2022

Microplastic Abundances in the Guana River Estuary in Northeast Florida

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MICROPLASTIC ABUNDANCE IN THE GUANA RIVER ESTUARY IN NORTHEAST FLORIDA

by

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A thesis submitted in partial fulfillment of the requirements
for the Honors Undergraduate Thesis Program in Biology
in the College of Sciences
and in the Burnett Honors College
at the University of Central Florida
Orlando, Florida

Spring Term, 2022

Thesis Chair: Linda Walters, PhD

ABSTRACT

Plastic never fully disappears, but instead breaks into smaller pieces referred to as microplastics (< 5 mm length). Microplastics are common worldwide, and more studies are needed to understand the accumulation and diversity of microplastics found in various environments. In this study, six locations were sampled for one year in the Guana River Estuary, a partially impounded system with heavily urbanized headwaters. This study was conducted in conjunction with the Guana Tolomato Matanzas National Estuarine Research Reserve (GTM NERR) and the Florida Fish and Wildlife Conservation Commission (FWC) at their water sampling stations. The objectives of this study were to investigate: 1) distribution patterns across sample sites over time, 2) the color, size, and shape of microplastics found, and 3) polymer composition. Water samples were collected from surface waters in 1-L bottles, with five replicates at each site. Sampling occurred once a month, at the beginning of each month from August 2020-August 2021. Samples were analyzed for abundance and characteristics of microplastics using a dissecting microscope and the polymer composition was determined using Fourier-transform infrared spectroscopy. No pattern was found in the distribution of microplastics from north to south or over time. Variations in plastic color, size, and polymer composition suggests that there are multiple sources of pollution into the Guana River Estuary.

DEDICATION

For my mother, thank you for your being my number one fan and always encouraging me to achieve my dreams. For my family, thank you for the continuous support and encouragement. For all my mentors, thank you for helping me develop the skills I need to succeed and encouraging my curiosity and ambition. To Mr. Joseph H. McCoy, thank you for you for instilling my passion for science and going above and beyond as an educator.

ACKNOWLEDGEMENTS

Funding for this project was provided by the Indian River Lagoon National Estuary Program to Dr. L. Walters, UCF Burnett Honors College, UCF Office of Undergraduate Research, UCF Research and Mentoring Program, and the UCF Biology Department. Special thanks to Wade Brenner with the Florida Fish and Wildlife Commission for air boat transportation, Guana Tolomato Matanzas National Estuarine Research Reserve staff with field assistance, Sidney Busch for lab assistance, and Dr. Melinda Donnelly for help with statistical analysis. Additional thanks to Dr. Matthew Rex and Katie Abrams with Fourier Transform Infrared Spectroscopy assistance.

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INTRODUCTION

Plastic pollution has become a topic of international interest due to increased research reporting accumulations in every habitat on Earth, as well as the harm plastics can cause to many organisms (e.g., Andrady 2011; Barboza et al. 2018; Browne et al. 2011). Plastics are commonly found in many products such as facial cleaners, toothpaste, synthetic fabric, disposable packaging, and tires (e.g., Andrady 2011; Schwarz et al. 2019). Plastics enter the environment directly from run-off or improper disposal of debris (e.g., Andrady 2011; Browne et al. 2011). Plastic pollution can also enter the marine environment via sewage and stormwater outfalls (e.g., Browne et al. 2011; Conley et al. 2019). While wastewater treatment facilities are able to catch some plastics, there are no cost-effective filters in sewage treatment or wastewater plants that remove small particles, particularly fibers (Freeman et al. 2020). Therefore, when products that contain plastics are used and not properly disposed, nothing is there to prevent them from entering the environment (e.g., Browne et al. 2011; Freeman 2020).

Microplastics are defined as plastics that are 5 mm or smaller (e.g., Andrady 2011; Hidalgo-Ruz et al. 2012) and can be categorized as either primary or secondary based on origin (De Sá et al. 2018). Primary microplastics enter the environment as they were originally produced, whereas secondary microplastics result from the degradation and fragmentation of larger plastics (e.g., Andray 2011; Freeman et al. 2020). Microplastics can be further classified by type: fibers, fragments, beads, foams, and films (Marine and Environmental Research Institute 2019). Jambeck et al. (2015) estimated that 225 metric tons of plastic waste entered our oceans in 2010. A few years later, Lebreton et al. (2017) used a regression model to document that 1 to 2.5 million tons of plastic waste were transported annually across the globe from rivers

to oceans. These numbers are expected to increase by approximately 50% by 2050 unless proper action is taken to mitigate plastic pollution (Van Wijnen et al. 2019).

Increased plastic pollution is concerning since studies have shown that microplastics may contain or attract chemicals and other pollutants that can accumulate throughout the food web (e.g., Barboza et al. 2018; Dong et al. 2020; Smith et al. 2018). The biological and toxicological effects of ingesting these plastics are not fully understood and more research is needed to understand the negative impacts of microplastic pollution (Andrady 2011). For example, microplastics can carry bacteria and other microbes with antibacterial resistance (e.g., Browne et al. 2011; Dong et al. 2021). Agriculture and aquaculture practices often introduce antibiotics into the marine environment, which can then cause antibiotics to enter the aquatic, terrestrial or combination food webs via microplastics (Dong et al. 2020).

