Microplastic Pollution and its Occurrence In The Diet of Juvenile Fish and Shrimp in Texas Bays

Final Report

GLO Contract No. 19-044-000-B078

September 2021

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A report funded by a Texas Coastal Management Program Grant approved by the Texas Land Commissioner pursuant to National Oceanic and Atmospheric Administration Award No. NA18NOS4190153

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EXECUTIVE SUMMARY

Commercial and recreational fisheries in Texas contribute billions to the state's economy while supporting thousands of jobs. The long-term health of this economic engine could be threatened by the growing amount of plastic, specifically microplastic (< 5 mm diameter), that is now ubiquitous in aquatic ecosystems. The degree to which microplastic is entering in aquatic food webs was investigated by this baseline study that assessed the ingestion of microplastic by juvenile stages of finfish collected in seagrass nursery habitats in four bay systems along the central Texas coast (Baffin Bay, Aransas Bay, San Antonio Bay, and Matagorda Bay) for the first time. Microplastic concentrations in the adjacent water column and microplastic categories ingested by juvenile fish were compared between the bay systems as it was hypothesized that different surrounding population densities and land use types may lead to different pollution levels. Seasonal differences in microplastic ingestion were tested as the loading of the water column may be influenced by changes in precipitation rates to either increase the runoff of microplastic into bay systems with higher precipitation. If this effect is low, alternatively microplastic densities may be elevated during dry periods, as the same amount of microplastics would then concentrate in a lower amount of water. Third, we tested for different levels of microplastic ingestion between different species/taxa associated to different feeding guilds, as the exposure and ingestion likelihood to microplastic pollution may differ from pelagic to benthic feeders. And lastly, we tested for correlation between fish health indices and amount of microplastic detected in the digestive tracts as microplastic ingestion has been shown to negatively affect fish health in mainly laboratory studies.

For this study 735 digestive tracts of juvenile fish were analyzed for microplastic ingestion collected between Winter 2018 and Summer 2019. The majority of fish belonged to one of two taxonomic groups for which members were encountered ubiquitously in most of the 3 sampling stations within each of the 4 bays during 3 seasons (winter, spring and summer): Silversides (*Menidia* sp.) represented the pelagic feeding mode foraging in the water column and underneath the water surface, and Sciaenidae (Drums and Croakers) were represented by species that are primarily benthic feeders associated to the bottom or other surfaces. Results from these two taxonomic groups were used to compare for differences between bay systems and sampling season to minimize the effect of an unbalanced design in terms of foraging type as good as possible. In addition, a total of 13 species/taxa that were encountered in only some sampling stations were included for analyzing differences in microplastic ingestion between species/taxa. The original plan to include brown shrimp as well could not be realized due to low specimen numbers collected. In addition to juvenile fish, standard water parameters as well as microplastic pollution levels in the adjacent water body was collected during each sampling trip.

A multi-step analysis in the lab under cleanroom conditions was subsequently conducted to accurately assess microplastic pollution in the samples that was followed by a 2-step process to correct data for (i) airborne pollution and (ii) to confirm visually identified “suspected microplastic" by a chemical composition analyses as either "confirmed microplastic" or as "man-made natural", which is a category that we decided to include due to its prevalence in the juvenile fishes' digestive tracts. We decided to report both "suspected" and "confirmed microplastic" results as there has been no standardized procedure for correcting and reporting the data in the field, which means that you can find that results reported in the literature provide corrected data equivalent to our "confirmed microplastic" level, that provide uncorrected date for ingestion levels equivalent to our "suspected microplastic" level and report results from chemical confirmation separately, and earlier studies may only report data equivalent to "suspected microplastic" level without accompanying chemical confirmation.

The majority of "suspected" and "confirmed microplastic", as well as "Man Made Natural" were in the form of fibers, with less particles and rare films, which reflected the order found for "suspected microplastic" in the parallel water samples. Feeding incidence, the number of juvenile fish that contained at least one item in their digestive tract, was high at 77% and 71% respectively for the categories "suspected microplastic" and "man-made natural fibers", whereas it was only 24% for "confirmed microplastic". Clear, white, blue, black and red were the dominant colors in "suspected microplastics" from both water samples and fish digestive tracts as well as from ingested "manmade natural fibers", whereas white, brown, purple and orange dominated for the ingested "confirmed microplastic".

There were no differences found in microplastic ingestion levels between the four bay systems which may be explained by their relative similarity in terms of population densities along their coastlines, with Aransas Bay being the one with highest exposure compared to the rural environment of the other three bays, but all four bays ranking comparatively low compared to for example Galveston and Corpus Christi Bays in terms of human impact.

Interestingly, Silverside and Sciaenid juvenile fish collected during winter had a significantly higher amount of ingested "suspected and confirmed micro plastic" as well as "Manmade Natural fibers" than those collected during summer. Precipitation levels during the winter period were lower which may have led to a higher concentration of microplastic particles in the environment, although the limited and snapshot data from parallel water column "suspected microplastic" did not show a similar pattern. Different food availability between the seasons may also have led to a seasonally higher ingestion of microplastic during winter.

No clear pattern was found for ingestion of microplastic or manmade natural fibers in relation to feeding guilds, but species-specific differences were detected. Silversides was the taxon with high numbers of ingested "Man-Made Natural Fibers" leading to its first rank in the "suspected microplastic category" as well. For "confirmed microplastics", that were ingested at a much lower level throughout, the three sciaenid taxa, Seatrouts, Red Drum, and Atlantic Croaker, joined Silversides as species with higher ingestion numbers. Of those three sciaenid taxa, especially Red Drum and Seatrouts occupy a higher trophic level and are voracious predators in the bay systems as adults which makes them popular sportfish. In their juvenile fish they already make this transition including documented cannibalism when reared in controlled environments. So for these taxa, biomagnification which is the ingestion of prey that had microplastics ingested, may have contributed to their elevated position in the interspecific/taxa comparison. Silversides on the other hand feed in the water column and underneath the surface, so their high rank supports the hypothesis that pelagic foraging types are more prone to the ingestion of man-made materials. Less selective feeding and bulk ingestion of prey during filter feeding in opposition to more selectively picking prey from surfaces may have contributed to our results. Interestingly the second pelagic, filter-feeding taxon, Anchovies, showed much lower ingestion of man-made items.

Lastly, we did not find any correlation of number of ingested "confirmed microplastic" nor "man-made natural" fibers with one of the two integrative indicators of fish health, the nutritional condition factor and the Hepatosomatic index. This may be interpreted that there is no effect of ingestion load of man-made items on fish health at the observed levels for juvenile fish. However, we noted a decrease in variance and a tapering off to lower condition scores with higher number of both "confirmed microplastic" and "man-made natural fibers", which may warrant further investigations.

In conclusion, we found prevalence of "confirmed microplastic" pollution in digestive tracts of fish in all four bay systems with a quarter of fish containing at least one item, but overall load in digestive tracts appeared to be low. In addition, we detected a much higher number of fibers belonging to a newly established category, "man-made natural fibers", which may warrant more focus potential effects on aquatic food webs and health in the future.

CAPACITY BUILDING and OUTREACH EFFORTS

Capacity Building

This project funded one M.S. graduate student's thesis work (Mx. Gray Ryan). 8 undergraduate research assistants were associated to the project and were trained and worked both in field and lab methods (Cindy Hielscher, Michaela Rust, Rachel Moy, Ashleigh Campbell, Raina Watts, Angelina Weigand, Kaylee Sparkman, Elizabeth Birdwell). Additional 9 graduate and 4 undergraduate students gained field sampling experience (Shannan McAskill, Elizabeth Dibona, Carol Hailey, Kaylin McKinley-Zipp, Emily Miller, Polly Hajovsky, Matthew Watford, Jennifer Whitt, Nicolette Beeken, Roy Roberts, Annika Heising-Huang, Lisa Gignac, Stormy Paxton).

