MICROPLASTIC INGESTION OF JUVENILE FISH
IN CORPUS CHRISTI BAY AND UPPER LAGUNA MADRE, TEXAS

A Thesis

By

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This thesis meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

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ABSTRACT

Microplastic pollution and the negative effects of microplastics entering the marine food web has come into the focus of research in recent years. For early life stages of fish this is of particular concern due to their high energy demand, since sufficient food availability is necessary for fast growth and survival. However, not much is currently known about the extent of microplastic pollution in Corpus Christi Bay and the Upper Laguna Madre, and whether it presents a possible risk to juvenile fishes who may ingest microplastic together with similarly sized prey items. This study presents the first baseline information on microplastic pollution in Corpus Christi Bay and the Upper Laguna Madre, which are important nursery areas for fish species such as, Red Drum (*Sciaenops ocellatus*), Atlantic Croaker (*Micropogonias undulatus*) and Mullet (*Mugil* spp).

Juvenile fish from Corpus Christi Bay and Upper Laguna Madre showed several unique differences and spatial patterns. Over 81% of the juveniles had one or more ingested pieces of suspected microplastics. Several species (*Leiostomus xanthurus, Brevoortia* spp. and *Menidia* spp.) had higher mean amounts of suspected microplastics in their digestive tracts, likely due to a difference in feeding guilds or prey preferences. In addition, juveniles collected from highly urbanized areas (Ingleside and Oso Bay) had larger or higher amounts of suspected microplastic in them. This thesis showed that microplastic fibers are regularly found in the digestive tracts of early juveniles of eight species of fish in the Corpus Christi Bay and Upper Laguna Madre area. Further studies are needed to evaluate potential health and survival concerns caused by the documented microplastic ingestion of early juvenile fish.

DEDICATION

This work is dedicated to my parents, James and Debra, for inspiring me to chase after my dreams no matter where they take me. It is also dedicated to Tiffany, Daniel, Jeanna, Clint, Emma, and Tristan, who were a constant source of support, laughter, and inspiration to me during my studies at Texas A&M University-Corpus Christi.

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INTRODUCTION

Plastic production started to become popular in the 1950's for its durability and cost efficiency. Unfortunately, because of its durability, improper disposal of plastic meant it would take longer for it to breakdown and therefore accumulate in the environment. This study investigates the ingestion of suspected microplastic (MP) in the stomach content of eight species of juvenile fish (*Micropogonias undulatus*, *Sciaenops ocellatus*, *Lagodon rhomboides*, *Leiostomus xanthurus, Mugil* spp., *Menidia* spp., *Anchoa* spp., and *Brevoortia* spp.) collected in a South Texas bay and lagoon. The goals of this study were to determine (1) the presence of MP in the diet of juvenile fish and (2) the differences between feeding guilds, species and area. This thesis will serve as a baseline study for this highly urbanized bay system as the cities and towns continues to grow and develop their urbanized areas.

General Early Life History of Fish

In general, there are four life stages of a fish (Houde, 1987). First a fertilized egg which can vary in size and shape but is generally spherical. In this stage, pigmentation and morphology of the embryo starts to develop (Kendall *et al.*, 1983; Richards, 2005). Once hatched, the fish enters the "larval period" (Searcy *et al.*, 2007). Some larval fish can have a yolk sac that provides nourishment over time until it is used up and they switch to feeding on nutritious zoo-/plankton (Houde, 2008). During this transition period, the larval fish starts to develop several body systems (sensory, circulatory, muscular, and digestive systems), pigmentation patterns, and specialized structures (spines and fins) (Kendall *et al.*, 1983). As the larval fish continues to grow, it loses larval characteristics and gains adult-like features (Kendall *et al.*, 1983). This transition is known the juvenile stage and typically occurs after a fish has settled into suitable nursery grounds (Searcy *et al.*, 2007). Transition into the juvenile stage can happen gradually or abruptly depending on what habitat the late larval fish is recruiting to (Kaufman *et al.*, 1992; Hoey and McCormick, 2004). During this period, the juvenile starts to exhibit key characteristics of its adult form, including pigmentation, body shape, fin position, loss of long specialized structures, and later in the stage reproductive system starts to develop (Kendall *et al.*, 1983). The full development of the digestive tract occurs during the transition to the juvenile phase as well (Lazo *et al.*, 2011). This energy intensive process may make the early juvenile stage sensitive to disruptions in feeding success and diet compromised by plastic fibers. Lastly, during the adult stage, the fish shift their energy intake from stomatic growth to gametic growth and may migrate to spawning habitats.

Factors Affecting Survival

There are a variety of physical and biological factors that can influence survivorship during the early life stages of fishes. Different combinations of environmental and biological factors influence survival of early life history stages (Houde, 1987; Anderson, 1988; Leggett and Deblois, 1994). Environmental factors such as temperature (Malloy and Targett, 1991), salinity (Peterson *et al.*, 1999), storms/turbulence (Dower *et al.*, 1997), and currents/flow features (Miller and Kendall, 2009) have been shown to not only influence individual growth, but influence prey production, encounters and accumulations. The biological factors: predation and feeding (Claramunt and Wahl, 2000), then influence growth, survival, and recruitment (Bergenius *et al.*, 2002; Graeb *et al.*, 2004). An individual's growth rate is strongly associated with the amount of food available. The 'critical-period' hypothesis suggests that failing to find suitable feeding conditions shortly after yolk absorption results in mass mortality in a relatively short period of time (1914). Although there are a multitude of factors influencing survivorship during the early life stages of fish, predation and starvation are two of the most common sources of mortality (Graeb *et al.*, 2004).

Both starvation and predation are size-dependent variables and have a strong correlation with growth-rate. It is widely accepted that faster-growing individuals have a greater chance of survival (Houde, 1987; 2008; Do Souto *et al.*, 2019). Currently there are three major contributing factors that provide supporting evidence for this paradigm: size, stage duration, and growth-rate. The 'bigger-is-better' hypothesis (size-based) found that mortality is size-selective (Miller *et al.*, 1988). In other words, smaller fish exhibit a higher mortality rate. The 'stage duration' hypothesis (time based) expands upon this concept by suggesting that faster growing larvae experience a cumulative decrease in mortality because the amount of time they spend in their early life history stages where they are most vulnerable to predation is shortened (Leggett and Deblois, 1994). The 'growth-mortality' hypothesis suggests that risk of predation decreases with an increase in size (Anderson, 1988). Additionally, Hare and Cowen found that increased feeding success led to increased growth that lowered an individual's probability of mortality due to starvation and predation (1997). The longer an individual is in an early life history stage, the more vulnerable they are. Each species in each life stage relies on different prey sources. Cushing found that prey source productions are dependent upon physical conditions (1990). In some cases, juvenile fish can deplete their food source decreasing their growth rate but increase overwintering mortality (Sogard, 1997; Hurst and Conover, 1998; Hales and Able, 2001).

Fish species with Estuarine Dependent Life Cycle

Estuaries are shallow semi enclosed coastal systems where freshwater and saltwater come together and mix (Pritchard, 1967). These systems have high levels of turbidity and can consist of multiple types of habitats within them such as mangroves, salt marshes, oyster reefs and seagrass beds. Seagrass provides various ecological services from increased primary production and water quality to stabilizing the bottom sediment and buffering wave action (Fry and Parker, 1979). Multiple studies have shown seagrass beds to have higher density and diversity of early life stage fish than bare bottom (Heck and Thoman, 1981; Ara *et al.*, 2011). Juvenile reef fish are known to use seagrass beds in the estuaries during their gradual habitat transition from mangroves to reefs (Dorenbosch *et al.*, 2005).

Estuarine dependent species of fish rely on the bay and estuary resources (food and habitat availability) for survival during at least one stage of their life cycle (Tolan *et al.*, 1997). Many commercial and recreational fish species use these systems as nurseries during their late larval and juvenile life stage. Most of these species, like the redfish (*Sciaenops ocellatus*), leave the estuaries and bays to spawn offshore (Pearson, 1929). The larvae will return to the bays and estuaries through coastal inlets along the barrier islands (Boehlert and Mundy, 1988). Once in the bays and estuaries, the larvae will transform or metamorphose into juveniles and settle in a suitable habitat. The juvenile stage in fishes is characterized by being morphologically similar to their adult aspect and by transitioning into the feeding behaviors similar of that in their adult stage (Miller and Kendall, 2009). Benthic and epibenthic feeders will settle out of the water column that their planktonic larvae inhabited onto the bottom, while pelagic species remain to occupy the open water column, but their diet may change compared to their larval stages (Kendall *et al.*, 1983).

General Plastic Pollution

Over the past decade, plastic production and use has dramatically increased by 20-fold, peaking at 311 million metric tons in 2014 (Derraik, 2002; Andrady, 2011; 2016). This increase has led to the introduction of a new type of anthropogenic impact on the environment called plastic pollution. Around 10-20 million tons of plastic find their way into the ocean each year (Raynaud, 2014). Recently, the negative effects of plastic pollution on fisheries and the environment has come into the focus of research (Wright *et al.*, 2013; Kaposi *et al.*, 2014). Each year, plastic pollution causes around $13 billion in damages to the marine ecosystem (Raynaud, 2014). The larger plastics, known as macroplastics (> 500 µm), are known to cause harm to marine life through entanglement (Allen *et al.*, 2012), introduction of invasive species (Rech *et al.*, 2016), and ingestion (Phillips and Bonner, 2015; Poon *et al.*, 2017). However, the physiological consequences of MP (< 500 µm) pollution are much less understood (Setälä *et al.*, 2014; Phillips and Bonner, 2015; Nadal *et al.*, 2016; Vendel *et al.*, 2017).

There are two types of MP: primary and secondary. Primary plastics were purposely made to be small for example in facial-cleaners and other cosmetics (Zitko and Hanlon, 1991) or air-blasting media (Gregory, 1996). Secondary plastics are small pieces that originate from the breakdown of larger plastic (Cole *et al.*, 2011). Microplastic pollution is introduced into waterways and the ocean by runoff, through sewage treatment plants, or the breakdown of large plastic into small pieces (Andrady, 2011). Once MP enters aquatic and marine environments, it is found in bays (Ashton *et al.*, 2010), estuaries (Browne *et al.*, 2010), beaches, coral reefs (Donohue *et al.*, 2001), and the pelagic ocean (Cózar *et al.*, 2014), where it accumulates in rotating currents, gyres (Law *et al.*, 2010; Eriksen *et al.*, 2013), and the deep sea (Galgani *et al.*, 2000). In addition, MP attract harmful chemicals and heavy metals to bind at their surface (Carpenter *et al.*, 1972; Nakashima *et al.*, 2012).

Previous laboratory-based studies have looked at the uptake of MP particles by copepods (Cole *et al.*, 2014), and how mistaken ingestion of MP can transfer up trophic levels (Farrell and Nelson, 2013). A fairly recent study looked at harmful chemicals that bonded to MP, transferring to copepods before transferring to fish larvae (Katzenberger, 2015). A similar study looking at juvenile gobies found that the chemicals that leached out from the MP decreased the predatory performance as well as efficiency of the fish (de Sá *et al.*, 2015). For early life stages, this is of particular concern due to their high energy demand, since sufficient food availability is necessary for fast growth and survival (Thorson, 1950; Hunter, 1981).

Gulf of Mexico and Texas Estuaries and Studies on Fish and Plastic

Several studies have looked at marine debris and MP in the Gulf of Mexico (GoM). The studies range from coastal to offshore and surface water to deep sea. Macroplastic was found along multiple barrier islands in the GoM (Wessel *et al.*, 2019) and in deep sea trawls, with Mississippi Canyon being a focal point of debris (Wei *et al.*, 2012). Similar patterns of MP being found throughout all habitats are emerging. Off the coast of Louisiana on the continental shelf, MP was found with a size range that overlaps with various zooplankton size ranges (Di Mauro *et al.*, 2017). Microplastics have been found in multiple beaches along the gulf coast from the tip Florida (Yu *et al.*, 2018) up the coast to a sea turtle nesting area in Florida (Beckwith and Fuentes, 2018) to Mobile Bay estuary in Alabama (Wessel *et al.*, 2016), to South Padre Island in TX (https://missionaransas.org/nurdle-patrol).

In Texas, Wessel et. al found 10 times more marine debris than the other gulf states (Wessel *et al.*, 2019). Already in the mid to late 1980's, a report mentioned strandings of a *Kogia breviceps* (Pygmy Sperm Whale) on Galveston Island and a *Balaenopter acutorostrata* (Minke Whale) on Matagorda Peninsula, both with a variety of plastic bags and material in their stomachs (Tarpley and Marwitz, 1993). During this time, 42% of the 215 sea turtles that were necropsied were found with various types of plastic in their stomachs (Plotkin and Amos, 1990). In recent years, different species of fish were collected from various watersheds and two bay systems throughout Texas (Fig. 1). Urbanized watersheds were found to have a higher percentage of MP (Phillips and Bonner, 2015) and majority of MP found in the digestive tract of adult fish were fibers (Peters and Bratton, 2016; Peters *et al.*, 2017). However, none of these studies focused on the Texas Coastal Bend (TCB) and on examining juvenile fish

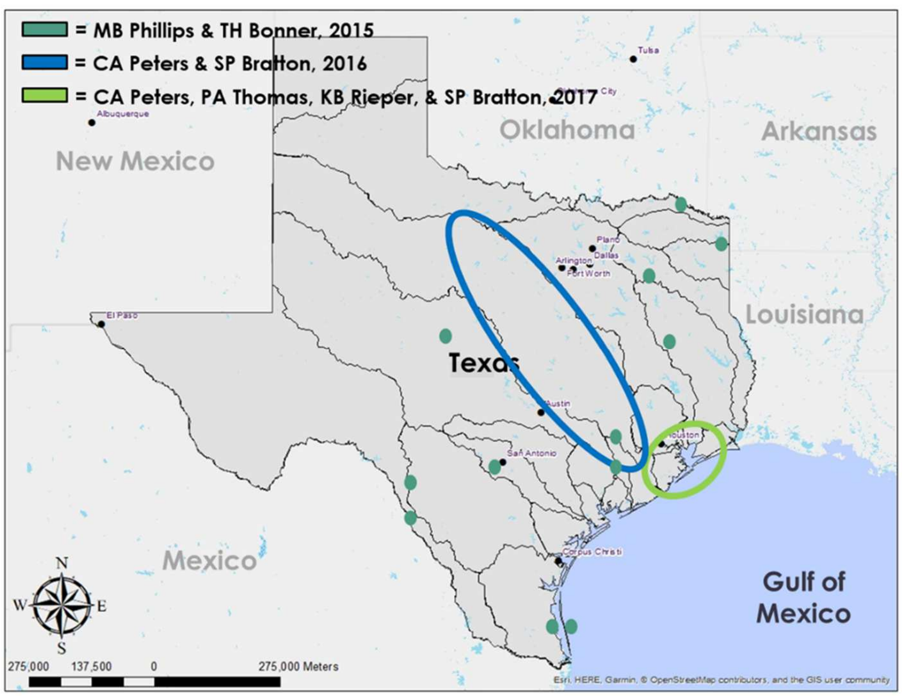

Figure 1. Overlay map showing the study areas from three recent studies on MP ingestion by fish conducted in Texas.

Objectives / Research Questions

This study fills an important knowledge gap on the ingestion of suspected MP pollution by juvenile fish, which has the potential to affect their survival rate. Several species of estuarine dependent juvenile fish were examined from Corpus Christi Bay (CCB) and Upper Laguna Madre (ULM) located in the TCB.

This thesis addressed research question in relation to four study objectives:

Objective 1: Assess the presence of MP in the diet of juvenile fish.

RQ 1.1) Do juvenile fish ingest MP and to what amount?

RQ 1.2) Is the number of ingested MP correlated with body size? and

RQ 1.3) Is maximum and median size of ingested MP increasing with body size?

It is hypothesized that: a) if MP is present in the environment, it will then be present in the fish, and b) a difference in MP ingestion frequency and MP size is correlated with fish size with larger juveniles showing the highest ingestion frequency and largest pieces of MP to smaller juveniles.

Objective 2: Compare differences between two feeding guilds (benthic and planktivore).

RQ 2.1) Is there a difference in MP ingestion between feeding guilds?

