Spatial and Temporal Levels of Microplastics in Tampa Bay Surface Waters

by

Laurinda Kinsley McEachern

A thesis submitted in partial fulfillment
of the requirement for the degree of
Master of Science
Department of Environmental Science, Policy, and Geography
College of Arts and Sciences
University of South Florida St. Petersburg

Co-Major Professor: Henry Alegria, Ph. D.
Co-Major Professor: David Hastings, Ph. D.
Committee Member: Michael Martinez Colon, Ph. D.

Date of Approval:
March 2nd, 2018

Keywords: estuary, plastics, persistent organic pollutants, pollution, emerging contaminant, wastewater treatment

DEDICATION

For my parents, who have supported me and pushed me from the beginning of my life in any and every single way they could. Thank you for your unconditional love and support, and for teaching me that learning, curiosity, and compassion to give back to others are the greatest gifts we are given in our life. You have truly done everything in your power to give me the opportunities to pursue my passions and protect what I love. You both have enriched my life in countless ways as my parents, teachers, mentors, and support system- teaching me to challenge myself, encouraging me to believe in myself, supporting my love to explore the outdoors, and empowering me to help change our world in an impactful way.

And for my best friend, Lindsey, who has been like a sister to me and been there by my side to remind me of my abilities to complete tasks and help effect change in our world and natural environment. Thank you for all of your unconditional encouragement and love as my coworker, peer facilitator, and friend in such an impressionable time in my life. You have always been so kind to remind me of my ability to give back to our world and connect youth and other individuals to the environment while offering positive affirmations through all of my endeavors.

ACKNOWLEDGMENTS

I wish to formally acknowledge and most graciously thank my co-major professors, Dr. Henry Alegria and Dr. David Hastings, for imparting their invaluable expertise, support, and guidance throughout the journey of this graduate program. Thank you Dr. Alegria, for your extensive help with meeting the thesis and graduate program requirements and keeping me within the guidelines and timeline. I owe a great appreciation to Dr. David Hastings for imparting interest and support from the very beginning of my graduate career and ensuring my success and ultimate completion of the microplastics field work and lab project. I would also like to thank my committee member, Dr. Michael Martinez-Colon, for his assistance, interest in the project, and his generous gifts of time and assistance with editing and review of my thesis, including expertise regarding my statistical analysis. Thank you for coming on board when you were needed most.

Much gratitude is due to the Tampa Bay Estuary Program for their encouragement to apply for funding through the Tampa Bay Estuary Environmental Restoration Fund. I am deeply indebted to Tom Ash, Dave Karlen, Barbara Goetting, Ethan Mathiak, and Michael Schuman from Hillsborough County Environmental Protection Commission for the use of their boat, staff, and time to help with my project. I am grateful for the opportunity to accompany them on field runs and for their assistance in helping with the logistics of planning my project and collecting my samples. I am sincerely grateful to

Eckerd College for the generous use of their chemical oceanography lab, microscope, laboratory supplies, and facility. Sincere thanks to Dave Bennett and Bren Ross-Greene for laboratory and logistical assistance at Eckerd College. Thank you to Cypress Hansen from Eckerd College for her expertise and teaching of microplastic identification skills and laboratory methods. And, special thanks to Amelia Kalagher and Tessa Bolon for their laboratory assistance with processing my samples.

I am also grateful for the assistance of my professors Dr. Rebecca Johns, Dr. Chris Meindl, Dr. Barnali Dixon, Dr. Danny Smoak, and Dr. Richard Mbatu for their generous guidance and excellent teaching as well as academic expertise throughout my graduate program. I am grateful to have had the opportunity to take a well-rounded curriculum and enroll in classes by exceptional professors and experts in diverse fields. I am humbly grateful for the opportunity to be a teaching assistant and to be mentored by the Biology and Environmental Science department professors, including Dr. David John and Dr. Jimmy Ivey. Much gratitude is due to every member of my 2015 Environmental Science and Policy cohort, especially Amanda Chappel, Jess Jacobs, Ashley Huber, Tim McColgan, Solanke Adebayo, Leo Meirose, and Kyle Flanagan for their friendship, comradeship, GIS assistance and knowledge, and support.

Last, but definitely not least, I owe endless gratitude to my parents, my family, and my close friends for all their love, understanding, patience, support, and encouragement throughout my schooling and life.

TABLE OF CONTENTS

List of Tables iii

List of Figures vi

Abstract viii

Chapter One: Background 1
- Introduction 1
- Plastics as Marine Pollutants 4
- Formation and Properties of Microplastics 8
- Plastic Toxicity and Organic Pollutants 10
- Microplastics in Oceans, Rivers, and Estuaries 12
- Wastewater Treatment Plants and Agriculture 16
- Microplastics in Ground Water 19
- Microplastics in Air 19
- Tampa Bay 21
  - Old Tampa Bay and Old Tampa Bay Tributaries 23
  - Hillsborough Bay and Hillsborough River Tributary 24
  - Middle Tampa Bay 24
  - Lower Tampa Bay 25
- Contaminants and Wastewater Treatment in Tampa Bay 26

Chapter 2: Determining Spatial and Temporal Distribution of Microplastics in Tampa Bay 29
- Background 29
- Objectives 29
- Methodology 30
  - Site Selection 30
  - Sample Collection 33
  - Sample Processing 34
    - Discrete Samples 34
    - Plankton Tow Samples 34
  - Microplastic Identification 38
  - Quantification of Microplastics 41
    - Discrete Samples 41
    - Plankton Tow Samples 41
- Results and Discussion 42
  - Bay Stations and Regions 42

Tests of Normality 43
Volume and Rate of Flow of Freshwater Sources 53
Distance from Freshwater Input and Proximity to Gulf of Mexico 54
Salinity 58
Circulation of Tampa Bay: Depth and Tides 63
Sampling Period, Month, Season, and Precipitation 65
Wastewater Treatment Facilities 71
Kruskal-Wallis Test between Stations and Bay Regions 79
Welch's Anova and One-Way Anova between Stations and Bay Regions 80
Types of Microplastics 83
Discrete Versus Plankton Tow Samples 86
Contamination and Identification 88
Global Microplastic Evaluation 89
Conclusion 91

Literature Cited 96

Appendix I: Additional Text 102

Appendix II: Additional Tables 106

Appendix III: Additional Figures 120

About the Author .... End Page

LIST OF TABLES

Table 1.1: Plastic Polymer Identification and Characteristics 3

Table 2.1: Sampling Station Locations 32

Table 2.2: Microplastic Lab Blank Data 36

Table 2.3: Microplastic Concentrations: Discrete Samples (count/L) 45

Table 2.4: Microplastic Concentrations: Plankton Tow Samples (count/L) 46

Table 2.5: Average Microplastic Concentrations: Discrete Samples.....46

Table 2.6a: Average Microplastic Concentrations: Plankton Tow Samples 47

Table 2.6b: Adjusted Average Microplastic Concentrations: Plankton Tow Samples 47

Table 2.7: Annual Average Flow for Freshwater Sources into Tampa Bay 54

Table 2.8: Tampa Bay Domestic Wastewater Treatment Plants Greater than or Equal to 1 MGD Plant Capacity 74

Table 2.9: Total Tampa Bay Wastewater Treatment Facility Discharge by Bay Region (MGD) (1995) 76

Table 2.10: Summarized Results of Statistical Analysis 81

Table 2.11: Global Microplastic Counts.....90

Table A1: Microplastic Concentrations for fall/winter 2016 Plankton Tow Samples 107

Table A2: Microplastic Concentrations for spring 2017 Plankton Tow Samples 108

Table A3: Microplastic Concentrations for summer 2017 Plankton Tow Samples 109

Table A4: Average Monthly Streamflow of Tampa Bay Freshwater Inputs ($feet^3/sec$) 110

Table A5: Salinity at Surface 111

Table A6: Mean Depth (Meters below MLW) by Bay Region versus Discrete and Plankton Tow Average Concentrations 112

Table A7: Average Monthly Rainfall (inches) at Seven Rainfall Recording Stations over Specified Periods of Record.....112

Table A8: Precipitation Values (in) from One Day Prior to Sampling: Discrete Samples 113

Table A9: Precipitation Values (in) from Two Days Prior to Sampling: Discrete Samples 114

Table A10: Precipitation Values (in) from Three Days Prior to Sampling: Discrete Samples 115

Table A11: Precipitation Values (in) from One Day Prior to Sampling: Plankton Tow Samples.....116

Table A12: Precipitation Values (in) from Two Days Prior to Sampling: Plankton Tow Samples.....117

Table A13: Precipitation Values (in) from Three Days Prior to Sampling:

Plankton Tow Samples 118

Table A14: Flowmeter Calculations for summer 2017 Plankton Tow Sampling.....119

LIST OF FIGURES

Figure 1.1: Map of Tampa Bay with Major Cities and Counties and Bay Regions 21

Figure 2.1: Sampling Stations in Tampa Bay 31

Figure 2.2: Flow Chart Indicating Steps for Plankton Tow Sample Processing 37

Figure 2.3: A Small Red Microplastic Bead 39

Figure 2.4: A Blue Microplastic Fragment beside Diatoms 39

Figure 2.5: Blue and Clear Microplastic Threads Tangled in Plankton 40

Figure 2.6: A Desiccated and Digested Sample.....40

Figure 2.7a: Average Microplastic Concentrations: Discrete Samples 48

Figure 2.7b: Average Regional Microplastic Concentrations: Discrete Samples.....48

Figure 2.8a: Average Microplastic Concentrations: Plankton Tow Samples 49

Figure 2.8b: Average Regional Microplastic Concentrations: Plankton Tow Samples 49

Figure 2.8c: Adjusted Regional Averages: Plankton Tow Samples 50

Figure 2.9: Average Microplastic Concentrations: Discrete Samples 51

Figure 2.10: Average Microplastic Concentrations: Plankton Tow Samples 52

Figure 2.11: Average Microplastic Concentration by Bay Region versus Distance from Freshwater Source: Discrete Samples 56

Figure 2.12: Average Microplastic Concentration by Bay Region versus Distance from Freshwater Source (km): Plankton Tow Samples 57

Figure 2.13: Average Microplastic Concentration versus Salinity for Bay Stations:

Discrete Samples 61

Figure 2.14: Microplastic Concentration versus Salinity for Bay Stations:
Plankton Tow Samples 62

Figure 2.15: Average Microplastic Concentrations versus Wastewater Treatment
Facilities MGD (Millions of Gallons/Day): Discrete Samples.....77

Figure 2.16: Average Microplastic Concentrations versus Wastewater Treatment
Facilities MGD (Millions of Gallons/Day): Plankton Tow Samples 78

Figure 2.17a: Type of Microplastics: Discrete Samples.....84

Figure 2.17b: Type of Microplastics: Plankton Tow Samples 85

Figure A1: Average Microplastic Concentration versus Mean Depth of Tampa
Bay in 2000 by Bay Region: Discrete Samples.....121

Figure A2: Average Microplastic Concentration versus Mean Depth of Tampa
Bay in 2000 by Bay Region: Plankton Tow Samples 122

Figure A3: Concentration versus Precipitation (one day prior to sampling):
Discrete samples 123

Figure A4: Concentration versus Precipitation (one day prior to sampling):
Plankton Tow Samples 124

ABSTRACT

This study focused on collecting microplastics in Tampa Bay estuarine surface waters in order to identify temporal and spatial differences. While there have been many studies of microplastic contamination in the marine environment over the past decade, quantifying microplastics in urban estuaries is constrained. This study provides an understanding of the abundance, distribution, and composition of microplastics at twenty sites in Tampa Bay as well as four ancillary tributary stations. The objectives of the study were to identify how surrounding land use type, freshwater sources, seasonal changes in precipitation, and wastewater treatment plant discharge affect microplastic concentrations. Water samples were collected at 1 m below the surface by using a Beta Van Dorn discrete sampler, as well as a plankton net, to capture appropriately-sized microplastics (less than 5.0 mm) in four different regions of Tampa Bay as well as surrounding rivers. Samples were taken during different seasons from June 2016 through July 2017. Microplastics were identified through visual inspection under a dissecting microscope and a hot needle test was utilized to confirm that the particles were plastic.

Eighty-five out of 182 (47%) discrete samples contained microplastics. Ninety-three out of 97 (96%) plankton tow samples contained microplastics. Concentrations for discrete samples in the Bay and tributary stations ranged from 0.25-7.0 particles/ L with an average of 0.95 particles/ L. Samples taken with a 330 μm plankton net yielded a range of concentrations from 0.0012-0.018 particles/L with an average of 0.0047 particles/ L. For both discrete and plankton tow samples there were no statistically

significant differences in concentration between stations or bay regions at the 95% confidence level. Discrete microplastic concentrations demonstrated statistically significant differences between sampling periods potentially due to higher than average rainfall and storm water runoff during sampling period 10, July 2017, than other sampling periods. The highest average concentrations of microplastics for discrete samples in all bay regions were recorded in June 2016 or June or July 2017 (summer seasons) and the highest concentrations of microplastics for two bay regions (OTB) and (LTB) for the plankton tow samples were recorded in June or July 2017 when rainfall and runoff were elevated. June 2016 had higher than average rainfall for the month at (11.48 inches) as did June 2017 (7.9 inches) and July 2017 (8.99 inches).

Future studies can improve accuracy by sampling different depths of the water column as well as the vegetation and sediment. Sampling monthly over several years would help confirm seasonal differences and trends in concentrations in relation to precipitation levels and significant rainfall events. Possible contamination can be reduced by taking replicate field samples and taking larger volumes for discrete water samples. Furthermore, the identification procedure can be improved by confirmation of plastic polymers using fluorescent microscopy and Raman and Infrared spectroscopy. Modeling of microplastic movement using the numerical Estuarine Coastal Ocean Model (ECOM-3D) with the Lagrangian particle tracking method could help with prediction of spatial and temporal changes in microplastic distribution as well as determination of the transport pathways.

CHAPTER ONE: BACKGROUND

Introduction

With the rise of urbanization, industrialization, and an innately convenience- and commodity-based society in the 21st century, a new contaminant has emerged that pervades the marine and estuarine environment. This contaminant is in the form of plastic pollution. Plastic pollutants can take many forms: microplastic particulates which result from the break down over time of large plastic material; nurdles, the plastic pellet raw material used for producing plastic goods that can spill from ships or enter surface waters from land based sources; "microbeads" found in personal care products such as skin cleansers, toothpastes, and shampoos; and microfibers from synthetic textiles that can escape filtration in water treatment facilities and make their way into natural ecosystems along with treated water. In 1975, 48 million tons of plastic were produced annually (Jambeck et al., 2015); fast forward to 2016 and production of plastic reached 322 million metric tons, a 600% increase in production (Gasperi et al., 2018). In the 1960s, plastic made up less than 1% of the waste stream by mass in the U.S. (Jambeck et al., 2015). By the year 2000, the percentage of plastic in the waste stream had increased by an order of magnitude. In 2005, plastic made up at least 10 % of the solid waste stream by mass in 61 out of 105 countries with accessible data (Jambeck et al., 2015). This high production rate stems from the low cost of production, and the high demand and utility of plastic. The largest market sector for plastic resin is in single-use packaging (50%) which is

designed for almost immediate disposal, 20% is in long term infrastructure such as structural materials, and the remainder of plastics generated are used in furniture and other goods with intermediate lifespans (Hopewell et al., 2009). Out of the current production rate, 85% of the plastic produced is in the form of plastic pellets that are later transformed into different types of plastic; 12% is in the form of synthetic textiles and 2% is used in synthetic tires (Boucher and Friot, 2017). To date, more than 9 billion tons of plastic have been made since the 1950s. Globally, approximately 75% of plastics are discarded, only 15% are recycled, and 10% are incinerated for energy (Jambeck et al., 2015). Because of the magnitude of the implications of plastic production, use, mismanagement, and subsequent release into waterways, the atmosphere, and soil; and transport throughout our environment, the current period of time in history is now commonly referenced as the "Plasticene era" (Reed, 2016).

Today, plastics are considered by the United Nations Environmental Program to be one of the top environmental issues facing our species (Mason et al., 2016). A recent report compiled following six years of research from *The 5 Gyres Institute* estimates that 5.25 trillion plastic particles influence our ocean ecosystem and marine hydrology (Seltenrich, 2015).The ubiquity and far reaching effects of plastic can be felt throughout every biome on our planet. Particles have been quantified in remote marine environments, including the deep sea, as well as in coastal habitats, freshwater lakes, and rivers. Particles have also been identified in Arctic sea ice, coral reefs, ambient air and the digestive tract of sea birds, aquatic mammals, fish and deep sea invertebrates (Tyree and Morrison, 2017; Reichert et al., 2017).

Plastics are durable synthetic polymers that include polypropylene, polyethylene, polyethylene terephthalate, polyvinylchloride, polystyrene, and polyamide. Lighter, more buoyant plastic polymers, like polypropylene and polyethylene, are more commonly found on the sea surface, whereas other microplastics like acrylic are denser than sea water and probably accumulate on the sea floor (Table. 1.1) (Boucher and Friot, 2017). Organic plastic additives in the plastic polymers include benzophenone, 1,2-benzenedicarboxylic acid, dimethyl phthalate, diethylhexyl phthalate, dibutyl phthalate, diethyl phthalate, phenol and 2,4-di-tert- butylphenol. These additives can leach out of the plastic matrix over time and present a toxic and endocrine disruptive effect on marine organisms (Dekiff et al., 2014).

Table 1.1: Plastic Polymer Identification and Characteristics.

Polymer Name	**Common Name**	**Density (g/cm^3)**
Polyethylene	(PE)	0.98
Polypropylene	(PP)	0.89-0.91
Polystyrene	(PS)	1.04-1.11
Expanded polystyrene	(EPS)	0.02-0.06
Polyvinyl chloride	(PVC)	1.20-1.55
Polytetrafluroethylene	Teflon	2.20
Polymethyl methacrylate	Plexiglas	1.18
Polyacrylonitrile	Acrilan	1.18
Polyvinyl Acetate	(PVA)	1.19
Polyamides	Nylon	1.14
Polyesters	Mylar	1.37
Polyethylene terephthlate	(PET)	1.38-1.40
High density polyethylene	(HDPE)	0.94-0.96
Low density polyethylene	(LDPE)	0.91-0.93

(Source: Chemical Heritage Foundation)

Plastics as Marine Pollutants

Over the last half century, plastics have entered our marine environment in numbers that parallel production (Cozar et al., 2014). Most recently, deposition of plastics in the environment has accelerated past production, and plastics are now a persistent and common source of pollutants in beaches and oceans throughout the world. Jambeck et al. (2015) used worldwide data on solid waste, population density, and economic status to estimate the mass of plastic entering the ocean from land-based sources. The team calculated potential releases of plastic waste to the oceans that range substantially larger than the current estimate of floating plastic, anywhere from 4.8 Mtons/years to 12.7 Mtons/year with an average value of 8.0 Mtons/year.

Today, 60-80% of marine litter is plastic in some form, with this number potentially increasing to reach upwards of 90-95% (Moore, 2008). Derraik (2002) indicated that 62% of the total litter in a harbor study site originated from recreation and land-based sources. On the other hand, in remote beaches away from urban areas, the litter was mostly fishing debris. Lima et al. (2014) and Andrady (2011) qualify that plastic pollution enters estuaries through land runoff and from the ocean through wind, waves and tidal flow. Plastic pollution can enter sanitary systems and, although some may be trapped during sewage treatment, the majority will be discharged into marine water and concentrate on surface waters and be dispersed by currents (Derraik, 2002).

Although littering and mismanaged waste are often identified as the main source of plastic entering the ocean, primary microplastics are quantified as a major source of

concern especially in the developed world. In an analysis conducted by the International Union for Conservation of Nature and Natural Resources (2017) researchers developed a model that concludes that between 15-31% of the plastic found in the oceans originates from primary sources. These primary microplastics include microbeads from personal care products, micro fibers from textiles made of polyester, polyethylene, acrylic, or elastane, styrene butadiene rubber microplastics coming from tires and polyurethane, epoxy, vinyl and lacquer microplastics stemming from the abrasion of marine coatings, as well as microplastic from the paint and preformed polymer tape on roadways (Boucher and Friot, 2017).

Plastic pellets are the only form of primary microplastics that result in losses that occur during the production, transport, or recycling phase whereas most primary microplastics are lost during the use (wearing, driving, or abrasion) or maintenance phase (washing). The pathways of greatest loss include roadways which account for 66% of loss of primary microplastics, and wastewater treatment systems which account for 25% of loss of primary microplastics. About 34% of primary microplastic releases are due to the laundry of synthetic textile and about 28% of the releases of primary microplastics are due to the abrasion of tires while driving (Boucher and Friot, 2017). The global release of primary microplastics into the ocean was estimated at 1.5 million tons per year. The estimate ranged between 0.8 and 2.5 million tons/year, corresponding to one plastic grocery bag thrown into the ocean per person per week worldwide (Boucher and Friot, 2017). The largest regional releases occur in India, South Asia, and North America, followed closely by Europe, Central Asia, China, and East Asia. Releases in the different

regions depend upon population, economic development, and technological capability to process the microplastic losses and releases (Boucher and Friot, 2017).

Plastic pollution can have significant adverse effects on marine wildlife and on human health. In addition, plastic pollutants on beaches and in coastal waters and oceans may have negative impacts on the economy and aesthetics of coastal regions (Moore, 2008). Microplastics' hydrophobic nature helps attract persistent organic pollutants such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), endocrine-disrupting compounds (EDCs), and insecticides like DDT which, like microplastics, are also detected in effluent of wastewater treatment systems and marine ecosystems. Plastics can also act as a substrate for heavy metals, and they may transport invasive or pathogenic species, including bacteria, throughout ecosystems and thus, disrupt the natural ecology (Yonkos et al., 2014). Bisphenol A (BPA), along with many other monomers and polymers that make up polycarbonate plastics like polyvinyl chloride, along with phthalates or other plasticizers, and flame retardants, as well as antimicrobials and other chemicals used in plastic manufacturing can leach from the plastic and migrate to the marine environment where they bioaccumulate, presenting toxic effects to organisms in water bodies (Seltenrich, 2015). The weathering processes, along with the negative buoyancy of plastics, leads to far-ranging dispersal of plastics to remote areas where plastic litter does not originate (Cozar et al., 2014; Eriksen et al., 2014).

Research across the board indicates that 44% of seabirds, 26 species of cetaceans, and 267 species of marine organisms have been affected by plastics. The number

affected has been estimated at 500 million individuals a year, and this number will only increase as the microplastic effects on zooplankton and filter feeders at the base of the food web is further quantified. The ingestion of microplastics by organisms such as zooplankton, fish, and aquatic invertebrates results in physical and chemical complications such as gastric blockages, lacerations, and inflammation, the consumption of empty nutrients, cellular necrosis, as well as adsorption and assimilation of the harmful organic pollutants and chemical additives into the tissues of the organisms, oxidative stress, and even death (Cole et al., 2015) (Dekiff et al., 2014) (Rochman et al., 2015) (Sany et al., 2014). Identification of plastic debris in wildlife is meaningful, not only due to the environment consequences, but also in terms of human health threats which are becoming a widespread concern. Plastics have been identified and counted in the stomachs of fish and shellfish species marketed for human consumption in many countries throughout the world like Brazil, Indonesia, and California. (Lima et al., 2014; Rochman et al., 2015). Micro fibers and threads are potentially the most dangerous microplastics in terms of ingestion because of their shape and ability to hook around parts of the digestive tract (Boucher and Friot, 2017; Bakir et al., 2014; Carr et al., 2016).

Furthermore, plastics can affect sources of drinking water for human consumption. Drinking water in the Tampa Bay region comes from aquifer systems (groundwater), and surface water supplies (e.g., lakes, reservoirs, and streams) like the Hillsborough River, Alafia River, and Tampa bypass canal, which together account for 32% of the region's drinking water. If the rivers are polluted with microplastics, then ground and drinking water sources also have the potential to be polluted (Shober, 2009).

Formation and Properties of Microplastics

Plastic is made up of synthetic organic polymers derived from petroleum, most commonly polyethylene or polypropylene, which both show a high resistance to aging (Rios et al., 2007). The influence of plastics on marine organisms depends on the characteristics of the plastic particles in terms of size, shape, and chemical composition (Law, 2017). Plastic marine debris includes macro-plastic in the form of fishing nets and lines that entangle millions of fish, birds, and mammals (Eriksen et al., 2014; Moore, 2008). Through photodegredation (UV) and bacterial degradation, along with shore deposition, wave, and wind action macro plastics can degrade into microplastics. The effects of UV radiation on the degradation of plastics in sea water is slow due to the relatively lower temperatures and lower oxygen concentration in water compared to land (Andrady, 2011).

Plastic pollution is classified similarly across research studies, although the exact number of size classes identified, as well as the name given to the type and genre of microplastic, differ. For example, Eriksen et al. (2014) classify plastics into two microplastic size classes as well as one class of meso and one class of macroplastics. Microplastics are classified as plastic particles less than 5 mm in diameter that enter the aquatic environment as either primary or secondary sources. Microplastic particles vary in size, shape, and chemical composition. Microplastics formed from the breakdown of degraded plastics can be separated from natural debris by sieves. Plastics directly released into the environment in the form of small particulates are classified as primary

microplastics. They can be a voluntary addition to products such as microbeads and scrubbers from personal care products and cleaners, or they may take the form of plastic powders, nano-particles, and industrialized preproduction plastic pellets, or "nurdles." Primary microplastics can also originate from the abrasion of large plastic objects during manufacturing, use, or maintenance, the erosion of tires while driving, and the abrasion of synthetic textiles during use or washing. Larger plastic sources that are degraded by photo oxidation or mechanical action through wave and wind action in the marine environment produce secondary microplastics. Secondary microplastics are derived from discarded waste like styrofoam, plastic bags, bottles, wrappers, cigarette butts, and tires or unintentional plastic losses like those that accompany the use of fishing nets.

Scientific studies indicate that small-size microplastics may exist at higher amounts in aquatic ecosystems than what is being quantified due to loss of the plastics through net mesh during sampling. For instance, Estahbanati and Fahrenfeld (2016) identified the distribution of microplastics smaller than 355 μm, which is close to the size of a typical microplastics sampling net, in the fresh water environment in New Jersey Rivers. Throughout their study, the smallest class size of plastics was most prevalent. Furthermore, the laboratory analysis indicated that microplastics in the 63–125 μm and 125–250 μm size categories dominated the personal care products tested. These smaller classes of microplastics might not be adequately filtered out through drinking water or wastewater filters.

Plastic Toxicity and Organic Pollutants

There exists concern about toxic substances such as dyes, plasticizers, and antimicrobials on microplastics desorbing and leaching into the aquatic environment and becoming bioavailable. The presence of microplastics and persistent organic pollutants (POPs) in the aquatic environment is another toxicological concern since the hydrophobic POPs readily adsorb onto polymers of plastic. These contaminants are then potentially ingested and bioconcentrated, leading to bio-magnification through the trophic cascade (Rios et al., 2007). Examples of persistent organic pollutants (POPs) include flame retardants and pesticides. Both microplastics and POPs in the marine environment have extended residence times, which means their effects in the aquatic environment may persist over long periods of time (Dekiff et al., 2014).

Many research studies discuss the hydrophobic nature of plastic monomers of polyethylene, the most common polymer type used in manufacture, as well as toxic chemical additives like bisphenol A and phthalate plasticizers. The compounds added to plastics at the time of manufacture increase the adsorption of other toxic compounds in the environment. These contaminants can be transferred to filter feeding organisms and other invertebrates (Derraik, 2002) and pose adverse effects in terms of pathological conditions and physiological responses that are identified through measurable environmental degradation and toxicity experienced by individual wildlife (Sany et al., 2014).

Research regarding organic pollutants and microplastics in the Great Lakes and tributaries indicates that persistent organic pollutant concentrations are 10^5 to 10^6 higher associated with microplastics than in the surrounding water columns (Eriksen, 2014). Additionally, this study, as well as others on freshwater systems, indicate that microplastic concentrations were just as high, or higher, than those concentrations in oceanic gyres. The Great Lake tributaries study indicates that trace metals and pathogens accumulate on microplastics as well (Eriksen, 2014).

Microplastics are known to accumulate at the sea surface and animals with long life spans are chronically exposed to contaminants leaching from microplastics as a result of ingestion and transfer through the food chain. More information about microplastics and their associated contaminants, as well as their spatial and temporal distributions, including transport dynamics, interactions with biota (especially larger and long lived vertebrates), and information on potential accumulation areas, are needed for both marine and estuarine environments (Fossi et al., 2014). Anthropogenic sources of pollution like microplastics pose a great stress for aquatic ecosystems, especially since these ecosystems are the ones that are constantly threatened by persistent and toxic organic and inorganic chemicals (Sany et al., 2014). Finally, both Rios et al. (2007) and Andrady (2011) suggest a need for future studies that identify the probability of the transfer between POPs on plastic particulates and tissues found in living organisms.

Microplastics in Oceans, Rivers, and Estuaries

Research has been conducted in various locations throughout the United States as well as the world to document the threat of organic pollutants and microplastic pollution and their synergistic effects in marine and, more recently, brackish and freshwater ecosystems (Moore, 2008; Andrady, 2011; Lima et al., 2014). Land-based sources, as opposed to marine based sources, are considered the dominant source of plastics in the oceans (Besseling, 2017).