Microplastic transport via food webs is an important area of research. Setälä et al. (2014) found that the transfer of microplastics in food webs was possible by various zooplankton. This was the first study to demonstrate that microparticles can transfer between planktonic trophic levels from mezozooplankton to macrozooplankton (Setälä et al. 2014). Carlin et al. (2020) found microplastics in birds of prey, which are top predators in many food webs. In their study, an average of 12 microplastics per bird was documented. The diversity of the plastics found suggested that top predators were consuming plastics both indirectly and directly (Carlin et al. 2020). One way that plastics may be consumed indirectly is when a predator eats prey that has already ingested plastic. Plastics can be directly ingested if a predator mistakes plastic debris for an edible food source. The presence of microplastics in top predators demonstrates how plastic pollution in the environment or lower on the food web can transfer between different organisms.

Similar to birds of prey, microplastics have also been found in humans (e.g., Barboza et al. 2018; Smith et al. 2018). Smith and their team (2018) completed a literature review examining evidence from peer-reviewed articles to determine the extent of human exposure to microplastics through seafood and discussed the possible implications of ingesting microplastics. Shellfish and other marine organisms contain microplastics and pose a threat to human health (Smith et al. 2018; Waite et al. 2018). Similarly, Barboza et al. (2018) found microplastics in seafood and other food items. With the presence of microplastics in marine species that humans consume, it may compromise human food safety and health (Barboza 2018).

Rivers, Estuaries, and Microplastics

Estuaries are one of the habitats most vulnerable to pollution, including microplastics (e.g., McEachern et al. 2019; Wu et al. 2019; Zhao et al.2019), since estuaries function as a filter for rivers and streams, removing sediments and pollutants before reaching the ocean (Office of Habitat Conservation 2020). Pollutants from waste or run-off can easily build up in the estuary, impacting the water quality in the estuary and preventing the estuary from fulfilling its role in the environment (Rodrigues et al. 2019). One study conducted in Florida found that microplastics are widely dispersed and abundant in the surface waters in the Tampa Bay, with fibers being the most common type of microplastic (McEachern et al. 2019). Researchers estimated that 4 billion microplastics contaminate the surface waters in the Tampa Bay, with higher concentrations near industrial facilities and the mouths of rivers (McEachern et al. 2019). More research is needed to understand how microplastics travel throughout riverine and estuarine systems (Wu et al. 2019). Another study from a Florida estuary aimed to identify the magnitude of microplastics in the tissues of oysters and crabs and found that microplastic levels were much higher than found in studies conducted in other areas across the globe (Waite et al. 2018). Researchers did not directly

study the causes but suggested that microplastic abundance varied across sites due to urbanization, intense recreational use, weather conditions, and limited flushing in the area.

Study Location

The Guana Tolomato Matanzas National Estuarine Research Reserve (GTM NERR; Figure 1) is in northeast Florida and is comprised of three rivers and two inlets that connect to the Atlantic Ocean. These rivers are the Guana, Tolomato, and Matanzas (GTM) Rivers. The Guana River flows parallel with the Tolomato River for 19.3 kilometers and converges with it north of the St. Augustine Inlet, where the Tolomato and Matanzas Rivers merge (Frazel 2009). Both the Tolomato and Matanzas Rivers are a part of the Atlantic Intracoastal Waterway. These rivers flow through Florida cities including Ponte Vedra Beach, St. Augustine, and Marineland. Several species of birds, fish, and reptiles depend on this riverine and estuarine ecosystem for survival and reproduction, as well as the people who live there, as the estuary acts as a natural buffer against storms (Frazel 2009). Maintaining the health and integrity of this ecosystem is also important for the regional economy since many people depend on its resources for food, tourism, and recreational activities.

One area that GTM NERR monitors closely is the Guana River; this river is the focus of this study. Portions of the Guana River Estuary fall within the GTM NERR, the Guana River Wildlife Management Area managed by the Florida Fish and Wildlife Conservation Commission (FL FWC), and the Guana River Marsh Aquatic Preserve managed by Florida Department of Environmental Protection. The Guana system is comprised of three waterbody types: Class III freshwater lagoons and streams; Guana Lake, a Class III Estuary; and Guana River, a Class II Estuary. Water control systems, including weirs, dikes, inland wells, drainage ditches, and a dam

alter the natural hydrology of the Guana (Dix et al. 2019). The headwaters of the Guana River Estuary start in the Diego Plains Drainage in Ponte Vedra Beach and enter Guana Lake through the weir at Micklers Landing (e.g., Dix et al. 2019; Frazel 2009). In the southernmost point of the lake, the Guana Dam serves as the output to this system, exchanging water with the Guana River.

Guana Dam was constructed in 1957 to create Lake Ponte Vedra (Guana Lake) to help improve fishing and to stabilize the wintering waterfowl population in the northern region of the Guana (e.g., FL FWC 2020; Frazel 2009). In a water quality report for the Guana, Dix and her team (2019) describe the physical structure of the Guana Dam. The dam structure acts as a partial barrier to tidal surge and can alter normal flow patterns of the surface and ground waters through controlled discharge and recharge via the swing gates inside the dam. The swing gates allow for two-way flow between the lake and river portions of the Guana (Dix et al. 2019). Dix et al. (2019) noted that this water exchange between the lake and river occurs occasionally based on water-level management and tidal conditions. Furthermore, the report notes that water levels in the lake start to draw down in mid-February and extend throughout April until desired water levels are reached as determined by FL FWC resource managers. This drawdown is to mimic the drought periods of a natural shallow lake. Lake water is discharged through the dam to lower water levels approximately 15.2 centimeters per month (Dix et al. 2019). Salinity and water levels on both sides of the dam are influenced by the water exchange via the dam. Dix et al. (2019) reported that after a water discharge event, water salinity levels dropped an average 20.3% across all river-side sampling sites while salinity levels on the lake side had a slight increase. The only study site on the lake side to be noticeably impacted by this event was Guana Lake South, which is 0.09 km north of the dam (Dix et al. 2019).

