in comparison, or not as accessible to high volume human traffic and direct human contact.

3. Urban watersheds fed by groundwater and/or springs will have less microplastic particles per sample, on average, than run-off fed urban watersheds.

4. Still water micro-habitats where microplastic particles have the potential to deposit (i.e. pool, deposition bend, debris and open water) will have more microplastics per sample, on average, than running water micro-habitats (i.e. riffle and cut bank).

5. Sampling intervals occurring in sequence with a known seasonal pulse event (i.e. rainfall, Baylor students returning to campus, summer break, etc.) will result in higher microplastic pollution levels than will intervals not associated with known pulse events.

CHAPTER THREE

Methods

Study Locales

This research will examine and compare microplastic pollution levels in the surface waters of five different freshwater systems in the Central Texas, USA, inclusive of two creeks, one spring, one pond and a river, with a total number of eighteen sample sites upstream and downstream, consisting of varying degrees of urbanization across study locales (Table 3.1). All study locales and their respective sample sites were selected based on geographical and hydrological comparativeness relative to other study locales, accessibility, permissibility and were established to reflect the most accurate overall cross-sectional representation of the stream profile while also encompassing a variety of land use and types of development along each stream channel. The affects of rainfall events on microplastic pollution levels is not a part of this investigation, therefore, sample collection sequences were scheduled to intentionally avoid rain by at least a 48-hr window, to collect as close to base flow conditions as possible.

Site Descriptions

Waco Creek

Waco Creek (31.515, -97.184) is a highly urbanized, run-off fed perennial waterway located in Waco, Texas (TX), USA. Waco Creek's headwaters begin in northwest Waco and flows both underground and on the surface to the southeast, eventually conjoining with Lake Waco and the Brazos River at the Baylor Marina Basin. Although originally recognized as a natural limestone stream system, Waco Creek is now classified as a micro-watershed by the state of Texas and is the largest of seven primary drainage basins for the city of Waco with a drainage area of approximately 10.5 mi^2 (Figures 3.1 & 3.2). Waco Creek drains some of the most highly urbanized sectors of the city, inclusive of residential, industrial and commercial areas as primary land use (Spencer, 1966). As a result, Waco Creek has undergone high levels of urbanization over time such as roads, storm drain and culvert construction, and other floodwater management projects beginning as early as the 1950's (Gately, 2017). One of the main construction projects on Waco Creek is a man-made diversion constructed by the City of Waco about 3.5 miles downstream from the headwaters. This diversion largely affects that natural drainage of the system, resulting in stretches of dry creek bed and underground flow.

Six sample sites were selected along Waco Creek, three of which are above the diversion and three below (Figure 3.3; Table 3.2). The most downstream site, *Site 1 – BSB Bridge*, is located adjacent to the Baylor Science Building (BSB), just above the Baylor Marina Basin. This part of the creek is largely surrounded by campus development (i.e. buildings, mowed lawns, sidewalks and parking lots) and is proximal to both a footbridge and a road bridge of the major high traffic road, University Parks Drive. This site is geographically considered part of Lake Brazos and is below sewage effluents on the Bosque and Brazos Rivers, whereas the other five Waco Creek sites are above any sewage outfall.

Table 3.1. Study locales and general descriptives of sampling sequences and basic hydrology for each respective locale.

General Descriptive	Waco Creek (WC) – Locale #1	Wilson's Creek (WsC) – Locale #2	Proctor Springs (PS) – Locale #3	Buena Vista Pond (BVP) – Locale #4	San Marcos River (SMR) – Locale #5
Water Source	Run-off	Run-off, low discharge spring	Groundwater	Run-off	High discharge spring
Catchment Size	10.5 mi^2	-	-	-	522 mi^2
No. of Sample Sites	6	2	3	1	6
No. of Sampling Rounds	7	4	5	4	3
Sampling Dates	Sept. 2017, Oct. 2017, Mar. 2018, Apr. 2018, June 2018 & July 2018	July 2017, Mar. 2018, Apr. 2018 & June 2018	July 2017, Mar. 2018, Apr. 2018, June 2018 & July 2018	July 2017, Mar. 2018, Apr. 2018, & June 2018	Apr. 2018, June 2018, & July 2018

Figure 3.1. Map of West Waco, TX defining major drainage basin divides, where Wilson's Creek catchment area is denoted by 1 and Waco Creek catchment area is denoted by 4 (Spencer, 1966).

Figure 3.2. Map of East Waco, TX defining major drainage basin divides, where Wilson's Creek catchment area is denoted by 1 and Waco Creek catchment area is denoted by 4 (Spencer, 1966).

Site 2 - Baylor Bookstore, is a completely concrete channelized portion of the creek that flows through the heart of Baylor University's campus between the bookstore and the student center. *Site 3 - Common Grounds*, is located below Interstate-35 in a highly populated area. Here, the creek flows in a steep ditch between a large parking garage and a strip of small businesses. *Site 4 - Bell's Hill Park*, is a municipal park located just above the diversion and also contains a low water dam. This site is widely utilized for recreation by local residents including but not limited to fishing, wading in the creek and use of the playground and surrounding open fields. *Site 5 - Floyd Casey*, is a relatively undisturbed portion of Waco Creek flowing in a ditch between residential development and the large parking lot of what was once Baylor's University's football stadium. This site is nearest Valley Mills Drive, another main, high traffic road in the city. The most upstream point of the sampling sites, *Site 6 – Beverly Drive*, is located 2.5 miles downstream from the headwaters, flows under a road bridge and is surrounded largely by residential development.

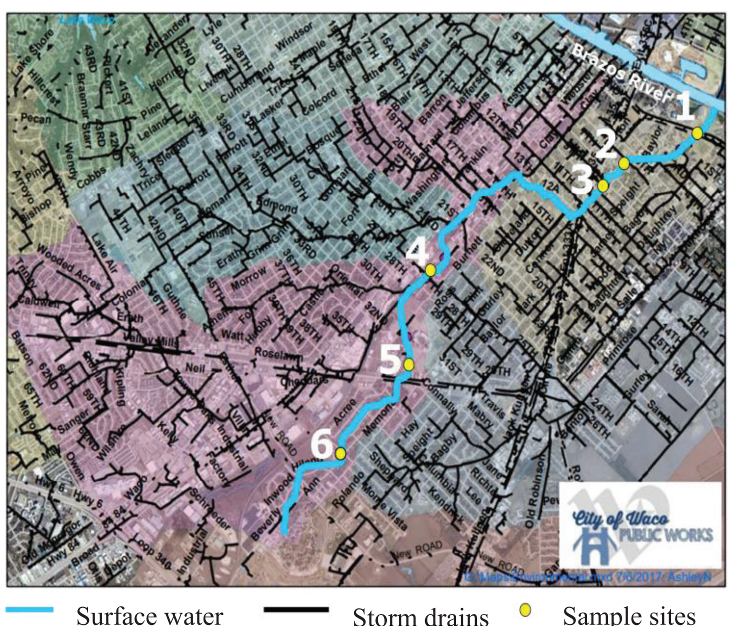


Figure 3.3. Waco Creek sampling map. Note: Sample site number increases in upstream direction.

Table 3.2. Waco Creek sampling sites and general descriptives for each site.

Sample Site	Water body type	Stream width (min-max) (m)	Overall macroplastic abundance within 5m	Above or below sewage effluent	No./Type of Dams	Type of Drains	% of Bank Disturbance (open, trampled, mowed, etc.)	Type of Development
Site #1 – BSB Bridge (BSB)	Lake	5.49-6.10	Largely scattered throughout with debris entanglement	Below	0	Road surface drains & pipes, concrete culvert	100%	Commercial - Roads, sidewalks, parking lots, buildings, bridges
Site #2 – Baylor Bookstore (BB)	Stream	3.00	Sparsely scattered (<10 pieces)	Above	0	Road surface drain & pipes, stream in concrete channel	100% (paved)	Commercial – Sidewalks & buildings
Site #3 – Common Grounds (CC)	Stream	1.10-7.20	Largely scattered throughout with debris entanglement	Above	0	Road surface drain & pipes	30-40%	Commercial - Roads, sidewalks, bridges, buildings
Site #4 – Bell's Hill Park (BHP)	Stream	4.00-12.50	Largely scattered (>10 pieces)	Above	1 – low barrier	Drain pipes	0%	Recreational - Fishing access points
Site #5 – Floyd Casey (FC)	Stream	3.20-6.00	Largely scattered (>10 pieces)	Above	0	Concrete culvert	1-10%	Residential - Roads, sidewalks, parking lot
Site #6 – Beverly Drive (BD)	Stream	0.30-2.40	Largely scattered (>10 pieces)	Above	0	Road surface drain	0%	Residential - Roads, sidewalks, bridges

Wilson's Creek

Wilson's Creek (31.574, -97.148) is a low discharge urban creek in Waco, TX fed primarily by a spring in addition to local run-off. Wilson's Creek also serves as a primary drainage basin for the city of Waco. Wilson's Creek is surrounded largely by residential development and flows through Waco's largest municipal park, Cameron Park, where it subjected to recreation and fishing use by residents just before conjoining with the Brazos River. Two sample sites were selected for collecting at Wilson's Creek: Lower Creek, downstream just above the confluence with the Brazos River and Upper Creek, both of which are near road bridges (Table 3.3).

Proctor Springs

Proctor Springs (31.573, -97.149) is one of the last remaining natural limestone groundwater springs in Waco, TX. Proctor Springs is a partially channelized waterway that flows adjacent to a drainage ditch through Cameron Park right above the Brazos River and is the main water source for Wilson's Creek. Three sample sites were selected at Proctor Springs: two seeps (upper and lower) and the channelized surface flow (Table 3.3).

Buena Vista Pond

Buena Vista Pond (31.570, -97.074) is an urban, residential stock pond located in a municipal park in Waco, TX. Buena Vista Pond is largely surrounded by residential and recreational urbanization as well as agricultural land (Table 3.3).

Table 3.3. Wilson’s Creek, Proctor Springs and Buena Vista Pond sampling sites and general descriptives for each site.

Sample Site		Water body type	Stream width (min-max) (m)	Overall macroplastic abundance within 5m	Above or below sewage effluent	No./Type of Dams	Type of Drains	% of Bank Disturbance (open, trampled, mowed, etc.)	Type of Development
Wilson’s Creek	Site #1 – Lower Creek (LC)	Stream	1.11-4.23	Largely scattered (>10 pieces)	Above	0	0	91-100%	Recreational – walking trails, roads, bridges
	Site #2 – Upper Creek (UC)	Stream	0.28-3.23	Largely scattered (>10 pieces)	Above	0	0	91-100%	Recreational – picnic area, walking trails, parking lot, roads, bridges
Proctor Springs	Site #1 – Upper Seep (US)	Spring	-	None	Above	0	0	-	Recreational – picnic area, walking trails, parking lot
	Site #2 – Lower Seep (LS)	Spring	-	None	Above	0	0	-	Recreational – picnic area, walking trails, parking lot
	Site #3 – Surface Flow (SF)	Stream	0.62-1.82	Sparsely scattered (<10 pieces)	Above	0	Stream in concrete channel	100% - paved	Recreational – picnic area, walking trails, parking lot
Buena Vista Pond	Site #1 – Buena Vista Pond (BVP)	Stock Pond	~14.63	Sparsely scattered (<10 pieces)	Above	0	Road surface drains	100% - mowed	Recreational/Residential – sidewalks, roads, fishing access points, benches

San Marcos River

The San Marcos River (29.888, -97.934), a part of the Edwards Aquifer system, is a high discharge limestone spring-fed river located in San Marcos, TX. The San Marcos River headwaters begin at the perpetual, clear flowing San Marcos Springs of Spring Lake and flows in a southeastern direction through eight cities before coflowing with the Blanco River just outside of the city of San Marcos and ultimately emptying into the Guadalupe River outside of Gonzales, TX. The river is split into two segments, the Upper San Marcos River (Segment 1814) and the Lower San Marcos River (Segment 1808) totaling to approximately 79 miles long, with a watershed drainage area of 522 mi^2 (Figure 3.4). Additionally, the river also serves as a public water supply for the city of San Marcos and has six wastewater treatment plants (WWTP) (4 domestic and 2 land application) along the river channel, three of which are permitted to discharge treated wastewater into the river (Guadalupe-Blanco River Authority (GBRA), 2008).

Upper San Marcos River. The upper San Marcos River is 4.5 miles in length and extends from the headwaters at the San Marcos Springs downstream to the confluence of the San Marcos and Blanco Rivers. The springs discharge a mean flow of 169 cfs, which heavily influences the hydrology of the upper segment as its main water source. Primary land and water use include urban, residential, industry, recreation (fishing, swimming, canoeing and tubing), agricultural and cattle, poultry and oil production. Two of the three permitted WWTPs are in the upper segment of the river: the city of San Marcos's WWTP (permitted discharge = 9 million gallons per day (mgd)) and the Texas Parks and Wildlife Department's A.E. Wood Fish Hatchery. The upper segment of the river is subjected to substantially higher levels of urbanization in comparison to the lower river. The visual aesthetic of clear waters, stable temperature year-round and multiple easy access points in parks throughout the city make the Upper San Marcos a hot spot for tourism traffic of both locals and out-of-towners alike typically between late May and early September (GBRA, 2008).

Lower San Marcos River. The lower San Marcos River is 75 miles in length and extends from the confluence with the Blanco River downstream to the confluence with the Guadalupe River. This segment of the river is smoother flowing with a median instantaneous flow of 272 cfs. Primary land and water use include recreation (swimming, canoeing and tubing), farm and ranchland. The third permitted WWTP is the city of Luling, TX south WWTP (permitted discharge = 500,000 mgd) and is in the lower segment of the river (GBRA, 2008).

Six sample sites were selected along the San Marcos River, three of which are above the city of San Marcos WWTP in the upper segment and three below in the lower segment (Figure 3.4; Table 3.4). *Site 1- Southside Park,* located in Luling, TX is a municipal park with a large dam (all water samples at this site were collected above the dam) in which the river flows through. This segment of the river is relatively slow flowing, resembling that of a lake. Southside Park is utilized primarily for fishing and canoeing and is also equipped with picnic areas. The park is surrounded by dirt roads except for the state highway that runs perpendicular to the river channel through the city of Luling. Of the six sample sites, Southside Park is the most downstream site in the lower segment and the least populated in terms of tourist use. *Site 2 – Luling Paddling Trail* is in Luling, TX approximately 0.3 miles upstream from Site 1. At this site, the river is relatively shallow allowing easy launching access for paddlers, tubers, swimmers and fishermen and thus is subjected high traffic, especially after the official establishment of the Paddling Trail. Despite the organized establishment of the Paddling Trail, this area of the riverbank remains largely undisturbed and is surrounded by dirt roads except for the state highway that runs perpendicular to the river channel through the city of Luling. *Site 3 – San Marcos Scout Camp* is a large, private scout camp located in San Marcos, TX where groups of locals and/or visitors may utilize the land and the 1,500 ft section of the San Marcos River that runs through it for a fee. This section of the river has a generally higher flow and multiple sections of rapids throughout the river channel. This riverbank is relatively undisturbed and farthest removed from paved roads than any of the other sample sites. In addition to small buildings on the land, agricultural fields and an RV park surround the camp. Water related recreational activities inclusive of tubing, canoeing and fishing occur at this site. *Site 4 – John Stokes Park* is a six-acre municipal park centered around the river with walking trails, picnicking space and access for water recreation. Stokes Park is the most downstream site in the upper segment, just above the WWTP, is one of the three sites located in the highly urbanized part of the city of San Marcos and is primarily surrounded by roads and residential development, specifically large apartment complexes. *Site 5 – Rio Vista Park* is a municipal park located in the heart of San Marcos, TX and as a result is subjected to heavy recreational use ranging from swimming in the rapids to large events such as birthday parties. This site also marks the end of a popular tubing route along the river. The park is surrounded by high levels of urbanization such as roads, parking lots, swimming pools, a community center, picnic areas, railroad tracks and houses and restaurants directly off of one side of the riverbank. *Site 6 – Sewell Park* is a municipal park where the river flows through and is located near

Texas State University campus. This site is approximately 1.8 miles downstream from the headwaters and is the most upstream sampling site in the upper segment. Sewell Park marks the beginning of the aforementioned popular tubing route, as the tube rental company is located adjacent to the river. This section of the river is partially channelized by concrete with easy access points for fishing, swimming and tubing, resulting in high traffic and heavy recreational use. It is also in this section of the river where the endangered Texas Wild Rice can be found. Urbanization and human development surrounding the river include roads, sidewalks, and parking lots. It is my assumption that the aforementioned waterways are all subjected to varying levels of pollution by anthropogenic debris via both point and non-point sources, primarily inflow from the city streets during heavy rain events, agricultural runoff, improper discarding of fishing and swimming equipment and/or direct deposit of litter into the storm drain or waterway by residents.

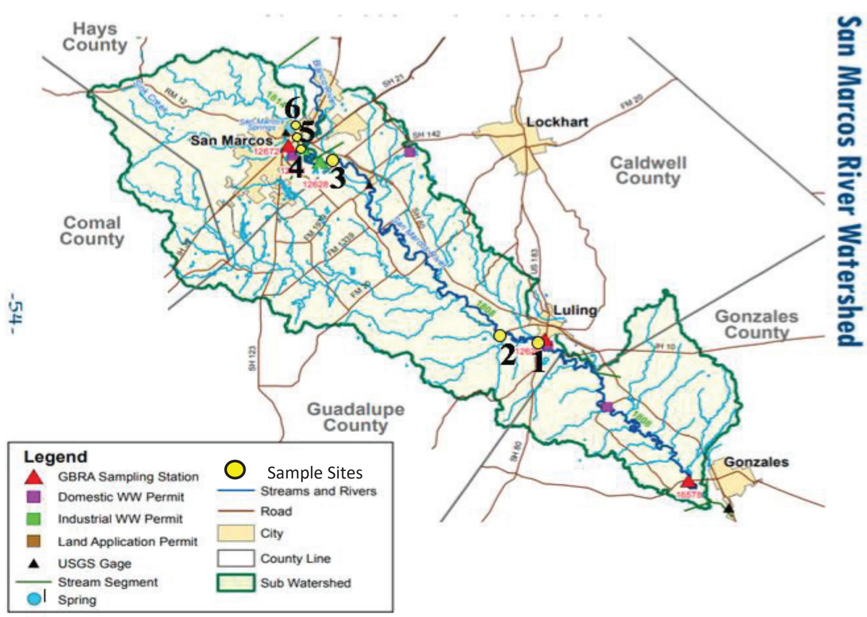


Figure 3.4. San Marcos River watershed and sampling site map. Note: Sample site number increases in upstream direction, (GBRA, 2008).

