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AN ASSESSMENT ON MICROPLASTIC POLLUTION IN THE RESACAS OF THE LOWER RIO GRANDE VALLEY, TEXAS

A Thesis

by

WESLEY D. FRANKLIN

Submitted to the Graduate College of The University of Texas Rio Grande Valley In partial fulfillment of the requirements for the degree of MASTER OF SCIENCE

May 2021

Major Subject: Ocean, Coastal, and Earth Sciences

AN ASSESSMENT ON MICROPLASTIC POLLUTION IN THE RESACAS OF THE LOWER RIO GRANDE VALLEY, TEXAS A Thesis

by WESLEY D. FRANKLIN

COMMITTEE MEMBERS

Dr. Elizabeth Heise Chair of Committee

Dr. Alejandro Fierro-Cabo Committee Member

> Dr. Jude Benevides Committee Member

> > May 2021

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ABSTRACT

Franklin, Wesley D., <u>An Assessment on Microplastic Pollution in the Resacas of the Lower Rio</u> <u>Grande Valley, Texas</u>. Master of Science (MS), May, 2021, 61 pp., 2 Tables, 29 Figures, references, 29 titles.

Microplastic pollution is a serious issue that impacts most aquatic environments. Microplastics have the potential to cause numerous detriments to the fitness and reproductive capability of many organisms. The resacas of the Lower Rio Grande are an under-studied area that could be sinks for large congregations of microplastics. We analyze microplastic pollution in two species of fish as well as the sediment and water of eleven different pools from four resaca systems. We find that microplastics are present in every pool sampled.

Altogether 218 microplastic pieces are found from the water, sediment and fish samples. We find that microplastics are most likely to be found in the water followed by sediment and then fish. Microfibers were the most prevalent microplastic found in every parameter from all resacas. We employed Fourier Transform Infrared Spectrometry to further analyze our plastic samples and identify polypropylene (PP), polyethylene (PE), and polyethylene terephthalate (PET).

DEDICATION

I dedicate this research to two of my closest friends, Tanner Guidry & Adriene Lasko, for their love, support, and friendship during this season of my life. Also, I dedicate this research to my brother Tyler Franklin for motiving me to continue developing and refining myself as a research scientist. I am beyond grateful for each of you.

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CHAPTER 1

INTRODUCTION

Microplastic pollution is a very severe problem with many known and even more unknown hazards present for many species including humans. We survey two species of fish, *Dorosoma cepedianum* and *Pterygoplichthys disjunctivus* in the resacas (former distributaries) of the Lower Rio Grande Valley located in and around Brownsville, South Texas. We also collected sediment samples and water samples from eleven different pools from these rural and urban resacas near Brownsville, TX. We described the different classes of microplastics and identified which class is most prevalent in the resacas. We also used Fourier Transform Infrared Spectrometry (FTIR) to determine the composition of the microplastic pieces found. The results from this paper will raise awareness to the plastic pollution problem in the Rio Grande Valley as well as the rest of Texas and the United States.

Microplastics

The concern of microplastics is growing more and more as information about their presence, abundance, and potential hazard is being made available to science as well as the public. Plastic pollution was first reported in the early 1960s in marine ecosystems and the term microplastic was first introduced to scientific literature in 2004 (Law et al. 2014; Peters et al. 2016). Microplastics are typically defined as any plastic that is under 5mm in length (Barnes et al. 2009). There are two criteria of microplastics – primary and secondary microplastics (Cole et al.

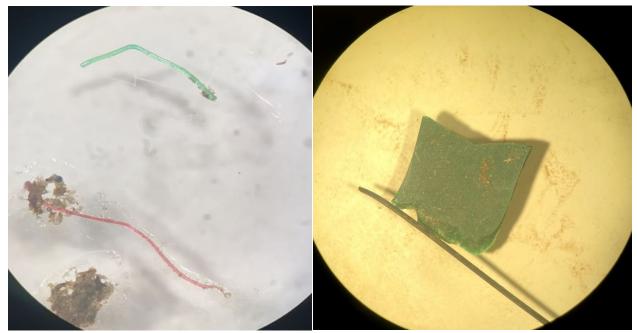


Figure 1. Two photographs of microplastic specimens taken from sediment samples. The plastics on the left are microfibers and the one on the right is a chunk of a larger plastic.

2011; Lasee et al. 2017). The difference between the two is quite simply that primary microplastics are manufactured to be small, for instance materials like tiny beads and pellets found in cosmetics and nurdles used to make larger plastics, and secondary microplastics are those that were not manufactured to be microscopic. Microplastics in this criterion are those that are the degraded forms of larger plastics and polymers, as well as fibers that come from clothing (Law et al. 2014). The larger plastics are broken down into these microplastics by both physical events as well as fragmentation due to UV light (Law et al. 2014; Lasee et al. 2017). Among these two classes of microplastics, the materials can be further specified into foams, pellets, fibers, fragments, and films, with fibers being the most abundant type found in all ecosystems (Miller et al. 2017). It is now common knowledge that microplastics have invaded many if not all ecosystems, but why do we care? Microplastics are more of a concern than other debris because of their durability in nature and their tendency to adhere toxins to their surfaces. Many aquatic animals have been found with plastics (microplastic & macroplastic) in their digestive

systems, including marine mammals, sea turtles, and fish (Tuetan et al. 2009). The macroplastics cause problems such as entanglement of fish, marine mammals, and turtles as well as choking, blockage of digestive tracts, and starvation. Although these macroplastics are generally more publicized because of their visibility and effect on macrofauna, there are more microplastics present in our waters that could be having the same or worse effects in every trophic level, not just macrofauna (Phillips et al 2015). Microplastics enter the digestive system of aquatic organisms directly as they are ingested by animals that mistake the microplastic for prey items such as phytoplankton (Sigler, 2014). They also enter the digestive tracts of these fish indirectly when the predator ingests a fish or other animals that already have microplastics present in their digestive systems, or when they ingest microplastics from the water column and/or sediments in their habitats. Microplastics that are ingested can either remain in the gut, pass through the GI tract, be excreted as feces, or be translocated through the epithelium into body tissues (Phillips et al. 2015). There are several harmful qualities of these microplastics, and one of these effects is known as biomagnification (Tuetan et al. 2009). This occurs when microplastics are passed from prey to predator up the food chain so that the plastics accumulate at the higher trophic levels. Another effect is the transfer of toxic chemicals into the hosts digestive system from the breakdown of the plastic in the digestive tract as well as the bioaccumulation of the chemicals as these fish are eaten by larger predators (Neves et al. 2015). Microplastics are made of synthetic organic polymers which transport persistent organic pollutants (POPs). They also absorb many toxins such as pesticides, carcinogens, and dioxins (Phillips et al 2015). The presence of microplastics in the stomach can also allow for a sense of a 'false-fullness' which means that the animal thinks it is full when in fact there is just plastic in its stomach that is of no nutritional value, this leads also to lower consumption rates which can starve the animal (Lusher et al.

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2012). Other effects of microplastics include symptoms such as inhibited growth and development, oxidative stress, loss of energy, endocrine and neurotransmission dysfunction, and genotoxicity (Yifeng et al. 2016). These effects can ultimately lower fitness and survival rates in fish and other animals that have consumed plastic. Annual plastic production is 192 times higher than it was in the early 1950s with 288 million tons of plastic being produced per year (Phillips et al. 2015). Of the 29.6 million tons of plastic being produced in the United States, not even 10% of it is being recycled which means this plastic is either going into landfills or is being released into the environment and breaking down into microplastics (Phillips et al. 2015; Peters et al. 2016). As this trend continues, we will continue to see a prevalence of microplastics in our ecosystems. Although the size of individual plastics decreases over time, the polymers are far less likely to degrade as they are very resistant to biological degradation (Lasee et al. 2017). This study will add substance to the limited knowledge of how microplastics impact the animals that ingest them.

Resacas

We chose to sample 11 of the resacas in and around the municipality of Brownsville, TX. Resacas are defined as former channels and distributaries of a river, in this case, the Rio Grande river. These resacas were formed because of floodwaters and changes during the Rio Grande and its distributaries. Many resacas eventually dry up but over time, humans have converted them into floodwater relief areas for municipal drinking water as well as irrigation (Robinson, 2010). These resacas are very important in the Rio Grande Valley since they are sources of freshwater in an area that is dominated by marine habitat (Lessley, 2016). Also, these areas provide resting habitat to many of the migratory birds that migrate through Texas. Many of these resacas are supplied with water so that they do not dry up throughout the year. Since these resacas are supplied by managed water sources, their reduced flow, and because of their location in urban settings, they have potential to be very promising study sites for microplastic pollution. Since many resacas do not have any inflow or outflow except for runoff from agriculture and rainfall, it is possible that these bodies of water become sinks for contamination (Mora et al. 2001). Also, there hasn't been much research or funding for the wildlife in these water systems or many studies done on the conditions of these systems in general.

This study includes developed resacas, undeveloped resacas, flowing resacas, nonflowing resacas, large, and small resacas. More developed resacas are those that include bridges, weirs, artificial walls, etc. Undeveloped resacas are those that are more or less wild. There were many parameters to consider when drawing conclusions from the data we retrieved. This study focused on quantifying the microplastic pollution in the sediment, water, and fish in these resacas. We draw correlation between the feeding strategies of the fish and the amount of microplastics found in their digestive tracts. We also compared the amount and types of microplastics found in rural and urban resacas.

Study Organisms

This study sampled, members of two different species of fishes, *Dorsoma cepedianum* (Lesueur, 1818) (gizzard shad) and *Pterygoplichthys disjunctivus* (Weber, 1991) (vermiculated sailfin catfish). We selected these two species to compare the microplastic impacts with the differences in where/how they feed in their habitats. These two fish are from similar trophic levels, but have different feeding strategies, *D. cepedianum*, water column feeder and *P*.

disjunctivus, benthic forager. We analyzed the presence and abundance of microplastics in the digestive tract of the fish. We assessed the environments in which these organisms live as well. For us to successfully understand the microplastics found in these fish, we also observed abiotic factors, such as the sediments under the water as well as on the banks of the water systems. We also sampled the water in which these fish live. Since water is the way that these microplastics are transported to the fish, sediments, and larger bodies of water, it is important that we understand how the microplastics are transported by water and whether they settle out or stay suspended in the water. It is also important for us to find out ways to limit the transport of plastic polluted water by wastewater facilities and other industries as some studies show that anywhere from 70-100% of microplastics that enter wastewater facilities leave the facilities and are pumped back into natural systems (Estahbanati, 2016). In water systems, microplastics with higher densities will settle to the bottom and become a part of the benthic environment and those microplastics with densities that are lighter than that of water will either stay in the water column or be washed ashore (Karlsson, 2017). Therefore, we surveyed both benthic sediment and shore sediment of the systems we are observed.