Figure 1- Guana River Dam

Dix et al. (2019) found evidence of human related pollution in the Guana River Estuary. Traces of sucralose were found across the Guana River, with highest concentrations in the north near the weir at Micklers Landing and declining in a linear gradient throughout the river from north to south (Dix et al. 2019). Dix et al. (2019) used sucralose as an indicator of human wastewater since sucralose is often found in wastewater but only in trace amounts in ambient water (Oppenheimer et al. 2011). Since there is evidence of treated wastewater infiltrating the Guana River Estuary, it is likely that microplastics, including microfibers, are accumulating in similar patterns to sucralose since both substances are found in treated wastewater and are resistant to degradation.

Figure 2- Map of Sampling Locations

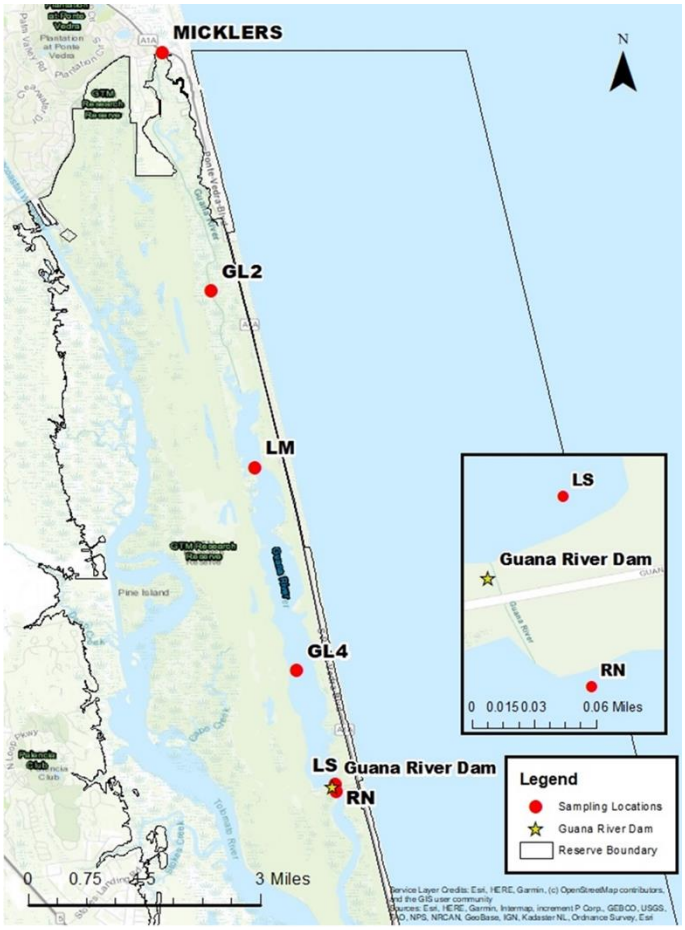


Table 1- Sampling Sites

Site No.	Site Name	Abbreviation	Latitude	Longitude
1	Micklers	MK	30.16074	-81.3603
2	Guana Lake 2	GL2	30.1161	-81.3511
3	Lake Middle	LM	30.08302	-81.3429
4	Guana Lake 4	GL4	30.0451	-81.3351
5	Lake South	LS	30.02376	-81.3279
6	River North	RN	30.02242	-81.3277

HYPOTHESES

The purpose of this study was to identify microplastic abundances in the Guana River Estuary. Microplastics research has not previously been conducted in the Guana River Estuary, and this is the first study to record the distribution of microplastics in the Guana River Estuary system. I hypothesize that if microplastics are accumulating in the Guana River Estuary, then the abundance of microplastics will follow a linear gradient from north to south due to the natural flow of the river.

H_0: The abundance of microplastics will be similar throughout the Guana River Estuary system and show no spatial pattern.

H_a: The abundance of microplastics will follow a linear gradient from north to south following the natural flow of the river.

Additionally, the water levels of the Guana River Estuary decrease from February to April, which could affect microplastic abundance. I hypothesize that microplastic abundance will decrease across all sites from February to April in relationship to the lake drawdowns that happen in the Guana River Estuary during that period.

H_0: The abundance of microplastics will be similar throughout the Guana River Estuary system and show no temporal pattern.

H_a: The abundance of microplastics will decrease across all sites from February to April.

Furthermore, I hypothesize that the microplastics observed in water samples collected from the Guana River Estuary will be dominated by fiber microplastics. Since fibers are common in

wastewater and there is evidence of wastewater intrusion in the Guana River Estuary, then fibers should dominate microplastic collections.

H_0: There will be no dominant type of microplastic in the Guana River Estuary.

H_a: The dominant type of microplastic found in the Guana River Estuary will be fibers.

Identifying the polymer composition of the microplastics recorded would provide a better understanding of where microplastics could be infiltrating this system. One of the most common types of polymers are polyethylene terephthalate (PET) and can be associated with fishing nets, boat rope, and synthetic clothing. Therefore, I hypothesize that the dominant polymer composition of microplastics observed in the Guana River Estuary will be PET.

H_0: There will be no dominant type of polymer in the Guana River Estuary.