The majority of microplastic studies have focused on their presence and implications on the marine environment; much less is known about their movement on land, storage in soils and sediments, and transport by rivers to the marine environment. Implementing mitigation strategies for reducing plastic emissions to the world's oceans requires an understanding and quantification of the riverine sources taking into account spatial and temporal variability. Both Schmidt et al. (2017) and Lebreton et al. (2017), in their respective comprehensive modeling approaches, estimate the numerical quantity of plastic debris exported by rivers globally into the sea.

The model by Lebreton et al. (2017) aligns with field studies' data and indicates that between 1.15 million and 2.41 million tons of plastic waste currently enters the ocean every year from riverine sources. Seventy-four percent of emissions occur during the rainy seasons; and 20 rivers in the world, mostly located in Asia, account for about two-thirds of the world's plastic emissions. The Chinese Yangtze River is the largest contributing river catchment with an annual input of 0.33 million tonnes of plastic.

Factors responsible for this high contribution from Asian rivers include high population density, high mismanagement of plastic waste production rates, and episodes of heavy rainfall. A similar empirical study by Schmidt et al. (2017) aimed to estimate the amount of plastic exported by river catchments but utilized a larger data set and identified microplastics and macroplastics separately. Their study also confirmed that Asian rivers accounted for most of the input of plastics into the sea and in fact, 10 rivers in the world accounted for a greater percentage of the global load, up to 95%. Data was compiled from the water column (sediment was not included) and a median estimate of river inputs into the oceans for two different models were calculated: 0.48×10^6 tonnes per year and 2.75×10^6 tonnes per year.

Estuaries provide vital linkages between freshwater sources and marine environments. The connectivity and interaction between estuarine and ocean habitats results in variability of hydrological circulation patterns where the denser marine water flows below the freshwater influx, creating a stratified water column. The most critical result of this stratification is the influx of key nutrients for a diverse planktonic community, which is the basis of the estuarine food web. Estuaries are thus some of the most productive marine environments and serve as vital habitats for seabirds, fish, and mammals. Furthermore, estuaries are important marine coastal ecosystems used as settlement, feeding,and nursery grounds by many fish species. Many species of fishes spawn in estuaries at times that result in protection and food availability for their eggs and larva. These actions are influenced by seasonal variations of salinity, temperature, oxygen, turbidity, and availability of food resources. In turn, the distribution and

abundance of fish larvae and other planktonic organisms differ throughout space and time in estuaries worldwide (Lima et al., 2014).

While the hydrodynamic complexities of estuaries influence the biotic components of the marine environment, they also shape and play a role in the movement, transfer, and dispersal of inanimate material such as microplastics. Estuaries can serve as great sinks, conveyor belts, and sources of microplastic contamination. Due to the varied buoyancy of plastics, they can be found at the sea surface, in the water column, or in sediments throughout estuaries, all readily available for ingestion by marine animals. Studies of plastic pollution indicate that there is a wide range of marine organisms exposed to microplastics.

Microplastics floating over water are transported by ocean currents and are known to be concentrated in regions where water circulation is relatively stationary, on seashores, or in estuaries (Fossi et al., 2014). In a study by Lima et al. (2014) in the Goiana Estuary in Northeast Brazil, researchers found that the seasonal migration of a salt wedge and rainfall were the main contributory factors influencing the spatial and temporal distribution of microplastics along the main channel. Furthermore, high rainfall rates were found to be associated with microplastics flushing out and into the estuary and the rainfall rates were more influential on the presence of microplastics than seasonal variations were (Lima et al., 2014). The study reported the presence of microplastics in the estuary in Brazil at levels of half of the number of fish larvae. Moreover, since microplastics and fish larvae exist at comparable densities in the water column, the fish are prone to interaction with the microplastic particles, leading to increased incidence of

direct ingestion of microplastics (Lima et al., 2014).

Research in the Chesapeake Bay indicated that concentrations of microplastics are influenced by such parameters as proximity to human population centers, river mouths, prevailing ocean currents, and residences times of aquatic ecosystems. The study concluded that the potential for larger sources of plastics to degrade or break down and to be transported into the estuarine systems is great, especially near shorelines of densely populated urban areas. The study also found that secondary sources of microplastics were more prevalent in aquatic systems. Furthermore, all sites had peak mean microplastic concentrations during September sampling. In this particular study, Raman microspectroscopy was used to confirm the polymer composition of ten samples (Yonkos et al., 2014).

Understanding the fate and transport of microplastics in estuaries is invaluable to understanding the effects of a ubiquitous and persistent water quality contaminant. Moreover, these transport mechanisms and pathways affect water quality in larger bodies of surface water connected to the estuaries. This new field is in its infancy and limited studies exist to indicate the full spectrum of chemical and biological implications of microplastics on estuarine ecosystems. Furthermore, there are few large-scale studies that document the comprehensive effects of microplastics on estuarine water quality. These studies are critical to determine an average concentration of microplastics entering surface waters as well as the range of biological, chemical, and physical effects (Mason et al., 2016).

Wastewater Treatment Plants and Agriculture

A large fraction of the total emissions of microplastics from land is anticipated to originate in urban and industrial environments through household and industrial effluents and runoff. These microplastics are lost in use or maintenance of a product and directed through the drainage system and subsequently taken to industrial/municipal wastewater treatment plants and released along with treated wastewater or as sludge. Conventional wastewater treatment plant processes are designed to reduce the amount of organic matter, pathogens, and nutrients from the incoming influent. However, the processes are not as effective in removing micropollutants, including microplastics and organic pollutants.

In a study by Sutton et al. (2016), wastewater treatment facilities with varying levels of discharge, locations, and treatment technologies were tested for microplastic concentrations in San Francisco Bay. In another study on microplastics and wastewater treatment plants in several states throughout the U.S., including New Jersey, California, and Florida, the results indicated that wastewater treatment plants' effluents are a key source of microplastics and organic pollutants throughout the country. After averaging the data across all 17 facilities and sampling dates, 0.05 ± 0.024 microparticles were found per liter of effluent. This is a conservative value, but after taking into account that even the smallest municipal wastewater treatment plants discharge millions of gallons of water a day, the data was extrapolated for each wastewater treatment plant to predict

daily microplastic concentration for the individual plants. Approximately 50,000 to nearly 15 million particles of microplastics were discharged daily from different wastewater plants. Averaging across the 17 facilities, the results indicate that wastewater treatment facilities are releasing over 4 million microparticles per facility per day (Mason et. al., 2016).

Studies of wastewater treatment facilities throughout the U.S. documented smaller particles as being more prevalent than larger particles. These studies indicated an increasing concentration of primary microplastics in the <63, 125, and 250 μm categories downstream of all wastewater treatment plants (WWTPs) sampled, highlighting the role of WWTPs as a source of primary microplastics (Estahbanati and Fahrenfeld, 2016). Understanding the size class of microplastics is critical since the potential for these plastics to travel increases with decreasing size class of microplastics. Low density plastics tend to remain buoyant and float on the water's surface resulting in them traveling longrt distances (Mason et al., 2016).

The prevalence of micro fibers making their way into the environment has been confirmed in several studies, including one by Rochman et al. (2015) in which researchers found a prevalence of fibers within the stomachs of fish and shellfish caught and sold in Princeton, California, fish markets. This coastal area is surrounded by more than 200 wastewater treatment facilities discharging billions of gallons of final effluent of wastewater every day, thus serving as a major pathway for synthetic fibers to readily enter bay and ocean waters.

Nizzetto et al. (2016) highlighted the widespread application of sewage sludge coming from wastewater treatment plants and its use as nutrient-rich organic material or bio solids applied as fertilizer on crop lands in industrialized countries serves as a major input of microplastics to agricultural soils, which in turn has consequences for sustainability and food security. Effectiveness of microplastic retention in the sludge is dependent on particle density and size. Microplastics with densities greater than water are usually retained in sewage sludge during primary and secondary treatment. Tertiary treatment removes the larger floating particles, but smaller lighter particles are released with wastewater effluents. Over 90% of microplastics coming through wastewater treatment plants are filtered from being released in the effluent but retained in sewage sludge (USEPA, 2017).

Approximately 390,000 acres across the U.S. receive annual biosolids application. The majority of biosolids are applied to row and forage crops grown for animal feed and a smaller percentage is utilized for fertilizing vegetable crops. Even with a small percentage of land being fertilized with biosolids, Nizzetto et al. (2016) estimated that between 63,000-430,000 tons of microplastics are released onto North American farm lands yearly. Although regulations monitor the use of sludge containing harmful substances including heavy metals and organic substances, the European and U.S. regulations fail to include microplastics in their standards for biosolids. Microplastics can impact soil ecosystems directly or through the toxic and endocrine-disruptive properties of their chemical additives and substances that bind to microplastics in the environment. The study of microplastics in agricultural sludge and their movement and transport

through soil is in its infancy and more lab studies that focus on evaluating common types of microplastic waste like microfibers are needed in order to determine the risk to the soil ecosystem as well as groundwater sources (Rillig et al., 2017).

Microplastics in Ground Water

In a global study led by prominent microplastic researcher, Sherri Mason, a global survey of tap water from six regions of five continents was conducted from January to March 2017. Eighty-three percent of the samples were found to contain plastic particles. Most of the plastic was documented as fibers between 0.1 and 5 mm in length. The highest density of particles per volume of tap water was found in North America. This study exists as the first survey of tap water and though it was not comprehensive nor long-term, it provides background information on the implications of the use of plastic on our water sources and human health. Future studies that quantify and investigate plastic particles in drinking water should account for the source of the drinking water, as well as the filtration utilized, when analyzing samples. More extensive, longer term, and repeat analyses within particular regions would also be useful (Kosuth et al., 2017).

Microplastics in Air

Throughout the globe more than 90 million metric tons of textile fibers were produced in 2016. More than half of this annual production is comprised of synthetics. More importantly, production rates of plastic fibers has increased 6.6% every year over the last ten years, totaling 60 million metric tons. Fibers can be lost from clothing or textiles indirectly or directly either through wear or during the washing and drying process. The shedding of fibers from household textiles including carpets, cables, paint,

vinyl flooring, and cables made up of plastic polymers like acrylic, nylon, and polyester can be laden with polybrominated diphenyl ethers or other plasticizers that are endocrine-disrupting. Moreover, over 1,412.6 million vehicles in use globally contribute to potential losses of microplastics through the abrasion of the tires as well as the markings on the road that are made up of plastic derivatives (Boucher and Friot, 2017). Furthermore, city dust, derived from the abrasion of objects and infrastructure, contributes 24.2% of global releases of primary microplastics. If these micro plastic particles are respired, their harmful toxins can desorb and lead to genotoxicity as well as potential reproductive, carcinogenic, and mutagenicity effects on respiratory organs and cells (Gasperi et al., 2018).

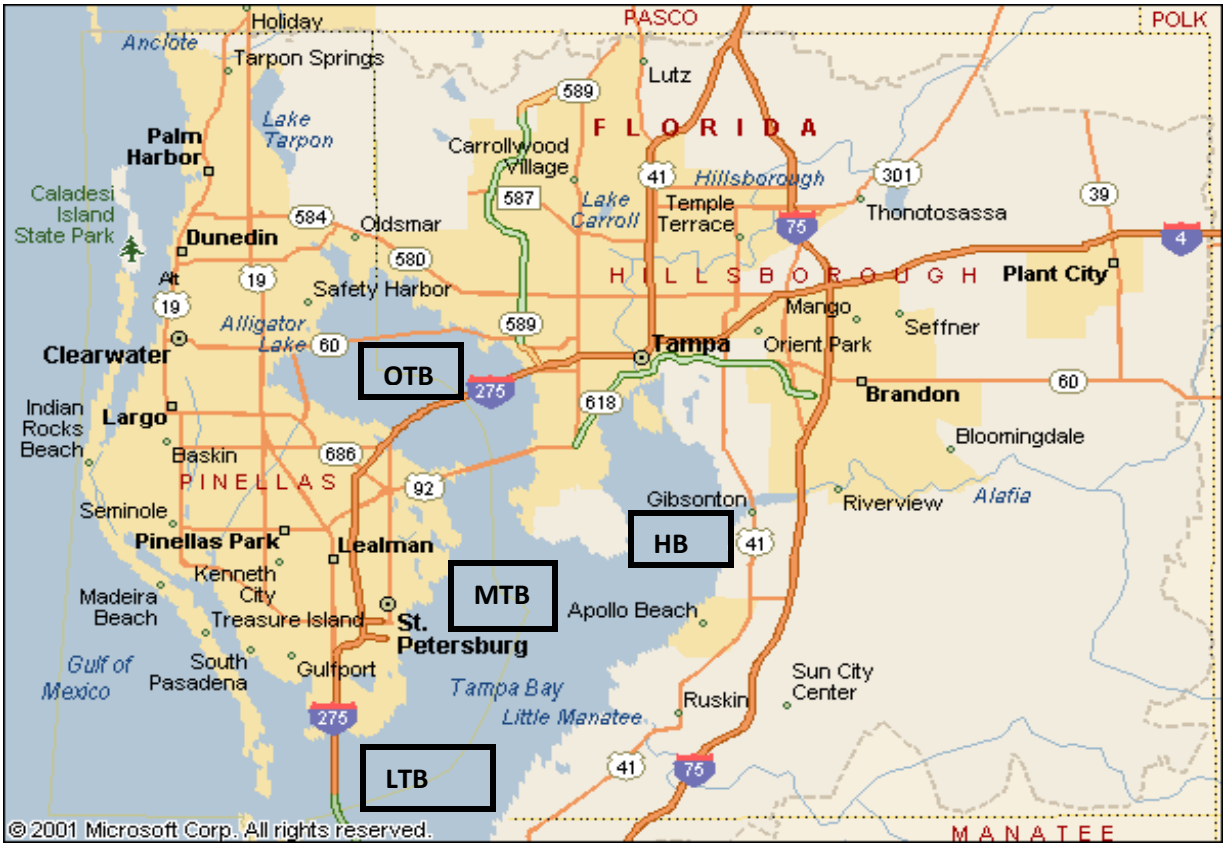

Figure 1.1: Map of Tampa Bay with Major Cities and Counties and Bay Regions. (Source: http://4.bp.blogspot.com/_pu-m0jtbjuy/tkkrhjd9syi/aaaaaaaab4i/naalf1r-bua/s1600/alt)

Tampa Bay

Tampa Bay is Florida's largest open water estuary, located on the West-Central Coast of the Florida Peninsula (Fig. 1.1). It consists of several interconnected bays and lagoons. Overall, the Tampa Bay watershed encompasses an area of approximately 2,200 square miles, providing essential habitat for a highly diverse composition of flora and fauna. The bay is divided into four different regions based on bathymetry and input of freshwater sources including the Hillsborough River, Alafia River, Manatee River and Little Manatee River, as well as additional fresh water inputs by Lake Tarpon and the

Palm River. Most of the coastal lands within the Bay's watershed are developed. Approximately 43% of the watershed area has been converted to urban/suburban land use, about 20% has been converted to agriculture, and about 5% of the landscape has been converted to extractive/mining practices. The first region of the Tampa Bay estuary is known as Old Tampa Bay (OTB), the region immediately west of the Interbay Peninsula. The second region is Hillsborough Bay (HB), the region east of the Interbay Peninsula. The third region is Middle Tampa Bay (MTB), extending from the Skyway Bridge to the Interbay Peninsula (IP). The fourth region is Lower Tampa Bay (LTB) and extends from the Gulf of Mexico to the Skyway Bridge (Tampa Bay Estuary Program).

The Bay's average depth is approximately 4 m and estuarine circulation within the Bay is driven by rivers, tides, and wind. The Bay is also characterized by a gradient of fresh to salt water. The mean calculated residence time of the Tampa Bay estuary is 150 days and is exponentially related to freshwater inflow, decreasing dramatically with increases in precipitation or point sources of freshwater. Previous studies surrounding estuarine circulation indicate that higher freshwater inflow equates to shorter residence times, with some exceptions to this relationship evident in different locations within the Bay (Meyers & Luther, 2008).

The Tampa Bay estuary serves as a regionally-significant environmental resource providing resources in the form of tourism, fisheries, and recreation for the regional area ($55 billion to the Tampa Bay area economy) and the State of Florida as a whole. The Tampa Bay estuary is home to a myriad of wildlife and extensive ecosystems such as freshwater, salt marsh, mangrove and sea grass habitats, and spawning and juvenile fish

habitat. Moreover, these precious habitats are home to more fish and bird species than in many other areas of the country. Together, these animals all depend upon good water quality to survive and thrive (Yates et al., 2011; Tampa Bay Estuary Program, 2006).

Old Tampa Bay and Old Tampa Bay Tributaries

Old Tampa Bay consists of the highly urbanized Pinellas County to the west, including the cities of Safety Harbor, Largo, Clearwater, and St. Petersburg. The eastern side of the Coastal Old Tampa Bay Basin encompasses the western side of the urbanized Interbay Peninsula, which includes the City of Tampa. Brooker Creek fuels Lake Tarpon, which is the closest freshwater source to Old Tampa Bay. The heavy urbanization of the area that surrounds Old Tampa Bay increases the impervious-ness of the land areas which means less percolation of water back into the surficial aquifer and more transport of storm water flows to Old Tampa Bay. Moreover, the eastern side of the Old Tampa Bay Basin was developed prior to the adoption of the modern storm water management regulations and technology in the late 1970s to mid-1980s, so there are greater volumes of storm water runoff of poorer quality entering this bay area in comparison to areas that were developed more recently. Additionally, there are twelve domestic categories and two industrial facilities located in the surrounding land areas with more than half of them permitted to have daily capacity greater than 1 M gal/d (Yates et al., 2011).

Hillsborough Bay and Hillsborough River Tributary

The Hillsborough River Basin originates from the Green Swamp, which includes the headwaters of the Withlacoochee River. The Hillsborough River is the largest source of freshwater inflow into the Bay, with an annual mean rate of 15 m^3/sec. Crystal Springs contributes its discharges of the Upper Floridan groundwater providing 85 to 100 percent of the Hillsborough River's flow during dry periods, although groundwater discharges have been declining due to natural and anthropogenic factors. Surface runoff from the highly urbanized eastern side of the Interbay Peninsula could influence the amount of primary and secondary microplastics entering the Bay waters. The lower eastern part of Hillsborough Bay encompasses the Alafia River Basin, whose upper section originates in Polk County and converges in eastern Hillsborough County where the Alafia River is formed. Lithia and Buckhorn Springs provide discharges from the Floridan aquifer to the river. The Alafia River contributes an average flow rate of 13 m^3/ second every year to Tampa Bay. Urbanized land is rapidly increasing in both coastal and inland areas of Hillsborough Bay. Phosphate mining has greatly affected the headwater area of the Alafia River. Agriculture has a great influence on the lower eastern section of Hillsborough Bay. Furthermore, over 162 wastewater discharges are permitted to discharge into surface waters on the surrounding land areas of this bay region (Yates et al., 2011).

Middle Tampa Bay

Coastal Middle Tampa Bay Basin is defined largely by the urban city of St. Petersburg, located on the west side of the Bay, as well as the agricultural area on its east side. Furthermore, a desalination facility withdraws up to 44 Mgal/d of surface water

from Middle Tampa Bay to produce potable water for regional distribution before discharging 19.5 Mgal/d of brine concentrate into the cooling water discharge at the TECO Big Bend Power Station. This power station circulates more than 1000 Mgal/d of cooling water to and from the northeastern shoreline. The Little Manatee River Basin, encompassed by Middle Tampa Bay, is a 220-mi^2 watershed with a predominately agricultural land use. This part of the Bay is the least urbanized in all of Tampa Bay, however, groundwater discharges from agricultural irrigation impact the surrounding wetlands (Yates et al., 2011).

Lower Tampa Bay

Lower Tampa Bay is composed of several different basins. Eastern lower Tampa Bay is made up of Coastal Lower Tampa Bay and the Terra Ceia Bay basins, which are in northern Manatee County expanding over an area of about 56-mi^2. The predominant land uses here are agricultural and urban. There is a former phosphate processing facility located in the land area surrounding Lower Tampa Bay that is closed and managed by the State of Florida. Manatee River Basin includes a 1,900 acre instream reservoir (Lake Manatee) that supplies water for surrounding Manatee County. Within this watershed the land use is mostly agricultural, with some rangeland, urban, wetlands, and upland forest. The cities of Bradenton and Palmetto, and their surrounding suburbs, account for the urban land use. There are also 14 domestic and industrial facilities that discharge wastewater within this sub basin. Finally, Boca Ciega Bay Basin is surrounded by a highly urbanized landscape (83%). The municipalities of Gulfport, St. Petersburg, St. Pete Beach, and South Pasadena surround this basin, making it a high density residential, commercial, and industrial land use area. Similar to the land surrounding Old Tampa

Bay, much of the urbanization here occurred before modern storm water management regulations, therefore, there is a lot of untreated storm water discharge from this land area that can discharge into the bay (Yates et al., 2011).

Contaminants and Wastewater Treatment in Tampa Bay

From the 1950s to the 1970s, extensive habitat destruction continued to occur due to industrial, commercial, and residential growth and development around Tampa Bay (Florida Department of Environmental Protection, 2015). The expansion is attributed to the great increases in population density. As a result, since the 1950s, Tampa Bay has been impacted by point and nonpoint sources of contaminants, varying from inorganic and organic pollutants, coming from wastewater discharge from municipal sewage treatment facilities, phosphate transport and processing sources, storm water runoff, power generating systems, fertilizers, pesticides, as well as waste and urban discharge (Lewis and Russell, 2015). The urban impacts often stem from the industrial areas as well as the most populated cities in the Tampa Bay Area, which include Tampa in Hillsborough County, and the cities of St. Petersburg and Clearwater in Pinellas County (McCain et al., 1996). Tidal currents, estuarine circulation, wind driven flow, and turbulence all make up the hydrodynamic advection which controls the residence time of contaminants and organisms throughout Tampa Bay. These contaminants include chemical tracers, nutrients, and pollutants. Tidal currents account for 95% of the current energy transfer and movement in Tampa Bay. Estuarine circulation, which is driven by freshwater flowing into the north end of the Bay, creates a density gradient with the salty ocean water of the Gulf of Mexico at the south end of the Bay. As a result, the freshwater

flows on the surface heading to the mouth of the Bay, whereas, the salty water flows in at a greater depth (Meyers and Luther, 2008).

Although the 1970s brought improvements in municipal sewage treatment practices and levels of treatment to reduce concentrations of nutrients and other contaminants going into the Bay, studies suggest that current wastewater treatment facilities are not equipped to completely filter out microscopic, highly mobile and long-lasting contaminants like microplastics or POPs which act as endocrine-disrupting chemicals (EDCs) (Yates et al., 2011; Cook, 2015). Conventional wastewater treatment plant processes are equipped to remove organic matter, pathogens, and nutrients, but even in advanced treatment plants, POPs and microplastics have been found in effluents. The combination of POPs and microplastics represents a critical combination and a collective threat to the Tampa Bay estuary in terms of water quality.

The North County sewer system collects and transmits domestic wastewater to the William E. Dunn Water Reclamation Facility, whereas the South sewer system collects and transmits domestic wastewater to the South Cross Bayou Water Reclamation Facility. The South Cross Bayou Water Reclamation Facility is an Advanced Wastewater Treatment Facility that releases treated water as reclaimed water for irrigation, as well as surface water discharge directly into Joe's Creek, treating 21.04 million gallons of water per day. Joe’s Creek runs into Cross Bayou which eventually discharges into Lower Tampa Bay. The Dunn facility, located directly west of Tarpon Lake, is an Advanced Wastewater Treatment (AWT) plant with treatment achieved through the Bardenpho process. It releases its 6.41 million gallons per day of effluent through the county's

reclaimed water system (The Pinellas County Planning Department, 2013). Furthermore, two City of Clearwater treatment plants, one Oldsmar plant, and the Largo treatment plant, discharge effluent directly into Tampa Bay. Additional plants discharge into creeks and tributaries that are interconnected with the Tampa Bay watershed. There is also great potential for a large source of microplastics to be released indirectly to the Bay through reclaimed water which eventually returns as runoff to surface water (Cook, 2015). Furthermore, Tampa Bay is characterized by surrounding agricultural lands (27% of watershed surface area) that are adjacent to the freshwater rivers that flow into the Bay. Even if the majority of microplastics are being removed from the wastewater treatment effluent or reclaimed water being discharged, the microplastics concentrated in the sludge have a high chance of becoming eventual runoff into the surface waters of Tampa Bay (Yates et al., 2011).

CHAPTER 2: DETERMINING SPATIAL AND TEMPORAL DISTRIBUTION OF MICROPLASTICS IN TAMPA BAY

Background

This work is the first assessment of levels and distribution of microplastics in Tampa Bay. This information is needed to understand the health of the Bay with respect to these pollutants and is critical to the development of sound management strategies for the Bay. It is also necessary to know the concentrations and distribution of microplastics as a first step to pursuing further research on potential impacts in the Bay.

Objectives and Hypotheses

Specific objectives of this study are: (1) quantification of concentrations of microplastics in Tampa Bay; (2) determination of spatial and temporal patterns in microplastic concentrations in Tampa Bay; and, (3) comparison of two different methods for microplastic sampling: discrete water vs. plankton tow. The specific hypothesis of this study is that concentrations of microplastics will be correlated with proximity to urban sources, freshwater sources, periods of higher rainfall, and bay region wastewater treatment plant effluent.

Methodology

Site Selection

The Environmental Protection Commission of Hillsborough County (EPC-HC) has conducted comprehensive water quality sampling in Tampa Bay for over 40 years. The agency conducts monthly sampling at over 200 stations to record bacteria, nutrients, plankton, algal blooms, water temperature, salinity, turbidity, wind speed, wave height, and tidal stage, all relevant to our investigation of microplastics. Their data can be accessed at ftp://ftp.epchc.org/epc_erm_ftp/wqm_reports/.

Twenty-four stations were chosen from the EPC-HC monthly sampling locations to collect discrete and plankton tow samples over the course of ten months (Table. 2.1 and Fig. 2.1). Five sites are located in Old Tampa Bay (stations 65, 42, 60, 61, and 50); six sites in Hillsborough Bay (44, 52, 7, 8, 55, and 80); five sites are in Middle Tampa Bay (84, 28, 19, 33, and 14); and four sites are in Lower Tampa Bay (91, 22, 92, and 94). Four sites were selected in rivers that flow into Tampa Bay. Stations 141, 102, and 103 are in Old Tampa Bay tributaries; station 2 is in the Hillsborough River Tributary. The 24 sites were chosen to represent different parts of the Tampa Bay system, specific urban population densities, industrial and wastewater outputs, as well as proximity to pristine bodies of water and healthy mangrove, oyster, and seagrass ecosystems.

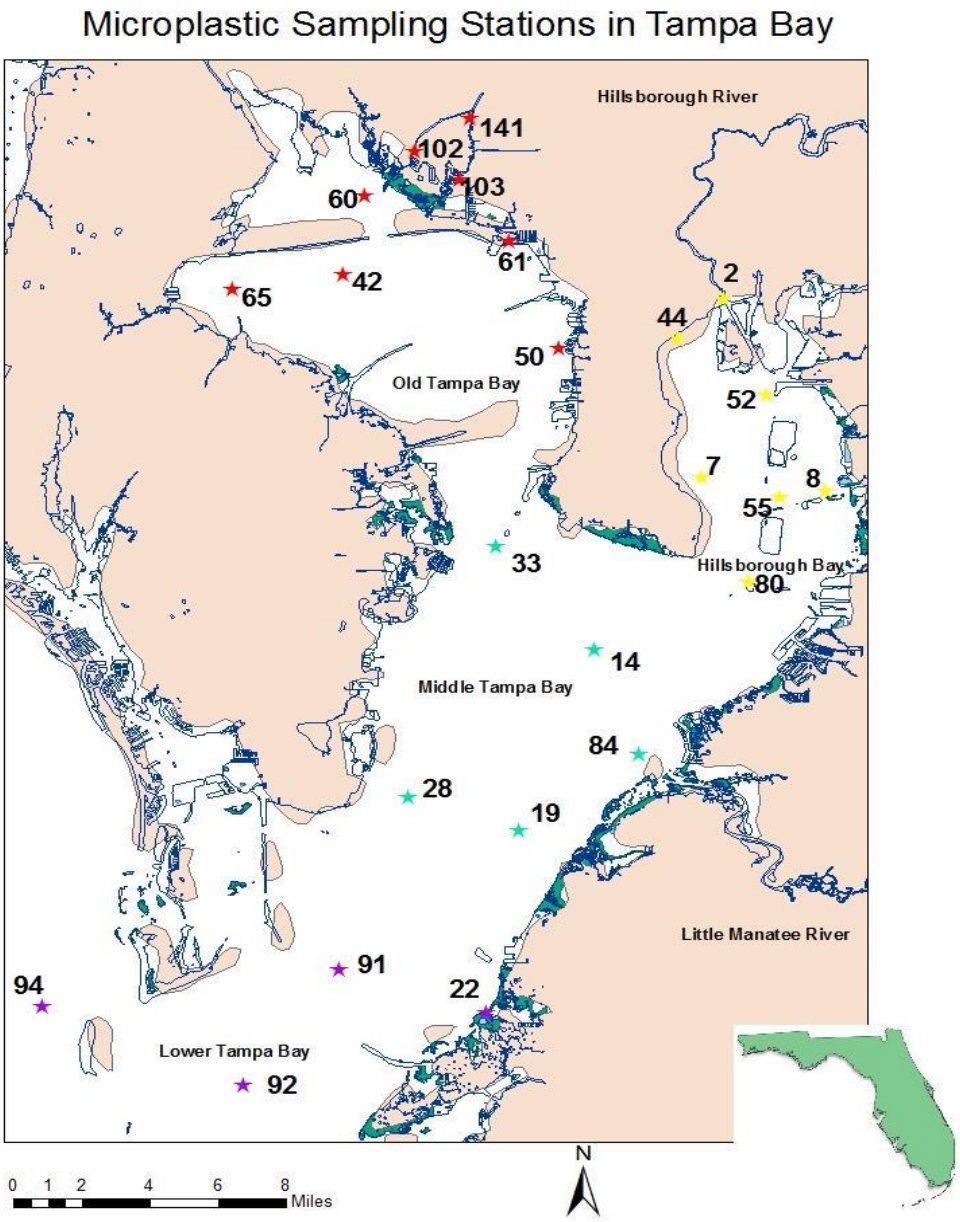

Figure 2.1: Sampling Stations in Tampa Bay.
Sampling locations for discrete and plankton tow samples are marked by a star.
(Red: Old Tampa Bay (OTB) and Old Tampa Bay Tributaries (OTB Tributaries); yellow: Hillsborough Bay (HB) and Hillsborough River tributary (HB Tributary); blue: Middle Tampa Bay (MTB); purple: Lower Tampa Bay (LTB))

Table 2.1: Sampling Station Locations.