Table 3.4. San Marcos River sampling sites and general descriptives for each site

Sample Site	Water body type	Stream width (Approx. min-max) (m)	Overall macroplastic abundance within 5m	Above or below sewage effluent	No./Type of Dams	Type of Drains	% of Bank Disturbance (open, trampled, mowed, etc.)	Type of Development
Site #1 – Southside Park (SSP)	River	7.32-19.51	Sparsely scattered (<10 pieces)	Below	1 – large dam	0	0%	Recreational – Fishing access points, picnic areas, boat launches
Site #2 – Luling Paddling Trail (PT)	River	5.00-7.00	Sparsely scattered (<10 pieces)	Below	0	0	0%	Recreational – Fishing access points, picnic areas, boat launches
Site #3 – San Marcos River Scout Camp (SC)	River	12.00-15.00	Sparsely scattered (<10 pieces)	Below	0	0	0%	Recreational – Fishing access points, picnic areas
Site #4 – John Stokes Park (STP)	River	8.00	Largely scattered (>10 pieces)	Above	1 – low barrier	0	0%	Recreational - Fishing access points, walking trails, roads
Site #5 – Rio Vista Park (RVP)	River	2.00-17.07	Sparsely scattered (<10 pieces)	Above	0	0	41-50%	Recreational/Commercial - Fishing access points, picnic areas, parking lots, sidewalks, roads
Site #6 – Sewell Park (SWP)	River	9.75	Sparsely scattered (<10 pieces)	Above	0	0	100% - paved	Recreational/Commercial - Fishing access points, boat launch, parking lots, sidewalks, roads

Field Sampling Preparation and Collection Methods

Between May 2017 and July 2018, a total of 779 surface water samples were collected across study locales. Samples were collected along the stream gradient of each study locale, beginning at the furthest point downstream and moving in an upstream direction. Two replicates of surface water samples of 800mL each were collected at each study locale from five different micro-habitat types per designated sample site using long-handled steel dippers. Micro-habitat types examined in this study were categorized as follows: riffle, pool, cut bank, deposition bend, debris, open water 3’ and 8’ from the bank, well or seep, and vegetation/roots. Following collection, the water was then filtered through a 53 μm mesh filter on a 3-inch diameter wooden embroidery hoop into a 400mL glass beaker. Once filtered, the sample was covered with a 4-inch diameter glass round and sealed in aluminum foil to prevent contamination from handling and transport. The steel dippers, glass round and foil wrapping were rinsed in the water at the associated sample site prior to use. The filtered water in the beaker was discarded on site. Prior to sample collection, all filters, glass covers, and foil wrappings were triple rinsed with deionized water and visually examined via microscopy to ensure the absence of contamination and stored covered until use.

Visual observation was used to assess the overall plastic abundance within 5m, bank vegetation composition 15m upstream and downstream, canopy cover, bank disturbance, presence of algae, recreation development, presence of dams and presence of drains of each sample site and was recorded using scalars. Depth, distance from the bank, plastic abundance and type of debris were measured, observed and recorded using scalars for each micro-habitat type where the sample was collected. A HANNA Instruments water meter and Swoffer current meter were used to record temperature, pH, conductivity, total dissolved solids, dissolved oxygen and current at each sample site.

Outline of Sampling Method. This method is to be performed at each of the two replicates of the sample site, where replicate 1 is downstream and replicate 2 is upstream.

1. Using HANNA Instruments water meter and dissolved oxygen meter, insert probes into water and record temperature (°C), pH, conductivity (mS), total dissolved solids (ppt), dissolved oxygen (ppm).

2. Using Swoffer current meter, measure and record current at sample site in m/s.

3. Measure maximum and minimum stream width and max depth (cm).

4. Record the color of clothing and shoes for all team members handling samples.

5. Utilize visual observation to assess and record the overall plastic abundance within 5m, bank vegetation composition 15m upstream and downstream, canopy cover, bank disturbance, presence of algae, recreation development, presence of dams and presence of drains using previously defined scalars

6. Remove filter from foil wrapping and place atop a clean, glass 400 mL beaker.

7. Using a long-handled steel dipper, obtain surface water from the designated micro-habitat type and pour 800 mL of water through the filter into the beaker. Triple rinse dipper in site water before use.

8. Using metal forceps, triple rinse glass cover and foil wrapping in creek water, place glass round over the filter, securely wrap in foil and return to aluminum pan for transport.

9. Using a meter stick, measure and record depth, distance from the bank and use visual observation to assess plastic abundance and type of debris and record using previously defined scalars for each micro-habitat type where the sample was collected.

10. Discard beaker water on site and repeat the procedure for each additional micro-habitat as well as the second replicate.

Laboratory Analysis

All samples were removed from the foil wrapping upon return the laboratory to prevent mold, however, the glass rounds remain covering the filter to eliminate air contamination from the laboratory environment. Each filter was visually analyzed for the presence of microplastics via stereomicroscopy utilizing polymer identification and quantification protocol established by Hidalgo-Ruz et al., 2012. Microplastics were extracted by hand via metal tweezers, transferred to a microscope slide and sealed with a plastic cover slip. Total microplastics extracted was enumerated per sample and each particle was characterized individually by size, particle form, color and condition (i.e. frayed ends and body). Microplastic forms included fibers, spheres (microbeads) and fragments (Hidalgo-Ruz et al., 2012). Microplastic color was characterized using the Munsell Color System, a three-dimensional color matching system that identifies colors by three attributes; hue, value and chroma. Hue defines major color families (i.e. red, green, blue), value measures the relative lightness or darkness of a color and chroma measures the intensity of a color.

Quality Assurance and Quality Control (QA/QC)

Contamination prevention measures were practiced throughout the entirety of the project. As a result of failed sample blanks performed in the laboratory environment (long-term open exposure of the filters in various parts of the lab), the use of glass round covers was employed to minimize airborne background contamination from the hood and ventilation systems when the samples are in the laboratory. All filters and glass round covers were tripled rinsed with deionized water and preliminarily examined via stereomicroscopy to ensure cleanliness before being used in the field, triple rinsed while in the field, in addition to all sampling instruments, and were stored covered until use. Water probes and beakers were rinsed in deionized water between sampling sequences. Lab benches and other work areas were cleaned regularly. The colors of each team member's clothing and shoes were recorded during each field sampling event as a measure of cross-checking if contamination from the researchers was suspected. Additionally, wearing bright colors that are easily detectable in samples was encouraged. Samples were stored, transported, processed and analyzed with glass covers on at all times, with the exception of fiber extraction in which only small portions of the filter were exposed to air for small amounts of time. Any occurrences of contamination in field from ambient air were considered part of the sample.

Statistical Analysis

Statistical analysis was performed using non-parametric tests (i.e. Kruskall-Wallis and Mann-Whitney) and regressions via IBM SPSS Statistics for Windows, Version 23, to examine differences in microplastic concentrations within and between study locales, sampling rounds, micro-habitat types, current (i.e. still vs. running water), mean depth and rainfall. The Community Analysis Program was used to analyze similarities in particle hue across sample sites and sampling intervals. Statistical results $p \leq 1$ will be investigated as a trend. Statistical tests were considered significant at p-value < 0.05.

CHAPTER FOUR

Results

Waco Creek

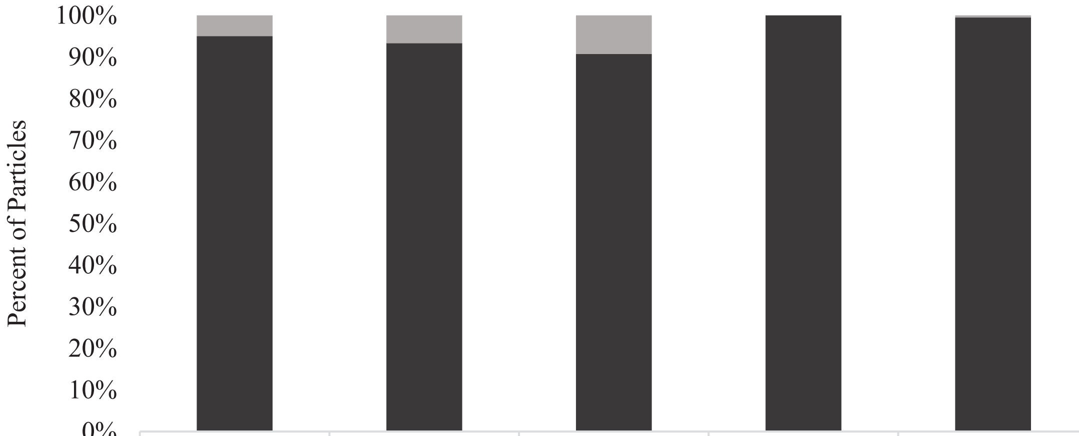
A total of 663 microplastic particles were extracted from 420 samples across all six Waco Creek sampling sites, inclusive of fibers (95%) and fragments (5.0%) (Figure 4.1; Table 4.1). Approximately 60% of all 800-mL samples were contaminated with microplastics, ranging from 55.7%-64.3% per sample site (Table 4.2). Site 4, Bell's Hill Park (BHP), contained the highest raw count of contaminated samples. However, there was no statistically significant difference in the number of samples with microplastics present between sites (X^2=0.902; p=0.839). Elevated levels of contaminated samples at BHP may be attributed to two factors. The first being that BHP is the sample site located just above the mid-creek main diversion of Waco Creek and contains a small, low barrier dam. The diversion and the dam collectively may decrease flow, subsequently causing the particles to accumulate as the water in the impoundment builds up before flowing over the dam and thus resulting in more particles within the system. Secondly, of the six sampling sites along Waco Creek, BHP is the only site repeatedly subjected to human recreation and, in comparison with the other sites, would arguably have the highest likelihood of frequent direct physical contact with humans in both bathing suits and street clothes wading in the stream and depositing loose fibers from their clothing into the water column. This, in addition to runoff and visual evidence of trash input at the park, may result in a higher frequency of microplastic presence within individual samples at BHP.

Table 4.1. Total microplastics counts across sample sites, sampling intervals and micro-habitat types for Waco Creek.

Sample Site Number	Sample Site Name	No. of Samples	Total Plastics	Sample Interval	No. of Samples	Total Plastics	Micro-habitat Type	No. of Samples	Total Plastics
1	BSB Bridge (BSB)	70	95	Sept. 2017	60	75	Riffle	70	125
2	Baylor Bookstore (BB)	70	144	Sept. 2017	60	132	Pool	70	102
3	Common Grounds (CC)	70	104	Oct. 2017	60	151	Cut bank	84	154
4	Bell's Hill Park (BHP)	70	111	Mar. 2018	60	103	Deposition Bend	70	117
5	Floyd Casey (FC)	70	118	Apr. 2018	60	50	Debris	84	110
6	Beverly Drive (BD)	70	91	Jun. 2018	60	122	Open Surface Water (3' from bank)	14	25
				Jul. 2018	60	30	Open Surface Water (8' from bank)	14	20
							Vegetation	14	10

Table 4.2. Cross tabulation of microplastic presence within samples of Waco Creek sites.

Waco Creek		Site 1 - BSB	Site 2 - BB	Site 3 - CC	Site 4 - BHP	Site 5 - FC	Site 6 - BD	Total
Microplastics Absent	Number of Samples	28	26	30	25	31	29	169
	% Within Sample Site	40.0%	37.1%	42.9%	35.7%	44.3%	41.4%	40.2%
	% of Total	6.7%	6.2%	7.1%	6.0%	7.4%	6.9%	40.2%
Microplastics Present	Number of Samples	42	44	40	45	39	41	251
	% Within Sample Site	60.0%	62.9%	57.1%	64.3%	55.7%	58.6%	59.8%
	% of Total	10.0%	10.5%	9.5%	10.7%	9.3%	9.8%	59.8%
Total	Number of Samples	70	70	70	70	70	70	420



	Waco Creek	Wilson's Creek	Proctor Springs	Buena Vista Pond	San Marcos River
Microbead	0	0	0	0	0
Fragment	33	7	19	0	1
Fiber	630	98	185	39	186

Figure 4.1. Frequency and percent of microplastic particle forms recovered in samples across all study locales.

Microplastic Pollution Across the Stream Profile: A Spatial Comparison Between Sample Sites

Out of all six sites sampled, Site 2, Baylor Bookstore (BB) contained the highest mean number of microplastics per sample (Table 4.3). However, overall, there was no statistically significant difference between mean number ($p=0.330$) or total number ($p=0.778$) of microplastics across Waco Creek sample sites (Figure 4.2). Higher totals of microplastics per sample at Baylor Bookstore may be a result of its geographic location and local land use. Baylor Bookstore is in the heart of campus between the student union and the university bookstore, both of which are high traffic areas with large parking lots surrounding them that may result in higher levels of plastic trash being deposited locally. This area of the creek is also channelized with a large surface of concrete, which could be influencing deposition. The relatively weak linear correlation between Sites 2-6 and mean number of microplastics (r^2=0.5449; Site 1 excluded from regression since it is a part of Lake Brazos) suggest widespread pollution and relatively uniform spatial distribution across the stream profile.

Table 4.3. Sample means, size, standard deviation and variance for total plastic counts per sample at each Waco Creek sample site.

Sample Site	Mean	N	Std. Deviation	Variance
BSB Bridge (BSB)	1.3571	70	1.67709	2.813
Baylor Bookstore (BB)	2.0571	70	2.90370	8.431
Common Grounds (CC)	1.4857	70	2.00538	4.022
Bell's Hill Park (BHP)	1.5857	70	1.68963	2.855
Floyd Casey (FC)	1.6857	70	2.45270	6.016
Beverly Drive (BD)	1.3000	70	1.77217	3.141
Total	1.5786	420	2.13417	4.555

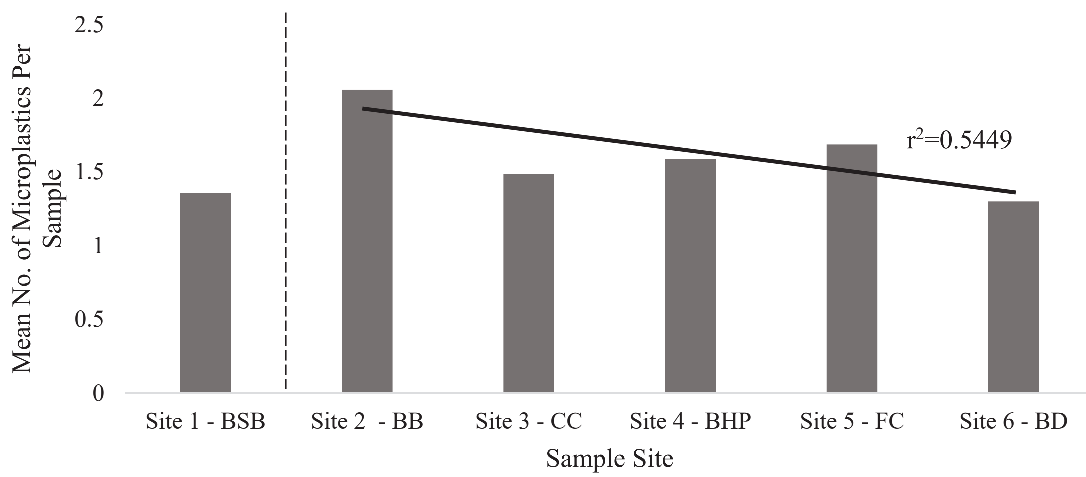


Figure 4.2. Mean number of microplastics per sample at each Waco Creek sample site. The dotted line indicates the exclusion of Site 1, BSB Bridge, from the regression analysis as it is part of Lake Brazos.

Microplastic Pollution Comparison Between Micro-Habitat and Water Type

The riffle, cut bank and open water (3' from the bank) micro-habitats contained the most microplastics per sample on average based on the raw data (Table 4.4), however, overall there was no statistically significant difference between mean number of microplastics for micro-habitats across all sample sites (p=0.537) (Figure 4.3). The running water micro-habitats (riffle and cut bank) had more microplastics per sample than the still water micro-habitats (pool, deposition bend, debris and open water) on average, however the differences were not statistically significant (p=0.089) (Table 4.5; Figure 4.4). The micro-habitat types sampled at each study locale were close in proximity and therefore drastic differences were not expected. However, the increased levels of microplastics in the running water sections of the stream may indicate active transport of particles. Due to the sampling method, which does not collect from the base of the plants, vegetation may be serving as a filter. Further research is needed to draw any conclusions; however, these findings do reject the originally stated hypothesis pertaining to differences in microplastic levels between micro-habitat type.

Table 4.4. Sample means, size, standard deviation and variance for total plastic counts per sample at each Waco Creek micro-habitat type.

Micro-habitat Type	Mean	N	Std. Deviation	Variance
Riffle	1.7857	70	2.24508	5.040
Pool	1.4571	70	2.13790	4.571
Cut bank	1.8333	84	2.37879	5.659
Deposition Bend	1.6714	70	2.50056	6.253
Debris	1.3095	84	1.59045	2.530
Open Water (3' from bank)	1.7857	14	2.39161	5.720
Open Water (8' from bank)	1.4286	14	1.39859	1.956
Vegetation	0.7143	14	0.72627	0.527
Total	1.5786	420	2.13417	4.555

Table 4.5. Sample means, size, standard deviation and variance for total plastic counts per sample for each water type at Waco Creek.