Outreach

Educational booth at the Texas State Aquarium World Ocean Day 2019 including two marine plastic pollution educational displays/activities and one marine plastic pollution-related videogame, June 8, 2019 by PI and students.

Microplastic themed Teen STEM café event for high-school students was held by Matt Watford of Dr. Conkle's lab on October 28, 2019.

Video documenting module showcasing the rationale different steps of the project was produced and submitted to TGLO. It will also be hosted at the lab's website www.geistlarvallab.com.

Session Organizer and Chair (scientific conference)

Dr. Geist organized and chaired a conference session on "Marine plastic pollution from nano- to macro-scale: fate, effects, solutions" at the 25th Biennial Coastal and Estuarine Research Federation conference in November 2019, Mobile, AL.

Presentations (scientific conferences)

Ryan, M.G., Geist S. (2021) Occurrence of microplastics in the diet of juvenile fish in five Texas Coastal Bend bays. 3rd Texas Plastic Pollution Symposium. March 4, 2021. (virtual, talk), Ryan et al. talk starts at 2:22:38: https://www.youtube.com/watch?v=wxAHxZoe2w0

Ryan, G., Geist, S. (2020) Microplastic ingestion by juvenile silversides (*Menidia* spp.) in seven bays and estuaries along the Mid-Texas coast. 2020 Annual Meeting of the Texas Chapter of the American Fisheries Society, Waco, TX. Jan 24, 2020. (talk), https://units.fisheries.org/tx/tc-meetings/2020-meeting-home-page/2020-meeting-podcasts/

Ryan, M.G., Geist S. (2019) Occurrence of microplastics in the diet of juvenile fish in five Texas Coastal Bend bays. 25th CERF Biennial Conference, Mobile, AL. Nov 5, 2019. (talk)

Presentations (public)

Geist, S. "Juvenile Fish and Microplastic in Texas bays". Public talk in the Science on the Bayou series at Jefferson Street Pub, Lafayette, Louisiana on March 13, 2019.

Presentations (university)

Geist, S. "Larval and juvenile fish research in the Early Life History lab at Texas A&M University Corpus Christi. Ichthyoplankton Food web, Microplastic Pollution and a Hurricane." Marine Biology Department Seminar Series, University of Louisiana at Lafayette, Lafayette, Louisiana. March 14, 2019

Geist, S. "Effects of microplastics on organisms". Guest lecture for undergraduate class "Microplastics in Coastal Environments", Dr. Zhanfei Liu at the University of Texas Marine Science Institute, Port Aransas, TX on June 2, 2020.

Manuscript

Ryan, G., Rust, M., Hielscher, C., Weigand, A., Geist, S.J. "Microplastic in the diet of juvenile fish across five Texan Coastal bay systems." (*In Prep*)

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ACKNOWLEDGEMENTS

Firstly, we would like to thank the Coastal Management Program of the Texas General Land Office for providing support for this project.

Numerous Individuals helped throughout the duration of the project, with whose support, dedication and enthusiasm this research would not have been possible.

For field work we would like to thank Chriss Shope and Joshua Martin for driving the trucks and boats to the field sites and lending a hand to pull seine nets. We would like to thank the following students, volunteers and colleagues for their help during field sampling trips: Elizabeth Dibona, Carol Hailey, Kaylin McKinley-Zipp, Emily Miller, Polly Hajovsky, Roy Roberts, Matthew Watford, Jennifer Whitt, , Dr. Frauke Seemann, Mathieu Grancher, Annika Heising-Huang, Nicolette Beeken, Lisa Gignac, Shannan McAskill and Stormy Paxton.

Our team of dedicate undergraduate project research assistants that did a tremendous job in both the field and the lab Michaela Rust and Rachel Moy; and our excellent and dedicated lab work team Cindy Hielscher, Raina Watts, Angelina Weigand, Kaylee Sparkman, Ashleigh Campbell, and Elizabeth Birdwell. We would also like to thank Matthew Watford for his help and expertise on the FTIR instrument, and Jason Selwyn and Shannan McAskill for support and discussions for data analysis.

We would also like to thank TAMU-CC College of Science and Engineering and Department of Life Sciences staff for direct administrative support: Luis Hernandez, Lisa Garza, Heather Calderon, Estevan Espinoza, Sarah Khan, Jessica Ramon, Suzann Burgess, Kenneth Brown.

And last but not least this study would not have been possible without a Texas Parks and Wildlife Department Scientific Sampling Permit to Dr. Geist (SPR-0316-065) for which Christopher Maldonado, TPWD was the point of contact, and without Animal Use Protocols AUP 01-18 and 03-19 approved and administrated by the TAMU-CC Compliance Office led by Rebecca Ballard and the TAMU-CC IACUC Committee.

1. INTRODUCTION

1.1 Background Information

Commercial and recreational fisheries in Texas contributed billions to the state's economy while supporting thousands of jobs. The long-term health of this economic engine could be threatened by the growing amount of plastic, specifically microplastic (< 5 mm diameter), that is now ubiquitous in aquatic ecosystems. Both the effect of microplastic on the nursery function and its loading to Texas's coastal bays and estuaries had not been investigated until this study.

This baseline study assessed the ingestion of microplastic by juvenile stages of finfish that use Texas bay systems as nursery habitat in four bay systems along the central Texas coast (Baffin Bay, Aransas Bay, San Antonio Bay, and Matagorda Bay), none of which have been assessed for microplastic pollution.

1.2 Microplastics

Microplastics are defined as plastic pieces smaller 5 mm in size (Andrady. 2003) and microplastic pollution is found in many aquatic ecosystems both marine and fresh (Barnes et al., 2009). Microplastics can be classified into two distinct categories based on the process they were formed. Primary microplastics are particles manufactured to be microscopic and are or were found in products with abrasive properties such as air-blasting media, hand or facial cleaners, and cosmetic products (Gregory, 1996; Zitko and Hanlon, 1991). Plastic pellets (nurdles) used in the manufacturing of macroplastic also fall into this category (Isobe, 2016). Secondary microplastics are formed from the fracturing and break-down of larger plastic items (Cole et al., 2011), and appear in a variety of forms, including fibers, films, and fragments (Zhu et al., 2018; Zobkov and Esiukova, 2017).

Two main categories of pollution sources can be distinguished. "Point-sources" are those that can be traced back to a single location or a distinct time period, which can include waste spills, storage tank seepage, industrial and municipal waste outfalls, smaller-scale runoff events, and wastewater systems (Loague and Corwin, 2005). Point sources of marine microplastic pollution are typically coastal areas associated with higher human activity. For example, sewage disposal areas, including those that haven't been actively used for over a decade, consistently had over 250% higher microplastic concentrations than similar reference sites, much of which likely entered the environment as microplastics (Browne et al., 2011). Machine washing and drying of polyester and acrylic clothing is another likely source for microplastic pollution that can enter aquatic systems over wastewater and sewage outflows (Browne et al., 2011). Nonpoint pollution sources are those that are diffuse in nature and cannot be traced back to a single, distinct location (Corwin and Wagenet, 1996). Examples include large-scale activities like agriculture irrigation, urban and industrial runoff, erosion associated with anthropogenic activities like construction, and atmospheric deposition (Loague and Corwin, 2005). Terrestrial nonpoint source pollutants typically enter the environment over an extended time and large area and its amplitude is typically associated with natural meteorological processes such as rainwater runoff which can lead to seasonal differences in microplastic pollution loads (Loague and Corwin, 2005; Doyle et al., 2011). Commercial and recreational fishing activities can also add nonpoint microplastic pollution, through for example shedding fibers and fragments from netting material as well as improver disposal of fishing line (Dantas et al., 2012).