Bay Region	Sta #	Type of Sample	Lat	Long	Freshwater Source	Distance (km)	Surrounding Land Use
OTB	50	D, P	27.91850	-82.53790	Lake Tarpon	20.4	Heavily urbanized
OTB	61	D, P	27.96870	-82.56210	Lake Tarpon	15.2	Heavily urbanized
OTB	60	D	27.98990	-82.63160	Lake Tarpon	8.2	Heavily urbanized
OTB	65	D	27.94560	-82.69430	Lake Tarpon	10.1	Highly urbanized
OTB	42	D, P	27.95280	-82.64160	Lake Tarpon	10.7	Highly urbanized
HB	44	D	27.92370	-82.48070	Hillsborough R.	2.9	Ag, Urban
HB	7	D	27.85890	-82.46860	Alafia River	6.8	Urban, Ag, mining
HB	80	D	27.80960	-82.44600	Alafia River	6.5	Urban, Ag, mining
HB	8	D, P	27.85240	-82.40930	Alafia River	0.9	Urban, Ag, mining
HB	52	D	27.89700	-82.43820	Palm River	5.7	Urban, Ag
HB	55	P	27.84930	-82.43140	Alafia River	3.1	Urban, Ag, mining
MTB	14	D	27.77800	-82.52030	Little Manatee R.	7.3	Urban, Ag
MTB	84	D	27.72900	-82.49870	Little Manatee R	1.6	Urban, Ag
MTB	19	D	27.69340	-82.55590	Little Manatee R	7.2	Urban, Ag
MTB	33	D	27.82610	-82.56750	Lake Tarpon	26.6	Urban, Ag
MTB	28	D, P	27.70840	-82.60920	Little Manatee R	11.9	Urban, Ag
LTB	22	D	27.60810	-82.57120	Manatee River	11.5	Urban, Ag
LTB	92	D	27.57370	-82.68680	Manatee River	6.4	Urban, Ag
LTB	94	D, P	27.61000	-82.78320	Manatee River	16.3	Urban, Ag
LTB	91	D	27.62790	-82.64150	Manatee River	11.0	Urban, Ag
OTB Trib	141	D	28.02610	-82.58120	Old Tampa Bay Trib	0.0	Heavily Urbanized
OTB Trib	102	D	28.01060	-82.60780	Old Tampa Bay Trib	0.0	Heavily Urbanized
OTB Trib	103	D	27.99760	-82.58630	Old Tampa Bay Trbi	0.0	Heavily Urbanized
HR Trib	2	D	27.94183	-82.45854	Hillsborough R	0.0	Urban, Ag

OTB= Old Tampa Bay, HB= Hillsborough Bay, MTB=Middle Tampa Bay, LTB= Lower Tampa Bay, OTB Trib=Old Tampa Bay Tributaries, HR Trib= Hillsborough River Tributary; D= discrete sample; P= plankton tow sample; Ag= agricultural, mining= phosphate mining

Sample Collection

Microplastic particles were collected using two different methods: discrete water samples and plankton tow. The discrete samples were captured using a Beta Van Dorn sampler. The discrete samples were taken at five sites in Old Tampa Bay (65, 42, 60, 61, and 50); five sites in Hillsborough Bay (44, 52, 7, 8, and 80); five stations in Middle Tampa Bay (33, 14, 84, 19, and 28); four sites in Lower Tampa Bay (22, 92, 94, and 91); three sites in the Old Tampa Bay tributaries surrounding Tampa Bay (141, 102, and 103); and one site in the Hillsborough River Tributary (2). The Beta Van Dorn sampler was opened, triple-rinsed in seawater, and then lowered to one meter below the water surface to collect the discrete sample. The sample was poured into a one-liter HDPE collection bottle that was cleaned in the lab prior to field sampling and triple-rinsed in sea water at the site, then stored at 4C.

Plankton tow samples were taken with a 333 µm plankton net with a diameter of 0.5 m at two stations in Old Tampa Bay (50 and 42); two stations in Hillsborough Bay (55 and 8); one station in Middle Tampa Bay (28);and,one station in Lower Tampa Bay (94). The net and collection bottle was triple-rinsed with seawater at the station before being deployed, towed at ~2 knots (1 m/s) one to two meters below the sea surface for 3 minutes outside of the research vessel's wake. After detaching the first cod end collection bottle, the net was thoroughly washed in the field with seawater from the location using an additional 1 L triple rinsed collection bottle to collect the overflow sample. This

ensured no microplastics were stuck on the net. The plankton tow and overflow samples were frozen until processed.

Sample Processing

Discrete Samples

In the laboratory, discrete water samples were vacuum-filtered through a 1.2 µm 47 mm diameter gridded cellulose nitrate (CN) filter paper with grid spacing of 1/8 inch. Microplastic particles on the filter were counted under a 33x dissecting microscope. Characteristics including shape, color, and type of each plastic piece were recorded. In order to avoid air borne or water borne procedural contamination of microplastics, cotton lab coats were always worn and clothing that easily shed microplastic fibers was not worn. The laboratory counter surface was wiped down before each use and filter parts were rinsed thoroughly in the sink before each use, and then a complete rinse followed using deionized water in a squeeze bottle. Furthermore, a cover was placed over the top of the vacuum filter during use. The filter papers were removed with tweezers and then placed directly in a covered petri dish each time that a water sample was filtered. Blanks of deionized water were taken to provide a baseline comparison for any amounts of possible contamination in the deionized water, glassware, or lab environment. The deionized blanks were processed using the same method as that used for discrete and plankton tow samples (Table 2.2).

Plankton Tow Samples

Plankton tow samples contain a large amount of organic material which were removed by a digestion process in order to facilitate counting of the microplastic particles. First, visual separation and removal of large pieces of material, such as sea

grass, from the sample was conducted. Next, samples were split five times with a Folsom plankton splitter. Samples were then filtered through a 212 µm wire mesh into a beaker and rinsed with distilled water (Fig. 2.2). Filtrate was filtered again through a 1.2 µm CN filter. The material that did not pass through the mesh and that remained on top of it was rinsed with distilled water into a 45 mL glass vial. The sample vials were desiccated at 65- 80° C. After the samples were fully desiccated, protein content of the samples was reduced following the digestion protocol outline by Cole et al. (2014). 15 mL of a 250-mL homogenizing solution, containing 15.77 g of Tris HCl, 4.38 g of EDTA, 1.53 g of NaCl, and 1.26 g of SDS, was added to each vial. Samples were then vortexed for 30 seconds and homogenized using an 18-gauge needle and syringe. The samples were heated at 50 C for 20 minutes in a hot water bath. Finally, 375 µl of Proteinase K (500 µg/ml) was added and vials were incubated for 2-4 hours at 50 C in a hot water bath. After incubation, 5 mL of 5M $NaClO_4$ was added to each vial and they were vortexed for 30 seconds. The solutions were filtered onto a 1.2 µm 47 mm diameter gridded cellulose nitrate filter paper. Microplastic particles on the filter paper were counted under a dissecting microscope (Fig. 2.2). In addition to following the protocols to avoid contamination used in the discrete sample methodology, plankton tow sample processing also involved covering the beakers during the rinsing process and sample vials were covered with a fine mesh to ensure that no particles entered the vials during desiccation in the oven. Furthermore, blanks were utilized to ensure that the plankton splitter procedure did not result in contamination of the samples with outside plastics (Table 2.2).

Table 2.2: Microplastic Lab Blank Data.

Type of Blank	Date	# of microplastics counted
Discrete	8/29/16	0
Discrete	9/13/2016	0
Plankton tow (splitter)	10/4/16	0
Plankton tow (splitter)	10/6/16	0

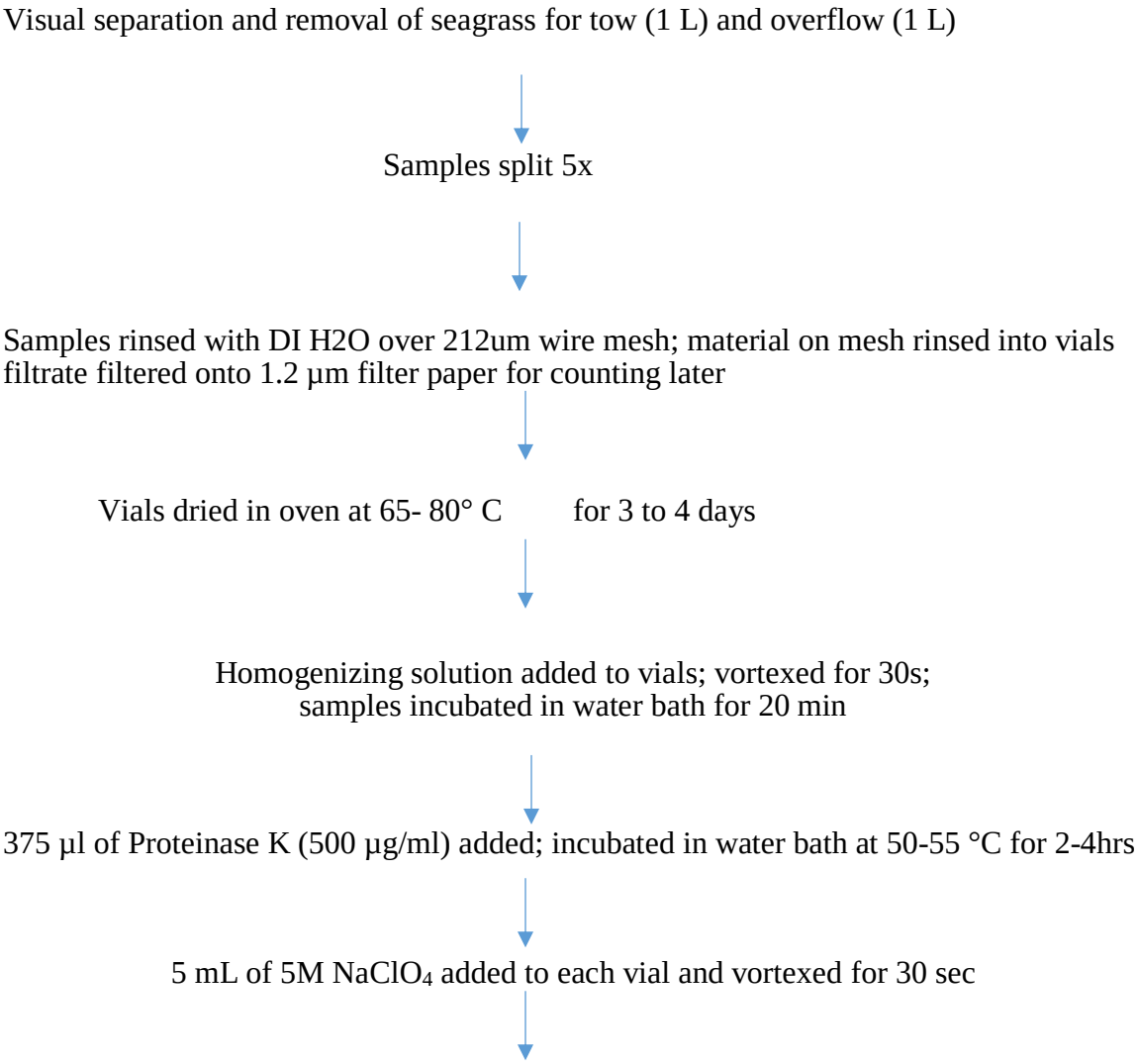

Figure 2.2: Flow Chart Indicating Steps for Plankton Tow Sample Processing.

Microplastic Identification

Microplastics were identified as pieces ≤ 5mm possessing no cellular structures, equally thick throughout their entire length, and either clear or homogeneous color throughout (Hidalgo-Ruz et al., 2012). Any pieces believed to be plastic were probed with a heated dissecting needle. If the material quickly melted or changed shape, the sample was classified as plastic. Biological materials do not melt but rather burn and react less to heat (Hidalgo-Ruz et al., 2012). Microplastics were categorized as either

- fibers/ threads/ filaments/lines;
- beads/pellets;
- fragments/shards;
- film, flakes; or,
- foam (Figs. 2.3- 2.6).

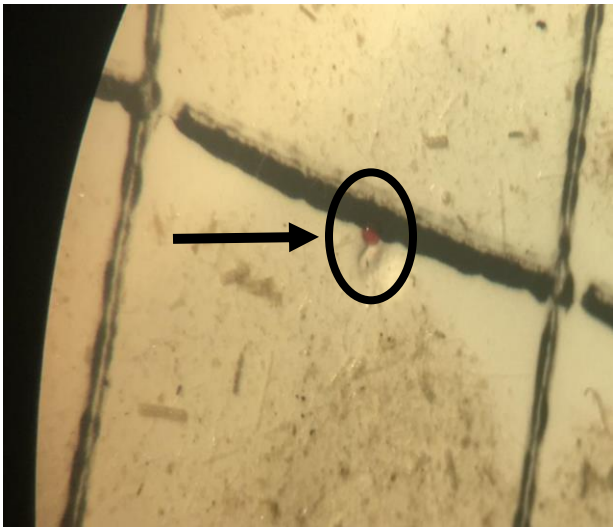

Figure 2.3: A Small Red Microplastic Bead.
Indicated by the arrow and encompassed by the circle. (grid square =1/8 inch)

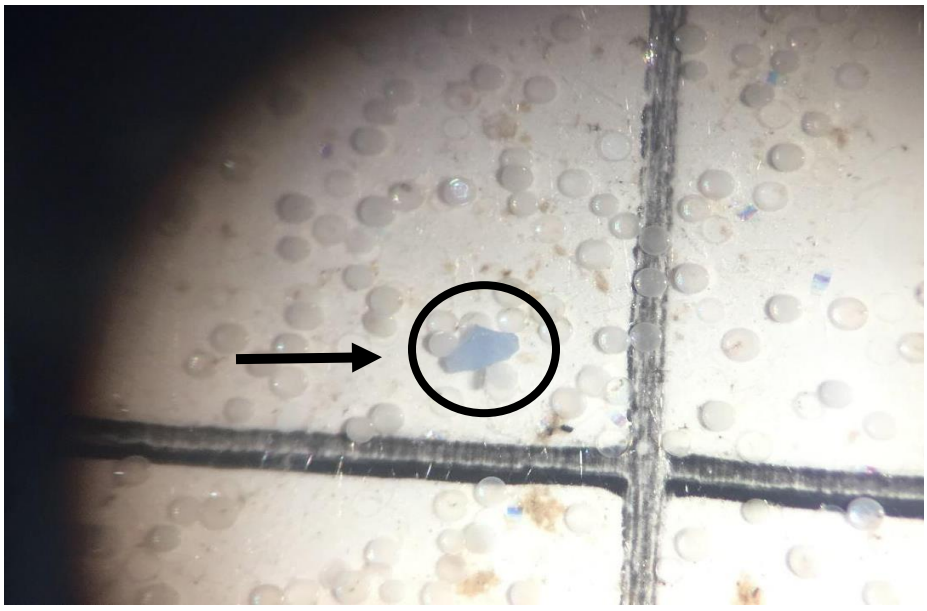

Figure 2.4: A Blue Microplastic Fragment beside Diatoms.
Indicated by the arrow and encompassed by the circle. (grid square =1/8 inch)

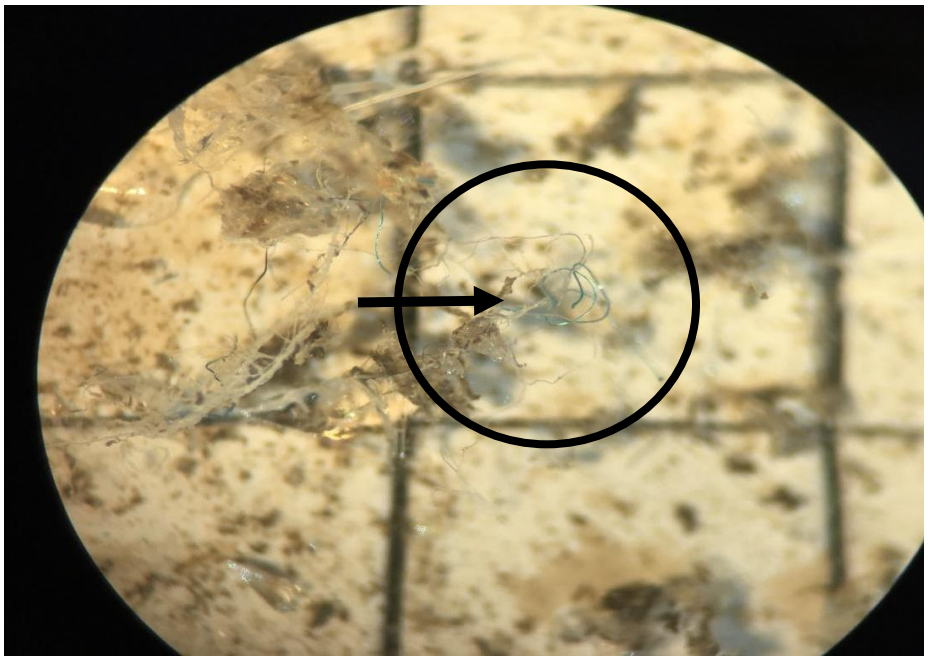

Figure 2.5: Blue and Clear Microplastic Threads Tangled in Plankton. Indicated by the arrow and encompassed by the circle. (grid square =1/8 inch)

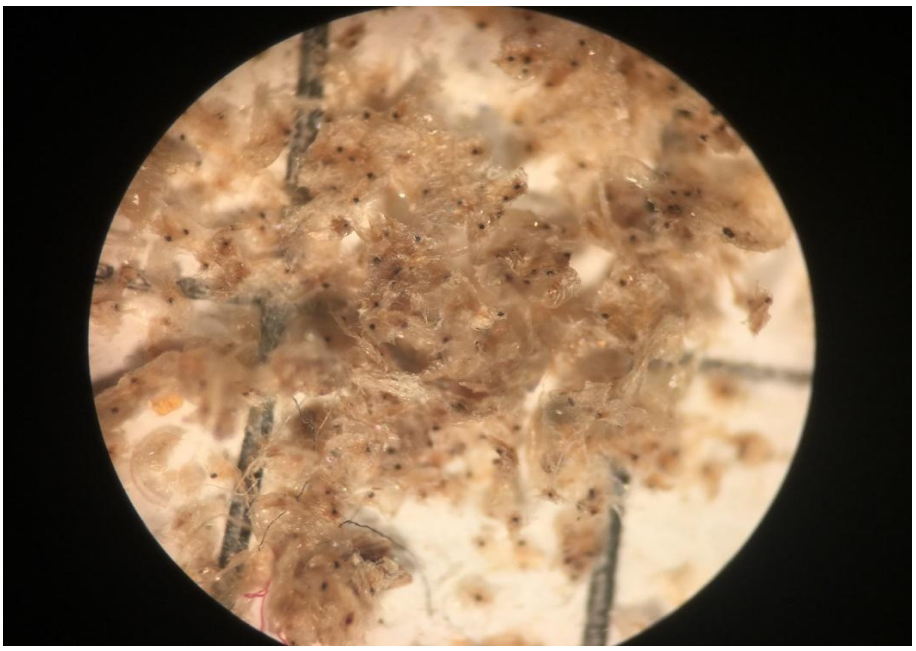

Figure 2.6: A Desiccated and Digested Sample. (grid square = 1/8 inch)

Quantification of Microplastics

Discrete Samples

Discrete water samples were filtered through a gridded 1.2 µm cellulose nitrate filter paper for counting under a dissecting microscope. Microplastics were identified as explained above and quantified.

Plankton Tow Samples

The number of microplastics counted after 5 splits was multiplied by 32 to get the number present in the original unsplit sample. Geographical coordinates at the beginning and end of each tow were used to calculate the distance traveled, which was used in combination with the area of the opening of the plankton net (19.625 cm^2) to calculate the volume of water sampled.

A calibrated flowmeter was incorporated at the end of the field study in June and July 2017. The flowmeter was used for stations in Middle and Lower Tampa Bay in June 2017 and for all stations in July 2017. This distance calculated using the flowmeter provides greater accuracy in determining the distance traveled when towing the net accounting for the influence of the net being weighted down with plankton, as well as wave action, and currents. This volume was compared to the volume determined by using geographical coordinates and was found to be within a similar range; therefore, the original volumes and concentrations for the plankton tow samples were maintained (Table A14). Additional data, including air temperature and water temperature at the surface, were recorded. Other chemical parameters, such as salinity, were determined in the laboratory at EPC-HC and included in this report.

RESULTS & DISCUSSION

Bay Stations and Regions

The average discrete microplastic concentration for bay regions was 0.94 particles/L; the average discrete microplastic concentration for tributaries was 0.96 particles/L. The average plankton tow microplastic concentration for bay regions was 0.0047 particles/L.

The average discrete microplastic concentration in Old Tampa Bay was 1.03 ± 1.63 (counts/L), the second highest concentration for the four bay regions. The average plankton tow microplastic concentration was 0.0038 ± 0.0015 (counts/L), the lowest average value for the four bay regions for plankton tow samples. The average discrete microplastic concentration for Old Tampa Bay Tributaries was 1.67 ± 1.41 (counts/L) which was the highest average concentration compared to the other bay regions and Hillsborough River Tributary. Hillsborough Bay's overall microplastic concentration for discrete samples was the lowest with an average of 0.68 ± 0.93 counts/ L. The Hillsborough River Tributary's average microplastic concentration for discrete samples was 0.25 ± 0.50 (counts/L), the lowest discrete concentration out of all the bay regions and tributaries. On the other hand, Hillsborough Bay's average plankton tow concentration was 0.0046 ± 0.0020 counts/ L which was the second highest average regional value. It would have been the highest average regional value had it not been for

Lower Tampa Bay's elevated concentrations for the sampling events in June 2017 and July 2017, directly following heavy rain events that caused increased storm water runoff to enter Lower Tampa Bay. Middle Tampa Bay had the highest average microplastic concentrations for discrete samples at 1.14 ± 1.64 (counts/L) and the second lowest average microplastic concentration for plankton tow samples for station 28 at 0.0044 ± 0.0026 counts/ L. Lower Tampa Bay's average microplastic concentration for discrete samples was the second lowest average out of the bay regions at 0.92 ± 1.05 counts/L. The average concentration for the plankton tows for station 94 in Lower Tampa Bay was 0.0058 ± 0.0071 counts/ L. Both Hillsborough and Lower Tampa Bays are located near urban and agricultural areas; the fact that these two regions have the higher average plankton tow concentrations aligns with the original hypothesis that increased urban and agricultural land use increases microplastic concentrations. The large standard deviations for both discrete and plankton tow sample average concentrations indicate that the variability between stations, and within sampling periods, is high. This high variability suggests that the average concentrations for both discrete and plankton tow samples are not significantly different from one another. Further statistical analysis was utilized to compare the bay and tributary stations and regional concentrations for significant differences (Tables. 2.3-2.6b) (Figs. 2.7a and 2.7b) (Figs. 2.8a-2.8c) (Figs. 2.9 and 2.10).

Tests of Normality

In order to assess the data for normality, histograms, Q-Q plots, and the Shapiro Wilk test of normality were utilized on the discrete microplastic concentrations, plankton tow concentrations, distance to freshwater source, salinity, bay depth, precipitation, and wastewater treatment discharge data. The discrete microplastic data did not follow a

normal distribution. After log transformation of the data set, the data did not follow a normal distribution; therefore, it was analyzed with non-parametric statistical methods. The plankton tow microplastic data was log transformed with a $\log_{10}(x+1)$ transformation; data then fit a normal distribution so parametric tests were utilized. The other data sets utilized with tests of correlation between microplastic concentration and the respective parameter did not follow normal distributions nor result in a linear relationship so the Spearman's correlation test was utilized to test for monotonic correlation (Table 2.10).

Table 2.3: Microplastic Concentrations: Discrete Samples (count/L).

Bay Region	Sta #	Jun-16	Jul- 16	Aug-16	Sep-16	Oct-16	Nov-16	Apr-17	May-17	Jun-17	Jul-17	Avg	Std. Dev
		6/8		**8/1**		**10/3**	**11/1**	**4/10**		**6/5**	**7/5**		
OTB	65	0		0		0	2	0		0	2	0.57	0.98
	42	0		0		1	4	0		0	5	1.43	2.15
	60	0		0		0	0	1		5	1	1.00	1.83
	61	0		0		0	0	4		1	3	1.14	1.68
	50	4		2		0	1	0		0	0	1.00	1.53
	mean											**1.03**	**1.63**
		6/14	**7/11**	**8/9**	**9/12**	**10/11**	**11/7**	**4/11**	**5/8**	**6/13**	**7/10**		
HB	44	0	2	0	1	2		0	0	1	4	1.11	1.36
	52	0	0	0	0	1	2	0	1	1	1	0.60	0.70
	7	0	2	0	0	0	1	0	0	0	1	0.40	0.70
	8	0	2	0	0	0	0	1	1	0	2	0.60	0.84
	80	2	0	1	0	3	0	0	0	1	0	0.70	1.06
	mean											**0.68**	**0.93**
		6/8, 6/14, 6/20	**7/5, 7/117/25**	**8/1, 8/9, 8/15**	**9/6, 9/12, 9/20**	**10/3. 10/11. 10/24**	**11/1, 11/7, 11/15**	**4/10,4/11, 4/17**	**5/2, 5/8, 5/16**	**6/5/, 6/13, 6/19**	**7/10, 7/18 & 7/24**		
MTB	84	2	0	0	0	0	0	0	1	7	1	1.10	2.18
	28	4	0	0	1	0	2	0	0	0	1	0.80	1.32
	19	0	0	0	0	2	2	0	0	4	0	0.80	1.40
	14	0	2	0	1	2	0	1	0	3	1	1.00	1.05
	33	6		2		1	1	0		0	4	2.00	2.24
	mean											**1.14**	**1.64**
		6/20	**7/25**	**8/15**	**9/20**	**10/24**	**11/15**	**4/17**	**5/16**	**6/19**	**7/18, 7/24**		
LTB	91	0	0	1	0	5	3	0	0	2	2	1.30	1.70
	22	2	0	0	0	1	2	1	0	0	1	0.70	0.82
	92	2	2	1	0	0	0	1	1	1	1	0.90	0.74
	94	2	0	0	0.67	0	2	1	0	0	2	0.77	0.92
	mean											**0.92**	**1.05**
		6/13		**8/2**									
OTB Trib	141	4		0								2.00	2.83
	102	2		2								2.00	0.00
	103	0		2								1.00	1.41
	mean											**1.67**	**1.41**
		6/9	**7/13**	**8/23**	**9/14**								
HR Trib	2	0	0	0	1							0.25	0.50
	mean											**0.25**	**0.50**

OTB= Old Tampa Bay, HB= Hillsborough Bay, MTB=Middle Tampa Bay, LTB= Lower Tampa Bay, OTB Trib=Old Tampa Bay Tributaries, HR Trib= Hillsborough R. Tributary

Table 2.4: Microplastic Concentrations: Plankton Tow Samples (count/L).

Bay Region	Sta #	Oct-16	Nov-16	Dec-16	Apr-17	May-17	Jun-17	Jul-17	Avg	Std. Dev
OTB		**10-3-16**	**11-1-16**		**4-10-17**		**6/5/17**	**7/5/17**		
	50	0.0041	0.0026		0.0054		0.0082	0.0026	0.0046	0.0023
	42	0.0023	0.0039		0.0031		0.0024	0.0035	0.0031	0.0007
	mean								**0.0038**	**0.0015**
HB		**10-11-16**	**11-7-16**	**12-13-16**	**4-11-17**	**5-8-17**	**6/13/17**	**7/10/17**		
	55	0.0103	0.0018	0.0058	0.0019	0.0052	0.0065	0.0029	0.0049	0.0030
	8	0.0049	0.0044	0.0052	0.0042	0.0022	0.0040	0.0049	0.0043	0.0010
	mean								**0.0046**	**0.0020**
MTB				**12-19-16**	**4-17-17**	**5-16-17**		**7/24/17**		
	28			0.0066	0.0034	0.0063		0.0012	0.0044	0.0026
	mean								**0.0044**	**0.0026**
LTB				**12-19-16**	**4-17-17**	**5-16-17**	**6/19/17**	**7/18/17**		
	94			0.0019	0.0020	0.0016	0.0181	0.0054	0.0058	0.0071
	mean								**0.0058**	**0.0071**

OTB= Old Tampa Bay; HB= Hillsborough Bay; MTB= Middle Tampa Bay; LTB=Lower Tampa Bay

Table 2.5: Average Microplastic Concentrations: Discrete Samples.