Water Type	Mean	N	Std. Deviation	Variance
Still	1.4436	266	2.01659	4.067
Running	1.8117	154	2.31158	5.343
Total	1.5786	420	2.13417	4.555

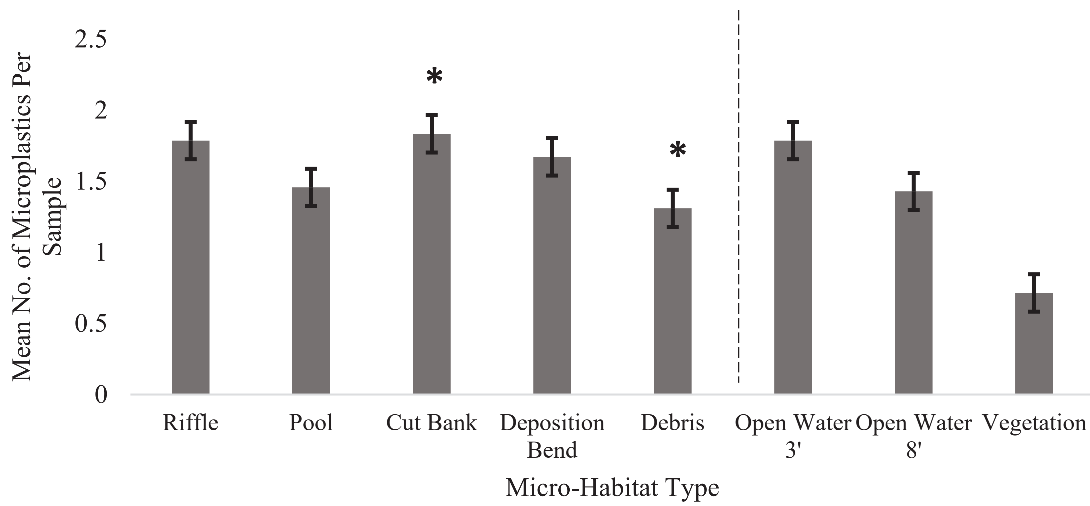


Figure 4.3. Mean number of microplastics per sample at each Waco Creek micro-habitat type. The dotted line separates micro-habitats sampled exclusively at the lake sample site, Site 1; BSB Bridge (right). Asterisks indicate micro-habitat types sampled at all six samples sites.

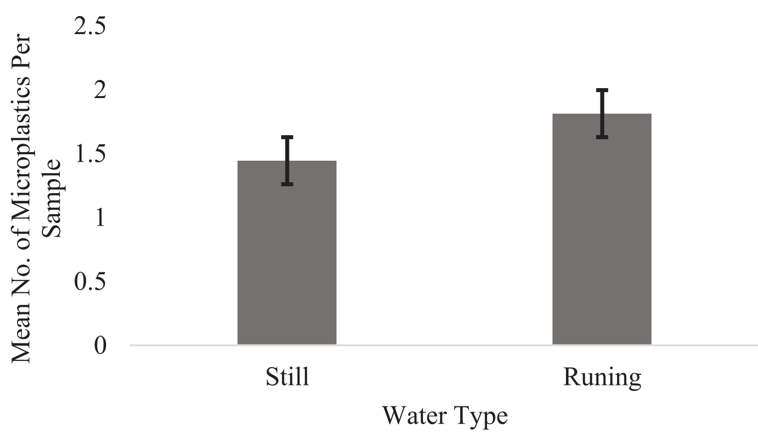


Figure 4.4. Mean number of microplastics per sample for running and still water micro-habitats at Waco Creek.

Microplastic Pollution Comparison Above and Below Local Sewage Effluent

The five sample sites above the local sewage effluent had more particles per sample, on average, in comparison to the sample site below the sewage effluent (Table 4.6). However, there was no statistically significant difference between contamination levels ($p=0.342$) (Figure 4.5). The higher levels of microplastic pollution in the upper portions of the watershed suggest that localized inputs via sources such as surface run-off and aerial transport may be influencing microplastic pollution levels in said areas that are not receiving sewage discharge.

Table 4.6. Sample means, size, standard deviation and variance for total plastic counts per sample at Waco Creek sites above and below local sewage effluent.

Sewage Effluent	Mean	N	Std. Deviation	Variance
Above	1.6229	350	2.21367	4.900
Below	1.3571	70	1.67709	2.813
Total	1.5786	420	2.13417	4.555

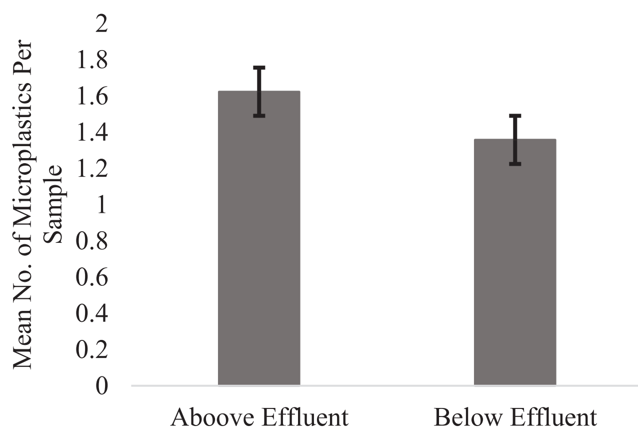


Figure 4.5. Mean number of microplastics per sample at Waco Creek sites above and below local sewage effluent.

Microplastic Pollution Levels Across a Temporal Scale: A Comparison Between Sampling Intervals

The overall average microplastic pollution levels differ significantly between sampling intervals ($p=.000$) (Table 4.7; Figure 4.6), as does the mean number of microplastics per sample between rounds ($p<0.05$) for all individual sample sites except for Site 3, Common Grounds (CC) ($p=0.143$), suggesting that seasonality and localized pulse events may influence short-term fluxes of microplastic levels over time throughout the stream profile (Figure 4.7). Common Grounds is below Interstate 35 and flows through a ditch just above the Baylor University campus between a large parking garage and a small cluster of local businesses. Aside from minimal activity from trash clean ups and road maintenance, this sample site is rarely subjected to regular, direct human activity primarily due to decreased aesthetic and inaccessibility because of roads, fences and steep banks. These characteristics combined may cause Common Grounds to be less susceptible to human induced pulse events (i.e. wading and swimming) resulting in less drastic fluctuations in microplastic contamination levels over time. The results suggest that temporal distribution, seasonality and the associated local human activity have a stronger influence on overall microplastic pollution of the stream than does the actual spatial positioning within the watershed (i.e. upstream vs. downstream) and the associated localized effects.

Table 4.7. Sample means, size, standard deviation and variance for total plastic counts per sample at each Waco Creek sampling interval.

Sampling Interval	Mean	N	Std. Deviation	Variance
Sept. 2017	1.2500	60	1.82845	3.343
Sept. 2017	2.2000	60	3.04096	9.247
Oct. 2017	2.5167	60	2.21315	4.898
Mar. 2018	1.7167	60	1.90532	3.630
Apr. 2018	.8333	60	1.35505	1.836
Jun. 2018	2.0333	60	2.31404	5.355
Jul. 2018	.5000	60	.81303	.661
Total	1.5786	420	2.13417	4.555

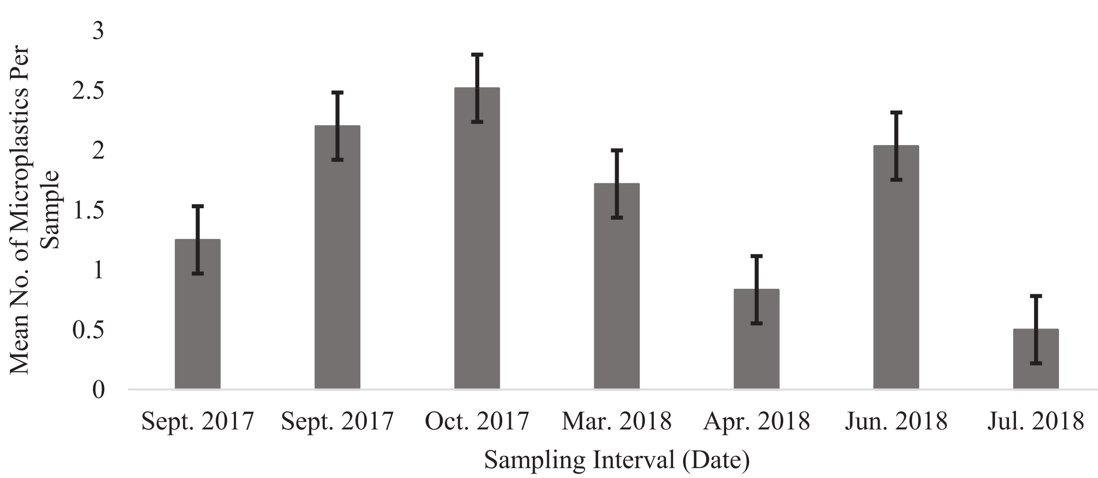


Figure 4.6. Mean number of microplastics per sample for each sampling interval of Waco Creek.

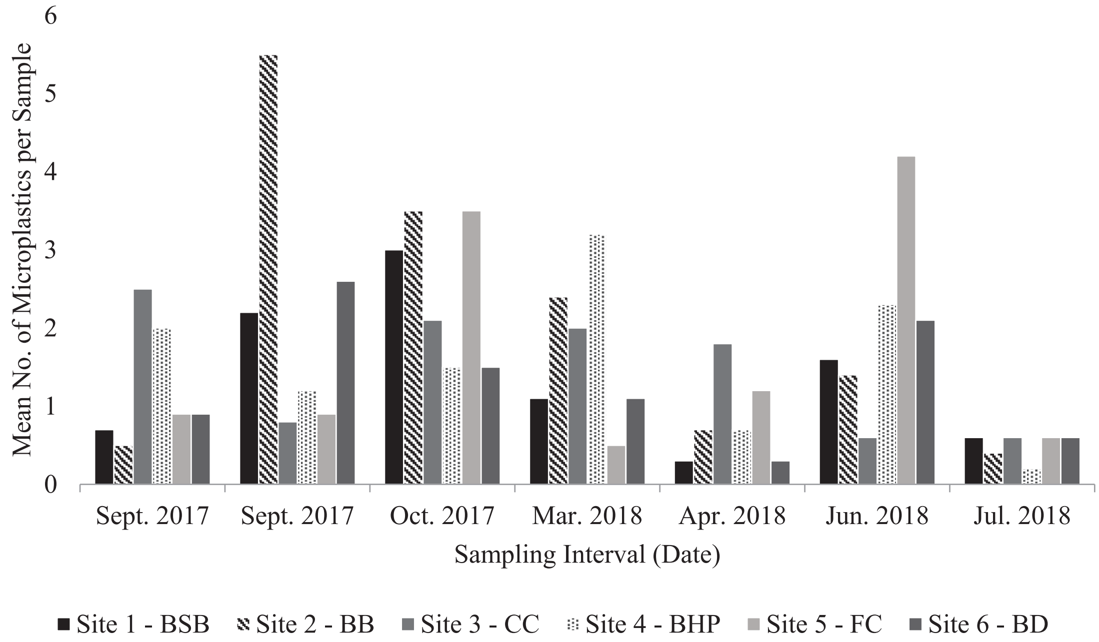


Figure 4.7. Mean number of microplastics per sample for each sampling interval across all Waco Creek sites.

Wilson's Creek

A total of 105 microplastic particles were extracted from 79 samples across two sampling sites along Wilson's Creek, inclusive of fibers (93.3%) and fragments (6.7%) (Table 4.8; Figure 4.1). Approximately 62% of all samples were contaminated with microplastics, ranging from 57.5%-66.7% per sample site (Table 4.9). The upper end of the creek, Site 2, contained a higher total count of contaminated samples than the lower end of the creek, Site 1, however the difference was not statistically significant (X^2=0.704; p=0.401).

Table 4.8. Total microplastics counts across sample sites, sampling intervals and micro-habitat types for Wilson's Creek.

Sample Site Number	Sample Site Name	No. of Samples	Total Plastics	Sample Interval	No. of Samples	Total Plastics	Micro-habitat Type	No. of Samples	Total Plastics
1	Lower Creek (LC)	40	54	Jul. 2017	20	15	Riffle	15	28
2	Upper Creek (UC)	39	51	Mar. 2018	20	53	Pool	16	23
				Apr. 2018	20	9	Cut bank	16	24
				Jun. 2018	19	28	Deposition Bend	16	11
							Debris	16	19

Table 4.9. Cross tabulation of microplastic presence within samples of Wilson's Creek sites.

Wilson's Creek		Site 1 – Lower Creek	Site 2 – Upper Creek	Total
Microplastics Absent	Number of Samples	17	13	30
	% Within Sample Site	42.5%	33.3%	38.0%
	% of Total	21.5%	16.5%	38.0%
Microplastics Present	Number of Samples	23	26	49
	% Within Sample Site	57.5%	66.7%	62.0%
	% of Total	29.1%	32.9%	62.0%
Total	Number of Samples	40	39	79

Microplastics Pollution Across the Stream Profile: A Spatial Comparison Between Sample Sites

In contrast with the presence versus absence results of the two sample sites, Site 1, Lower Creek, had more microplastics per sample on average than did Site 2, Upper Creek, however there was no statistically significant difference in the means ($p=0.909$) (Table 4.10). Elevated levels of microplastics at the lower end of the creek, as indicated in the raw data results, may be due to the active transport of particles from upstream to downstream or the geographic location of Site 1 in comparison to Site 2. Site 1, Lower Creek, is located near the mouth of Wilson's Creek that empties into the Brazos River. The river itself frequently backlogs into the lower portion of the creek bed. Therefore, Wilson's Creek's interaction with the river water and its associated inputs may influence microplastic levels at the downstream site (Site 1), while the upper part of the creek (Site 2) may not be directly influenced by the backlogging.

Table 4.10. Sample means, size, standard deviation and variance for total plastic counts per sample at each Wilson's Creek sample site.

Sample Site	Mean	N	Std. Deviation	Variance
Lower Creek	1.3500	40	1.84738	3.413
Upper Creek	1.3077	39	1.39838	1.955
Total	1.3291	79	1.63081	2.660

Microplastic Pollution Comparison Between Micro-habitat and Water Types

The riffle and pool micro-habitats had the most microplastics per sample, on average, while the deposition bend micro-habitats contained the least (Table 4.11). Overall, the differences in mean number of microplastics per sample was not statistically significant across habitats ($p=0.351$), suggesting widespread microplastic pollution throughout the cross-sectional profile (Figure 4.8). The running water habitats had a higher microplastic count per sample, on average, than the still water habitats (Table 4.12). However, the difference was not statistically significant ($p=0.128$) (Figure 4.9). Despite the lack of significance, it is important to note the consistency in the results of higher microplastic counts in running water habitats versus still water habitats for both Waco Creek and Wilson's Creek.

Table 4.11. Sample means, size, standard deviation and variance for total plastic counts per sample at each Wilson's Creek micro-habitat type.

Micro-habitat Type	Mean	N	Std. Deviation	Variance
Riffle	1.8667	15	2.16685	4.695
Pool	1.4375	16	1.93111	3.729
Cut bank	1.5000	16	1.54919	2.400
Deposition Bend	0.6875	16	0.94648	0.896
Debris	1.1875	16	1.27639	1.629
Total	1.3291	79	1.63081	2.660

Table 4.12. Sample means, size, standard deviation and variance for total plastic counts per sample for each water type at Wilson's Creek.

Water Type	Mean	N	Std. Deviation	Variance
Still	1.1042	48	1.44752	2.095
Running	1.6774	31	1.85089	3.426
Total	1.3291	79	1.63081	2.660

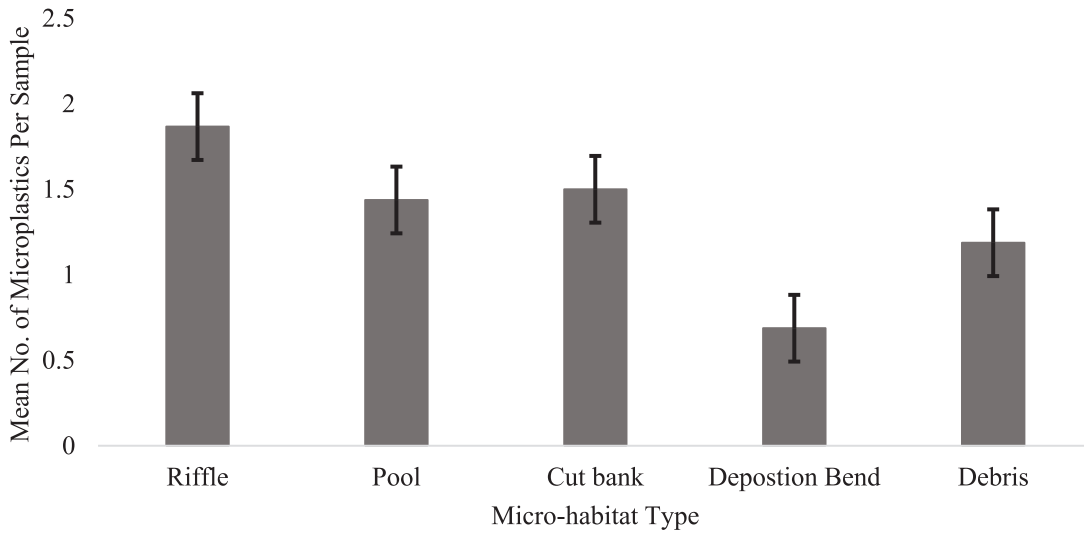


Figure 4.8. Mean number of microplastics per sample at each Wilson's Creek micro-habitat type.

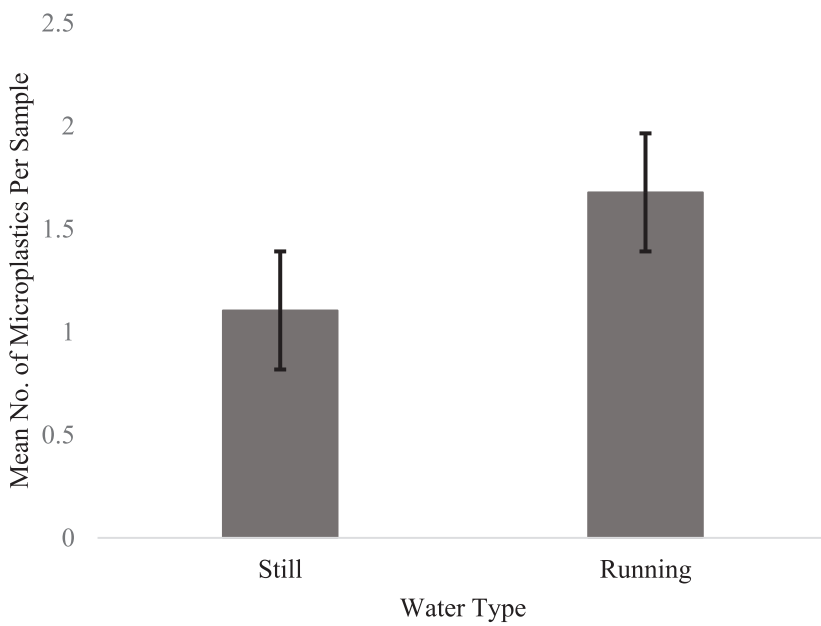


Figure 4.9. Mean number of microplastics per sample for running and still water micro-habitats at Wilsons' Creek.