Table 1 Examples of specific densities of fresh- and seawater and different plastic materials according to Callister and Rethwisch, 2011; Oberg et al., 2016, GESAMP 2015

Water and Plastic Materials	Specific Density (g cm^{-3})
Freshwater at 0°C	1
Freshwater at 30°C	0.95
Seawater	1.02-1.03
Styrene	0.94
Low-density polyethylene (LD-PE)	0.925
Polytetrafluoroethylene (PTFE)	2.17
Polyvinyl chloride (PVC)	1.384
Polyethylene (PE) terephthalate	1.39
Nylons	1.135 – 1.163
Polystyrene	1.09

Vertical position of microplastic pollution in the water column depends on its buoyancy, and microplastics can be broadly categorized into three types (floating, suspended, or sinking) dependent on their density in relation to the density of the water (GESAMP, 2015). The density of brackish and seawater is a function of salinity and temperature (Table 1).

Floating microplastics are less dense than the surrounding water and therefore aggregate on the water surface, for example styrene and low-density polyethylene (Table 1). Sinking microplastics, on the other hand, are denser than the surrounding water and tend to settle over time, for example polytetrafluoroethylene, polyvinyl chloride and polyethylene terephthalate (Table 1). Microplastic types with densities similar to that of seawater may remain suspended in the water column, with their exact position influenced by densities of surrounding water as well as water movements. Types of plastic that tend to remain suspended somewhere in the water column with certain nylons and polystyrene as examples (Table 1). Additional factors influencing microplastic buoyancy include size and shape, as well as biofouling (Kowalski et al., 2016).

1.3 Ingestion of microplastics by fish

Ingestion of macro- and microplastics by fish has been observed across a variety of fish taxa and feeding types, which may include direct ingestion as well as trophic transfer by eating something that had ingested microplastic (Ferreira et al., 2019). No clear pattern has emerged yet about the role of purpose and intent in microplastic ingestion and different studies reported both positive and negative selection. The potential role of microplastics in the diets of juvenile fish got into focus over the past 5 years with studies being conducted around the world. Based on laboratory and field studies it seems that when exposed to suspended microplastics, many species of juvenile fish will ingest them. Juvenile gobies in a laboratory setting ingested each of three types of microplastic spheres when exposed (de Sá et al., 2015). Anemonefish in a laboratory setting ingest a variable number of microplastics, depending in part on the activity level of the fish, and in part on the fish phenotype (Nanninga et al., 2020). On the coastline of South Africa, 52% of juvenile fish had ingested microplastics at an average frequency of 0.79 ± 1.00 microplastics particles per fish (Naidoo et al., 2020). Two species of drums in the Goiana estuary demonstrated a lower degree of microplastic ingestion in the juvenile stage than in the adult stage (Dantas et al., 2012). The majority of sympatric snooks (*Centropomus undecimalis* and *Centropomus mexicanus*) examined had recently ingested microplastics regardless of ontogenetic stage, but microplastic contamination rates tended to be lower in the juvenile stage than in the adult stage (Ferreira et al., 2019). 100% of the sciaenid species *Micropogonias furnieri* sampled from the Bahia Blanca Estuary in Argentina ingested microplastics (Arias et al., 2019). A mean of 12% of the particles composing diets of juvenile blueback herring in the Hudson River were microplastics, and these fish demonstrated a selective avoidance of microplastics as a food type (Ryan et al., 2019). In the nearshore marine environments of the east coast of Vancouver Island, BC, juvenile Chinook Salmon digestive tracts contained an average of 1.2 pieces of plastic per fish, most of which were fibers (Collicutt et al., 2019).

Few microplastics ingestion studies focused on juvenile fish exist in the Gulf of Mexico. In several watersheds feeding the Gulf of Mexico, 8% of freshwater and 10% of marine fishes examined contained microplastics in their digestive tracts (Phillips and Bonner, 2015). Even fewer studies examine the ingestion of microplastics in the bays and estuaries of the Texas coast. Within Corpus Christi Bay and the Upper Laguna Madre, over 81% of sampled juvenile fish had ingested suspected microplastics. The amount of "suspected microplastics" (based on visual identification without confirmation through chemical analysis) found in each digestive tract varied between species, with the most abundant microplastic counts occurring in the digestive tracts of Spot (*Leiostomus xanthurus*), Menhaden (*Brevoortia* spp.), and Silversides (*Menidia* spp.) (Hajovsky, 2019). Adult and juvenile fish belonging to six coastal species from Galveston Bay to Freeport, 42.4% of fish had ingested microplastics (Peters et al., 2017). In the Brazos River Basin, 45% of Bluegill and Longear sampled had ingested microplastics (Peters and Bratton, 2016). In Corpus Christi Bay, 36% of blue crabs contained fully or semisynthetic fragments and fibers at a frequency of around 0.87 items per individual (Waddell et al., 2020).

Despite what may be a disproportionate effect of microplastics on the diets of juvenile fish in Texas Coastal regions, ingestion of microplastics by juvenile fish of the bays and estuaries of Texas had until now remained unstudied, except for Corpus Christi Bay and the Laguna Madre. The present study fills this knowledge gap by providing baseline estimates of microplastic ingestion by juvenile estuarine fish of San Antonio Bay, Matagorda Bay, Baffin Bay, and Aransas Bay. As the incidence rates of microplastics ingestion were somewhat higher in previous Texas studies than in similar studies from other parts of the world, it is hypothesized that microplastics ingestion in the Texas Coastal Bend bays will be occurring in relatively high frequency.

1.4 Factors potentially affecting microplastic ingestion

1.4.1 Feeding Guild and Foraging Type – Floating and sinking plastic types

It is hypothesized the feeding guild to which a fish belongs influences its tendency to ingest microplastics. Planktivorous fish feeding in the water column may ingest more floating plastic types and may be less selective than demersal fish that feed on organisms in or on the sediment and other surfaces. A MS thesis on microplastic ingestion in Corpus Christi Bay, Texas found a tendency for planktivorous species to ingest higher quantities of "suspected microplastics" than other feeding groups (Hajovsky, 2019). Buoyancy characteristics of microplastics play a role in the accessibility for organisms, with sinking materials more accessible to benthic feeders and floating and suspended materials more available to pelagic feeders (Mc Neish et al., 2018). Microplastics ingested in the central North Pacific gyre were, on average, positively buoyant in seawater (Choy and Drazen, 2013). For this reason, it is hypothesized that microplastic is more readily ingested by fish species that feed at the water's surface, like for example silversides. However, significant relationships between feeding type and microplastics ingestion were not always apparent in microplastic ingestion studies with several that did not find a clear relationship (Dantas et al., 2020; Filgueiras et al., 2020; Lusher et al., 2013; Phillips and Bonner, 2015; Vendel et al., 2017).