Bay Region	Sta #	Avg (count/L)	Std. Dev
OTB	65, 42, 60, 61, 50	1.03	1.63
HB	44, 52, 7, 8, 80	0.68	0.93
MTB	84, 28, 19, 14, 33	1.14	1.64
LTB	91, 22, 92, 94	0.92	1.05
OTB Trib	141, 102, 103	1.67	1.41
HR Trib	2	0.25	0.50

Table 2.6a: Average Microplastic Concentrations: Plankton Tow Samples.

Bay Region	Sta #	Avg (count/L)	Std. Dev
OTB	50,42	0.0038	0.0015
HB	55, 8	0.0046	0.0020
MTB	28	0.0044	0.0026
LTB	94	0.0058	0.0071

Table 2.6b: Adjusted Average Microplastic Concentrations: Plankton Tow Samples.

Bay Region	Sta #	Avg (count/L)	Std. Dev
OTB	50,42	0.0038	0.0015
HB	55, 8	0.0046	0.0020
MTB	28	0.0044	0.0026
LTB	94	0.0018	0.0002

*without June and July 2017 samples in LTB

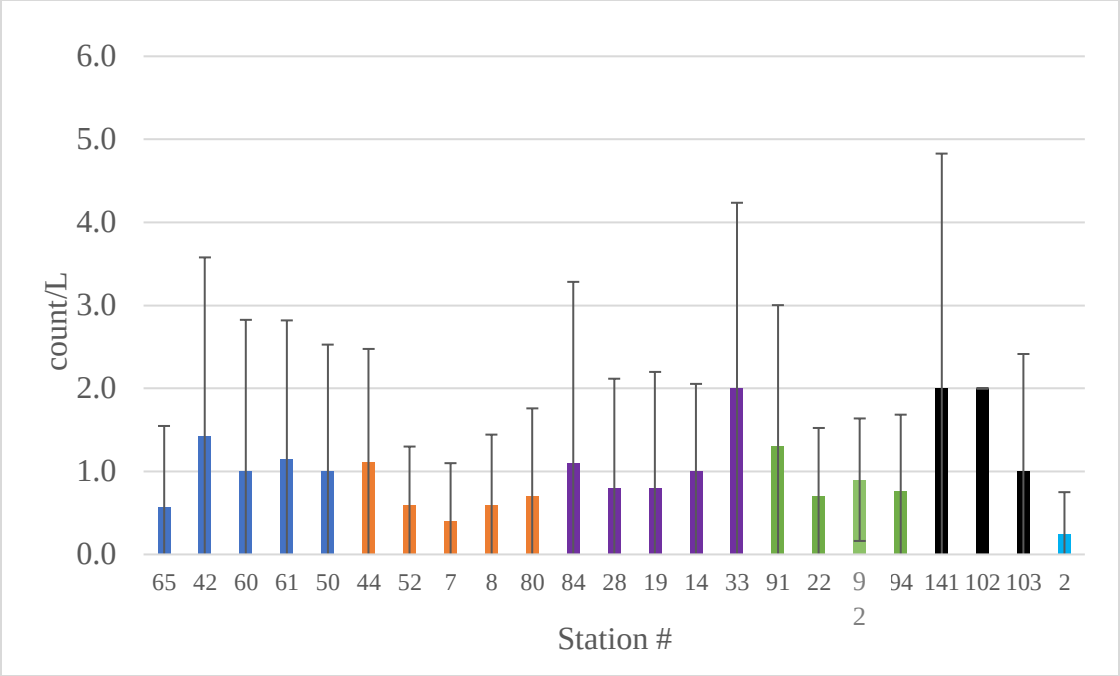

Figure 2.7a: Average Microplastic Concentrations: Discrete Samples.

Blue =Old Tampa Bay (OTB), orange=Hillsborough Bay (HB), purple=Middle Tampa Bay (MTB), green= Lower Tampa Bay (LTB), Black= Old Tampa Bay Tributaries (OTB Trib), light blue= Hillsborough River Tributary (HR Trib)

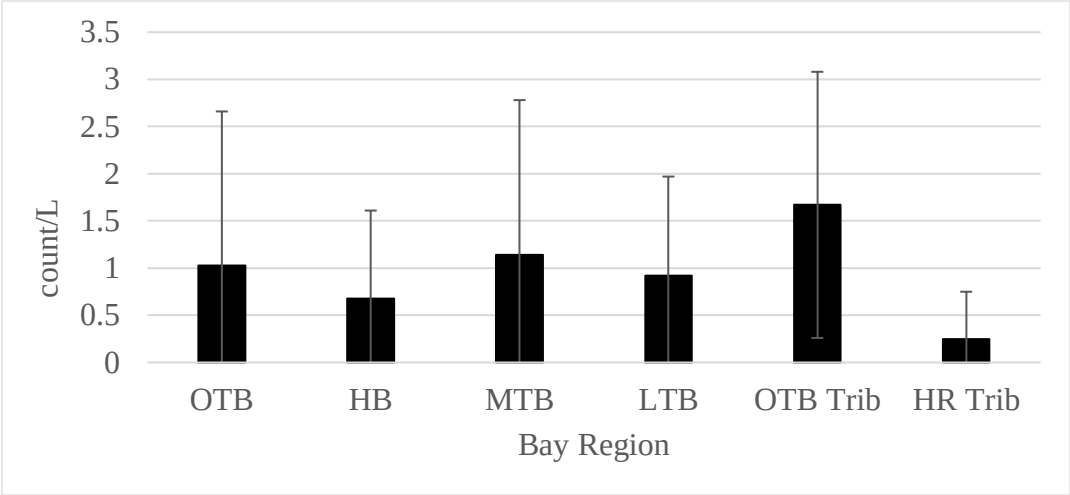

Figure 2.7b: Average Regional Microplastic Concentrations: Discrete Samples.

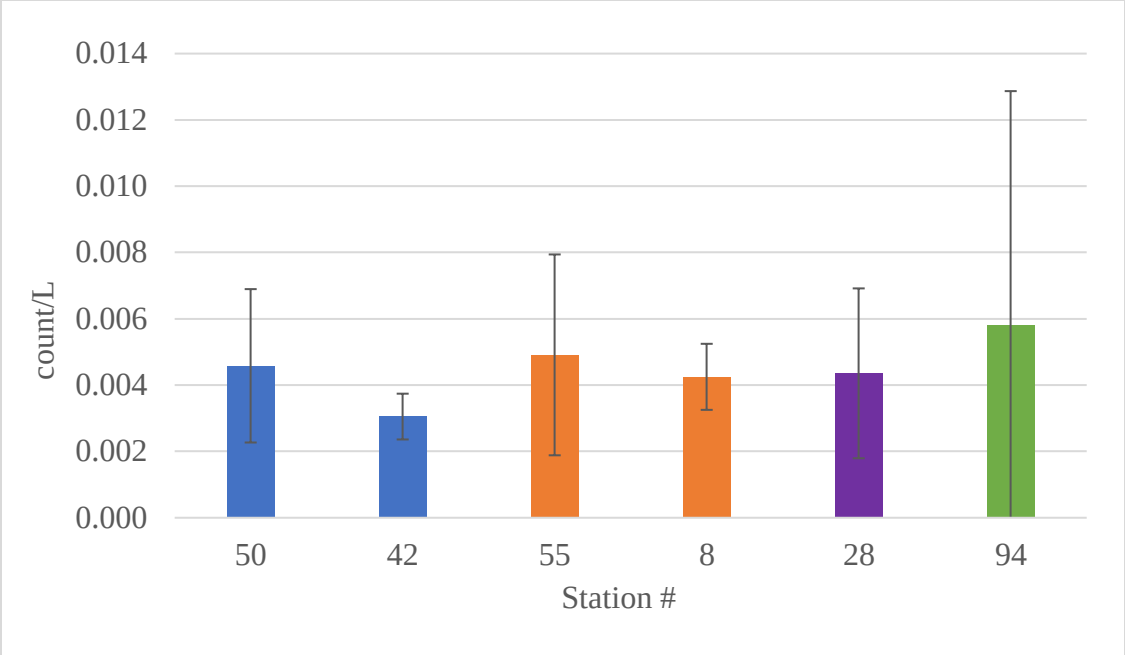

Figure 2.8a: Average Microplastic Concentrations: Plankton Tow Samples.
Blue =Old Tampa Bay, orange=Hillsborough Bay, purple=Middle Tampa Bay, green= Lower Tampa Bay

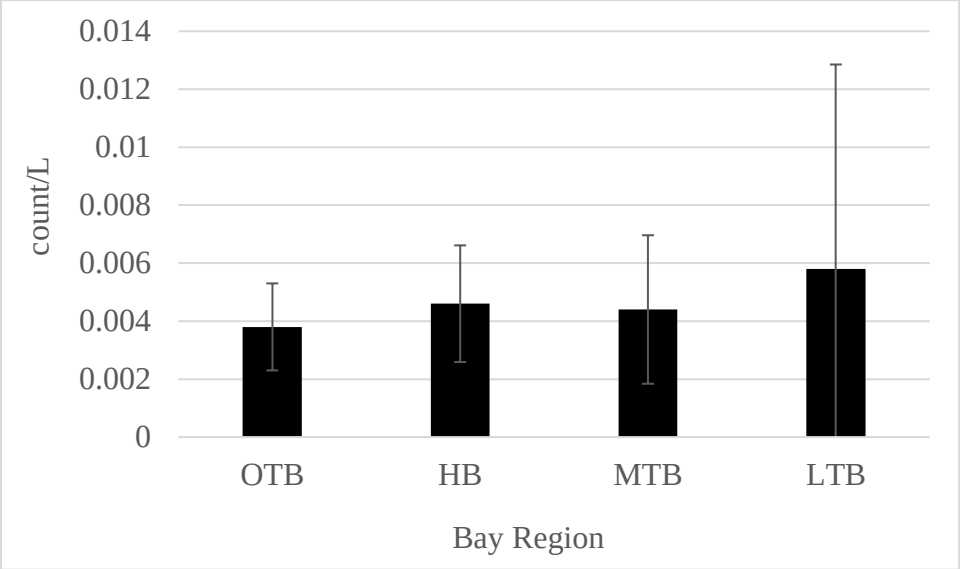

Figure 2.8b: Average Regional Microplastic Concentrations: Plankton Tow Samples.

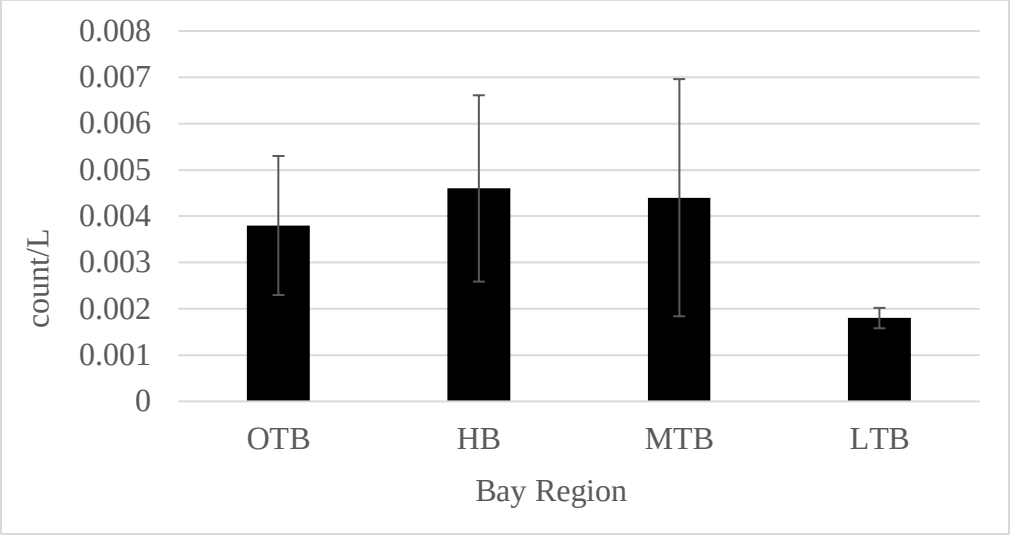

Figure 2.8c: Adjusted Regional Averages: Plankton Tow Samples.
(Without June and July sampling values for LTB)

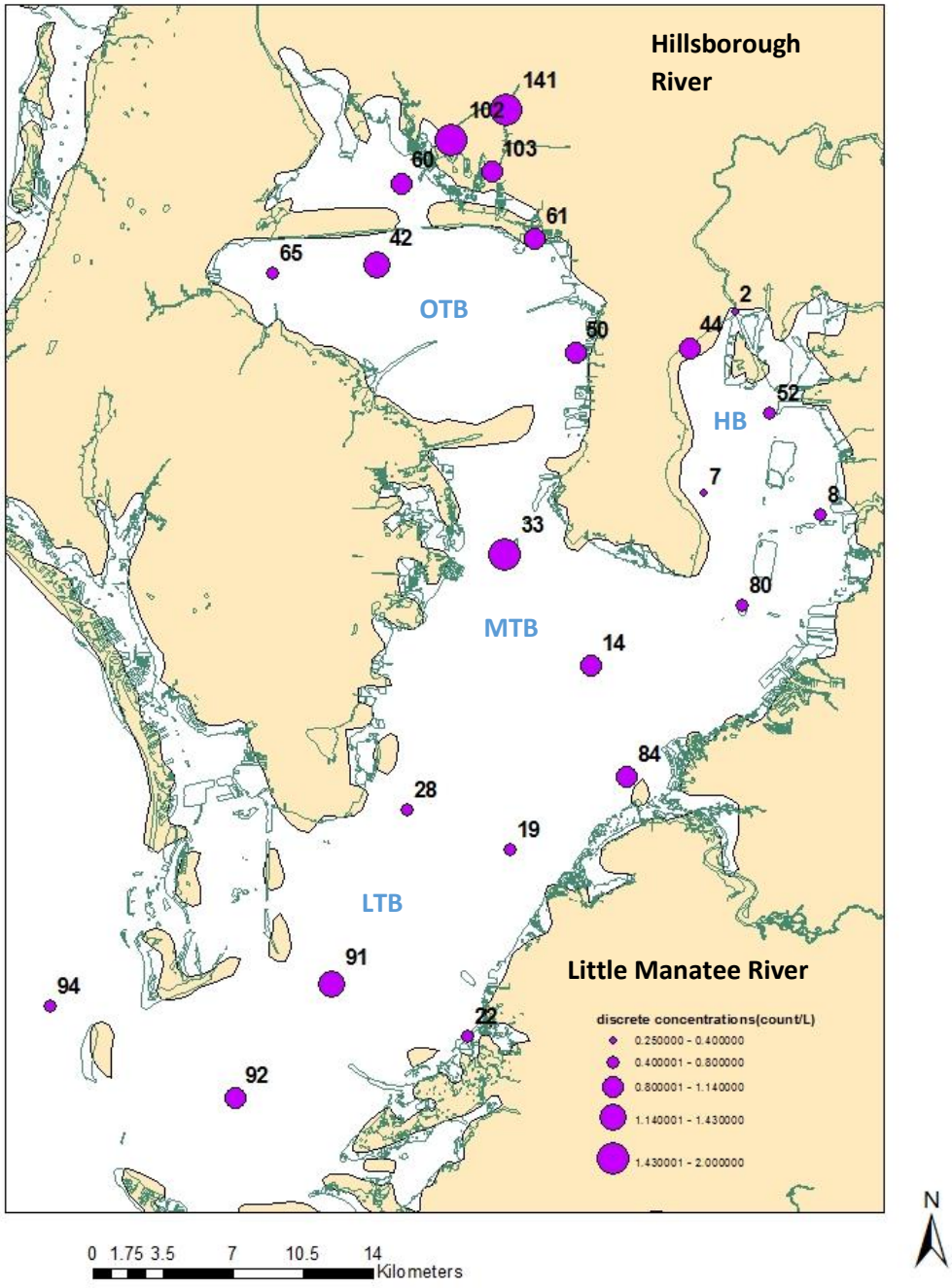

Figure 2.9: Average Microplastic Concentrations: Discrete Samples.

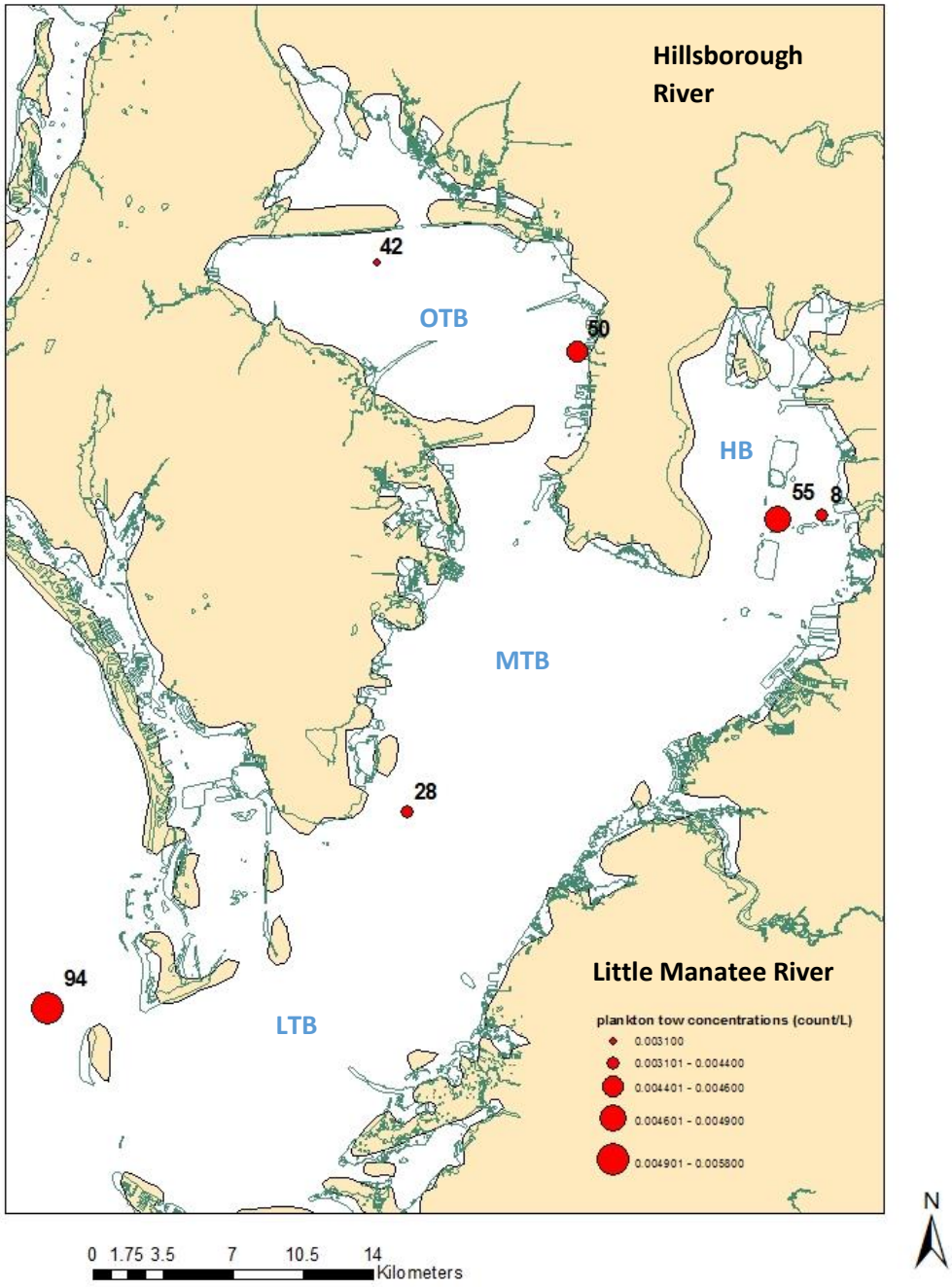

Figure 2.10: Average Microplastic Concentrations: Plankton Tow Samples.

Volume and Rate of Flow of Freshwater Sources

Freshwater inflow to Tampa Bay is about 2.0 x 10^9 m^3/yr, with four major rivers (Alafia, Hillsborough, Little Manatee, and Manatee) contributing 70%-85% of this inflow (Table 2.7) (Tampa Bay Estuary Program, 2006). The Hillsborough River, flowing into the Hillsborough Bay Region, contributes the most freshwater in Tampa Bay with the highest annual average rate of flow of 15 m^3/s. The Alafia River, which flows into the Hillsborough Bay region, is second in flow volume with an annual average flow rate of 13 m^3/s. Manatee River, which flows into Lower Tampa Bay, has the third highest flow rate of 10 m^3/s. Little Manatee River, which flows into Middle Tampa Bay, has the fourth highest singular source flow rate of 6 m^3/s. The remaining 19 m^3/s (out of the total 63 m^3/s annual average flow rate) is contributed by smaller streams, springs, and land drainage (Table 2.7).

For discrete samples the highest concentration (1.14 particles/L) of the four bay regions was found in Middle Tampa Bay which is close to the mouth of the Alafia River and encompasses the regions where Little Manatee River discharges into Tampa Bay. The Hillsborough Bay region had the second highest average microplastic concentration for the plankton tow samples at 0.0046 counts/L and would have had the highest value if the sampling months of June and July had not skewed Lower Tampa Bay's average with significantly higher concentration values. The Hillsborough Bay region is directly influenced by both the discharge of the Hillsborough and the Alafia Rivers which have the two highest annual average flow rates out of the freshwater sources inputting into Tampa Bay (Table 2.7 and Table A4).

Table 2.7: Annual Average flow for Freshwater Sources into Tampa Bay.

Freshwater source	Annual Average Flow (m^3/sec)
Hillsborough	15
Alafia	13
Little Manatee	6
Manatee	10
Other	19
Total	63

Freshwater source "other" refers to smaller streams, springs, and direct land drainage (Source: Weisberg and Zheng, 2006)

Distance from Freshwater Input and Proximity to Gulf of Mexico

The hypothesis tested was that stations closer to a freshwater source would have higher concentrations of microplastics. Based on the scatter plot of the relationship between microplastic concentration and distance from freshwater source (km) for discrete samples, there is no apparent trend for distance from freshwater input and microplastic concentration. Within the bay regions, there were noticeable correlations. Within Old Tampa Bay, station 61 (average 1.14 counts/L) is close to the Old Tampa Bay Tributaries and had the second highest concentration in Old Tampa Bay. Furthermore, Old Tampa Bay Tributary stations 141 and 102 had higher average values than most every other bay region stations (2.0 average counts/L). Hillsborough Bay station 44 (average 1.11 counts/L) had the highest concentration in the HB bay region, as well as one of the highest average concentrations in the entire Bay. Within Middle Tampa Bay stations, closer to freshwater sources meant increased microplastics in four out of the five stations. Station 33 had the highest concentration for the MTB region and out of all the bay stations (average 2.0 counts/L) (Fig. 2.11). These specific stations' higher values

could be linked to the significant amount of freshwater input from Old Tampa Bay tributaries and Hillsborough River carrying increased microplastics from land-based sources of microplastics such as wastewater effluent, reclaimed water, and storm water.

Based on the scatter plot for plankton tow samples within the different bay regions, there does not appear to be a trend between microplastic concentrations and distance to freshwater source or proximity to the Gulf of Mexico. Within regions, Lower Tampa Bay station 94, which is the closest bay station to the Gulf of Mexico, had the highest average microplastic concentration out of all the stations (average 0.0058 counts/L) (Fig. 2.12). Freshwater sources such as Joe's Creek and Cross Bayou carry microplastics from wastewater treatment effluent as well as storm water runoff from the middle of densely populated Pinellas County, down into Boca Ciega Bay, and finally out into Lower Tampa Bay. Furthermore, the Gulf of Mexico's surface water circulation is driven by winds and tides off the west coast of the Pinellas county peninsula, resulting in microplastics from land-based sources on the coast making their way into the mouth of the Bay. Furthermore, the residence time of Middle and Lower Tampa Bay is lower than that of upper Tampa Bay so the flow of microplastic contaminants is directed out of the Bay and can result in increased sampled microplastics aggregated closer to the Gulf of Mexico.

Upon running a Spearman's correlation test, the p value (r_s= .04, p=0.58) was not significant at alpha =0.05 and there is inconclusive evidence that discrete microplastic concentration and distance to freshwater source are positively correlated at the 95% confidence level. Furthermore, the p value (r_s = -0.06, p=0.76) was not significant at alpha =0.05 and there is inconclusive evidence that $\log_{10}(x+1)$ plankton tow microplastic concentration and distance

to freshwater source are negatively correlated at the 95% confidence level. Circulation within Tampa Bay distributes microplastics throughout so that concentrations are statistically homogenous regardless of their station location or bay region location.

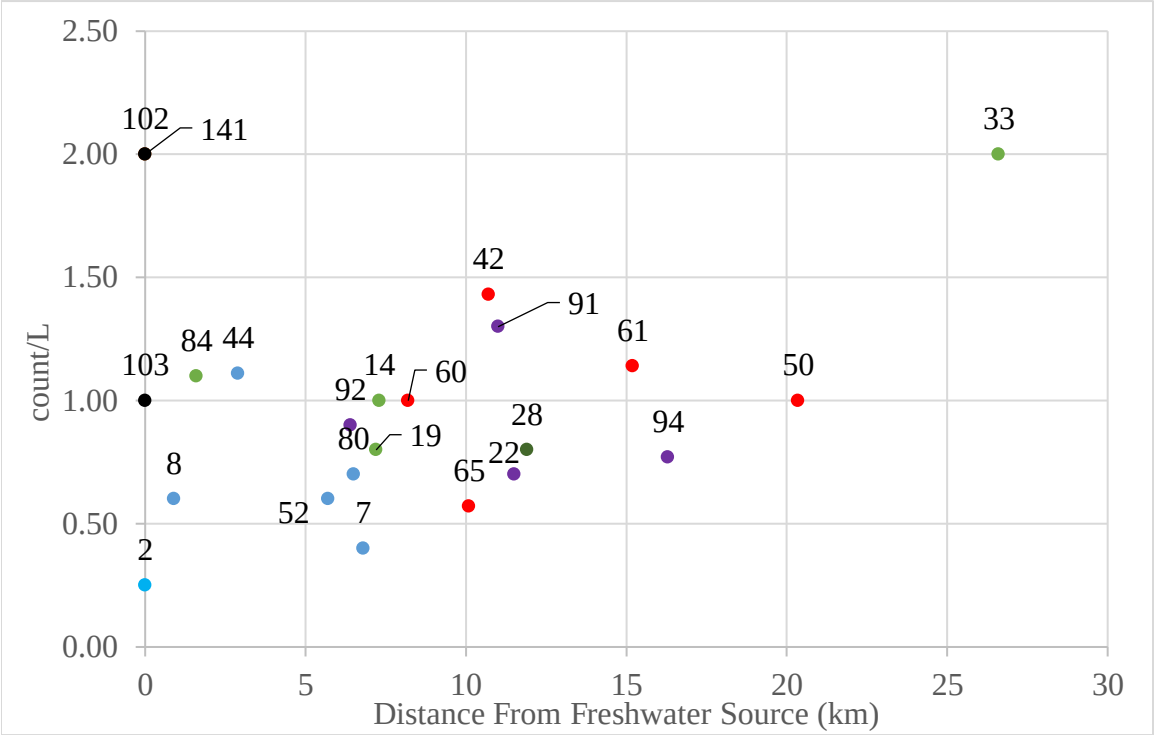

Figure 2.11: Average Microplastic Concentration by Bay Region versus Distance from Freshwater Source: Discrete Samples.

