Identification and Quantification of Microplastic Pollution in Water Samples and Four Species of Fish from the Mississippi River

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QUANTIFICATION AND IDENTIFICATION OF MICROPLASTIC POLLUTION IN WATER SAMPLES AND FOUR SPECIES OF FISH FROM THE MISSISSIPPI RIVER

A Thesis
Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science

in

The Department of Oceanography and Coastal Sciences

by
Kerrin Toner
B.S., University of Florida, 2017
August 2020
I would like to express my sincere appreciation to my advisor, Dr. Mark Benfield, who has offered me continued guidance and support throughout my time at Louisiana State University. Thank you for always including me in your microplastic research endeavors and teaching me to grow into the scientist and young professional I am today. I would also like to thank my committee members, Dr. Steve Midway and Dr. Cassie Glaspie, for their continued support throughout the planning and thesis writing process. Thank you for always listening and responding to my many questions and for offering me your R expertise, it was greatly appreciated.

I wish to show my gratitude to the numerous workers of the Louisiana Department of Wildlife and Fisheries, Arkansas Game and Fish Commission, Missouri Department of Conservation, Wisconsin Department of Natural Resources, and Minnesota Department of Natural Resources. Without them, the collection of fish samples for this study would not be possible. I am also indebted to King Chambers of ThermoFisher for his technical support using the FTIR Microscope system and the Maiti Lab for supplying liquid nitrogen.

I would like to thank Dr. Matthew Kupchik and Dr. Rosana Di Mauro for their help in the collection of water samples prior to me starting this project. Thank you, Ahmed Gad for being a great partner on the fish study and Josef Schuster and Emma Guidry for their help in the lab.

This course of work would also not be possible without the funding support of Louisiana Sea Grant and the Lamar Family Foundation and ThermoFisher for funding the FT-IR Microscope system. During the fall 2019 and spring 2020 semesters I was funded with a research assistantship from a grant from the National Fish and Wildlife Foundation.

To the friends I have made at LSU, thank you for being my family away from home. I am forever grateful for the lifelong friendships we have made.

Last, but definitely not least, a big thank you to my Mom, Dad, and sister for their unconditional love and support throughout this journey and for always believing in me. I love you all so much.
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ABSTRACT

Microplastics (≤5 mm) have become a persistent anthropogenic pollutant and a growing environmental concern with evidence of them being found throughout several ecosystems. In recent years, there has been a growing recognition that rivers play a major role in transporting plastics from the land to the sea. The Mississippi River is the largest river in North America, draining land populated by over 100 million people. With high numbers of microplastics found in the waters of the northern of the Gulf of Mexico, it is likely that the Mississippi River is the primary source of these plastics. This study quantifies and identifies microplastics, sizes 0.5–5.00 mm, in the lower Mississippi River, as well as documents microplastics in four species of fish from the river: bluegill (Lepomis macrochirus), flathead catfish (Pylodictis olivaris), largemouth bass (Micropterus salmoides) and shortnose gar (Lepisosteus platostomus). Microplastic concentrations (n m⁻³) and masses (mg m⁻³) measured within the river were combined with river discharge measurements to estimate the annual flux of microplastics from the lower Mississippi River, and to estimates inputs near Baton Rouge, New Orleans, and the petrochemical zone in between these two cities. Polymer composition of putative microplastic particles, both from water samples and fish, were confirmed using Fourier- transform infrared spectrometer (FTIR). The mean annual flux of microplastics out of the Mississippi River (0.5 mm–5.00 mm) is estimated to be 328 billion particles, weighting approximately 811,870 kg. Within the four species of fish there was an overall underlying trend of increasing polymer richness with distance downstream in the Mississippi River. However, distance downstream from the source did not have the same underlying positive effect on the number of fibers found within fish. Both polymer richness and fiber count varied
among the four fish species. The findings of this study provide baseline data on microplastic pollution within the Mississippi River and that fish are indeed ingesting microplastics. Further research is needed to understand whether ingestion was active or incidental and what, if any adverse effects this level of ingestion has on fish health and the potential for food web transfer.
CHAPTER 1. GENERAL INTRODUCTION

Since the start of the plastic industry in the 1950s, global production of plastic has continued to grow exponentially over the years (Clark et al., 2016), causing plastics to become ubiquitous pollutants in ocean and freshwater environments (Barnes et al., 2009; Li et al., 2018; Phillips & Bonner, 2015). The earliest reports of floating plastic pollution date back to the early 1970s, with debris found in the Sargasso Sea (Carpenter & Smith, 1972) and in coastal waters of the Northwest Atlantic (Colton et al., 1974).

Plastics consist of chains of polymeric molecules derived from hydrocarbons (Shah et al., 2008; Sul et al., 2014). These synthetic materials are lightweight, durable, and inexpensive (Sul et al., 2014), making them the perfect material for a wide variety of household and industrial products. However, the same characteristics that make plastics beneficial to society also makes them a long-lasting problem for the earth’s natural environments (Cauwenberghe et al., 2015).

Plastic debris comes in all sizes, ranging from meters (macroplastics) all the way down to millimeters (microplastics) and even microns (nanoplastics) (Barnes et al., 2009). Microplastics are defined as being ≤5 mm in diameter (Moore, 2008) and can be categorized into two groups; primary and secondary (Li et al., 2018). Primary microplastics are purposely manufactured to be small in size (Cole et al., 2011; Li et al., 2018). Examples of these include exfoliants in cosmetic products, raw resin pellets (nurdles) used in the plastic manufacturing process, and microbeads used for abrasive blast cleaning (Lehtiniemi et al., 2018). Secondary microplastics are tiny pieces of plastics derived from larger plastics breaking apart (Cole et al., 2011). This fragmentation is due
to physical, biological and chemical processes that weaken the structural integrity of the larger plastic over a prolonged period of time (Cole et al., 2011).

Primary plastic pollution has been linked to industrial outlets since the 1970s, when researchers first called for restrictions within the petrochemical industry (Hays & Cormons, 1974). Louisiana is home to hundreds of petrochemical plants, a number of which are located on the 85-mile stretch of land between Baton Rouge and New Orleans, in an area referred to as the Chemical Corridor or Cancer Alley (Singer, 2011). Some of these petrochemical plants are the production sites of raw resin pellets or nurdles, which are often spilled into the surrounding environment during the production and transportation process (Karlsson et al. 2018).

Compared to what is known about microplastic accumulation in marine environments, far less data are available on microplastics in freshwater systems (Lambert & Wagner, 2018). However, the concentrations that have been reported in estuaries, lakes (Fischer et al., 2016) and rivers (Klein et al., 2015; McCormick et al., 2016; Moore et al. 2011) reveal that these systems, rivers especially, are just as polluted with plastics as marine environments (Peng et al., 2017; Li et al., 2018). Lebreton et al. (2016) was among the first to model plastic concentrations in rivers and quantify how much plastics these rivers are inputting into oceans on a global scale. Lebreton et al. (2016) estimated rivers input 1.5–2.4 million tons of plastic debris into the ocean each year, using data from waste management, population density and hydrological information (Lebreton et al., 2016). Schmidt et al., (2017) estimated a wider but higher range of plastic concentrations in rivers, with concentrations being anywhere from 0.41–4.0 x 10^6 t y^-1.
High microplastic concentrations in aquatic environments are usually associated with densely populated areas. A study by Mani et al. (2015) measured 11 locations over an 820 km stretch along the Rhine River and found higher microplastics concentrations near very populated areas. Despite population densities along the northern Gulf of Mexico (Gulf) being relatively low, high microplastic concentrations were found in the waters of the northern Gulf (Di Mauro et al., 2017). This study area is highly influenced by the Mississippi River plume, suggesting that the river is a major conduit of these plastics (Di Mauro et al., 2017). With the Mississippi River being the largest drainage basin in North America, containing a population of over 100 million people, including 7 major metropolitan areas, the potential for large amounts of microplastics originating from within the basin and ending up in waters of the northern Gulf of Mexico is extremely high.

Louisiana is home to hundreds of petrochemical plants, a number of which are located on the 85-mile stretch of land between Baton Rouge and New Orleans, in an area referred to as the Chemical Corridor or Cancer Alley (Singer, 2011). Some of these petrochemical plants are the production sites of raw resin pellets or nurdles. During the production process nurdles Microp lastics are known to harbor and absorb persistent organic pollutants (POPs), both intrinsic to their manufacturing process and those present in aquatic systems (Teuten et al., 2009). POPs are highly toxic and may originate from herbicides, pesticides, solvents, and pharmaceuticals (Rochman, 2015). The hydrophobic surface chemistry of microplastics allows POPs to be easily absorbed. As microplastics become smaller in size their surface area to volume ratio increases, allowing them to absorb large quantities of POPs (Güven et al., 2017). All plastic polymers have the ability to absorb
POPs, however, polyethylene has been shown to absorb more than other polymers (Rios et al., 2007)

Microplastics can be very similar in size to planktonic organisms (Di Mauro et al. 2017), the natural food of zooplankton and numerous other marine organisms. Studies show that fish can potentially ingest microplastics when exposed to them in aquatic environments, either directly or indirectly when feeling on planktonic organisms (Cole et al., 2013). Research on microplastic ingestion in marine fish dates back to the 1970s (Colton et al., 1974), with a boom in research at the turn of the century. A study by Foekema et al. (2013) sampled more than 50 fish from 7 different species found in the North Sea and found microplastic particles in 5 of the 7 species with a percent occurrence rate of only 2.6%. However, a study looking at 10 species of marine fish in the English Channel had an occurrence rate as high as 36% (Lusher et al., 2017). While studies on marine fish still dominate, research is starting to expand its focus to include microplastic ingestion in freshwater species. Sanchez et al., (2014) studied gudgeon (Gobio gobio), a freshwater species in France, and found 12% of the fish to be contaminated with microplastics. A study by Phillips & Bonner (2015) found microplastics in 8% of fish sampled within freshwater drainages in South and Southeast Texas.