Microplastic Pollution Levels Across a Temporal Scale: A Comparison Between Sampling Intervals

The overall, average microplastic pollution levels differ significantly between sampling intervals (p=0.000) (Table 4.13; Figure 4.10), as does the mean number of microplastics per sample between intervals for both individual sample sites, Site 1 (p=0.002) and Site 2 (p=0.024) (Figure 4.11). These findings are parallel to those of Waco Creek and thus further support the concept that seasonality and associated land use drives microplastic pollution levels in a given system more so than spatial distribution within the system.

Table 4.13. Sample means, size, standard deviation and variance for total plastic counts per sample at each Wilson's Creek sampling interval.

Sampling Interval	Mean	N	Std. Deviation	Variance
Jul. 2017	0.7500	20	0.85070	0.724
Mar. 2018	2.6500	20	2.30046	5.292
Apr. 2018	0.4500	20	0.68633	0.471
Jun. 2018	1.4737	19	1.21876	1.485
Total	1.3291	79	1.63081	2.660

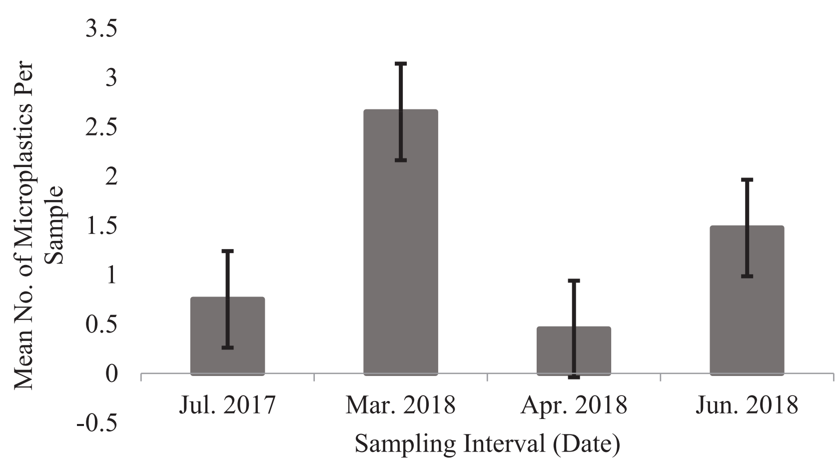


Figure 4.10. Mean number of microplastics per sample for each sampling interval of Wilson's Creek.

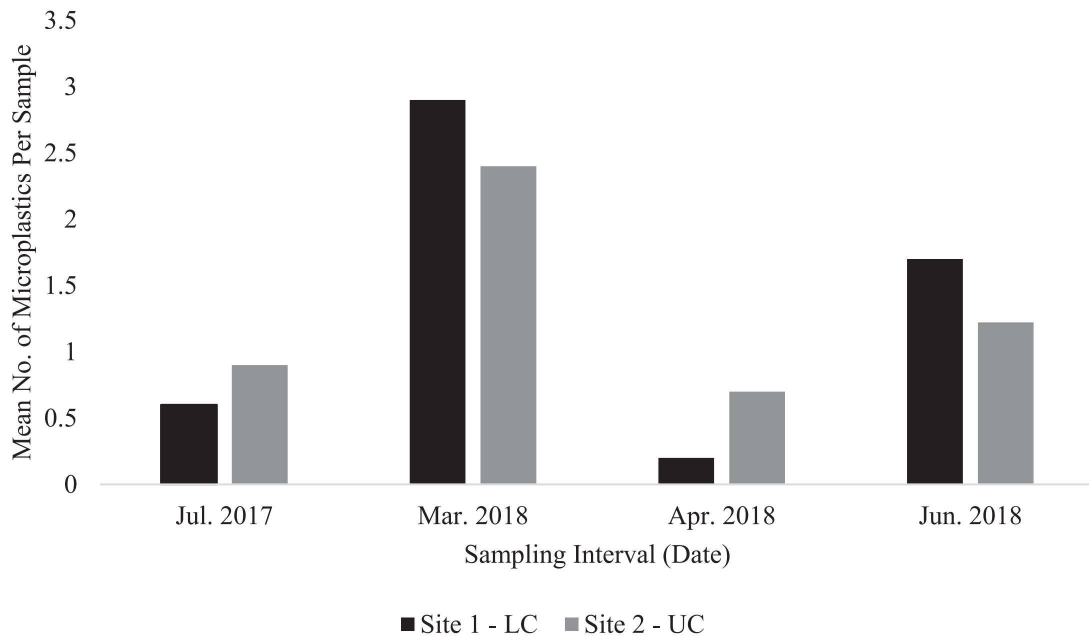


Figure 4.11. Mean number of microplastics per sample for sampling intervals at each Wilsons' Creek sample site.

Proctor Springs

A total of 204 microplastic particles were extracted from 60 samples across three sampling sites at Proctor Springs, inclusive or fibers (90.7%) and fragments (9.3%) (Table 4.15; Figure 4.1). Approximately 71% of all samples were contaminated with microplastics, ranging from 70%-80% per sample site (Table 4.14). Sample contamination levels were not significantly different between sites (X^2=0.410; p=0.814).

Table 4.14. Cross tabulation of microplastic presence within samples of Proctor Springs sites.

Proctor Springs		Site 1 – Lower Seep	Site 2 – Upper Seep	Site 3- - Spring Flow	Total
Microplastics Absent	Number of Samples	1	1	15	17
	% Within Sample Site	20.0%	20.0%	30.0%	28.3%
	% of Total	1.7%	1.7%	25.0%	28.3%
Microplastics Present	Number of Samples	4	4	35	43
	% Within Sample Site	80.0%	80.0%	70.0%	71.7%
	% of Total	6.7%	6.7%	58.3%	71.7%
Total	Number of Samples	5	5	50	60

Table 4.15. Total microplastics counts across sample sites, sampling intervals, and micro-habitat types for Proctor Springs.

Sample Site Number	Sample Site Name	No. of Samples	Total Plastics	Sample Interval	No. of Samples	Total Plastics	Micro-habitat Type	No. of Samples	Total Plastics
1	Lower Seep (US)	5	7	Jul. 2017	12	26	Riffle	10	9
2	Upper Seep (LS)	5	9	Mar. 2018	12	11	Pool	10	16
3	Spring Flow (SF)	50	187	Apr. 2018	12	23	Cut bank	10	11
				Jun. 2018	12	137	Deposition Bend	10	66
				Jul. 2018	12	6	Debris	10	85
							Seep	10	16

Microplastics Pollution Across the Stream Profile: A Spatial Comparison Between Sample Sites

The Spring Flow sample site, which is the concrete channelized surface flow section of the Springs, contained the greatest overall count of microplastics per sample on average in comparison to the other two sites, like the Baylor Bookstore, the concrete channelized section of Waco Creek (Table 4.16). Although the difference was not significantly higher than the seeps ($p=0.847$), it was my assumption that the water coming straight out of the bedrock, if not completely clean, would be distinctively cleaner than the exposed surface flow (Figure 4.12). Although minimal, contamination within the seeps raises questions regarding the source of origin for the microplastics contaminating the upper regions of watersheds.

Table 4.16. Sample means, size, standard deviation and variance for total plastic counts per sample at each Proctor Springs sample site.

Sample Site	Mean	N	Std. Deviation	Variance
Lower Seep	1.4000	5	1.14018	1.300
Upper Seep	1.8000	5	1.92354	3.700
Spring Flow	3.7400	50	11.59136	134.360
Total	3.3833	60	10.61034	112.579

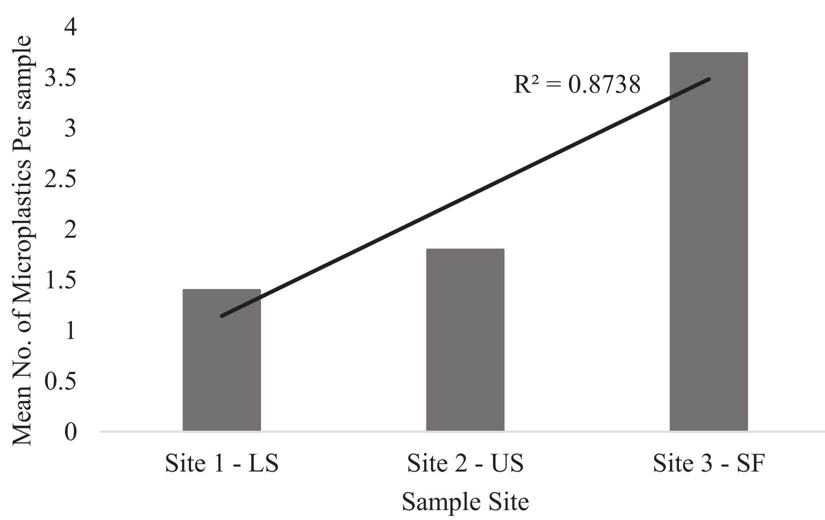


Figure 4.12. Mean number of microplastics per sample for each Proctor Spring sample site.

Microplastic Pollution Comparison Between Micro-habitat and Water Types

The debris and deposition bend micro-habitats contained distinctively more microplastics per sample, on average, than did the other micro-habitats examined (Table 4.17). Despite the higher count of microplastics calculated from raw data, the difference between micro-habitats was not statistically significant (p=0.450) (Figure 4.13). In agreement with the micro-habitat type comparison, the still water micro-habitats collectively had more microplastics per sample, on average, then the running water micro-habitats (Table 4.18). This difference was also not statistically significant (p=0.222) (Figure 4.14). These results are dissimilar to the previously discussed study locales in that the still water micro-habitats dominate plastic abundance, suggesting particle deposition within the stream profile, whereas running water micro-habitats dominating suggest active transport of particles.

Table 4.17. Sample means, size, standard deviation and variance for total plastic counts per sample at each Proctor Springs micro-habitat type.

Micro-habitat Type	Mean	N	Std. Deviation	Variance
Riffle	0.9000	10	1.28668	1.656
Pool	1.6000	10	1.42984	2.044
Cut bank	1.1000	10	0.87560	0.767
Deposition Bend	6.6000	10	12.47397	155.600
Debris	8.5000	10	22.70218	515.389
Seep	1.6000	10	1.50555	2.267
Total	3.3833	60	10.61034	112.579

Table 4.18. Sample means, size, standard deviation and variance for total plastic counts per sample for each water type at Proctor Springs.

Water Type	Mean	N	Std. Deviation	Variance
Still	4.5750	40	12.85997	165.379
Running	1.0000	20	1.07606	1.158
Total	3.3833	60	10.61034	112.579

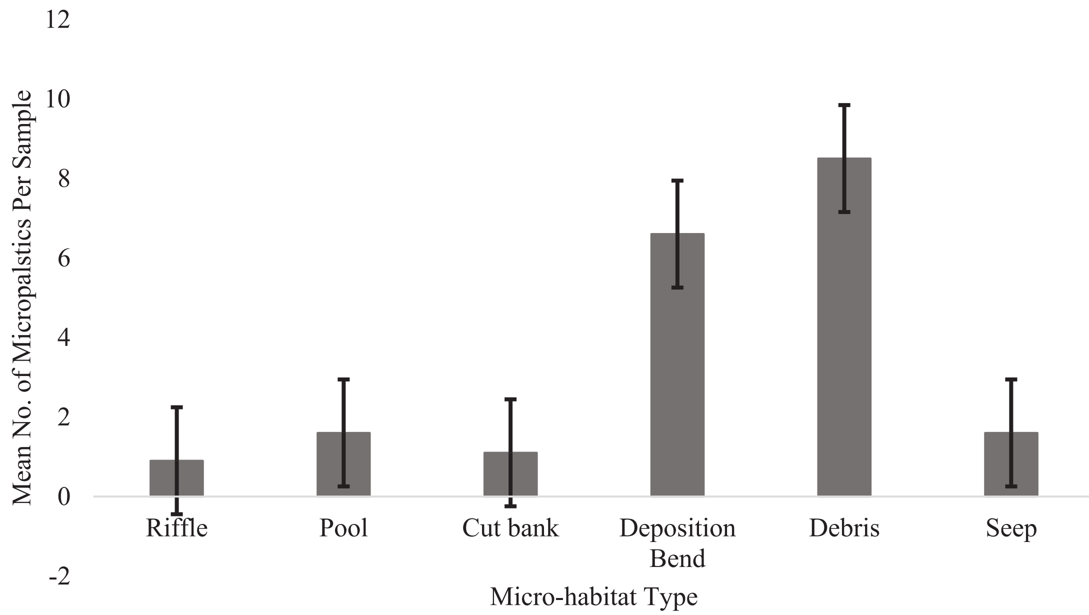


Figure 4.13. Mean number of microplastics per sample at each Proctor Springs micro-habitat type.

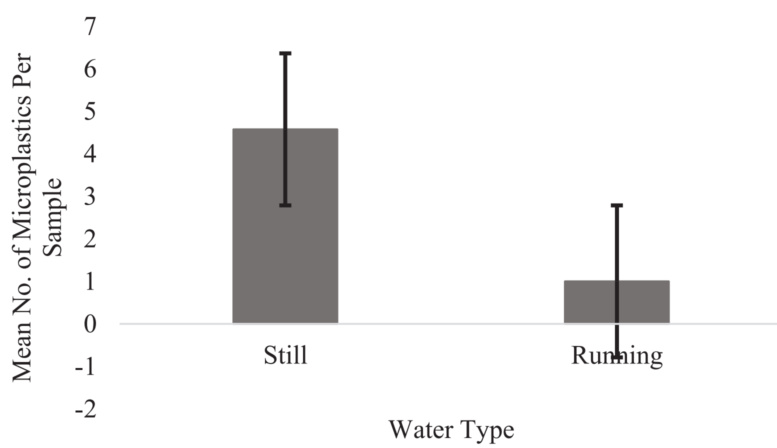


Figure 4.14. Mean number of microplastics per sample for running and still water micro-habitats at Proctor Springs.

Microplastic Pollution Levels Across a Temporal Scale: A Comparison Between Sampling Intervals

The average microplastic pollution levels did not differ statistically between overall sampling intervals (p=0.060) (Table 4.19; Figure 4.15) or between sampling intervals for the individual sites (Site 3; p=0.073) (Figure 4.16). These differences display a strong trend, however, and it is likely that a larger sample size would have found both significant. These results are also dissimilar to the results of the previously mentioned study locales. It is not clear why spatial variables are having more influence at the spring. One possibility may be that the mixture of strong spring flow and run-off inputs are generating a stronger pattern of deposition along the margins. Despite the lack of significance, I think it is important to draw attention to the peak in mean particles per sample during the June 2018 sampling interval. This large difference may be explained, in part, by high traffic use of the springs during the summer months. For this sampling interval particularly, high volumes of swimmers were observed in the pool area of the springs just days before the sampling event, which may have attributed to the unusually high amounts of particles recovered for this interval, comparison to the others.

Table 4.19. Sample means, size, standard deviation and variance for total plastic counts per sample at each Proctor Springs sampling interval.

Sampling Interval	Mean	N	Std. Deviation	Variance
Jul. 2017	2.1667	12	2.40580	5.788
Mar. 2018	0.9167	12	0.66856	0.447
Apr. 2018	1.9167	12	1.92865	3.720
Jun. 2018	11.4167	12	22.43965	503.538
Jul. 2018	0.5000	12	0.52223	0.273
Total	3.3833	60	10.61034	112.579

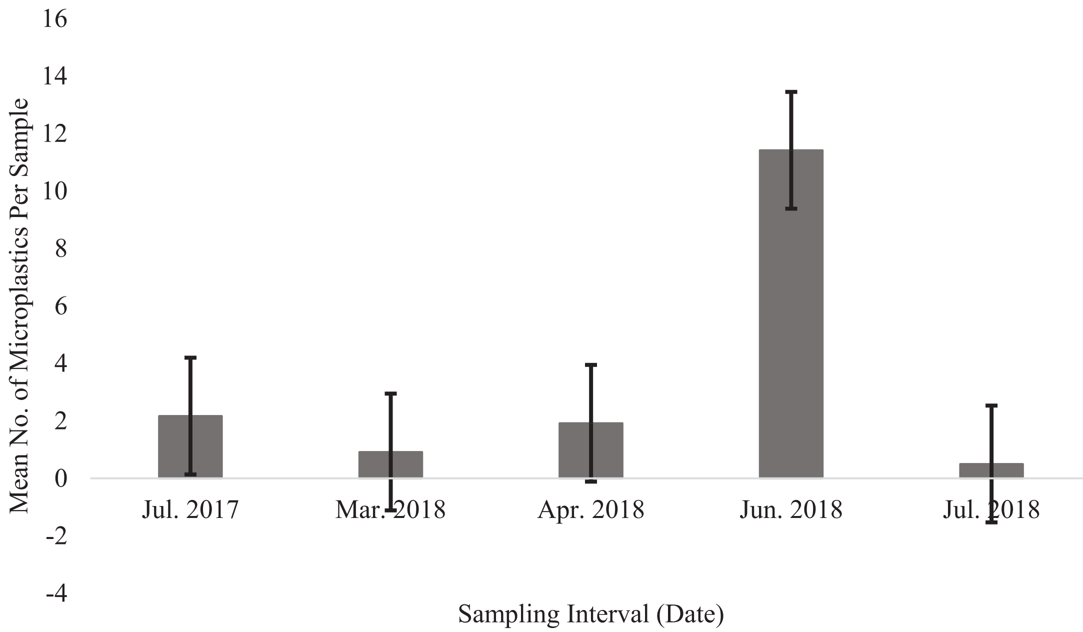


Figure 4.15. Mean number of microplastics per sample for each sampling interval of Proctor Springs.