RQ 2.2) Is there a difference in the size of the MP between feeding guilds? and

RQ 2.3) Is there color preference in the ingested MP between the feeding guilds?

It is hypothesized that: a) if there are differences in MP uptake between feeding guilds and types, the less selective filter feeders will show higher ingested MP numbers compared to pickers or predators, b) a difference in MP ingestion frequency and size is apparent between the fish sizes within the feeding guilds, with active feeders showing the lowest ingestion frequency but larger pieces of MP compared to passive feeders with smaller pieces of MP, and c) if MP in unselective planktivore feeders is apparent, the number of different colors of MP will be higher than the number of colors in benthic feeders.

Objective 3 Examine the differences between the eight species (M. undulatus, S. ocellatus, L. rhomboides, L. xanthurus, Mugil spp.*, Menidia* spp.*, Anchoa* spp.*, and Brevoortia* spp.*).*

RQ 3.1) Is there a difference in ingested MP between species?

RQ 3.2) Does the body size influence the number of the MP ingested among species?

RQ 3.3) Is there a difference in the size of the MP among species?

RQ 3.4) Is there a color preference in the MP ingested among species?

It is hypothesized that, if there are differences in MP uptake between species, the fish with a narrow diet spectrum will show a lower amount of ingested MP than one who has a broad spectrum, b) if there are differences in species between the relationship of MP size and fish size, the non-selective larger species will have larger pieces of MP, and c) if the ingestion of MP was higher in less selective fish, then the number of different colors of MP should be higher in them than species with a narrow prey spectrum.

Objective 4 Determine if there is a spatial difference in MP pollution between the six areas throughout CCB and ULM.

RQ 4.1) Is there an area, in which species are more prone to ingesting MP?

RQ 4.2) Is there a difference in size of the ingested MP among areas?

RQ 4.3) Are color preferences of a species similar for all areas?

RQ 4.4) Does area influence what type of MP is ingested in the same species?

It is hypothesized that MP pollution of water and in juvenile fish is apparent, and that concentrations are related to the proximity of these potential sources.

MATERIALS AND METHODS

Sampling Locations

Pilot Study Area

From November 2016 to March 2017, a pilot study to determine if MP were prevalent in the TCB bays. Six locations (major bay systems) were sampled (Fig. 2), three coastal inlets (Cedar Bayou, Port Aransas Shipping Channel and Packery Channel), two bays (CCB and Baffin Bay) and a shallow lagoon (ULM). The sampling locations were characterized by a gradient of potential MP pollution sources with a maximum in the central locations (CCB, Packery, Shipping Channel) characterized by high level of urbanization, industrial development, river inflow and exchange with the GoM, whereas the northern (Cedar Bayou) and southern-most locations (Baffin Bay) were surrounded by rural area with little to no settlements.

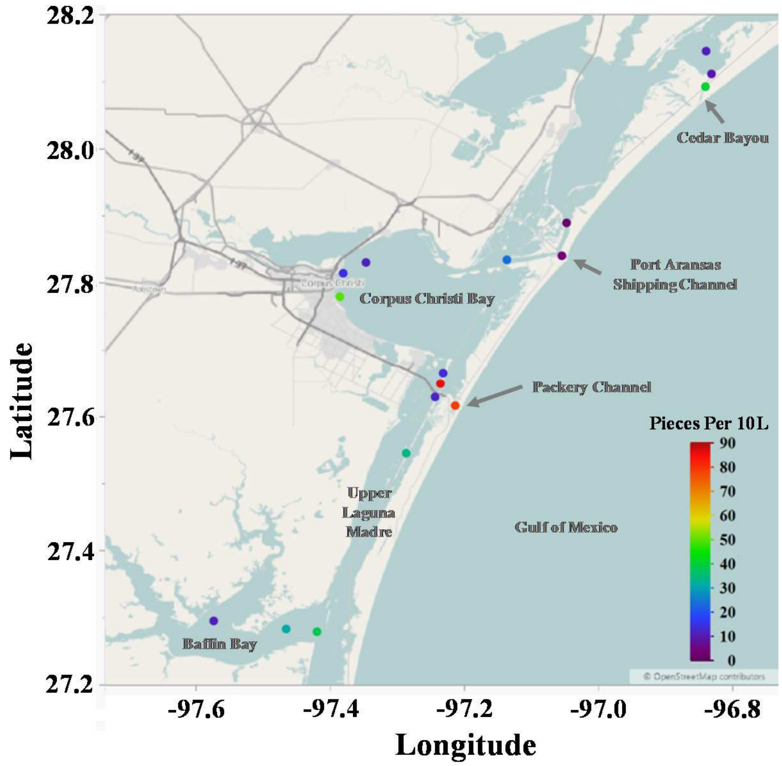

Figure 2. Suspected MP concentrations collected at six locations from November 2016 until March 2017 along the TCB during the pilot study.

Main Study Area

The main study focused on CCB, the central pilot study location. Corpus Christi Bay (Fig. 3) is a primary bay that covers 43,316 ha. It is a shallow bay with an average depth of three to four meters during low tide (Matlock *et al.*, 1982). The bay is surrounded by several cities and a sandy barrier island. Mustang Island is preventing water exchange with the GoM which is limited to two coastal inlets, Packery Channel and Port Aransas Shipping Channel. The residence time of bay water is about one year (Solis and Powell, 1999). The ULM is a shallow hypersaline lagoon that covers 41,040 ha (Matlock *et al.*, 1982) with an average depth of 0.8 meters (Tunnell and Judd, 2001). Unlike CCB, ULM is surrounded by rural area. Padre Island acts as barrier preventing water exchange with the GoM (Tunnell and Judd, 2001).

Six sampling areas (sections within a location) were selected along the shorelines of CCB and the ULM (Fig. 3) characterized by different proximity to potential MP pollution sources, including point sources such as stormwater outfalls and wastewater treatment plants in addition to non-point sources such as plastic waste caused through littering (Lusher, 2015). Central ULM (Area 1) was selected as hypothetically least affected, representing the area furthest away from urban settlements, industry, water exchange with the GoM and minimal freshwater inflow. Area 2 was located on the southeast shoreline in CCB south of Shamrock Island. Of the CCB areas it was the one the furthest away from major urban settlement and active industry, with a single storm water outfall in its vicinity (Fig. 3). Area 3, located near Ingleside by the Bay's public boat ramp, was close to a high density of industrial development (Cheniere Energy Incorporated, Tianjin Pipe Corporation, Voestalpine, Occidental Chemical, Chemours plant, Kiewit Offshore Services, Gulf Marine Fabricators, and Martin Marietta Materials) as well as two towns (Portland and Ingleside). Area 4, at CCB's northwest shoreline near the Indian

Point Pier is influenced is close to downtown Corpus Christ an area with high population density and tourism (North Beach, Lexington, and Texas State Aquarium), close to the Port of Corpus Christi with an agglomeration of petrochemical industry (Valero Bill Greehey Refinery and Corpus Christi Polymers) and wastewater treatment plants (Broadway and Allison). It was also the area closest to freshwater inflow from the Nueces River, whose watershed includes 15 counties including the town of Uvalde. Oso Bay (Area 5), located to the south of CCB, is characterized by a high level of surrounding urbanization (dense settlements, Texas A&M University-Corpus Christi, Naval Air Station, South Padre Island Causeway, wastewater treatment plants). It receives little freshwater inflow from Oso Creek and the Oso Wastewater Treatment plant empties out into Oso Bay. A second wastewater treatment plant, Greenwood Wastewater Treatment, is located right off Oso creek. Area 6 was located where CCB and ULM meet. This area is surrounded by a high level of development, two wastewater treatment plants and the John F. Kennedy Memorial Causeway. Tourism on Padre Island (Waves waterpark, Bob Hall Pier and Whitecap Beach), boat traffic (Intracoastal Waterway and four public boat ramps), and water exchange with the GoM (Packery Channel).

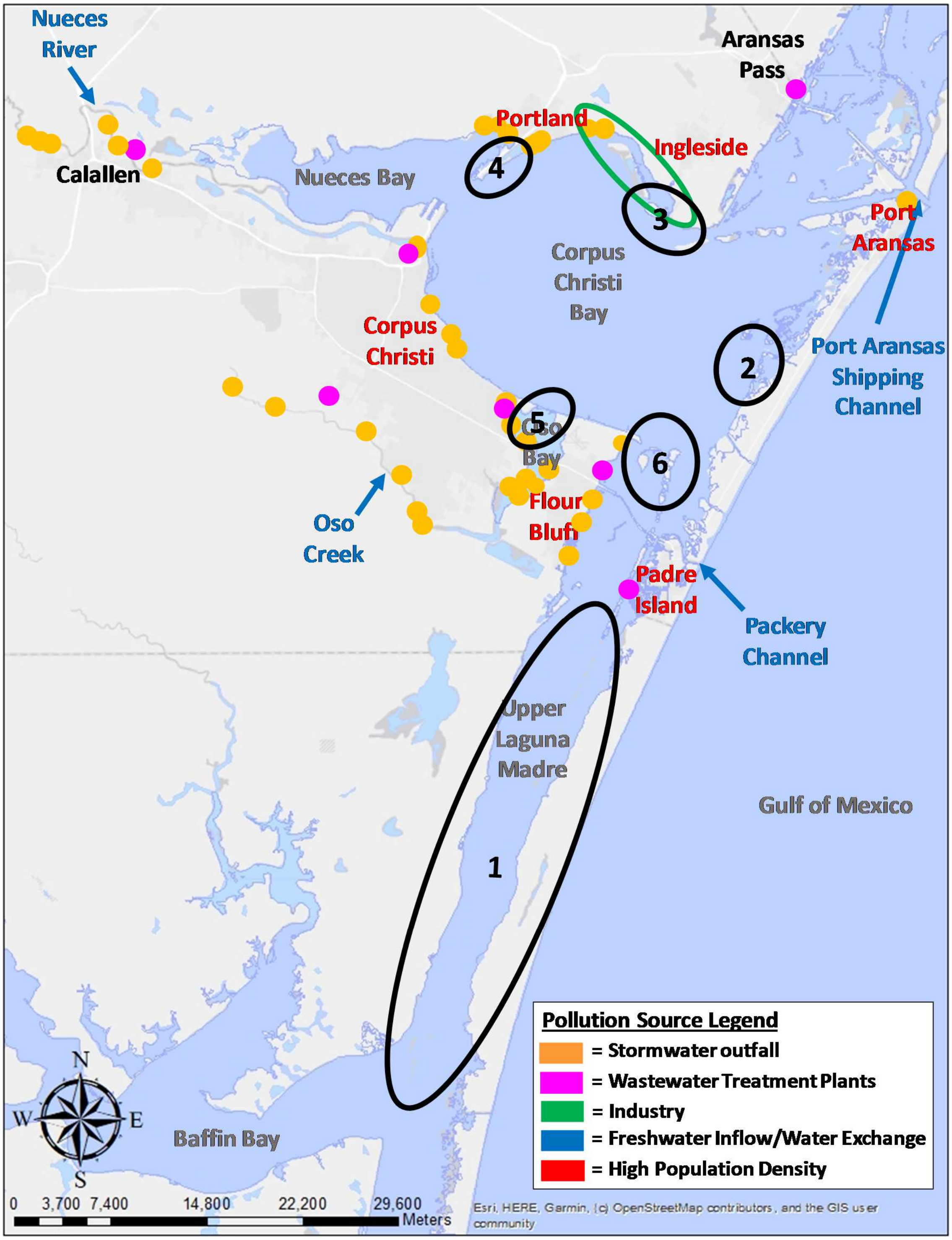

Figure 3. Map of the six study areas in CCB and ULM (black) sampled between February 2017 to May 2018, and different potential MP pollution sources (color coded).

Study Species

A total of eight different fish species/species groups were assessed for MP content in their digestive tracts: *Micropogonias undulatus* (Atlantic Croaker), *Sciaenops ocellatus* (Red Drum), *Lagodon rhomboides* (Pinfish), *Leiostomus xanthurus* (Spot Croaker), *Mugil* spp. (Mullet), *Menidia* spp. (Silverside), *Anchoa* spp. (Anchovy), and *Brevoortia* spp. (Menhaden) (Table 1). Each focus species resembled members of two different feeding guilds, benthic and planktivore feeders. The benthic feeding guild, feeds more toward the bottom of the water column. They feed either on benthic epifauna or infauna such as polychaetes, crustaceans, mollusks, and gastropods. The Planktivore feeding guild can be found feeding toward the top of the water column on zoo-/phytoplankton (Able and Fahay, 2011).

Table 1. Target species investigated for MP ingestion, respective feeding guilds, targeted size range, peak recruitment times of the year and sampling months.

#	Species Name	Common Name	Adult Feeding Guild	Standard Length Range (mm)	Recruitment Months	Sampling Months of Interest
1	*M. undulatus*	Atlantic Croaker	Benthic Pisc-/Carnivore	20 - 60	Feb.-May	Feb.-May
2	*S. ocellatus*	Red Drum	Benthic Pisc-/Carnivore	20 - 90	Nov.-Mar.	Nov.-May
3	*L. rhomboides*	Pinfish	Benthic Pisc-/Carnivore	10 - 40	Apr.-Jul.	Feb.-May
4	*L. xanthurus*	Spot Croaker	Benthic	20 - 90	Mar.-May	Feb.-May
5	*Mugil* spp.	Mullet	Benthic/ Planktivore	20 - 60	Jan.-Aug.	Feb.-May
6	*Anchoa* spp.	Anchovy	Planktivore	10 - 40	All year	Feb.-May
7	*Brevoortia* spp.	Menhaden	Planktivore	20 - 60	Apr.-Jun.	Feb.-May
8	*Menidia* spp.	Silverside	Planktivore	20 - 50	All year	Feb.-May

Field Sampling

Field sampling design

Pilot study sampling design

To assess the MP in the surface water in each location, one sample was taken at every area within the location. A minimum of three areas within each location were visited at least once.

Main study sampling design

To assess the MP in the surface water in each area, a total of 26 water samples were taken in close vicinity of where the juvenile fish sampling was done. Due to time constraint of MP analysis, 18 water samples were analyzed for MP. Water quality data were also collected at each of the water sampling sites within an area, for comparison with MP density at that same area.

To assess the occurrence of MP in juvenile fish, each area was visited at least twice (Table A1). Sampling was performed in seagrass/unvegetated habitats in late November-December for the recruitment of the fall spawners while sampling took place throughout February-May for the recruitment of the spring spawners. For each focus species a target number of 70 specimens per sampling area was set, based on the initial goal to process 35 specimen per species, an estimated ratio of 50:50 between full and empty stomachs lead to the target number. This target number was not always met dependent on the abundance of target species at the respective sampling days. Due to time constraints and time effort required for the processing steps (described below) the final number of analyzed specimens per species and area was reduced to a minimum of 8 individuals.

Field Sampling Methods

Pilot Study Field Sampling

At each of the locations, an 18.92 L carboy of agitated surface water was collected and then filtered using a 40 µm mesh sieve. The whole sample was then transferred into a clean glass jar (29.5 mL) and stored in clean deionized (DI) water for processing back in lab. Water quality was sampled at each site using a multiparameter sonde (YSI EXO) equipped with sensors for temperature (°C), salinity (ppt), dissolved oxygen (mg O_2 L^{-1}), pH and depth (m), that were calibrated before each sampling trip.

Main Study Field Sampling

Microplastic pollution in the first one meter of the water column was sampled by vertical casts of a plankton net (20.3 cm diameter, 20 µm mesh). The net was lowered to one m water depth four times in a row sampling 0.130 m^3 of water. Between each of the four casts the cod end was emptied into a concentrator sieve (40 µm mesh). After the fourth cast, the net was carefully rinsed over the sieve to ensure all the sample was collected. All four cast were condensed into one sample. The whole sample was then transferred into a clean glass jar (29.5 mL) and taken up in clean DI water for processing back in lab. Water quality was sampled at each site using a multiparameter sonde (see 2.3.2.1. for description of sonde) equipped with sensors for temperature (°C), salinity (ppt), dissolved oxygen (mg O_2 L^{-1}), and pH that were calibrated before each sampling trip.