H_a: The dominant type of microplastic found in the Guana River Estuary will be PET.

The results of this study will contribute to the understanding of microplastics in the Guana River Estuary and how microplastics are transported in estuarine and riverine environments.

RESEARCH METHODS

Sample Collection

Five replicate, 1-L water samples were collected in marine-grade, high density polyethylene terephthalate containers from surface waters (<5 cm) (Craig et al. 2021) at 6 sites throughout the Guana River system. One site was from the river portion and five sites in the lake portion, covering a total distance of 15.6 km (Figure 1). Sample bottles were rinsed in the laboratory with freshwater three times prior to field days with lids tightly secured and rinsed again with lake water three times prior to collection (Craig et al. 2021). Collection of samples was taken a minimum of 5 m away from rinsing locations (Craig et al, 2021, Cutroneo et al. 2020).

The duration of this study was one year starting in August 2020. Samples were collected monthly in conjunction with the GTM NERR water nutrients sampling schedule (Table 1). The sampling occurred at the beginning of each month, weather permitting. Involved agencies rescheduled to later in the month if conditions were not safe to sample on the planned collection day. Sample collections occurred in the morning, generally two hours after high tide. All samples were collected by boat. A jon boat was used on the river side, and an air boat was used on the lake side. Water quality data, including water temperature (˚C), specific conductivity, pH, dissolved oxygen (mg/L and %), salinity (ppt), and water depth (m) were collected at each site using the YSI ProDSS digital multiparameter meter. Additional abiotic data collected included air temperature (˚C, digital thermometer), wind speed (m/s, Kestrel wind gauge), and water transparency (cm, secchi disk and tube).

Filtration, Microscope Inspection, and Quality Control

Water samples were stored at room temperature in the laboratory for up to 4 weeks. Samples were then vacuumed-filtered through 0.45-micron nitrocellulose filters and analyzed under a dissecting microscope at 40X magnification (Cutroneo et al. 2020). Contamination was prevented throughout vacuum-filtration by rinsing all equipment and glassware with filtered deionized water (Cutroneo et al. 2020). Prior to rinsing, the deionized water was vacuum-filtered through a 0.45-µm pore nitrocellulose membrane filter paper (diameter: 47 mm) and stored in a microplastic-free carboy (Waite et al., 2017; Craig et al., 2021). All sample processing was consistent throughout the project, using the same pore size and diameter filter paper. Most water samples required one filter per liter, but some used up to six filters dependent upon the amount of sediment or organic material present. Each filter was stored in an individual petri dish previously rinsed with 0.45-µm filtered deionized water and secured with tape.

Figure 3- Vacuum Filtration Apparatus

Any aerial microplastic contamination during microscope inspection was accounted for by using blank filters to capture aerial microplastics around the microscope inspection area (Cutroneo et al. 2020). These blank filters were prepared by soaking 5 filter papers with 0.45

micron filtered, deionized water and leaving them open approximately 0.3 meters around the inspection area for the duration of the inspection (Craig et al. 2021). Blanks were inspected at the conclusion of every inspection session (Craig et al. 2021). Clothing made from natural fibers was worn to minimize microplastic fiber contamination.

Microplastics on filter papers were visually identified using the following guidelines: (1) plastics were not easily broken by forceps; (2) plastic color was uniform; (3) plastic had a uniform shape, meaning the dimensions of the plastic were consistent throughout; and (4) the margins and length of the plastic were uniform, meaning the plastic has distinct, non-frayed edges and a consistent thickness throughout (Marine and Environmental Research Institute, 2019). If at least three out of four of these characteristics were observed, potential microplastics were recorded (Marine and Environmental Research Institute, 2019). All potential plastic pieces were counted and classified by color, size (mm), and type (i.e., fiber, bead, fragment, film).

To correct plastic counts for potential contamination, contamination rates on blank filters were calculated then multiplied by the number of minutes that the sample filter was exposed to the air to determine the microplastic contamination per filter. The corrected number of microplastics per filter was then determined by subtracting the microplastic contamination per filter from the number of microplastics per filter observed.

To calculate the rate of contamination per minute of each sample, the mean number of microplastics per blank (MP_B) is divided by the total time (in minutes) the control blanks were exposed (T_B) during microscope inspection (Craig et al., 2021). This is demonstrated by the following formula:

$$C_M = MP_B / T_B$$

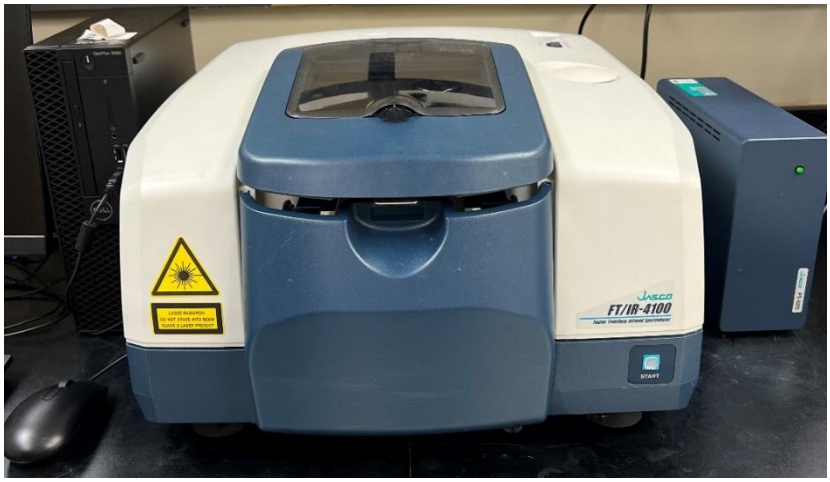
To quantify the contamination from microscope inspection, the following formula was used to calculate the contamination per sample (C_S) by multiplying the rate of contamination per minute (C_M) by the sample exposure time (T_E) (Craig et al., 2021):