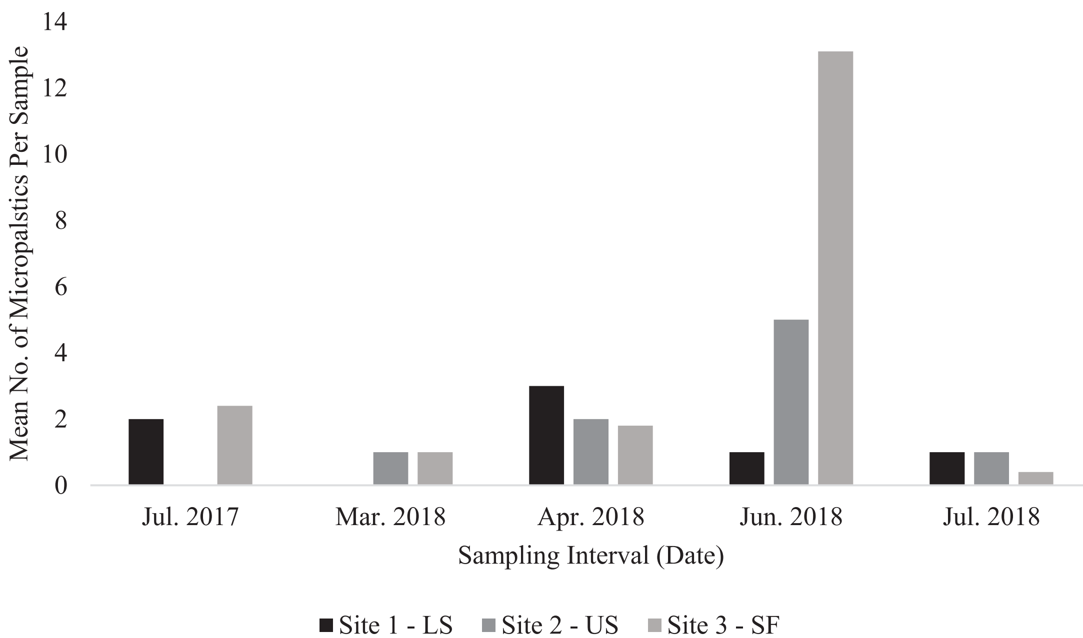


Figure 4.16. Mean number of microplastics per sample for sampling intervals at each Proctor Springs sample site.

Buena Vista Pond

A total of 39 microplastics particles were extracted from 40 samples at Buena Vista Pond, all of which were of the fiber form (Table 4.21; Figure 4.1). Approximately 42.5% of all samples collected were contaminated with microplastics Overall, Buena Vista Pond samples contained an average of 0.9750 microplastics per sample, ranging from zero to seven particles per sample (Table 4.20).

Table 4.20. Cross tabulation of microplastic presence within samples of Buena Vista Pond sites.

Buena Vista Pond		Site 1- Buena Vista Pond	Total
Microplastics Absent	Number of Samples	23	23
	% Within Sample Site	57.5%	57.5%
	% of Total	57.5%	57.5%
Microplastics Present	Number of Samples	17	17
	% within Sample Site	42.5%	42.5%
	% of Total	42.5%	42.5%
Total	Count	40	40

Microplastic Pollution Comparison Between Micro-habitat and Water Types

The debris micro-habitat had more microplastics on average than the other habitats examined, however, the difference was not statistically significant (p=0.880) (Table 4.22; Figure 4.17). Subsequently, the still water habitats contained more microplastics per sample on average than the running water habitats (Table 4.23). This difference was also not significant (p=0.849) (Figure 4.18). Since this site is a true pond as opposed to a stream-like system, it would be expected for deposition to be a more probable behavior of the microplastic particles deposited into the system rather that active transport simply due to decreased flow.

Table 4.21. Total microplastics counts across sample sites, sampling intervals, and micro-habitat types for Buena Vista Pond.

Sample Site Number	Sample Site Name	No. of Samples	Total Plastics	Sample Interval	No. of Samples	Total Plastics	Micro-habitat Type	No. of Samples	Total Plastics
1	Buena Vista Pond (BVP)	40	39	Jul. 2017	10	7	Open Surface Water (3' form bank)	8	8
				Mar. 2018	10	15	Open Surface Water (8' from bank)	8	7
				Apr. 2018	10	1	Cut bank	8	7
				Jun. 2018	10	16	Vegetation	8	5
							Debris	8	12

Table 4.22. Sample means, size, standard deviation and variance for total plastic counts per sample at each Buena Vista micro-habitat type.

Micro-habitat Type	Mean	N	Std. Deviation	Variance
Open Surface Water (3' from bank)	1.0000	8	2.44949	6.000
Open Surface Water (8' from bank)	0.8750	8	1.35620	1.839
Cut bank	0.8750	8	1.35620	1.839
Vegetation	0.6250	8	0.74402	0.554
Debris	1.5000	8	2.00000	4.000
Total	0.9750	40	1.62493	2.640

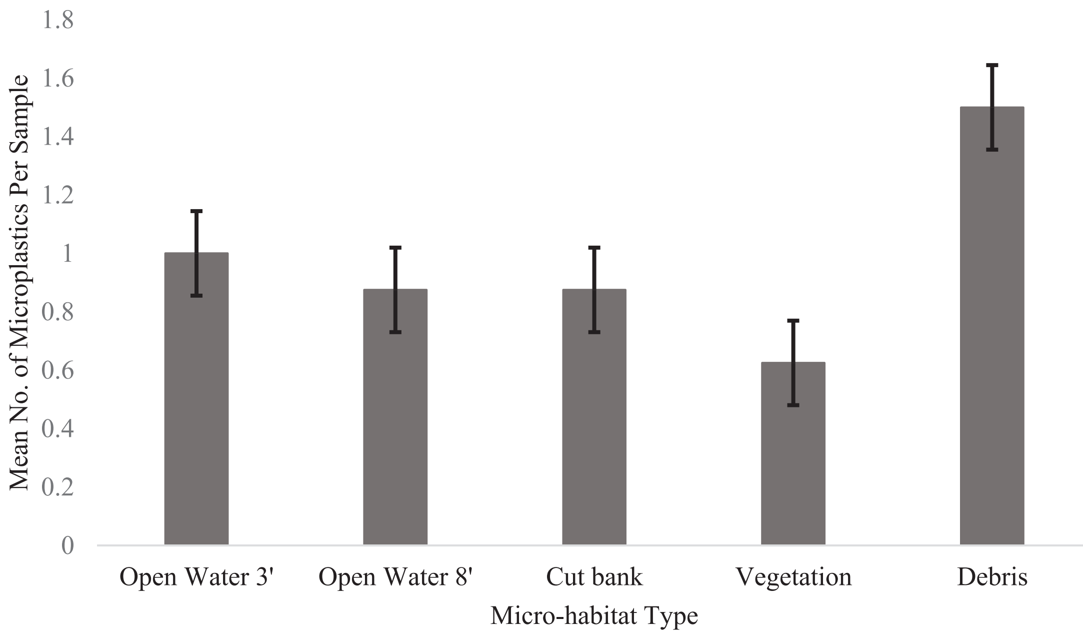


Figure 4.17. Mean number of microplastics per sample at each Buena Vista Pond micro-habitat type.

Table 4.23. Sample means, size, standard deviation and variance for total plastic counts per sample for each water type at Buena Vista Pond.

Water Type	Mean	N	Std. Deviation	Variance
Still	1.0000	32	1.70389	2.903
Running	0.8750	8	1.35620	1.839
Total	0.9750	40	1.62493	2.640

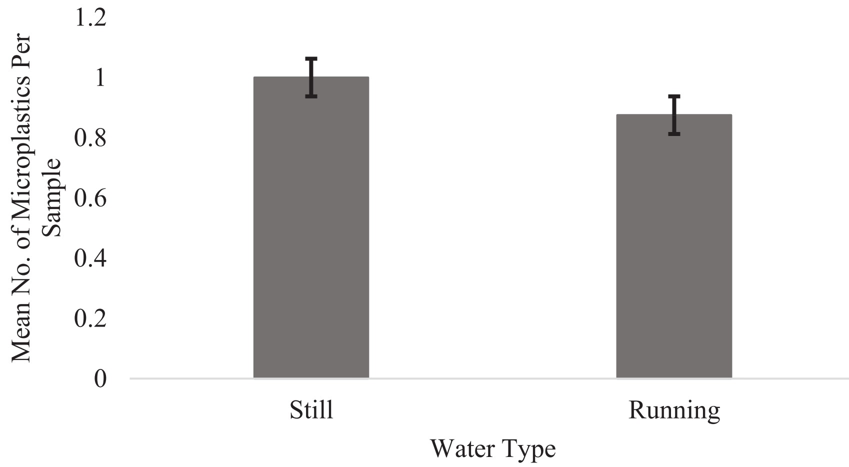


Figure 4.18. Mean number of microplastics per samples for running and still water micro-habitats at Buena Vista Pond.

Microplastic Pollution Levels Across a Temporal Scale: A Comparison Between Sampling Intervals

Overall, the average microplastic pollution levels did not differ significantly between sampling intervals (p=0.123) (Table 4.24). The highest levels of microplastics per sample were seen during the March 2018 and June 2018 sampling events (Figure 4.19). Buena Vista Pond was stocked with catfish by Texas Parks and Wildlife on three occasions during the month of June. This is the only month of the fish stocking calendar in which the pond is stocked three times as opposed to the usual once or twice per month. The June sampling event fell between the second and third stocking dates of the month. Elevated levels of microplastics during this sampling interval may be related to higher traffic and increased fishing activity by the local neighborhood due to the optimal seasonal fishing conditions, in addition to the increased stock.

Table 4.24. Sample means, size, standard deviation and variance for total plastic counts per sample at each Buena Vista Pond sampling interval.

Sampling Interval	Mean	N	Std. Deviation	Variance
Jul. 2017	0.7000	10	1.25167	1.567
Mar. 2018	1.5000	10	2.46080	6.056
Apr. 2018	0.1000	10	0.31623	0.100
Jun. 2018	1.6000	10	1.42984	2.044
Total	0.9750	40	1.62493	2.640

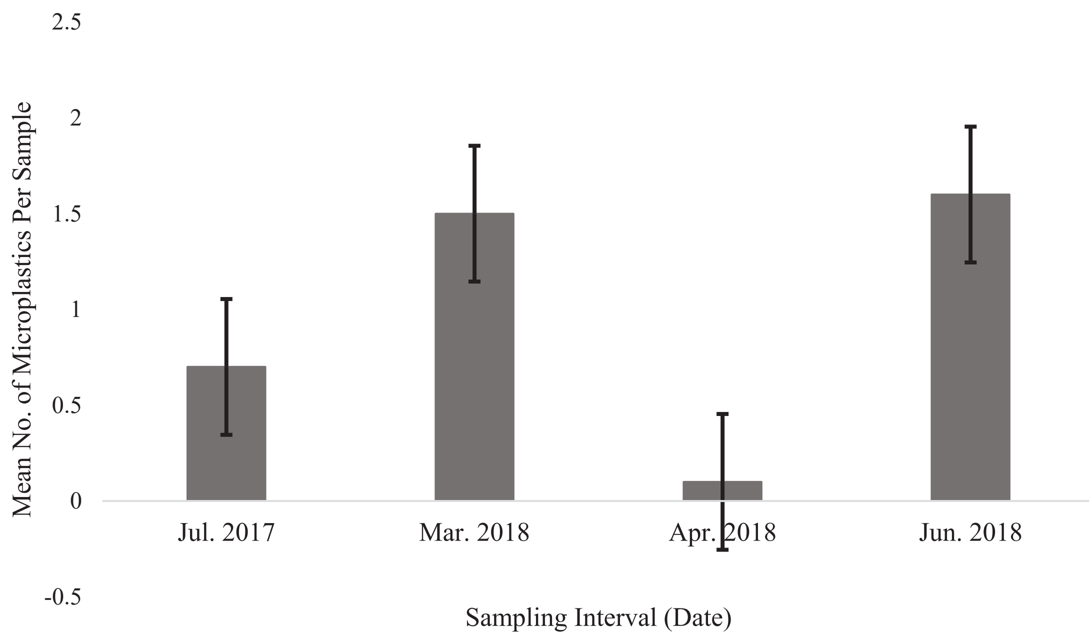


Figure 4.19. Mean number of microplastics per samples for each sampling interval of Buena Vista Pond.

San Marcos River

A total of 187 microplastic particles were extracted from 180 samples across six sample sites along the San Marcos River channel, inclusive of fibers (99.5%) and fragments (0.5%) (Table 4.25; Figure 4.1). Approximately 48% of all samples were contaminated with microplastics, ranging from 33.3%-66.7% per sample site (Table 4.26). Site 4, John Stokes Park, contained the highest overall count of contaminated samples, however, overall sample contamination levels were not statistically different between sample sites (X^2=7.125; p=0.211). Higher levels of microplastic contamination at John Stokes Park may be attributed to the high recreational traffic that this site is subjected to as a municipal park in the heart of the city. Additionally, this site is the most downstream point of the upper segment of the river, just above both the confluence with the Blanco River as well as the city of San Marcos WWTP. These factors combined may result in localized accumulation of microplastic particles that travel downstream from the upper parts of the river and thus resulting in higher levels in comparison to upstream sites and sites below the WWTP receiving treated discharge.

Microplastic Pollution Across the Stream Profile: A Spatial Comparison Between Sample Sites

Out of all six sites sampled, Site 4, John Stokes Park (STP) contained a significantly higher number of microplastics per sample, on average ($p=0.040$) and in total ($p=0.046$), than the other five sites (Table 4.27). As previously stated, significant differences between the sites and John Stokes Park being the outlier may be explained by the geographical location of the site, as well as the high recreational traffic and associated localized, direct influx of trash and land use. John Stokes Park is located adjacent to a large apartment complex and surrounded by paved roads, dirt trails and picnic areas. There is also a slight increasing trend ($r^2=0.304$) across sites, with higher levels of microplastics in the upstream sites, closer to the Springs, versus the downstream sites below the WWTP (Figure 4.20).

Table 4.25. Total microplastics counts across sample sites, sampling intervals, and micro-habitat types for San Marcos.

Sample Site No.	Sample Site Name	No. of Samples	Total Plastics	Sample Interval	No. of Samples	Total Plastics	Micro-habitat Type	No. of Samples	Total Plastics
1	Southside Park (SSP)	30	17	Apr. 2018	60	97	Riffle	24	18
2	Luling Paddling Trail (PT)	30	16	Jun. 2018	60	58	Pool	24	18
3	SM River Scout Camp (SC)	30	18	Jul. 2018	60	32	Cut bank	36	43
4	John Stokes Park (STP)	30	67				Deposition Bend	24	28
5	Rio Vista Park (RVP)	30	24				Debris	36	51
6	Sewell Park (SWP)	30	45				Open Surface Water (3' from bank)	12	6
							Open Surface Water (8' from bank)	12	7
							Vegetation	12	16

Table 4.26. Cross tabulation of microplastic presence within samples of San Marcos River sites.

San Marcos River		Site 1 – SSP	Site 2 – PT	Site 3 – SC	Site 4 – STP	Site 5 – RVP	Site 6 - SWP	Total
Microplastics Absent	Number of Samples	16	17	20	10	16	15	94
	% Within Sample Site	53.3%	56.7%	66.7%	33.3%	53.3%	50.0%	52.2%
	% of Total	8.9%	9.4%	11.1%	5.6%	8.9%	8.3%	52.2%
Microplastics Present	Number of Samples	14	13	10	20	14	15	86
	% Within Sample Site	46.7%	43.3%	33.3%	66.7%	46.7%	50.0%	47.8%
	% of Total	7.8%	7.2%	5.6%	11.1%	7.8%	8.3%	47.8%
Total	Number of Samples	30	30	30	30	30	30	180

Table 4.27. Sample means, size, standard deviation and variance for total plastic counts per sample at each San Marcos River sample site.

Sample Site	Mean	N	Std. Deviation	Variance
Southside Park (SSP)	0.5667	30	0.67891	0.461
Luling Paddling Trail (PT)	0.5333	30	0.73030	0.533
River Scout Camp (SC)	0.6000	30	0.96847	0.938
John Stokes Park (STP)	2.2333	30	4.85431	23.564
Rio Vista Park (RVP)	0.8000	30	1.21485	1.476
Sewell Park (SWP)	1.5000	30	2.96822	8.810
Total	1.0389	180	2.48878	6.194

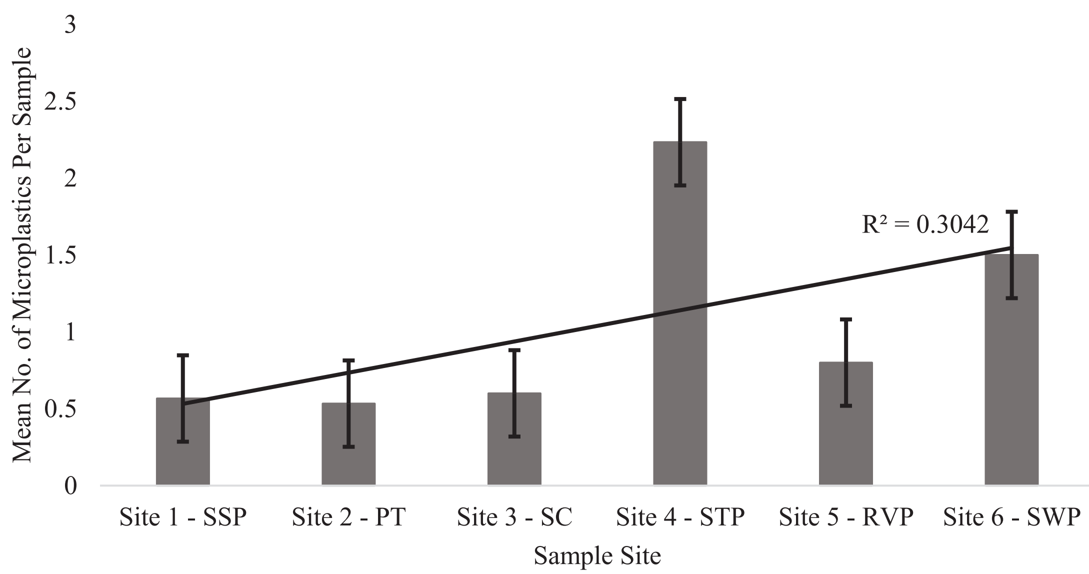


Figure 4.20. Mean number of microplastics per sample at each San Marcos sample site.