1.4.2 Microplastic Color

Microplastic color selection is hypothesized to be related to feeding types. Fish species that primarily use eyesight to detect food will have a higher degree of color selectivity than those that use non-visual cues and senses. Transparent(clear)/white, lack/gray, red, and blue microplastics (not necessarily in that order) tend to be the most frequently ingested microplastics colors, but this varies between species and sampling location. Species and locations in which transparent (clear) or white is the most frequently reported color include pelagic fish of the North Pacific gyre (Boerger et al., 2010), 21 marine species and 6 freshwater species of China (Jabeen et al., 2017), fish of Magdalena Bay on the Pacific Coast of Mexico (Jonathan et al., 2021), Indian Mackerel and Honeycomb Grouper of the Tuticorin coast of India (Kumar et al., 2018), coastal fish of the Guarapari Islands (Macieira et al., 2021), 46 species of the Amazon River estuary (Pegado et al., 2018), five species of the North and Baltic Seas (Rummel et al., 2016), and Japanese Anchovy of Tokyo Bay, Japan (Tanaka and Takada, 2016). Species and locations for which ingestion of black/gray microplastics is most common include six species of the Musa Estuary and Persian Gulf (Abbasi et al., 2018), ten species of mesopelagic fish of the North Atlantic (Lusher et al., 2016), European Flounder and European Smelt of the River Thames (McGoran et al., 2017), and Bluegill and Longear in the Brazos River Basin of Texas (Peters and Bratton, 2016). Locations and species for which blue is the most commonly ingested color of microplastic include Blackmouth Catshark of the Mediterranean Sea (Alomar and Deudero, 2017) and juvenile fish of the coast of KwaZulu-Natal, South Africa (Naidoo et al., 2020).

Species and locations of fish for which red is the most commonly ingested microplastic color in eleven species of the Rio de la Plata estuary in Argentina (Pazos et al., 2017).

1.4.3 Microplastic Shape and Size

The microplastic type that tends to be most frequently ingested in most ecosystems is fibers (Abbasi et al., 2018; Alomar and Deudero, 2017; Campbell et al., 2017; Garcia and Cardozo, 2020; Hajovsky, 2019; Jabeen et al., 2017; Kumar et al., 2018; Lusher et al., 2016; McGoran et al., 2017; Naidoo et al., 2020; Pazos et al., 2017; Peters et al., 2017). Some exceptions include 46 species of the Amazon River estuary for which pellets were the most common type of microplastic ingested (Pegado et al., 2018), and five species of the North and Baltic Seas (Rummel et al., 2016) and Japanese anchovy from Tokyo Bay for which fragments were most frequently ingested (Tanaka and Takada, 2016).

Microplastic size ingested by a fish is anticipated to be correlated with fish size, feeding type, and mouth shape. As feeding type and mouth shape are species-dependent, it is also expected that the size of ingested microplastics will vary between species. Average microplastic length and the size range of microplastics ingested by fish varies in the literature on the basis of habitat and fish species. Of studies that reported size ranges, those size ranges tended to be between ~0.1 and ~5.0 mm (Abbasi et al., 2018; Naidoo et al., 2020; Pazos et al., 2017; Pegado et al., 2018; Tanaka and Takada, 2016), but reported length could be as low as low as a only a couple hundredths of a mm (Foekema et al., 2013) or as high as over a centimeter (Lusher et al., 2016). As fish that visually identify prey for ingestion tend to do so in a size-selective manner, it’s likely that their ingestion of microplastics will also be of a relatively narrow size range. Non-selective feeding methods (i.e., filter feeding) are similarly expected to ingest a wider range of microplastic sizes, especially regarding extremely small microplastics. There may also be a relationship between feeding location and size selectivity as feeding method is often related to feeding location (e.g., feeding methods that involve filtering sediment occur exclusively on the benthos, whereas planktivorous fish can be expected to feed primarily within the water column).

1.4.4 Microplastic pollution levels in the water column

Degree of urbanization of the adjacent land area is expected to have an influence on microplastic pollution levels in adjacent water bodies, which then may reflect in the amount of microplastics taken up by fish. Specifically, highly urbanized areas and certain human activities tend to mean higher levels of microplastic ingestion by fish in nearby bodies of water. For example, the contamination of sympatric snooks by microplastics increased with heavy fishery activity and increased river basin runoff (Ferreira et al., 2019). Thirteen freshwater species in Southern Brazil demonstrated a positive correlation between high microplastics ingestion and more highly urbanized streams (Garcia and Cardozo, 2020). In the Gulf of Mexico, 29% of fish in urbanized regions ingested microplastics, whereas 5% of fish in non-urbanized regions did (Phillips and Bonner, 2015). Rain may lead to an increased run off from land washing in plastic pollution into waterways which may lead to an increase in plastic. At the same time, increased water levels may lead to lower densities in microplastic pollution levels than during a drought when microplastic may become more concentrated.

1.5 Possible effects of microplastic ingestion on fish body condition and behavior

Plastic ingestion by fish sometimes results in changes in fish behavior or condition. Some, but not all, gobies collected from the northwest Iberian coast that had ingested microplastic spheres showed decreased predation success and efficiency (de Sá et al., 2015). Reduction in food intake caused by microplastic ingestion may have the potential to decrease individual and population fitness (de Sá et al., 2015). Microplastic ingestion can negatively affect nutritional condition and survival rates of juvenile fish and shellfish species, resulting in poor stock recruitment and less fishable adults. However, the relationship between microplastic ingestion and body condition or behavior is not always clear. For example, of omnivorous intertidal fish, those that had ingested higher levels of microplastics tended to have lower body condition factors (Mizraji et al., 2017). However, *Acanthochromis polyacanthus* in a laboratory setting did not show a difference in fish growth, body condition, or behavior as a result of microplastic exposure (Critchell and Hoogenboom, 2018). Ecotoxicological effect studies on microplastic ingestion aimed to estimate predicted no-effect concentration (PNEC) of microplastics for several marine species, the mean predicted no-effect concentration (PNEC) for microplastics was 3.84 × 106 parts m^{-3} (Adam et al., 2021). The most sensitive species examined was *Oryzias melastigma*, a fish within family Adrianichthyidae, with a calculated no observed effect concentration (NOEC) of 3.9 × 106 parts m^{-3} (Adam et al., 2021). The global mean exposure concentration (MEC) is 1.5 × 103 parts m^{-3}, with the highest concentrations occurring in the Pacific and Atlantic Oceans (Adam et al., 2021).

In addition to the potential direct influence of microplastics on fish health and condition, they can also act as vectors for toxic chemicals that adhere to their surface. For example, plastics in the marine environment are capable of absorbing and concentrating persistent organic pollutants (POPs) and may even be a vehicle for trophic transfer of POPs (Andrady, 2011; Betts, 2008). Microplastics have similarly high sorption ability for hydrophobic organic contaminants (HOCs), including dioxin-like chemicals (DLCs), especially when the microplastic is older or is made of Styrofoam™ (Chen et al., 2019). Microplastics are also capable of absorbing polychlorinated biphenyls (PCBs) from the surrounding water (Carpenter et al., 1972). The concentration of PCBs within microplastics in the marine environment can be over a million time higher than the surrounding water (Betts, 2008). Pellets from a beach in Tokyo contained concentrations of absorbed PCBs of up to 1200ng/g, and high PCB concentrations were associated with higher degrees of microplastic discoloration (i.e. yellowing), which could be of ecological significance to marine organisms with color-selective feeding behaviors (Endo et al., 2005). Microplastics are also capable of absorbing heavy metals. Plastic production pellet samples recovered from Devon, England revealed enrichment on cadmium and lead in plastic pellets that had been exposed to sea water. The forms of these metals accumulated in these plastic pellets are also hypothesized to be relatively bioaccessible (Ashton et al., 2010). In fact, mercury has observable influence on bioaccumulation, the concentration of toxic compounds as they are passed upward tropically, of mercury in European seabass (Barboza et al., 2018).

1.6 Study Objectives

This baseline study aimed to assess the ingestion of microplastic by juvenile stages of finfish that use bay systems as nursery habitat in four bay systems along the central Texas coast (Baffin Bay, Aransas Bay, San Antonio Bay, and Matagorda Bay), none of which have been assessed for microplastic pollution during three seasons characterized by different precipitation patterns.