Once ingested microplastics may cause physical and chemical harm to fish. Physical harm includes false satiation, digestive blockage and choking (Derraik, 2002; Hoss & Settle, 1990). Chemical harm includes the leaching of POPs and the plastic's plasticizers, like bisphenol A (Güven et al., 2017; Teuten et al., 2009), which are then at risk to biomagnify throughout the food chain causing issues with growth rate (Lwange et
al., 2016), increased immune response (Moos et al., 2012), low fecundity (Sussarellu et al., 2016), and loss of appetite (Lusher et al., 2017; Rochman et al., 2013).

Despite the many research efforts focused on microplastics, less than 4% of the microplastic-related studies are associated with freshwater environments (Lambert & Wagner, 2018). This gap in the literature is the motivation behind this study. My research is designed to provide an estimate of the quantity and morphological types of microplastics flowing through the Mississippi River from the Baton Rouge to New Orleans area (Chapter 2). I also quantified microplastic ingestion within four species of fish common throughout the Mississippi River drainage basin (Chapter 3).
CHAPTER 2. THE MISSISSIPPI RIVER: A MAJOR CONDUIT FOR MICROPLASTICS INTO THE GULF OF MEXICO

2.1. Introduction
Since the 1950s, global production of plastic has grown exponentially (Clark et al. 2016). As a consequence, plastics have become ubiquitous pollutants in marine and freshwater environments (Barnes et al., 2009; Li et al., 2018; Phillips & Bonner, 2015). Plastic debris ranges in size from >5 mm (macroplastics) to ≤5 mm (microplastics and nanoplastics) (Moore, 2008; Barnes et al., 2009). Microplastics can be categorized into two groups; primary and secondary (Li et al., 2018). Primary microplastics are purposely manufactured to be small in size (Arthur et al., 2009; Cole et al., 2011; Li et al., 2018). Examples of these include exfoliants in cosmetic products (Zitko & Hanlon, 1991), raw resin pellets (nurdles) used in the plastic manufacturing process, and microbeads used for abrasive bead blasting (Sul & Costa, 2014). Secondary microplastics are tiny pieces of plastics derived from larger plastics breaking apart (Cole et al., 2011; Sul & Costa, 2014; Shah et al., 2008). This fragmentation is due to physical, chemical and biological processes weakening the structural integrity of the larger plastic over a prolonged period of time (Cole et al., 2011; Shah et al., 2008).

The earliest reports of floating plastic pollution dates back to the early 1970s, with debris found in the Sargasso Sea (Carpenter & Smith, 1972) and in coastal waters of the Northwest Atlantic (Colton et al., 1974). The majority of plastic pollution in the oceans originates from terrestrial sources (IUCN, 2018). Rivers are important conduits for this waste into the oceans (Lebreton et al., 2017; Schmidt et al., 2017). Most studies of riverine microplastic fluxes into oceans have examined rivers in Asia or Europe (Lahens et al., 2018; Lebreton et al., 2017). A list of the top 20 most plastic polluting rivers globally
Lebreton et al. (2017) does not include any North American waterways. A few studies have examined North American rivers, specifically the St. Lawrence River (Castañeda et al., 2014); although this study was based on sediment, rather than water column samples), the Ottawa River (Vermaire et al., 2017), and smaller systems (Moore et al., 2011; Yonkos et al., 2014; McCormick et al., 2016).

Semi-enclosed seas receiving riverine inputs or with substantial coastal populations have been reported to contain high concentrations of microplastics. Examples include the Mediterranean Sea (Collignon et al., 2012; Cózar et al., 2015), Black Sea (Aytan et al., 2016), and the Baltic Sea (Gewert et al., 2017). A study by Di Mauro et al. (2017) reported that the waters off the Louisiana coastline contained high concentrations of microplastics. The Gulf of Mexico receives runoff inputs from its coastal communities as well as water from the Mississippi River, which is the largest river system in North America and drains a catchment containing a population in excess of 100 million people. The samples collected by Di Mauro et al. (2017) were within the plume of the Mississippi River and they raised questions about the role of the river in transporting microplastics to the Gulf of Mexico. Despite being the largest river system in North America, the microplastics associated with the Mississippi have only recently been examined. A few studies have examined microplastics in smaller waterways that ultimately enter the Mississippi River. McCormick et al. (2014) documented high concentrations of microplastics in the North Shore Channel (NSC), Illinois, which flows into the Mississippi River via the Illinois River. High microplastic concentrations (15.99 n m\(^{-3}\)) appeared associated with the wastewater treatment plant based on differences in concentrations above and below the plant. The first study directly examining microplastics
in the Mississippi was an embargoed thesis by Martin (2018), whose abstract reported very high concentrations of microplastics in the Mississippi River. Martin (2018) included small fibers and reported a mean microplastics concentration of 11.6 n L\(^{-1}\) (11,600 n m\(^{-3}\)) resulting in an estimated annual flux into the Gulf of Mexico of over 4.6 quadrillion microplastic particles. A recently published study by Scircle et al. (2020) estimated microplastic concentrations in the Mississippi River and its tributaries. Concentrations from this study were even higher than Martin (2018), with estimates ranging from 15 microplastics particles per L (15,000 m\(^{-3}\)) for Greenville, MS to 38 microplastic particles per L (38,000 m\(^{-3}\)) for the New Orleans area (Scircle et al., 2020).

This paper describes the results of a microplastics sampling program conducted in the lower Mississippi River between Baton Rouge and New Orleans during 2016–2017. This study was designed to characterize the concentrations and masses of microplastics within the river and to combine these data with river discharge measurements to estimate the contributions by Baton Rouge, New Orleans, and the petrochemical corridor between these two cities to the riverine load of microplastics. The concentrations of microplastics and riverine discharge data were combined to estimate the annual flux of microplastics into the Gulf of Mexico, as well as seasonal patterns of microplastic concentrations within the river. The following hypotheses were evaluated: (1) there will be higher concentrations of microplastics in New Orleans water samples compared to Baton Rouge water samples, (2) there will be higher abundances of nurdles at New Orleans sample sites compared to Baton Rouge samples sites, and (3) there will be higher concentrations of microplastics during spring when the Mississippi River is at flood stage.
2.2. Methods

2.2.1. Microplastic Sampling

Samples were collected from the Mississippi River at five stations located between Baton Rouge and New Orleans, Louisiana (Fig. 2.1). Microplastics were collected from the river surface using a Manta net (Brown & Cheng, 1981) modified to be a smaller size fitted with a 333 μm mesh net and a flow meter (General Oceanics 203R). The net was towed behind a small boat during a series of cross-river transects. The vessel position was monitored with GPS. Four replicate tows were collected at each station with a single transit of the river constituting one sample. The volume filtered in each tow was estimated as the product of the linear distance recorded by the flow meter and the submerged frontal area (0.0659 m²) of the net (Table 2.1). Upon retrieval, the net was washed down using river water that was electrically pumped through a 20 μm pleated paper filter. The contents of the net were emptied into a 100 μm stainless steel mesh tray. Large pieces of woody debris were removed from the sample, rinsed with filtered water over the tray, and then discarded. Each microplastic sample from the Manta net was stored in a single, or multiple glass jars (depending on the sample volume), and the jars were placed on ice in a cooler until they were returned to the lab. Upon return to the lab, samples were stored in a refrigerator at 4°C. In total, 48 Manta net tows were taken from Baton Rouge sites and 36 from New Orleans sites. However, during processing several samples were contaminated or partially lost (Baton Rouge n=2 and New Orleans n=7) and these were omitted from further analysis.
Table 2.1. The average volume of water filtered at upstream, midstream and downstream locations for both the Baton Rouge (BR) and New Orleans (NO) sample sites for each sampling date. The volume filtered was estimated as the product of the linear distance recorded by the flow meter and the submerged frontal area (0.0659 m$^2$) of the net filtered and then averaged was taken for the four the tows.