Red: OTB; Blue: HB; Green: MTB; Purple: LTB; Black: OTB Tributaries; Light Blue: HR Tributary

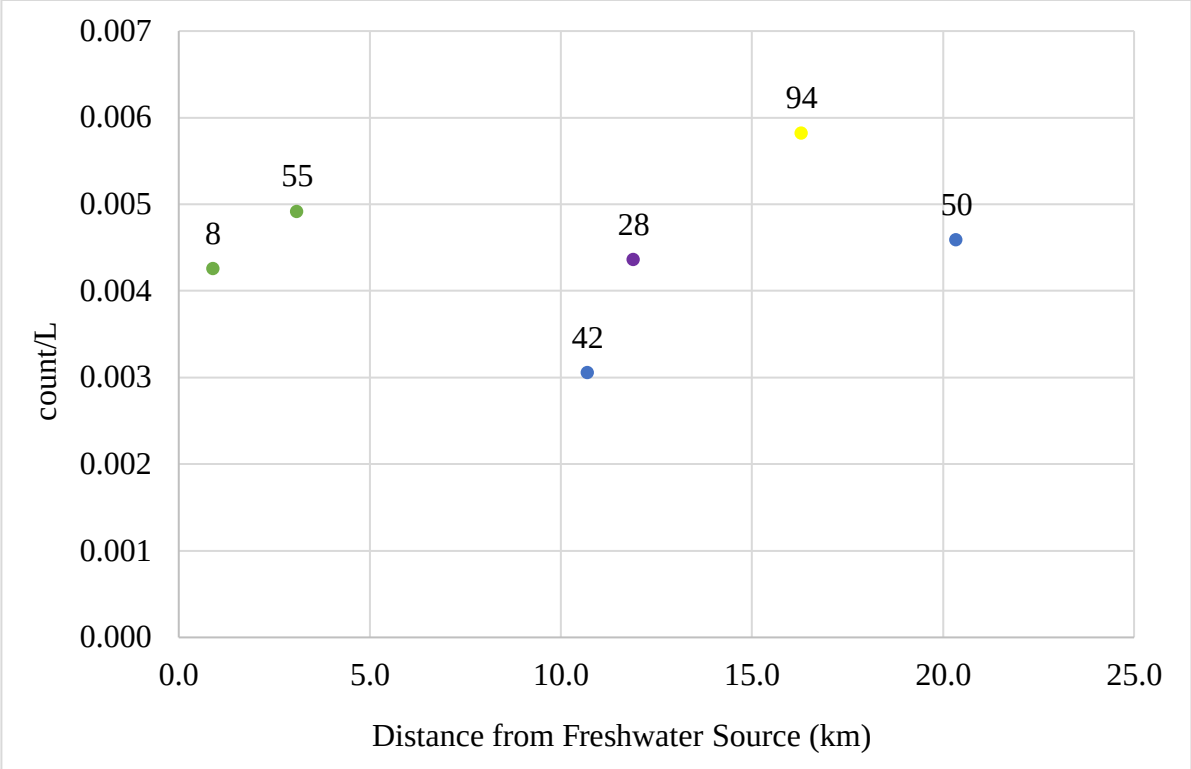

Figure 2.12: Average Microplastic Concentration by Bay Region versus Distance from Freshwater Source (km): Plankton Tow Samples.

Blue: OTB; Green: HB; Purple: MTB; Yellow: LTB

Salinity

Salinity varies substantially in Tampa Bay depending upon location. Near the head of Tampa Bay salinity varies from 12 ppt to 33 ppt depending upon freshwater inflow. At the Bay's mouth, salinity varies from 30 ppt to 36 ppt (Meyers et al., 2007). Salinity in Lower Tampa Bay ranges from 25-38 ppt. Old Tampa Bay, in the northern part of Tampa Bay, typically has salinities from 18-32 ppt; Hillsborough Bay has a salinity range of 15-30 ppt. The tributaries range in salinity from 0.2-8 ppt (Hillsborough County EPC) (Tampa Bay Estuary Program, 2006). Salinity data can be used to indicate how the input from the freshwater rivers impact microplastic concentration. Surface salinity measurements taken by HC-EPC throughout the sampling period were compared to microplastic concentrations (Table A5). Based on a scatter plot for discrete samples, there is no correlation between microplastic concentration and salinity. Within the regions of Old Tampa Bay and Middle Tampa Bay, some stations with lower salinities had higher microplastic concentrations relative to other stations within these individual regions. One possible explanation is that microplastic concentrations at station 42 (OTB), and stations 14 and 84 (MTB), are more influenced by closely located freshwater sources like rivers and creeks transporting microplastics from land-based sources into these two regions. This aligns with research that suggests that tributaries and rivers are sources of microplastics (Schmidt et al., 2017; Lebreton et al., 2017). On the other hand, within both Hillsborough Bay and Lower Tampa Bay some stations with higher salinities had higher average microplastic concentrations when compared with other stations within the region, indicating that freshwater input of microplastics was less impactful within these regions. The increased microplastic concentrations for these higher salinity stations 80 (HB) and

91 (LTB) might be more indicative of the effects of residence time, tides, or wind concentrating more microplastics at those locations (Fig. 2.13).

For plankton tow samples, station 94 with the highest average salinity of 33.2 ppt, had the highest average plankton tow concentration (0.0058 counts/L) and stations 8 and 42, with the lowest average salinities of 21.3 ppt and 22 ppt, respectively, had the lowest average plankton tow concentrations (0.0043 and 0.0031 counts/L, respectively). Still, there does not appear to be an overall correlation between plankton tow microplastic concentration and salinity. Station 94's increased microplastic concentration, relative to the other averages, could be due to residence times, winds, and tides having a greater influence on increased microplastic concentration than freshwater input at this station. Furthermore, the Gulf waters of the west coast of Pinellas County have higher rates of microplastic transfer with the increased salinity station closest to the mouth of Tampa Bay (LTB). If there were increased amounts of microplastics coming from the Coast of Pinellas County, they would resultantly pile up at station 94. On the other hand, the lower salinity station 8's location is adjacent to the mouth of the Alafia River. Its location right next to the direct output of the second highest input of freshwater into Tampa Bay could be the cause of dilution of the microplastic concentration at this station. Furthermore, station 42 is located directly south of the Courtney Campbell Causeway, close to the Old Tampa Bay tributaries. The causeway could be blocking microplastics from being carried in freshwater flow down to this station (Fig. 2.14).

Statistical analysis of the relationship between microplastic concentration and salinity for the discrete (r_s = 0.06, p=0.44) and $\log_{10}(x+1)$ plankton tow microplastic concentration (r_s= -0.09, p=0.64) reveals that they are not correlated at 95 % confidence

since the p values were greater than alpha at 0.05. This lends to the idea that although there are some stations with higher or lower microplastic concentrations based on their different salinity levels, circulation within Tampa Bay distributes microplastics throughout so that concentrations are statistically homogenous regardless of salinity levels in relation to distance to freshwater sources or the Gulf of Mexico.

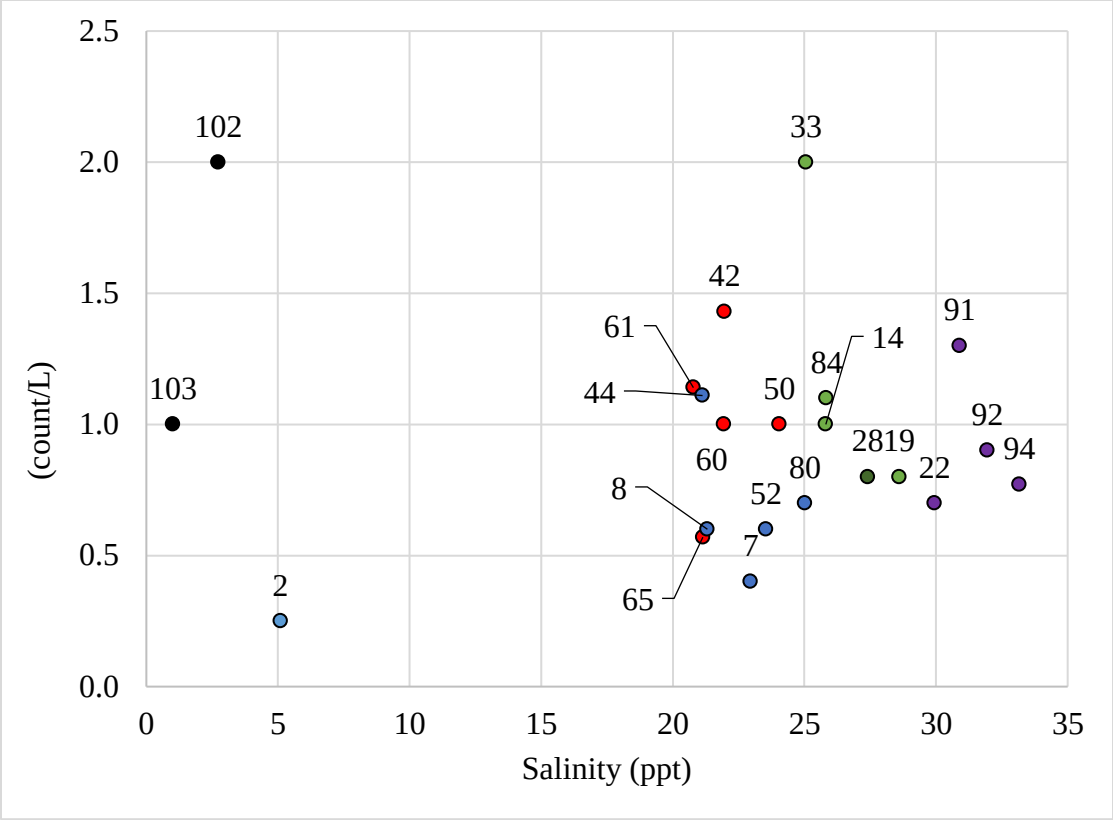

Figure 2.13: Average Microplastic Concentration versus Salinity for Bay Stations: Discrete Samples.

Red: OTB; Blue: HB; Green: MTB; Purple: LTB; Black: OTB Tributaries; Light Blue: HR Tributary

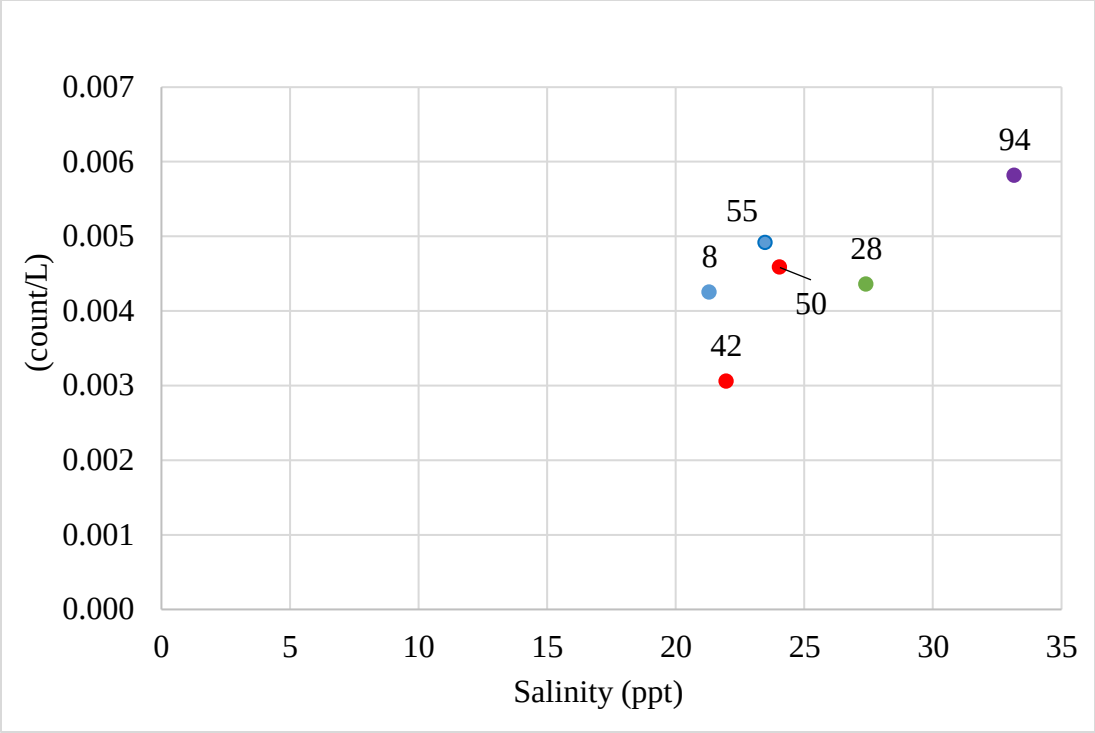

Figure 2.14: Microplastic Concentration versus Salinity for Bay Stations: Plankton Tow Samples.

Red: OTB; Blue: HB; Green: MTB; Purple: LTB

Circulation of Tampa Bay: Depth and Tides

The average depth of the main bay regions in Tampa Bay is 3.8 m. Old Tampa Bay's average depth is 2.73 meters below mean low water; Hillsborough Bay's average depth is 3.16 meters below mean low water; Middle Tampa Bay's average depth is 4.49 meters below mean low water; and, Lower Tampa Bay's average depth is 4.91 meters below mean low water, as measured in the year 2000 (Table A6) (Julian and Estevez, 2010).

The hypothesis is that the shallower bay regions, specifically Old Tampa Bay and Hillsborough Bay, allow for quicker settling of microplastics of higher density plastic polymers compared with other bay regions. The microplastics that will settle the fastest include polystyrene with a density range of 1.04-1/11 g/cm^3, polyvinyl chloride with a density range of 1.20-1.55 g/cm^3, polyacrylonitrile with a density of 1.18 g/cm^3, polyamides with a density of 1.14 g/cm^3, and polyesters with a density of 1.37 g/cm^3. All these plastic polymers have densities higher than the average density of seawater which is 1.027 g/cm^3. The settling of these microplastics into the sediment would occur more rapidly in shallower areas and fewer microplastics would be found in the surface waters in relation to other bay regions. This proved true in some instances but did not prove the same for both sampling methods. Old Tampa Bay did have the lowest plankton tow average concentration of 0.0038 counts/L (stations 42 and 50) but had the second highest discrete concentration of 1.03 counts/L, indicating that more shallow depth did not affect the average concentration of the discrete stations within this bay region. These particular discrete locations within Old Tampa Bay could have been hot spots for

microplastics at the surface where sampled, when compared with Hillsborough Bay and Lower Tampa Bay locations sampled at the surface. Hillsborough Bay, the second shallowest bay region, had the lowest overall average discrete concentration of 0.68 counts/L but had the second highest overall average plankton tow concentration of microplastics indicating that more shallow depth did not affect the average concentration of the plankton tow locations within this bay region. Stations 55 and 8 (from HB) could have been hot spots for microplastics at the surface when compared with Old Tampa Bay and Middle Tampa Bay plankton tow stations (Figs. A1 and A2). The two main channels within Tampa Bay are the only points for a two-layer circulation within Tampa Bay. These channels have significant vertical stratification because of deeper depth that prevents wind and tide from mixing the waters. Stations 14, 33, and 94 are located in much deeper Tampa Bay channels. Their increased discrete and plankton tow microplastic concentrations could be indicative of depth affecting microplastic concentrations.

After further statistical analysis using Spearman's correlation test, the correlation between microplastic concentration of discrete stations and average bay region depth was deemed not statistically significant since the p value ($r_s = 0.04$, $p=0.55$) is greater than alpha at 0.05. Furthermore, the correlation between $\log_{10}(x+1)$ plankton tow microplastic concentration and average bay region depth was also not statistically significant since the p value ($r_s = 0.006$, $p=0.97$) is greater than alpha at 0.05. According to Weisberg and Zheng (2006), Tampa Bay estuary's average depth is so shallow that the flow of materials is directed out of the estuary. The circulation and flow of materials in an estuary are highly influenced by tide and winds. Statistically, there is inconclusive evidence that

microplastic concentration and depth for discrete and plankton tow samples throughout Tampa Bay are positively correlated at alpha =0.05. There is no statistical significant correlation between microplastic concentration and depth, most likely because most of Tampa Bay is influenced by shallow depth and well-mixed layers. Therefore, diurnal and semi-diurnal tides, velocity, salinity and salt flux distributions are important other considerations for assessing microplastic transport and concentration throughout the Tampa Bay waters (Table A5).

Sampling Period, Month, Season, and Precipitation

The Tampa Bay area receives an average of 55 inches of precipitation each year and about 60% of the annual rainfall occurs during the summer months of June through September. The average monthly rainfall is 3.86 inches (Tampa Bay Estuary Program, 2006). Table A7 illustrates the average monthly rainfall at seven rainfall recording stations over specified periods of record. Bradenton, a site that is adjacent to Lower Tampa Bay, received the highest average summer precipitation value of 8.8 inches from the months of June through September in data gathered from 1911-2004. Bartow and Plant City, both closest to the Hillsborough Bay region, received the second greatest average summer precipitation over the course of the months of June through September- an average of 7.6 inches from 1901-2007. In a Part II 2008 study of the residual circulation of Tampa Bay, Meyers and Luther concluded that Hillsborough Bay receives 2.5 times the total (river + precipitation) freshwater than Old Tampa Bay, as well as 5 times the river input. The increased precipitation in Hillsborough Bay is due to the frequency of areas of higher elevation in the surrounding land region. Higher elevation results in lower air temperature than temperatures recorded in land areas closer to sea

level. As the air cools, it holds less moisture than warm air, and results in more precipitation (Tyler et al., 2007).

Seasons were defined for this sampling study based upon average monthly levels of precipitation in the Tampa Bay area and documented by the National Weather Service. For the discrete sampling events, summer was defined as the months of June, July, August, and September. Fall/winter was defined as the months of October and November. Spring was defined as the months of April and May. For the plankton tow sampling events, summer was defined as the months of June and July. Fall/winter was defined as the months of October, November, and December and spring was defined as the months of April and May. The results of the microplastic study suggest that months with increased precipitation levels might correlate with increased microplastic concentration. This could be due to the fact that increased precipitation increases runoff from surrounding land areas, and due to increased input of freshwater sources that empty into Tampa Bay, bringing more microplastic pollution from the roads, rivers, and storm water effluents (Lima et al., 2014; Yates et al., 2011).

In terms of overall average microplastic concentrations for discrete samples, all bay regions had the highest average concentration during summer 2017 noted as the months of June-July with the exception of Lower Tampa Bay, where the highest concentrations were recorded in the fall months of October and November of 2016. The overall mean concentrations for Old Tampa Bay Tributaries and the Hillsborough River Tributary were highest across the summer 2016 season (June through September 2016), although it should be noted that no sampling occurred after September 2016.

The Kruskal-Wallis H test showed that there was a statistically significant difference in discrete microplastic concentration between the sampling periods. The results were as follows: $\chi 2 = 21.14$, $p = 0.01$ with a p value which is less than alpha at 0.05, meaning there is enough evidence to reject the null hypothesis that the sum of the ranks of the discrete concentrations of the different sampling periods are all equal. Since the result from the Kruskal-Wallis test was significant there was justification to perform the Steel-Dwass Method for multiple comparisons to determine which of the sampling periods were significantly different from each other. Using this method, the differences in microplastic concentrations between sampling periods were statistically significant: $\chi 2 = 21.55$, $p = 0.001$ between sampling period 10 (July 2017) and sampling period 3 (August 2016); sampling period 10 (July 2017) and sampling period 4 (September 2016) $p=0.0007$; sampling period 10 (July 2017) and sampling period 7 (April 2017) $p=0.001$; and, sampling period 10 (July 2017) and sampling period 8 (May 2017) $p=0.0009$.

The Kruskal-Wallis H test showed that there was a statistically significant difference in discrete microplastic concentration between sampling months. The results were as follows: $\chi 2 = 15.96$, $p = 0.03$ with a p value which is less than alpha at 0.05, meaning there is enough evidence to reject the null hypothesis that the sum of the ranks of the discrete concentrations of the different sampling months are all equal. Since the result from the Kruskal-Wallis test was significant there was justification to perform the Steel-Dwass Method for multiple comparisons to determine which sampling months were significantly different from each other. Using this method, the differences in microplastic concentrations between months did not produce any p values less than alpha, indicating no statistically significant differences. If more sampling stations had been sampled per

sampling month the results might have indicated statistically significant differences between the different months using this multiple comparison method.

The Kruskal-Wallis H test indicated there was no statistically significant difference in discrete microplastic concentration between sampling seasons. The results were as follows: $\chi 2 = 5.38$, $p = 0.07$ with a p value which is greater than alpha at 0.05, meaning there is not enough evidence to reject the null hypothesis that the sum of the ranks of the discrete concentrations of the different sampling periods are all equal. Since the result from the Kruskal-Wallis test was not significant, there was no justification to perform the Steel-Dwass Method for multiple comparisons to determine which sampling seasons were significantly different from one other. If more sampling stations had been sampled per sampling season, the results might have indicated statistically significant differences between the different seasons when the Kruskal-Wallis test was used.

The results of the Bartlett's test of equal variances indicated that Welch's Anova should be used to test if there were statistically significant differences in $\log_{10}(x+1)$ plankton tow microplastic concentration (counts/ L) between sampling periods. The results did not indicate any significant differences between sampling periods ($F=0.74$, $p=0.63$). The results of the Levene's test of equal variances indicated that one-way Anova should be used to test if there were statistically significant differences in $\log_{10}(x+1)$ plankton tow microplastic concentration (counts/ L). The results did not indicate any significant differences between sampling periods ($F=1.51$, $p=0.22$).

The results of the Bartlett's test of equal variances indicated that Welch's Anova should be used to test if there were statistically significant differences in $\log_{10}(x+1)$

plankton tow microplastic concentration (counts/L) between sampling months. The results did not indicate any significant differences between sampling months (F=0.74, p=0.63). The results of the Levene's test of equal variances indicated that one-way Anova should be used to test if there were statistically significant differences in $\log_{10}(x+1)$ plankton tow microplastic concentration (counts/L) between sampling months. The results did not indicate any significant differences between sampling months (F=1.51, p=0.22).

The results of the Bartlett's test of equal variances indicated that Welch's Anova should be used to test if there were statistically significant differences in $\log_{10}(x+1)$ plankton tow microplastic concentration (counts/ L) between sampling seasons. The results did not indicate any significant differences between sampling seasons (F=1.11, p=0.35). The results of the Levene's test of equal variances indicated that one-way Anova should be used to test if there were statistically significant differences in $\log_{10}(x+1)$ plankton tow microplastic concentration (counts/ L). The results did not indicate any significant differences between sampling seasons (F=0.93, p=0.41).

Because the p values were all greater than alpha there was no need to conduct the Tukey post hoc test of multiple comparisons of $\log_{10}$ plankton tow concentration data to determine differences between sampling periods, sampling seasons, or sampling months.

Upon further statistical analysis of association between microplastic concentration and precipitation for the bay stations, there was no significant correlation for discrete concentrations and rainfall. A Spearman's correlation was run to determine if the relationship between discrete concentration and precipitation values one day (r_s= .02,

p=0.81), two days (r_s= 0.005, p=0.94), and three days (r_s= .03, p=0.68) before sampling was statistically significant. The results of the test were not significant since all p values were greater than alpha at 0.05. Although the June 2017 HB regional average concentration was 7 times the average concentration of the other sampling periods, and the July 2017 MTB regional average concentration was 9 times the average concentration of the other sampling periods, there was inconclusive evidence that discrete microplastic concentration and precipitation occurring one, two, and three days before sampling are correlated. More in-depth analysis of how and when precipitation events and increased monthly precipitation rates affect overall discrete microplastic concentration is needed (Tables A8-A13) (Figs. A3 and A4).

The average concentration for the plankton tows for station 94 in Lower Tampa Bay was 0.0058 ± 0.0071 counts/ L. Plankton tow concentration for station 94 in June 2017 was 0.0181 counts/L and the concentration in July 2017 was 0.0054 counts/L. This was 10 and 3 times, respectively, the average of the other monthly values collected for station 94. On June 19th, 2017, when station 94 was sampled, there was 0.21 inches of rainfall. Prior to this sampling date there was an intense rainfall event on June 17th, 2017 of 1.94 inches. On July 18th, 2017, when station 94 was sampled, there were 0.34 inches of rainfall. Prior to this sampling date there was intense rainfall on July 17th of 0.94 inches. On average, monthly precipitation values for St. Petersburg for the months of June and July are 6.69 and 7.09 inches, respectively. The amount of precipitation on the sampling day-and days leading up to the June and July sampling dates for the plankton tows for station 94- was significantly higher than the daily average for these months. The significant level of precipitation leading up to the sampling dates resulted in more storm

water runoff into Tampa Bay at the time of sampling, which in turn can result in higher concentrations of microplastics being collected in the plankton tows. After performing Spearman's correlation test, the r_s statistic indicating the relationship between $\log_{10}(x+1)$ plankton tow microplastic concentration and precipitation one day (r_s= .09, p=0.61), two days (r_s= .03, p=0.89), and three days (r_s= .08, p=0.67) prior to the sampling events, was not statistically significant since the p values were all greater than alpha at 0.05. Thus, although there were higher plankton tow microplastic concentrations at some stations in the summer months close to larger rainfall events, there is inconclusive evidence that plankton tow microplastic concentration and precipitation one, two, and three days before sampling were correlated. More in-depth analysis of how and when precipitation events and increased monthly precipitation rates affect overall microplastic concentration is needed (Tables A8-A13) (Figs. A3 and A4).

Wastewater Treatment Facilities

The effluent of wastewater treatment facilities are significant sources of microplastics (Mason et al., 2016). Furthermore, agricultural landscapes are often fertilized with the sludge and pellets formed at wastewater treatment facilities that hold the greatest percentage of the microplastics coming into wastewater treatment facilities (Nizzetto et al., 2016). Tables 2.8 and 2.9 outline the wastewater treatment facilities and effluent in the Tampa Bay area. As of 1995, both Hillsborough Bay and Lower Tampa Bay were influenced by 10 wastewater treatment facilities with significant discharge. Hillsborough Bay was influenced by 86 (MGD) of surrounding wastewater effluent in total and Lower Tampa Bay received 90 (MGD) total effluent from surrounding wastewater treatment facilities.

Figures 2.15 and 2.16 detail the domestic wastewater treatment facilities in the Tampa Bay area as well as their relationship to microplastic concentrations sampled in the bay area. For discrete samples, station 42 (1.43 counts/L) in Old Tampa Bay, stations 102 and 141 (2.0 counts/L) from the Old Tampa Bay Tributaries, and station 44 (1.11 counts/L) in Hillsborough Bay had elevated microplastic concentrations when compared to other stations sampled throughout the Bay. Old Tampa Bay has a large main facility, the William Dunn Facility, (formerly Northwest Pinellas), that services a significant percentage of the North Pinellas County population and that releases a large daily effluent of 9.0 MGD of reclaimed water (previously 5.4 MGD) that can bring microplastics back into the marine environment. For plankton tow samples, the overall average concentration of microplastics recorded in Lower Tampa Bay was the highest of all bay regions at 0.0058 counts/L. There may exist a positive correlation between the plankton tow samples' higher average concentrations originating in Lower Tampa Bay and the fourteen domestic and industrial wastewater facilities discharging wastewater in the surrounding land area. Furthermore, the land use surrounding the Manatee River and Lower Tampa Bay is highly agricultural (40%) which presents the potential for microplastic pollution to enter from the runoff of agricultural lands. Furthermore, Hillsborough Bay has the second highest average concentration of microplastics recorded for plankton tow samples at 0.0046 counts/ L. The significant number of adjacent wastewater treatment facilities (>189) could be driving the increased level of microplastics sampled there. Also, the Alafia River Basin surrounding Hillsborough Bay is a highly agricultural land area (Pinellas County Planning Department, 2013).

The Spearman's correlation test was run between average discrete microplastic

concentration and total wastewater treatment discharge (MGD) for the Bay region corresponding to the sampling stations, and was not statistically significant (r_s= -0.008, p=0.91) since the p value was greater than alpha at 0.05. Moreover, the correlation between average $\log_{10}$ (x+1) plankton tow microplastic concentration and total wastewater treatment discharge (MGD) for the Bay region corresponding to the sampling stations was not statistically significant (r_s= -0.02, p=0.90) since the p value was greater than alpha at 0.05. Therefore, there is no conclusive evidence that total wastewater treatment discharge (MGD) by Bay region is correlated with discrete or plankton microplastic concentrations.

Although wastewater treatment effluent could be a factor affecting microplastic concentration over time and space in the Bay, more detailed investigation into the rates of point (effluent) and non-point (reclaimed water) source transfer of microplastics in relation to individual area wastewater treatment facilities, as well as investigation into the exact discharge location and pathway of the surface water effluent, may be necessary (Estahbanati and Fahrenfeld, 2016) to determine any significant correlation. This might involve focusing on sampling the number of microplastics in both the inflow and effluent water at the largest wastewater treatment facilities and then comparing the values. Furthermore, additional investigation into the surrounding bay area lands that utilize agricultural sludge as fertilizer (and may be holding the majority of microplastics that come into wastewater treatment facilities) is critical (Nizzetto et al., 2016).

Table 2.8: Tampa Bay Domestic Wastewater Treatment Plants Greater than or Equal to 1 MGD Plant Capacity.