To collect the above outlined numbers of juvenile fish, specimens were sourced from different sampling activities: (1) by collaboration with Texas Parks and Wildlife Department Coastal Fisheries ecosystem teams who provided specimens collected during their routine monitoring trips (Corpus Christi Bay and Upper Laguna Madre), (2) three separate Texas A&M

University-Corpus Christi classes (Fisheries Techniques 2017 (TAMUCC IACUC AUP 02-17), Marine Ecology 2017 & 2018 (TAMUCC IACUC AUP 01-19), (3) collaboration with the Texas State Aquarium (Internal IACUC committee approval), and (4) dedicated sampling trips by the Geist Early Life History Lab (TAMUCC IACUC AUP 01-18).

Standard gears used to collect juvenile fish included bag seines, bottom trawl, epibenthic sled and ECOCEAN CARE light traps. Bag seines were pulled parallel to the shoreline for ~15.24 m before being pulled on shore. Bottom trawls were deployed by boats in water that was 1-2 m deep and pulled for 10 minutes before being pulled in. Epibenthic sleds were pulled by hand in water ~0.5 m deep for 16.6 m. Light traps were deployed at dusk to soak overnight on a new moon and retrieved at dawn the next morning. Target species were pulled from the net catches, sorted, killed humanely according to IACUC approved methods, bagged, tagged and stored on ice and at -20°C until processing in the Geist Early Life History Lab at Texas A&M University-Corpus Christi.

Laboratory Analyses

Water samples

Pilot Study

Samples were processed by filtering them through a sieve tower containing the mesh sizes: 500, 335, 200, 100, and 20 µm. All contents caught on each sieve were rinsed down and into a cleaned glass petri dish with a cover. Each dish was analyzed under a dissecting microscope (Zeiss Stemi 508) and for each suspected MP color and type were recorded.

Main Study

Samples were processed by filtering them through a sieve tower containing the mesh sizes: 500, 335, 200, 100, and 20 µm. All contents caught on each sieve were filtered individually onto a gridded mixed cellulose ester filter membrane with a pore size of 0.45 µm and a diameter of 47 mm. The vacuum filtration system consists of a 500mL graduated funnel with Ace-Thred bottom, 47 mm 70-100 µm fritted disc, nylon retaining ring, nylon adapter, one-liter vacuum flask, 678.18 mm of 12.7 mm diameter reinforced vinyl hose and Chemical Duty Vacuum Pressure Pump (MilliporeSigma). Filters were then placed in a closed 85 mm diameter non-vented petri dish after filtration was complete.

With the petri dish remaining closed, each filter was analyzed for MP under a dissecting scope (Zeiss Discovery V8). The type, abundance, and color of plastic was recorded. A picture of all plastic was taken using a camera (Zeiss Axio 506), which was attached to the dissecting scope. Using Imaging Software (Zeiss ZEN pro), the length (mm) was recorded for fibers, length and width (mm) was recorded for particles/films.

Juvenile fish samples

Samples from all the areas were processed for standard length, total wet weight, wet weight without the guts, and stomach/gut fullness. The stomach and intestine, digestive tract (DT), were separate from the rest of the guts. The DT was placed in one or more prelabeled 1.5 ml microtubes depending on the size/fullness and were then placed in the freezer until further processing.

To digest the organic matter, the DT was processed by using a modified technique of Karami *et al.* (2017). Briefly, once the sample was of out of the freezer, one milliliter of 10%

potassium hydroxide (KOH) will be pipetted into each microtube with the DT. The sample was sealed immediately afterward and placed in a thermal cycler (Eppendorf ThermoMixer C) for incubation at 40°C for two to three hours until most of the organic material was dissolved. After the digest is complete, the samples were diluted with DI water (100-150ml) to reach an approximate concentration of ~0.03% KOH to ensure that KOH would not harm the integrity of the filter membrane during the filtration process. The same filtration system was used for the digestion step as described under 2.4.1.2. Batches of up to 48 DTs were digested during one workday.

Filters were analyzed under a dissecting scope (see water samples main study for description of procedure). In addition, an extra picture was taken of the suspected MP but under a different lighting condition. A fluorescent light adaptor (NightSea Royal Blue Fluorescent) was used and a picture was taken. The fluorescent light was used to help tell the difference between suspected MP and organic material in two ways. First way was by shape. Under a regular light mollusk shells, fish scales/bones, copepod/amphipod antennas, and polychaete setae looked like suspected MP. However, when a picture was taken under the fluorescent light, the shape of the suspected MP became clearer. The second way the fluorescent light helped was by the fluorescent color of a suspected MP. This was helpful while telling the difference between a diatom and a bead. The diatom would fluorescence a reddish orange color around the outer edge and the color would fade towards the center. In a suspected MP, the color (if it fluoresced) would be consistent throughout it all.

Assessment of potential cross-contamination sources – controls for water- /airborne and cross-sample contamination

Contamination accountability

During the processing of the surface water samples and DT, special care was taken to ensure that sample contamination was kept at a minimum by different measures described here. First the number of researchers allowed within a close vicinity of the processing workbench was kept at a minimum and the color of the processor's shirt and pants were recorded for every single processing day. In addition, the ambient microplastic pollution in the laboratory was sampled using blank filters for three potential pollution sources as described in the following:

Waterborne MP contamination

To account for any contamination from the DI water system, three samples were filtered on the 25th September 2018, each consisting of one Liter DI water. The same filtration system and procedure in processing the surface water samples were used.

Airborne MP contamination

To account for airborne MP, the controls consisted of one gridded mixed cellulose ester filter membrane that was placed in a closed non-vented petri dish. This petri dish was then placed in close vicinity of the fish sample. Two separate filters were used for each the fish dissection and the DT digestions steps. Every time the sample was exposed during these processing steps, the petri dish lid was removed, and the control filter was exposed to ambient air in parallel.

Cross-sample MP contamination

To account for cross sample contamination, four control samples were taken in between individual *L. xanthurus*. This species was chosen for the high amounts of inorganic matter that did not dissolve in the digest process. The controls were taken by using the same filtration process as above and filtering 200mL of DI water between individuals that were being filtered on the 25th and 26th of September 2018. The same filtration system and procedure in processing the surface water samples were used for filtering/analyzing the cross-sample contamination.

Control correction of ingested suspected MP

From the three previously described potential cross-contamination sources, source B (airborne contamination during the digestion step) was regarded to have the highest influence on the results, since during the digestion step the exposure time of the DT content to ambient air was longest. A six-step contamination correction procedure was established (Fig. 5) and used to determine the necessary correction factor for each batch (processing day) of DT contents digestions.

First, the size range of the suspected MP length found in the DT was determined. Then, the standard error was either added or subtracted to the minimum or maximum length of suspected MP to account for an error margin (Fig. 6). This was done separately for each color found in the DT samples separate for each processing day. Second, the length of each airborne MP found on the respective control filter was determined separately for each color. If the length of a MP from the control filter was below or above the length threshold established in step one, the respective MP piece was considered as too small or too large to be ingested by the fish and excluded from the subsequent correction procedure. However, if the length of a MP from the control fell within the size range determined during step 1, then it was assumed that there was some airborne contamination in the DT sample. Third, all MP pieces on the control filter within the size range determined during step 1 were summed up, separately for each color. Fourth, the

DT sample(s) to be corrected for airborne MP contamination were selected. Assuming a higher likelihood of airborne contamination to have settled on DT filters containing a higher number of MP, the DT sample(s) with the largest number of ingested suspected MP for each processing day were selected. This was done separately for each color. Fifth and sixth, the lengths of the suspected MP on the control filter were compared with that of the suspected MP on the selected DT filter, and the MP piece that was closest in length to the control filter was then chosen to be excluded from further analyses on DT filter results.

If there was more than one MP that needed to be excluded from the DT samples, then the procedures described in step four through six were repeated until all MP pieces on the control filters were accounted for, always separately for each color and processing day.

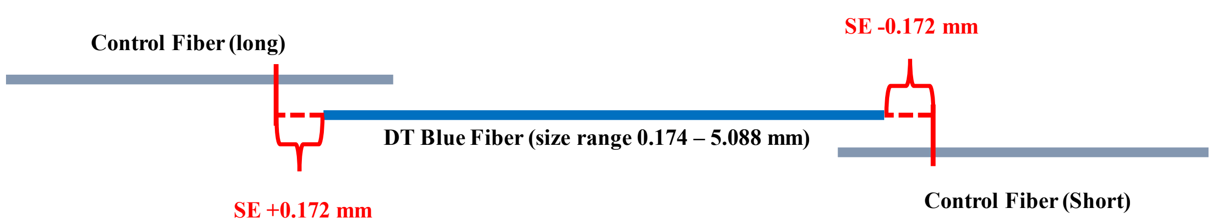

Figure 4. Determination of the upper and lower length thresholds of MP contamination correction. Using blue fibers from airborne contamination control filters during the digestion step for May 23, 2018 as example. The size range for blue fibers are 0.174 – 5.088 mm, ± 0.172 the standard error.

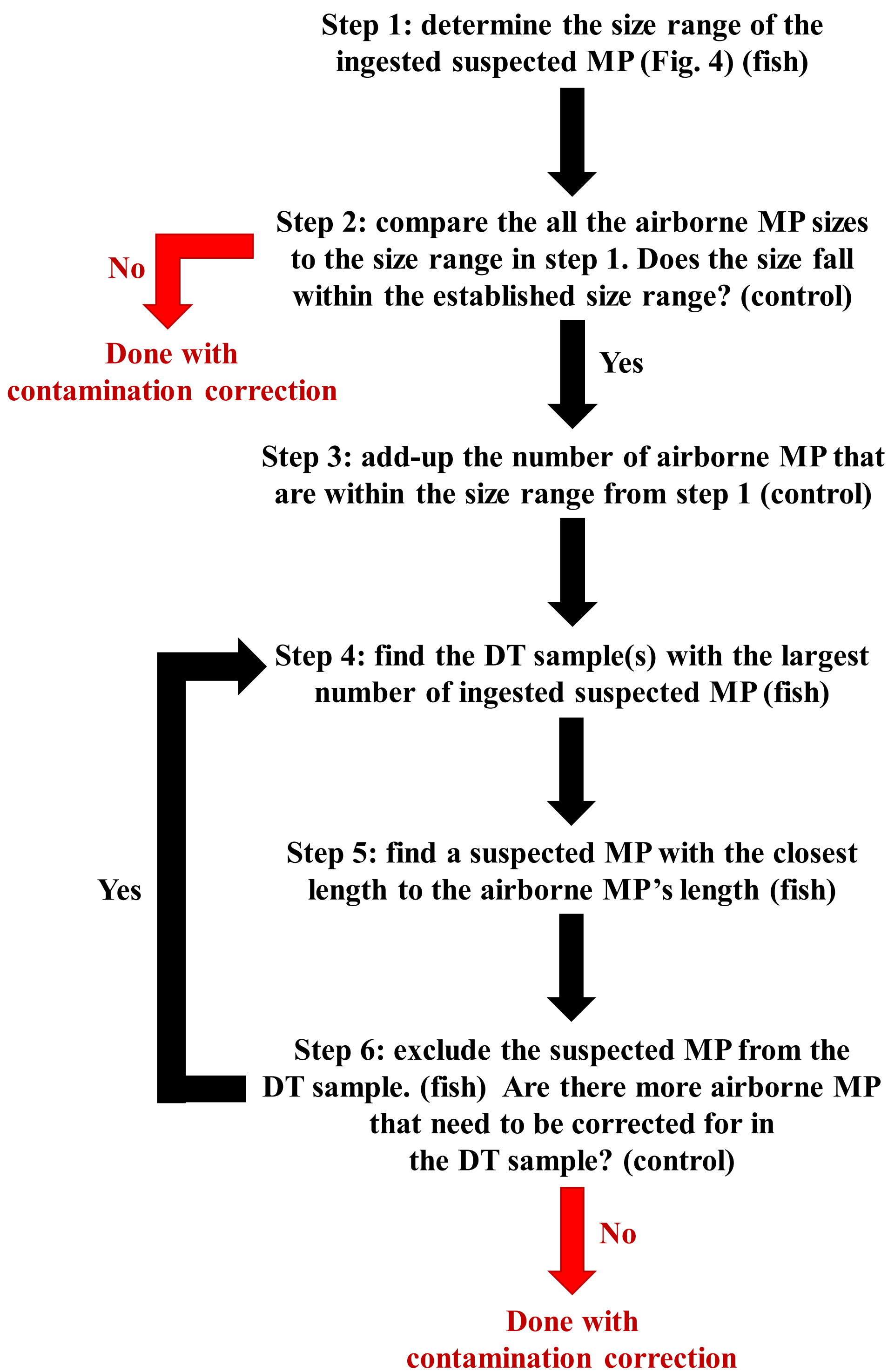

Figure 5. Flow chart of the contamination correction procedure.

Identification of plastic type

A Nicolet iN5 FTIR Micro-Fourier Transform Infrared Spectroscopy (FTIR) microscope (Thermo Scientific) equipped with a germanium crystal was used to examine the chemical composition of the ingested suspected MP. The MPs were placed on an Aluminum EZ-Spot Micro Mount Sample slide using ultra fine tweezers (Dumont SS-SA) and a dissecting microscope. The FTIR-ATR program (OMNIC software), was used to visualize data and identify the plastic type by comparing it to its spectrum database. All the suspected MPs used were stemming from *L. xanthurus* DT samples.

It should be noted, that during the processing of fibers for FTIR analyses several fiber samples were lost during the two main processing steps. The first delicate step was during handling the fiber to transfer it from the filter to the FTIR slide. The second risky step was during the actual FTIR analysis reading when either the fiber was crushed/stuck to the germanium crystal or machine error resulting in crushing the slide.

Statistical Analysis

All statistical analyses were done with the corrected data (see assessment of potential cross-contamination sources for description). To test for differences of ingested suspected MP numbers and MP lengths between feeding guilds, species and areas and the presence of a potential body size-effect several analyses of variance (ANOVA) were calculated. For these, the dependent variables (a) number of suspected plastic ingested, (b) the maximum and (c) the median length of the ingested plastic was log transformed to fit a normal distribution. Then generalized linear regression model were made, and all required assumptions were checked (normality, homoscedasticity and outliers). The Holm's post hoc test was performed for significant factors. This post hoc test was chosen due to the unbalanced sample numbers that were compared.

For examining color preference in ingested suspected MP color between feeding guilds, species and areas, a permutational multivariate analysis of variance (PERMANOVA) was used. This nonparametric statistical analysis method was necessary because the data were not normally distributed. Further post hoc tests were done to reveal significant differences within the feeding guilds, species and areas using Tukey contrast analyses with an $\alpha = 0.01$.

RESULTS

Water Quality

The range and average of the water quality measurements for temperature, salinity, pH, and dissolved oxygen were calculated (Table A2). In CCB, a total of 14 sampling trip were made and water quality data was collected for each of those trips. The overall temperature off CCB ranged from 13.82 to 30.63°C, salinity ranged from 11.00 to 40.08, pH ranged from 7.71 to 8.62 and dissolved oxygen ranged from 6.48 to 12.07mg O_2 L^{-1}. In ULM, at total of four sampling trips were made and water quality data was collected for each of those trips. The overall temperature off ULM ranged from 13.06 to 26.30 °C, salinity ranged from 33.19 to 50.75, pH ranged from 8.02 to 8.35. and dissolved oxygen ranged from 5.10 to 10.07mg O_2 L^{-1}.

Assessment of potential cross-contamination source

All three DI water filters were examined, an overall total of 10 suspected MP was found on the filters. An average concentration of 3.33 ± 0.58 suspected MP L^{-1} of DI water was found, which results in 0.33 MP pieces per 100mL. The majority of the suspected MP were blue (40%) followed by clear (30%).

During the 22 days of fish dissections, at total of 93 suspected MP were found on control filters. They averaged of 3.28 ± 3.046 suspected MP per dissection filter. As for color both blue and clear were the most abundant with a percent total of 33.33% each.

For the controls during the digestion step, a total of 79 suspected MP was found throughout the 30 days of processing. An average 2.56 ± 2.20 of suspected MP were found per filter. The most abundant color was clear, making up a total of 60.76% followed then by blue at 32.91%.

After airborne contamination correction MP size range and standard error was calculated, 34 fibers were assumed to be airborne contaminants from the digestive step and were excluded from the samples.