<table>
<thead>
<tr>
<th>Date</th>
<th>City</th>
<th>Location</th>
<th>Avg. Volume Filtered (m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/04/2016</td>
<td>BR</td>
<td>Upstream</td>
<td>46.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Midstream</td>
<td>59.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Downstream</td>
<td>43.81</td>
</tr>
<tr>
<td>04/04/2017</td>
<td>BR</td>
<td>Upstream</td>
<td>49.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Midstream</td>
<td>68.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Downstream</td>
<td>50.04</td>
</tr>
<tr>
<td>05/25/2017</td>
<td>BR</td>
<td>Upstream</td>
<td>47.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Midstream</td>
<td>60.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Downstream</td>
<td>46.24</td>
</tr>
<tr>
<td>07/18/2017</td>
<td>BR</td>
<td>Upstream</td>
<td>48.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Midstream</td>
<td>56.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Downstream</td>
<td>40.79</td>
</tr>
<tr>
<td>11/18/2016</td>
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<td>Upstream</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Downstream</td>
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<tr>
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<td>Downstream</td>
<td>41.27</td>
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<td>05/24/2017</td>
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<td>Upstream</td>
<td>32.67</td>
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<td></td>
<td></td>
<td>Downstream</td>
<td>49.52</td>
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<tr>
<td>08/23/2017</td>
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<td>Upstream</td>
<td>33.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Downstream</td>
<td>38.27</td>
</tr>
<tr>
<td>09/29/2017</td>
<td>NO</td>
<td>Downstream</td>
<td>62.31</td>
</tr>
</tbody>
</table>
Figure 2.1. Sample site locations within the Mississippi river. A: The white box shows the sample sites location within the Mississippi drainage basin. B: Sample sites locations within Louisiana and the close proximity to the Northern Gulf of Mexico. C: The shaded regions indicate Baton Rouge and New Orleans metropolitan areas. The three sample sites in Baton Rouge (upstream: BR-US; midstream: BR-MS; downstream: BR-DS) were chosen according to the city’s wastewater outfalls. The two sample sites for New Orleans (upstream: NO-US; downstream: NO-DS) were selected to bracket the city.
2.2.2. Sample Processing

Each manta net sample was size fractionated by washing the contents through interlocking stainless-steel mesh trays with mesh sizes of 4, 2, 0.5, 0.125, and 0.063 mm, allowing the shortest axis of each particle to pass through the mesh. Each size fraction was then examined visually under an illuminated magnifying lens (2–3.99 mm and 4–5.00 mm size fractions) or a dissecting microscope (0.5–1.99 mm). The two smallest size fractions (0.063–0.124 mm and 0.125–0.499 mm) were not processed since these size fractions were not fully retained in the 333 μm mesh of the net. The large number of plastic particles in the 0.5–1.99 mm fraction necessitated subsampling. I used a Folsom plankton splitter (McEwen et al. 1954) to split the sample into a series of equal aliquots, and one randomly selected aliquot was selected for processing. The number of splits performed depended upon the sample density. All putative plastic fragments from the 2–3.99 mm and 4–5.00 mm fractions were transferred into a vial containing 95% ethanol for storage. These were later dried at 40°C for 24 h and then weighed on an analytical balance (A&D GR-120). Within each size fraction, each putative plastic object was imaged with a digital camera/microscope system and the particles were classified into the following categories: fragments, nurdles, foams, films, and fibers (Fig. 2.2).

2.2.3. Polymer Analysis

Individual putative plastic particles from the 0.5–1.99 mm, 2.00–3.99 mm and 4.00–5.00 mm size fractions were further analyzed with an FTIR spectrometer (Thermoscientific iN-5) equipped with an attenuated total reflectance (ATR) accessory. FTIR analysis uses infrared radiation to characterize microplastics at the molecular level (Frais et al. 2014). The polymer composition of plastic particles was
determined by matching each spectrum with a library of polymer spectra. Non-plastic particles were excluded from further analysis. Due to the uncertainty of how long particles were exposed to natural elements, particles were considered to be plastic if their spectrum had over a 60% match with a polymer spectrum from the pre-existing OMNIC spectral library.

Once plastic polymer compositions were determined for each putative plastic for Baton Rouge and New Orleans sample sites, particles were then organized according to the American Chemistry Council (Undated) resin identification coding system. This system was used to summarize the polymeric composition of the samples (Fig. 2.3).
Figure 2.2. Examples of the five categories of microplastic particles that were found within the size fractions 0.5–1.99 mm, 2–3.99 mm and 4–5.00 mm. Note that the size fractionation method allows the shortest axis to pass through the mesh. The white horizontal scale bars are 1.0 mm and unique to each microplastic. The corresponding scale bar is the closest one to each microplastic.
2.2.4. Data Analysis

Mean microplastic concentrations (n m\(^{-3}\)) and masses (mg m\(^{-3}\)) were calculated for each sampling location for each of the three size fractions as well as a combined category that was the sum of the size fractions 0.5–1.99 mm, 2.00–3.99 mm and 4.00–5.00 mm. Mississippi River discharge data were obtained from the USGS gauges located at Baton Rouge (07374000) and Belle Chasse (07374525) for the 331 day period (Nov 4, 2016–Sep 29, 2017) that corresponded to the sampling period. The former is located near the east bank of the river between our Baton Rouge upstream and midstream sites while the latter gauge is located downriver of New Orleans near the New Orleans downstream station (Fig. 2.1). These gauges report discharge (ft\(^3\) s\(^{-1}\)) at 15 min intervals. Discharge data were converted from (ft\(^3\) s\(^{-1}\)) to (m\(^3\) s\(^{-1}\)) and all observations from each day were averaged at each location and the daily mean was then multiplied by 86,400 to estimate mean daily discharge (m\(^3\) d\(^{-1}\)).

The mean daily fluxes of microplastic abundance and mass from the Mississippi River into the Gulf of Mexico were calculated by multiplying the overall mean concentrations and mean masses of microplastic samples collected from both cities by the mean daily discharges (m\(^3\) d\(^{-1}\)) over a 331-day period. The mean daily discharge was the average of the New Orleans discharge and the Baton Rouge discharge after the latter had been corrected for losses at the Davis Pond and Caernarvon Diversion.

The cumulative flux of microplastics for both abundance and mass were calculated by summing the mean daily concentrations and masses over 331-day study period. Each of these sums were then corrected from the 331-day sampling period to a 365-day sampling period by multiplying by 365/331.
Statistical analyses were completed using R statistical software (R Core Team, 2020). Using the glm function in the stats library (R Core Team, 2020), Poisson generalized linear models (GLM) were used to model microplastic particle counts for Baton Rouge and New Orleans samples, polymer type abundances within Baton Rouge and New Orleans samples, nurdle abundances within the two cities, and seasonal patterns in microplastic concentrations. A Poisson GLM models count data and explains which independent variables have an effect on the dependent variables (R Core Team, 2020). Assumptions for this model included; dependent variable (microplastics) is count data, counts are positive integers, and the data is Poisson distributed (R Core Team, 2020). Using the emmeans library and function in R (Lenth, 2020), means comparisons were performed to determine statistical significance in microplastic counts between the two cities, polymer type abundance between Baton Rouge and New Orleans, and change in microplastic concentrations seasonally. To determine a significance difference in nurdle abundances between Baton Rouge and New Orleans, a Poisson $t$-test was implemented using the glm function in the stats library (R Core Team, 2020), in which the family argument was specified to be Poisson and the nature of the covariates resulted in the linear predictor of the model to be equivalent to a $t$-test.

2.3. Results

All of the 46 samples from Baton Rouge contained putative microplastic particles in at least one or more of the three size fractions (0.5–1.99 mm, 2.00–3.99 mm and 4.00–5.00 mm). Of the 29 samples from New Orleans, only one sample did not contain any putative microplastic particles, with the remaining 28 containing putative microplastic
particles in one or more of the three size fractions. A total of 719 putative microplastics were analyzed using a FTIR spectrometer to confirm polymer compositions.

All seven resin codes from The American Chemistry Council (Undated) resin identification coding system were present in the samples (Fig. 2.3). For Baton Rouge, high density polyethylene (HDPE, Code 2) was most abundant (31.83%), followed by polystyrene (PS, Code 6) (27.30%), polypropylene (PP, code 5) (20.44%), low density polyethylene (LDPE, code 4) (13.10%) and Other Plastic (Code 7) (11.86%). PS was significantly more abundant than PP within Baton Rouge water samples ($p=0.01$). For New Orleans, the most abundant polymer was HDPE at 36.17% followed by PP (24.87%), PS (16.17%), Other (12%), and LDPE (9.22%). There was no significance between polymer abundance within New Orleans water samples. Polyethylene terephthalate (PETE, Code 1) and polyvinyl chloride (PVC, Code 3) were least abundant in both Baton Rouge and New Orleans sample sites with percent occurrences all less than 1.5%. When looking at polymer abundances between sites, there was only a significant difference between Baton Rouge PS and New Orleans PP ($p=0.04$), with PS being more abundant in Baton Rouge.
Figure 2.3. Microplastic percent occurrences broken down by morphological plastic type (inner ring) and polymer composition (outer ring) for each sampling location for both Baton Rouge and New Orleans. Sampling location, mean microplastic concentration and mean microplastic mass located inside of each sunburst diagram.
Microplastics were divided into morphological types (foam, fiber, fragment, film, and nurdles) (Fig. 2.2). Nurdle concentrations were higher in both the New Orleans upstream (0.059 ± 0.053 n m⁻³) and downstream (0.042 ± 0.037 n m⁻³) sample sites, compared to the concentrations from the Baton Rouge upstream (0.033 ± 0.024 n m⁻³), midstream (0.023 ± 0.017 n m⁻³) and downstream (0.028 ± 0.024 n m⁻³) sample sites (Table 2.2). The amount of nurdles found in New Orleans samples sites was significantly different ($p=0.02$) than the amount of nurdles found within Baton Rouge samples.

Microplastic concentrations for all size classes remained relatively consistent throughout the year (Fig. 2.4). The collection dates for both Baton Rouge and New Orleans fell within fall, spring, and summer with no sampling dates falling within astronomical and meteorological winter. For fall, spring, and summer there was no significant difference in microplastic concentrations ($p$-values $> 0.05$).

Microplastic concentrations for the 0.5–1.99 mm size class were higher than microplastics concentrations (n m⁻³) reported for the the larger two size classes (Fig. 2.4). As the microplastics got smaller in size they also increased in abundance for both Baton Rouge and New Orleans microplastic samples (Fig. 2.5).