Water samples were collected near the collection sites of juvenile finfish to describe the range and mean of the microplastic concentrations measured at each sampling site and differences between bay systems and seasons.

Juvenile stages of fish were collected to determine the differences in microplastic ingestion between locations, foraging types and species.

2. STUDY AREAS

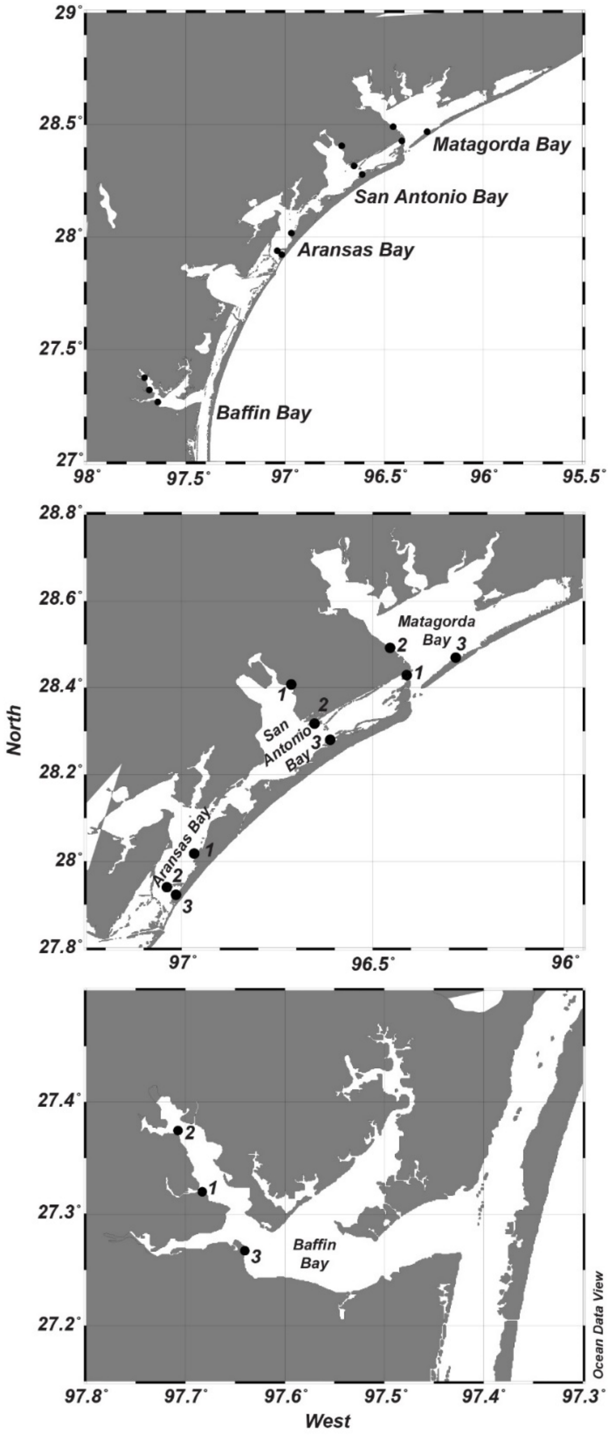

Figure 1 Map of sampled bays and three sampled sites per bay (black dots). Top: Overview, Center: Matagorda, San Antonio and Aransas Bay, Bottom: Baffin Bay

Sampling for this project took place at four Texas Coastal Bays (Matagorda, San Antonio, Aransas and Baffin Bay, Fig. 1). Three sites were sampled in each bay system during three different times of the year. Seagrass habitats were chosen as sample sites and site selection as aimed to reflect different distances to potential point-sources of microplastic pollution where possible with station 1 being the closest to a local population center (Table 2). Coordinates for all sampling stations are provided in Table 3.

Table 2 Population of local centers in each of the four studied bay systems (data from: https://www.texas-demographics.com/)

Bay	Population Center	Population (2020)
Matagorda Bay	Port O'Connor	954
San Antonio Bay	Seadrift	995
Aransas Bay	Rockport/Fulton	10,070/1,523
Baffin Bay	Loyola/Riviera Beach	unincorporated

Table 3 Seagrass habitat sampling station coordinates, short description of location, and approximate distance from population centers mentioned in Table 1 (distance estimated using Google Earth)

Station Code	Latitude (North)	Longitude (West)	Short Description	Approx distance from population center (miles)
MB-1	28.429452°	-96.411250°	Port O'Connor/ Little Mary's Cut	0.5
MB-2	28.492607°	-96.454791°	near LaSalle Bayou	6
MB-3	28.470290°	-96.283029°	Matagorda Peninsula past airstrip	8
SB-1	28.407709°	-96.713292°	Seadrift	0
SB-2	28.317681°	-96.652387°	Grass Island near Intracoastal Waterway	9
SB-3	28.280529°	-96.611316°	near Long Lake / Matagorda Island	12
AB-1	28.018093°	-96.967667°	San Jose Island across Rockport	5
AB-2	27.939574°	-97.039073°	Mud Island	6
AB-3	27.922527°	-97.015361°	San Jose Island behind Mud Island	9
BB-1	27.319796°	-97.683057°	Loyola Beach Boat Ramp	0
BB-2	27.374520°	-97.707490°	Cayo de Grullo/Drum Point	4
BB-3	27.267033°	-97.640325°	Pie de Gallo	5

3. METHODS

3.1 Sampling Frequency and Climatic Variation

Juvenile fish and water were sampled for microplastic pollution analysis during three different times of the year (Winter, December 2018-January 2019; Spring, March-April 2019; Summer, May-June 2019), to accommodate for spawning seasons of fall, winter and spring spawning species assuming an age of collected fish of around 60 days post hatch. Sites were also revisited to cover seasonally different rainfall which may contribute to the level of land-derived microplastic pollution washed into the bays. Average rainfall for the Texas Coastal Bend during the sampling period was highest during Summer 2019 and lowest during Spring 2018 (Table 4).

Table 4 Approximated rainfall for the Texas Coastal Bend Mean rainfall from July 2018 to June 2019 adapted from the National Weather Service's "Observed Monthly Rainfall for Southern Texas" (National Weather Service, 2019) online database by averaging the monthly rainfall for Kingsville, Rockport, and Port Lavaca.

Year	2018						2019					
Month	J	A	S	O	N	D	J	F	M	A	M	J
Sampling season						Winter			Spring		Summer	
Average monthly precipitation (inches)	2.8	1.8	14.6	3.6	2.4	2.0	2.7	1.3	1.6	1.8	3.9	6.1

3.2 Water Sampling

Microplastic in the nearby water column was sampled adjacent to juvenile fish sampling sites when a water depth of 1.5 m or greater was reached while moving along a perpendicular line away from fish collection site into deeper water. This was necessary to allow for vertical net samples of the water column of at least 1 m using a small ring net (diameter: 20 cm diameter; mesh size: 20 µm). The net was deployed vertically from a stationary boat for the top 1-2 m of the water column, and the depth (h) to which the net was deployed was recorded for each of four consecutive hauls. The net was rinsed thoroughly from the outside after each haul and each sample was filled in and stored in a labelled 8 oz glass jar and placed in a cool, dark location for long-term storage. The volume of water sampled (V) in each haul was calculated as volume of a cylinder: $V=\pi r^2 h$, with r = 10cm.