Microplastic in Water

Pilot Study

Results from three stations within each of the six different bays and inlets in the TCB, show suspected MP were present at all stations (Fig. 6). Packery Channel had the highest density of suspected MP with a mean of 55 pieces of plastic per 10 L. Although the ULM had the third highest level of suspected MP, only fibers were found there. Furthermore, suspected MP fibers were found at all sampled stations. For the main study the sampling region was narrowed down to CCB and ULM.

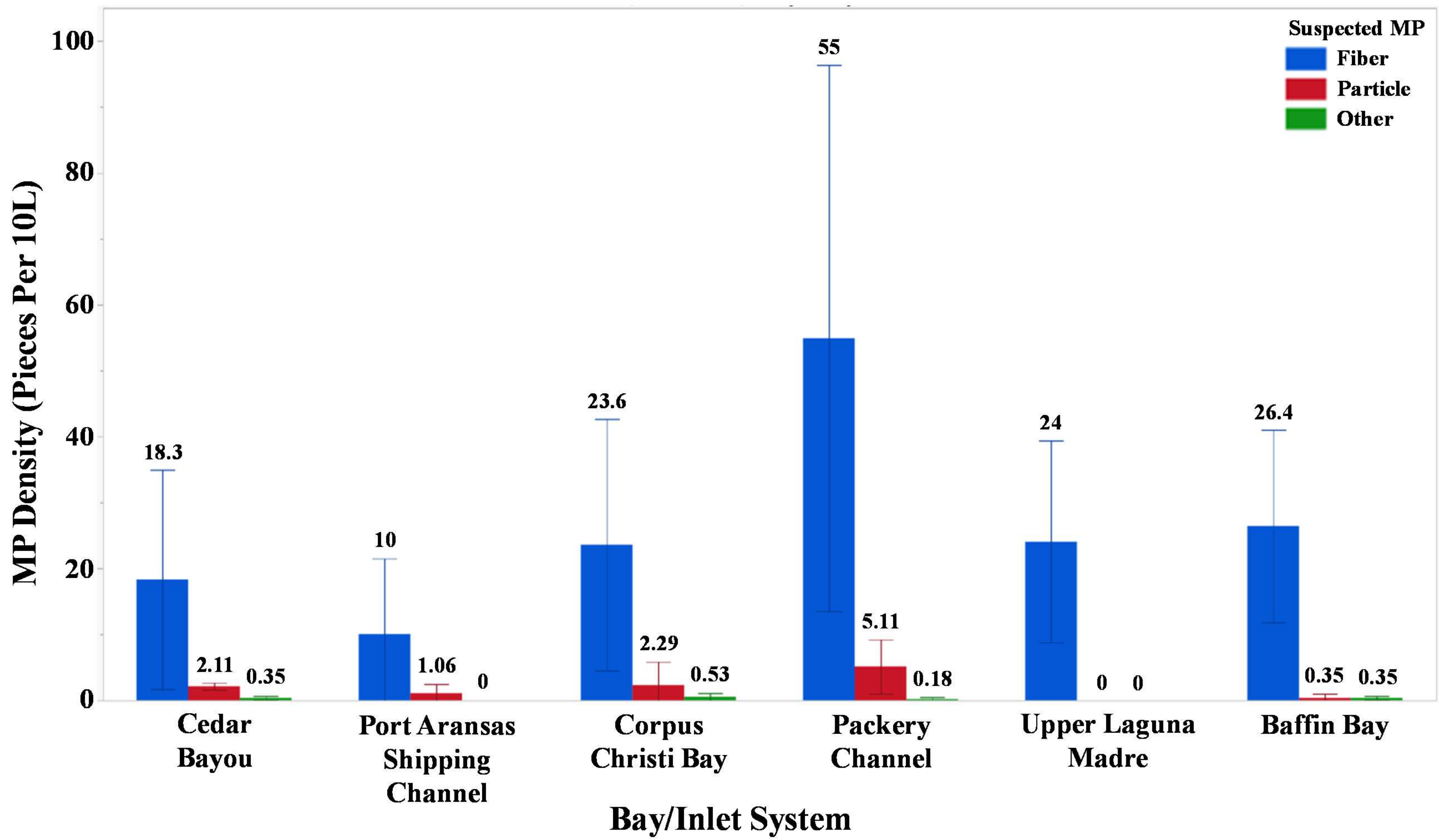

Figure 6. Microplastic concentrations determined in pilot study showing densities of three different suspected MP types based on 3 x 18.92L of surface water filtered for each of the six locations within the TCB. Numbers above each bar show the mean and bars the standard error from the 3 respective replicates.

Follow Up Study

In total, 18 water samples were taken spread over all the areas. Area 5 consisted of a total of 0.065 m^3 of filtered water and all the other areas consisted of a total 0.130 m^3 of filtered water. Area 6 had higher concentrations of suspected MP (Fig. 7). Overall, fibers were the most abundant out of 1,460 suspected MP. Blue fibers were the most abundant group making up 36.85% of the total number.

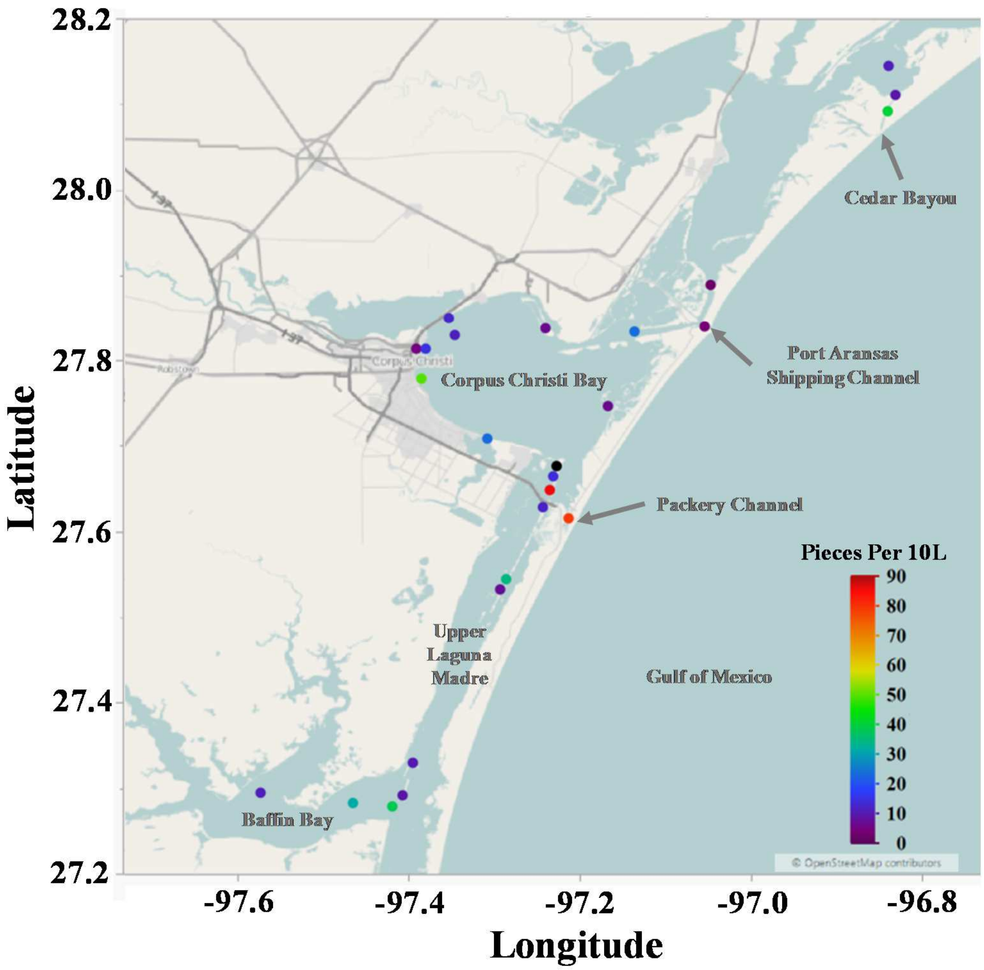

Figure 7. Microplastic concentrations (pieces $10L^{-1}$) in the surface water in the TCB. Combined results from pilot and follow up study collected between November 2016 until May 2018.

Microplastic in Juvenile Fish

Experimental Design

Numbers of Fish collected

In total, 1,768 fish were collected and identified from the bag seines, trawls, epibenthic sled and light traps during the 18 field sampling days (Table A1). Species were not distributed equally across the sampled area, therefore Due to gear restrictions, different resources that are available throughout the bay and seasonality of recruitment, not all species were encountered at each station. Due to varying densities the set target number for individuals to be collected per species was rarely met.

Numbers of fish processed and analyzed for ingested MP

In total, 345 fish that were processed and analyzed all the way for suspected MP ingestion (Table A2). Due to time constraint of MP analysis, a minimum of at least eight individuals per each species in each area were processed for MP. In some cases where there were less than eight, all individuals were processed for that species in that area. The shortest fish length recorded was 18 mm *Brevoortia* spp. and the largest fish length recorded was 96 mm in *S. ocellatus*.

Objective 1: Presence of MP in the diet of juvenile fish

A total of 81.45% of the fish had one or more suspected pieces of MP in them (RQ 1.1). The majority of the fish had one to three pieces of suspected MP (Fig. 8). Out of 1,087 pieces of MP, 96.94% of them were fibers. The average length of the ingested suspected MP was 1.309 ± 1.629 mm. The largest number of suspected MP found in one DT was 88 pieces in *S. ocellatus*.

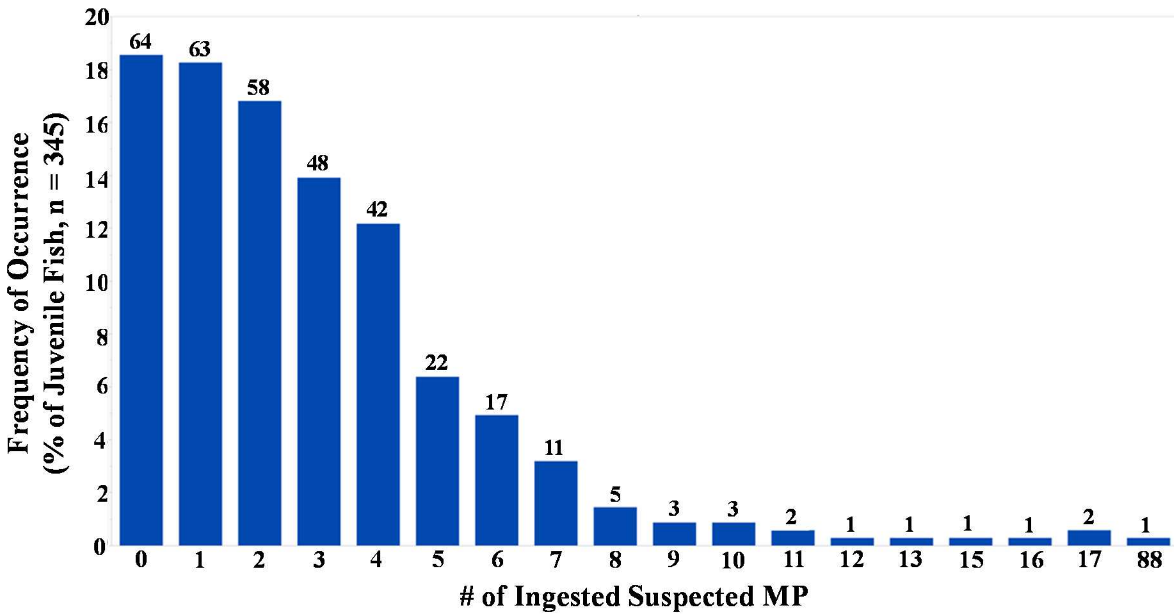

Figure 8. Frequency of occurrence of ingested suspected MP numbers per fish (n=345 fish analyzed)

A linear regression model showed a general relation of significantly increasing ingested suspected MP with fish standard length (ANOVA, p=3.368e-13; RQ 1.2). There was also a significant interaction between fish length and species. Therefore, the general relationship is also species specific (ANOVA, p=1.807e-06).

In addition to the number of ingested suspected MP, also the maximum MP length increased with the standard length of the fish based on 281measurements (p=8.144e-05; Fig. 9; RQ 1.2). The largest length of a suspected MP found in the DT was a brown fiber that measured 17.07 mm long. Similar to maximum length, the median length of the suspected MP increased with the standard length of the fish based on 118 individuals (p=0.0253; RQ 1.3). The median lengths of suspected ingested MP found in the DT ranged between 0.176 to 3.495 mm.

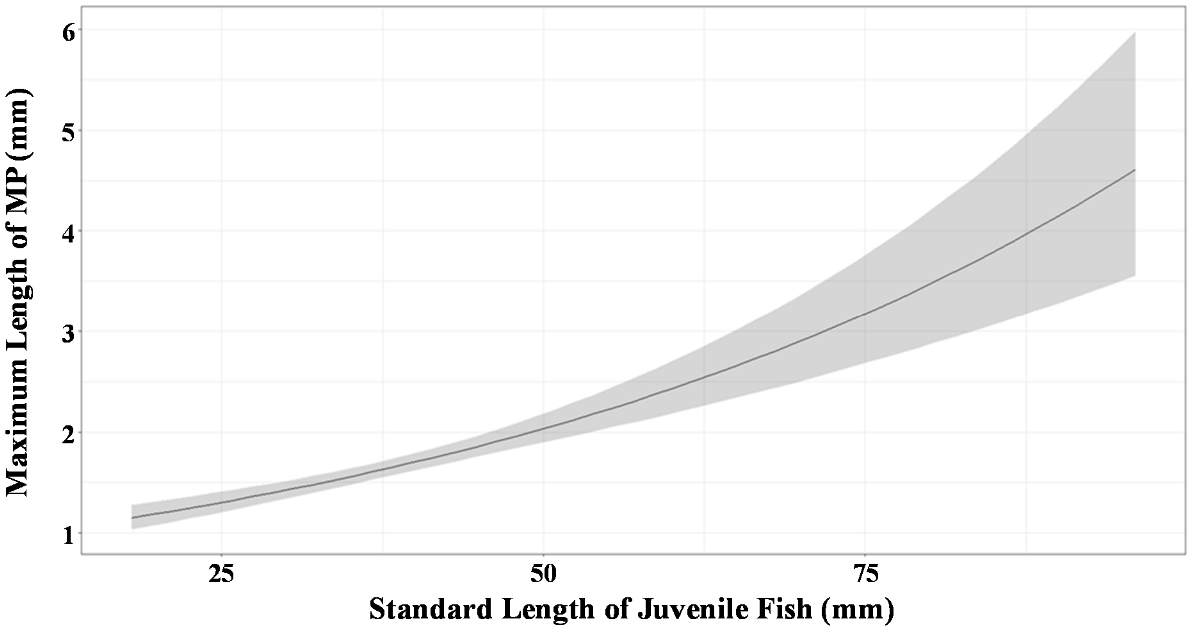

Figure 9. Correlation between maximum size of the suspected MP and the standard length of juvenile fish including all eight focus species (n=281).

Objective 2: Assessing the similarities within Feeding Guilds

To assess the differences between feeding guilds, the eight focus species were divided into two feeding guilds: a total of 125 planktivores and 220 benthic feeders. The linear regression model was used to compare if the number of suspected MP and the median length of suspect MP differed between the two different feeding guilds. The two one-way ANOVAs performed found a significant difference between the number of suspected MP (p=2.968e-07) and the median length of the ingested suspected MP (p=2.968e-07) in the feeding guilds. Further Holm's post hoc tests revealed that planktivores had a higher amount of suspected MP found in their DT than benthic feeders (p=0.0061; Fig. 10; RQ 2.1). The mean number of suspected MP for planktivores was 2.46 pieces while benthic feeders had 1.69 pieces. As for the median length of the ingested MP, benthic feeders were found to ingest larger pieces of suspected MP (p=0.0133; Fig. 11; RQ

2.2). The mean length of the suspected MP for benthic feeder was 1.01mm, compared to 0.785mm for planktivores.