Dorosoma cepedianum

D. cepedianum (Figure 2), is known by several other names such as gizzard shad, herring, skipjack, and hickory shad (TPWD, 2020). This fish feeds in the water column and is referred to generally as a topwater feeder which feeds on zooplankton, phytoplankton, and organic detritus (Aday et al. 2003). When feeding in the water column, they act as filter feeders, pulling water through their gills, and catching plankton on their gill rakers (Fuller et al. 2019). The gizzard shad's defining feature is a projection that protrudes from the last ray of its dorsal



Figure 2. D. cepedianum individual from one of the Resaca pools.

fin. This is also seen in another shad called a threadfin shad (*Dorosoma petenense*), but this shad is much smaller and usually has a yellow streak that runs along its lateral line into its caudal fin (Fuller et. al 2019). These two species of shad can be further distinguished from each other by the projection of the mouth, *D. cepedianum* has an inferior mouth whereas *D. petenense* has a superior mouth, and by the number of anal fin rays present (TPWD, 2020). These fish reproduce through broadcast spawning, and their females lay about 300,000 eggs which usually hatch in 2-3 days. The eggs once broadcasted, drift to the bottom of the water body and adhere to hard surfaces on the bottom (Fuller et al. 2019.) They aren't very hardy fish, they die easily from overstress, from temperature, and other stressors, and they usually can't survive in water with low dissolved oxygen content (Fuller et al. 2019). They travel in schools and are consumed by many gamefish and are a source of bait for recreational fishermen. These fish can have tremendous effects on other fishes based on their trophic level. Their foraging status can

influence phytoplankton and zooplankton communities as well as the abundance and structure of other piscivores that rely on them for prey. They do this by competing with other zooplanktivorous and phytoplanktivorous fish species that the piscivorous fishes rely on for prey (Aday et al. 2003).

Pterygoplichthys disjunctivus



Figure 3. P. disjunctivus individual from one of the resaca pools.

P. disjunctivus (Figure 3), also known as the sailfin suckermouth catfish or the vermiculated suckermouth catfish, feeds on the bottom of water bodies on dead organisms, invertebrates, algae, and detritus and is known as a benthic feeder. Armored suckermouth catfish (family Loricarridae) which include *P. disjunctivus* and other hypostomus species are readily identified by the presence of armored plates along their bodies as well as a ventral suctorial mouth which differs from Ictalurid species (Nico et al. 2020). *P. disjunctivus* uses this suctorial mouth to feed but it also can utilize its mouth to allow it to suction to hard surfaces. P.

discjunctivus is often confused with individuals from the Hypostomus genera but can be distinguished by the count of dorsal rays, Hypostomus individuals have 7-8 dorsal rays, and Pterygoplichthys individuals have from 9-14 dorsal rays (Nico et al. 2020). This species native range is the Madeira River basin in South America and it invasive to several locations in the U.S. but is limited to North San Antonio and the Rio Grande Valley in Texas (FWS, 2014). Figure 4 shows the nonindigenous occurrences of P. disjuntivus in the United States. As stated, the species is limited to San Antonio and the Rio Grande Valley in Texas but is also found in other states such as Florida, Mississippi, and Nevada (Nico et al. 2013). P. disjunctivus being an invasive species is another major influence for why we are targeting this species. This fish was most likely introduced through release from aquariums and aquaculture facilities (Nico et al. 2013). This fish is considered a very hardy fish as it can thrive in polluted water, alkaline to acidic water, soft and hard water, and can even be salt tolerant. They are limited only by cold temperatures starting at around 11 degrees Celsius. This species is known to breathe air and they have an enlarged stomach which is thought to be used as an accessory breathing organ (FWS, 2014). This species also has very few natural predators which makes them exceptionally threatening in our local waters. These fish are asynchronous multiple spawners whose males dig out burrows into banks of water bodies in which the females will burrow into and lay their eggs. This mating act contributes to siltation and higher rates of erosion (Nico et al. 2020). This burrowing activity can also cause the destruction of aquatic vegetation and lead to floating mats of vegetation that limits sunlight to the bottom of the water bodies. Because of the way this fish feeds, by removing algae and detritus, it is believed that they may be harming the invertebrates and vertebrates at the base of the food chain. This fish also competes with native species which

will limit their food selection and is thought to be a sink for nutrients such as nitrogen which they sequester in their bony plates (FWS, 2014).

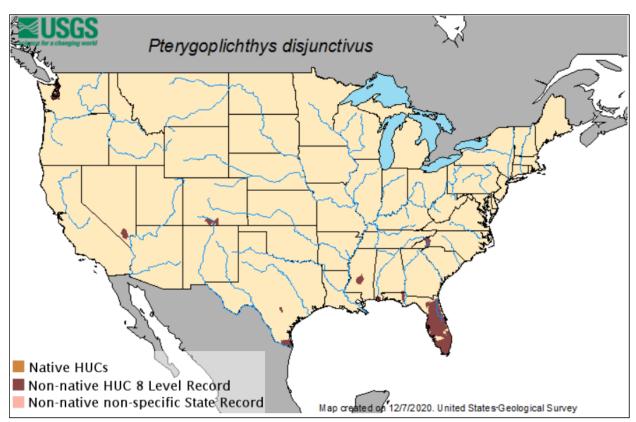


Figure 4. The non-indigenous reports of P. disjuntivus in the continental United States. P. disjunctivus can be found in Florida, Alabama, Mississippi, Texas, Colorado, Nevada, and Washington. In Texas this species is limited to the lower Rio Grande Valley as well as San Antonio. Source: United States Geological Survey.

Invasive Species

Invasive species are considered non-indigenous species that have varying negative effects on the ecosystem in which they are introduced (Zhang et al 2020). The presence of an invasive species in an ecosystem reduces the overall biodiversity of said ecosystem and can also cause a shift in community composition (Larkin et al 2020). Behind habitat loss, invasive species are one of the biggest threats to biodiversity (Zhang et al 2020). Once an invasive species is introduced into an ecosystem, there are many potential negative scenarios that can take place. These species like

stated earlier can reduce the biodiversity and cause composition shifts, but they also have been known to have other effects. They become competitors to the native species. They compete for food of the native fish, they may eat the native vegetation at higher rates than other species, they may be more attracted to native vegetation than to invasive vegetation which allows for a foothold for more invasive species to thrive in an ecosystem which further limits biodiversity. They can also have indirect effects such as decreasing the amount of sunlight that reaches aquatic vegetation through suspension of benthic materials and overloading systems with nutrients due to organic waste which leads to higher amounts of algae in the water. These species are usually more likely to thrive in their introduced habitats for several reason: they have not evolved in the local habitats and communities therefore they may not be affected by the diseases that effects the native plants and animals, they may not be parasitized by the parasites that have evolved with the native species, they may not be targeted by the predators that target the native species, and they may have evolved various anatomy and physiology in their native ranges that allow them to outcompete native species. In a study conducted by (Larkin et al 2020) it was found that an invasive species of carp (Cyprinus carpio) promoted the introduction, growth, and spread of invasive plant species. Invasive species not only negatively impact the ecology, environment, and native species, but they also have drastic impacts on the economy. Invasive species cause 1.4 trillion dollars of damage globally as well as 120 billion dollars of damage in the United States alone each year through containment, capture, property value decreases, and removal strategies (Zhang et al. 2020). I mention the economic side to say that this is a very important issue which drains 5% of the global economy. This money if utilized correctly could effectively work to end the issue of invasive species and could be used towards conservation of native habitats.

Hypotheses

- Microplastics would be more abundant in the sediment, water, and fish in the more urban resacas compared to rural resacas removed from human impact and alteration.
- *P. disjunctivus* would have more microplastics present compared to *D. cepedianum* since it is a benthic feeder along with it being larger.
- Microfibers would be the plastic found most frequently.
- Similar types of microplastics would be found in *P. disjunctivus* and the sediment and *D. cepedianum* and the water respectively based on these two species' feeding habits.
- Overall, the sediment samples would be most likely to have microplastics present.
- Heavier/longer fish will be more likely to have microplastics present and have higher numbers of microplastics in their digestive tracts.
- Different types and compositions of microplastics would be found in the sediment vs. the water column since some plastics should settle to the bottom, and some should stay suspended in the water column.

Objectives

- Discover if there is with microplastic pollution in the resacas of the Lower Rio Grande Valley.
- Determine if microplastics are in the fish, sediment, water, or all three.
- See if there are more microplastics located in fish compared to sediment and water or vice versa.
- Find out what plastic composition these microplastics are.

• Determine if there was any correlation between the plastic abundance or composition found in the fish, water, and sediment.

Purpose

Our purpose for conducting this research is to provide necessary insight to the health and condition of the resaca systems in the Lower Rio Grande Valley as well as the fish that live here. Additionally, we expect this study will also raise concern in the scientific community but also in the public community so that we can start taking strides towards preserving the resacas and the wildlife that live in them. Recent studies have shown high abundances of microplastics in freshwater bodies surrounding urban areas (Lasee et al. 2017). The area in which we have sampled is near the large metropolitan area of Brownsville, TX. Although there has been a surge in research on microplastics in recent years, most of this research has been focused on marine environments. There have been very few studies conducted on the status of microplastics in freshwater systems, where they are located, or how they affect wildlife (Lasse et al. 2017).

CHAPTER II

METHODOLOGY

We implemented many different methods and techniques in sampling, collecting, identifying, and analyzing microplastics found in the eleven resacas near the Brownsville area. We sampled from three different resaca systems in and around Brownsville area as well as an oxbow lake in the area. For the purposes of simplicity, we will also refer to this oxbow lake as a resaca. These are both rural and urban bodies of water. All of our samples were retrieved during two, one-week sampling excursions. The first occurred during January, 2020, and the second and last occurred during October, 2020.

Figure 5 shows the locations of the eleven resacas sampled. The resaca systems we sampled include Rancho Viejo, which includes four resacas: RV-54, RV-56, RV-68, and RV-85, the Town Resaca system which consists of two resacas: TR-05 and TR-14, Resaca De La Guerra containing four resacas: RG-42, RG-43, RG-31, and RG-39, and the oxbow lake Lozano Banco or LB-002 located on the University of Texas Rio Grande Valley Brownsville Campus. Figure 6 shows Lozano Banco, the only oxbow lake that was sampled. Figure 7 shows the four resacas sampled in the Rancho Viejo Resaca system. Figure 8 shows the three resacas sampled in the Resaca de la Guerra resaca system. And Figure 9 shows the two resacas sampled in the Town

Resaca system. We sampled several different components of these ecosystems including water, sediment, and fishes. Table 1 presents some information about the eleven resaca pools. It gives the name of each pool, the resaca system in which the pool is located, the sample number of *P*. *disjunctivus* and *D. cepedianum* for each pool, as well as the GPS coordinates for each of the eleven pools.