Water temperature, pressure, dissolved oxygen, conductivity/specific conductance, salinity, pH, and depth data were collected using a YSI EXO1 sonde (Xylem, USA) calibrated before each sampling day at three water depths at each site: at the water's surface where the fish collection occurred, and at the surface and bottom where water at the locations of the water sampling. Additionally, turbidity was measured using a Secchi disk at the location where the water column was sampled for microplastics.

3.3 Juvenile Fish and Shellfish Sampling

Fingerling-sized fish were collected in seagrass habitat near the shore and at shallow water depths under 1 m. At each site, a minimum of three bag seine net hauls (8.4 m length, 1.1 m height, 4 mm mesh) were completed. All juvenile fish were removed from the net and identified to the lowest taxonomic level possible with the naked eye. Several ecologically and economically important fish species that fill varying ecological niches were selected as target species for this study (Table 5). The first 20 individuals of each previously identified target taxa within the taxon-specific size range (Table 5) were euthanized via ice slurry, and all other fish were released following approved procedures (TPWD Sampling Permit SPR-0316-065; TAMU-CC IACUC protocols AUP 1-18 and 3-19). Bag seining continued until either 20 individuals of each target taxonomic group within the target size range were captured or additional net pulls failed to present new diversity. In addition to fish, Brown and White Shrimp were also targeted, but not caught in sufficient numbers that would have allowed to include them in the study. The euthanized fish were individually bagged in small sealable plastic bags and separated by taxonomic group into larger Ziplock® bags along with a water-proof label including the bay, site, date, and species. These Ziplock® bags were further separated by sampling site and placed into even larger Ziplock® bags labelled with the bay, site, and date. These bags were kept on ice until were returned to the university lab to be stored long-term in a -20°C freezer. The number of brown shrimps collected with the applied sampling method was minimal, therefore only juvenile fish could be used for the subsequent analyses.

Table 5 Target taxa listed by sceintific and common names, target size ranges and main feeding modes. Gray shaded taxa belong to the family Sciaenidae

Scientific name	**Common name**	**Target size range**	**Feeding Behavior**
Menidia spp.	Silversides	2-6 cm	pelagic
Bairdiella chrysoura	Silver Perch	2-6 cm	benthic
Cynoscion spp.	Seatrout	2-10cm	pelagic
Leiostomus xanthurus	Spot	2-6 cm	benthic
Micropogonias undulatus	Atlantic Croaker	2-6 cm	benthic
Pogonias cromis	Black Drum	2-10 cm	benthic
Sciaenops ocellatus	Red Drum	2-10 cm	benthic
Sciaenidae	Other sciaenidae	2-10 cm	
Anchoa spp.	Anchovy	2-6 cm	pelagic
Archosargus probatocephalus	Sheepshead	2-6 cm	benthic
Brevoortia spp.	Menhaden	2-6 cm	pelagic
Cyprinodon variegatus	Sheepshead Minnow	1-6 cm	benthic
Fundulus spp.	Killifish	1-6 cm	benthic
Gerreidae	Mojarras	2-6 cm	benthic
Gobiidae	Gobies	1-6 cm	benthic
Lagodon rhomboides	Pinfish	1-5 cm	benthic
Mugilidae	Mullet	2-10 cm	bentho-pelagic
Paralichthys lethostigma	Southern Flounder	2-6 cm	benthic

3.4 Fish Selection for Processing, definition of processing batches

Fish identification to genus and species level was confirmed using meristic characters with the aid of a dissecting microscope following standard identification literature (Hoese and Moore, 1977; Richards, 2006). For each individual, standard length was recorded to the nearest millimeter and a unique identification number was assigned. Any fish not suitable for further processing was sorted out (e.g., damage to the gut area). Fish taxa were not present at all sites and in varying numbers. To allow for a comparison of microplastic ingestion by specific taxa between bays and seasons it was decided that taxa/species were pooled into two larger taxonomic groups that consisted of taxonomically related species and that represented pelagic (*Menidia* sp./Silversides) and benthic (Sciaenidae/Drums and Croakers) feeding habits. For those two groups, 8 specimens were available for most sites per season. Thus, the individuals that were dissected and analyzed were organized into "*batches"* of approximately eight individuals each that were processed together. Each batch consisted of eight selected fish of the same taxonomic group, same site, and same season. If more than 8 fish for one of the groups were collected at a specific site, specimen for processing were selected to follow a normal size distribution with a mean and standard deviation approximately equal to that of all the sampled specimen of that particular group. Each batch was assigned a unique identifier and three parallel control filters to account for potential airborne microplastics contamination during processing. At four sampling sites the juvenile fish assemblage was more diverse and taxa-rich, which allowed for an additional higher taxonomic resolution comparison. A target number of 24 fish (eight from each season) of every available taxonomic group was processed when available for the following taxa: *Menidia spp.*, family *Sciaenidae*, *Lagodon rhomboides*, family *Clupeidae*, *Cyprinodon variegatus*, family *Fundulidae*, family *Gobiidae*, and *Mugil spp*. These were similarly divided into batches of eight following the specifications outlined above.

3.5 Clean Laboratory Procedures and Contamination Controls

Quantification of microplastics in digestive tracts and water samples may be confounded by the ubiquitous presence of microplastic fibers in our environment including surrounding air and water supplies and precautions need to be taken to avoid a negative influence on data quality (Hermsen et al., 2018). Therefore, thorough measures were taken to minimize potential cross-contamination as described subsequently in this section. For each batch, efforts were made to ensure consistency in laboratory conditions between each sample included in the batch, as well as the control filters. This included whenever possible, that (1) each sample underwent any given step of the preparation and analysis process on the same day, (2) the step was conducted by the same researcher(s) who were wearing the same clothes. Records were maintained for each batch detailing the date that each step was conducted, which names of researcher(s) and the color of their clothing.

3.5.1 Quality control measures

In an effort to minimize contamination of the samples by microplastics from the laboratory environment, several steps were taken to ensure a clean environment. Additionally, records were maintained detailing the potential sources of microplastic contamination as much as possible.

A biosafety hood (BioChemGARD 601, The Baker Company) was used for most laboratory protocols that required the samples to be open to the air for any period (i.e., dissection, addition of KOH for digestion, filtration) to minimize contamination of the samples by airborne microplastics. The exception was the FTIR microscopy, which took place within a designated clean room.

A clean room separated from the main laboratory by heavy-duty rubber curtains was designated exclusively for FTIR microscopy. Nothing that could be a source of microplastic contamination was permitted within the clean room, including clothes made of synthetic materials. The clean room was swept and dusted regularly, and only essential personnel were permitted into the clean room while the equipment was in use.

Diligent record-keeping accompanied all laboratory protocols that required the samples be open to the air for any period (i.e., dissection, addition of KOH for digestion, filtration, and FTIR spectroscopy). For each process, any factors that could influence the presence and amount of ambient microplastics in the laboratory environment were noted, including: the date on which the process was conducted, the name(s) of the researcher(s) conducting the process, the color of each garment worn by the involved researcher(s), and any other variation in the surrounding conditions noticed by the researcher(s). In any hypothetical instance in which unexplained variation in suspected microplastic counts indicated a potential source of microplastic contamination, this information was readily available, accurate, and organized to ensure rapid identification of the contamination source.

Researcher(s) that began a task followed it through to the end, as much as possible. If a change needed to be made in the middle of a laboratory procedure, it was noted at what point the new researcher(s) stepped in and what colors they were wearing. For any protocol that required some degree of subjective judgment-making, a single researcher was tasked with performing that protocol for all samples. Specifically, a single researcher oversaw determining gut and stomach fullness indices for all fish samples, and another researcher oversaw identifying suspected plastics for all fish samples. This ensured a minimal amount of variation in counts due to human error.