$$C_S = C_M \text{ X } T_E$$

Polymer Composition

Water samples with microplastics were organized into a list sorted by sample identification number. Those containing microplastic pieces that were 1 mm in size or larger were placed into a separate list for examination via Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (FTIR) (Walters and Craig 2021). This size minimum as necessary due to the limitations of the equipment. These microplastics were transferred to a blank filter before being analyzed in a FTIR spectrophotometer (JASCO FTIR 4100 Series). When analyzed, the machine produces a spectra graph detailing the chemical composition of the microplastic. Polymer composition was determined using reference literature (Jung et al. 2018) to identify peak characteristics of various polymers.

Figure 4- FTIR Spectrometer



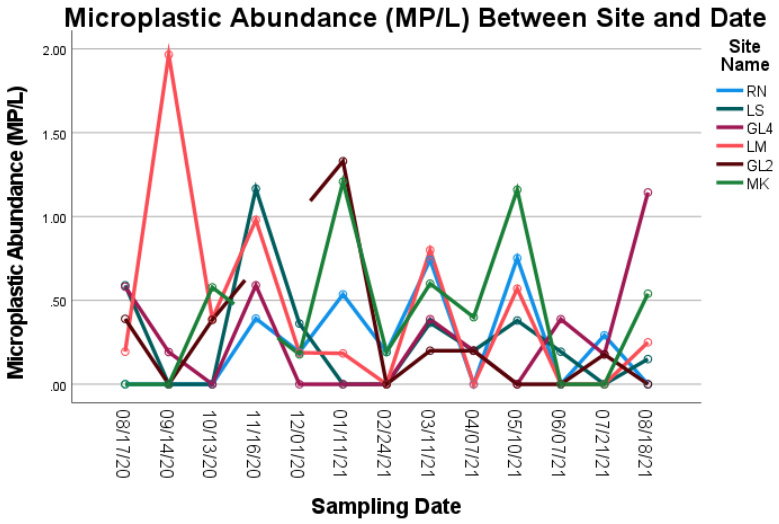
Data Analyses

To test for differences between the number of microplastics, microplastic type, and the temporal changes between sampling site and month, a two-way full factorial ANOVA (Time x Site) statistical analysis was used. A one factor ANOVA was used to determine if there were any significant differences in mean microplastic size across sampling sites. All statistical tests were performed in IBM Statistical Package for the Social Sciences (SPSS).

RESULTS

First, I calculated the total amount of microplastics collected at each sample site. In order from greatest to least, the total number of microplastics recorded at each sampling site (all months combined) was MK (n=27), LM (n=27), RN (n=18), LS (n=17), GL4 (n=17), and GL2 (n=14). The month with the most observed microplastics was January 2021 (n=18) and the months with the least observed microplastics was February 2021 (n=2) and June 2021 (n=2). There was a significant interaction between sampling site and sampling date (ANOVA: $p < 0.001$). This suggests that there was much variation in microplastic abundances among sampling sites over time. Due to this significant interaction, it was not appropriate to examine main effects.

Figure 5- A Summary of Mean Microplastic Abundance Between Sampling Site and Date



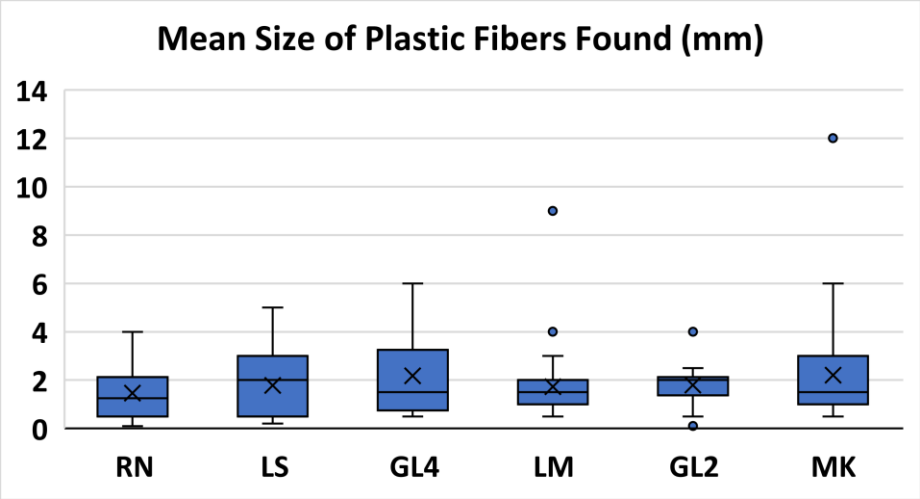
A total of 120 microplastics were found in 375 liters of water before considering the rate of contamination. The only type of microplastic found was fibers. The most common color recorded was black (n=50), followed by clear (n=24), then royal blue (n=15).