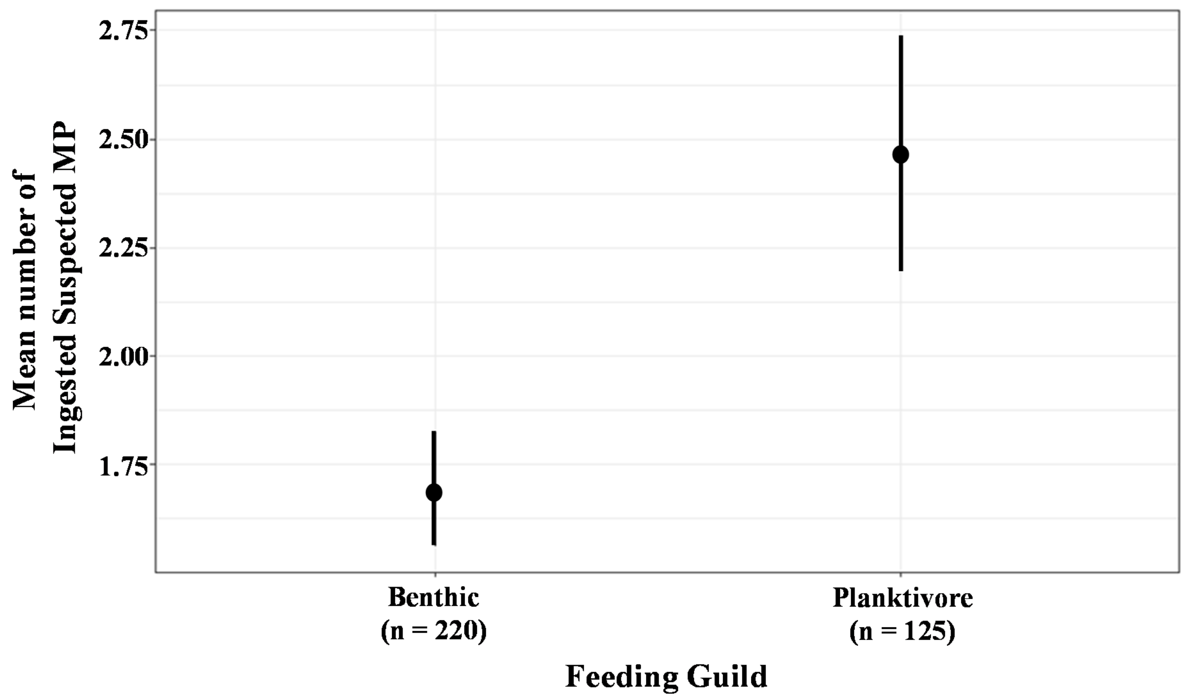

Figure 10. Difference in mean numbers of suspected ingested MP between feeding guilds.

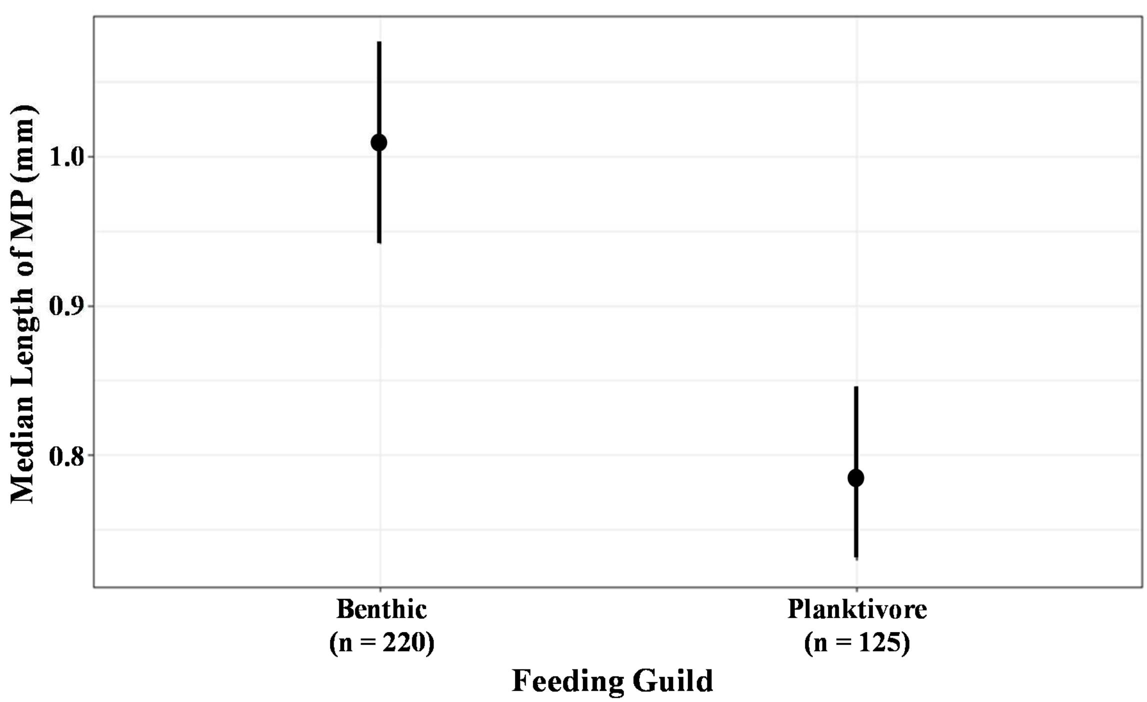

Figure 11. Difference in median lengths of suspected ingested MP between feeding guilds.

A total of 12 colors were found throughout the feeding guilds. The top four abundant colors found were blue (56.17%), black (26.60%), red (7.66%), and clear (7.46%). PEMANOVA was performed on the color preference of ingested suspected MP. There was no significant difference between the two feeding guilds were found (p=>0.05; RQ 2.3).

Objective 3 Examining the differences between species

All eight species in all the areas were analyzed for differences in ingested suspected MP. This included 45 *Anchoa* spp., 21 *Brevoortia* spp., 49 *L. rhomboides*, 57 *L. xanthurus*, 59 *Menidia* spp., 37 *M. undulatus*, 34 *Mugil* spp. and 43 *S. ocellatus*. A one-way ANOVA was performed to examine the differences between the species and the number of ingested suspected MP (p=2.968e-07; Fig. 12). A two-way ANOVA was performed to observe a difference between the slopes of the number of ingested suspected MP per species in relation to their fish length in species (p=0.025314; Fig. 12). There was also a significant interaction between the length of the fish and species (p=1.807e-06; Fig. 12). A one-way ANOVA was performed to assess the median length of the ingested suspected MP per species (p=0.009166).

A Holm's post hoc tests were performed to identify species specific differences in ingested suspected MP number, slope of ingested suspected MP number and body size, and length of ingested suspected MP. For the difference in number of ingested suspected MP, the Holm test found three species to have higher numbers of ingested suspected MP (RQ 3.1). The *L. xanthurus* had significantly higher amounts of ingested suspected MP (mean number of 3.42 ingested suspected MP pieces) than *M. undulatus* (p=0.0048), *Mugil* spp. (p=0.0001), *S. ocellatus* (p=<0.0001), and *Anchoa* spp. (p=0.0045). The *Brevoortia* spp. had a significantly higher amount of ingested suspected MP (mean number of 4.37 ingested suspected MP pieces) than *M. undulatus* (p=0.0077), *Mugil* spp. (p=0.0007), *S. ocellatus* (p=0.0005), and *Anchoa* spp. (p=0.0077). And, *Menidia* spp. (mean number of 2.13 ingested suspected MP pieces) had a significantly higher amount of ingested suspected MP than *S. ocellatus* (p=0.0355).

Three different types of slopes from correlations of the number of ingested suspected MP and fish length were identified (Fig.12). A negative correlation was shown for *Mugil* spp. *Anchoa* spp., and *Menidia* spp., whereas *L. xanthurus* showed a neutral relation, and the remaining species a positive correlation. The Holm's test identified two species that differed significantly in their slopes (Fig. 12; RQ 3.2). As *Anchoa* spp. grows in length the number of ingested suspected MP ingested decreased significantly slower compared to *L. rhomboides* (p = 0.0348) and *Brevoortia* spp. (p = 0.0112). In contrast, *S. ocellatus* has an increasing slope the larger it grows. The species *S. ocellatus* is significantly higher than are *Mugil* spp. (p = 0.0065), *Anchoa* spp. (p = 0.0007) and *Menidia* spp. (p = 0.0168).

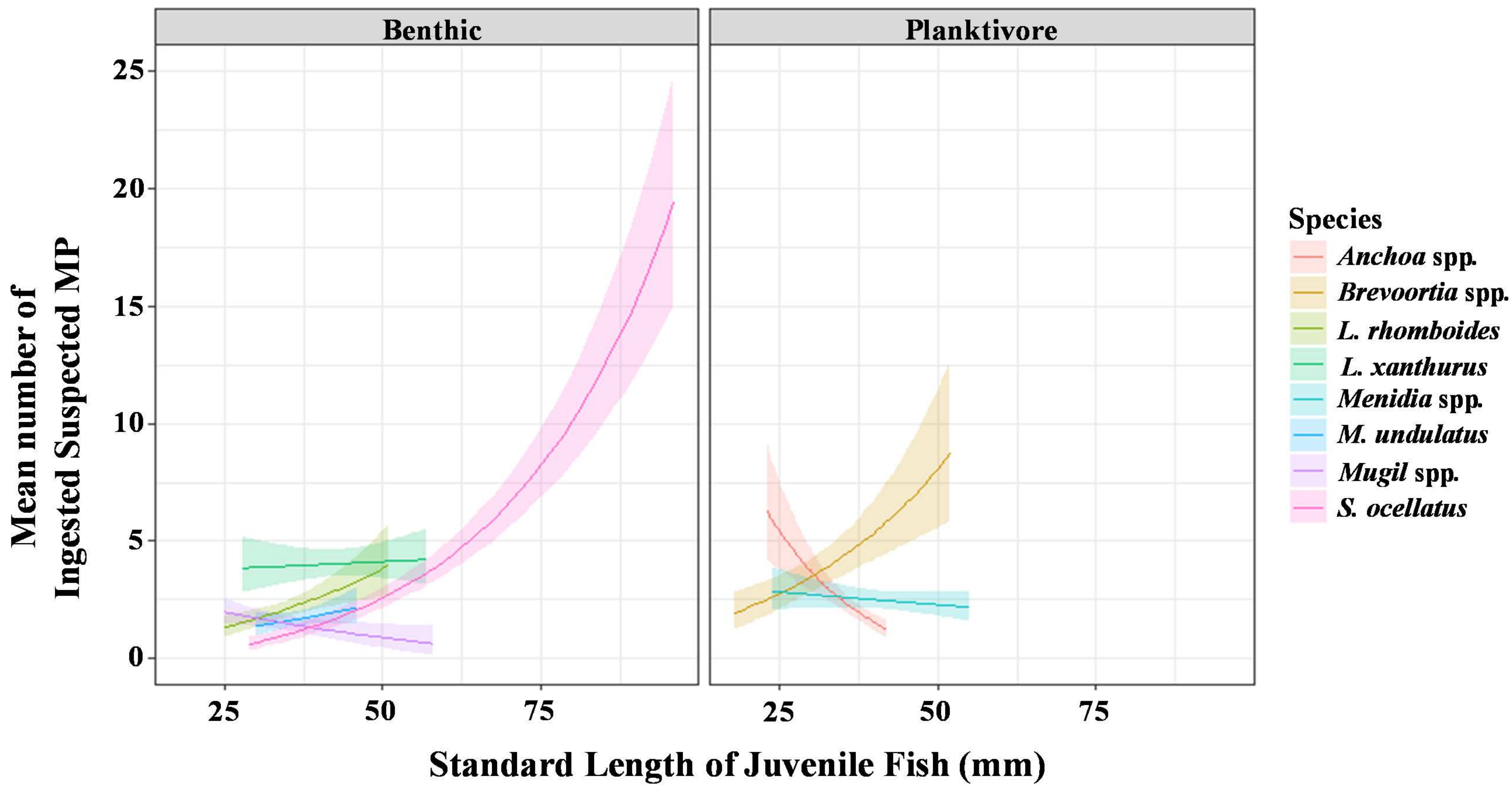

Figure 12. The mean difference in the ingested suspected MP per each species for each feeding guild. Each color represents a species. Species for the planktivores are red as *Anchoa* spp. (n = 45), orange as *Brevoortia* spp. (n = 21) and light blue as *Menidia* spp. (n = 59). Benthic feeding guild species are light green as *L. rhomboides* (n = 49), dark green as *L. xanthurus* (n = 57), dark blue as *M. undulatus* (n = 37), purple as *Mugil* spp. (n = 34) and pink as *S. ocellatus* (n = 43) was pink.

For the difference in median length of the ingested suspected MP, the Holm test found only one species with significantly larger MP than another species (Fig. 13; RQ 3.3), being *L. xanthurus* that ingested larger suspected MP than *Menidia* spp, (p=0.0378). The mean length of the suspected MP for *L. xanthurus* was 1.06mm while *Menidia* spp. were 0.699mm long.

In terms of color preference by species, a significant difference was found by the PERMANOVA, (p=0.003). Further analysis of the PERMANOVA showed two of the species had significantly different color preferences from each other (Fig. 14; RQ 3.4). The *Mugil* spp. were more prone to select for red/purple colors while *L. xanthurus* had a less specific color selection and had green/brown while (p = 0.003), and the remaining species did not differ significantly from each other.

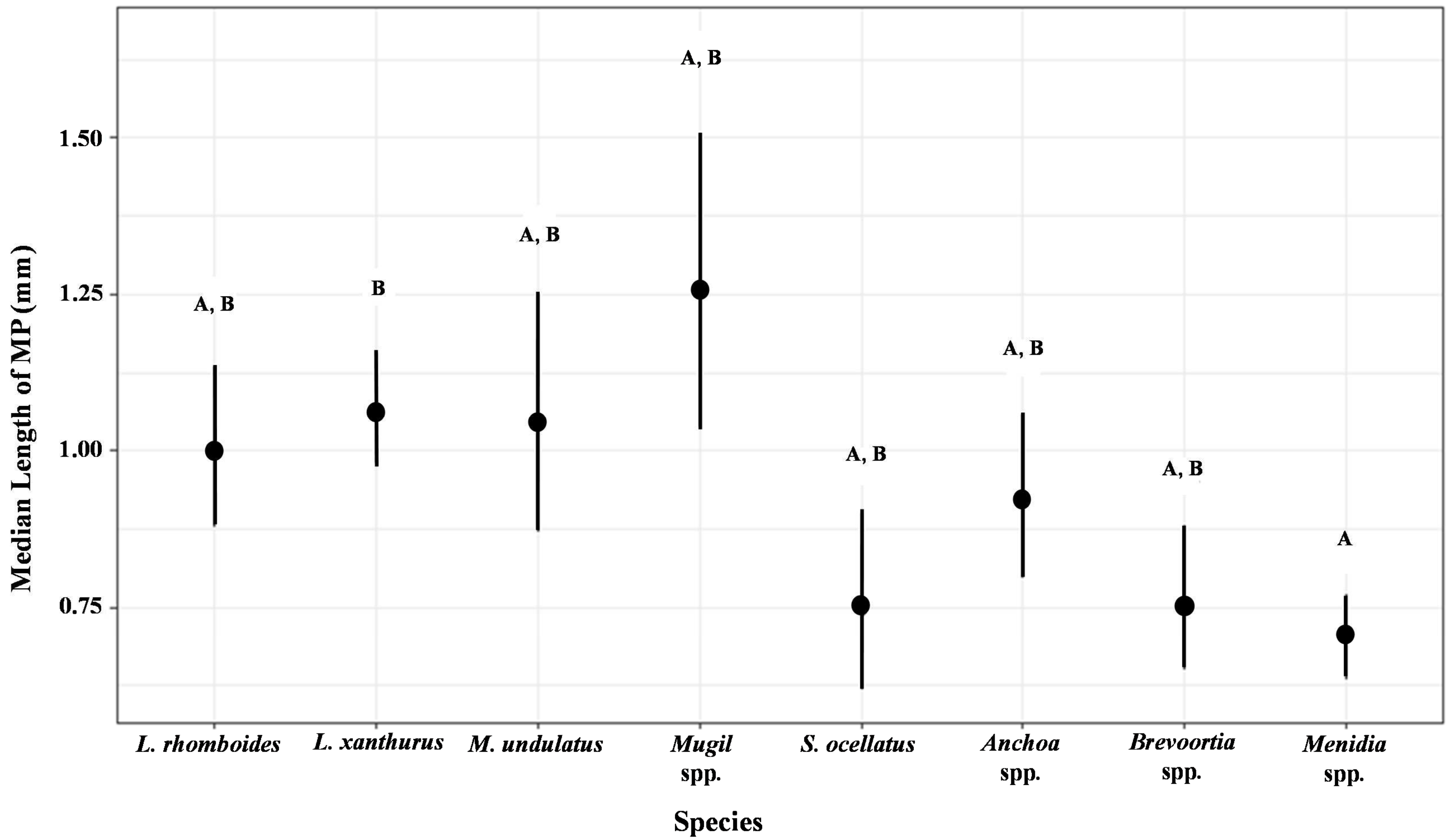

Figure 13. The medians size (mm) of suspected ingested plastic found in the DT of each species. The number above each line represents the similarities between the species. *Menidia* spp. has a one above it while *L. xanthurus* has a two. All the other species have both a one and a two which means they are similar to both *Menidia* spp. and *L. xanthurus.*