3.5.2 Negative controls for water contamination

Additional water control samples were analyzed to determine if any microplastic contamination may have come from the DI water used in the laboratory. Three water control runs were completed, one assessing the concentration of microplastics in water taken directly from the DI tap in the laboratory, another assessing the concentration of microplastics in laboratory DI water that had passed through an in-line filter, and a third assessing the concentration of microplastics in laboratory DI water that had been passed through an in-line filter and processed following a deep cleaning of all involved equipment. In a first run, DI water controls from laboratory tap were tested. Twelve clean eight-ounce glass jars with screw-on lids were rinsed three times and filled approximately 90% of the way with DI water directly from the laboratory tap. The contents of each jar were passed through separate 0.45 μm Whatman™ mixed cellulose ester membrane filters (diameter=47mm) using a three-channel PVC pipe filtration system following the previously described filtration protocol. Each filter was then placed in a covered, unvented petri dish and left to dry overnight. The dry filters were then analyzed for microplastics under a Zeiss Discovery dissecting microscope following the microplastics analysis protocol. In a second run, DI water controls that passed through an in-line filter were assessed. Instead of putting tap water through the mixed cellulose filters, tap water was first passed through a 20 μm in-line filter. Additionally, the filtration process followed a modification of the previously described filtration protocol in which every instance that DI water from the laboratory tap was used, DI water passed through a 20 μm in-line filter and subsequently stored in a large plastic jug with a spout was used instead. Finally, a third run identical to the second run was completed, with one major difference: Before beginning the water control processing and analysis procedure, every piece of equipment required for these procedures (filtration system, DI water squeeze bottles, large plastic jug with spout, glass jars, and forceps) was scrubbed thoroughly using a stiff-bristled brush and DI water that had been passed through a 20 μm in-line filter and then rinsed several times with filtered DI water. In addition, the aluminum foil used to cover the filtration system was replaced with new foil that had similarly been scrubbed and rinsed with filtered DI water. The third run revealed negligible levels of microplastics contamination, so this protocol was established as the laboratory standard.

3.5.3 Positive controls

To determine that microplastics in digestive tract samples were not being lost during the sample processing procedure or over-looked during the analysis phase, one batch (eight fish) of positive controls were analyzed. For each positive control, a randomly selected number of microplastic fibers (between zero and ten) were placed into clean 1.5 mL tubes, and the number of microplastics included was recorded by a laboratory assistant who did not disclose this information until the end of the analysis process. 1 mL of KOH was added to each tube, and each tube was placed into a thermocycler for two hours. Each positive control was subsequently filtered and analyzed, following the procedures outline above. The person analyzing the positive controls was given no prior knowledge regarding the quantity of microplastic particles in the positive controls.

3.5.4 Parallel negative controls for airborne contamination and correction procedure

For each batch, three control filters followed the eight fish through the entirety of the dissection, gut fullness indexing, and filtration processes. At any point during this process that the fish gut contents were exposed to the air, the control filters were also exposed to the air. These filters were subsequently analyzed in the same manner as the sample filters and used to correct for potential contamination from the air.

3.6 Fish Dissection and Basic Measures for Condition Indices

Fish selected for dissection were transferred to individually labelled glass petri dish filled with DI water for about 10 minutes to hydrate and thaw. The DI water was then then drained, the whole wet weight (WWW) in grams was recorded for each fish, and the fish was placed back in the dry petri dishes. Each fish was dissected within its petri dish under a biosafety hood to reduce airborne contamination. The entirety of the content of each fish's abdominal cavity was removed and placed in the corresponding labelled glass petri dish, and the remainder of the fish carcasses was weighed (i.e., gutted wet weight;

GWW) and returned to their individually labelled bags in the -20°C freezer. Each petri dish was covered and transported to a dissecting microscope (Hajovsky, 2019). The liver was separated from the rest of the digestive tract, and its wet weight was recorded. A gut fullness index was recorded for each stomach and each intestinal system following (Hajovsky, 2019) by the same research assistant (Table 6). The indexed digestive tracts were transferred into separate labelled 1.5 mL microcentrifuge vials and stored at -80°C until further processing.

Table 6 Digestive Tract (Stomach/Gut) fulness index scoring

% Fullness	Index Score
0%	0
1-25%	1
25-50%	2
50-75%	3
75-99%	4
100%	5

3.7 Digestive Tract Digestion, and Filtration

Individual digestive tracts stored in a microcentrifuge vial were thawed and 1 mL 10% KOH solution added (methodology modified after Karami et al., 2017). This mixture was then placed into a thermocycler at 40°C to digest any organic material in the sample until it appeared to no longer contain any large organic particles (maximum six hours). After being thoroughly digested, each sample was added to approximately 200 mL of DI water which was then filtered through a 47mm diameter mixed cellulose Whatman™ filter with a pore size of 0.45µm. Each filter had been priorly visually inspected under a dissecting microscope for contamination and manufacturing irregularities and discarded if any were discovered. Each was then placed in a separate labelled unvented plastic petri dish and stored flat to dry before undergoing further analysis.

3.8 Microscopic Enumeration, Shape Classification and Size Measurement, of Suspected Microplastic in Digestive Gut Samples

Each filter was visually analyzed for microplastic particles under a dissecting microscope. Visual identification of microplastics tends to be imperfect and usually requires verification via analysis of its chemical makeup. Thus, at this stage all particles that met all the visual qualifications of microplastic were identified as "*suspected microplastics*", which is used as term throughout the remainder of the report. For a particle to be counted as a suspected microplastic and further analyzed, it was required to meet the following conditions listed below (adapted from Norén, 2007):

- no visible organic structures within or on the surface of the particle or fiber
- fibers equally thick along the entirety of their length and not narrow to a taper at either end
- fibers have a three-dimensional bend as a perfectly straight fiber is indicative of biological origin
- particles must be homogenously colored and not opaque
- if the particle or fiber appears clear or white, extra care should be taken to ensure it is not of biological origin; this includes using higher microscope magnification and examining the particle/fiber under a fluorescent light

Each suspected plastic particle was recorded by type and color, marked on the petri dish to be easily located at a later date, and photographed using a Zeiss Imaging system to allow for determination of size. Suspected microplastics were measured from saved pictures using an Axiocam 506 microscope camera and Zen Professional application. Each picture file included the zoom at which it was taken (usually 8.0×) and the degree of magnification offered by the attached lens (usually 1.0×), which factored automatically into the utilized software's length calculation algorithm. Suspected microplastic particles were measured with the built-in length tool using the diameter from the widest point of the item. Predominately straight fibers were measured from one end to the other end of the fiber using the built-in length tool. More complex fibers with curvatures and overlaps were measured using the active curve tool in the Zen application, beginning at one end of the fiber, and tracing the entire fiber to the other end.

3.9 Ingested suspected microplastic confirmation and determination of plastic type using micro FTIR

Ten percent of the *"suspected microplastics"* were randomly selected to be processed using a Nicolet iN5 Micro-Fourier Transform Infrared Spectroscopy (FTIR) microscope from Thermo Scientific to verify their composition. The "*suspected microplastics"* were placed on an Aluminum EZ-Spot Micro Mount Sample slide and placed under the geranium crystal tip equipped to the FTIR microscope. The FTIR-ATR program (OMNIC software) was used to view the infrared spectrum of each suspected microplastic and determine its composition using the existing spectrum database. The color/size/type distribution of fibers and particles chosen for micro-FTIR analysis was representative of the color/size/type distribution of all suspected microplastics.