Resaca Pool	Resaca	n <i>P</i> .	n <i>D.</i>	Latitude	Longitude
	System	disjunctivus	cepedianum		
LB-002	Lozano Banco	10	11	25.89435	-97.48865
TR-14	Town Resaca	10	10	25.914853	-97.492246
TR-05	Town Resaca	1	10	25.921135	-97.507365
RG-31	Resaca De La	0	10	25.94088	-97.497175
	Guerra				
RG-39	Resaca De La	10	0	25.926544	-97.46018
	Guerra				
RG-42	Resaca De La	4	8	25.91372	-97.45731
	Guerra				
RG-43	Resaca De La	11	9	25.907104	-97.455674
	Guerra				
RV-85	Rancho Viejo	0	2	25.942868	-97.430366
RV-68	Rancho Viejo	0	7	25.982362	-97.536847
RV-54	Rancho Viejo	0	3	26.031149	-97.549526
RV-56	Rancho Viejo	3	0	26.030069	-97.544011

Table 1. The eleven resaca pools are presented here. Each pool is identified by resaca system. The sample number of both P. disjunctivus as well as D. cepedianum are shown for each pool as well as the GPS coordinates of each resaca pool. The locations of each of the eleven resaca pools are shown in figures 6 - 10.

For the water samples, we acquired samples using a dip bottle to retrieve water from 1 meter below surface. We retrieved two water samples from each resaca pool. We would take water samples from each gill net deployment location. Each of the bottles were labeled by location and sample number. Then we transported these samples back to the lab.

At the lab, the water samples were stored at room temperature, awaiting analysis. The water was sieved through a 125 and 63µm sieve. The sieve was then allowed to dry. Once the samples in the sieve were dry, the sieve was examined under a dissecting microscope. All

microplastics that were found were photographed and underwent further analysis using a Fourier Transform Infrared Spectrometer (FTIR) to determine the composition of said microplastics.

For the sediment samples, we used both a box core to get two sediment samples from the benthic environment of the resaca as well as a hand shovel to acquire a sediment sample from the bank of the resaca. We would take benthic samples from each gill net deployment location. The shore sample was taken from the boat input location. Then we transferred the sediment samples from the box core or the shovel into collection bags. Samples were then returned to the lab and individual samples were placed in a sieve system which contained a top sieve which had 4.00 mm mesh followed by a 2.00 mm, 1.00 mm, 500 µm, 250 µm, 125 µm, and a bottom sieve with a 63 µm mesh. This stack of sieves was then placed into a Humboldt MFG Co. Sieve Shaker and shaken for ten minutes. Upon completion, the sieves were taken out and analysis began on the sediments at each level starting with the 4.00 mm sieve and ending with the 63 µm sieve to determine if there were microplastics present in the sediments.

For the fish samples we used two methods of capture, cast nets as well as gill nets. We accessed the resacas by boat, and the gill nets and cast nets were both deployed by boat. Permits for this capture were acquired through Texas Parks and Wildlife, scientific permit number: SPR-0808-314. We initially planned to keep ten *D. cepedianum* and ten *P. disjunctivus* from each site; this would have given us a total of 110 individuals from each species. We did not collect this many samples at all of the locations. Gill nets were deployed twice for thirty minutes each time. We chose to do thirty-minute deployments to limit the mortality of non-fish bycatch such as diving birds and turtles. We used a monofilament gill net that was 36.58 m long and 2.13 m deep. It contained six, 6.09m panels. The top line was foamcore, and the bottom line was leadcore. The mesh of the six net panels follows: 2.54 cm, 5.08 cm, 7.62 cm, 7.62 cm, 5.08 cm,

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and 2.54 cm. The gill nets were set perpendicular to the bank of the resacas with the smaller mesh reaching to either side to capture the smaller fish in the sides, and with the larger mesh in the middle. The cast nets were deployed ten times for each gill net deployment and were taken during the time that the gill net was deployed. The cast net was a Fitec RS750 Series monofilament nylon net with a 121.92 cm radius and 0.95 cm mesh size. The gill nets were retrieved after thirty minutes and all fish caught were measured and then released if they were native species, terminated if invasive, or kept if they were the targeted species. The fish were identified on the boat. The fish caught were placed on ice and taken to the lab as soon as possible. The methodology used post capture of the fish is the same used in Neves et al. 2015. We completed one site at a time. Once at the lab, the fish were weighed (g) and measured (total length; cm). After this, an incision was made from the isthmus to the anal vent. We then removed the digestive tract from esophagus to the anal vent. We took a thirty cm section from the digestive tract to be used for observation. We chose to use only thirty cm to limit observation time which would limit the likelihood that foreign microfibers from the air would settle onto our sample. We moved this section to a sterile petri dish and dissected it using sterile utensils under a dissecting microscope. We observed the specimen and recorded any microplastics that were found. If the plastic was able to be retrieved, it was moved to a separate petri dish. Microplastics that were kept were photographed and kept for further analysis under the Fourier-Transform Infrared Spectrometer. Microplastics were also counted and were put into one of three morphological classes (fiber, chunk, or other). Other included microplastics that resembled

found microplastics that resembled foams, films, or oily plastics. Plastics that were larger than 5 mm were labeled "macroplastic" and were measured and photographed and analyzed under the

foams, oil-like plastics, or films. We chose to use "other" as a third category because we rarely

Fourier-Transform Infrared Spectrometer. We chose to include these macroplastics in our study because they are also plastic pollution, and they complement our research.

Statistical Analyses

Several variables were tested against each other using a student's t-test as well as an Rsquared linear regression to find significance. Variables tested against each other included: percent microplastic present in *D. cepedianum* vs. weight of *D. cepedianum*, percent microplastic present in *D. cepedianum* vs. length of *D. cepedianum*, weight of *D. cepedianum* from each pool vs amount of microplastic pieces in each pool, and length of *D. cepedianum* from each pool vs amount of microplastic pieces in each pool. Percent microplastic present in *P. disjunctivus* vs. weight of *P. disjunctivus*, percent microplastic vs. length of *P. disjunctivus*, weight of *P. disjunctivus* from each pool vs amount of microplastic pieces in each pool, and length of *P. disjunctivus* from each pool vs amount of microplastic pieces in each pool.

Fourier-Transform Infrared Spectroscopy

Fourier-Transform Infrared Spectrometry (FTIR) is a type of spectrometry that employs electromagnetic radiation to identify chemical samples (Peters et al 2018). FTIR uses vibrational modes to measure a particle's covalent chemical bonds. FTIR can identify covelant chemical bonds in plastics as small as 10 µm but is typically used in particles larger than 50 µm (Peters et

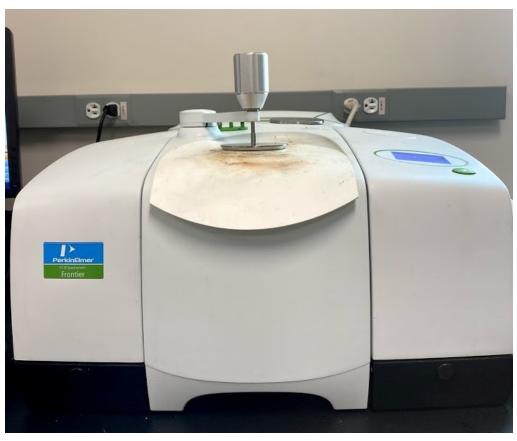


Figure 5. PerkinElmer FTIR used for analysis our plastic samples. It was used to identify the chemical composition of our plastic samples by sending infrared light through our samples. It also provided a spectrum of our sample as well as a search library where we could compare our samples to other known materials.

al. 2018). The FTIR instrument used in this study was a PerkinElmer Frontier FTIR (Figure 5). Once the microplastics had been removed from the digestive tracts of the fish and from the sediment and water samples, they were placed in petri dishes and moved to another lab for FTIR analysis. The FTIR sensor was sanitized using methanol and a chemwipe. We then conducted a background test on the FTIR which gives us a baseline to compare our samples. Samples were transported from the petri dish to a FTIR sensor and then analysis proceeded on the plastic. The FTIR sensor was sanitized following every test. The FTIR displayed a wavelength vs. amplitude graph. This graph showed a spectrum with several different peaks and valleys that is like a fingerprint for different molecules. Once the test was finished, the FTIR would search its spectral library and recommend the molecule that most aligned with our sample along with a "best hit score". The plastics from our samples rarely received a best hit score above 40% and the spectra from our samples did not match that of the preloaded library. The library for this FTIR did not have most polymers and plastics included. Because of this, we decided to build our own library.

This library consisted of plastics from resin codes one through six. Resin code one is Polyethylene Terephthalate (PET, PETE) and includes items such as soda bottles, water bottles, food containers, etc. Resin code two is High Density Polyethylene (HDPE) and includes items such as grocery bags, milk jugs, water jugs, etc. Resin code three is Polyvinyl Chloride (PVC, Vinyl) and includes items such as pvc pipes, vinyl gloves, shrink wrap, etc. Resin code four is Low Density Polyethylene (LDPE) and includes items such as bread bags, plastic toys, squeezable bottles, etc. Resin code five is Polypropylene (PP) and includes items such as bottle caps, yogurt containers, medicine bottles, etc. Resin code six is Polystyrene (PS) and includes items such as styrofoam containers, packing peanuts, building insulation, etc. (American Chemistry Council, 2021). Altogether together, twenty-three different plastics and other materials were used to build this library. Polyethylene Terephthalate samples acquired include a soda bottle as well as a dish soap bottle. The Polyethylene (both high density and low density) samples acquired include a shopping bag, a bread bag, and a section of a tarp. The polyvinyl chloride samples acquired include a pvc pipe as well as a vinyl glove. The polypropylene samples acquired include a plastic cup, a soda bottle cap, a plastic bucket, a disposable mask, and a soda bottle label. The polystyrene sample acquired was a foam cup. Other plastics and fibers were also gathered that we assumed are common in the environment such as a 100% polyester blend from a pair of athletic shorts, a 80%/20% polyester/nylon blend from a microfiber towel, a 85%/15% polyester nylon blend from a microfiber towel, a nylon rope, nylon fishing line, tire

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rubber, a 98%/2% cotton/spandex blend from a pair of hiking shorts, a 50%/50% cotton/polyester blend from a jacket, a nitrile glove, and another grocery bag.

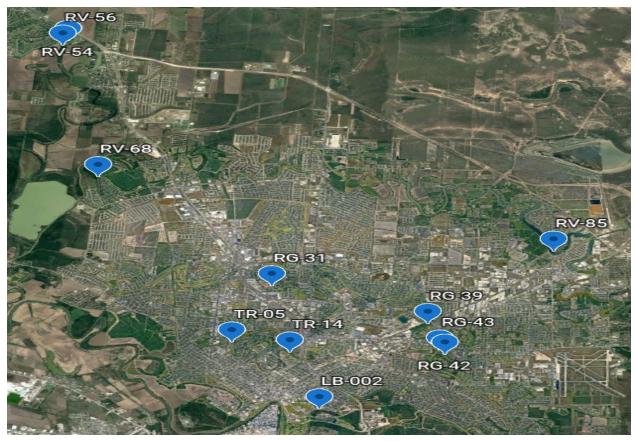


Figure 6. This image taken from Google Earth shows the locations of 11 bodies of water including three resaca systems and one oxbow lake from the lower Rio Grande Valley.