Overall, there were higher total concentrations of microplastics in New Orleans (0.773 ± 0.230 n m⁻³) compared to Baton Rouge (0.554 ± 0.098 n m⁻³). However, the difference in the total abundance of microplastics between the two cities was not statistically significant ($p=0.19$).
Table 2.2. Concentrations of microplastics by morphological plastic-type for Baton Rouge and New Orleans sample sites. Margin of error are the 95% C.I. for each plastic size fraction at each city’s sample locations.

<table>
<thead>
<tr>
<th>City</th>
<th>Sample Location</th>
<th>Foam</th>
<th>Fiber</th>
<th>Fragment</th>
<th>Film</th>
<th>Nurdle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baton Rouge</td>
<td>Upstream</td>
<td>0.105 ± 0.060</td>
<td>0.025 ± 0.013</td>
<td>0.262 ± 0.067</td>
<td>0.043 ± 0.026</td>
<td>0.033 ± 0.024</td>
</tr>
<tr>
<td></td>
<td>Midstream</td>
<td>0.112 ± 0.056</td>
<td>0.025 ± 0.024</td>
<td>0.241 ± 0.128</td>
<td>0.082 ± 0.035</td>
<td>0.023 ± 0.017</td>
</tr>
<tr>
<td></td>
<td>Downstream</td>
<td>0.088 ± 0.063</td>
<td>0.036 ± 0.022</td>
<td>0.402 ± 0.148</td>
<td>0.146 ± 0.056</td>
<td>0.028 ± 0.024</td>
</tr>
<tr>
<td>New Orleans</td>
<td>Upstream</td>
<td>0.111 ± 0.054</td>
<td>0.028 ± 0.022</td>
<td>0.496 ± 0.090</td>
<td>0.036 ± 0.031</td>
<td>0.059 ± 0.053</td>
</tr>
<tr>
<td></td>
<td>Downstream</td>
<td>0.136 ± 0.072</td>
<td>0.078 ± 0.052</td>
<td>0.488 ± 0.196</td>
<td>0.066 ± 0.044</td>
<td>0.042 ± 0.037</td>
</tr>
</tbody>
</table>
Figure 2.4. Microplastic concentrations from Baton Rouge (—) and New Orleans (—). From top to bottom: microplastics 0.5–1.99 mm; microplastics 2–3.99 mm; microplastics 4.0–5.00 mm. Error bars = 95% confidence intervals on the mean. No samples were taken during the winter (December- early March) due to weather and logistical issues.
Figure 2.5. Inverse exponential relationship between microplastic size and abundance for both Baton Rouge and New Orleans microplastic samples. As the size of microplastics decreases, abundances of microplastics increase.

The mean daily discharge of the Mississippi River was highest during the months of May and June (Fig. 2.6). The mean daily flux of microplastics from the Mississippi River fluctuated with changing river discharge (Fig. 2.7), with higher estimates of microplastic particles being discharged during May and June when the river is in flood stage (Fig. 2.7A). Masses of microplastics in the Lower Mississippi River were also estimated to be highest during these months (Fig. 2.7B).
Annually, the Mississippi River discharged an estimated $3.2758 \times 10^{11}$ microplastic particles within the size range 0.5–5.0 mm into the Gulf of Mexico (Fig. 2.8A). This represents a mass of approximately 811,870 kilograms each year (Fig. 2.8B). Baton Rouge contributed an estimated $1.1803 \times 10^{11}$ particles annually (177,190 kg annually). New Orleans contributed about $3.8029 \times 10^{10}$ microplastic particles each year. However, mass estimates (Table 2.3) in downstream New Orleans were less than that of the upstream sample site, making it impossible to calculate the New Orleans microplastic mass contribution. To calculate Chemical Alley’s (the petrochemical corridor between Baton Rouge and New Orleans) microplastic contribution, the New Orleans Upstream concentration was subtracted from Baton Rouge Downstream concentrations (Table 2.4) then multiplied by the mean annual river discharge. The same was done to calculate mass
contribution. Chemical Alley contributes about $1.4315 \times 10^{10}$ (688,440 kg) microplastic particles within our measured size range annual
Figure 2.7. The mean daily flux (abundance (A) and mass(B)) of microplastics, sizes 0.5mm–5.00 mm, from the Mississippi River into the Gulf of Mexico over a 331-day period. The 95% C.I. is the product of the mean daily discharge and the upper and lower confidences on microplastic abundance and mass, respectively.
Figure 2.8. Cumulative flux of total microplastic abundance (A) and mass (B), sizes 0.5–5.00 mm from the Mississippi River over a 331-day period. The 95% C.I. is the product of the mean daily discharge and the upper and lower confidences on microplastic abundances (A) and mass (B), respectively. Annual flux estimated by correcting the sum from our 331-day sampling period to a 365-day period.
Table 2.3. Microplastic mass (mg m$^{-3}$) for Baton Rouge and New Orleans sample sites. Margin of error are the 95% C.I. for each plastic size fraction at each city’s sample locations.

<table>
<thead>
<tr>
<th>City</th>
<th>Sample Location</th>
<th>Plastic Size Fraction</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5 – 1.99 mm</td>
<td>2.0 – 3.99 mm</td>
<td>4.0 – 5.0 mm</td>
</tr>
<tr>
<td>Baton Rouge</td>
<td>Upstream</td>
<td>0.016 ± 0.002</td>
<td>0.139 ± 0.103</td>
<td>0.425 ± 0.420</td>
<td>0.580 ± 0.524</td>
</tr>
<tr>
<td></td>
<td>Midstream</td>
<td>0.014 ± 0.002</td>
<td>0.459 ± 0.217</td>
<td>1.307 ± 1.247</td>
<td>1.780 ± 1.467</td>
</tr>
<tr>
<td></td>
<td>Downstream</td>
<td>0.023 ± 0.007</td>
<td>0.374 ± 0.230</td>
<td>0.533 ± 0.528</td>
<td>0.930 ± 0.765</td>
</tr>
<tr>
<td>New Orleans</td>
<td>Upstream</td>
<td>0.023 ± 0.005</td>
<td>0.934 ± 0.777</td>
<td>1.378 ± 0.832</td>
<td>2.335 ± 1.614</td>
</tr>
<tr>
<td></td>
<td>Downstream</td>
<td>0.028 ± 0.012</td>
<td>0.469 ± 0.363</td>
<td>1.582 ± 1.370</td>
<td>2.078 ± 1.745</td>
</tr>
</tbody>
</table>

Table 2.4. Mean microplastic concentrations (n m$^{-3}$) for Baton Rouge and New Orleans sample sites. Margin of error are the 95% C.I. for each plastic size fraction at each city’s sample locations.

<table>
<thead>
<tr>
<th>City</th>
<th>Sample Location</th>
<th>Plastic Size Fraction</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5 – 1.99 mm</td>
<td>2.0 – 3.99 mm</td>
<td>4.0 – 5.0 mm</td>
</tr>
<tr>
<td>Baton Rouge</td>
<td>Upstream</td>
<td>0.391 ± 0.040</td>
<td>0.053 ± 0.019</td>
<td>0.023 ± 0.013</td>
<td>0.467 ± 0.071</td>
</tr>
<tr>
<td></td>
<td>Midstream</td>
<td>0.342 ± 0.046</td>
<td>0.082 ± 0.039</td>
<td>0.059 ± 0.041</td>
<td>0.483 ± 0.126</td>
</tr>
<tr>
<td></td>
<td>Downstream</td>
<td>0.569 ± 0.016</td>
<td>0.084 ± 0.035</td>
<td>0.047 ± 0.028</td>
<td>0.700 ± 0.078</td>
</tr>
<tr>
<td>New Orleans</td>
<td>Upstream</td>
<td>0.566 ± 0.116</td>
<td>0.105 ± 0.071</td>
<td>0.059 ± 0.042</td>
<td>0.729 ± 0.229</td>
</tr>
<tr>
<td></td>
<td>Downstream</td>
<td>0.690 ± 0.305</td>
<td>0.088 ± 0.022</td>
<td>0.041 ± 0.022</td>
<td>0.809 ± 0.349</td>
</tr>
</tbody>
</table>
2.4. Discussion

Draining a population of over 100 million people, the Mississippi River is the largest basin in North America, making it a potential conduit of plastic pollution into the Gulf of Mexico. While similar studies have been conducted to quantify microplastic in major rivers (Mani et al., 2016, Vermaire et al., 2017, Moore, 2011, Lebreton et al., 2017) (Table 2.5), this is the first study conducted to quantify microplastics in the lower Mississippi River between Baton Rouge and New Orleans. Previous predictions by Di Mauro et al. (2017) noted that high concentrations of microplastics in the northern Gulf of Mexico are highly influenced by the input of the Mississippi River. Annually, the Mississippi River discharges an estimated $3.2758 \times 10^{11}$ microplastic particles, (0.5–5.0 mm), into the Gulf of Mexico (Fig. 2.7).
Table 2.5. Average microplastic concentrations and average river discharge rates from rivers all over the world, including the Mississippi River.