Table 2- Count of Fiber Color

Color	Count of Fiber Color	Total Percent
Black	50	41.7%
Clear	24	20.0%
Royal Blue	15	12.5%
Light Blue	10	8.33%
Dark Blue	9	7.50%
Red	10	8.33%
Pink	2	1.67%

The plastic size ranged from 0.1-12 mm and anything over 5 mm was recorded as a macroplastic. Size of plastics ranged across site locations. A one-factor ANOVA was used to determine if there were any significant differences in mean size across sampling sites. There were no significant differences between sampling sites (ANOVA: $p = 0.726$).

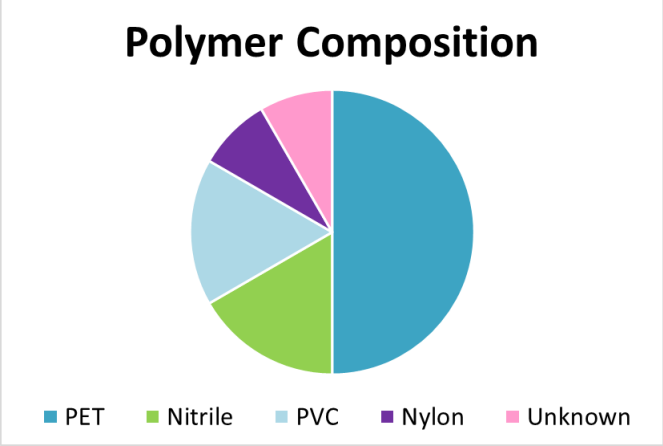
Figure 6- Mean Size of Microplastics Versus Site



Twelve randomly selected plastics (10% of the 120 total) from various sampling sites and dates were examined using ATR FTIR. Most common polymer identified was polyethylene

terephthalate (PET, n=6), followed by polyvinyl chloride (PVC, n=6), nitrile (n=2), and nylon (n=1). One sample could not be determined and is labeled unknown.

Figure 7- Polymer Composition



Contamination rate was calculated for the 15 microscope inspection sessions. The average microplastic contamination rate was 0.13 per sample, with a range of 0 to 0.01818 contamination per minute. A total of 120 microplastics were recorded in this study, but taking contamination into account, this value was corrected to 117 microplastics. The table below lists inspection date and contamination rate.

Table 3-Contamination Rate Results

Inspection Date	Contamination Rate (MP contamination/min)
3/4/21	0.007
3/5/21	0.006
3/18/21	0
3/20/21	0.010
3/21/21	0.014

9/23/21	0.010
9/24/21	0.004
9/28/21	0.018
10/11/21	0.013
10/12/21	0.017
10/22/21	0
11/2/21	0.011
11/5/21	0.008
11/10/21	0.012
11/11/21	0

The mean ± standard deviation of the abiotic data is shown below in Table 4, sorted by sampling site.

Table 4- Abiotic Data

Abiotic Data	RN	LS	GL4	LM	GL2	MK
Air Temp (˚C)	22.91 ± 6.03	23.12 ± 6.28	23.82 ± 5.87	22.32 ± 8.50	26.01 ± 4.32	26.25 ± 6.67
Wind Speed (m/s)	1.76 ± 1.14	2.01 ± 1.05	2.60 ± 1.23	2.85 ± 1.50	2.36 ± 1.45	0.73 ± 0.34
Secchi (m)	0.61 ± 0.28	0.59 ± 0.24	0.48 ± 0.18	0.35 ± 0.15	0.41 ± 0.18	0.86 ± 0.26
Water Depth (m)	1.22 ± 0.27	0.92 ± 0.21	0.91 ± 0.16	0.82 ± 0.16	0.62 ± 0.24	0.98 ± 0.17
Water Temp (˚C)	22.8 ± 5.65	23.25 ± 6.26	23.38 ± 5.67	23.33 ± 5.71	24.38 ± 6.01	24.24 ± 6.00
Specific Conductivity	33753.00 ± 10242.70	31392.69 ± 31392.69	27454.31 ± 10422.50	17538.15 ± 10666.69	9722.08 ± 12031.44	808.92 ± 204.43

pH	7.66 ± 0.20	7.75 ± 0.38	8.00 ± 0.24	8.04 ± 0.23	7.88 ± 0.27	7.58 ± 0.36
DO (mg/L)	5.79 ± 1.48	12.87 ± 20.83	7.66 ± 1.58	7.61 ± 1.83	6.20 ± 1.86	5.20 ± 3.33
DO (%)	74.34 ± 11.03	87.48 ± 9.21	94.91 ± 11.06	93.02 ± 15.03	76.86 ± 23.57	59.29 ± 34.67
Salinity (ppt)	21.30 ± 7.00	19.65 ± 6.57	17.01 ± 6.99	10.54 ± 6.90	5.76 ± 7.71	0.40 ± 0.10

DISCUSSION

Microplastic research in the Guana River Estuary is limited; this study was one of the first to document the distribution of plastics in this region. The primary goal of this study was to identify microplastic abundances in the surface waters of the Guana River Estuary. This study specifically investigated the type, size, color, and chemical composition of the microplastics recorded. I hypothesized that microplastic abundance in the Guana River Estuary would follow a linear gradient from north to south and that there would be a decrease in microplastic abundance from February to April; however, no spatial or temporal pattern was found in the distribution of microplastics. With the impediment of the Guana River Dam to the south, I expected microplastic abundance to increase linearly towards the south since the dam could act as a reservoir for microplastics. Microplastic abundance variation could be due to recreational activities or changes in the water depth due to opening and closing of the Guana River Dam floodgates from dam maintenance or Guana Lake drawdowns that begin in February each year and extend until April (Dix et al. 2019). Hurricane Eta in November of 2020 could have also affected microplastic abundance for that month since sampling was conducted after the storm. Despite these findings, there could still be more microplastics accumulating in this system.