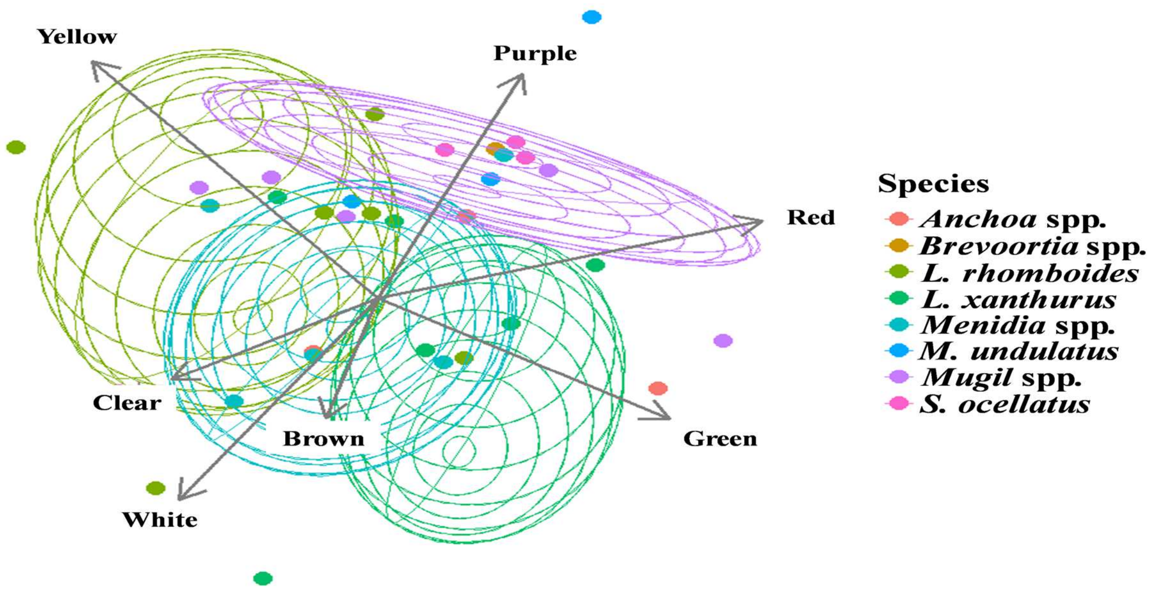

Figure 14. A NMDS plot of the difference in color preference between species. Species for the planktivores are red as *Anchoa* spp. (n = 45), orange as *Brevoortia* spp. (n = 21) and light blue as *Menidia* spp. (n = 59). Benthic feeding guild species are light green as *L. rhomboides* (n = 49), dark green as *L. xanthurus* (n = 57), dark blue as *M. undulatus* (n = 37), purple as *Mugil* spp. (n = 34) and pink as *S. ocellatus* (n = 43) was pink.

Objective 4 Determine if there is a spatial difference in MP pollution among the areas

A total of 64 fish were analyzed for Area 1, 46 fish for Area 2, 66 fish for Area 3, 54 fish for Area 4, 74 fish for Area 5 and 41 fish for Area 6 (Table A4). A one-way ANOVA showed that there was a significant difference in the number of suspected MP ingested between the areas (p=4.545e-05), as well as in the length of suspected MP ingested between the areas (p=0.0394). No differences in color preferences of ingested suspected MP between the areas were identified by the PERMANOVA (p=>0.05; RQ 4.3).

A Holm's post hoc test revealed that fish from Area 2 had a higher number of suspected MPs ingested than both Area 1 (p=0.0001), Area 3 (p=0.0001), and Area 6 (p=0.0490). Similar to Area 2, Area 5 also had a high number of ingested suspected MP than Area 1 (p=0.0481) and Area 3 (p=0.0481). The mean number of the suspected MP for Area 2 was 3.44 and Area 5 was 2.44 pieces (Fig. 15; RQ 4.1).

In terms of length of ingested suspected MP, the Holm's test showed that fish from Area 3 had larger pieces of suspected MP ingested than those from Area 6 (p=0.0241; Fig. 16; RQ 4.2). The mean length of the suspected MP for Area 3 was 1.15 while Area 6 was 0.658 mm long.

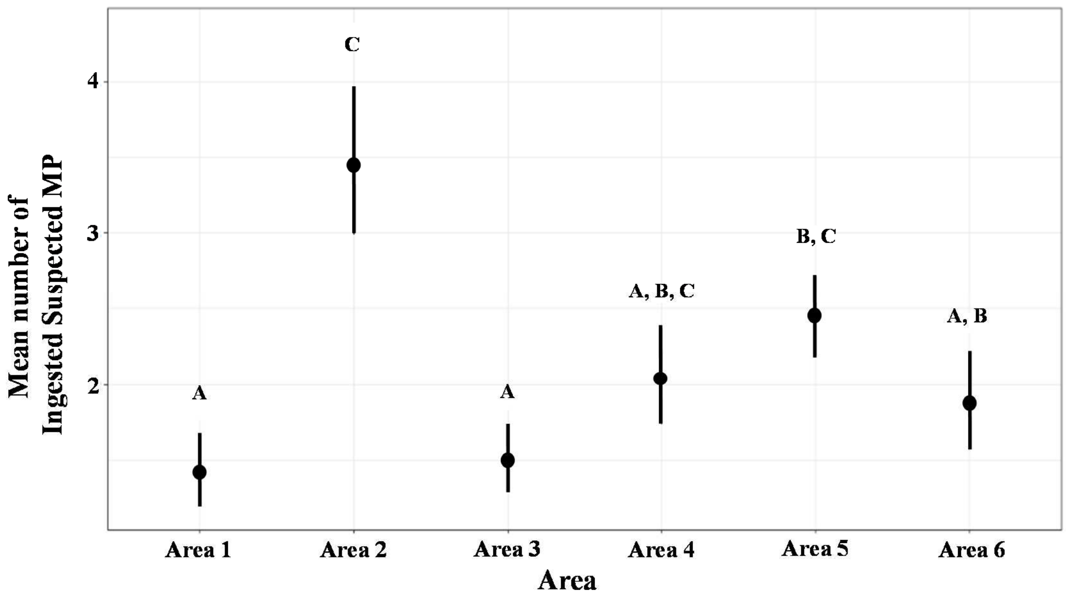

Figure 15. The mean number of ingested suspected plastic per area. The number above each area represents the similarities between areas. Area 1 and Area 3 has a one above it while Area 2 has a three. Area 5 has a two plus a three. All the other areas have a two with a combination of or both a one and a three above them which means they are similar to both Area 1, Area 2, Area 3 and Area 5.

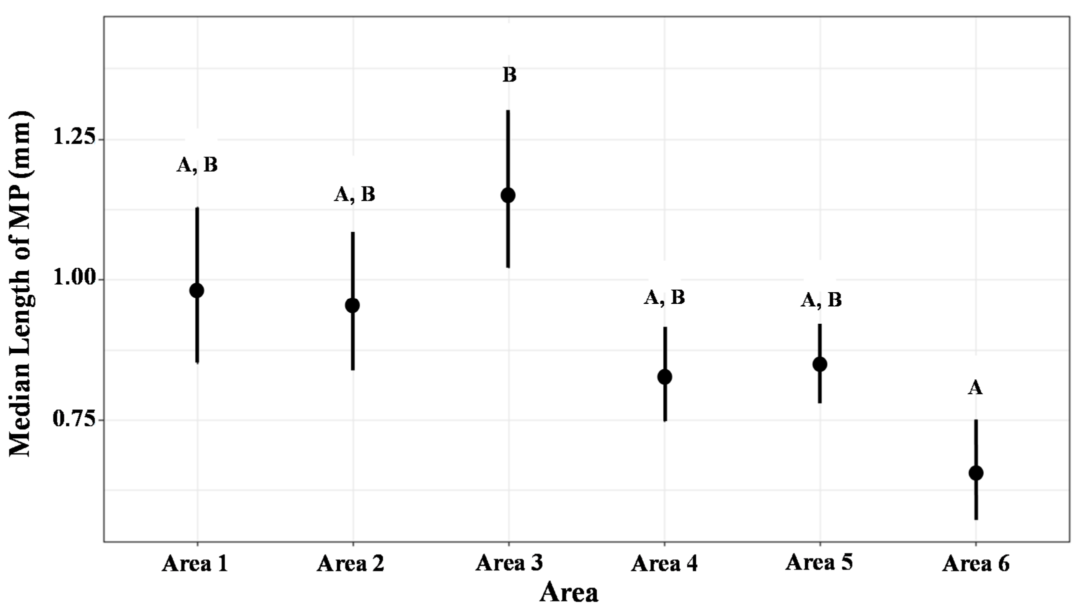

Figure 16. The median size of the plastic per area. The number above each area represents the similarities between areas. Area 6 has a one above it while Area 3 has a two. All the other areas have both a one and a two above them which means they are similar to both Area 3 and Area 6.

Does area influence what type of plastic is ingested in the same species?

Three fibers from the top four abundant colors for *L. xanthurus*. In some cases, there were less than three fibers for one of the colors which in that case the fibers for that color were not analyzed. A total of 45 suspected MP fibers from *L. xanthurus* were analyzed on the FTIR. The majority of the fibers analyzed (n = 41) were identified as some type of MP (93.18%). Fully synthetic fibers made up 13.64%, and semi synthetic fibers made up 79.55%, whereas fibers from natural and miscellaneous material made up 4.55% each of the ingested suspected MP that were processed (Table 2).

Based on these results the composition of all ingested MP was extrapolated, assuming that the relative composition of suspected ingested MP type was similar for all eight species. This extrapolation suggested that the top three types of ingested materials were semi-synthetic cotton blend (36.05 %), cellophane (29.34 %), and rayon (15.56 %) (Fig. 17).

Table 2. The breakdown of the chemical composition from FTIR analysis for the suspected MP that were ingested by *L. xanthurus* in different areas. In parentheses are the three types of material, fully synthetic (FS), semi synthetic (SS), and natural (N).

Area	**Acrylic (FS)**	**Neoprene (FS)**	**Polyester (FS)**	**Cellophane (SS)**	**Cotton Blend (SS)**	**Rayon (SS)**	**Cotton (N)**	**Misc.**	**Total by Area**
1	0	0	1	2	3	0	0	0	6
2	1	0	1	2	1	5	0	0	10
3	0	0	1	2	4	1	0	0	8
4	0	1	0	1	2	1	2	1	8
5	0	0	0	3	4	1	0	0	8
6	0	0	1	2	1	0	0	1	5
Total by Material =	1	1	4	12	15	8	2	2	
% Total by Material =	2.22	2.22	8.89	26.67	33.33	17.78	4.44	4.44	

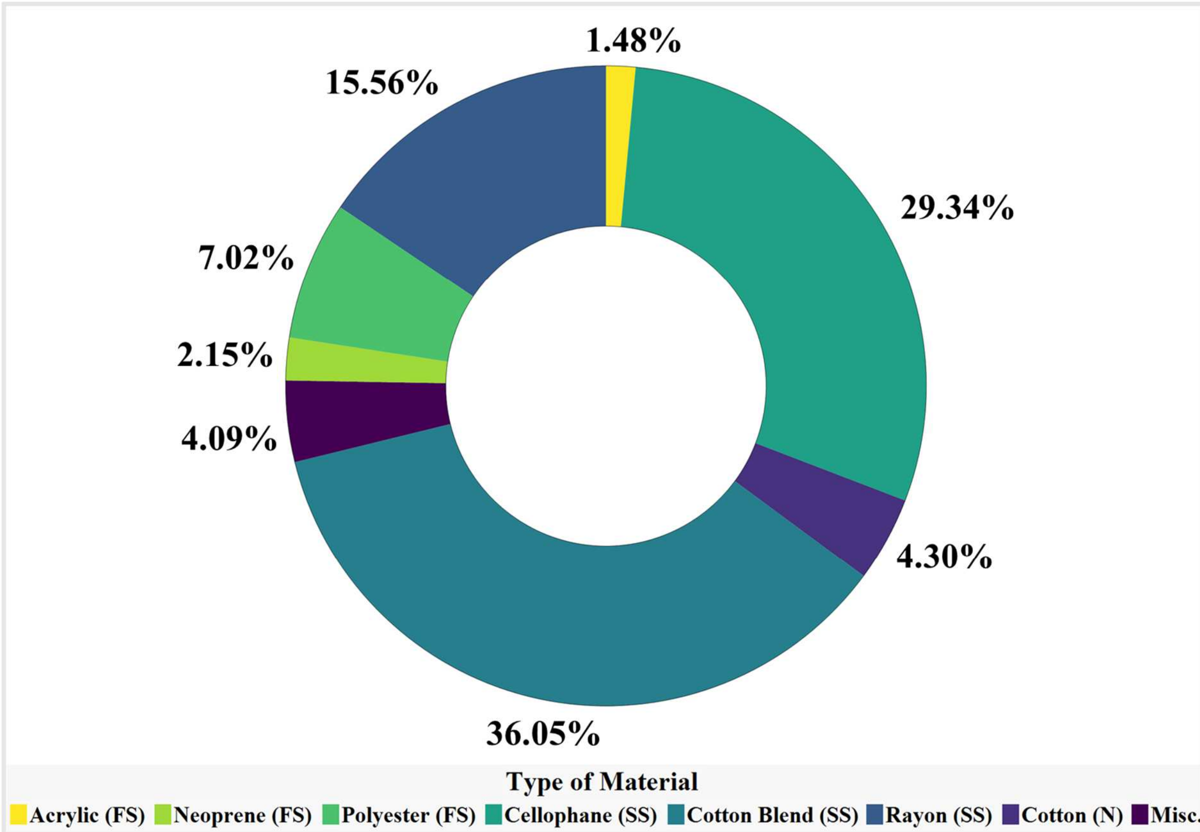

Figure 17. Assumed chemical composition of the overall number of ingested suspected MP found in the DT of all eight species from different areas. In parentheses are the three types of material, fully synthetic (FS), semi synthetic (SS), and natural (N).

Divided by color, a total of 20 blue fibers, 13 black fibers, six red fibers and five clear fibers were analyzed with the FTIR (Table 3). Based on these results the composition of all ingested MP was extrapolated based on color, assuming that the ratios from Table 3 carried over to the overall top popular color of ingested suspected MP found in the DT of all the eight species. Red was made up of completely all semi-synthetic MP while colors clear, black and blue had either or both fully synthetic and natural MP in them (Fig. 18). For Black and red semi-synthetic cotton blend (black = 38.5%, red = 71.4%) made up the majority of the semi-synthetic material while cellophane made of the majority of blue (55.0%) and rayon made up the majority of the clear semi-synthetic (60.0%). Blue was the only color that had natural cotton fibers.

Table 3. The breakdown of the chemical composition from FTIR analysis for the top popular ingested suspected MP colors. In parentheses are the three types of material, fully synthetic (FS), semi synthetic (SS), and natural (N).