County	Facility Name	Closest Bay Region	Discharge (Mgal/d) (1995)[1]	Discharge Type	Existing Land use[2]	Future land use [2]
Pinellas	City of Mainland Dunedin	OTB	1.00; 3.68	Ground: reuse system Surface: St. Joseph Sound	Single family, multi family, industrial	Recreation/residential
Pinellas	City of Clearwater East WWTP	OTB	3.04	Surface: Clearwater Bay		
Pinellas	City of Clearwater Marshall Street WRF	OTB	0.13; 7.00	Ground: reuse system; Surface: Stevenson Creek	Single family, multi family	Residential urban
Pinellas	Clearwater: Northeast WWTP	OTB	0.50; 5.67	Ground: Reuse System; Surface: Tampa Bay		
Pinellas	City of Largo	OTB	6.00; 7.66	Ground: reuse system Surface: Feather Sound	Industrial	Industrial
Pinellas	City of Oldsmar	OTB	1.15	Surface: Old Tampa Bay	Single family	Recreation, residential urban
Pinellas	City of Tarpon Springs	OTB	1.00; 0.45	Surface: Anclotte River; Ground: Reuse System	Single family, multi family	Residential urban
Pinellas	South Cross Bayou WRF	LTB	0.22; 21.66	Ground: spray field; Injection well; Surface: Joe's Creeek	Single family, industrial	Residential urban
Pinellas	Mckay Creek[3]	LTB	4.91	Injection Well; Surface: Boca Ciega Bay		
Pinellas	Northwest Pinellas[4]	OTB	5.54	Ground: Reuse System/spray field		
Pinellas	St. Petersburg Northeast WWTP	MTB	7.35; 3.30	Ground: reuse system; Ground: Injection well		
Pinellas	St Petersburg Northwest WWTP	LTB	1.80; 11.30	Ground: Reuse System Ground: Injection well		

Pinellas	St. Petersburg Southwest WWTP	LTB	11.00; 4.10	Ground: Reuse System; Ground: Injection well
Pinellas	St. Petersburg Whited WWTP[5]	LTB	2.00; 7.75	Ground: Reuse system; Ground: Injection well
Manatee	WWTP City of Palmetto	LTB	0.08; 1.25	Ground: reuse system Surface: Tierra Ceia Bay
Manatee	WWTP City	LTB	0.67; 5.68	Ground: reuse system Surface: Manatee River
Manatee	Manatee County Southeast	LTB	2.41	Ground: Percolation pond/ reuse system
Manatee	Manatee County Southwest	LTB	2.21; 11.17	Ground: spray field/reuse system; Ground: injection well
Manatee	Manatee County North Regional WRF	LTB	2.34	Ground: Spray field/reuse
Hills.	Howard F Curren AWTP	HB	57.44	
Hills.	Plant City WRF	HB	4.09	Surface: Baker/ Mill/ Pemberton Creeks
Hills.	River Oaks AWWTP	HB	7.15	Surface: Canal A
Hills.	MacDill AFB WWTP	HB	0.68	Surface: Hills. Bay
Hills.	Falkenburg Road AWTP	HB	1.06; 2.88	Ground: reuse system; Surface: Tampa Bypass Canal/ Palm River
Hills.	Dale Mabry AWWTP	HB	1.08; 1.41	Ground: reuse system; Surface: Brushy Creek
Hills.	Valrico WWTP	HB	2.39; 0.45	Ground: reuse system Surface: Turkey Creek
Hills.	South County Regional AWWTP	HB	2.11	Ground: reuse system
Hills.	Northwest Regional	HB	0.42; 3.90	Ground: reuse system Surface: Hills. Bay
Hills.	Van Dyke WWTP	HB	0.65	Ground: reuse system

[1] Source: Marella RL. 1999. Water withdrawals, use, discharge, and trends in florida, 1995. Tallahassee, Fla. (227 North Bronough St., Ste. 3015, Tallahassee 32301-1372): U.S. Dept. of the Interior, U.S. Geological Survey; Denver, CO: U.S. Geological Survey, Branch of Information Services distributor], 1999.

[2]Source: Potable water supply, wastewater, and reuse element. Chapter 2. 2013. In: The Pinellas county planning department Pinellas county comprehensive plan.

[3] Mckay Creek WWTP has been closed and flow directed to South Cross Bayou plant; source: updated 2/1/2018; Florida Department of Environmental Protection Wastewater Facilities Information

[4] Northwest Pinellas WWTP has been closed and flow directed to William E. Dunn facility; source: updated 2/1/2018; Florida Department of Environmental Protection Wastewater Facilities Information

[5] Whited wastewater treatment facility has been closed down and flows directed to City of St.Petersburg Southwest plant; source: updated 2/1/2018; Florida Department of Environmental Protection Wastewater Facilities Information

Hills=Hillsborough

Table 2.9: Total Tampa Bay Wastewater Treatment Facility Discharge by Bay Region (MGD) (1995).

Bay Region	Discharge (MGD)
OTB	43
HB	86
MTB	11
LTB	90

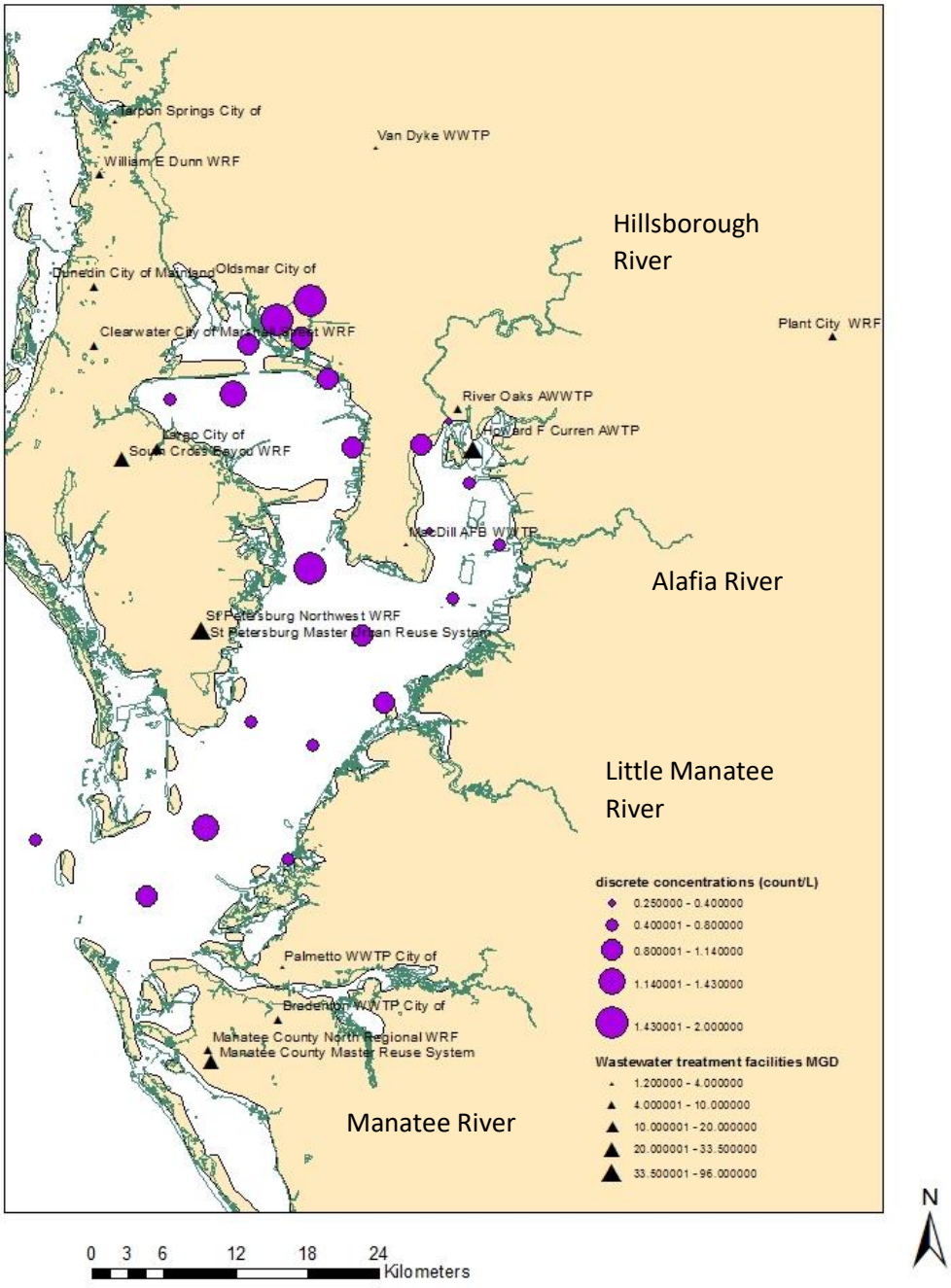

Figure 2.15: Average Microplastic Concentrations versus Wastewater Treatment Facilities MGD (Millions of Gallons/Day): Discrete Samples.

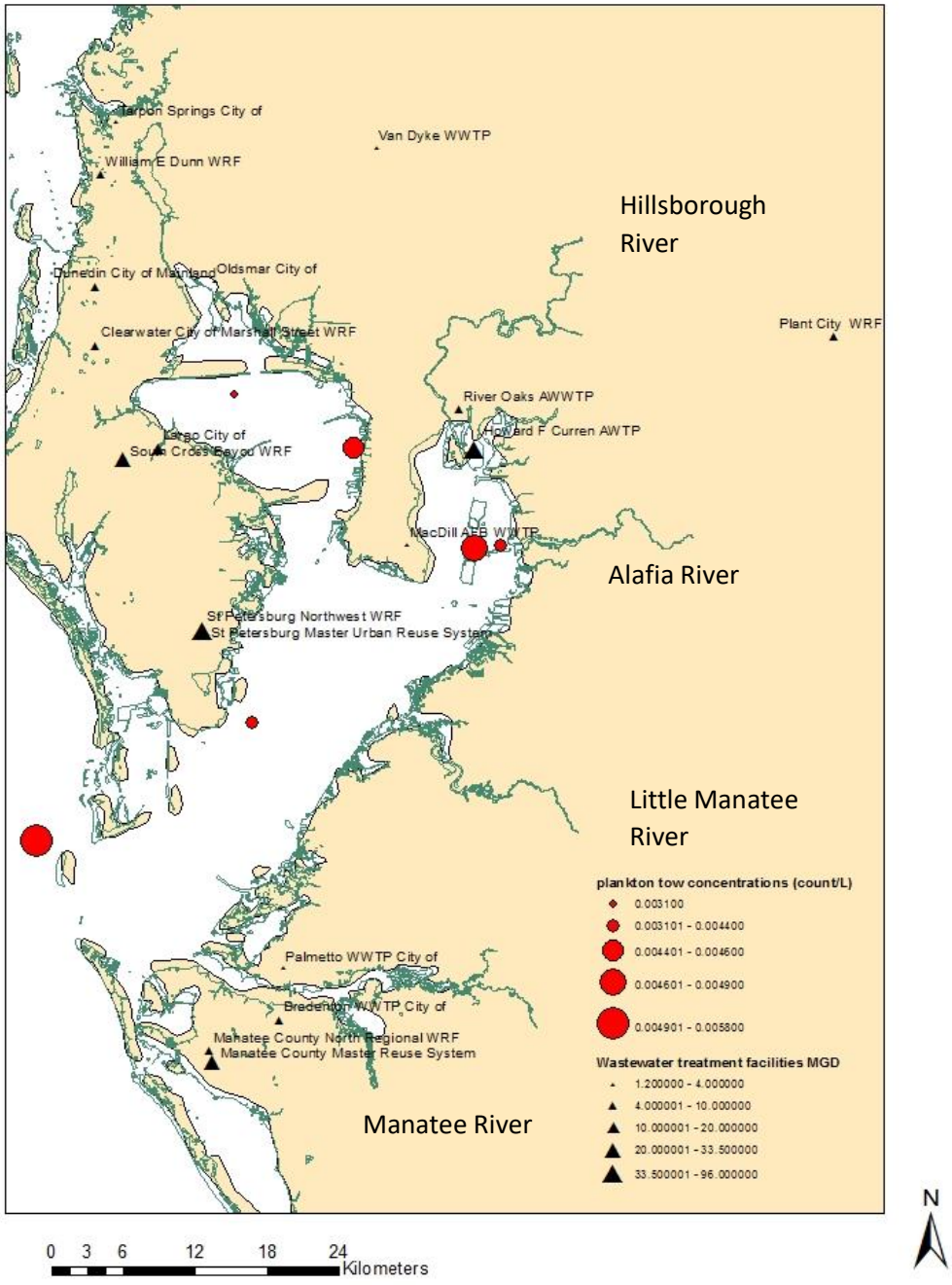

Figure 2.16: Average Microplastic Concentrations versus Wastewater Treatment Facilities MGD (Millions of Gallons/Day): Plankton Tow Samples.

Kruskal-Wallis Test between Stations and Bay Regions

The Kruskal-Wallis test is a nonparametric equivalent of the one-way between-subjects ANOVA that does not require that the dependent variable (microplastic concentration) have a normal distribution. However, it does assume that the distribution of the dependent variable has approximately the same shape in each of the groups tested (bay regions), which implies that the variance is approximately equal across groups. Since the discrete microplastic concentration data failed to meet the assumption of normality for the ANOVA test, the Kruskal Wallis test was utilized to assess for significant differences among the discrete microplastic concentrations by station and by bay region. The null hypothesis is that there are no differences among the sum of ranks for the stations or bay regions for discrete microplastic concentrations other than by random chance. The alternative hypothesis is that there are differences among the sum of ranks for the stations or bay regions for discrete microplastic concentrations.

A Kruskal-Wallis H test showed no statistically significant difference in discrete microplastic concentration between the stations $\chi 2 = 12.19$, $p = 0.96$ or between bay regions $\chi 2 = 4.74$, $p = 0.58$. With p values greater than alpha at 0.05 there is not enough evidence to reject the null hypothesis that the sum of the ranks of the microplastic concentrations of the different stations or bay regions are all equal. Since the result from the Kruskal-Wallis test was not significant, there was no justification to perform a set of uncorrected Mann-Whitney U tests to determine which of the stations or bay regions may be significantly different from one other (Table 2.10).

Welch's Anova and One-Way Anova between Stations and Bay Regions

Following the Levene and Bartlett's tests for assessing variances for the $\log_{10}$ (x+1) plankton tow microplastic concentration data, the Welch's Anova test, which allows for variances to be unequal, was utilized. The hypothesis is that the means are equal between stations. The results indicated an F ratio of 1.48 and a p value of 0.27. The null hypothesis that the means among stations are equal for the plankton tow microplastic data fails to be rejected. Therefore, there was no statistically significant differences in plankton tow concentrations per station based on the data set at hand. Since the result from the Welch's Anova test was not significant there was no justification to perform a Tukey post hoc test to determine which of the stations were significantly different from one other in terms of microplastic concentration.

Following the Levene and Bartlett's test results for assessing variances for the $\log_{10}$ (x+1) plankton tow $\log_{10}$ microplastic concentration data, the Welch's Anova test, which allows for variances to be unequal, was utilized to test for differences among bay regions. The F ratio was 0.34 and the p value was 0.80 which is greater than alpha at 0.05, and the null hypothesis stating that the means are equal among bay regions for the plankton tow concentration data, fails to be rejected. There was no statistically significant difference in plankton tow concentration per bay region. Since the result from the Welch's Anova test was not significant, there was no justification to perform a Tukey post hoc test to determine which of the bay regions were significantly different from one other (Table 2.10).

Table 2.10: Summarized Results of Statistical Analysis.

Data Set	Shapiro Wilk Normality	Levene's test	Bartlett's test	Kruskal Wallis	One-Way Anova	Welch's Anova	Multiple comparison post hoc	Spearman's correlation
Discrete by station	Non normal	Non equal variances	Non equal variances	Not sig	N/A	N/A	N/A	N/A
Discrete by bay region	Non normal	Non equal variances	Non equal variances	Not sig	N/A	N/A	N/A	N/A
Discrete by time period	Non normal	Non equal variances	Non equal variances	Sig	N/A	N/A	period 10, July 2017, & period 3, August 2016; period 10, July 2017, & period 4, Sept 2016; period 10, July 2017, & period 7, April 2017; period 10, July 2017, & period 8, May 2017	N/A
Discrete by month	Non normal	Non equal	Non equal	Sig	N/A	N/A	No sig differences	N/A
Discrete by season	Non normal	Non equal	Non equal	Not sig	N/A	N/A	N/A	N/A
Plankton by station	Log transformed: normal	Non equal	Non equal	N/A	N/A	Not sig	N/A	N/A
Plankton tow by bay region	Log transformed: normal	Non equal	Equal variances	N/A	Not sig	Not sig	N/A	N/A

Plankton tow by time period	Log transformed: normal	Equal variances	Equal variances	N/A	Not sig	N/A	N/A	N/A
Plankton tow by month	Log transformed: normal	Equal variances	Equal variances	N/A	Not sig	N/A	N/A	N/A
Plankton tow by season	Log transformed: normal	Equal variances	Equal variances	N/A	Not sig	N/A	N/A	N/A
Distance from freshwater source (km)	Discrete: non normal Plankton: non normal	N/A	N/A	N/A	N/A	N/A	N/A	Not sig
Salinity (ppt)	Discrete: non normal Plankton: non normal	N/A	N/A	N/A	N/A	N/A	N/A	Not sig
Average depth of bay region (m)	Discrete: non normal Plankton: non normal	N/A	N/A	N/A	N/A	N/A	N/A	Not sig
Precipitation (1, 2, 3 days prior) (in)	Discrete: non normal Plankton: non normal	N/A	N/A	N/A	N/A	N/A	N/A	Not sig
Total Wastewater treatment effluent per bay region (MGD)	Discrete: non normal Plankton: non normal	N/A	N/A	N/A	N/A	N/A	N/A	Not sig

Types of Microplastics

For discrete samples collected, all the bay regions indicated that the type of microplastics most commonly present were threads/ fibers (Fig. 2.17a). Threads and fibers are indicative of plastic polymers, including polypropylene, polyacrylonitrile, and polyester. These thread-like plastics are most likely coming from synthetic clothing washed and transported through the wastewater treatment facilities and released in effluent. These threads might also be synthetic threads broken down from household textiles or tires being transported to the bay via wind or sewage transport (Boucher and Friot, 2017). The second and third most common categories of microplastics identified in discrete samples were fragments and flakes, which can be secondary microplastics stemming from the breakdown of larger plastics.

For plankton tow samples the most common type of microplastics found were threads/fibers (Fig. 2.17b). The second and third most common categories of microplastics identified in plankton tow samples were the same as those found in discrete samples: fragments and flakes. Middle and Lower Tampa Bay plankton tow samples contained the only foam microplastics identified in the entire study. Foam microplastics originate from expanded polystyrene that is used in fast food packaging, egg boxes, and meat packaging trays. Polyethylene is the most common plastic polymer (Table 1.1) and is used to make bags, wire insulation, and squeeze bottles. Many of the microplastic fragments and flakes could have originated from this very common polymer of plastic.

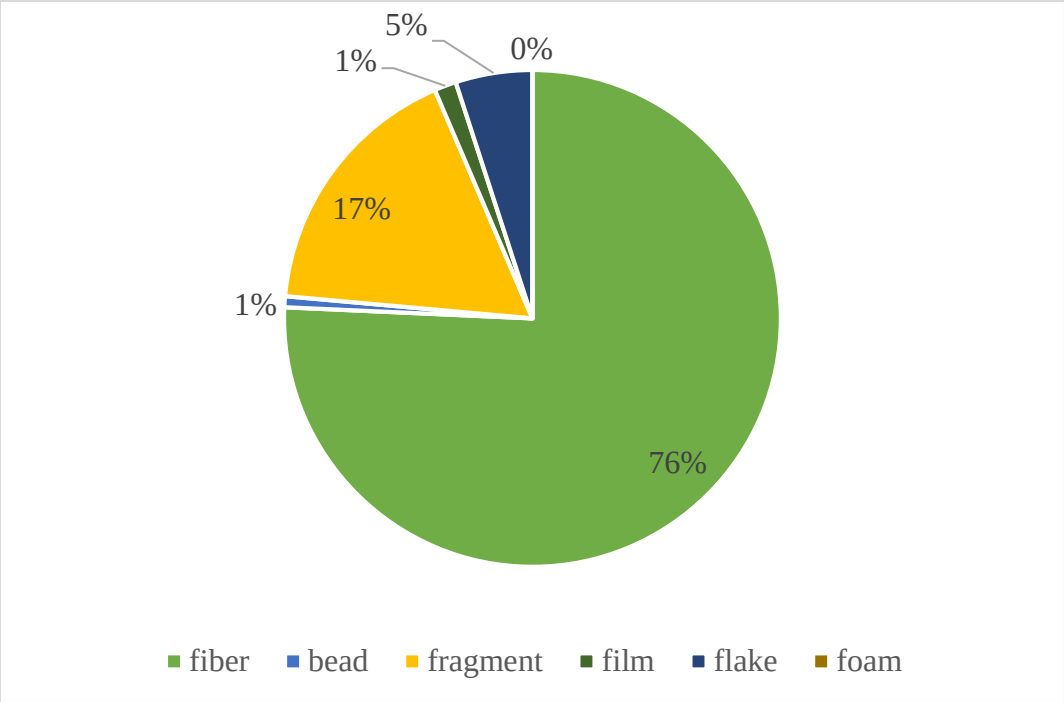

Figure 2.17a: Type of Microplastics: Discrete Samples.

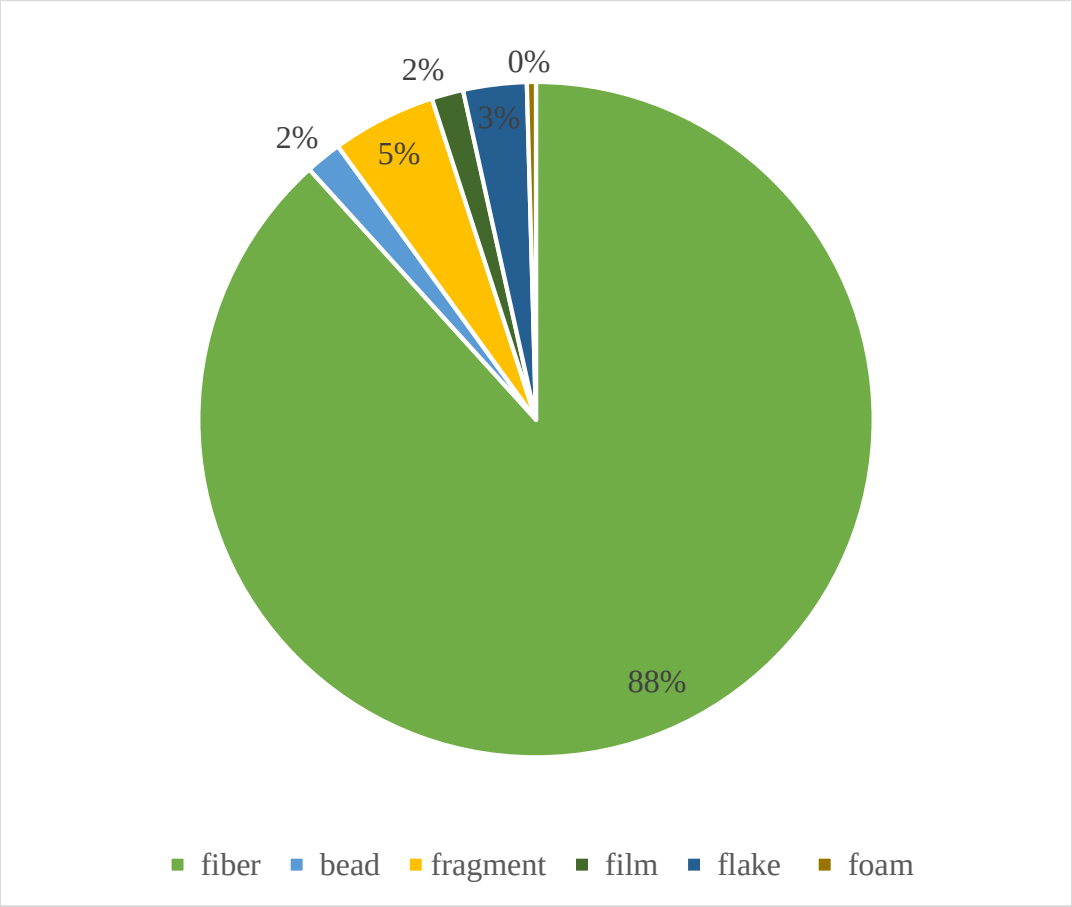

Figure 2.17b: Type of Microplastics: Plankton Tow Samples.

Discrete Versus Plankton Tow Samples

Microplastics were present in 85 out of 182 discrete water samples that ranged from 0.25 to 2 L. Therefore, 47% of all discrete samples contained microplastics. There were microplastics present in 93/97 plankton tow (1L) replicate samples. Therefore 96% of plankton tow replicate samples contained microplastics. Therefore, discrete sampling resulted in a lower detection rate but higher efficiency in the collection of microplastics. The plankton net resulted in a higher detection rate but lower efficiency in the collection of microplastics. Both methods involved sampling one to two meters below the surface which will collect only the microplastics that are distributed there since they might not be uniformly distributed across the entire water column (Schmidt et al., 2017).

The differences in microplastic concentrations between the discrete samples are, on average, 200 times more than plankton tow concentrations. The difference could be attributed, in part, to a lack of accuracy when volumes were calculated for the plankton tow. The volumes for the plankton tow were calculated based upon the area of the plankton tow net and the amount of water flowing through the net. For purposes of this study, the distance traveled from the first geographical coordinate to the end geographical coordinates was used to determine the amount of water flowing through the net. The distance calculated by using the geographical coordinates that is used in combination with the area of the plankton tow net to calculate volume does not account for wave and current action that could create resistance or push more water through faster depending upon whether the tidal current is going with, or against, the towing of the net. Therefore, the tide flow affects the amount of water flowing through the plankton net. Moreover, the

distance calculated to determine the amount of water that flows through the net did not account for the net clogging with plankton throughout the tow which could also affect the amount of water that flows through the net.

Furthermore, the plankton tow concentrations could be considered a conservative estimate since the net is 333 µm in mesh size; therefore, any microplastics smaller than 333 µm can escape through the net. The discrete samples were taken with a Beta van Dorn sampler that simply collects the water directly without any restriction on microplastic size. Microplastics in the size range (<0.333 mm) may have been lost from the plankton tow but collected in the discrete samples. Furthermore, there could have been contamination in the discrete samples; even one thread of contamination could affect the value since the volumes of water involved in discrete samples (0.25- 2 L) were much lower when compared to the volume of 10,000-40,000 L of the plankton tow samples. Sampler contamination of 1.02, 0.68, 1.14, and 0.91 threads for Old Tampa Bay, Hillsborough Bay, Middle Tampa Bay, and Lower Tampa Bay discrete station samples, respectively, could account for this large concentration difference between discrete and plankton tow samples. Wearing cotton clothing in the field and cotton lab coats in the laboratory reduces the chance for contamination. An increase in the volume of water sampled in the field at each station, as well as taking field replicates at each station during each sampling event, would decrease the chance of bias due to contamination. The discrete samples were typically 1 L and required upwards of an hour to filter. One could sample upwards of 20 – 100 L of water at a time for a discrete sample to reduce the influence of contamination. Filtering larger volumes of water through the vacuum pump

would take significantly more time and so one would need to consider using a filter larger than 1.2 μm to filter the higher volume.

Contamination and Identification

Contamination could be a factor influencing the measured concentration of microplastics in both discrete and plankton tow samples. Contamination from sampler's clothing, from the air, or contamination in the sampling equipment, laboratory equipment, or distilled water used to clean the sampling bottles and laboratory equipment, are all potential problems that can introduce microplastics to the samples.

Visual identification of microplastics on the filters using characteristics from Hildalgo Ruz (2012), are somewhat subjective. Visual identification risks include the possibility of missing some microplastics or an under- or over-estimation caused by confusing biological organisms or non-plastic polymers as plastics. Moreover, enzyme digestion of plankton tow samples leaves chitin or exoskeletons that are densely clumped on the filter after digestion and filtration and can result in microplastics that are hidden from visual and hot needle identification. This could account for errors in quantification.

Another factor for consideration is the hot needle probing protocol. Secondary identification confirmation is typically not feasible since the particle is commonly burned, melted, or lost. This is a significant consideration since particle verification and confirmation as plastic is important.

Global Microplastic Evaluation

Discrete sample concentrations in this study are orders of magnitude higher than the average concentrations found in other studies. The average plankton tow concentration was similar to that measured in other estuarine and tributary studies. In comparison to the concentrations reported by Eriksen et al. (2014), in their global ocean study, the Tampa Bay surface waters have significantly higher concentrations of microplastics when sampled through discrete or plankton tow methods (Table 2.11). At the regional and global scale, the key sources of microplastic releases differ among regions. Factors to consider when determining microplastic releases include population size, affluence, and economic development, including the activities that can generate losses, and the technology that exists to prevent and mitigate losses and releases into the ocean.

Table 2.11: Global Microplastic Counts.

Study Location	# (µ plastics)	Sampling period	Size Class	Seasonal # of microplastics
Goiana Estuary, NE Brazil[1]	Surface waters average density Range: 3.1/m^3-260/m^3	11 months	avg= 2.23 mm; (all <5 mm)	Surface waters: 350/m^3 (maximum) rainy season
29 Great Lake Tributaries, U.S.[2]	Sample range: 0.05/m^3-32/m^3	12 months	98% <4.75 mm	
Global Ocean[3]	Total counted: 3.6 X 10^{-5}/m^3	6 years	0.33-200 mm; >200mm	
San Francisco Bay[4]	Sample range: 25,000/km^2-3,000,000/km^2; Avg: 700,000/ km^2	2 days	0.355-4.75 mm; >4.75 mm	
Four estuarine Chesapeake Bay Rivers[5]	Sample range: 5,534-297,803/ km^2	6 months	0.3-5.0 mm	259,803/km^2 maximum after hurricane and tropical storm
Tampa Bay, FL	Avg discrete sample:940/m^3; avg plankton tow sample: 4.5/m^3	13 months	<5mm	Discrete: sample max (June 2017) 7000 counts/ m^3; plankton tow: sample max (June 2017) 18.1 counts/ m^3

[1] Lima et al., 2014; [2] Baldwin et al., 2016; [3] Eriksen et al., 2014; [4] Sutton et al., 2016; [5] Yonkos et al.,2014

Global ocean estimate based on total plastics sampled in microplastic classes and volume of ocean

CONCLUSION

This study serves as a foundation for spatial and temporal microplastic research in Tampa Bay. It is integral to the development of field techniques to survey surface waters of Tampa Bay and to identify key areas of the Bay to study and focus for further microplastic research. Research conducted for this study aimed to investigate the influences of the bay regions, sampling method, salinity, precipitation, wastewater treatment, and land use on microplastic concentrations measured in surface waters. This study was integral to developing laboratory techniques and the evaluation of the use Enzyme Proteinase K in digesting biological materials in water samples processed for microplastic identification and concentration measurement.