Microplastic Pollution Comparison Between Micro-habitat and Water Type

The debris and vegetation micro-habitat types contained the most microplastics per sample on average (Table 4.28), however, overall there was no statistically significant difference between mean number of microplastics across micro-habitats (p=0.906) (Figure 4.21). The still water micro-habitats had more microplastics per sample than the running water micro-habitats on average (Table 4.29), however the differences were also not significant (p=0.933) (Figure 4.22). These results are in contrast with the other major comparison study locale, Waco Creek.

Table 4.28. Sample means, size, standard deviation and variance for total plastic counts per sample at each San Marcos River micro-habitat type.

Micro-habitat Type	Mean	N	Std. Deviation	Variance
Riffle	0.7500	24	1.15156	1.326
Pool	0.7500	24	0.98907	0.978
Cut bank	1.1944	36	4.48374	20.104
Deposition Bend	1.1667	24	1.40393	1.971
Debris	1.4167	36	2.51140	6.307
Open Water (3’ from bank)	0.5000	12	0.67420	0.455
Open Water (8’ from bank)	0.5833	12	0.51493	0.265
Vegetation	1.3333	12	2.34844	5.515
Total	1.0389	180	2.48878	6.194

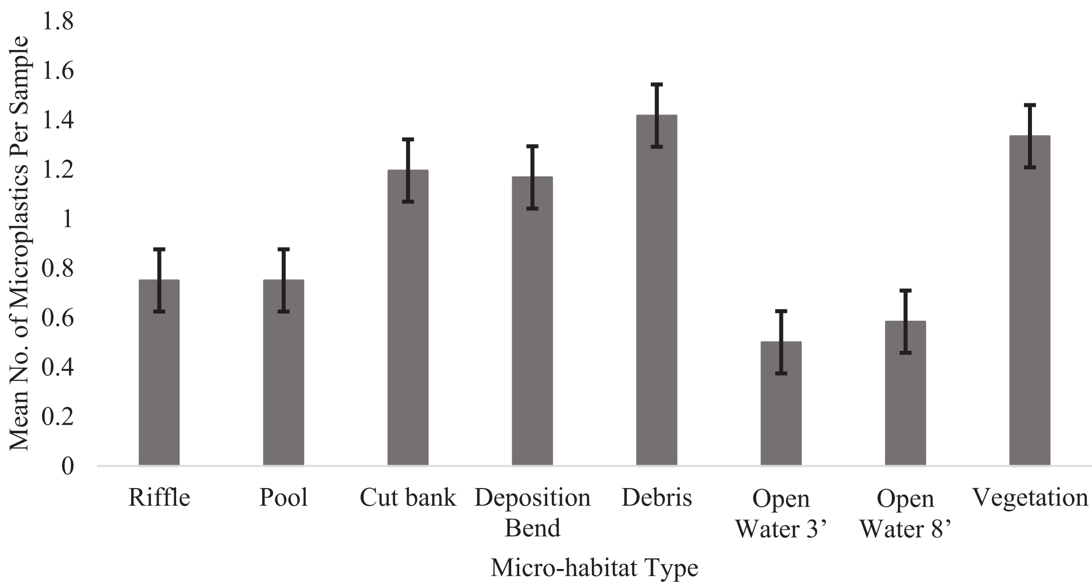


Figure 4.21. Mean number of microplastics at each San Marcos micro-habitat type.

Table 4.29. Sample means, size, standard deviation and variance for total plastic counts per sample for each water type at San Marcos.

Water Type	Mean	N	Std. Deviation	Variance
Still	1.0500	120	1.76735	3.124
Running	1.0167	60	3.53430	12.491
Total	1.0389	180	2.48878	6.194

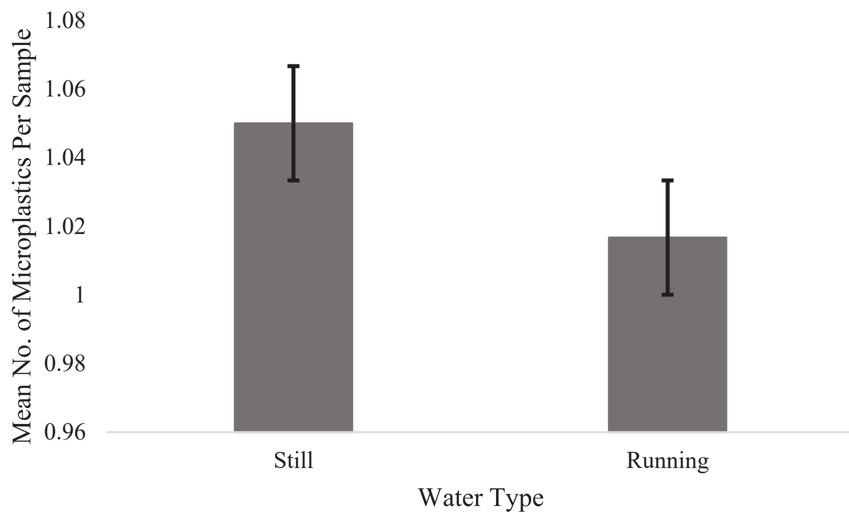


Figure 4.22. Mean number of microplastics per sample for running and still water micro-habitats at San Marcos.

Microplastic Pollution Comparison Above and Below Local Sewage Effluent

The mean and total number of microplastics per sample was significantly higher in those sites above the local sewage effluent versus the sites below the sewage effluent ($p=0.011$; $p=0.018$) (Table 4.30; Figure 4.23). These results indicate overall higher levels of contamination in the upper end of the watershed, possibly including the Springs, however further research would be necessary to confirm microplastic contamination of the Springs. These results also bring about the question of the role and influence that WWTPs may have in the removal and overall decrease of microplastic particle concentration in water during the treatment process before discharge. The significant differences between sample sites above the sewage effluent versus sites below both the sewage effluent and the confluence with the Blanco River may also be attributed to differences in land use for the upper and lower segments of the river. As previously mentioned in the site descriptions, the upper segment is subjected primarily to recreational and development within a larger city and subsequently higher levels of urban surface run-off and direct contact with humans whereas the lower segment of the river is primarily subjected to farmland and rural development and is thus more isolated in terms of human disturbance. There may also be a dilution effect because of the high levels of urbanization around the upper sample sites.

Table 4.30. Sample means, size, standard deviation and variance for total plastic counts per sample at San Marcos sites above and below local sewage effluent.

Sewage Effluent	Mean	N	Std. Deviation	Variance
Above	1.5111	90	3.37287	11.376
Below	0.5667	90	0.79394	0.630
Total	1.0389	180	2.48878	6.194

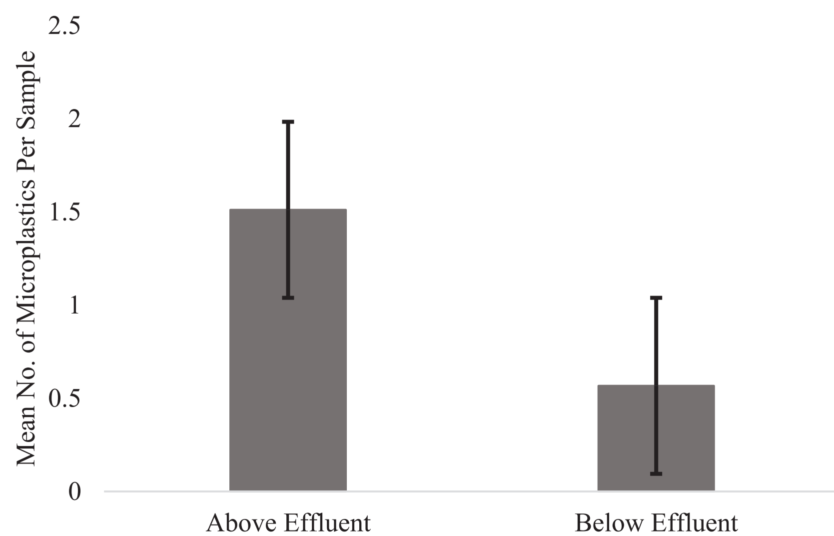


Figure 4.23. Mean number of microplastics per sample at San Marcos sites above and below local sewage effluent.

Microplastic Pollution Levels Across a Temporal Scale: A Comparison Between Sampling Intervals

Neither the average nor total microplastic pollution levels differed significantly between sampling intervals (p=0.055; p=0.171) (Table 4.31), however, there is a strong decreasing linear trend present as the sampling intervals move later into the summer months, which may be a results of decreased water recreation activity due to dangerously high summer temperatures (r^2=0.9869) (Figure 4.24). The mean number of microplastics per sample between rounds is significantly different (p<0.05) for all individual sample sites except for Site 2, Luling Paddling Trail (PT) (p=0.381), Site 3, River Scout Camp (SC) (p=0.490), Site 4, John Stokes Park (STP) (p=0.115) and Site 6, Sewell Park (SWP) (p=0.360), suggesting that both geographic location and differences in land uses as well as seasonality may influence short-term fluxes of microplastic levels over time throughout the stream profile (Figure 4.25).

Table 4.31. Sample means, size, standard deviation and variance for total plastic counts per sample at each San Marcos River sampling interval.

Sampling Interval	Mean	N	Std. Deviation	Variance
Apr. 2018	1.6167	60	3.76465	14.173
Jun. 2018	0.9667	60	1.88632	3.558
Jul. 2018	0.5333	60	0.67565	0.456
Total	1.0389	180	2.48878	6.194

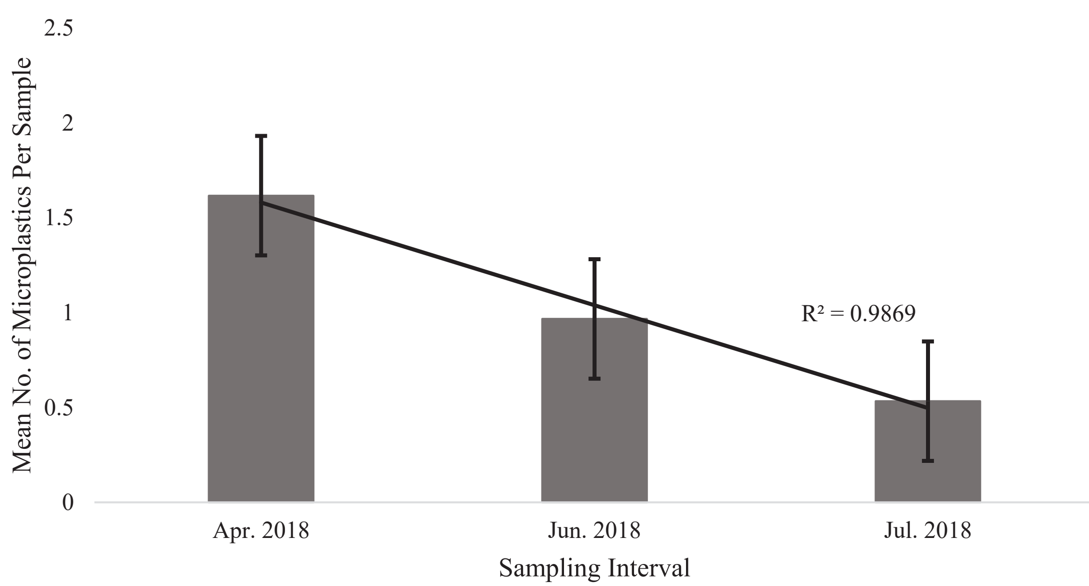


Figure 4.24. Mean number of microplastics per sample for each sampling interval of the San Marcos River.

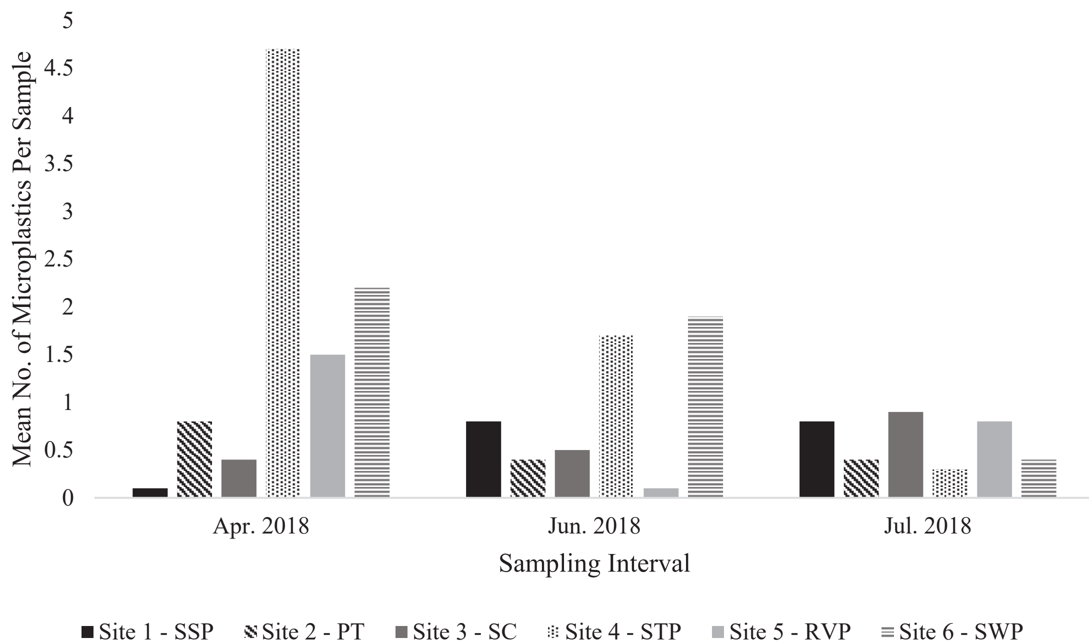


Figure 4.25. Mean number of microplastics per sample for each sampling interval across all San Marcos River sites.

Comparative Analysis Between Study Locales

Total Microplastic Pollution Levels: A Comparison Across Study Locales

Individual samples collected from all study locales most frequently contained between zero and three particles per sample (Figure 4.26), with at least 30% of all samples per study locale containing zero microplastics (Figure 4.27). The number of particles per sample across study locales ranged from a minimum of zero to a maximum of 73 particles per sample, which was found in a single sample at Proctor Springs. A comparative analysis was performed using the Mann-Whitney non-parametric statistical test to assess significant differences in overall total plastic levels between study locales. Tests were conducted comparing each of the five study locales in pairs as well as all sites combined.

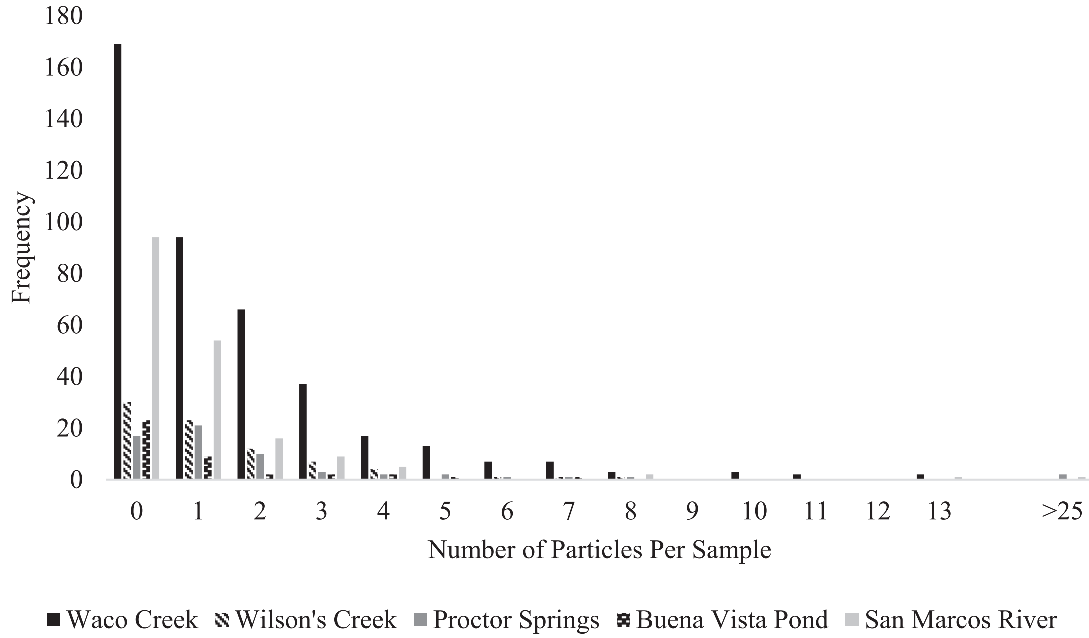


Figure 4.26. Microplastic frequency per sample across study locales.

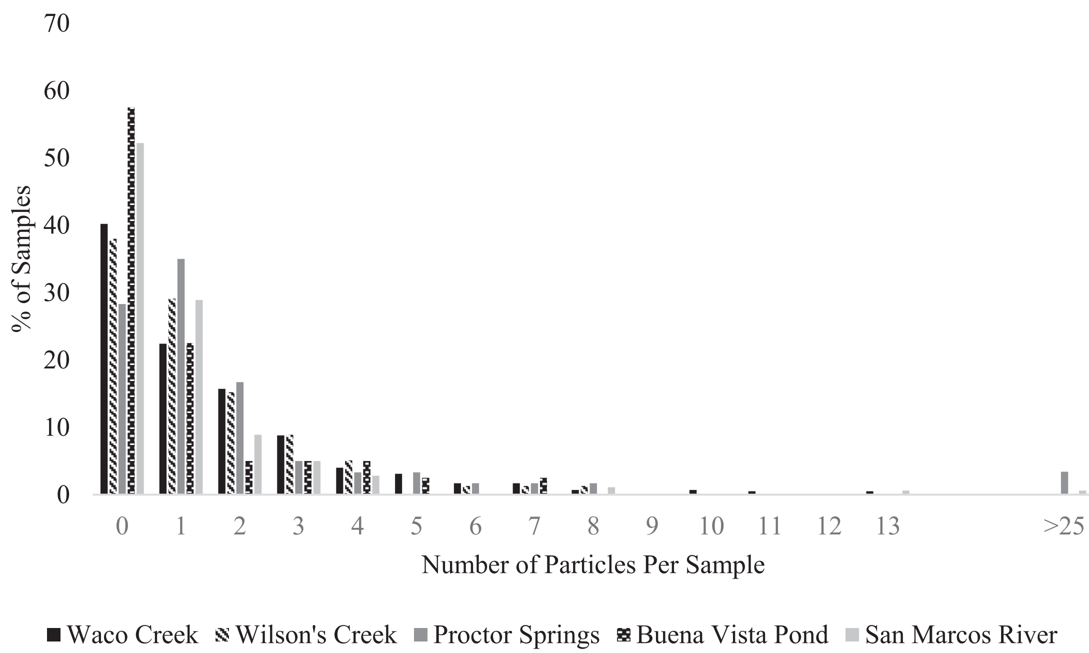


Figure 4.27. Percentage of samples within each particle frequency category for all study locales.