Microplastic abundance in the Guana River Estuary ranged from 0-5 total microplastic pieces per liter, which is lower than in other estuaries in Florida. A possible reason for this could be that the water in the Guana River Estuary has a higher flow rate compared to the Tampa Bay Estuary and Indian River Lagoon systems. Water flow data would need to be collected in order to make that comparison. Samples collected from the surface waters of the northern region of the Indian River Lagoon found 15-30 microplastic pieces per liter (Waite et al. 2018). Surface waters from the Tampa Bay Estuary reported an average of 0.94 (±0.52) microplastic particles per liter

with samples ranging from 0.25 to 7.0 microplastic particles per liter (McEachern et al. 2019). A possible reason as to why the Tampa Bay Estuary and the Indian River Lagoon might have a higher concentration of microplastics is that there are more people living on those estuary systems compared to the Guana River Estuary. The presence of septic tanks could also increase the microplastic abundance in both the Tampa Bay Estuary and Indian River Lagoon since there are more septic systems present along those estuaries in relation to the Guana River Estuary. What was consistent among the Guana River Estuary, the Tampa Bay Estuary, and the Indian River Lagoon, was the dominance of fibers (McEachern et al. 2019; Waite et al. 2018).

The only type of microplastic found in the Guana River Estuary was fibers. There was variation in fiber color, size, and polymer composition (Figure 6, 7). This suggests that there are multiple sources of microplastics entering the Guana River. Possible sources of these fibers could be from runoff, fishing materials, synthetic clothing from laundry wastewater, and boat rope. I hypothesized that PET would be the dominant polymer in the Guana River Estuary and it was the most abundant. Similarly, PET was the dominant polymer recorded in microplastic samples throughout the Indian River Lagoon (Craig et al. 2021). Common sources of PET fibers include consumer goods such as clear water bottles, food packaging, and synthetic clothing (Andrady, 2011; Jung et al. 2018). Potential sources of PET in the Guana River could be from wastewater runoff, littering, or fishing nets, and boating rope.

One of the limitations of this study is that only water samples were collected. Collecting both water and sediment samples would have presented a more complete profile of how microplastics are being transported and deposited through the Guana River Estuary system. Another limitation regarding this research were the COVID-19 related restrictions. These

restrictions limited access to the laboratory and restricted the number of sites that could be sampled due to how many people could be present in the boat during sampling collection. My recommendations for future research regarding microplastics in the Guana River Estuary would be to collect both sediment and water samples to determine where microplastics are being deposited in this system. Furthermore, future microplastics research in the Guana River Estuary should consider the presence of the Guana River Dam and how it could be affecting microplastic transportation. Dams and other water control structures affect flow by altering the natural hydrology of a water body (Hübner et al. 2020; Watkins et al. 2019). Dams have been identified as a gap in microplastic research since studies that assess how reservoirs could act as sinks for plastics is very limited (Blettler et al. 2018). To better account for the presence of the Guana River Dam and its effect on microplastic transportation in the Guana River Estuary, more sites need to be added on the Guana River portion, south of the dam. In this project there was only one site beyond the Guana River Dam. Originally there were ten sites, six in the Guana Lake portion and four in the Guana River but to COVID-19 restrictions, I was not able to able to sample in three of the Guana River sites. One of the sites from the lake portion of the Guana River Estuary was removed since there were consistently issues with collecting abiotic data from that site. From the data that was collected, the microplastic abundance was similar at the sites immediately before and after the Guana Dam, with one more microplastic recorded south of the dam (LS=17 total microplastics and RN= 18 total microplastics). This data suggests that microplastic abundance could be the same on either side of the Guana River Dam. The recommendations I have provided would give researchers and policy makers in this region a better understanding of how plastics are affecting the Guana River Estuary. It is my hope that the results of this study

will bring more awareness to microplastic pollution in this region and that more initiative will be taken to mitigate plastic pollution.

REFERENCES

Andrady, A. L. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin*, 62(8), 1596-1605.

Barboza, L. G. A., Vethaak, A. D., Lavorante, B. R., Lundebye, A. K., & Guilhermino, L. (2018). Marine microplastic debris: An emerging issue for food security, food safety and human health. *Marine Pollution Bulletin, 133, 336-348.*

Blettler, M. C., Abrial, E., Khan, F. R., Sivri, N., & Espinola, L. A. (2018). Freshwater plastic pollution: Recognizing research biases and identifying knowledge gaps. *Water Research*, 143, 416-424.

Browne, M. A., Crump, P., Niven, S. J., Teuten, E., Tonkin, A., Galloway, T., & Thompson, R. (2011). Accumulation of microplastic on shorelines worldwide: sources and sinks. *Environmental Science & Technology*, 45(21), 9175-9179.

Carlin, J., Craig, C., Little, S., Donnelly, M., Fox, D., Zhai, L., & Walters, L. (2020). Microplastic accumulation in the gastrointestinal tracts in birds of prey in central Florida, USA. *Environmental Pollution*, 264, 114633.

Craig, C. (2021). Correlations in Microplastic Abundance Between Water, the Eastern Oyster, Crassostrea virginica, and Their Biodeposits in a Dynamic Florida Estuary. https://stars.library.ucf.edu/etd2020/488

Cutroneo, L., Reboa, A., Besio, G., Borgogno, F., Canesi, L., Canuto, S., ... & Capello, M. (2020). Microplastics in seawater: sampling strategies, laboratory methodologies, and identification techniques applied to port environment. *Environmental Science and Pollution Research*, 27(9), 8938-8952.