The results indicate that there were no significant differences among microplastic concentrations from discrete samples collected at stations throughout the four different regions of the Bay on an individual or Bay region scale. Furthermore, there were no significant differences found among microplastic concentrations from plankton tow samples collected at stations throughout the four different regions of the Bay on an individual or Bay region scale. Since there were no significant differences among microplastic concentrations for discrete or plankton tow samples, the results suggest that the impact of urban land use on microplastic concentration is distributed homogeneously throughout Tampa Bay surface waters.

Since there were no significant correlations between microplastic concentrations for discrete or plankton tow samples and salinity or distance to freshwater source the results suggest that these are not controlling factors for microplastic concentrations in Tampa Bay and that other parameters such as tidal influence, wind transport, residence times, and storm water discharge should be investigated further to determine if these factors significantly influence microplastic concentrations in the Tampa Bay estuary.

There were temporal differences among microplastic concentrations for both discrete and plankton tow samples. The summer months of June 2016 and June 2017 and July 2017 with higher precipitation amounts, proved to contain the highest concentration of microplastics for both discrete and plankton tow samples. There were statistically significant differences for discrete microplastic concentrations depending on sampling period. The effects of increased precipitation and seasonal changes in storm water and other sources of microplastics on microplastic concentration measured in Tampa Bay surface water should be furthered explored.

Some stations and bay regions had higher microplastic concentrations when closer to wastewater treatment centers, as well as when agriculture dominated land use areas. For discrete samples, station 42 (1.43 counts/L) in Old Tampa Bay, stations 102 and 141 (2.0 counts/L) from the Old Tampa Bay Tributaries, and station 44 (1.11 counts/L) in Hillsborough Bay had elevated microplastic concentrations compared to other stations sampled throughout the Bay. Furthermore, microplastic concentrations were high for plankton tow stations located in Hillsborough Bay and Lower Tampa Bay: stations 55 and 8 had average concentrations of 0.0049 and 0.0043 counts/ L, respectively, and

station 94 within Lower Tampa Bay had an average concentratation of (0.0058 counts/L). In Lower Tampa Bay there are 10 wastewater treatment facilities that discharge over 1 Mgal/day of effluent and in Hillsborough Bay there is high potential for microplastic contamination from effluent due to the proximity to large amounts of total discharge (MGD) from treatment facilities on the surrounding land. The regions with higher concentrations of microplastics are surrounded by land areas characterized by domestic wastewater treatment facilities that provide a significant source of effluent (HB 86 MGD and LTB 90 MGD) and agricultural sludge, both sources of microplastics. Using Spearman's Correlation test there was no significant correlation found among microplastic concentrations for discrete or plankton tow samples and total bay region wastewater treatment effluent, suggesting that the impact of wastewater effluent and agricultural sludge sources of microplastics are possibly distributed evenly throughout Tampa Bay surface waters. A further investigation into the levels of microplastics in Tampa Bay wastewater influent and different effluent releases, such as reclaimed water, sludge, and effluent, at individual facilities is necessary to better understand the entry and transport of microplastics coming from wastewater within the estuary. Finally, although wastewater treatment impacts on microplastics should be further investigated, there are additional potential pathways for microplastics such as atmospheric deposition and dust from urban areas, which should be further investigated as potential significant sources.

There were substantial differences of two orders of magnitude between discrete water samples and plankton tow sample concentrations, indicating that the different methods result in differing levels of efficiency and detection. Selecting sites in a grid

pattern for discrete samples, and increasing the efficiency of plankton tow sampling, would be most beneficial for improving data collection.

Important considerations resulting from this study include the potential risk of contamination in the field and in the laboratory, the loss of microplastics through the mesh of a plankton tow net, and the lack of uniform distribution of microplastics throughout the water column due to bio fouling and organismal influence. Daily and seasonal changes in tides, precipitation, wind, freshwater inflow, and storm water runoff may influence the settling and spatial and temporal distribution of microplastics. Moreover, another consideration that resulted from this study is the potential loss of microplastics due to organismal ingestion and loss to sediments.

In terms of microplastic field sampling, lab methodology, and identification techniques, future research will focus on utilizing Raman and Infrared spectroscopy to identify the plastic polymers, as well as the use of Nile Red to identify and to determine the size of microplastics under fluorescent microscopy (Maes et al., 2017; Yonkos et al., 2016). For improving the ability to ascertain statistical significance of changes of microplastic concentration for discrete and plankton tow methods over space and time and the relationship to bay area parameters, more stations, sampling events, and fully overlapping seasons (as well as multiple year data comparison) is necessary. Lack of significance in the data can be attributed, in part, to short-term data analysis and an isolation of comparison of spatial and temporal concentrations by individual independent variable bay parameters. Modeling of microplastic movement, using the numerical Estuarine Coastal Ocean Model (ECOM-3D) with the Lagrangian particle tracking

method to map the effects of spatial and temporal changes in estuarine circulation due to environmental and anthropogenic variables on microplastic distribution, would provide a more holistic understanding of the movement of microplastics throughout the estuary. Model selection of the best fit model, encompassing the main variables and interactions that affect microplastics throughout the estuary, would allow for an improved understanding of the distribution of microplastics throughout time and space in Tampa Bay. It would also help with understanding how future land use or input of sources of microplastics will affect distribution levels (Meyers and Luther, 2008).

This study will help raise questions and interest in studying the direct impacts of microplastics on the Tampa Bay estuarine habitat and water quality, as well as the specific impacts of microplastics on the endocrine and gastric health, reproductive success, and survival of organisms. It can be compared to other studies throughout the globe that are working to document, describe, and quantify microplastics in estuarine and ocean environments. Plankton tow values were similar to other estuaries (Lima et al., 2014) and greater than the concentration of microplastics per m^3 in the open ocean (Eriksen et al., 2014). Moreover, this study can serve as an example of field and laboratory methods for future studies that are working to document the input of plastic waste to the marine environment or to determine concentrations of microplastics throughout Tampa Bay, as well as their implications throughout the ecosystem. Future studies relating to this one coupled with the heightened interest in microplastics in Tampa Bay, have the potential to stir policy changes regarding plastic production, use, and recovery in the surrounding Bay municipalities.

LITERATURE CITED

Andrady, A. 2011. Microplastics in the marine environment. Marine Pollution Bulletin 62:1596-605.

Bakir, A., Rowland, S.J., Thompson, R.C. 2012. Competitive sorption of persistent organic pollutants onto microplastics in the marine environment. Mar Pollut Bull (12):2782.

Baldwin, A.K., Corsi, S.R., Mason, S.A. 2016. Plastic debris in 29 great lakes tributaries: Relations to watershed attributes and hydrology. Environ Sci Technol 50(19):10377-85.

Besseling E, Quik J, Sun M, Koelmanss AA. 2017. Fate of nano- and microplastic in freshwater systems: A modeling study. Environmental Pollution: 540.

Besseling E, Foekema EM, Van Franeker JA, Leopold MF, Kühn S, Bravo Rebolledo EL, Heße E, Mielke L, IJzer J, Kamminga P, et al. 2015. Microplastic in a macro filter feeder: Humpback whale megaptera novaeangliae. Mar Pollut Bull 95(1):248.

Biosolids [Internet]: United State Environmental Protection Agency; cDecember 6, 2017. Available from: https://www.epa.gov/biosolids/frequent-questions-about-biosolids.

Boucher J and Friot D. 2017. Primary microplastics in the oceans: A global evaluation of sources. International Union for Conservation in Nature.

Bowmer, T. and Kershaw, P. 2010. Proceedings of the GESAMP international workshop on microplastic particles as a vector in transporting persistent, bioaccumulating and toxic; 06-28-2010-06-30-2010; Paris. UNESCO-IOC Paris.

Carr, S.A., Liu, J., Tesoro, A.G. 2016. Transport and fate of microplastic particles in wastewater treatment plants. Water Res: 174.

Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., Galloway, T.S. 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. Scientific Reports: 1.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S. 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod calanus helgolandicus. Environ Sci Technol 49(2):1130-7.

Cook, M.M. 2016. Endocrine-disrupting compounds: Measurement in Tampa Bay, removal from sewage and development of an estrogen receptor model. US: ProQuest Information & Learning.

Cózar, A., Echevarría, F., González-Gordillo, J.I., lrigoien, X., Úbeda, B., Hemández-León, S., Palma, Á.T., Navarro, S., García-de-Lomas, J., Ruiz, A., et al. 2014. Plastic debris in the open ocean. Proc Natl Acad Sci U S A 111(28):10239-44.

Dekiff, J.H., Remy, D., Klasmeier, J., Fries, E. 2014. Occurrence and spatial distribution of microplastics in sediments from Norderney. Environmental Pollution: 248.

DelCharco, M.J. 1998. Tidal flow in selected areas of tampa bayy and charlotte harbor, florida, 1995-96. Tallahassee: U.S. Geological Survey. Report nr 97-4265.

Derraik, J. 2002. The pollution of the marine environment by plastic debris: A review. Marine Pollution Bulletin 44:842-52.

Endo, S., Takizawa, R., Okuda, K., Takada, H., Chiba, K., Kanehiro, H., Ogi, H., Yamashita, R., Date, T., 2005. Concentration of polychlorinated biphenyls (PCBs) in beached resin pellets: variability among individual particles and regional differences. Mar. Poll. Bull. 50, 1103–1114.

Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J. 2014. Plastic pollution in the world's oceans: More than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. Plos One (12).

Estahbanati, S. and Fahrenfeld, N.L. 2016. Influence of wastewater treatment plant discharges on microplastic concentrations in surface water. Chemosphere 162:277-84.

Eriksson, P., Jakobsson, E., Fredriksson, A. 2001. Brominated flame retardants: A novel class of developmental neurotoxicants in our environment? Environ Health Perspect (9):903.

Fisher, W., Oliver, L., Winstead, J., Long, E. 2000. A survey of oysters *Crassostrea Virginica* from Tampa Bay, Florida: Associations of internal defense measurements with contaminant burdens. Aequatic Toxicology 51:115-38.

Fossi, M.C., Coppola, D., Baini, M., Giannetti, M., Guerranti, C., Marsili, L., Panti, C., de Sabata, E., Clò, S. 2014. Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: The case studies of the mediterranean basking shark (cetorhinus maximus) and fin whale (balaenoptera physalus). Mar Environ Res 100:17-24.

Gasperi J, Wright S, Dri R, Collard F, M, C., Guerrouache M, Langlois V, Kelly, F., Tassin, B. 2018. Microplastics in air: Are we breathing it in? Environmental Science and Health.

Grabe, S.A. and Barron, J. 2004. Sediment contamination, by habitat, in the Tampa Bay estuarine system (1993–1999): PAHs, pesticides and PCBs. Environmental Monitoring & Assessment 91(1-3):105.

Hartline NL, Bruce NJ, Karba SN, Ruff EO, Sonar SU, Holden PA. 2016. Microfiber masses recovered from conventional machine washing of new or aged garments. Environ Sci Technol 50(21):11532-8.

Hastings David and Sharp Katherine. Microplastics in Tampa Bay: Abundance, spatial and temporal variability. Basis 6. St. Petersburg, Florida: Eckerd College.

Hemond, H.F. and Fechner-Levy, E. 2000. Chemical fate and transport in the environment. San Diego, Calif.: Academic, c2000; 2nd Ed.

Hidalgo-Ruz V, et al. 2012. Microplastics in the marine environment: A review of the methods used for identification and quantification. Environ Sci Technol 46(6):3060-75.

Hoellein, T., Kelly, J., McCormick, A. Wastewater treatment plants significant source of microplastics in rivers, new research finds. American Geophysical Union/ Loyola University.

Hopewell J, Dvorak R, Kosior E. 2009. Plastics recycling: Challenges and opportunities. Philosophical Transactions: Biological Sciences (1526):2115.

Identifying Polymers [Internet]: Learn Chemistry: Enhancing,Learning, and Teaching [cited 2017 . Available from: http://www.rsc.org/learn-chemistry/resource/res00000385/identifying-polymers?cmpid=CMP00005147.

[Internet]: Hillsborough County Environmental Protection Commission; c2017 [cited 2017. Available from: ftp://ftp.epchc.org/EPC_ERM_FTP/WQM_Reports/.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L. 2015. Plastic waste inputs from land into the ocean. Science (6223):768.

Julian P and Estevez ED. 2010. Historical bathymetric analysis of tampa bay. Florida Gulf Coast University.

Kosuth M, Wattenberg E, Mason S, Tyree C, Morrison D. 2017. Synthetic polymer contamination in global drinking water. Orb.

Land Application of Biosolids: Human Health Risk Assessment Related to Microconstituents [Internet]: Water Environment Federation; c2017. Available from: http://biosolidsresources.org/oe/wp-content/uploads/2016/11/wef-fact-sheet-microconstituents-v11-jul-2017.pdf.

Land use, land value and tenure [Internet]: United States Department of Agriculture Economic Research Service; August 28, 2017. Available from: https://www.ers.usda.gov/topics/farm-economy/land-use-land-value-tenure/major-land-uses/.

Law, K.L. Plastics in the Marine Environment. Annual Review of Marine Science 9.1 205-29.

Learn about your watershed: Tampa bay watershed. Florida's waters, ours to protect. 2015. Florida Department of Environmental Protection.

Lebreton, L. C. M., van der Zwet, J., Damsteeg, J.-W., Slat, B., Andrady, A., & Reisser, J. (2017). River plastic emissions to the world's oceans. Nature Communications, 8, 15611.

Lewis, R.R., Clark, P.A., Fehrings, W.K., Greening HS, Johansson RO, and Paul RT.

1998. The rehabilitation of the Tampa Bay estuary, Florida, USA, as an example of successful integrated coastal management. Marine Pollution Bulletin 37(8-12):468-73.

Lewis, M.A. and Russell, M.J. 2015. Contaminant profiles for surface water, sediment, flora and fauna associated with the mangrove fringe along middle and lower eastern Tampa Bay. Mar Pollut Bull (1):273.

Lima A, Costa M, Barletta M. 2014. Distribution patterns of microplastics within the plankton of a tropical estuary. Environmental Research 132:146-55.

Maes T, Jessop R, Wellner N, Haupt K, Mayes AG. 2017. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with nile red. Scientific Reports : 44501.

MacDonald, D.D., Carr, R.S., Eckenrod, D., Greening, H., Grabe, S., Ingersoll, C.G., Janicki, S., Janicki, T., Lindskoog, R.A., Long, E.R., et al. 2004. Development, evaluation, and application of sediment quality targets for assessing and managing contaminated sediments in Tampa Bay, florida. Archives of Environmental Contamination & Toxicology 46(2):147-61.

Marella RL. 1999. Water withdrawals, use, discharge, and trends in florida, 1995. Tallahassee, Fla. (227 North Bronough St., Ste. 3015, Tallahassee 32301-1372): U.S. Dept. of the Interior, U.S. Geological Survey; Denver, CO: U.S. Geological Survey, Branch of Information Services distributor], 1999.

Mason, S.A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J., Fink, P., Papazissimos, D., Rogers, D.L. 2016. Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. Environmental Pollution 218:1045-54.

McCain BB, Brown DW, Tom Hom a, Myers MS, Pierce SM, Collier TK, Stein JE, Sin-Lam Chan a, Usha Varanasi a. 1996. Chemical contaminant exposure and effects in four fish species from Tampa Bay, florida. Estuaries (1):86.

Meyers S, Luther M, Wilson M, Havens H, Linville A, Sopkin K. 2007. A numerical simulation of residual circulation in tampa bay. Part I: Low-frequency temporal variations. Estuaries and Coasts 30(4):679-97.

Meyers SD and Luther ME. 2008. A numerical simulation of residual circulation in tampa bay. Part II: Lagrangian residence time. Estuaries and Coasts (5):815.

Moore CJ. 2008. Synthetic polymers in the marine environment: A rapidly increasing, long-term threat. Environmental Research 108(2):131-9.

Nizzetto L, Futter M, Langaas S. 2016. Are agricultural soils dumps for microplastics of urban origin? Environmental Science & Technology (50):10777.

Nizzetto L, Bussi G, Futter MN, Butterfield D, Whitehead PG. 2016. A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. Environmental Science Processes and Impacts 18(8):1050.

Pinellas County, Florida 2014 waste characterization study. 2015. Tampa: Kessler Consulting.

Pinellas County Planning Department, Potable water supply, wastewater, and reuse element. Chapter 2. 2013. In: The Pinellas county planning department Pinellas county comprehensive plan.

Protecting and restoring America's watersheds: Status, trends, and initiatives in watershed management. 2001. United States Environmental Protection Agency Office of Water. Report nr EPA-840-R-00-001.

Rillig M, Ingraffia R, de Souza Machado A. 2017. Microplastic incorporation into soil in agroecosystems. Frontiers in Plant Science 8.

Rios L, Moore C, Jones P. 2007. Persistent organic pollutants carried by synthetic polymers in the ocean environment. Marine Pollution Bulletin 54:1230-7.

Rochman C, Tahir A, Williams S, Baxa D, Lam R, Miller J, Teh F, Werorilangi S, Teh S. 2015. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Nature 5(14340):1-10.

Schmidt C, Krauth T, Wagner S. 2017. Export of plastic debris by rivers into seas. Environmental Science & Technology.

Schnoor, J.L., 1996. Chapter 1. Environmental modeling: Fate and transport of pollutants in water, air, and soil. New York: Wiley, 1-16. Print.

Science of Plastics [Internet]: Chemical Heritage Foundation; c2017 [cited 2017. Available from: https://www.chemheritage.org/science-of-plastics.

Seltenrich, N., 2015. New link in the food chain? Marine plastic pollution and seafood safety: Investigators are researching whether consumption of plastic debris by marine organisms translates into toxic exposures for people who eat seafood. Environ Health Perspect (2).

Sherwood, E.T., H. Greening, A. Janicki and D. Karlen. 2015. Tampa Bay estuary: Monitoring long-term recovery through regional partnerships. Regional Studies in Marine Science 4:1-11.

Shober, A.L., 2009. Drinking water source protection in the Tampa Bay region: A guide for homeowners. EDIS New Publications RSS. Soil and Water Science.

Sigler, M., 2014. The effects of plastic pollution on aquatic wildlife: Current situations and future solutions. Water, Air, & Soil Pollution (11).

Sutton R, et al. 2016. Microplastic contamination in the san francisco bay, california, USA. Marine Pollution Bulletin 109(1):230-5.

Tampa Bay Estuary Program. 2006. Charting the course: The comprehensive conservation and management plan for Tampa Bay. Tampa Bay Estuary Program.

Tavakoly Sany, SB, Hashim, R., Rezayi, M., Salleh, A., Safari, O. 2014. A review of strategies to monitor water and sediment quality for a sustainability assessment of marine environment. Environmental Science and Pollution Research (2):813.

Taylor, M.L., Gwinnett, C., Robinson, L.F., Woodall, L.C. 2016. Plastic microfiber ingestion by deep-sea organisms. Scientific Reports:33997.

Tidmore, S., 2016. Perils of plastic. Bay Soundings.

Tyler, Dean, Zawada, D.G., Nayegandhi, Amar, Brock, J.C., Crane, M.P., Yates, K.K., and Smith, K.E.L., 2007, Topobathymetric data for Tampa Bay, Florida: U.S. Geological Survey Open-File Report 2007-1051.

Tyree, C. and Morrison, D. 2017. Invisibles The Plastic inside Us [Internet]: Orb; [cited 2018. Available from: https://orbmedia.org/stories/Invisibles_plastics.

Weisberg RH and Zheng L., 2006. The circulation of tampa bay driven by buoyancy, tides and winds, as simulated using a finite volume coastal ocean model. J Geophys Res.

Woodall, L., Sanchez-Vidal, A., Canals, M., L.J. Paterson, G., Coppock, R., Sleight, V., Calafat, A, Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. open sci. 1(140317).

Xian, G., Crane, M., Su, J., 2007. An analysis of urban development and its environmental impact on the Tampa Bay watershed. Journal of Environmental Management 85(4):965-76.

Yates, K., Greening, H., Morrison, G., 2011. Integrating science and resource management in Tampa Bay, Florida [electronic resource]. Reston, Va.: U.S. Dept. of the Interior, U.S. Geological Survey, 2011.

Yonkos, L.T., Friedel, E.A., Perez-Reyes, A., Ghosal, S., Arthur, C.D., 2014. Microplastics in four estuarine rivers in the chesapeake bay, U.S.A. Environ Sci Technol 48(24):14195-202.

APPENDIX I: ADDITIONAL TEXT

Appendix I

To assess, monitor, and compare the full realm of potential risk by microplastics throughout the globe, there is a need to define, understand, and quantify emissions both geographically and over time. Much of plastic research utilizes different methods and quantification scales, or perhaps only conducts single season or year per site data. Microplastic lab and field studies can be expanded to better quantify and detect microplastic waste by focusing on more long-term studies with better standardization, classification of plastics into categories, and recording of mass estimates to help reduce the "missing plastic problem" by relating the mass of plastic production and mass of uncaptured waste estimates to the mass sampled in different locations (Schmidt et al., 2017).

It is of benefit to conduct field studies along different pathways in which the release of plastics into the marine environment occurs. This will require conducting more studies of the movement, transport, and storage of microplastics through freshwater ecosystems, soil, groundwater, and air. This knowledge will help in working towards an estimated global budget for ocean plastics and will result in more cost-effective monitoring and source mitigation efforts.

Solutions to the plastic problem are going to require a multi stakeholder approach to radically alter our relationship with plastic on technological, behavioral, and policy platforms. Losses of primary microplastics are mostly unintentional besides the intentional inclusion of primary microplastics in cosmetic products. These losses occur in the product use and maintenance phase such as when transporting nurdles, washing

Appendix I (Continued)

textiles, and utilizing boats and cars with synthetic paints and tires. The releases of these microplastics into the ecosystem are far reaching and have to be addressed with a design, technology, and life cycle management approach to lessen the impacts of microplastics lost from use and maintenance of products. This can involve using metrics and indicators for products that measure and monitor positive reduction of loss or releases of microplastics.

Intentional losses such as those stemming from the use of microbeads in cosmetic products can be easily reduced by removing them and replacing them with a natural alternative. As of 2015, congress amended the Federal Food, Drug, and Cosmetic Act (FD&C Act) by passing the Microbead-Free Waters Act of 2015 that prohibits the manufacturing, packaging, and distribution of rinse-off cosmetics containing plastic microbeads such as tooth paste and body wash. There still needs to be further development of regulation of microplastics in products in the U.S. that are not considered rinse off like deodorant and makeups. Furthermore, clothing can be designed with natural materials such as hemp or the innovative new protein found in spider silk. Products like in line water filters and the Cora Ball, can reduce the release of microplastic particles in the maintenance phase and prevent the loss of them through wastewater and into the

ecosystem.

Reducing secondary microplastics occurs through reducing mismanaged waste which will involve strategic collaboration between consumers and waste and water infrastructure engineers. Collaboration between epidemiological and air and water quality stake holders is required to implement critical research initiatives and monitoring strategies (Gasperi et al., 2018). Real solutions depends on individuals, companies, and governments taking responsibility for the plastic waste generated by their purchases, their product designs, and their attitude to environmental protection (Tyree and Morrison, 2017).

APPENDIX II: ADDITIONAL TABLES

Appendix II

Table A1: Microplastic Concentrations for fall/winter 2016 Plankton Tow Samples.

Sampling date	Sta #	Replicate #	# / L	Std. Dev.
10-3-16	42	1	0.002	
10-3-16	50	1	0.004	
10-11-16	55	1	0.016	0.005
	55	2	0.007	
	55	3	0.007	
10-11-16	8	1	0.004	0.002
	8	2	0.004	
	8	3	0.007	
11-1-16	42	1	0.003	0.004
	42	2	0.008	
	42	3	0.001	
11-1-16	50	1	0.004	0.002
	50	2	0.003	
	50	3	0.001	
11-7-16	55	1	0.003	0.002
	55	2	0.003	
	55	3	0.000	
11-7-16	8	1	0.008	0.003
	8	2	0.002	
	8	3	0.003	
12-13-16	55	1	0.009	0.003
	55	2	0.004	
	55	3	0.004	
12-13-16	8	1	0.009	0.003
	8	2	0.003	
	8	3	0.003	
12-19-16	94	1	0.002	0.000
	94	2	0.002	
	94	3	0.001	
12-19-16	28	1	0.012	0.005
	28	2	0.005	
	28	3	0.002	

Appendix II (Continued)

Table A2: Microplastic Concentrations for spring 2017 Plankton Tow Samples.

Sampling date	Sta #	Replicate #	#/ L	Std.Dev.
4-10-17	42	1	0.002	0.003
		2	0.001	
		3	0.006	
4-10-17	50	1	0.002	0.005
		2	0.003	
		3	0.011	
4-11-17	55	1	0.003	0.001
		2	0.002	
		3	0.001	
4-11-17	8	1	0.003	0.001
		2	0.006	
		3	0.004	
4-17-17	94	1	0.001	0.001
		2	0.003	
		3	0.002	
4-17-17	28	1	0.003	0.001
		2	0.003	
		3	0.004	
5-8-17	55	1	0.003	0.003
		2	0.009	
		3	0.003	
5-8-17	8	1	0.003	0.000
		2	0.002	
		3	0.002	
5-16-17	94	1	0.001	0.001
		2	0.002	
		3	0.002	
5-16-17	28	1	0.006	0.000
		2	0.007	
		3	0.007	

Appendix II (Continued)

Table A3: Microplastic Concentrations for summer 2017 Plankton Tow Samples.

Sampling date	Sta #	Replicate #	# / L	Std. Dev.
	50	1	0.005	0.003
6/5/17		2	0.009	
		3	0.010	
6/5/17	42	1	0.001	0.003
		2	0.004	
6/13/17	55	1	0.009	0.003
		2	0.004	
	8	1	0.002	0.002
6/13/17		2	0.006	
		3	0.004	
6/19/17	94	1	0.008	0.009
		2	0.023	
		3	0.023	
7/5/17	42	1	0.005	0.001
		2	0.002	
		3	0.004	
7/5/17	50	1	0.005	0.002
		2	0.002	
		3	0.001	
7/10/17	55	1	0.000	0.004
		2	0.008	
		3	0.001	
7/10/17	8	1	0.010	0.005
		2	0.004	
		3	0.000	
7/18/17	94	1	0.006	0.004
		2	0.009	
		3	0.001	
7/24/17	28	1	0.001	0.000
		2	0.001	
		3	0.001	

Appendix II (Continued)

Table A4: Average Monthly Streamflow of Tampa Bay Freshwater Inputs (feet^3/sec).

Freshwater Input and Period of Record	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	Avg
Alafia River, 1932-2008	400	425	500	300	200	500	900	1200	1300	575	300	325	577
Little Manatee River, 1939-2008	180	190	200	150	75	225	300	500	600	200	175	160	246
Manatee River, 1966-2008	30	35	40	20	20	75	100	200	200	35	20	20	66

*(Data from U.S. Geological Survey)

Appendix II (Continued)

Table A5: Salinity at Surface.

Bay Region	Sta #	Jun-16	Jul-16	Aug-16	Sep-16	Oct-16	Nov-16	Dec-16	Apr-17	May-17	Jun-17	Jul-17	Avg	Std. Dev
OTB	65	21.0	21.2	20.5	13.2	14.5	16.5	19.7	26.3	27.4	26.8	25.5	21.1	4.99
	42	21.9	21.4	21.2	14.7	15.4	16.9	20.6	27.0	27.7	28.4	26.5	22.0	4.94
	60	23.0	21.9	21.4	11.2	13.9	18.0	20.9	27.1	28.2	29.2	26.7	21.9	5.81
	61	14.1	20.7	18.0	11.3	15.4	19.1	21.3	27.5	28.4	27.3	25.6	20.8	5.85
	50	23.6	22.9	22.8	18.6	17.6	21.2	23.0	28.0	28.7	30.2	28.0	24.1	4.19
	Mean												**22.0**	**5.16**
HB	44	21.8	20.2	15.4	13.4	15.9	23.8		27.4	28.5		23.7	21.1	5.34
	52	19.0	20.8	18.4	17.5	21.2	24.8	25.7	28.4	28.9	28.6	25.7	23.5	4.31
	7	19.7	21.7	22.2	15.7	17.6	24.4	19.1	28.1	29.3	28.1	26.7	23.0	4.67
	8	9.3	20.8	19.7	15.6	14.1		23.2	26.9	28.8	28.1	26.6	21.3	6.63
	80	18.4	23.3	23.9	20.7	20.2	25.0	25.9	28.9	31.1	29.6	28.1	25.0	4.16
	55	18.1	21.0	22.0	17.4	18.5	24.3	25.0	28.0	29.2	28.1	27.0	23.5	4.35
	Mean												**22.9**	**4.91**
MTB	84	23.4	24.5	24.1	20.5	23.4	24.4	26.3	29.3	31.0	28.9	28.5	25.8	3.20
	28	27.8	26.0	26.1	22.3	23.2	26.3	26.8	30.8	31.6	30.5	30.2	27.4	3.07
	19	27.8	28.0	27.7	22.8	26.4	26.3	26.9	32.0	32.6	32.5	31.6	28.6	3.17
	14	23.4	24.2	25.1	20.0	21.1	25.3	26.5	29.5	30.3	30.2	28.5	25.8	3.53
	33	24.3	24.5	25.3	19.1	18.6	21.6	23.9	29.3	29.8	30.7	28.7	25.1	4.21
	Mean												**26.5**	**3.44**
LTB	91	32.4	30.1	31.4	26.0	28.0	30.1	27.9	33.7	34.4	33.9	32.0	30.9	2.74
	22	29.1	30.6	28.7	25.8	26.3	28.8	30.0	32.7	34.2	32.6	30.8	29.9	2.61
	92	32.7	31.9	29.7	28.7	30.2	30.7	31.8	33.9	35.1	34.4	32.3	32.0	2.01
	94	34.2	33.9	33.1	30.4	32.8	31.7	31.3	34.0	35.0	34.6	33.9	33.2	1.48
	Mean												**31.5**	**2.21**
OTB Trib	102	0.7	8.0	2.5	0.4	2.2							2.7	3.09
	141													
	103	0.7			0.2	2.2							1.0	1.03
	Mean												**1.9**	**2.06**
HR Trib	2	6.4	6.8		3.3	3.9							5.1	1.76
	Mean												**5.1**	**1.76**

OTB= Old Tampa Bay, HB= Hillsborough Bay, MTB=Middle Tampa Bay, LTB= Lower Tampa Bay, OTB Trib=Old Tampa Bay Tributaries, HR Trib= Hillsborough River Tributary

*Data from HC-EPC

Appendix II (Continued)

Table A6: Mean Depth (Meters below MLW) by Bay Region versus Discrete and Plankton Tow Average Concentrations.