Color	**Acrylic (FS)**	**Neoprene (FS)**	**Polyester (FS)**	**Cellophane (SS)**	**Cotton Blend (SS)**	**Rayon (SS)**	**Cotton (N)**	**Misc.**	**Total by Color**
Black	0	1	3	1	5	2	0	1	13
Blue	1	0	0	11	4	1	2	1	20
Clear	0	0	1	0	1	3	0	0	5
Red	0	0	0	0	5	2	0	0	7
Total by Material =	1	1	4	12	15	8	2	2	
% Total by Material =	2.22	2.22	8.89	26.67	33.33	17.78	4.44	4.44	

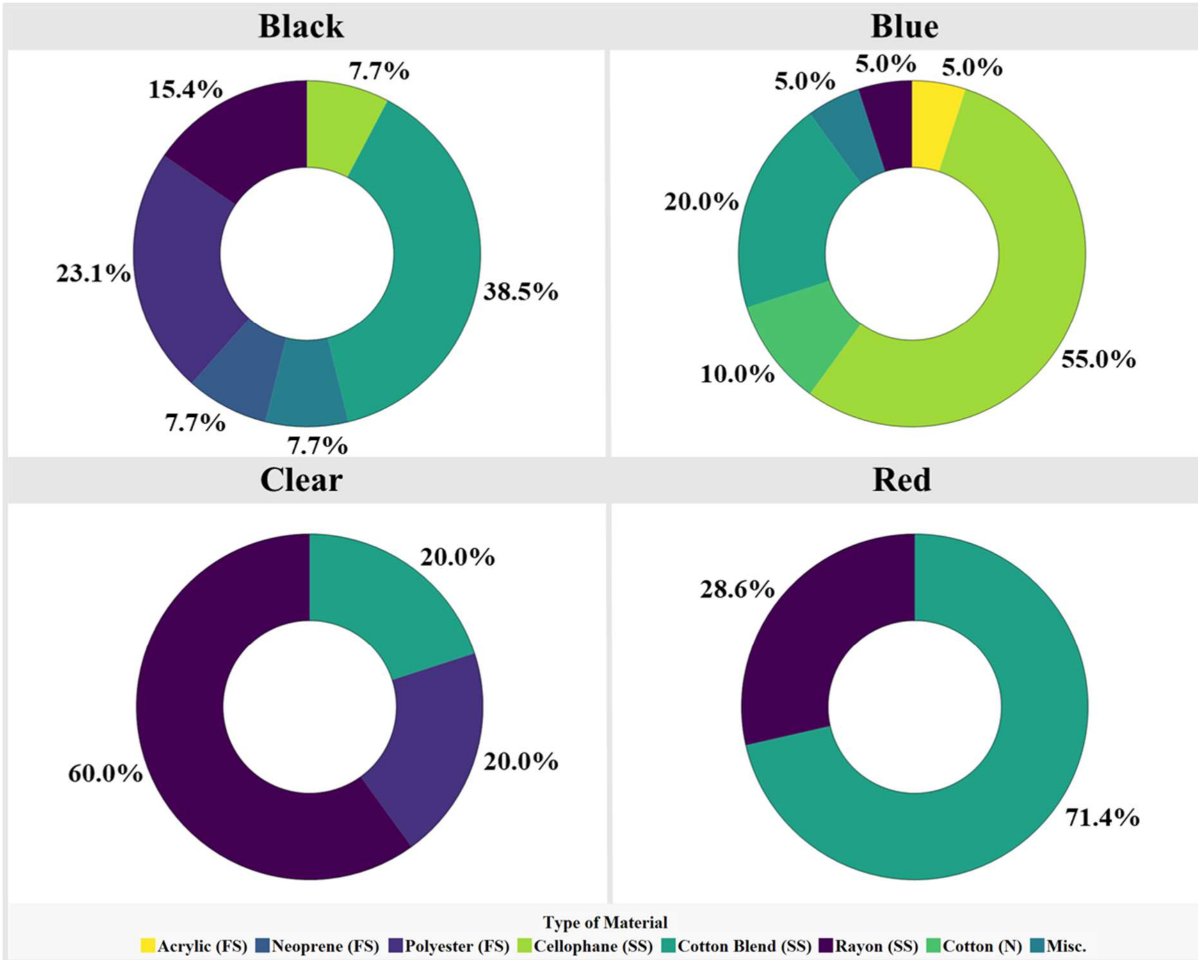

Figure 18. Assumed chemical composition of the overall top popular colors ingested suspected MP colors found in the DT of all the eight species. In parentheses are the three types of material, fully synthetic (FS), semi synthetic (SS), and natural (N).

For the determination of spatial differences among the areas a total of six fiber from Area 1, 10 fibers from Area 2, eight fibers from Area 3, eight fibers from Area 4, eight fibers from Area 5 and five fibers from Area 6 were processed from *L. xanthurus.* It was assumed that the ratios mentioned earlier (Table 6) carried over to the overall amount of MP found in the DT of *L. xanthurus* from each area. This resulted in a 100% dominance of semi-synthetic material in Area 5, while the other areas were made up of either, both or all fully synthetic and natural material (Fig. 19). Semi-synthetic Cotton blend material composed 50.0% of the material processed for Area 1, Area 3, and Area 5. Area 2 was mainly composed by the semi-synthetic rayon material (50.0%) while Area 6 was mainly composed of the semi-synthetic cellophane material (40.0%). Area 4 was composed equally mainly with the semi-synthetic cotton blend (25.0%) and the natural cotton material (25.0%). Area 4 was the only area that had natural cotton fibers.

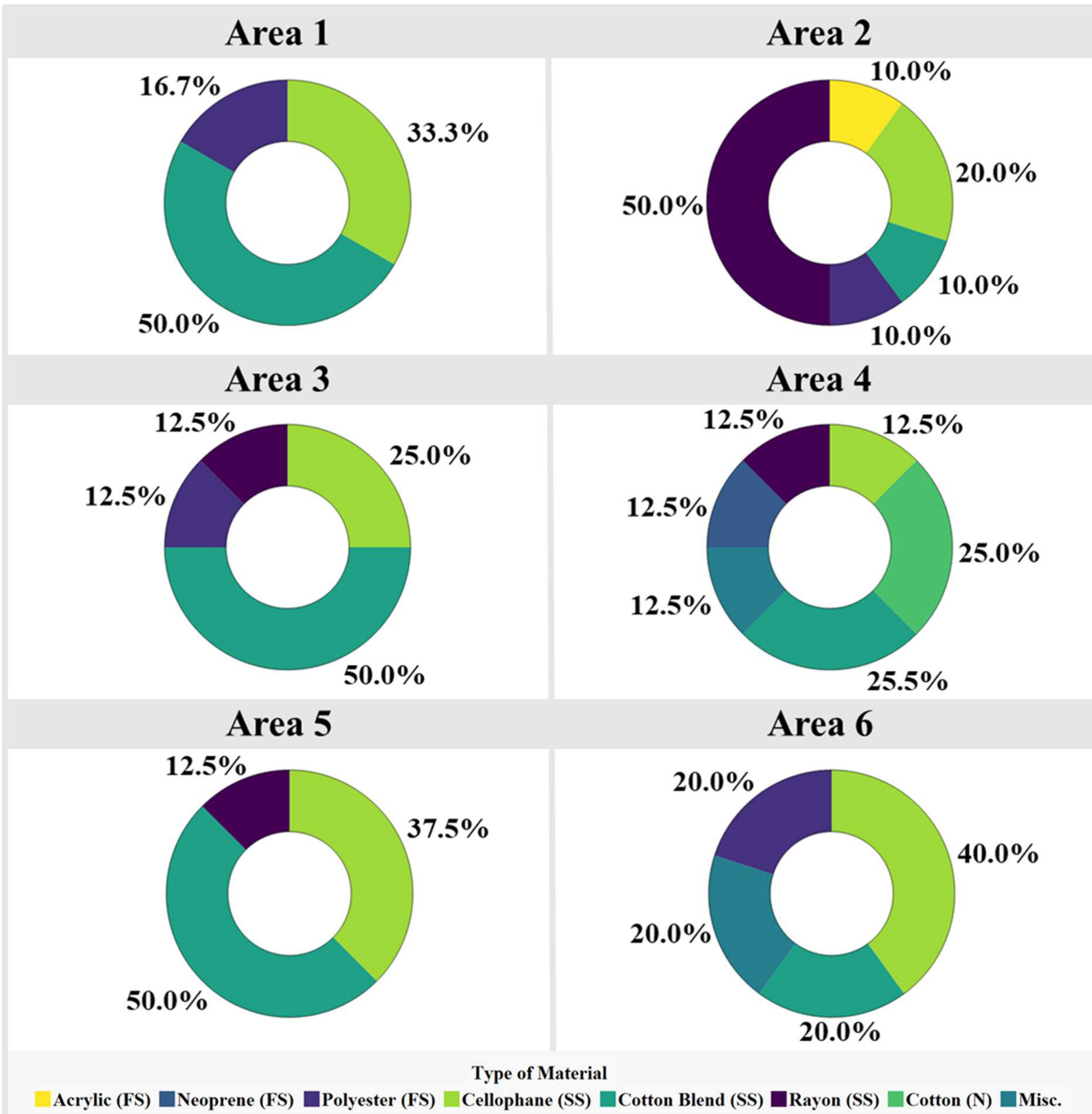

Figure 19. Assumed chemical composition of the suspected MP that were ingested by *L. xanthurus* from different areas. In parentheses are the three types of material, fully synthetic (FS), semi synthetic (SS), and natural (N).

DISCUSSION

This study differed from the other studies in several ways. One way that differed was by life stage. The primarily life stage of the fish were juveniles. Then the few studies that did look at juveniles, only looked at one species (de Sá *et al.*, 2015; Kazour *et al.*, 2018; Ory *et al.*, 2018). This was another way this study differed. Instead of looking at just one family or species of fish, this study looked at five families or eight different species. Lastly, this study looked at the difference in the colors of the MP that were ingested. The majority of the studies do not report the color and if they do, it's just an overall finding not used to compare differences.

Suspected Microplastic in Water

This study found suspected MP at all sampled locations in the Texas Coastal Bend, throughout CCB and ULM. This finding adds to the general observation that MP contamination is present in all marine environments nowadays (Barnes *et al.*, 2009; Browne *et al.*, 2010). For the GoM, the presence of MP was reported in plankton nets from Galveston (Texas), Cape San Blas (Florida) and the Mississippi River (Hoss and Settle, 1990; Di Mauro *et al.*, 2017). As well as the shelf waters of the Louisiana coast (Di Mauro, 2017).

A worldwide analyses of sandy beach sediment (Browne *et al.*, 2011) and a caging experiment with juvenile flounders in estuaries along the English Channel (Kazour *et al.*, 2018) suggest that coastal and estuarine areas are hotspots of MP pollution. In a Portuguese estuary, the density of MP in the water can even be higher than the density of larval fish (Rodrigues *et al.*, 2019).

The observed suspected MP concentrations in the TCB (up to ~90 MP per 10L = 9000 MP/m^3) are lower than those reported for water samples from Niskin bottles off Lousiana (Di Mauro et al. 2017, up to 60,000 MP/m^3), but higher by one order of magnitude than those reported for a Chinese river (Zhao *et al.*, 2019). It must be noted that different sampling methodologies are limiting these quantitative comparisons, and it can be assumed that the confirmed MP content after FTIR analyses would reduce the concentrations reported here significantly from 20-70% (Zhao *et al.*, 2019).

In addition to the classic sampling methodology of MP by collecting water or plankton net samples, it was recently suggested to use the stomach content from fish as an indicator of water quality both fresh and saltwater environments (Silva-Cavalcanti *et al.*, 2017).

Abundance and concentration of MP ingested by juvenile fish

In addition to the classic sampling methodology of MP by collecting water or plankton net samples, it was recently suggested to use the stomach content from fish as an indicator of water quality both fresh and saltwater environments (Silva-Cavalcanti *et al.*, 2017). In this study, 81% of the analyzed fish contained suspected MP in their digestive tracts. This frequency of occurrence appears high compared to values reported from previous studies in Texas. Peters *et. al* found reported a 50% lower amount of MP compared to this study (42%) in adult marine fish caught in Galveston Bay (2017). A third study from the Lower Laguna Madre and adjacent GoM found even less with only 10% of adult fish analyzed that had MP ingested (Phillips and Bonner, 2015). These large differences suggest that juvenile fish may be more susceptible to MP ingestion than adult fish. In addition, the influence of different sample processing and analyses protocols differences needs to be kept in mind when comparing different studies in this field. Nevertheless, the large difference, 80% vs 42% vs 10%, seems unlikely to be only explained by methodological error. In addition, site specific differences may play an important role as well, as a similarly high ingestion frequency (83%) was reported from a study on adult river fish from

Brazil (Silva-Cavalcanti *et al.*, 2017). For the three Texas studies, at least the 10% that found only 10% frequency of occurrence was conducted in an area with much fewer potential MP sources compared to the areas investigated here.

The ingestion of MP may dependent on the foraging type with less selective feeders being more prone to MP ingestion compared to more selective feeders. Thus, a difference in MP ingestion frequency and amount may exist between members of different feeding guilds. A study conducted in two Brazilian estuaries found MP commonly ingested by small and juvenile fish caught with seine net (8mm mesh size) throughout five different feeding guilds (Vendel *et al.*, 2017). In this study, a similar observation was made with MP ingested by members of both feeding guilds. However, the number of ingested suspected MP was higher in the planktivorous feeders compared to the benthic feeders, whereas the benthic feeders had on average larger pieces of suspected MP in their DT. This result suggests that planktivorous feeders are more susceptible to ingest MP, probably due to a less selective feeding mode which may be more passive, compared top more actively feeding benthic feeders. A high frequency of ingestion by juveniles of a planktivorous fish species has been reported from lab study conducted in Chile as well, which found that 93% of their fish ingested MP (Ory *et al.*, 2018). Vendel *et al.* (2017) reported differences between “Zooplanktivorous“ and “Benthivorous” species as well, whereas Phillips and Bonner’s study (2015) from south Texas did not find significant differences between freshwater benthic, herbivore/omnivore and invertivore feeding guilds. This suggests that factors such as active vs passive feeding mode and feeding at different positions within the water column are likely important factors to explain the differences between planktonic and benthic feeders. Availability of MP plastic pollution in the respective location and its type is another important factor. If the MP composition at a location is dominated by plastic types heavier than (sea)water then MP will accumulate at the bottom making it more available for demersal feeder, whereas lighter, floating plastic will be more available to pelagic, planktivorous feeders. The effect of plastic type buoyancy and a high amount of MP accumulated at the bottom may help to explain the contrasting results from Jabeen *et. al.* (2017), who studied 21 marine fish species from China, reported higher numbers of ingested MP for demersal compared to pelagic fish. The shallow water of coastal environments as sampled in this study allows for a fast resuspension of settled MP back into the water column through wind and current induced turbidity, which are common features in the TCB. Hence, this process may have contributed to the presence of floating and sinking MP types in the study areas increasing the availability of MP in the water column to be ingested by planktivorous fish.

At species/taxon level, it became apparent that single species within the feeding guilds differed significantly from each other in the amount of ingested MP with the benthic feeder *L. xanthurus,* and the two planktivorous taxa *Brevoortia* spp., and *Menidia* spp. having elevated amounts of suspected MP ingested. In addition to the arguments raised in the discussion about feeding guild differences, the diversity of the typical diet composition can differ between species and may contribute to the observed species-specific differences. In general, species within the same feeding guild with a reportedly broader diet spectrum had more suspected MP ingested in this study (Able & Fahay, 2011). For example in the benthic feeding guild, *L. xanthurus* tend to have a broader spectrum (copepods, polycheates, oligocheates, nematods, bivalves, mysids, tanaids, and detritus) than *S. ocellatus* who has a narrow spectrum (mysids, small shrimp, small crabs, and small fish) (Able & Fahay, 2011). This observation is similar to the result reported by Peters *et. al.* (2017), who served *Orthopristis chrysoptera* had not only a narrow diet spectrum, but the lowest number of MP in them compared to other benthic species who had a broader diet spectrum.

The highest number of confirmed MP found in a juvenile fish, after the contamination correction and the assumed FTIR ratio was applied, was 87.09MP in one DT of a *S. ocellatus*. This was higher than the highest recorded number of 83 pieces found in an adult fish collected by a trawl in the North Pacific Central Gyre (Boerger *et al.*, 2010). On average 3.01 pieces of MP was found per juvenile fish which was slightly higher than the 2.1 pieces per adult planktivores in the northwestern Pacific Gyre (Boerger *et al.,* 2010) and 2.04 pieces per juvenile European flounder in the English Channel in France (Kazour *et al.*,2018).