Based on the mean ranks of the Mann-Whitney test, the study locales in order from overall most polluted to least polluted are as follows: Proctor Springs, Waco Creek, Wilson's Creek, San Marcos River and Buena Vista Pond. Despite the use of Buena Vista Pond for recreational fishing, factors such as the site being relatively geographically isolated compared to the other study locales in combination with minimal high traffic use, lower levels of urbanization, lack of direct inputs from sewage effluent and the absence of regular, direct contact with humans (i.e. swimmers, boaters and tubers) may all be valid explanations as to why it is statistically regarded at the cleanest site.

Out of all five study locales, Proctor Springs contained significantly higher levels of microplastics in total ($p=0.000$) and per sample, on average ($p=0.000$) (Table 4.32; Figure 4.28). Aside from Proctor Springs, the remaining four study locales contained approximately 1 microplastic per sample, on average. Significantly higher levels of microplastic pollution at Proctor Spring may be attributed to its concrete channelized urbanization, high traffic use, seasonal pulse events and frequent direct contact with humans in combination with low water volume and a relatively small stream channel area. That is, higher levels of plastics being deposited at higher frequencies into lower volumes of water would likely result in more microplastics present per unit volume. The raw data results also indicated that Proctor Springs had the highest percentage of contaminated samples, followed by Wilson's Creek (Table 4.33). However, in comparison, the percentage of individual samples with microplastics present was not significantly different between study locales ($X^2=0.328$).

Table 4.32. Sample means, size, standard deviation, minimum, maximum and variance of particles per sample at each study locale.

Study Locale	Mean	N	Std. Deviation	Minimum	Maximum	Variance
Waco Creek	1.5786	420	2.13417	.00	13.00	4.555
Wilson’s Creek	1.3291	79	1.63081	.00	8.00	2.660
Proctor Springs	3.3833	60	10.61034	.00	73.00	112.579
Buena Vista Pond	0.9750	40	1.62493	.00	7.00	2.640
San Marcos River	1.0389	180	2.48878	.00	27.00	6.194
Total	1.5366	779	3.62718	.00	73.00	13.156

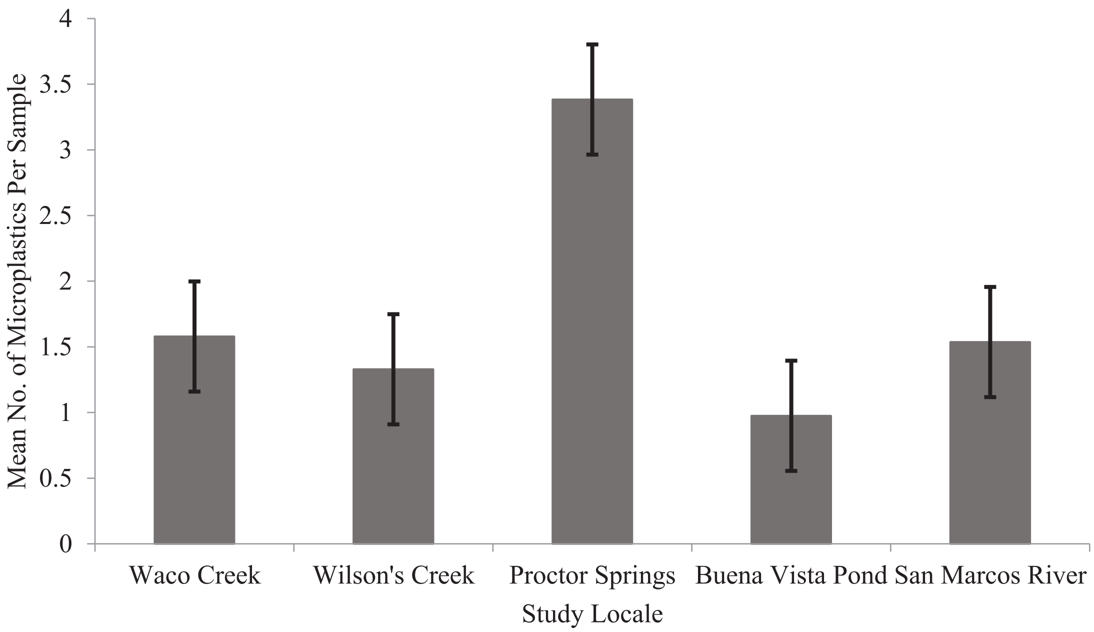


Figure 4.28. Mean number of microplastics per sample at each study locale.

Table 4.33. Frequency and percent of presence of microplastic contamination within individual samples for all study locales.

Study Locale	Present		Absent	
	Frequency	Percent	Frequency	Percent
Waco Creek	251	59.8	169	40.2
Wilson’s Creek	49	62.0	30	38.0
Buena Vista Pond	17	42.5	23	57.5
Proctor Spring	43	71.7	17	28.3
San Marcos River	86	47.8	94	52.2

Microplastic Hue Classification: A Comparison Across Study Locales

The highest percentage of microplastic particles recovered across all study locales were classified within the transparent and purple-blue hues in color followed by blue, purple, red-purple and red, respectively (Figure 4.29). Less than ten percent of particles at each study locale were classified as 'Other', inclusive of yellow-red, yellow, green-yellow, green, blue-green, black, white and iridescent (Figure 4.30). Overall, the color variety of the microplastics was not significantly different between study locales (X^2=0.442) (Table 4.34).

A principal component analysis (PCA) of microplastic hue per sample round at each study locale resulted in a DECORANA ordination plot with two main cluster groups with the late spring and summer sampling intervals at Proctor Spring, Wilson's Creek and Buena Vista Pond as outliers (Figure 4.31). The largest cluster, inclusive of the 15 out of the 23 total data points, consisted mainly of summer (June/July) and fall (September/October) sampling intervals, suggesting that the color variety of microplastics recovered in the samples of said intervals are most similar across the indicated study locales. The second cluster is inclusive of one spring (April) and two summer (July) sampling intervals from Waco Creek and Proctor Springs. It is possible that similar input sources across locations as well as common colors associated with seasonal summer and fall land use activities (i.e. bathing suits) may explain the consistency and similarities across study locales. PCA was also performed for microplastic hue per sample site at each study locale to assess the influence of spatial distribution on color variety; however, there were no apparent cluster patterns present.

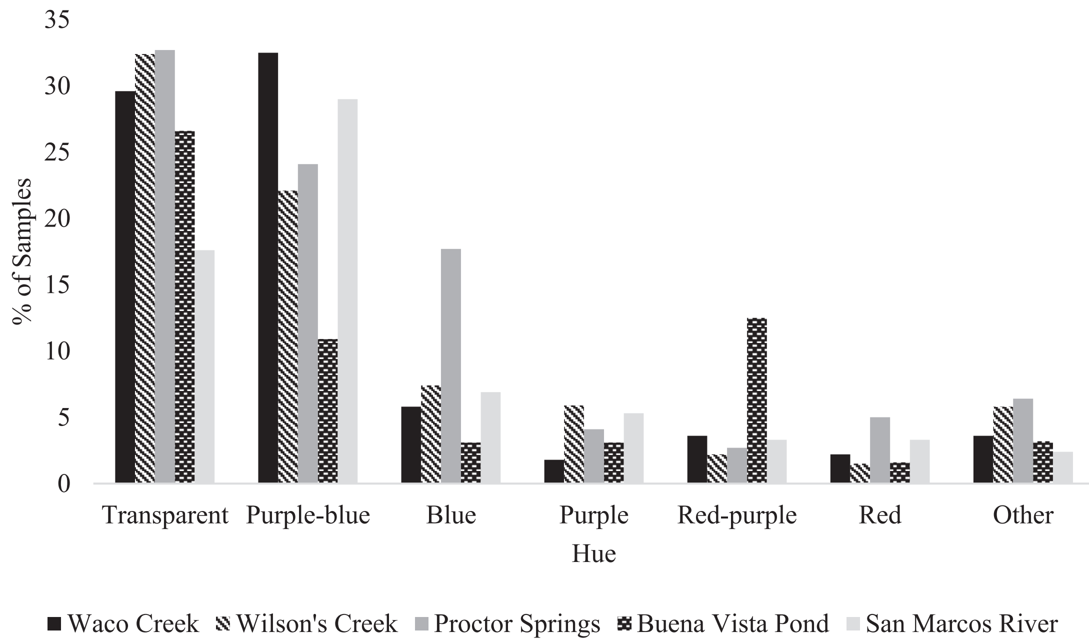


Figure 4.29. Microplastic color classification by hue for all study locales.

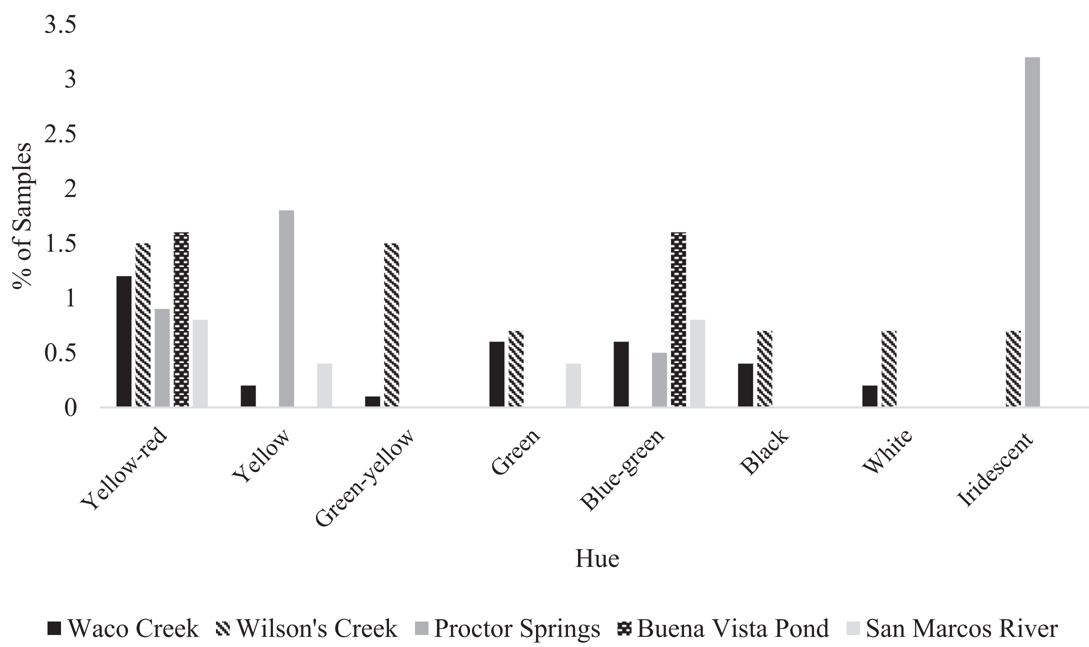


Figure 4.30. Breakdown of the 'Other' color classification category by hue for all study locales.

Table 4.34. Frequency and percent of microplastic particles within each major hue category for all study locales.

Hue Category	Waco Creek		Wilson's Creek		Buena Vista Pond		Proctor Spring		San Marcos River	
	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent
Transparent	246	29.6	44	32.4	17	26.6	72	32.7	43	17.6
Purple-blue, Blue, Purple	333	40.1	48	35.4	11	17.1	101	45.9	101	41.2
Red-purple, red	48	5.8	5	3.7	9	14.1	17	7.7	16	6.6
Others	30	3.6	8	5.8	2	3.2	14	6.4	6	2.4
Total	657	79.1	105	77.3	39	61.0	204	92.7	166	67.8

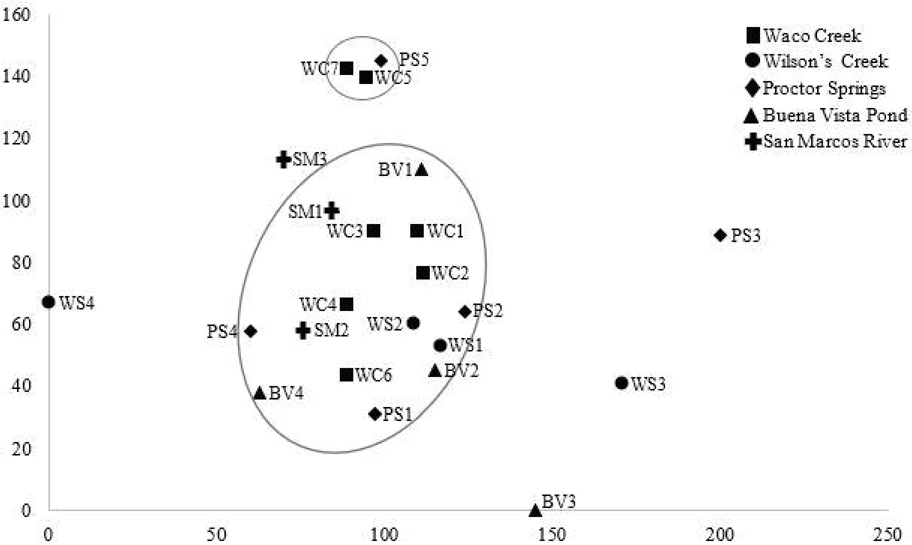


Figure 4.31. Community analysis program PCA DECORANA ordination plot of microplastic hue by sampling interval for all study locales.

The PCA DECORANA ordination plot of microplastic hue categories show the hues that were most abundant within the samples clustered together and centralized within the plot (i.e. transparent, purple-blue, red-purple, red, blue) as they are similar in frequency (Figure 4.32). Interestingly, this figure also displays a gradient of darker to lighter/brighter colors from left to right. This pattern suggests that time, weathering and/or proximity to source influences the groupings (i.e. lighter hues fade and weather easily through time vs. darker colors or vice versa). The outlying data points are those of white, iridescent, yellow and gray belonging to particles found in a single or only a few samples at one or two study locales exclusively. White microplastics were recovered at Waco and Wilson's Creek, iridescent at Wilson's Creek and Proctor Spring, yellow at Waco Creek, Proctor Springs and San Marcos River, and gray at Waco Creek. Outliers such as these and the rarity of certain colors may be best explained by a one-time or infrequent occurrence of an input source that is specific to the respective locale and the corresponding sampling interval. Higher frequencies of the rarer colors found in select places in addition to the possibility of said colors being less weathered may be explained by proximity to the source. These results are parallel with the color classification results shown in Figures 4.29 and 4.30.

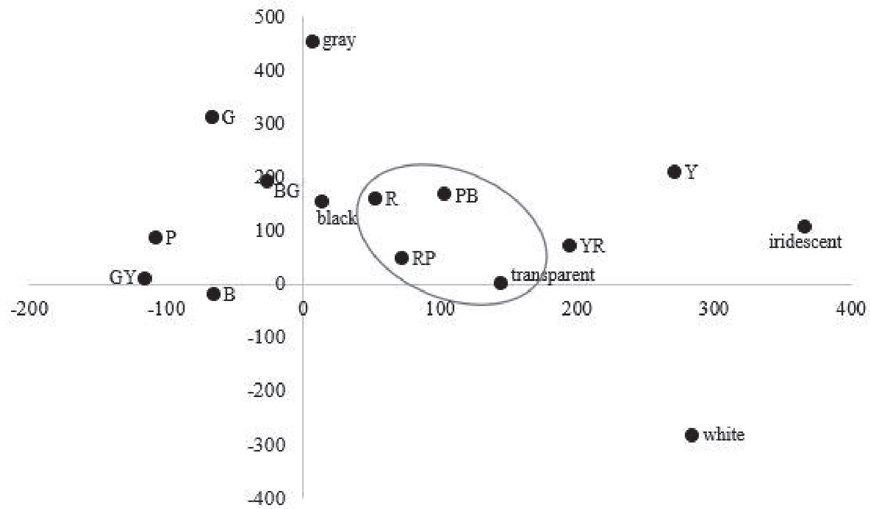


Figure 4.32. Community analysis program PCA DECORANA ordination plot of microplastic hue by overall frequency where G=green, BG=blue-green, P=purple, GY=green-yellow, B=blue, R=red, RP=red-purple, PB=purple-blue, YR=yellow-red and Y=yellow.

A color cluster analysis that groups together similarities in microplastic hue by both sample site and sample round was conducted using the Community Analysis Program. The resulting dendrograms (Figures 4.33 and 4.34) serve as supplemental results to the DECORANA ordination plots. The results indicate possible clustering by potential inputs as the upper watershed and springs sample sites are more closely clustered (i.e. Waco Creek’s uppermost site Beverly Drive, Proctor Springs seeps, Buena Vista Pond, Wilson’s Creek and San Marcos’ uppermost site Sewell Park), while the lower watershed and highly urbanized sites are clustered together (i.e. Waco Creek’s Floyd Casey, Bell’s Hill Park, BSB Bridge and Common Grounds). The higher recreation sites in both the upper and lower segments of the San Marcos River were also closely related, which further supports the idea that similar land use type may result in analogous input sources, plastic types and colors (i.e. swimsuits, plastic innertubes, rubber flip flops, etc.) Sample sites with higher mean levels of microplastics, or 'hot spots' (i.e. Waco Creek Baylor Bookstore, San Marcos Stokes Park and Proctor Springs surface flow) were found to be similar in hue as well (Figure 4.33). This may be a result of a wider variety of hues recovered in the samples due to a higher particle count. In contrast to the hue vs. sample site dendrogram and unlike the ordination plot for hue vs. sampling interval (Figure 4.31), there were no apparent patterns of temporal clustering by hue across sampling intervals using the dendrogram analysis method (Figure 4.34).