<table>
<thead>
<tr>
<th>River Name</th>
<th>Continent</th>
<th>Mean Microplastic Concentrations (n m$^3$)</th>
<th>Mean Discharge Rate (m$^3$ s$^{-1}$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamsui</td>
<td>Asia</td>
<td>10.10–70.40</td>
<td>209.10</td>
<td>Wong et al. 2020</td>
</tr>
<tr>
<td>Danube</td>
<td>Europe</td>
<td>0.04–10.60</td>
<td>6498.70</td>
<td>Lebreton et al. 2017</td>
</tr>
<tr>
<td>Ofanto</td>
<td>Europe</td>
<td>0.90–13.00</td>
<td>162.99</td>
<td>Campanale et al. 2020</td>
</tr>
<tr>
<td>Rhine</td>
<td>Europe</td>
<td>1.85–4.92</td>
<td>2300.00</td>
<td>Lebreton et al. 2017</td>
</tr>
<tr>
<td>Los Angeles</td>
<td>North America</td>
<td>3,473</td>
<td>6.40</td>
<td>Moore et al. 2011</td>
</tr>
<tr>
<td>Mississippi *</td>
<td>North America</td>
<td>0.47–0.81</td>
<td>16791.89</td>
<td>Present Study</td>
</tr>
<tr>
<td>Mississippi</td>
<td>North America</td>
<td>38,000</td>
<td>16791.89</td>
<td>Scircle et al. 2020</td>
</tr>
<tr>
<td>Ottawa</td>
<td>North America</td>
<td>1.35</td>
<td>1950.00</td>
<td>Vermaire et al. 2017</td>
</tr>
<tr>
<td>San Gabriel</td>
<td>North America</td>
<td>169.54</td>
<td>5.38</td>
<td>Moore et al. 2011</td>
</tr>
<tr>
<td>Maipo</td>
<td>South America</td>
<td>0.65</td>
<td>92.31</td>
<td>Lebreton et al. 2017</td>
</tr>
</tbody>
</table>
A recent study by Scircle et al. (2020) estimated microplastic concentrations in the Mississippi River to be 38 microplastic particles per L (38,000 m$^{-3}$) for the New Orleans area. Although they saw a similar pattern of microplastic concentrations increasing downstream, the concentrations calculated by Scircle et al. (2020) included microplastic particles that were much smaller (30 μm) than the 500 μm lower limit used in the present study. Scircle et al. (2020) also used a different sampling method that used a very small sample volume (~1L). Their estimates of the microplastic concentrations were much higher than the concentrations of microplastics found within this study. The present study's estimates of microplastic concentrations to be 0.729 ± 0.229 n m$^{-3}$ and 0.809 ± 0.349 n m$^{-3}$ for upstream and downstream New Orleans (Table 2.2), respectively, with all of our sample sites averaging less than 1 microplastic particle per m$^3$ (~0.001 microplastic particle per L). Given the use of Scircle et al. (2020) small sample volume (~1L) it is difficult to compare the two studies. Size spectrum theory can be applied to microplastics and it is clear from the present study that the abundances increase exponentially with decreasing size. The extremely high concentrations of very small particles down to 30 μm in the Scircle et al. (2020) study are quite possible, though it is unlikely that they were able to quantitatively measure the particles in the same size range as the present study due to the low volume of water sampled. Di Mauro et al. (2017) reported concentrations of very small particles, that were primarily fibers, in the northern Gulf in the range 60,000–174,000 m$^{-3}$. Combining data from both Scircle et al. (2020) and the present study supports that contention that the Mississippi River is supplying microplastics to the northern Gulf of Mexico.
It is important to note that particles smaller than the 333um sampling net were not accounted for as they likely passed through the Manta net. This may also explain why fibers had relatively low concentrations in Baton Rouge and New Orleans (Table 2.4). The long narrow shapes and flexible nature of fibers likely allowed for them to slip through the sampling net. Our sampling method also focused on microplastics floating at the surface of the water, which would have generally collected more buoyant polymers. Plastics with densities greater than the density of freshwater water, such as polyethylene terephthalate (PET) and polyvinyl chloride (PVC) found in our samples were likely resuspended in the water column due to the turbulent nature of the water. These polymers are likely underrepresented in our samples. Sampling methods that allow samples to be taken at various depths in the water column could potentially increase estimates of microplastic concentrations in the river.

The petrochemical corridor located between Baton Rouge and New Orleans likely influences the abundances of microplastics, especially nurdles, in water samples taken from the New Orleans sample sites. This hypothesis was supported and there was in fact a significant difference in nurdles found within New Orleans samples compared to the Baton Rouge samples. The increase in their abundance in New Orleans relative to Baton Rouge does provide evidence of their discharge within this industrialized zone of the river between the two cities. Pinpointing where nurdle pollution comes from can help implement tighter pollution regulations on factories creating these plastics. Long term, stricter regulations will hopefully lead to a decrease in the amount of nurdles ending up in the river.
Baton Rouge contributes an estimated $1.1803 \times 10^{11}$ microplastic particles annually, which was higher than New Orleans, $3.8029 \times 10^{10}$ microplastic particles annually. There are at least three potential explanations: (1) the calculated mean annual river discharge for Baton Rouge ($5.0570 \times 10^{11} \text{ m}^3 \text{ y}^{-1}$) was higher than that of New Orleans ($4.7477 \times 10^{11} \text{ m}^3 \text{ y}^{-1}$); (2) the waste water outfalls in Baton Rouge were closer to the sampling sites and microplastics may not have had a chance to fully mix and disperse in Baton Rouge compared to New Orleans; and (3) New Orleans may have lost microplastic particles to the Davis Pond Diversion and the Caernarvon Diversion. Losses at the two diversions were factored into the calculation of overall discharge.

There was no significant difference in concentrations of microplastics seasonally, with concentrations for each size class remaining relatively constant throughout the year (Fig. 2.4). Microplastic concentrations are likely affected by particle distribution within the river. Particle distribution can be influenced by microplastic characteristics, river flow, and water depth (Eerkes-Medrano et al., 2015; Klein et al., 2015). With higher Mississippi River discharge rates during the spring months (May and June) there was more room for the microplastics to disperse throughout the surface waters, hence microplastic concentrations being diluted during this time. Months where river discharge rates were lower, the microplastics had less room to disperse and concentrations were not as diluted. There were no sampling dates that fell within astronomical and meteorological winter for this study. To determine more conclusively whether or not there is any seasonal change to microplastic concentrations, sampling should be conducted in the winter months as well.
With microplastic concentrations remaining relatively constant within the Mississippi River, organisms in the river may be at risk for constant exposure throughout the year. This constant exposure could leave organisms more vulnerable to ingesting these microplastics which could potentially lead to adverse effects on their health (Derraik, 2002; Hoss and Settle, 1990; Sussarellu et al., 2016), however more research is needed to say for certain.

Although microplastic concentrations were higher in downstream New Orleans samples compared to upstream, microplastic mass was not. This may be a result of downstream New Orleans samples having higher abundance of plastics that are less dense such as polystyrene, polypropylene, and low-density polyethylene. A lower mean microplastic mass downstream may also be a result of microplastics having to travel a longer distance to reach downstream sample sites, leaving microplastics exposed to environmental stressors for a longer period of time, thus causing them to be more brittle and break apart into smaller plastics. To calculate the mean microplastic mass contribution from New Orleans, samples <0.5 mm, the lower limit used for this study need to be collected.

Jambeck et al. (2015) estimated the United States plastic input into the sea to be 0.04–0.11 million metric tons per year (4.0x10^7–1.10x10^8 kg y\(^{-1}\)). This estimate includes coastal populations located 50 km within the United States coastline which excludes a large population located within the drainage basin of the Mississippi River that also contributes to plastic inputs into the ocean. These coastal zones produce 32 x 10^6 MT y\(^{-1}\) of mismanaged plastic waste each year (Jambeck et al., 2015). However, river drainage basins produce about 76 x 10^6 MT y\(^{-1}\) of mismanaged plastic waste each year (Schmidt
et al., 2017). Jambeck’s et al. (2015) estimate of US plastic input would likely increase with added plastic loads from the Mississippi River drainage basin. The Mississippi River carries 811.87 MT y\(^{-1}\) (811,870 kg y\(^{-1}\)) of microplastics alone. This estimate does not account for plastics larger than 5.0 mm in size (macroplastics) nor smaller than 0.5 mm. If macroplastics and smaller microplastics were to be included in this estimate, plastic inputs from the Mississippi would be considerably higher. Lebreton et al. (2017) predicted that on average, river plastic export is anywhere from 1.15–2.41x10\(^6\) MT y\(^{-1}\).

There is a need for standardized monitoring of plastic pollution in freshwater ecosystems because comparisons among studies and catchments can be challenging (Lebreton et al., 2017). Some studies estimate concentrations per unit area (n m\(^{-2}\) or n km\(^{-2}\)), while some use per unit volume (n m\(^{3}\)). This difference, combined with the use of different sampling gear and techniques, makes data comparisons complicated.

This study provided an underestimation for microplastic abundances and flux in the Mississippi River. Microplastics less than 0.5 mm and >5 mm were not represented in our samples. Microplastics in the Mississippi River are being discarded directly into the northern Gulf of Mexico. Long-term monitoring of plastic concentration and mass (both macro and micro) in the river would be very beneficial and allow a better understanding of the effects of plastic pollution; identification of top contributors/sources; and planning to help polluters reduce their plastic footprint.
CHAPTER 3. MICROPLASTICS IN MISSISSIPPI RIVER FISHES

3.1. Introduction

Plastic production has increased 560-fold since its start in the 1950s (Plastics Europe, 2012; Rochman et al., 2013). This increase in production is consistent with the prevalence of plastics in aquatic ecosystems (Cole et al., 2011; Rochman et al. 2013). Plastic debris in marine ecosystems was first discovered decades ago and has since been dominated by research focused on marine environments (Cole et al., 2011). However, in more recent years, there has been a growing interest in understanding the dynamics of plastics in freshwater environments and the subsequent effects on the fish that live there (Eerkes-Medrano et al., 2015).