Dix, N., Dunnigan, S., Lee, J., Tomazinis, J. (2019). Guana Water Quality Two-Year Summary

Report July 2017-June 2019. 10.13140/RG.2.2.20601.83042.

Dong, H., Chen, Y., Wang, J., Zhang, Y., Zhang, P., Li, X., ... & Zhou, A. (2020). Interactions of microplastics and antibiotic resistance genes and their effects on the aquaculture environments. *Journal of Hazardous Materials*, 123961.

Frazel, D. (2009). *Site Profile of the Guana Tolomato Matanzas National Estuarine Research Reserve.* Guana Tolomato Matanzas National Estuarine Research Reserve. https://coast.noaa.gov/data/docs/nerrs/Reserves_GTM_SiteProfile.pdf

Freeman, S., Booth, A. M., Sabbah, I., Tiller, R., Dierking, J., Klun, K., ... & Angel, D. L. (2020). Between source and sea: The role of wastewater treatment in reducing marine microplastics. *Journal of Environmental Management*, 266, 110642.

Hübner, M. K., Michler-Kozma, D. N., & Gabel, F. (2020). Microplastic concentrations at the water surface are reduced by decreasing flow velocities caused by a reservoir. *Fundaments of Applied Limnology*, 194(1), 49-56.

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., ... & Law, K. L. (2015). Plastic waste inputs from land into the ocean. *Science*, 347(6223), 768-771

Jung, M. R., Horgen, F. D., Orski, S. V., Rodriguez, V., Beers, K. L., Balazs, G. H., ... & Lynch, J. M. (2018). Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. Marine Pollution Bulletin, 127, 704-716.

Lebreton, L. C., 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(1), 1-10.

Marine and Environmental Research Institute. (2019). Guide to Microplastic Identification

University of Florida, *Institute of Food and Agricultural Sciences* (2019) https://sfyl.ifas.ufl.edu/media/sfylifasufledu/flagler/sea-grant/pdf-files/microplastics/MERI_Guide-to-Microplastic-Identification.pdf

McEachern, K., Alegria, H., Kalagher, A. L., Hansen, C., Morrison, S., & Hastings, D. (2019). Microplastics in Tampa Bay, Florida: Abundance and variability in estuarine waters and sediments. *Marine Pollution Bulletin*, 148, 97-106.

Office of Habitat Conservation. (2020, April 4). *Estuary Habitat*. NOAA. https://www.fisheries.noaa.gov/national/habitat-conservation/estuary-habitat

Oppenheimer, J., Eaton, A., Badruzzaman, M., Haghani, A. W., & Jacangelo, J. G. (2011). Occurrence and suitability of sucralose as an indicator compound of wastewater loading to surface waters in urbanized regions. *Water research*, 45(13), 4019-4027.

Rodrigues, S. M., Almeida, C. M. R., Silva, D., Cunha, J., Antunes, C., Freitas, V., & Ramos, S. (2019). Microplastic contamination in an urban estuary: Abundance and distribution of microplastics and fish larvae in the Douro estuary. *Science of the Total Environment*, 659, 1071-1081.

Schwarz, A. E., Ligthart, T. N., Boukris, E., & van Harmelen, T. (2019). Sources, transport, and accumulation of different types of plastic litter in aquatic environments: A review study. *Marine Pollution Bulletin*, 143, 92-100.

Setälä, O., Fleming-Lehtinen, V., & Lehtiniemi, M. (2014). Ingestion and transfer of microplastics in the planktonic food web. Environmental Pollution, 185, 77-83.

Smith, M., Love, D. C., Rochman, C. M., & Neff, R. A. (2018). Microplastics in seafood and the implications for human health. *Current Environmental Health Reports*, *5*(3), 375-386.

Van Wijnen, J., Ragas, A. M., & Kroeze, C. (2019). Modelling global river export of microplastics to the marine environment: Sources and future trends. *Science of the Total Environment*, 673, 392-401.

Waite, H. R., Donnelly, M. J., & Walters, L. J. (2018). Quantity and types of microplastics in the organic tissues of the eastern oyster Crassostrea virginica and Atlantic mud crab Panopeus herbstii from a Florida estuary. *Marine Pollution Bulletin*, 129(1), 179–185. doi: 10.1016/j.marpolbul.2018.02.026

Walters, L., & Craig, C. (2021). Microplastics, Oysters, and the Indian River Lagoon – Final Report to IRL NEP, March 2021. *CEELAB Research Data*.

Watkins, L., McGrattan, S., Sullivan, P. J., & Walter, M. T. (2019). The effect of dams on river transport of microplastic pollution. *Science of the Total Environment*, 664, 834-840.

Wu, N., Zhang, Y., Zhang, X., Zhao, Z., He, J., Li, W., ... & Niu, Z. G. (2019). Occurrence and Distribution of Microplastics in surface water and sediments of two typical estuaries in Bohai Bay, China. *Environmental Science: Processes & Impacts*.

Zhao, J., Ran, W., Teng, J., Liu, Y., Liu, H., Yin, X., ... & Wang, Q. (2018). Microplastic pollution in sediments from the Bohai Sea and the Yellow Sea, China. *Science of the Total Environment*, 640, 637-645.