Comparative Analysis of Physical and Chemical Characteristics Across Sites

A series of regression analyses were performed to investigate whether relationships exist between the mean number of microplastics per sample site at Waco Creek and San Marcos River and the physical and chemical characteristics of each site, inclusive of mean depth, mean current, conductivity, current, temperature, total dissolved solids (TDS), pH, and dissolved oxygen (DO). For the purposes of statistical validity, Wilson's Creek, Proctor Springs and Buena Vista Pond were excluded from the these analyses due to at small number of sample sites. There is a slight decreasing linear trend between mean depth and mean microplastics per sample site (r^2=0.204) as well as a slight increasing linear trend between mean current and mean microplastics (r^2=0.319), both of which occur at Waco Creek (Figures 4.35 and 4.36). The positive relationship between current and microplastic levels suggests that active transport of particles may be occurring. Aside from these two findings, there were no other significant correlations observed for depth and current.

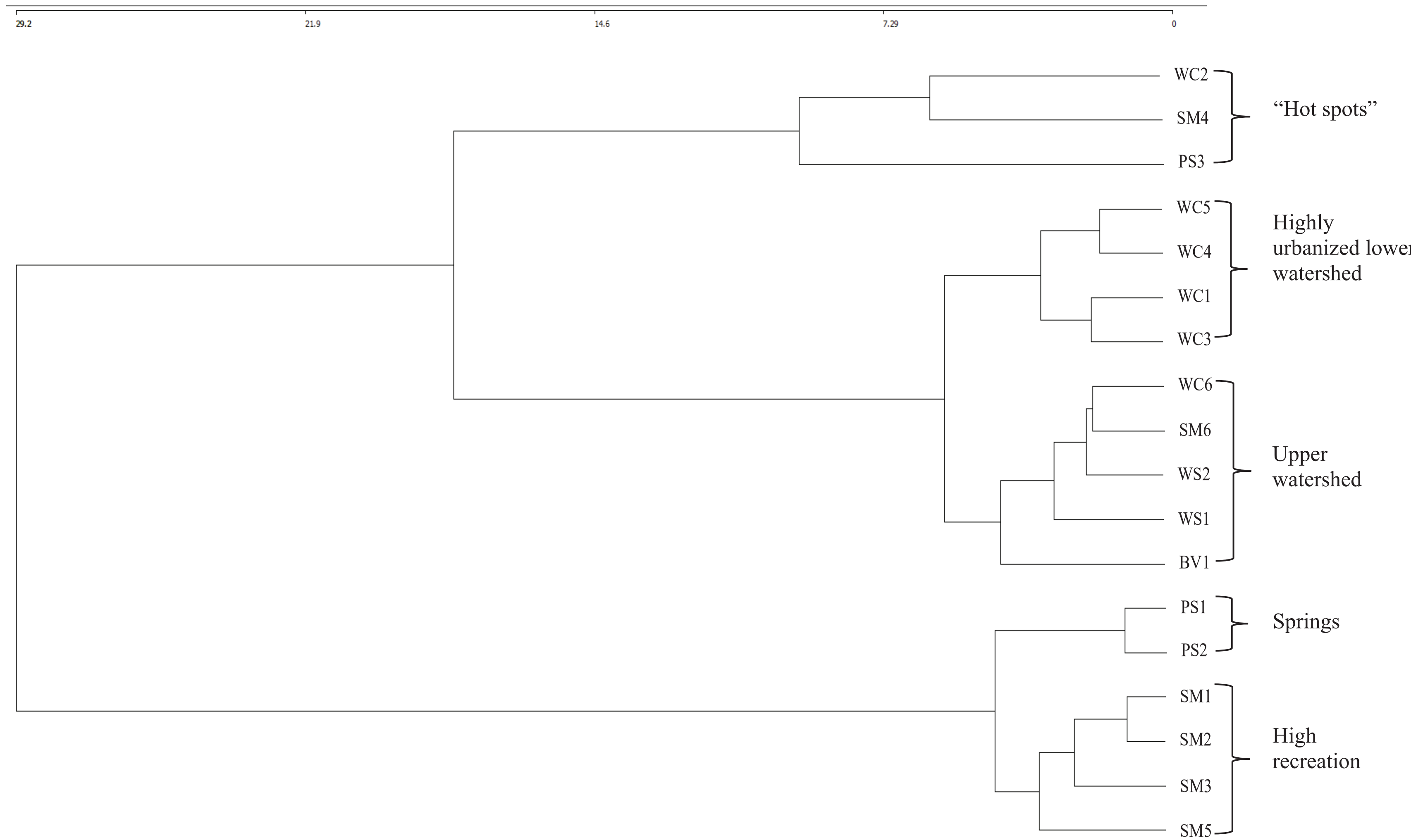


Figure 4.33. Community Analysis Program microplastic hue vs. sample site dendrogram. The clusters, as noted by the brackets, indicate similarity of potential inputs categorized into five main groups: the high frequency microplastic "hot spots", the lower watershed urban sites, the upper watershed sites, the springs and the high recreation sites.

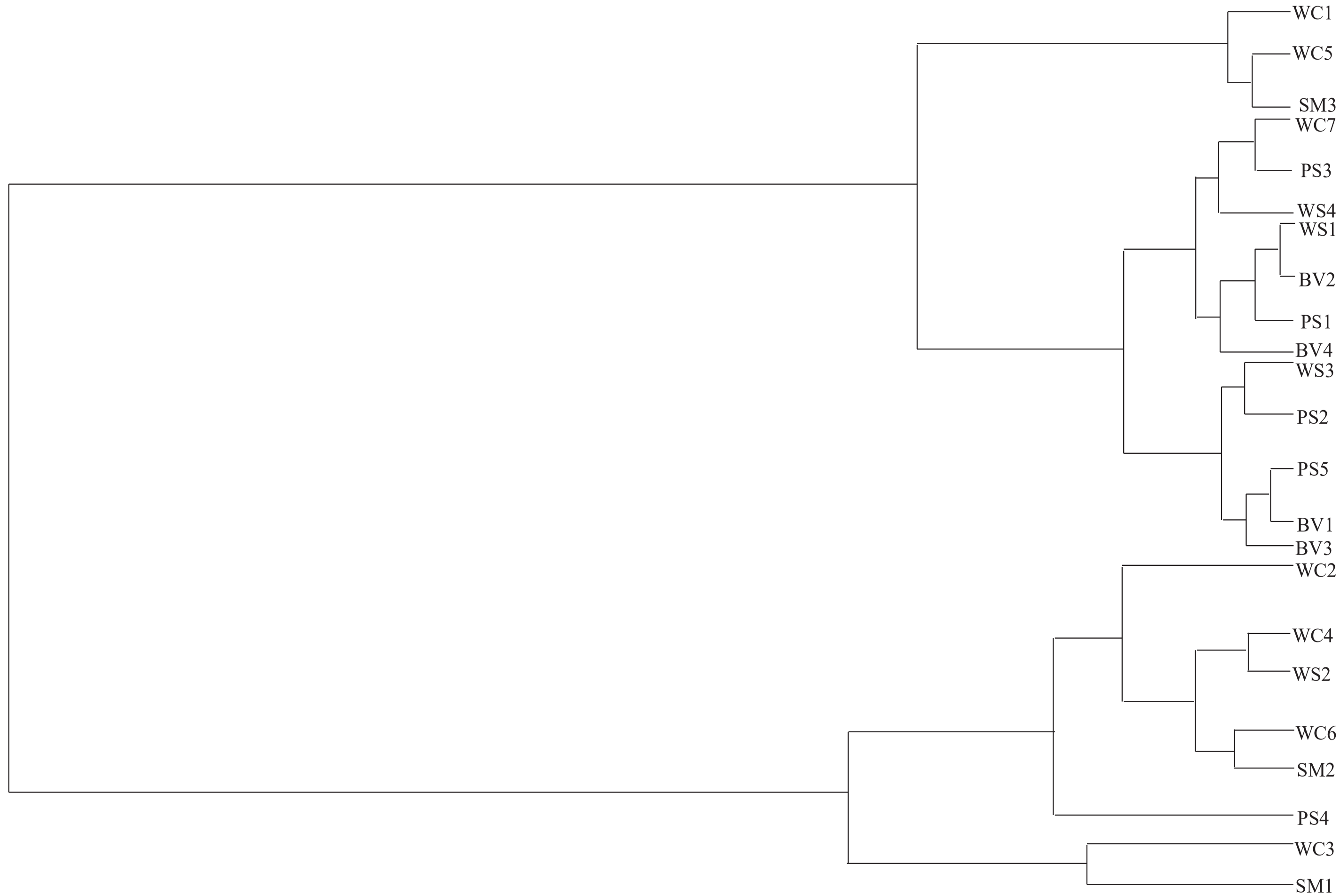


Figure 4.34. Community Analysis Program microplastic hue vs. sampling interval dendrogram. There were no apparent patterns of temporal clustering by hue across sampling intervals using the dendrogram analysis method.

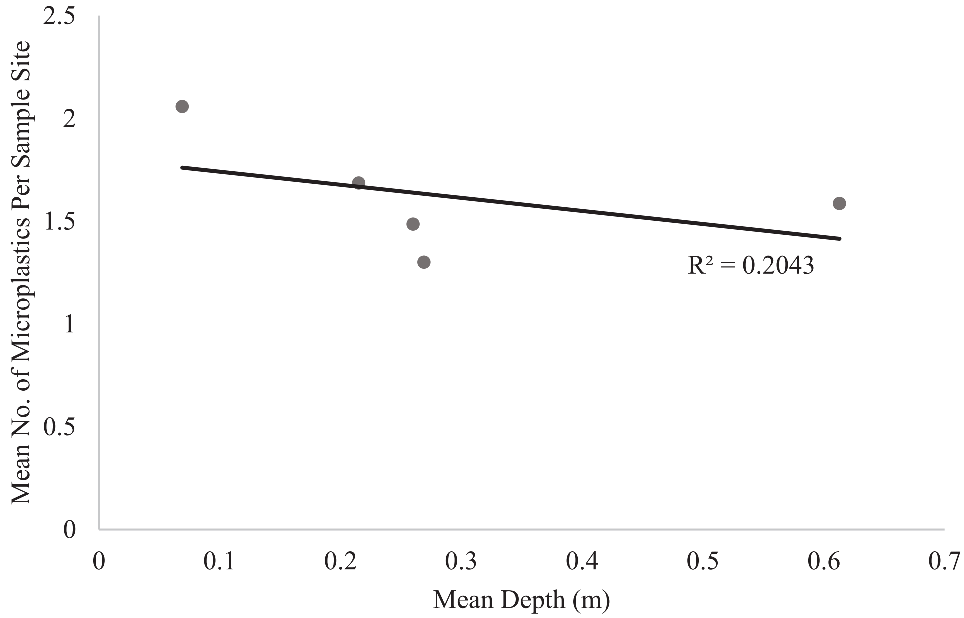


Figure 4.35. Mean depth vs. mean number of microplastics per sample site at Waco Creek. There is a slight decreasing linear trend apparent.

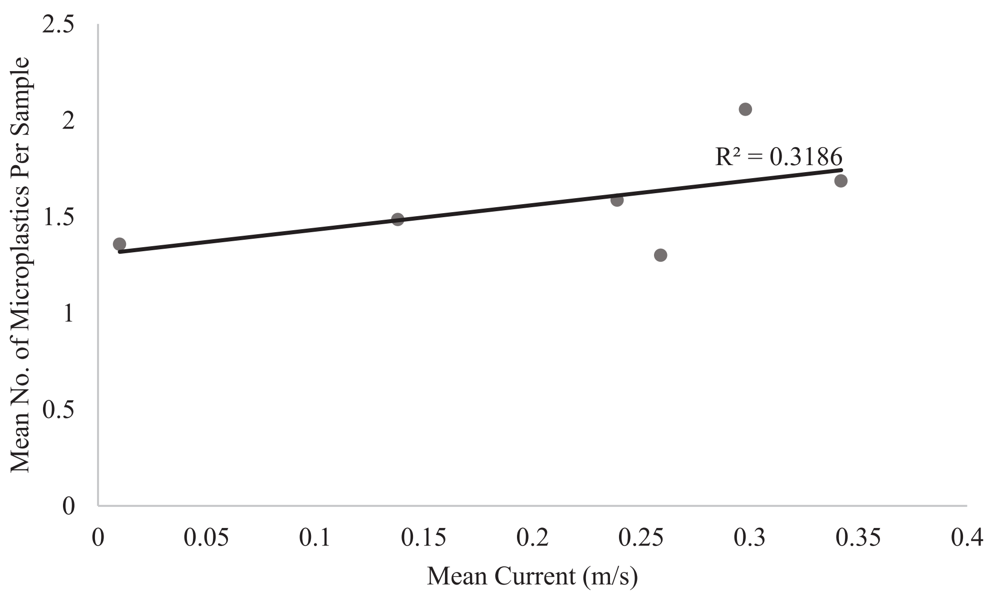


Figure 4.36. Mean current vs. mean number of microplastics per sample site at Waco Creek. There is a slight increasing linear trend apparent.

Regarding the regressions with chemical characteristics, significant correlations were found (i.e. conductivity vs. current, conductivity vs. TDS, conductivity vs. total plastic and TDS vs. total plastic) but were inconsistent among variables in both significance and positive or negative across sample sites within the same study locale and thus no comprehensible patterns were established. The qualitative chemical characteristic data collected in this study, in addition to more information regarding the chemistry of the plastics may, however, be useful in a future study. An additional regression analysis was conducted to investigate the role of rainfall events and its potential influence on microplastic levels at each sampling interval for all study locales. The total daily rainfall 1, 3 and 7 days prior to the sampling date was plotted against the mean number of microplastics per sample for each interval. Overall, the r^2 values did not yield any significant relationships between these two variables.

CHAPTER FIVE

Discussion

This study investigated the effects of seasonality, run-off and urbanization on microplastic pollution levels as well as the spatial distribution of particles within different types of freshwater systems on a small watershed scale through field sampling and visual analysis of urban surface water samples. In total, 1,198 microplastics were found, with fibers being the most common particle form, which is comparable to the results of urban surface water studies conducted by Dris et al., (2015b) and Wang et al., (2017). A strong, decreasing trend in the upstream direction at Waco Creek indicated higher particle counts, on average, in the lower end of the watershed. These findings are in contrast with those of the San Marcos River where significantly higher particle counts occurred in the upper end of the watershed, which consists mainly of the high traffic recreational sites. The lack of significance between micro-habitat types, water types and sample sites across study locales, except for the San Marcos River, suggest widespread pollution throughout the systems, with localized effects of land use and human activity driving subtle changes in microplastic influx levels. Although the micro-habitats sampled were relatively proximal, the lack of significance between running and still water micro-habitats across study locales indicates that there was no evident active transport or deposition of particles occurring within the systems. In the case of the San Marcos River, significantly higher levels of microplastic pollution in the upper portions of the watershed suggest that localized inputs via sources such as surface run-off and aerial transport may be influencing microplastic pollution levels in areas that are not receiving sewage discharge. These findings, parallel to those of Dris et al. (2015b), bring about question of the role of WWTP's as a source of input or removal of micrplastics during the treatment process.

A statistical ranking of all five study locales from most to least polluted found Proctor Springs to be the most polluted, while Buena Vista Pond was the least polluted. Significantly higher levels of microplastics at Proctor Springs demonstrate the variation in pollution levels between a low discharge spring and the other study locales. However, I am inclined to believe that this difference is more of a result of local land use differences and seasonality and less attributed to differences in water source. Hot spotting at high traffic recreational sites, in addition to the dendrogram results showing that similar land use type may result in analogous input sources, plastic types and colors are both findings that support the concept of localized effects being a major influence on the type of materials. For example, fibers may be prominent where plastic bags and clothing are the main inputs, whereas fragments may be more common where there is more ground up plastic items being deposited, such as plastic bottles. Overall, the results suggest that seasonality, land use and the associated local human activity have a stronger influence on overall microplastic frequency within the system, while the actual spatial positioning within the watershed likely influences particle color and form.

Several limitations and accessory variables were present throughout the course of this study, such as inclement weather, limited funding, time constraints and limited manpower, which prevented the possibility of a simultaneous sampling sequence of equal size across study locales, which ultimately would have been ideal. However, despite these limitations, the results of the study have set a strong foundational framework for gaining a better understanding of the behavior of microplastics within the urban surface waters of small watershed systems within the central Texas region. With relatively simple methodology, this study design can be easily adapted to similar aquatic systems for future comparison studies. The preliminary data collected may be used for future research that focuses more on temporal variables and seasonal events, such as rainfall, rather than spatial variables such as micro-habitats. Sampling individual micro-habitats at each site for all sampling intervals was a substantially time-consuming element of the sample collection process and ultimately did not yield any valid conclusions. Therefore, with careful consideration, this step may be omitted for future studies in similar small stream environments. A follow up project in which the recovered microplastics are chemically analyzed and identified by polymer type via methods such as Fourier-transform infrared spectroscopy (FTIR) or Pyr-GCMS may be beneficial in obtaining more information regarding possible input sources and common plastic types associated with land use type (i.e. recreational, residential, industrial, etc.). Finally, as more information regarding polymer type of the particles is obtained, further studies investigating possible relationships between chemical characteristics of stream, such as total dissolved solids and conductivity, and chemical properties of plastics within the system would also be a project worth undertaking.

Although research regarding microplastics as an emerging contaminant in freshwater systems is still in its infancy stages, this type of monitoring study makes a worthwhile contribution to the existing knowledge gaps. It also gives a first look at microplastic presence in understudied, yet equally important areas particularly urban streams and small watersheds Recent findings of microplastics in human fecal matter

(Wüstneck, 2018) extend the issue of microplastic pollution beyond the realm of ecological risk by providing evidence showing that microplastics may also pose a risk to human health. The global increase of plastic production and use, in addition to the continued overall mismanagement of waste further support the conclusion that more research, effective mitigation practices, governmental attention and public awareness are still very much urgent needs.

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