Figure 7. This image taken from Google Earth shows Lozano Banco, the only oxbow lake that was sampled. It is located on the Brownsville campus of The University of Texas Rio Grande Valley.



Figure 8. This image taken from Google Earth shows the four resacas of the Rancho Viejo resaca system. On top we have RV-54 & RV-56. In the middle, RV-68, and at the bottom RV-85.

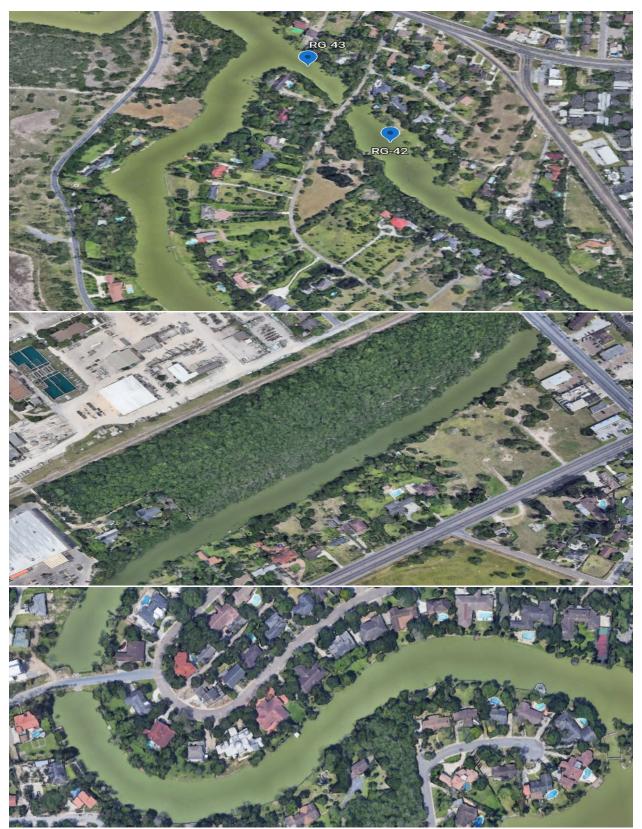


Figure 9. This image take from Google Earth shows the four resacas sampled from the Resaca de la Guerra resaca system. Top: RG-43 & RG-42 Middle: RG-39 Bottom: RG-31.

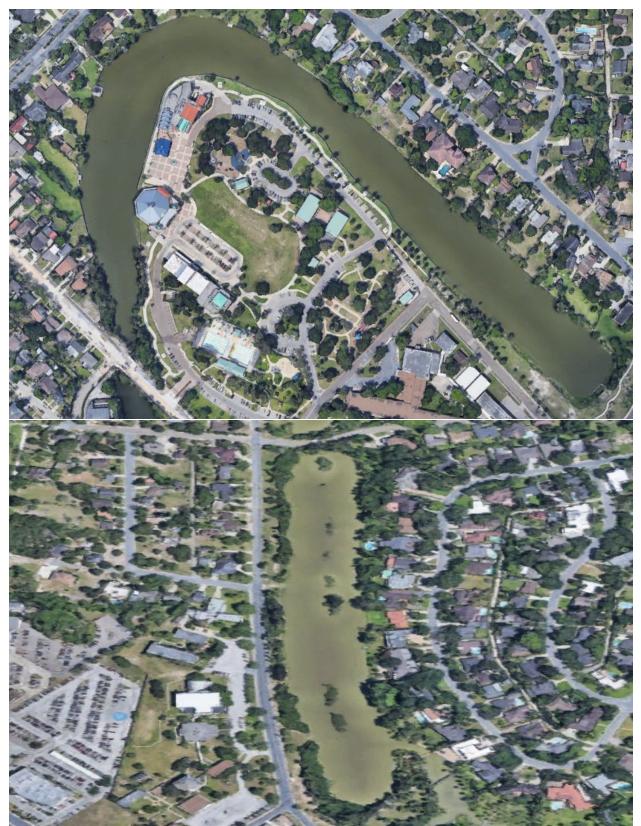


Figure 10. This image taken from Google Earth shows the two resacas from the Town Resaca system. Top: TR-14 Bottom: TR-05

CHAPTER III

RESULTS

Fish Analysis

Dorosoma cepedianum

Seventy *D. cepedianum* individuals were taken from three different resaca systems as well as Lozano Banco. Of the seventy *D. cepedianum* individuals, sixty had at least one microplastic present in their digestive system (85.7%). 108 microplastic pieces were found in the sixty *D. cepedianum* individuals that had at least one microplastic present in their digestive tract.

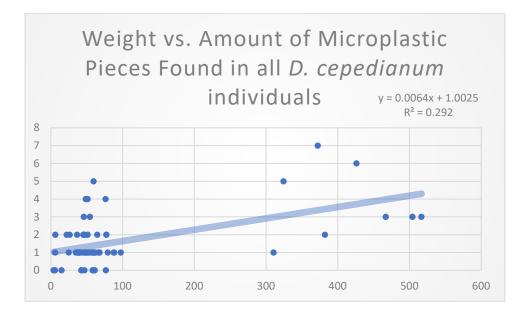


Figure 11. There was a slight positive correlation between the weight of D. cepedianum individuals and the number of microplastics in their digestive tracts. The data points to the far right are the individuals from Lozano Banco. Lozano Banco had the heaviest individuals of any pool. When the data points from Lozano Banco were removed, there was no correlation between weight and number of microplastics present.

Figure 12 illustrates the percentage of each category of microplastic found in the *D. cepedianum* individuals from across the eleven sampling sites. Seventy-seven were microfibers (71.3%), twenty-five were chunks (23.1%), and six were labeled other (5.6%). Four (5.7%) of the plastic pieces found were large enough to be considered macroplastics. Figure 11 shows the relationship between the weight of all *D. cepedianum* individuals and the amount of microplastics found from each pool. There is a slight positive correlation with an R² value of 0.29. The noticeable outlier here is the individuals from Lozano Banco whose individuals were much larger and had more microplastics present. When the data points from Lozano Banco were removed, there was no correlation present between the weight of *D. cepedianum* and the number of microplastics found. The average number of microplastic pieces found in D. cepdianum 1.54 pieces per individual. The data of the amount of microplastics as well as the amount of each category (microfiber, chunk, and other) from all pools where microplastics were present in *D. cepedianum* can be found in Figure 12.

Location	Individuals	Microplastic Presence %	Microplastic Pieces	Fibers	Chunks	Other	Avg Weight (g)	Avg Length (cm)
Resaca De La Guerra	27	86.4	34	23	7	4	54.226	17.537
Town Resaca	20	75	28	24	3	1	26.483	12.717
Rancho Viejo	12	80	16	12	4	0	57.565	17.965
Lozano Banco	11	100	30	18	11	1	321.627	29.891

Table 2. D. cepedianum data from all locations. There is an unequal sampling size from the locations which can be seen in the individual's column. Also, the microplastic presence % and microplastic pieces from Lozano Banco are noticeably high considering it has only one pool. The weight and length from Lozano Banco are also noticeably larger than the other locations. Rancho Viejo has the least amount of microplastic pieces and has the smallest D. cepedianum individuals. It is also worth noting that fibers are the most prevalent microplastic in all these systems.

Table 1 shows the data for *D. cepedianum* across three resaca systems and one oxbow lake. Twenty-seven individuals were collected from the Resaca De La Guerra system. Resaca De La Guerra consisted of four different resaca pools (RG-31, RG-39, RG-42, & RG-43). The

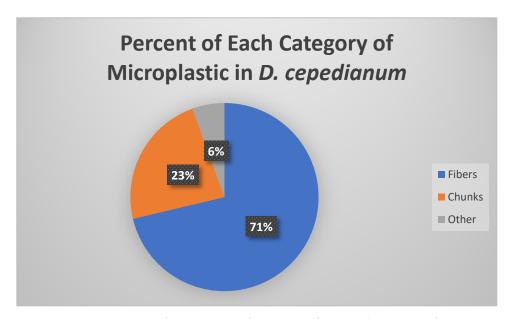


Figure 12. The percentage of each category of microplastic found in all individuals of D. cepedianum. Blue represents fibers, orange represents chunks, and gray represents other microplastics. We see that fibers are the most prevalent microplastic in D. cepedianum followed by chunks and lastly, other microplastics.

microplastic presence average was 86.4%. Thirty-four microplastic pieces were collected from the *D. cepedianum* individuals from Resaca De La Guerra. Of the thirty-four microplastic pieces, twenty-three were fibers (67.6%), seven were chunks (20.6%), and four were termed other (11.8%). The average weight of the twenty-seven individuals from Resaca De La Guerra was 54.226g and the average length was 17.537 cm.

The data from each of the four resacas from Resaca De La Guerra follows. Ten individuals were captured from RG-31, eight from RG-42, nine from RG-43, and zero from RG-39. The microplastic presence average from RG-31 was 70%, RG-42 was 100%, and RG-43 100%. The number of microplastic pieces found in RG-31 was thirteen (eight fibers, four chunks, and one other). The number of microplastic pieces found in RG-42 was nine (six fibers and three chunks), and the number of microplastic pieces found in RG-43 was twelve (nine fibers and three others). The average weight of the individuals from RG-31 was 54.18 g, RG-42 was 63.4 g, and RG-43 was 46.2 g. The average length of the individuals from RG-31 was 17.7 cm, RG-42 was 18.4 cm, and RG-43 was 16.7 cm.

The Town Resaca system contained two resaca pools (TR-14 & TR-05). We collected twenty individuals from these two pools. The microplastic presence average here was 75%. Twenty-eight microplastic pieces were collected in this system, twenty-four of which were fibers (85.7%), three were chunks (10.7%), and one was termed other (3.6%). The average weight of the twenty individuals from the Town Resaca system was 26.5 g and the average length was 12.7 cm. Two macroplastics were also found in the individuals from this system.

The data from the two resacas in the Town Resaca system are as follows. Ten individuals were captured from TR-14 and ten from TR-05. The microplastic presence average was 90% for TR-14 and 70% for TR-05. Seventeen microplastic pieces (two of which were macroplastics) were found in TR-14 (sixteen fibers and one other), and eleven microplastic pieces were found in TR-05 (eight fibers and three chunks). The average weight for TR-14 was 55.5 g and for TR-05 59.6 g. The average length for TR-14 was 18.1 cm and for TR-05 17.8 cm. The largest microplastic found in TR-14 was 2.6 cm long.