Bay Region	Depth (m)	Discrete (count/L)	Plankton tow (count/L)
OTB	2.7	1.03	0.0038
Hillsborough Bay	3.2	0.68	0.0046
Middle Tampa Bay	4.5	1.14	0.0044
Lower Tampa Bay	4.9	0.92	0.0058

Depth is measured as mean depth in meters at the mean low water in the year 2000
*Data from (Julian and Estevez, 2010)

Table A7: Average Monthly Rainfall (inches) at Seven Rainfall Recording Stations over Specified Periods of Record.

Location and Period of Record	Bay Region	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec
Bartow, 1901-2007	HB	2.5	2.5	3.3	2.5	4.3	8	8.2	7.3	6.8	3	1.7	2.1
Lakeland, 1915-2006	HB	2.25	2.3	3.4	3.3	3.7	7.2	7.4	6.9	6	2.3	1.6	2
Plant City, 1901-2007	HB	2.3	2.7	3.2	3.3	3.7	7.7	8.1	8.2	6.3	2.6	1.65	2.2
Tarpon Springs, 1901-2004	OTB	2.6	2.6	3.3	3.3	2.6	5.7	7.7	8.4	7	3	1.8	2.3
Tampa,1901-2007	OTB/HB	2.1	2.5	2.9	2.6	2.7	6.3	7.2	7.5	6	2.4	1.5	2.2
St. Petersburg,1914-2004	MTB/LTB	2.4	2.6	3.2	3.2	2.6	6	8	8.2	7.6	2.9	1.7	2.25
Bradenton, 1911-2004	LTB	2.5	2.55	3.15	3.1	2.7	7	8.8	9.5	7.9	3.1	1.8	2.1

*(Data from National Weather Service)

Appendix II (Continued)

Table A8: Precipitation Values (in) from One Day Prior to Sampling: Discrete Samples.

Bay Region	Sta #	Jun-16	Jul-16	Aug-16	Sep-16	Oct-16	Nov-16	Apr-17	May-17	Jun-17	Jul- 17
		6/7		**7/31**		**10/2**	**10/31**	**4/9**		**6/4**	**7/4**
OTB	65	2.78		1.26		0.70	0	0		0.11	0
	42	2.78		1.26		0.70	0	0		0.11	0
	60	2.78		1.26		0.70	0	0		0.11	0
	61	2.78		1.26		0.70	0	0		0.11	0
	50	2.78		1.26		0.70	0	0		0.11	0
		6/13	**7/10**	**8/8**	**9/11**	**10/10**	**11/6**	**4/10**	**5/7**	**6/12**	**7/9**
HB	44	0.97	0	0.51	0	0		0	0	0.87	0.9
	52	0.97	0	0.51	0	0	0	0	0	0.87	0.9
	7	0.97	0	0.51	0	0	0	0	0	0.87	0.9
	8	0.97	0	0.51	0	0	0	0	0	0.87	0.9
	80	0.97	0	0.51	0	0	0	0	0	0.87	0.9
		6/7, 6/13, 6/19	**7/10, 7/24**	**7/31, 8/8, 8/14**	**9/11, 9/19**	**10/2. 10/10. 10/23**	**10/31, 11/6, 11/14**	**4/9, 4/10, 4/16**	**5/7, 5/15**	**6/4, 6/12, 6/18**	**7/5, 7/9, 7/17 & 7/23**
MTB	84	0.04	0.02	0.06	0.13	0	0	0	0	2.01	0.01
	28	0.04	0.02	0.06	0.13	0	0	0	0	2.01	0.7
	19	0.04	0.02	0.06	0.13	0	0	0	0	2.01	0.01
	14	0	0	3.07	0	0	0	0	0	2.01	0
	33	3.76		0.17		0.08	0	0		0.46	1.45
		6/19	**7/24**	**8/14**	**9/19**	**10/23**	**11/14**	**4/16**	**5/15**	**6/18**	**7/17 & 7/23**
LTB	91	0.05	0.11	0	0	0	0	0	0	0	0.08
	22	0.05	0.11	0	0	0	0	0	0	0	0.45
	92	0.05	0.11	0	0	0	0	0	0	0	0.45
	94	0.05	0.11	0	0	0	0	0	0	0	0.45
		6/12		**8/1**							
OTB Trib	141	1.28		0							
	102	1.28		0							
	103	1.28		0							
		6/8	**7/12**	**8/22**	**9/13**						
HR Trib	2	0.25	0.52	0	0.02						

Source: NOAA National Centers for Environmental Information: Climate Data Online

Appendix II (Continued)

Table A9: Precipitation Values (in) from Two Days Prior to Sampling: Discrete Samples

Bay Region	Sta #	Jun-16	Jul-16	Aug-16	Sep-16	Oct-16	Nov-16	Apr-17	May-17	Jun-17	Jul- 17
		6/6		**7/30**		**10/1**	**10/30**	**4/8**		**6/3**	**7/3**
OTB	65	2.76		0.05		0.02	0	0		0.34	0
	42	2.76		0.05		0.02	0	0		0.34	0
	60	2.76		0.05		0.02	0	0		0.34	0
	61	2.76		0.05		0.02	0	0		0.34	0
	50	2.76		0.05		0.02	0	0		0.34	0
		6/12	**7/9**	**8/7**	**9/10**	**10/9**	**11/5**	**4/9**	**5/6**	**6/11**	**7/8**
HB	44	0.59	0	0.04	0	0		0	0.02	0.12	0
	52	0.59	0	0.04	0	0	0.01	0	0.02	0.12	0
	7	0.59	0	0.04	0	0	0.01	0	0.02	0.12	0
	8	0.59	0	0.04	0	0	0.01	0	0.02	0.12	0
	80	0.59	0	0.04	0	0	0.01	0	0.02	0.12	0
		6/6, 6/12 6/18	**7/9, 7/23**	**7/30, 8/7, 8/13**	**9/10, 9/18**	**10/1, 10/9, 10/22**	**10/30, 11/5, 11/13**	**4/8, 4/9, 4/15**	**5/6, 5/14**	**6/3, 6/11, 6/17**	**7/3, 7/8, 7/16 & 7/22**
MTB	84	0.44	0	0.06	0	0	0	0	0	0.48	0.02
	28	0.44	0	0.06	0	0	0	0	0	0.48	0.16
	19	0.44	0	0.06	0	0	0	0	0	0.48	0.02
	14	0.42	0.07	0.59	0	0	0	0	0	0.48	1.05
	33	3.53		0.26		0	0	0		0.49	0.01
		6/18	**7/23**	**8/13**	**9/18**	**10/22**	**11/13**	**4/15**	**5/14**	**6/17**	**7/16 & 7/22**
LTB	91	0.15	0.21	0.02	0	0	0	0	0.05	0.03	0.34
	22	0.15	0.21	0.02	0	0	0	0	0.05	0.03	0.02
	92	0.15	0.21	0.02	0	0	0	0	0.05	0.03	0.02
	94	0.15	0.21	0.02	0	0	0	0	0.05	0.03	0.02
		6/11		**7/31**							
OTB Trib	141	0.21		1.26							
	102	0.21		1.26							
	103	0.21		1.26							
		6/7	**7/11**	**8/21**	**9/12**						
HR Trib	2	2.78	0.35	0	0						

Source: NOAA National Centers for Environmental Information: Climate Data Online

Appendix II (Continued)

Table A10: Precipitation Values (in) from Three Days Prior to Sampling: Discrete Samples.

Bay Region	Sta #	Jun-16	Jul-16	Aug-16	Sep-16	Oct-16	Nov-16	Apr-17	May-17	Jun-17	Jul- 17
		6/5		**7/29**		**9/30**	**10/29**	**4/7**		**6/2**	**7/2**
OTB	65	0.77		0.03		0.07	0	0		0.15	0.01
	42	0.77		0.03		0.07	0	0		0.15	0.01
	60	0.77		0.03		0.07	0	0		0.15	0.01
	61	0.77		0.03		0.07	0	0		0.15	0.01
	50	0.77		0.03		0.07	0	0		0.15	0.01
		6/11	**7/8**	**8/6**	**9/9**	**10/8**	**11/4**	**4/8**	**5/5**	**6/10**	**7/7**
HB	44	0.22	0	0	0	0.04		0	0.48	0	0
	52	0.22	0	0	0	0.04	0	0	0.48	0	0
	7	022	0	0	0	0.04	0	0	0.48	0	0
	8	0.22	0	0	0	0.04	0	0	0.48	0	0
	80	0.22	0	0	0	0.04	0	0	0.48	0	0
		6/5, 6/11, 6/17	**7/8, 7/22**	**7/29, 8/6, 8/12**	**9/9, 9/17**	**9/30. 10/8. 10/21**	**10/29, 11/4, 11/12**	**4/7, 4/8, 4/14**	**5/5, 5/13**	**6/2, 6/10, 6/15**	**7/2, 7/7, 7/15 & 7/21**
MTB	84	0	0.25	0	0	0	0	0	0.4	0.22	0.11
	28	0	0.25	0	0	0	0	0	0.4	0.22	0.05
	19	0	0.25	0	0	0	0	0	0.4	0.22	0.11
	14	0.05	1.42	0.01	0	0	0	0	0.4	0.22	0.82
	33	1.01		0.01		0.14	0	0		1.15	0
		6/17	**7/22**	**8/12**	**9/17**	**10/21**	**11/12**	**4/14**	**5/13**	**6/16**	**7/15 & 7/21**
LTB	91	0.01	0	0.58	0	0	0	0	0	0	0.45
	22	0.01	0	0.58	0	0	0	0	0	0	0.14
	92	0.01	0	0.58	0	0	0	0	0	0	0.14
	94	0.01	0	0.58	0	0	0	0	0	0	0.14
		6/10		**7/30**							
OTB Trib	141	0.52		0.05							
	102	0.52		0.05							
	103	0.52		0.05							
		6/6	**7/10**	**8/20**	**9/11**						
HB Trib	2	2.76	0	0	0						

Source: NOAA National Centers for Environmental Information: Climate Data Online

Appendix II (Continued)

Table A11: Precipitation Values (in) from One Day Prior to Sampling: Plankton Tow Samples.

Bay Region	Rain Date	Sta #	Precip (in)
OTB	10-2-16	42	0.7
OTB	10-2-16	50	0.7
HB	10-10-16	55	0.00
HB	10-10-16	8	0.00
OTB	10-31-16	42	0.00
OTB	10-31-16	50	0.00
HB	11-6-16	55	0.00
HB	11-6-16	8	0.00
HB	12-12-16	55	0.01
HB	12-12-16	8	0.01
LTB	12-18-16	94	0.00
MTB/LTB	12-18-16	28	0.00
OTB	4-9-17	42	0.00
OTB	4-9-17	50	0.00
HB	4-10-17	55	0.00
HB	4-10-17	8	0.00
MTB/LTB	4-16-17	94	0.00
MTB/LTB	4-16-17	28	0.00
HB	5-7-17	55	0.00
HB	5-7-17	8	0.00
MTB/LTB	5-15-17	94	0.00
MTB/LTB	5-15-17	28	0.00
OTB	6-4-17	42	0.46
OTB	6-4-17	50	0.46
HB	6-12-17	55	2.01
HB	6-12-17	8	2.01
MTB/LTB	6-18-17	94	0.14
OTB	7-4-17	42	1.45
OTB	7-4-17	50	1.45
HB	7-9-17	55	0.00
HB	7-9-17	8	0.00
MTB/LTB	7-17-17	94	0.01
MTB/LTB	7-23-17	28	0.70

Source: NOAA National Centers for Environmental Information: Climate Data Online

Appendix II (Continued)

Table A12: Precipitation Values (in) from Two Days Prior to Sampling: Plankton Tow Samples.

Bay Region	Rain Date	Sta #	Precip (in)
OTB	10-1-16	42	0.02
OTB	10-1-16	50	0.02
HB	10-9-16	55	0
HB	10-9-16	8	0
OTB	10-30-16	42	0
OTB	10-30-16	50	0
HB	11-5-16	55	0.01
HB	11-5-16	8	0.01
HB	12-11-16	55	0
HB	12-11-16	8	0
MTB/LTB	12-17-16	94	0
MTB/LTB	12-17-16	28	0
OTB	4-8-17	42	0
OTB	4-8-17	50	0
HB	4-9-17	55	0
HB	4-9-17	8	0
MTB/LTB	4-15-17	94	0
MTB/LTB	4-15-17	28	0
HB	5-6-17	55	0.05
HB	5-6-17	8	0.05
MTB/ LTB	5-14-17	94	0.05
MTB/LTB	5-14-17	28	0.05
OTB	6-3-17	42	0.34
OTB	6-3-17	50	0.34
HB	6-11-17	55	0.25
HB	6-11-17	8	0.25
MTB/LTB	6-17-17	94	0.03
OTB	7-3-17	42	0
OTB	7-3-17	50	0
HB	7-8-17	55	0.07
HB	7-8-17	8	0.07
MTB/LTB	7-16-17	94	0.02
MTB/LTB	7-22-17	28	0.16

Source: NOAA National Centers for Environmental Information: Climate Data Online

Appendix II (Continued)

Table A13: Precipitation Values (in) from Three Days Prior to Sampling: Plankton Tow Samples.

Bay Region	Rain Date	Sta #	Precip (in)
OTB	9-30-16	42	0.07
OTB	9-30-16	50	0.07
HB	10-8-16	55	0.04
HB	10-8-16	8	0.04
OTB	10-29-16	42	0.00
OTB	10-29-16	50	0.00
HB	11-4-16	55	0.00
HB	11-4-16	8	0.00
HB	12-10-16	55	0.02
HB	12-10-16	8	0.02
LTB	12-16-16	94	0.00
MTB/LTB	12-16-16	28	0.00
OTB	4-7-17	42	0.00
OTB	4-7-17	50	0.00
HB	4-8-17	55	0.00
HB	4-8-17	8	0.00
MTB/LTB	4-14-17	94	0.00
MTB/LTB	4-14-17	28	0.00
HB	5-5-17	55	0.57
HB	5-5-17	8	0.57
MTB/LTB	5-13-17	94	0.00
MTB/LTB	5-13-17	28	0.00
OTB	6-2-17	42	0.15
OTB	6-2-17	50	0.15
HB	6-10-17	55	0.00
HB	6-10-17	8	0.00
MTB/LTB	6-16-17	94	0.00
OTB	7-2-17	42	0.01
OTB	7-2-17	50	0.01
HB	7-7-17	55	0.00
HB	7-7-17	8	0.00
MTB/LTB	7-15-17	94	0.14
MTB/LTB	7-21-17	28	0.05

Source: NOAA National Centers for Environmental Information Climate Data Online

Appendix II (Continued)

Table A14: Flowmeter Calculations for summer 2017 Plankton Tow Sampling.

Date	Sta	Vol calculated by geo distance (L)	Vol calculated by flowmeter (L)	Avg concentration dep on vol by geo distance	Adj. avg concentration dep on vol by flowmeter
6/19/17	94	16,485	29,559	0.018	0.010
7/5/17	50	49,063	42,091	0.003	0.003
7/5/17	42	33,363	28,676	0.004	0.004
7/10/17	55	29,438	33,549	0.003	0.003
7/10/17	8	37,288	31,314	0.005	0.006
7/18/17	94	21,588	65,455	0.005	0.002
7/24/17	28	64,763	42,585	0.001	0.002

APPENDIX III: ADDITIONAL FIGURES

Appendix III

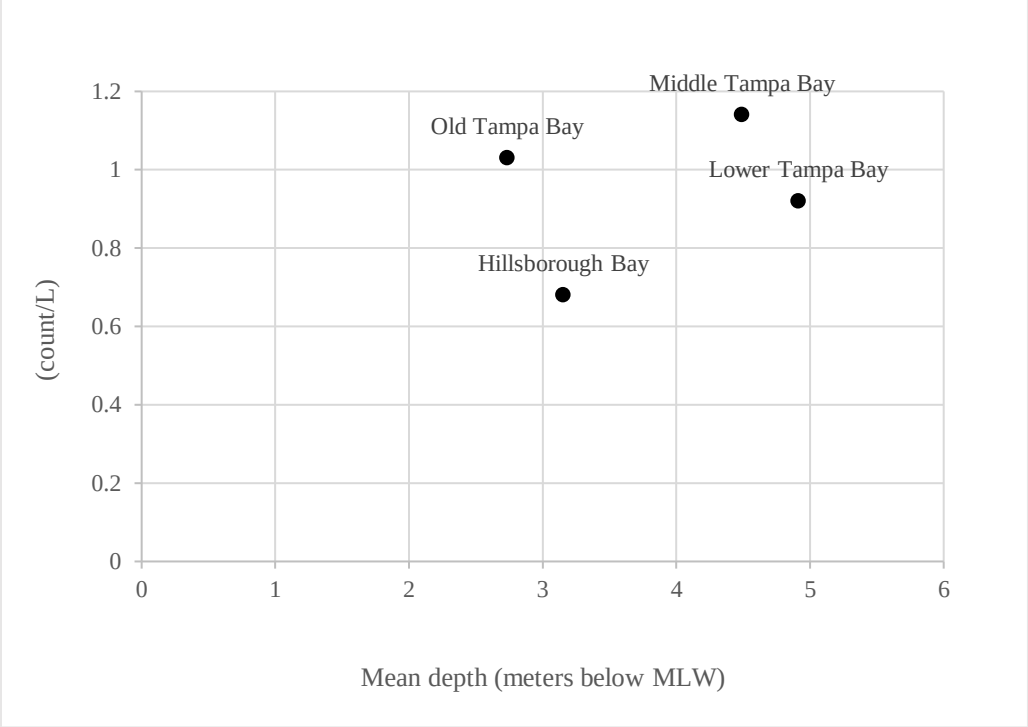

Figure A1: Average Microplastic Concentration versus Mean Depth of Tampa Bay in 2000 by Bay Region: Discrete Samples.

Appendix III (Continued)

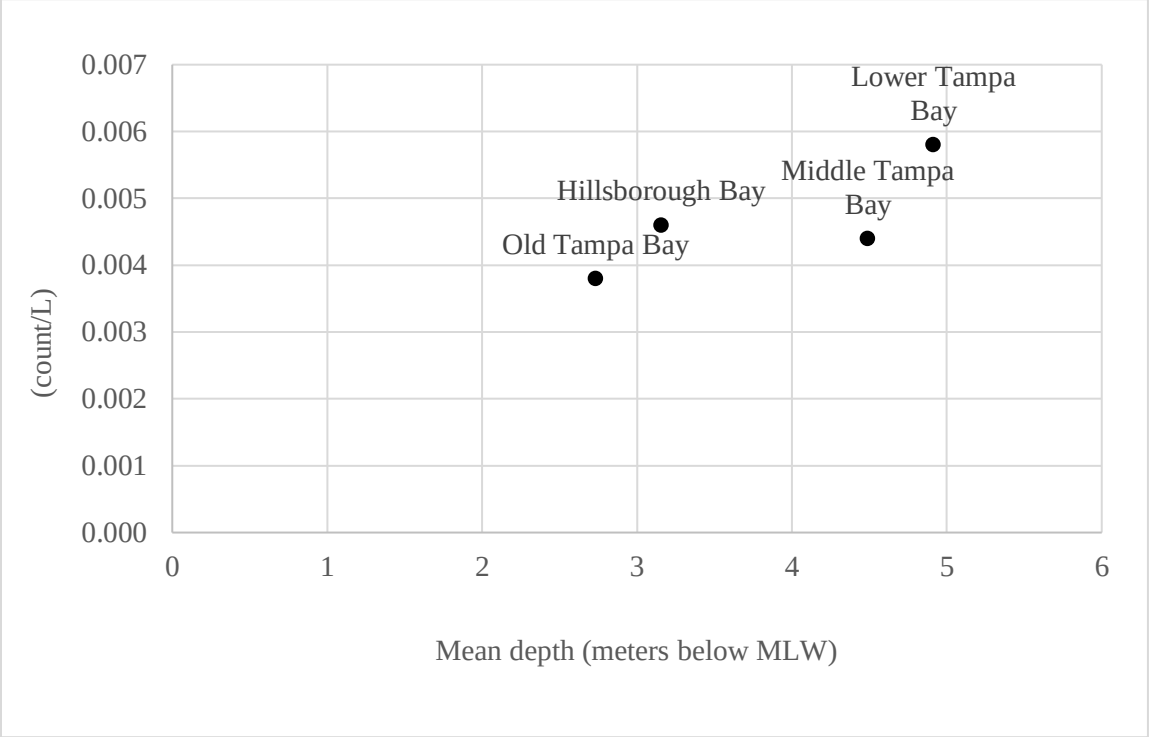

Figure A2: Average Microplastic Concentration versus Mean Depth of Tampa Bay in 2000 by Bay Region: Plankton Tow Samples.

Appendix III (Continued)

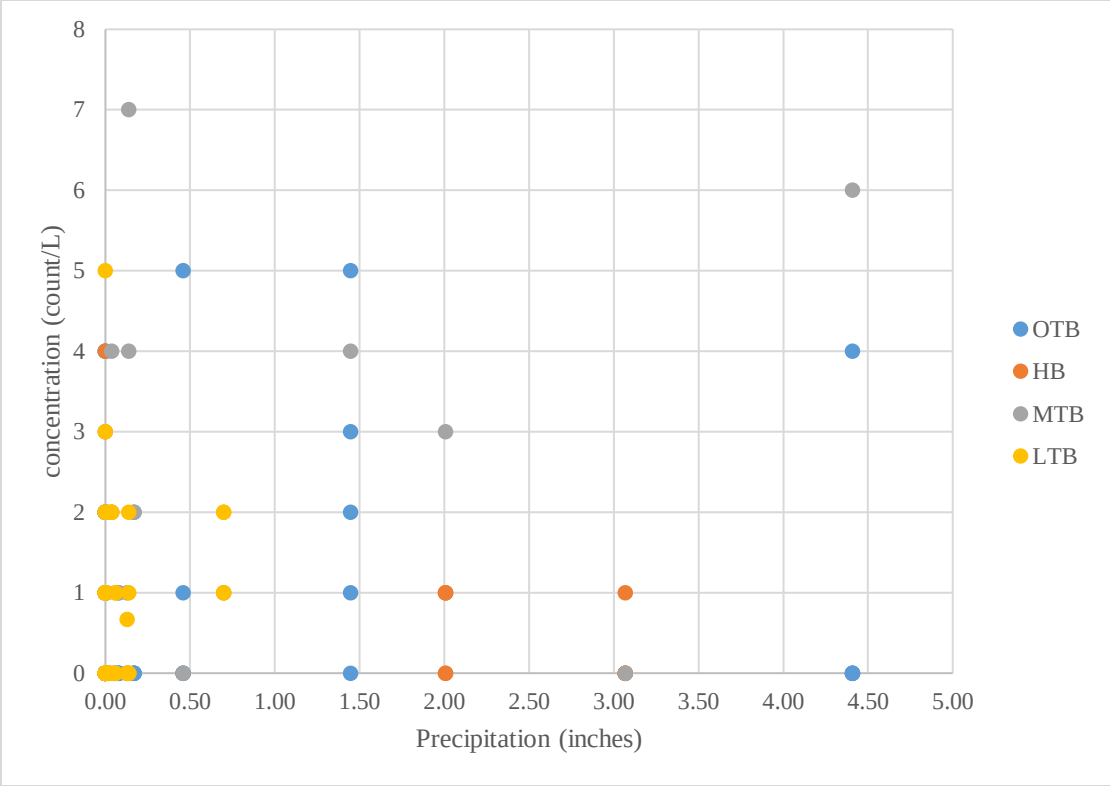

Figure A3: Concentration versus Precipitation (one day prior to sampling): Discrete samples.

Appendix III (Continued)

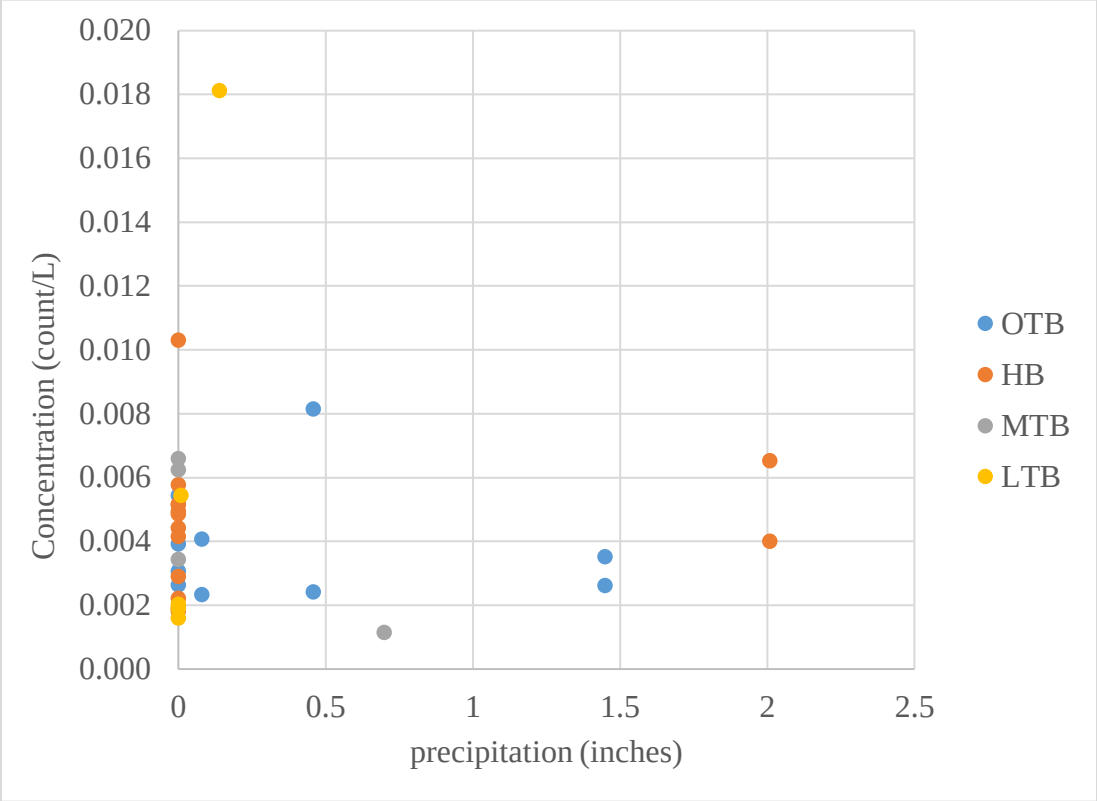

Figure A4: Concentration versus Precipitation (one day prior to sampling): Plankton Tow Samples.

ABOUT THE AUTHOR

Kinsley is a graduate student in the USF Environmental Science and Policy master's program. She graduated with a Bachelor's of Science in Wildlife Ecology and Conservation and minors in environmental science and sustainability studies from the University of Florida in 2012. Since then she has worked in different sectors of the environmental field from helping with rehabilitation of sea turtles at Mote Marine Lab, assisting with field work, laboratory work, and analysis regarding manatee genetics at Florida Fish and Wildlife, and serving as an environmental educator at nonprofits in the Tampa Bay area. She entered the program in 2015 and has served as a teaching assistant for the Biology and Environmental science labs throughout her attendance in this program.

She feels strongly that scientific evidence should be a part of the policy process as well as the importance of communicating to nonscientists. She is passionate in pursuing a career within the junction of science and communications, hopefully involving marine debris in aquatic ecosystems, and hopefully helping develop compelling outreach and effective mitigation to reduce the anthropogenic impacts on local and global ecosystems.