The word plastic means “malleable” or “workable.” Plastics’ ability to be molded into nearly any shape, combined with their high thermal and electrical insulation characteristics and low production cost, make them extremely practical for a variety of uses (Sul & Costa, 2014; Thompson et al., 2009). Plastics consist of chains of artificial polymers made from organic and inorganic raw materials originating from oil, natural gas and coal (Shah et al., 2008). Plastics often have plasticizers added such as bisphenol A and phthalates (Gewert et al., 2015; Shah, et al., 2008). These plasticizers give polymers specific characteristics such as ductility and texture. When plasticizers break down under ultraviolet light, plastics may become brittle and break into smaller fragments. Nonetheless, most polymers have very long lives and can take hundreds of years or more to degrade. This allows for them to persist in aquatic environments for extended periods of time (Gewert et al., 2015; Murphy, 2001). Some of the most common polymers include polyethylene (low density: LDPE) and high density: HDPE), polypropylene (PP),
polystyrene (PS), polyvinyl chloride (PVC), and polyethylene terephthalate (PETE) (Güven et al., 2017; Sul & Costa 2014). Densities of these polymers vary, which determines their position in the water column and their potential availability to aquatic organisms (Browne et al., 2011).

Microplastics are plastics that are ≤5 mm in diameter (Moore, 2008; Neves et al., 2015). These plastics can further be divided into two categories; primary and secondary microplastics (Li et al., 2018). Primary microplastics are raw resin pellets (nurdles) used in the plastic manufacturing process and are purposely manufactured to be small in size (Arthur et al., 2009; Cole et al, 2011; Li et al., 2018). Primary microplastics are also found in facial cleansers and used as bead-blasting media (Fendall & Sewell, 2009; Thompson et al., 2009). Secondary microplastics originate from larger plastics breaking apart over time. When exposed to physical, biological and chemical processes such as UVB radiation in sunlight or the hydrolytic properties of water, the plasticizers in the polymers weaken, causing the plastic’s structural durability to decay (Cole et al., 2011; Moore, 2008).

Fibers are secondary microplastics that are often overlooked but are most abundant in land and aquatic environments. Synthetic fibers shed from clothing during the laundering process and from everyday use (Miller et al., 2017). Microplastic fibers have been reported in all type of aquatic and marine ecosystems including oceans (Lusher et al., 2014), estuaries (Naidoo et al., 2015), lake and rivers (Faure et al., 2015; Mani et al., 2015). Microplastic fibers are three times more prevalent in aquatic systems close to urban centers (Dris et al., 2015) and downstream of wastewater outfalls (Browne et al., 2010), making the Mississippi River a likely hotspot for them.
Microplastics can span the size ranges of phytoplankton and zooplankton, the natural food of zooplankton and other marine organisms. When these small plastics enter aquatic environments, they become bioavailable to the organisms that ingest them, along with any absorbed contaminants (Jabeen et al., 2017). Ingestion of microplastics has been documented in multiple species of pelagic and demersal marine fishes (Lusher et al., 2013; Miranda & Carvalho-Souza, 2016; Neves et al., 2015; Romeo et al., 2015; Vendel et al., 2017) as well as other marine organisms such as mussels (Browne et al., 2008), copepods (Cole et al., 2013), and sea cucumbers (Graham & Thompson, 2009). There has been a growing amount of available data regarding microplastic ingestion in freshwater fishes in recent years. A study by Park et al. (2020) investigated microplastic pollution within six species of fish from the Han River and its tributaries in South Korea. Among the six species of fish were common carp (Cyrinus carpio), Japanese crucian carp (Carassius cuvieri), bluegill (Lepomis macrochirus), largemouth bass (Micropterus salmoides), Amur catfish (Silurus asotus) and northern snakehead (Channa argus), with all species of fish containing 4–48 microplastic particles fish⁻¹ (Park et al., 2020). The variation in concentration of microplastics between fish was explained by the fish’s preferred feeding habits, with certain feeding habits, such as bottom feeding, leading to higher concentrations of microplastics within the fish’s intestines (Park et al., 2020). Similar findings were seen in a study on the bottom feeding European flounder (Platichthys flesus), with 75% of the sampled flounder contained microplastics compared to only 20% of the pelagic European smelt (Osmerus eperlanus) that were sampled (McGoran et al., 2017).
The percent occurrences of microplastics within freshwater fish species have varied greatly among studies. A study including bluegill (*Lepomis macrochirus*) and longear sunfish (*Lepomis megalotis*), found 45% of the fish to contain microplastics in their stomachs (Peters & Bratton, 2016). A study looking at gizzard shad (*Dorosoma cepedianum*) and largemouth bass (*Micropterus salmoides*) within two hypereutrophic drinking water reservoirs in Illinois, found 100% of the fish to be contaminated with microplastics (Hurt et al., 2020). The percent microplastic occurrence for these two studies are higher compared to past studies researching freshwater species, such as the French study, looking at gudgeon (*Gobio gobio*) in urbanized streams, that found 12% of the sampled fish to be contaminated with microplastics (Sanchez et al., 2014). And another study looking at 44 different species of freshwater fish within freshwater drainages that enter the Gulf of Mexico from Texas and found that only 8% had microplastic contamination (Phillips & Bonner, 2015).

The ingestion of microplastics can lead to both physical and chemical hazards. Physical hazards include choking, digestive blockages and false satiation (Derraik, 2002; Hoss & Settle, 1990), while some chemical hazards include decreased growth rate (Lwanga et al., 2016), increased immune response (Moos et al., 2012), low fecundity (Sussarellu et al., 2016), and a decrease in food consumption (Lusher et al., 2017, Rochman et al., 2013). Microplastics are known to collect and produce persistent organic pollutants (POPs), the former can be inherent to their manufacturer and the latter, waterborne in aquatic environments (Teuten et al., 2009). POPs include herbicides, pesticides, solvents and pharmaceuticals, all originating from agriculture and industrial processes. Non-polar POPs are readily adsorbed to the hydrophobic surface chemistry.
of plastics and since surface area to volume ratios of microplastics increase inversely with size, they have the potential to transfer large quantities of POPs to the fish that ingest them (Moore, 2008).

The aim of this chapter is to evaluate the morphological types of plastic and plastics polymers found in the stomachs of four species of fish common to the Mississippi watershed. Two hypotheses were evaluated: (1) there will be an increase in polymer richness in fish moving downstream in the Mississippi River and (2) fiber abundance within fish will increase moving downstream in the Mississippi River.

3.2. Methods

3.2.1. Fish Sampling

Four species of fish were chosen for this study based on their widespread availability throughout the Mississippi watershed, their ecological importance, and variability in trophic guilds (Table 3.1). Fish samples were collected by states agencies located along the Mississippi River watershed including Louisiana Department of Wildlife and Fisheries (LDWF), Arkansas Game and Fish Commission (AGFC), Missouri Department of Conservation (MDC), Wisconsin Department of Natural Resources (WDNR), and Minnesota Department of Natural Resources (MNDNR). Sampling of fishes took place between spring and fall for the 2018 season. Due to high water levels during the 2019 sampling season, samples were only able to be collected in late fall from all agencies except LDWF. No samples were received from LDWF during 2019 sampling season.

Fish were collected from locations throughout the mainstem of the Mississippi River. Sampling locations included Minneapolis, MN, La Crosse, WI, Cape Girardeau,
MO, Caruthersville, MO, and St. Francisville, LA (Fig. 3.1). All fish were collected using electrofishing, except for flathead catfish in St. Francisville, LA and shortnose gar in Caruthersville, MO (Table 3.2). Those fish were caught using hoop nets (1.2m and 4.5m) and gillnets (1.5m), respectively. Frozen fish samples were either shipped via the mail or collected in person. For agencies located further away from Louisiana (WDNR and MNDNR), fish samples were packed in coolers with dry or regular ice and shipped overnight or within two days. For the agencies located closer to Louisiana (LDWF, AGFC, and MDC), a road trip was made to collect fish samples during both the 2018 and 2019 sampling season. Upon collection, fish were separated into labeled bags and immediately placed in ice filled coolers. Once back at the lab, fish remained in the labeled bags and were immediately placed in a chest freezer until they were ready to be processed.

Table 3.1. Species collected for microplastic sampling in the Mississippi River. All species are commonly found throughout the watershed.

<table>
<thead>
<tr>
<th>Species</th>
<th>Common Name</th>
<th>Trophic Guild</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pylodictis olivaris</td>
<td>Flathead catfish</td>
<td>Benthic piscivore</td>
</tr>
<tr>
<td>Lepomis macrochirus</td>
<td>Bluegill</td>
<td>Insectivore/Particulate zooplanktivore</td>
</tr>
<tr>
<td>Lepisosteus platostomus</td>
<td>Shortnose gar</td>
<td>Piscivore</td>
</tr>
<tr>
<td>Micropterus salmoides</td>
<td>Largemouth bass</td>
<td>Piscivore</td>
</tr>
</tbody>
</table>

Table 3.2. Sampling locations and methods used to collect fish samples.