The Rancho Viejo system contained four resaca pools (RV-56, RV-54, RV-85, & RV-68). Twelve individuals were collected from these four pools. Of these twelve individuals, 80% of them contained at least one microplastic in their GI tract. Sixteen microplastic pieces were found in these individuals, twelve of which were microfibers (75%) and four which were chunks (25%). The average weight of these individuals was 57.6 g and the average length was 18.0 cm. Two macroplastics were also found in the individuals from these systems. The data from the four Rancho Viejo resaca pools follows. Two individuals were captured from RV-85, three were captured from RV-54, seven from RV-68, and zero from RV-56. The microplastic presence average from RV-85 was 100%, for RV-54 100%, and for RV-68 57.1%. Three microplastic pieces were found in RV-85, all of which were fibers (one of these were a microplastic), three in RV-54 (two fibers (one microplastic) and one chunk)), and ten were found in RV-68 (seven fibers and three chunks). The average weight for RV-85 was 23.6 g, for RV-54 49.2 g, and for RV-68 17.6 g. The average length for RV-85 was 13.2 cm, for RV-54 16.5 cm, and for RV-68 11 cm. The largest microplastic found in the Rancho Viejo system was 1.4 cm long.

Lozano Banco was a single oxbow lake which was sampled. Eleven individuals were collected from this pool, all of which had microplastics present in their GI tract (100%). Thirty

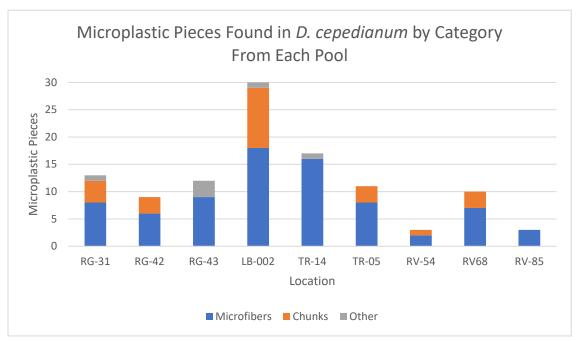


Figure 13. Microplastic pieces found in each site where microplastics were present in D. cepedianum. Each column has microfibers, chunks, and other microplastics which are colored blue, orange, and gray respectively. We see that microfibers are the most common microplastic followed by chunks and other. We also see here that Lozano Banco has the highest amount of microplastics followed by TR-14 and RG-31.

microplastic pieces were collected from these eleven individuals. Eighteen were microfibers (60%), eleven were chunks (36.7%), and one was considered other (3.3%). The average weight of these eleven individuals was 321.627g and the average length was 29.891cm. Lozano Banco had the largest *D. cepedianum* of any system. Figure 13 illustrates the microplastics by category from each pool.

P. disjunctivus

Forty-nine *P. disjunctivus* individuals were captured from the three resaca systems as well as Lozano Banco. Of the forty-nine individuals, twenty-two had at least one microplastic present in their digestive tract (44.9%).

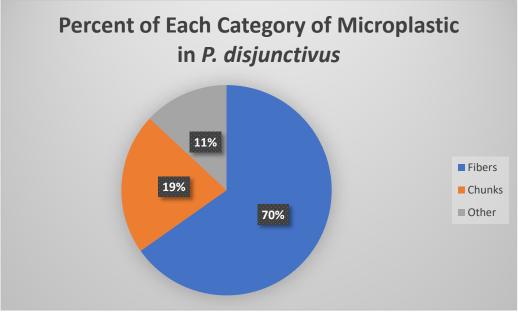


Figure 14. Percentage of each type of microplastic found in all individuals of P. disjunctivus. Blue represents microfibers, orange represents chunks, and gray represents other microplastics. Microfibers are the most prevalent type of microplastic in P. disjunctivus.

Figure 14 illustrates the amount of microplastic pieces found in each category. Twenty-seven microplastic pieces were found in the twenty-two individuals that showed microplastic presence.

Of the twenty-seven microplastic pieces, nineteen were fibers (70.4%), five were chunks (18.5%), and three were other (11.1%). The average number of microplastic pieces per *P*. *disjunctivus* 0.55 pieces per individual. There was no significant correlation between the weight of *P. disjunctivus* individuals and the number of microplastics found in their digestive tract as seen in Figure 15 (R^2 =0.005). There also was not a significant correlation between the length of *P. disjunctivus* and the number of microplastics found in the digestive tract.

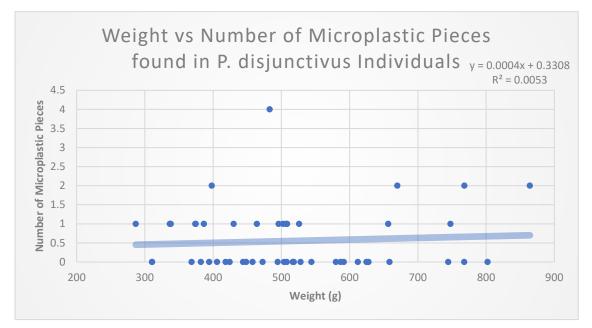


Figure 15. The weight vs number of microplastics per P. disjunctivus relationship is shown here. There is no correlation between the weight of P. disjunctivus and the number of microplastics found in the digestive tract. All P. disjunctivus individuals are shown here.

Twenty-five *P. disjunctivus* were caught from the four resacas in the Resaca De La Guerra system. The microplastic presence average of Resaca De La Guerra for *P. disjunctivus* was 36% meaning nine of the twenty-five individuals had at least one microplastic present in their digestive tract. Five microplastic pieces were found in the individuals from this system. Three were fibers (60%), one was a chunk (20%), and one was other (20%). The average weight of these *P. disjunctivus* individuals were 448.87 g and the average length was 39.04cm.

The data for the *P. disjunctivus* individuals from the four pools from the Resaca De La Guerra system follows. Zero individuals were captured from RG-31, four from RG-42, eleven from RG-43, and ten from RG-39. The microplastic presence average from RG-42 was 0%, RG-43 36.4%, and RG-39 50%. Three microplastic pieces were found in RG-43, all of which were fibers, and five were found in RG-39 (three fibers, 1 chunk, and 1 other). The average weight of *P. disjunctivus* from RG-42 was 397.9 g, for RG-43 425.9 g, and for RG-39 494.5 g. The average length for *P. disjunctivus* from RG-42 was 37.5 cm, for RG-43 38.3 cm, and for RG-39 40.5 cm.

Eleven *P. disjunctivus* were caught from the two resacas in the Town Resaca system. The microplastic presence average of the Town Resaca System was 54.5% meaning six of the eleven *P. disjunctivus* individuals had at least one microplastic in their digestive tract. Nine microplastic pieces were found in the individuals from this system. Six were microfibers (66.7%), one was a chunk (11.1%), and two were other microplastics (22.2%). The average weight for this system was 651.2 g, and the average length was 41.9 cm. The Town Resaca system had the heaviest *P. disjunctivus* of all systems.

The data of the two pools from the Town Resaca system follows. Ten individuals were captured from TR-14 and one from TR-05. The microplastic presence average from TR-14 was 50% and the one individual from TR-05 had microplastics present in its GI tract. Eight microplastic pieces were found in TR-14 (five fibers, one chunk, and two other), and one fiber was found in TR-05. The average weight of TR-14 was 670 g, and the one individual from TR-05 weighed 464.3 g. The average length from TR-14 was 42.2 cm and the length of the one individual from TR-05 was 39.2 cm.

Three *P. disjunctivus* were captured from RV-56 in the Rancho Viejo system. This was the only resaca which *P. disjunctivus* were found in the Rancho Viejo system. The microplastic

presence average of the Rancho Viejo system was 66.7%, 2 individuals had microplastics present. Three plastics were found in these individuals, two fibers (66.7%) and one chunk (33.3%). The average weight for this system was 513.7 g and the average length was 39 cm.

Ten *P. disjunctivus* were captured from the oxbow lake Lozano Banco. Five of the fish from this system had at least one microplastic present in their GI tract (50%). Six microplastic pieces were found in these individuals. Four were microfibers (66.7%) and two were chunks (33.3%). The average weight and length from this system were 524.4 g and 42.3 respectively.

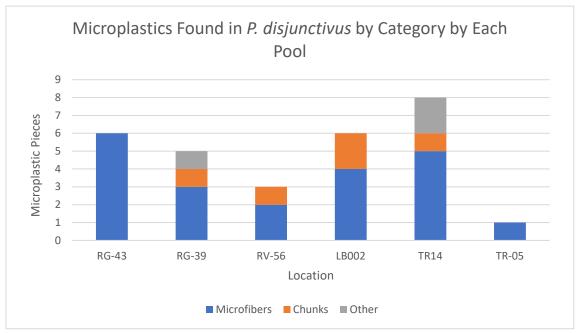


Figure 16. The number of microplastic pieces in P. disjunctivus by category for each pool. Blue represents microfibers, orange represents chunks, and gray represents other microplastics. TR-14 has the most microplastics of any pool. Microfibers are the most prevalent plastic in the pools.

Total Fish Results

119 fishes from *D. cepedianum* and *P. disjunctivus* were sampled from eleven different bodies of water. Of the 119 specimens taken, seventy individuals were *D. cepedianum* (58.8%)

and forty-nine individuals were *P. disjunctivus* (41.2%). Eighty-one individuals of the 119 had at least one microplastic present in their gastrointestinal tract (68.1%) (Figure 17).

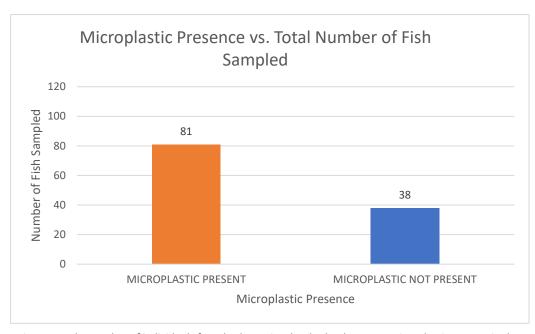


Figure 17. The number of individuals from both species that had at least one microplastic present in the orange column (81) and the number of individuals without a microplastic present in the blue column (38). There are significantly more fish with microplastics present than those without.

135 plastic pieces were found in the eighty-one individuals that had microplastics present in their digestive systems. Of these 135 pieces, 80% of these were found in *D. cepedianum* (108 pieces) and 20% were found in *P. disjunctivus* (twenty-seven pieces) (Figure 18). Microplastic pieces that were found in both species of fish were categorized by fiber, chunk, or other. Other included pieces that resembled foams, films, and oily substances. Of the 135 plastic pieces found, ninety-two pieces were fibers (70.2%), thirty pieces were chunks (22.9%), and nine pieces were assigned as other (6.9%).

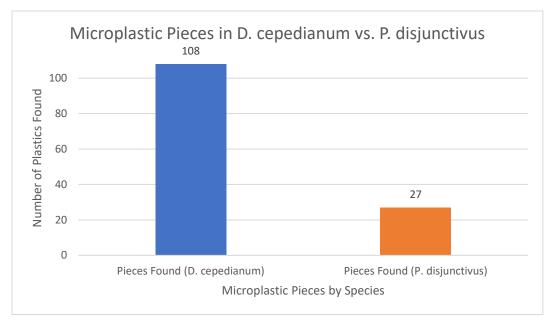


Figure 18. The number of plastic pieces found in D. cepedianum vs P. disjunctivus. D. cepedianum is in blue and P. disjunctivus is in orange. There were many more pieces found in D. cepedianum than P. disjunctivus.