This study showed that the relation between the amount of ingested MP and body size is species specific, and that three types exists. The amount of MP either increased, stayed constant or decreased with body size. The positive correlation, as seen for *S. ocellatus*, can be explained by the combination of two factors: Increased food demand with increasing size may increase the ingested amount of MP in parallel, and a change in foraging mode towards a more piscivorous diet together with an increased size of prey items may lead to the ingestion of prey who in turn may had ingested .

This study showed that the relation between the amount of ingested MP and body size is species specific, with three different types identified. The amount of MP either increased, stayed constant or decreased with body size. The positive correlation type, as seen for *S. ocellatus*, can be explained by the combination of two factors: Increased food demand with increasing size may increase the ingested amount of MP in parallel. And, a change towards a more piscivorous diet and larger prey items with increasing body size may enhance a bioaccumulation effect, by ingesting MP indirectly through the diet of the prey item. A positive correlation was observed before already for sunfish collected in the Brazos River Basin, Texas (Peters and Bratton, 2016). The mechanism of a trophic cascade was also put forward in a study on snook species from Brazil (Ferreira et al., 2019). The opposite type with decreasing MP ingested with size was observed for the planktivorous Anchoa spp.. In contrast to the changing prey types and sizes in *S. ocellatus*, they keep the same feeding mode throughout their life, a combination of filtering and picking planktonic organisms. An increase in target size of prey items may likely occur during ontogeny but is comparatively small. This change however may be large enough that smaller MP pieces are passed through the filter apparatus and therefore not ingested with increasing body size. The third type of a neutral response, as identified for *L. xanthurus* in this study, matches the observations reported by Vendel *et al.* (2017).

Size of MP ingested by juvenile fish

In addition to the correlation between the body size of the fish and number of MP, this study additionally investigated the correlation between size of the ingested MP and fish body size, which has not been looked at in previous studies. Both, the maximum and median sizes of the suspected MP overall increased with the length of the fish. A reason for this significant correlation can be the basic morphological principle, that mouth gape width and by that accessible prey size increases with body size, allowing for larger pieces of MP to be ingested (Keast and Webb, 1966). A more active feeding by benthic feeders may explain the observation of the ingestion of larger pieces of suspected MP compared to planktivorous species. The effect of turbidity, that holds smaller plastic pieces longer in suspension than larger plastic pieces may in addition contribute to a higher availability of larger MP fibers at the bottom compared to the

water column that planktivorous species exploit. To date no data on MP loading and characterization of sediments are available for the study region to back up this hypothesis.
At species level, the median size of ingested MP was larger in *L. xanthurus* compared to *Menidia* spp.. In addition to the argumentation brought forward in the feeding guild discussion another process may explain the observed difference. Both species have a broad diet spectrum, but the size of the prey could explain why we seen larger plastic in *L.* xanthurus. According to Able and Fahay (2011), *L. xanthurus* feeding of larger prey items such as polychaetes, bivalves and crustaceans, while *Menidia* spp. feed smaller prey on insects, algae, diatoms, fish eggs and larval mollusk/decapods.

Color of MP ingested by juvenile fish

In addition to the amount and size of the MP found in the DT of juvenile fish, this study also looked at color preferences. The most abundant colors found in all areas was blue, black, red, and clear. These colors are commonly found in the digestive tract of other studies from South America (Dantas *et al.*, 2011; Mizraji *et al.*, 2017), Canada (Hipfner *et al.*, 2018), Europe (Kazour *et al.*, 2018) and Asia (Jabeen *et al.*, 2017). No significant differences were detected when planktivorous and benthic feeders were compared. This contradicts the hypothesis that more selective feeders such as the benthic feeder group would also select stronger for color. Assuming that color preference of MP is linked to color of preferred prey items a broad color spectrum of benthic feeders could be an explanation for the missing distinction between the two feeding guilds.

However, two of the eight study species showed more distinct color preferences, *Mugil* spp. and *L. xanthurus*. *Mugil* spp. preferred more red and purple colors whereas *L. xanthurus* preferred more green and brown colors. This difference may be explained by the following hypothesis. One suggestion is that the colors ingested are similar to the colors of their prey. In a laboratory, juvenile *Seriolella violacea* were shown to go after black colored MP because it was similar to their food pellets (Ory *et al.*, 2018). Reddish amphipods and copepods may be higher up on the diet list of mullets compared to *L. xanthurus*. A second explanation could be that the colors contrasted with the background the most are the ones that were ingested (Palm, 2001). Further studies on the importance of visual and color cues on the feeding ecology of these species are needed to confirm this field observation.

Spatial difference of suspected MP pollution and ingestion

This study found spatial difference in MP ingestion between the six sample areas, following the approach to use the stomach content from fish as biosamplers and indicator of MP pollution (Silva-Cavalcanti *et al.*, 2017). Such spatial differences can originate in the distance to MP sources/pathways for MP to enter the marine environment (Browne, 2015). A literature review found higher MP pollution in prominent coastal regions adjacent to large population centers (Barnes *et al.*, 2009). Similar to this observation, the two areas with highest MP ingestion numbers were located in CCB and the lowest mean number was found in the ULM. Other studies have found a similar pattern, with fish from highly urbanized areas containing elevated MP amounts in their stomachs from Texas (Phillips and Bonner, 2015; Peters and Bratton, 2016; Peters *et al.*, 2017) and Brazil (Silva-Cavalcanti *et al.*, 2017). However, this relation does not always show, as the results from another study that compared two estuaries with different anthropogenic pressures found no differences between the two locations (Vendel *et al.*, 2017). To explain the observed differences between sampling areas, the water currents in CCB are important as they may lead to accumulation of floating MP in certain areas. A coastal model of

CCB showed currents flowing towards Area 2 from highly urbanized areas (Huang *et al.*, 2012), which may explain the elavated amount of ingested MP found for this area. This explanation is similar to the one for an estuary in Brazil, where higher MP density from river runoff was found downstream of in the lower part of the estuary (Ferreira *et al.*, 2019). The elevated values in Area 5 may be caused by the close vicinity to potential MP sources such as wastewater treatment plants. Kazour *et. al.* (2018) reported similar results from France, with fish that were in close vicinity of a wastewater treatment plant having higher amounts of MP ingested.

Apart from the general present MP throughout the study areas, was there an area with larger MP? The size of plastic was found to be larger in Area 3 than in Area 6. One explanation for this could be the vicinity of the sample in relation to the pollution source. A study done in an estuarine with wastewater discharge found larger pieces MP near the treatment plant than in the other areas (Dantas *et al.*, 2011). However, this was not the case with both Area 3 and Area 6 since they are roughly the same distance from their pollution sources. Another explanation, in regard to MP size, could be the speed of the currents at the two areas. In the predicted model by Huang *et. al.* (2012), Area 3 has stronger currents than Area 6. These stronger currents would allow for the larger MP to settle down out of the water column to the bottom, while the smaller MP remained suspended.

Lastly, did the type of MP ingested by the same species differ between areas? Overall, CCB had more diversity of types of MP than ULM. Within CCB, areas 2 and 4 had the highest level of diversity with five different MP types (Area 2 = acrylic, polyester, cellophane, cotton blend, and rayon; Area 4 = neoprene, cellophane, cotton blend, and rayon). None of the studies referenced in this discussion who included an FTIR analyses reported the presence of any type of cotton blend, which is in strong contrast to the presented results which showed cotton blend to be the most abundant MP type, 36.05%, (33% cotton, 49% rayon, 16% nylon, and 2% elastane). The second most abundant MP type in this study was Cellophane (29.34%), which was even more dominant type (49.10%) in fish collected in China (Jabeen *et al.*, 2017). Both cotton blends and cellophane belong to the semisynthetic materials, which have not been reported as dominant group in any other of the referenced studies, which reported MPs to largely consist of fully synthetic materials (Phillips and Bonner, 2015; Kazour *et al.*, 2018)or the majority (Hipfner *et al.*, 2018; Peters *et al.*, 2018). The cotton blends are very likely stemming from clothing, which indicates a high contribution of sewage treatment plants as source for ingested MP in the CCB areas. A reason for the underreporting of semi-synthetic MP by previous studies may be the very recent developments towards the establishment of a standardized protocol for MP processing. Earlier this year, a new definition framework was made suggesting heavily modified natural polymers (cellophane and rayon) can be included as plastic debris (Hartmann *et al.*, 2019).

Summary

Overall this study differed from previous MP studies by focusing primarily at the comparison of juvenile stage fish of multiple species. During the year 2017 and 2018, suspected MP was found in both the water and juvenile fish in CCB and ULM. The number of suspected MP, the median MP size and color differs between feeding guilds, species and areas. The FTIR analysis revealed that majority of the ingested MP was made of a semi synthetic cotton blend material. The potential effects of ingesting MP could compromise the energy uptake, cause physical damage to the DT membranes, and chemical poison juvenile fish and decrease their chance of survival.

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LIST OF APPENDICES

Appendix 1: Supplemental Field Sampling Days Data.

Table A1. List of all field sampling days performed at every site in each area. [1] only water samples and hydrological water data was taken. [2] only water samples were taken. [3] no data was collected because no fish were caught.

Area	Latitude	Longitude	Date	Crew
1	27°32'38.19"N	97°17'04.12"W	12/11/2017	GELH Lab
1	27°17'29.20"N	97°24'20.80"W	2/23/2018	TWPD
1	27°17'19.54"N	97°23'49.10"W	2/23/2018	TWPD
1	27°21'59.10"N	97°22'58.70"W	2/23/2018	TWPD
1	27°26'03.00"N	97°20'30.00"W	3/2/2018	TWPD
1	27°23'45.00"N	97°20'35.00"W	3/2/2018	TWPD
1	27°26'19.00"N	97°22'01.00"W	3/2/2018	TWPD
1	27°32'38.19"N	97°17'04.12"W	5/10/2018	GELH Lab
1	27°32'12.49"N	97°17'22.03"W	5/10/2018	GELH Lab
1	27°31'37.97"N	97°17'39.32"W	5/10/2018	GELH Lab
2	27°44'11.70"N	97°09'43.81"W	12/11/2017	GELH Lab
2	27°44'11.70"N	97°09'43.81"W	5/11/2018	GELH Lab
3	27°50'17.25"N	97°14'38.89"W	11/15/2017	GELH Lab
3	27°50'18.39"N	97°14'18.16"W	11/15/2017	GELH Lab
3	27°52'36.83"N	97°15'18.12"W	11/15/2017	GELH Lab
3	27°50'17.25"N	97°14'38.89"W	12/9/2017	GELH Lab
3	27°50'18.39"N	97°14'18.16"W	12/9/2017	GELH Lab
3	27°49'26.54"N	97°13'19.12"W	3/13/2018	TPWD
3	27°50'04.46"N	97°13'38.32"W	3/13/2018	TPWD
3	27°50'13.77"N	97°14'13.42"W	3/13/2018	TPWD
3	27°52'38.01"N	97°16'07.92"W	3/13/2018	TPWD
3 [1,2]	27°49'26.54"N	97°13'19.12"W	3/29/2018	GELH Lab
3 [1,2]	27°52'38.01"N	97°16'07.92"W	3/29/2018	GELH Lab
3 [1,2]	27°50'13.35"N	97°14'06.18"W	3/29/2018	GELH Lab
3	27°50'18.39"N	97°14'18.16"W	5/9/2018	GELH Lab
4	27°48'50.22"N	97°23'27.92"W	4/18/2018	GELH Lab
4	27°51'06.10"N	97°21'41.19"W	5/14/2018	GELH Lab
4	27°51'34.27"N	97°20'48.58"W	5/14/2018	GELH Lab
4 [2]	27°50'59.30"N	97°21'9.38"W	5/16/2018	GELH Lab
5	27°42'32.17"N	97°18'45.24"W	2/17/2017	Marine Ecology
5	27°42'32.17"N	97°18'45.24"W	2/16/2018	Marine Ecology
5	27°42'32.17"N	97°18'45.24"W	5/14/2018	GELH Lab
5 [2]	27°42'34.40"N	97°18'28.69"W	5/16/2018	GELH Lab
6	27°40'54.56"N	97°13'45.76"W	2/22/2017	Fisheries Techniques
6	27°40'28.22"N	97°13'26.11"W	2/22/2017	Fisheries Techniques
6	27°42'01.02"N	97°12'53.57"W	4/19/2017	Fisheries Techniques
6	27°40'28.22"N	97°13'26.11"W	4/19/2017	Fisheries Techniques
6	27°40'28.22"N	97°13'26.11"W	11/20/2017	GELH Lab
6 [3]	27°37'52.55"N	97°13'03.40"W	11/20/2017	GELH Lab
6	27°40'28.22"N	97°13'26.11"W	5/11/2018	GELH Lab

Appendix 2: Supplemental YSI Data.

Appendix 2: Supplemental YSI Data.

Table A2. The range and average of the water quality data for every sample area.

Area	Temperature Range (°C)	Temperature Average (°C)	Salinity Range (ppt)	Salinity Average (ppt)	pH Range (s.u.)	pH Average (s.u.)	DO Range (mgL^{-1})	DO Avera (mgL
1	13.06 - 26.30	20.33	33.19 - 50.75	39.45	8.02 - 8.35	8.18	5.10 - 10.24	6.8
2	13.85 - 25.26	19.56	30.63 - 31.44	31.04	7.93 - 7.98	7.96	7.14 - 8.90	8.0
3	13.82 - 24.09	20.66	29.10 - 33.01	30.81	7.71 - 8.12	7.93	7.09 - 9.06	8.0
4	19.90 - 29.70	24.33	32.33 - 35.25	33.73	7.93 - 8.35	8.10	7.75 - 8.47	7.9
5	16.75 - 30.63	21.25	11.00 - 32.90	24.78	7.80 - 8.62	8.13	6.48 - 12.07	8.3
6	17.77 - 26.52	23.36	32.50 - 40.08	35.13	8.06 - 8.37	8.26	7.48 - 11.27	8.8

Appendix 3: Supplemental Juvenile Fish Catch Data.

Appendix 3: Supplemental Juvenile Fish Catch Data.

Table A3. The breakdown of the total number of juvenile fishes caught by the effort of GELH Lab, TPWD, Fisheries Techniq
Class 2017 and Marine Ecology Class 2017 and 2018.

	Benthic Pisc-/Carnivore	Benthic Pisc-/Carnivore	Benthic Pisc-/Carnivore	Bethic	Bethic/ Planktivore	Planktivore	Planktivore	Planktivore	
	M. undulatus	*S. ocellatus*	*L. rhomboides*	*L. xanthurus*	*Mugil* spp.	*Menidia* spp.	*Anchoa* spp.	*Brevoortia* spp.	
Area 1	14	6	70	68	60	70	0	0	
Area 2	0	28	60	55	0	59	0	0	
Area 3	14	70	70	70	3	70	18	0	
Area 4	0	0	60	61	60	60	9	70	
Area 5	70	7	70	38	40	38	23	6	
Area 6	50	0	55	39	25	0	70	0	
Total	**251**	**120**	**385**	**331**	**188**	**297**	**120**	**76**	**1**

Appendix 4: Supplemental Juvenile Fish Processing Data.

Appendix 4: Supplemental Juvenile Fish Processing Data.

Table A4. The breakdown of the total amount of juvenile fish processed and examine for MP in for each of the areas.

	Benthic Pisc-/Carnivore	Benthic Pisc-/Carnivore	Benthic Pisc-/Carnivore	Benthic	Bethic/ Planktivore	Planktivore	Planktivore	Planktivore
	M. undulatus	*S. ocellatus*	*L. rhomboides*	*L. xanthurus*	*Mugil* spp.	*Menidia* spp.	*Anchoa* spp.	*Brevoortia* spp.
Area 1	14	6	8	8	8	20	0	0
Area 2	0	15	8	8	0	15	0	0
Area 3	8	15	9	8	3	8	15	0
Area 4	0	0	8	15	8	8	0	15
Area 5	15	7	8	8	7	8	15	6
Area 6	0	0	8	10	8	0	15	0
Total	**37**	**43**	**49**	**57**	**34**	**59**	**45**	**21**