<table>
<thead>
<tr>
<th>State</th>
<th>City</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Collection Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minnesota</td>
<td>Minneapolis</td>
<td>44.8312</td>
<td>-93.0049</td>
<td>Electrofishing</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>La Crosse</td>
<td>43.8138</td>
<td>-91.2519</td>
<td>Electrofishing</td>
</tr>
<tr>
<td>Missouri</td>
<td>Cape Girardeau</td>
<td>37.3059</td>
<td>-89.5191</td>
<td>Electrofishing</td>
</tr>
<tr>
<td>Missouri</td>
<td>Caruthersville</td>
<td>36.1931</td>
<td>-89.6556</td>
<td>Electrofishing and gillnets</td>
</tr>
<tr>
<td>Louisiana</td>
<td>St. Francisville</td>
<td>30.7358</td>
<td>-91.4960</td>
<td>Electrofishing and hoop nets</td>
</tr>
</tbody>
</table>
Figure 3.1. Location of sample sites within the Mississippi River. Size of the circles represents the number of total fish collected at that site from both the 2018 and 2019 sampling season. Locations listed from top to bottom: Minneapolis, MN, La Crosse, WI, Cape Girardeau, MO, Caruthersville, MO, and St. Francisville, LA.
3.2.2. Sample Processing

Each fish was thawed for 24 hours prior to dissection. Once thawed, each individual fish was given a sample ID, measured for total length (mm), and weighed (g). Individual fish were dissected by removing their stomach, intestine, otoliths, liver and a piece of white muscle tissue. The stomach and intestines were weighed (g) and then placed in separate clean glass jars and stored in a freezer. Once ready to be digested, stomachs were removed from the freezer and broken down using a method by Foekema et al. (2013). The jars were filled with a 10% potassium hydroxide (KOH) solution, sealed tightly and placed in a warm water bath set to 60°C for 24 hours. After the 24 hours or until the stomach was completely liquified. After the 24 hours, contents of the sample were filtered through one or more (depending on sample density) 20μm nylon net filter paper using a vacuum filtration system. Excess contents of the jar were rinsed using filter deionized (DI) water into the filtration system. Filter paper was placed directly into a petri dish, sealed tightly and labeled. The petri dishes were then placed in a drying oven set at 60°C for 24 hours.

3.2.3. Polymer Analysis

Individual putative plastic particles found within each fish sample were further analyzed using Fourier transform infrared (FTIR) spectroscopy. FTIR analysis uses infrared radiation to characterize microplastics at the molecular level (Frais et al., 2014). Each filter paper was first examined visually under a dissecting microscope. If a putative plastic was visually identified it was then removed using stainless steel forceps and processed through the FTIR spectrometer. Depending on the size of the plastic either the ThermoScientific iS5 equipped with an attenuated total reflection (ATR) accessory or the
ThermoScientific Nicolet iN10 was used. In the latter, particles were placed on a gold-plated slide. To determine polymer composition of plastic particles, spectra taken from each particle were compared to a library of polymer spectra from the OMNIC (ThermoFisher) software library. Particles were considered to be plastic if their spectrum had over a 60% match with a polymer spectrum from the pre-existing OMNIC spectral library. Total numbers of fibers and their polymeric composition were summarized for each fish.

3.2.4. Data Analysis

Two separate multinomial logistic regressions were used to model polymer richness and fiber count within fish moving downstream. A multinomial logistic regression explains the relationship between the possible output of the dependent variables, polymer richness and fiber count (categorical: 0, 1, 2), and the independent variables, fish species and location in the river (river mile). Two-tailed z-tests were performed to determine statistical significance between polymer richness within species and river location as well as fiber count within species and river location. Predicted probabilities of polymer richness and fiber count were plotted against river mile and compared among species moving downstream in the Mississippi River.

Due to the large number of zeros in the data (no microplastics in a fish), the relationship between total microplastics and river discharge rate was modeled using a zero-inflated Poisson regression. The river discharge data were obtained from the USGS water gauges located along the Mississippi River (USGS, 2020). For sample locations that did not have a USGS gauge, data was taken from the next closest gauge on the river. These gauges report discharge (ft³ s⁻¹) at 15 min intervals. Data was averaged for each
day at each location to provide daily mean discharges. This was done for each of five sampling sites, Minneapolis, MN, La Crosse, WI, Cape Girardeau, MO, Caruthersville, MO and St. Francisville, LA (Fig. 3.1).

3.3. Results

A total of \(n=229\) fish were sampled from the mainstem of the Mississippi River with \(28\) (12.23\%) of those fish containing one or microplastic particle in their stomach. Microplastics were found in all four species of fish. Microplastics were present in 12 out of 89 bluegills (13.48\%), 3 out of 31 flathead catfish (9.68\%), 7 out of 55 largemouth bass (12.73\%), and 6 out of 54 shortnose gar (11.11\%) (Table 3.4).

Table 3.4. Species, number of fish collected, number of fish with microplastics present, percent occurrence of microplastics within each species, and the average number of microplastics found within each species.

<table>
<thead>
<tr>
<th>Fish Species</th>
<th>Number of Fish</th>
<th>Number of fish with Microplastics</th>
<th>% of fish with Microplastics</th>
<th>Mean Microplastics per fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluegill</td>
<td>89</td>
<td>12</td>
<td>13.48%</td>
<td>0.16</td>
</tr>
<tr>
<td>Flathead catfish</td>
<td>31</td>
<td>3</td>
<td>9.68%</td>
<td>0.10</td>
</tr>
<tr>
<td>Largemouth bass</td>
<td>55</td>
<td>7</td>
<td>12.73%</td>
<td>0.25</td>
</tr>
<tr>
<td>Shortnose gar</td>
<td>54</td>
<td>6</td>
<td>11.11%</td>
<td>0.13</td>
</tr>
<tr>
<td>Total</td>
<td>229</td>
<td>28</td>
<td>12.23%</td>
<td>0.17</td>
</tr>
</tbody>
</table>

The morphological types of plastic found within the fish were fibers, fragments, and foams (Fig. 3.2). Bluegills had the highest overall count of fibers at 9 fibers, followed by largemouth bass with 6, and shortnose gar with 2 (Fig. 3.3). No fibers were found within flathead catfish. Microplastic fragments were found in all four species. Largemouth bass had the highest overall counts of fragments at 7, followed by bluegill and shortnose gar with 5, and flathead catfish with an overall count of 3. Foam particles were only found in bluegill and largemouth bass.
Figure 3.2. Microplastics found in fish stomachs from the Mississippi River. Fiber (A) and fragment (B) found within shorthose gars, black bar = 1mm for scale. C-G are fibers and fragments found within in largemouth bass, black bar = 1mm for scale.
Figure 3.3. Total counts of the morphological plastic type found within bluegill (BG), flathead catfish (FHC), largemouth bass (LMB), and shortnose gar (SNG) sampled from the Mississippi River.

All the major categories of plastic polymers from The American Chemistry Council (Undated) resin identification coding system were found in at least one or more of the four fish species being studied (Fig. 3.4). Polyethylene terephthalate (PETE) and low-density polyethylene (LDPE) were present in bluegill and flathead catfish, respectively. Polypropylene was the only polymer found within all four species. High-density polyethylene (HDPE) was present in bluegill, flathead catfish, and largemouth bass but absent in shortnose gar. Bluegill, largemouth bass, and shortnose gar all had plastics
present from the polymer category labeled “Other.” This category was a catch-all category for all other types of plastic polymers outside of the six major polymers.

Figure 3.4. Total counts of microplastic polymer composition found within bluegill (BG), flathead catfish (FHC), largemouth bass (LMB), and shortnose gar (SNG) sampled from the Mississippi River.

There was an overall trend of increasing polymer richness with distance downstream. Moving downstream in the Mississippi River, the odds of finding multiple polymer types in fish vs. only finding one polymer type increased by a factor of 1.0016 for each mile traveled ($p=0.01$; Fig. 3.5). There was some evidence that moving downstream in the Mississippi River was associated with increased odds of finding one polymer type in fish vs. finding no polymers, though this was not significant ($p=0.06$; Fig. 3.5). Species type also had an effect on polymer richness. For bluegill and largemouth bass, the odds of finding multiple polymer types in fish vs. only finding one polymer type increased by a
factor of 6.65 \((p=0.0054)\) and 18.78 \((p=0.0003)\), respectively. For flathead catfish and shortnose gar, the odds of finding multiple polymer types in fish vs. only finding one polymer type decreased by a factor of 0.004 \((p<0.0001)\) and 0.004 \((p<0.0001)\), respectively. There was no significant impact of species on the odds of finding one polymer type in fish vs. finding no polymer types, for any of the fish species studied here.

Location in the river did not have the same overall underlying effect on fiber count as it did polymer richness. The odds of finding a single fiber in fish vs. finding no fibers increased by a factor of 1.0016 for each mile traveled downstream the Mississippi \((p=0.01; \text{Fig. 3.6})\) but there was no significant impact of river mile on the odds of finding multiple fibers in fish vs. finding one fiber in fish \((p=0.64)\). Species type also had an effect on fiber count. For bluegill and largemouth bass, the odds of finding multiple fibers in fish versus only finding one fiber increased by a factor of 7.55 \((p=0.04)\) and 24.17 \((p=0.0008)\), respectively. For shortnose gar, the odds of finding multiple fibers in fish vs. finding one fiber decreased by a factor of 0.004 \((p<0.0001)\). The odds of finding one fiber in fish versus finding no fibers in fish was not significant in the species largemouth bass \((p=0.18)\) and shortnose gar \((0.76)\) but was significant in bluegill \((p=0.0020)\). Flathead catfish were left out of the multinomial logistic regression because zero fibers were found within their stomachs.