Figure 19 shows the microplastic presence percentage in the three resaca systems and one oxbow lake from our study. Resaca De La Guerra had microplastic present in 88.89% of its *D. cepedianum* individuals and 53.1% of its *P. disjunctivus* individuals. The Rancho Viejo system had microplastics present in 75% of its *D. cepedianum* individuals and 63.7% of its *P. disjunctivus* individuals. The Town Resaca system had microplastics present in 80% of its *D. cepedianum* individuals. The Town Resaca system had microplastics present in 80% of its *D. cepedianum* individuals. And Lozano Banco had microplastics present in 100% of its *D. cepedianum* individuals and 50% of its *P. disjunctivus* individuals.

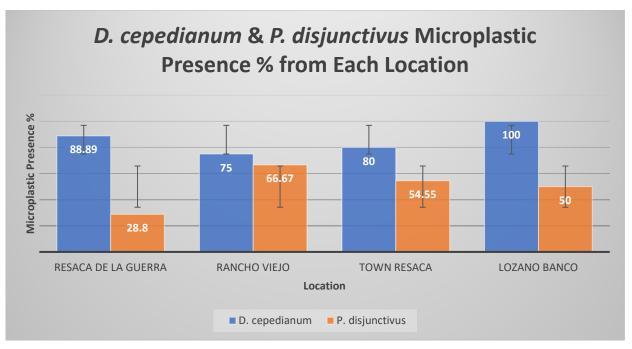


Figure 19. The microplastic presence average in both species of fish from all four systems. Blue represents D. cepedianum and orange represents P. disjunctivus. Lozano Banco has the highest % presence from any D. cepedianum, and Rancho Viejo has the highest from P. disjunctivus. The microplastic presence average is always higher in D. cepedianum.

Sediment

Thirty-three total samples were retrieved across the eleven pools from our four systems. Two benthic sediment samples were taken at each location as well as a shore sediment sample. There were microplastics present in all but two of the sediment samples and one of the shore samples. The microplastic presence average for all eleven pools was 90.9%. The Rancho Viejo system and Resaca De La Guerra system were the only two with resacas lacking microplastics in some of their samples. RV-85 did not have microplastics present in one of its three benthic sediment samples. RG-39 did not have microplastics in one of its three benthic sediment samples, and RG-42 did not have microplastics in its shore sample.

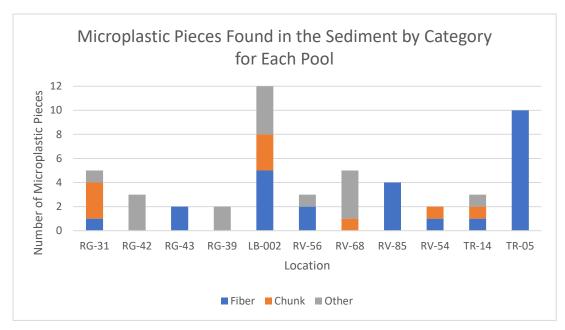


Figure 20. The number of microplastic pieces found in each category in the sediment of the eleven pools is shown here. Blue represents microfibers, orange represents chunks, and gray represents other microplastics. Lozano Banco has the most present followed by TR-05. Microfibers are most prevalent followed by other microplastics and then chunks last.

Figure 20 illustrates the number of microplastics found in each category from each of the eleven pools. Fourteen were found in the Rancho Viejo system, four from RV-85, five from RV-68, two from RV-54, and three from RV-56. Thirteen were found in the Town Resaca system, three in TR-14 and ten in TR-05. Twelve were found in the Resaca De La Guerra system, five from RG-31, two from RG-39, two from RG-43, and two from RG-43. Twelve were found in Lozano Banco. Of the fifty-one pieces found, twenty-six were microfibers, nine were chunks, and sixteen were other microplastics. Fifty-one microplastic pieces were found in total from these three resaca systems and one oxbow lake. Fourteen of the plastics were large enough to be considered macroplastics with the largest being 15.4 cm.

The data for the individual resaca pools from the Rancho Viejo system follows. RV-85 contained four microplastics, two from the benthic samples and two from the shore sample (both were microfibers). RV-68 contained five microplastics, three from the shore sample and two

from the sediment samples, one was a chunk and four were other microplastics. RV-56 contained three microplastics, two from the shore sample and one from the sediment samples. There were two microfibers and one other in this resaca. RV-54 contained two microplastics, one which was from the sediment sample and one from the shore sample (both were microfibers). There were two macroplastics in the Rancho Viejo system. The largest microplastic found in this system was 1.5 cm.

The data for the two resaca pools from the Town Resaca system follow. TR-14 contained three microplastics, one from the benthic sediment samples and two from the shore sediment sample. One was a microfiber, one was a chunk, and the last was an other microplastic. TR-05 contained ten microplastics all of which were found in the shore sample and all of which were microfibers. The Town Resaca system contained three macroplastics total, the largest of which was 1.2 cm.

The data from the four resacas in the Resaca De La Guerra system follows. RG-39 contained two microplastics, both found in the benthic sediment samples, and both of which were other microplastics. RG-31 contained five microplastics, three of which were from the shore sediment sample, and two of which were from the benthic sediment sample. One of the plastics was a microfiber, three were chunks, and one was an other. RG-42 contained three microplastics, both of which were in the benthic sediment samples. Both microplastics were in the other category. RG-43 contained two microplastics, one from the shore sediment sample and one from the benthic sediment samples. Both were microfibers. Resaca De La Guerra contained six plastics large enough to be considered macroplastic, the largest of which was 15.4 cm. This was the largest plastic found in all our samples.

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The data found in Lozano Banco follows. There were twelve microplastics found in Lozano Banco, nine of which were found in the benthic sediment samples and three of which were found in the shore sample. Five of the microplastics were microfibers, three were chunks, and four were other microplastics. Five of the plastics found were large enough to be considered macroplastic with the largest being 1 cm.

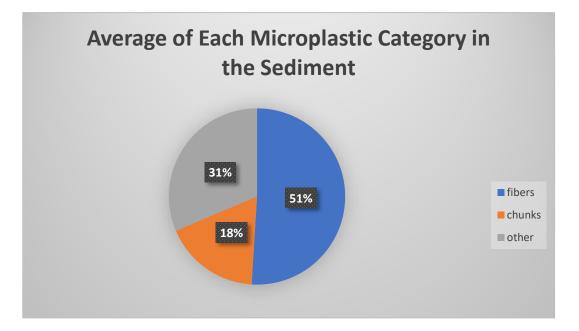
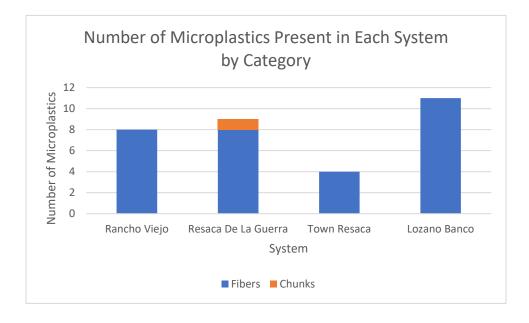


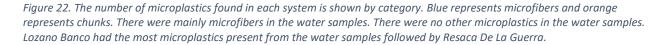
Figure 21. The percentage of each microplastic category found in all the sediment samples from all eleven pools is displayed here. Blue represents microfibers, orange represents chunks, and gray represents other microplastics. Microfibers were the most prevalent in the sediment samples. The sediment samples are the only samples where other contained more microplastics than chunks.

Water

Twenty-two water samples were retrieved from the eleven pools across our four different systems. Two samples were collected from each pool. There were microplastics present in all but two of the samples from the eleven pools and the microplastic presence average of the twenty-two samples was 87.5%. Figure 22 shows the microplastic presence by percentage of each of the three resaca systems as well as Lozano Banco. The Resaca De La Guerra and the Rancho Viejo

system were the only two systems which had microplastics missing from one of their water samples each. RV-68 and RG-43 were the two pools where microplastics were only present in one of their samples.





Thirty-two microplastics were found in total across these four systems (Figure 22). Thirty-one of these plastics were microfibers, and one was a chunk. Resaca De La Guerra contained nine fibers and one chunk. The Town Resaca system contained 4 microfibers, Rancho Viejo contained eight microfibers, and Lozano Banco contained eleven microfibers, one of which was large enough to be labeled a microplastic. Lozano Banco had the most microplastics present followed by Resaca De La Guerra.

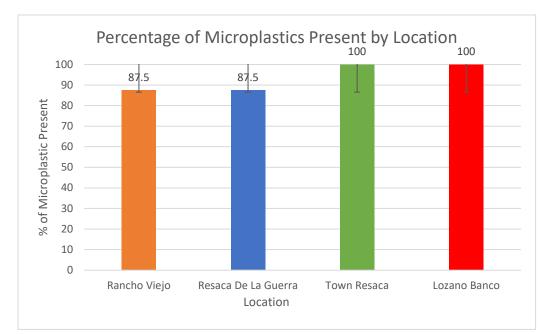


Figure 23. The percentage of microplastics present in each location is shown by each bar. Orange represents Rancho Viejo, blue represents Resaca De La Guerra, green represents Town Resaca, and red represents Lozano Banco. Lozano Banco and Town Resaca were the systems with the highest microplastic presence percentage.

FTIR Analysis

Figure 24 shows the FTIR results of a microplastic chunk found in a *D. cepedianum* individual from Lozano Banco. The box on the top left contains two columns, one labeled "Sample Name" which provides a list of all the samples being tested at a given time. In this case, all the plastic samples found in *D. cepedianum* individuals from Lozano Banco were being tested. "Search Best Hit Description" matches the sample with the best hit from the preloaded plastic library. The top right box contains three columns. "Search Score" provides us with a score of how similar the plastic sample from Lozano Banco is to one of the preloaded plastic

Irce	Spectra Search Re	sults:		Search	n Hit List		
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•	LB002 CHUNK	Sample 284 By Analyst Date Tuesday, February 09 2021		2	0.960203	C:\WES_L.K(PP).sp	Sample 281 By Analyst Date T
	LB002 FIBER	Sample 284 By Analyst Date Tuesday, February 09 2021		3	0.946982	C:\COKE LABEL.sp	Sample 283 By Analyst Date T
	LB002 FIBER	Sample 276 By Analyst Date Tuesday, February 09 2021		4	0.929256	C:\bUCKET (PP).sp	Sample 280 By Analyst Date T
	LB002 CHUNK	Sample 287 By Analyst Date Wednesday, February 10 2021		5	0.927793	C:\WES LL\nylon.sp	Sample 274 By Analyst Date T
	LB002OTHER	Sample 276 By Analyst Date Tuesday, February 09 2021		6	0.920854	C:\WES L. CUP).sp	Sample 291 By Analyst Date V
	LB002 FIBER	Sample 276 By Analyst Date Tuesday, February 09 2021		7	0.180577	C:\WES_L.T_NET.sp	Sample 276 By Analyst Date T
	LB002 FIBER	Sample 276 By Analyst Date Tuesday, February 09 2021		8	0.120949	C:\WES L. BAG).sp	Sample 287 By Analyst Date V
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Figure 24. Screenshot taken from a sample during FTIR analysis. This display shows the spectra, identification, and search score of a microplastic taken from a D. cepedianum individual from Lozano Banco. The top left box shows the sample name of the plastic. The top right box shows the search score as well as the search reference. And the box on the bottom left shows the spectra of both the plastic from Lozano Banco (red) and the known polypropylene reference plastic (green).

samples. "Search Reference" provides the plastic sample from the library that most closely matches the plastic sample from Lozano Banco. And the "Search Reference Spectrum Detail" shows the sample number for the Best Hit Plastic. The box on the bottom left displays the infrared spectra for both the unknown sample from Lozano Banco (top/red) as well as the known sample from the plastic library (bottom/green). Both the search score and the spectra comparison were used to obtain the identity of the unknown plastic. A search score of 0.90 or higher is preferred, but it is still possible to identify unknown plastics that have a lower search score than this by comparing the spectra of the unknown plastic to that of the known plastic. This microplastic had a search score of 0.962 = 96.2% that matched with a polypropylene sample from our plastic library. The spectra from this unknown plastic also matched very closely with the spectra of our polypropylene sample. PP plastics have four distinctive peaks right past the 3000 cm-1 wavelength that distinguishes PP plastics from PE and PET plastics. By comparing both the search score and the spectra of the unknown and known plastic side-by-side, we

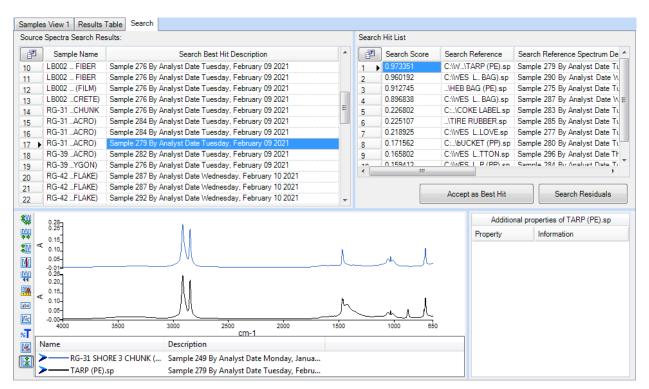


Figure 25. Display of FTIR analysis on a PE plastic from a sediment sample. This display shows the spectra, identification, and search score of a microplastic taken from a shore sediment sample from Resaca De La Guerra. The top left box shows the sample name of the plastic. The top right box shows the search score as well as the search reference. And the box on the bottom left shows the spectra of both the plastic from Resaca De La Guerra (blue) and the known polyethylene reference plastic (black). conclude that this unknown sample from Lozano Banco is polypropylene.

The three major plastics found in all our samples were polypropylene (PP), polyethylene (PE), and polyethylene terephthalate (PET). Figure 24 displayed the FTIR analysis for a polypropylene (PP) plastic. Figure 25 displays the analysis for a polyethylene (PE) plastic, and figure 26 displays the FTIR analysis for a polyethylene terephthalate (PET) plastic. The layout for both is the same as for the layout of figure 24. Figure 25 is the FTIR display of a PE plastic taken from the shore sediment of RG-31. It had a search score of 97.3% with one of the plastic samples from the plastic library. The spectra for the PE plastics have a distinctive fingerprint, around the 3000 cm-1 two steep peaks can be seen that are different from that in PP and PET

plastics. Figure 26 is the FTIR display of a PET plastic taken from the benthic sediment of RG-39. It had a search score of 92.8% with one of the PET plastics from the plastic library. This spectrum has a distinctive fingerprint after the 1800 cm-1 wavelength that distinguishes it from

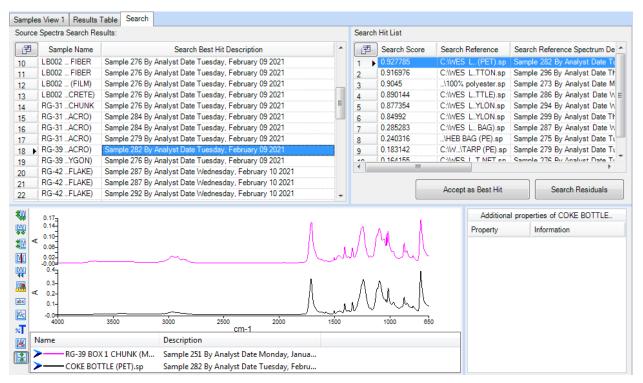


Figure 26. Display of FTIR analysis on a PET plastic from a sediment sample. This display shows the spectra, identification, and search score of a microplastic taken from a benthic sediment sample from Resaca De La Guerra. The top left box shows the sample name of the plastic. The top right box shows the search score as well as the search reference. And the box on the bottom left shows the spectra of both the plastic from Resaca De La Guerra (pink) and the known polyethylene terepthalate reference plastic (black).

PP and PE plastics.

FTIR analysis was conducted on all the micro and macroplastics that were collected from the two species of fish as well as the sediment and water. Significant readings were obtained from thirty-four of the plastic analyses conducted. Eighteen of these significant readings were from plastics found in *D. cepedianum*, seven were from plastics found in *P. disjunctivus*, and nine were from sediment samples. No significant readings were found in the plastics from the water samples. Of these thirty-four, it was found that thirteen were polypropylene (PP), ten were low density and high-density polyethylene (PE), and ten were polyethylene terephthalate (PET) (Figure 28). One of the samples matched with a 98/2% cotton/spandex material. 54% of the PP plastics were fibers and 46% were chunks. 20% of the PE plastics were fibers, 40% were chunks, and 40% were other microplastics. 80% of the PET microplastics were fibers, and 20% were chunks.

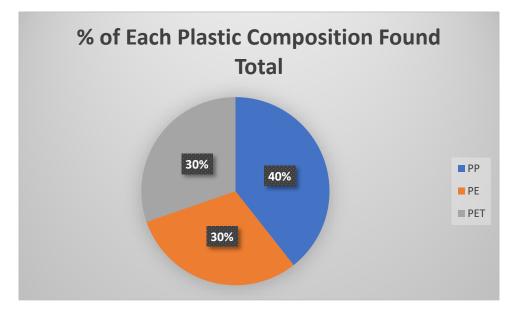


Figure 27. The plastic composition by percentage of all systems. Blue represents polypropylene (PP), orange represents polyethylene (PE), and gray represents polyethylene terephthalate (PET). PP were the most common plastics identified by FTIR for all parameters.

The plastic composition by system follows. Resaca De La Guerra contained five polypropylene (PP), three polyethylene (PE), and 7 polyethylene terephthalate (PET). There was one PE plastic found in the *D. cepedianum* individuals from Resaca De La Guerra, five PET plastics, and two PP plastics. There was one PP plastic found in *P. disjunctivus* individuals from Resaca De La Guerra. And there were two PE plastics found in the sediment from Resaca De La Guerra, two PP plastics, and two PET plastics. The Resaca De La Guerra system contained more PET plastics than any other plastic (Figure 28).

The plastic composition of Lozano Banco follows. Lozano Banco contained four PE plastics, seven PP plastics, and one PET plastic. There was two PE plastics found in *D*.

cepedianum individuals from Lozano Banco, and five PP plastics. There were two PE plastics found in *P. disjunctivus* and one PP plastic. And there was one PP and one PET found in the sediment of Lozano Banco. The Lozano Banco pool contained more PP plastics than any other plastic (Figure 28).

The plastic composition of the Town Resaca system follows. Town Resaca contained one PP plastic, three PE plastics, and one PET plastic. There was one PE and one PET plastic found in the *D. cepedianum* individuals from the Town Resaca system. There were two PE and one PET found in the *P. disjunctivus* individuals from the Town Resaca system. The Town Resaca system contained more PE plastics than anything else (Figure 28). The Rancho Viejo only contained one identified plastic which was a PET plastic found in the sediment.

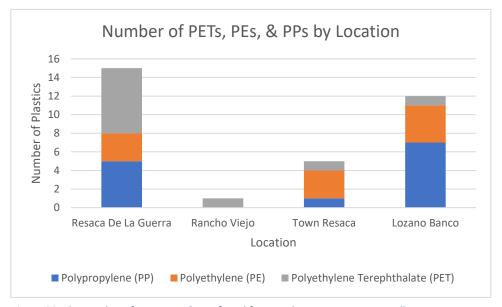


Figure 28. The number of PETs, PEs, & PPs found from each resaca system as well as Lozano Banco. Blue represents PPs, orange represents PEs, and gray represents PETs. Resaca De La Guerra had the largest number of PETs, and Lozano Banco had the most PP's and PE's. Rancho Viejo had the least amount of plastics identified by FTIR.

CHAPTER IV

DISCUSSION

Our results show us that microplastics are present in every resaca system and oxbow lake sampled and that microfibers were the most common type of microplastic found throughout. This was seen across both species of fish as well as the sediment and water of every resaca. This complements previous research that suggests microfibers are the most common microplastic in aquatic environments (Miller et al. 2017). Mason et al. 2016 & Browne et al. 2011 among others, suggests microfibers can be introduced to freshwater systems through stormwater runoff, treated wastewater effluent, etc. They also state that sewage sludge which is applied to terrestrial sources, and abrasives and scrubbers from cleaning products could also be introducing microplastics into the environment. Considering that resacas function as stormwater runoff and municipal drinking water storage, this along with trash pollution is a likely way in which microfibers and other types of microplastics are being introduced into the resacas.

The next most common microplastic found in the resacas were chunks and lastly other microplastics which include microplastics such as foams, films, etc. These three criteria of microplastics were chosen based on morphological observation of what was most common in our samples. *D. cepedianum* had higher microplastic presence percentages per pool than did *P. disjunctivus*. *D. cepedianum* also contained more microplastics overall as well as more microplastics per individual than did *P. disjunctivus*.

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We expected *P. disjunctivus* to have higher numbers of microplastics in their digestive tracts both because they are larger than *D. cepedianum* and they feed in benthic environments (FWS, 2014). This however was not supported by our results. This could have been influenced by the fact that P. disjunctius have larger digestive systems than do D. cepdianum and we only surveyed thirty cm of digestive contents from each species. Some of the D. cepdianum digestive tracts were smaller than thirty cm, so we could look at all the some of the *D. cepedianum* digestive tracts yet only observe a fraction of the *P. disjunctivus* digestive tract. However, to eliminate the risk of long observation periods where microfibers from the air or nearby surfaces could adulterate our samples, we realized the was a necessary implication.