There was no statistical difference between river discharge rate at each sampling location and the amount of microplastics found within the fish \((p=0.13)\).
Figure 3.5. Probability of polymer richness within bluegill (BG), flathead catfish (FHC), largemouth bass (LMB), and shortnose gar (SNG) moving downstream in the Mississippi River.
Figure 3.6. Probability of fiber count within bluegill (BG), flathead catfish (FHC), largemouth bass (LMB), and shortnose gar (SNG) moving downstream in the Mississippi River
3.4. Discussion

Although the percent occurrences of microplastics were low, microplastic particles were found within all four species of fish from sample sites throughout the Mississippi River. Of 229 fish collected, 12.23% contained microplastics. This proportion of microplastic contamination is consistent with microplastic proportions reported in a river fish study by Sanchez et al. (2013), as well as other studies done on estuarine species (Dantas et al., 2012; Ramos et al., 2012) and marine fishes (Possatto et al., 2011; Romeo et al., 2015). However, the percent occurrence for this study is lower than those reported in other studies done on freshwater species, which range from 20%–70% (Hurt et al., 2020; McGoran et al., 2017; Peters & Bratton, 2016). These studies varied in species being sampled, laboratory methods, and polymer analysis on putative microplastics, complicating comparisons with these studies.

The fish used in this study were collected during periods of record flooding for the Mississippi River (US Department of Commerce & NOAA, 2019). The highwater levels in 2018 and 2019 could have potentially diluted microplastic concentrations within the river (Scricle et al., 2020). Diluted microplastic concentrations mean less microplastic particles bioavailable to the fish in the river. This could help explain the low percent occurrence of microplastics (12.23%) within the 229 fish used in this study. However, further research would be needed to say this for certain.

The hypothesis that polymer richness and fiber counts would increase downstream due to an increasing number and diversity of sources was supported, and there was an increase in probability of having higher polymer richness or higher fiber counts in fish downstream compared to upstream in the Mississippi River. However, these findings
should be treated with caution, as most of the fish in the study had polymer richness of zero (no polymers or fibers found in the stomach). The sample size of fish with microplastics in their stomachs was low, with only 28 fish having confirmed plastics present. Only one bluegill and one largemouth bass had multiple polymers in their stomachs (polymer richness of two). The highest number of fibers found within a fish was two fibers in a largemouth bass stomach, and two fibers in a bluegill’s stomach. To say with complete confidence that polymer richness and fiber counts increase within fish downstream in the Mississippi, this study would need to be repeated, with a larger sample size.

The polymers found within the stomachs of the fish included PETE, HDPE, LDPE, PP, and PS. Similar polymers were reported in stomachs of fish sampled from three major tributaries of Lake Michigan (McNeish et al., 2018), the Amazon River estuary (Pegado et al., 2018) and several marine fish studies (Lusher et al., 2013; Neves et al., 2015; Steer et al., 2017). The exact sources of the polymers are unknown as samples were taken from multiple locations throughout the river. However, the large urban areas located along the river are likely a major source. A study about microplastic pollution in the Rhine River by Marni et al. (2015) showed sections of the Rhine River near highly populated cities having high microplastic concentrations. Along with surface runoff during storm events, urban areas are home to large wastewater treatment facilities that have been reported to be a source of microplastic pollution (Browne et al., 2011).

Some studies show different trophic guilds linked to differences in microplastic abundances within fish (McNeish et al., 2018; Park et al., 2020). However, the fish in this study belong to different trophic guilds and use different feeding strategies (Table 3.1),
yet microplastics were still found in each fish species. This may indicate that microplastic consumption is unrelated to the feeding habits of the fish. Other studies show similar evidence of trophic guild having no effect on microplastic consumption (Chan et al., 2019; Garnier et al., 2019). It is also possible that the fish were not selectively feeding on microplastics. The small sizes of the microplastics found in the stomachs would make them difficult for a fish to detect given the generally low visibility of Mississippi River water. It is therefore possible that the fish unintentionally consumed microplastics either directly when trying to consume prey or indirectly through the prey they consumed. Further research would be needed to say this for certain. Moreover, data on the abundance, sizes, and types of microplastics potentially available to each species would be needed.

Only secondary microplastic were found within the stomachs of the fish from this study. The fish stomachs contained plastic fragments, fibers, and foams that likely broke apart from larger plastics over time. Foams were only found in bluegill and largemouth bass. Foams have a density less than 1 g cm$^{-3}$ and will consequently float. Both bluegills and largemouth bass are known to feed on floating prey (Keast, 1978; McNaught & Hasler, 1961). Fibers were also found in bluegills. Other studies have documented a high proportion of fibers in bluegills. A study of bluegills from the Brazos River Basin (Texas) reported 96% of the particles sampled to be microplastic fibers (Peters & Bratton, 2016) and 100% of the particles sampled in bluegills from the Han River in South Korea were fragments (Park et al., 2020). Fiber and fragments were also the only types of microplastics found in the largemouth bass sampled by Park et al. (2020). No primary microplastics were found within the fish even though the Mississippi River is used as a transportation system for many industries, with petrochemical products being one of its
most popularly moved goods (Howard & Norrell, 2020). Primary microplastics such as raw resin pellets or nurdles are one of these petrochemical products. The reason that no nurdles were found within the fish stomachs is unclear; however, it may be because primary microplastics are less abundant in aquatic environments compared to secondary microplastics (Hale et al., 2020).

Evidence suggests that persistent organic pollutants (POPs) absorbed by microplastics can be transferred to fish (Besseling et al., 2012; Browne et al., 2013; Rochman et al., 2013); however, the effects of microplastic pollution on the health of fish and the humans consuming these fish is still not fully understood. The toxicity level of the chemical transfer depends on the sorption capacity of the plastic ingested, the surrounding environment, the organism that ingests the microplastic and the plastics’ residence time within the organism (Eerkes-Medrano et al., 2015; Phillips & Bonner, 2015). Though all of the polymers analyzed within this study contain crude oil derived chemicals and are potentially hazardous to fish (Lithner et al., 2011), the residence time of microplastics within fish has been reported to be low, thus minimizing the fish’s exposure to the absorbed chemicals within the plastic and lessening the likelihood of potential food web transfer (Phillips & Bonner, 2015).

This study along with a companion thesis (Gad, 2020) provided the first examination and confirmation of microplastic pollution in the stomach of fish from the Mississippi River. Microplastics were present in four species of fish from the Mississippi River however, further research is needed to determine if microplastics are having an adverse effect on fish health, if there is potential for food web transfer, and to determine the exact residence time of microplastics in a fish’s gastrointestinal tracts.
CHAPTER 4. CONCLUSIONS

Overall, this study is designed to provide an estimate of the quantity and morphological types of microplastics flowing through the lower Mississippi River (Chapter 2) and investigate microplastic ingestion within four species of fish common throughout the Mississippi River drainage basin (Chapter 3). With a majority of research efforts focused on microplastic pollution in marine environments, it is important to understand microplastics in freshwater environments, particularly rivers, as rivers are likely major conduits of microplastics into oceans.

The following hypotheses were evaluated for Chapter 2 of this study: (1) there will be higher concentrations of microplastics in New Orleans water samples compared to Baton Rouge water samples, (2) there will be higher abundances of nurdles at New Orleans sample sites compared to Baton Rouge samples sites, and (3) there will be higher concentrations of microplastics during spring when the Mississippi River is at flood stage. The results from this chapter suggest that the Mississippi River is a source of microplastic pollution into the northern Gulf of Mexico with an estimated 328 billion microplastic particles being discharged each year. There were higher concentrations of microplastics in New Orleans compared to Baton Rouge, however this difference was not significant. Not only are microplastic concentrations in the river relatively constant between cities, they are also relatively constant throughout the year, with no significant difference in spring concentrations compared to the other seasons studied. Nurdle abundances within New Orleans compared to Baton Rouge were significantly higher, indicating that the petrochemical corridor between the two cities likely influences nurdle abundance within the river.
The hypotheses tested for Chapter 3 were: (1) there will be an increase in polymer richness in fish moving downstream in the Mississippi River and (2) fiber abundance within fish will increase moving downstream in the Mississippi River. Percent occurrences of microplastics within the fish were low, with only 12.23% of the fish collected containing FTIR confirmed microplastics. Both hypotheses were supported as there was an increase in probability of having higher polymer richness or higher fiber counts in fish downstream compared to upstream in the Mississippi River. However, due to the small sample size of fish with confirmed microplastic, this study would have to be repeated to be able to say with complete confidence that polymer richness and fiber counts increase within fish downstream the Mississippi River.

This study provides some of the first evidence of microplastics in water samples and four species of fish from the Mississippi River. This research is essential in understanding the river’s role in the transportation of microplastics into the Gulf of Mexico as well as microplastics presence in the diets of fish from the river. Long-term monitoring of microplastics in the Mississippi River would be beneficial in helping to identify top microplastic contributors and work toward lessening polluter’s plastic footprint. More research is needed to determine if microplastics are having an adverse effect on fish health and if there is any potential for food web transfer.
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Kerrin Toner was born and raised on Long Island, New York. She graduated from West Babylon Senior High School in 2013 and earned a Bachelor of Science Degree in Marine Sciences in 2017 from the University of Florida. After graduation she worked as a marine science instructor at Seacamp Association Inc. until Hurricane Irma hit the Florida Keys in September 2017. She then returned to the University of Florida and worked as a marine fisheries technician in their Institute of Food and Agricultural Science Laboratory before joining the Master of Science program in the Department of Oceanography and Coastal Sciences at Louisiana State University in August 2018. Kerrin will receive her Master of Science Degree in August 2020. She has recently been accepted as NOAA Coastal Management Fellow and will be working with the US Virgin Islands Coastal Zone Management Program after graduation.