We hypothesized that more microplastics would be found in larger fish, which was not supported by our data. We found that there was a slight positive correlation between the weight of *D. cepedianum* and the number of microplastics present in their digestive tract. But when we removed individuals from Lozano Banco from the regression, we found that there was no correlation between the weight of *D. cepedianum* and the number of microplastics found. We removed Lozano Banco from the regression to see if this would change the outcome since Lozano Banco is a clear outlier in both weight and the number of microplastics found. So, it is likely that weight is not a key indicator of plastic presence, rather Lozano Banco could just simply be more polluted than the other systems, and this is why there was more microplastics present in its individuals.

We found that polypropylene (PP) was the most abundant plastic followed by polyethylene (PE) and polyethylene terephthalate (PET). It is interesting to note that only PET, PE, and PP plastics were the only plastic types found. No PVC or PS plastics were identified by FTIR. Only thirty-four of the 218 plastics analyzed by FTIR resulted in significant readings. We think this is the case because many of our microplastics were too minute to produce meaningful FTIR spectra. Also, there may have been unknown plastics from our samples that were of different composition than the plastics from our library. Both could have been reasons why we did not have more significant search scores and spectral matches. There is limited research conducted that shows how different compositions of microplastics (PET, PE, PVC, PP, PS, etc.) are being introduced into the environment or how each one of these are affecting fish populations and individuals. It would be very interesting to know the pollution source of each of these types of plastics. It would also be very helpful to know how each plastic type effects the organisms in the environment rather than assuming all plastics are the same and have the same impacts.

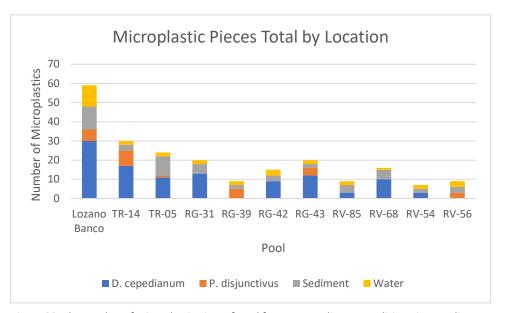


Figure 29. The number of microplastic pieces found from D. cepedianum, P. disjunctivus, sediment, and water by pool location. Blue represents D. cepedianum, orange represents P. disjunctivus, gray represents sediment, and yellow represents water. Lozano banco had the most pieces present followed by TR-14 and TR-05. Rancho Viejo had the least number of microplastics present per pool.

Lozano Banco

Lozano Banco was the only oxbow lake used in this study. Lozano Banco was the most polluted pool of this study (Figure 29). This location contained the most plastics of any pool with fifty-nine found (thirty in *D. cepedianum*, six in *P. disjunctivus*, twelve in the sediment, and eleven in the water). Lozano Banco contained the largest *D. cepedianum* individuals of any other pool.

The reason *D. cepedianum* individuals may be so much larger here than in other systems may be due to the lack of predators in Lozano Banco which would allow the *D. cepedianum* individuals to grow to uncommon sizes. We also caught our limit of *P. disjunctivus* at Lozano Banco.

Lozano Banco has the highest microplastics present of any resaca, their *D. cepedianum* are overgrown, and they have high number of *P. disjunctivus* which are invasive. These are all signs of an unhealthy/unbalanced system. Lozano Banco had the highest % microplastic presence for *D. cepedianum* and the highest number of plastics present in *D. cepedianum* compared to all other pools. The microplastic presence % was 100% for the water, sediment, and *D. cepedianum* samples, and 50% for the *P. disjunctivus* samples. There were 5.5 microplastic pieces per water sample, four per sediment sample, 2.71 per *D. cepedianum* sample, and 0.55 per *P. disjunctivus*. Microplastics in this system were most likely to be found in the water & sediment, then *D. cepedianum*, and lastly *P. disjunctivus*. Microplastic abundances were most likely to be higher in the water followed by the sediment, *D. cepedianum*, and lastly *P. disjunctivus*. Lozano Banco contained seven PP, four PE, and one PET plastic. Lozano Banco is located near downtown Brownsville, TX, and is on the campus of the University of Texas Rio Grande Valley. This resaca is also subject to noticeable trash pollution.

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Town Resaca

The two pools from the Town Resaca system were the next most polluted following Lozano Banco. This system contained fifty-four plastics total (twenty-eight in *D. cepedianum*, nine in *P. disjunctivus*, thirteen in the sediment, and four in the water). Thirty of the plastics were from TR-14 and twenty were from TR-05. This system contained the heaviest *P. disjunctivus* individuals. The *D. cepedianum* from this system were significantly smaller than that of Lozano Banco but were similar in size to the *D. cepedianum* individuals of Resaca De La Guerra.

The microplastic presence percentage was 80% for D. cepedinum, 54.5% for *P*. *disjunctivus*, 100% for the sediment, and 100% for the water samples. There were 1.4 plastics per *D. cepedianum*, 0.81 per *P. disjunctivus*, 1 per water sample, and 2.2 per sediment sample. Microplastics in this system are most likely to be found in the water & sediment followed by *D. cepedianum* and lastly *P. disjunctivus*. Higher abundances of microplastics are most likely to be found in sediment followed by *D. cepedianum*, water samples, and lastly *P. disjunctivus*. Three PE plastics, one PET plastic, and one PP plastic were identified from this system. The Town Resaca system is located in urban Brownsville and is surrounded by several roadways, residential housing, a zoo, city parks, etc.

Resaca De La Guerra

The Resaca De La Guerra system ranked three out of four of our four systems for microplastic pollution. This system is located on the outskirts of Brownsville and is surrounded by both residential housing, roadways, and wild riparian vegetation and habitat. This system contained sixty-four total plastics. Twenty were from RG-31, twenty from RG-43, fifteen from RG-42, and nine from RG-39. Thirty-four of the plastic pieces were from *D. cepedianum*, nine from *P. disjunctivus*, twelve from the sediment, and nine from the water.

The microplastic presence percentage was 88.9% for *D. cepedianum*, 28.9% for *P. disjunctivus*, 83.3% for the sediment, and 87.5% for the water. There were 1.3 plastics per *D. cepedianum*, 0.36 per *P. disjunctivus*, 1 per sediment sample, and 1.1 per water sample. Resaca De La Guerra had the lowest microplastic presence percent and number of microplastics per sample for *P. disjunctivus*. It is also the only system where microplastics are more likely to be found and likely to be found in higher numbers in *D. cepedianum* over both the water and sediment. Seven PET, five PP, and three PE plastics were identified from this system.

Rancho Viejo

The Rancho Viejo system was the least polluted system according to our data and had the least amount of the invasive *P. disjunctivus* present of all the other systems. Only three *P. disjunctivus* were found in all four of its pool. The Rancho Viejo system is located several miles away from urban Brownville, TX. It is surrounded by agricultural fields, wild riparian vegetation and wild habitat, and some residential housing. It is the most wild and rural Resaca system of the four systems sampled. The *D. cepedianum* here were much smaller than the *D. cepedianum* in any other system. All these factors may have contributed to the low amounts of microplastics found in its resacas.

Rancho Viejo contained forty-one plastic pieces (sixteen from RV-68, nine from RV-85, nine from RV-56, and seven from RV-54). Sixteen were found in *D. cepedianum*, three in *P. disjunctivus*, fourteen in the sediment, and eight in the water. The microplastic presence average

was 75% for *D. cepedianum*, 66.7% for *P. disjunctivus*, 91.7% for the sediment, and 87.5% for the water. There were 1.3 per *D. cepedianum*, 1 per *P. disjunctivus*, 1.2 per sediment sample, and 1 per water sample. Microplastics are most likely to be found in this system in the sediment followed by the water, *D. cepedianum* and lastly *P. disjunctivus*. Higher numbers of microplastics are most likely to be found in *D. cepedianum* followed by the sediment and water and *P. disjunctivus*. There was only one plastic identified by FTIR from this area and it was a PET.

CHAPTER V

CONCLUSION

According to our data, microplastics are most likely to be found in the water then sediment, then *D. cepedianum*, and lastly *P. disjunctivus*. This makes sense given that water is the transport system for pollution and that pollution will usually settle into the sediment or stay suspended in the water column. It is logical that there would be more microplastics in the environment than in the organisms that live in the environment just as it is more logical that there is more vegetation in the environment than in the stomachs of organisms that consume vegetation.

As shown in Figure 29, the more urban resacas were the pools with higher microplastic pollution. Lozano Banco located near downtown Brownsville and on the campus of the University of Texas Rio Grande Valley was the most polluted followed by the Town Resaca system which is also located in downtown Brownsville and surrounded by residential housing, roadways, a zoo, city parks, etc. Resaca De La Guerra was the next most polluted, it is located on the outskirts of Brownsville and is surrounded by residential housing, roadways, and some riparian vegetation and wild habitat. And the least polluted resaca system was also the most rural resaca system Rancho Viejo was located several miles away from urban Brownsville and is surrounded by agricultural fields, wild habitat and riparian vegetation and some residential housing.

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We found that microplastics were found in fish regardless of their size. Going into this study we assumed that larger fish would contain more microplastics, but our data suggests that microplastics are ubiquitous in fish regardless of size.

We were not able to discover if there is any correlation related to the plastic composition and the resaca location or the sediment, water, or fish species. This was not one of the goals of our study, our purpose was to determine if microplastics were present, and if so, what types were present, what compositions, and where were they located. We hope to know one day if there is a correlation between the plastic types and the areas they are found as well as the different effects different plastic types have on organisms.

Going forward, it is important for future studies to utilize invasive species in their research. By using invasive species when possible, we limit the mortality of native individuals by science. This will help eliminate some of the stress native populations experience. The research conducted here could also spur future research involving microplastics in freshwater systems in the Lower Rio Grande Valley. Because of the differing flow rates of the resacas, it would be interesting to see if microplastics are congregating at higher numbers in resacas with lower or no flow. It is also important that further research be done on the Rio Grande since some of the resacas contain diverted stormwater from the Rio Grande. Another avenue which could be pursued is collaboration between the United States and Mexico in observing microplastics in resacas on both sides of the border. Mora et al. 2001, conducted a study involving heavy metals in resacas in Matamoros, Mexico and Brownsville, Texas so there is precedent for a study of this degree. Our research will add to the limited literature available of the microplastic situation in freshwater systems of South Texas and will hopefully catalyze more scientific research in this

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area as well as awareness in both the public and scientific community of this very serious problem.

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BIOGRAPHICAL SKETCH

Wesley D. Franklin earned his B.S. in marine biology from Southern Arkansas University in 2019. Wesley was awarded his M.S. in Ocean, Coastal, and Earth Sciences in May of 2021 and plans on pursuing a career as a fisheries biologist.

Email: wesleyfranklin0889@gmail.com