3.10 Microplastic in adjacent water column

Suspended microplastic water samples collected via plankton net from the water columns of each bay were filtered through a 47mm diameter mixed cellulose Whatman™ filter with a pore size of 0.45µm. Each filter was first visually inspected under a dissecting microscope for contamination and manufacturing irregularities and discarded if any were discovered. Each was then placed in a separate labelled unvented plastic petri dish and stored flat to dry before undergoing further analysis that included the same steps as described for the filters with juvenile fish digestive tract content.

3.11 Statistical Analyses

3.11.1 Juvenile Fish Condition Indices

The standard length, whole wet weight, gutted weight, and liver weight were used to calculate a couple condition indices that served as one approximation of the fish's nutritional wellbeing. The first, Fulton's condition index (CI; Heincke, 1908), relates the fish's body weight to its length in a manner that allows comparison between relative weights of a fish to others of the same species and same length, and was calculated as

$K = 1000 \times W / L^3$,

where K was Fulton's condition index, W was the fish's weight in grams, and L was the length of the fish in cm. Fulton's index allows for the use of any body weight or length measurement desired; for the present study, standard length was used for L, and gutted weight and whole wet weight were used for W for independent analyses. A second index, the hepatosomatic index (HSI) approximates nutrient storage in the fish's liver, and was calculated as

$HSI = 100 \times LW / W$,

where HSI was the hepatosomatic index, LW was the liver weight in grams, and W was the body weight in grams. The hepatosomatic index allows for the use of any body weight measurement desired; for the present study, gutted weight and whole wet weight were used for w for independent analyses.

3.11.2 Statistical Correction of "suspected microplastic" for airborne "suspected microplastic" contamination

Using a modified procedure after Ryan et al. 2019, the average control filter count for each batch was calculated from the three airborne control filters (separately for each color, type, and batch) This average was then divided by the number of samples in that batch and the resulting number was subtracted from the applicable sample counts.

3.11.3 Statistical Corrections for "confirmed microplastic"

To correct the "suspected microplastic" to "confirmed microplastic" and "man-made natural fibers (cellulosic)", coefficients for samples were calculated based on the FTIR results, separate for each color, type, species group (sciaenids, silversides, others). For each sample, the "suspected microplastics" count was then multiplied by the respective FTIR coefficient. Then FTIR coefficients were calculated for "suspected microplastics" on control filters (separate for each color and type) and each control count was multiplied by the applicable FTIR coefficient. The same method as above was used for the step to correct for airborne microplastic contamination.

3.11.4 Univariate comparisons of "suspected" and "confirmed" microplastic and "Man-Made Natural Fiber" ingestion

Ingested "Suspected Microplastic" (SMP) after visual identification and airborne contamination correction, "Confirmed Microplastic" (CMP) after FTIR and airborne contamination correction, and "Man-Made Natural Fibers" (MMN) after FTIR confirmation and airborne contamination correction were compared for differences between a) bay systems and b) seasons including data from the two ubiquitous taxa groups Silversides and Sciaenidae. Differences between taxa including species to family level resolution were done on the complete dataset.

As the datasets did not fulfill the requirements for parametric statistical analyses, included many zeroes for the CMP data, and were unbalanced, one-way non-parametric tests were chosen that were followed by Steel-Dwass Nonparametric Multiple Comparison Post – Hoc Tests using the statistical software JMP Pro 16.1.0.

4. RESULTS

4.1 Water Parameters

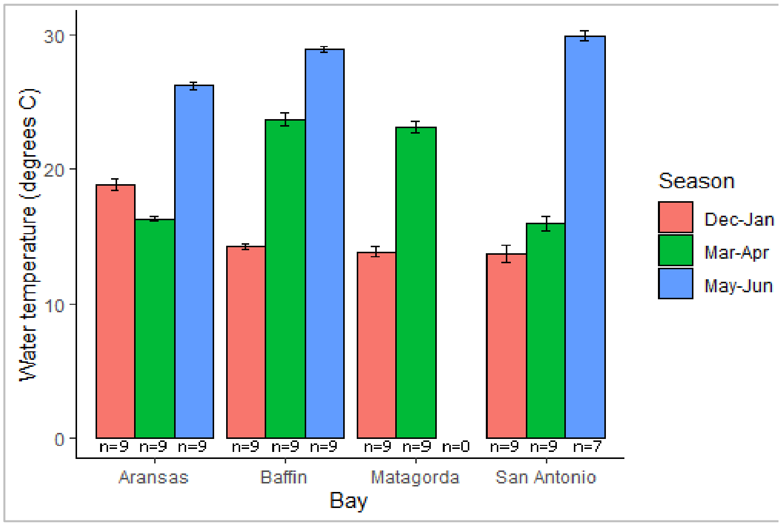

Figure 2. Mean temperature of the waters of each bay at the time of each sampling event (error bars = SE). Calculated from YSI EXO1 sonde measurements taken at each of three locations within each site of each bay in each sampling season. Nine measurements were missed from Matagorda Bay and two from San Antonio Bay in the May-June sampling

4.1.1 Water Temperature

The water temperature during the Dec-Jan sampling season was lower than the water temperature during the Mar-Apr sampling season, which was lower than the water temperature during the May-Jun sampling season (15.18 °C, 95% confidence interval (CI) [14.39, 15.97]; 19.79 °C, 95% CI [18.48, 21.11]; and 28.34 °C, 95% CI [27.80, 28.89], respectively; Figure 2). The mean water temperature was highest in Baffin Bay, followed by Aransas Bay, San Antonio Bay, and Matagorda Bay, but not a significant degree (22.32 °C, 95%CI [19.84, 24.79]; 20.48 °C, [18.74, 22.21]; 19.07 °C, 95% CI [16.11, 22.03]; and 18.48 °C, 95% CI [16.05, 20.92], respectively; Figure 2).

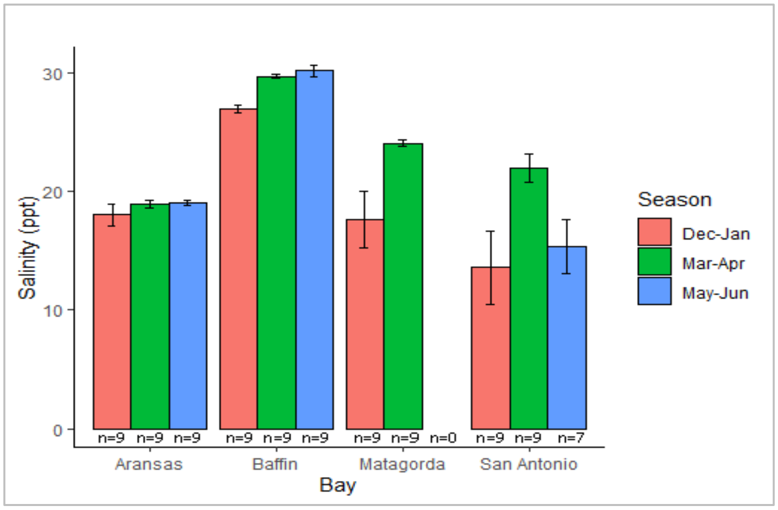

Figure 3. Mean salinity of the waters of each bay at the time of each sampling event (error bars = SE). Values calculated from YSI EXO1 sonde measurements taken at each of three locations within each site of each bay in each sampling season. Nine measurements were missed from Matagorda Bay and two from San Antonio Bay in the May – June sampling season.

4.1.2. Salinity

The mean salinity of these bays at the time of each sampling event was 20.26 ppt (95% CI [18.87, 21.65],3). Mean salinity was higher in the Mar-Apr sampling season than in the May-Jun or Dec-Jan sampling seasons, in that respective order (23.64 ppt, 95% CI

[22.17, 25.11]; 19.39 ppt, 95% CI [16.76, 22.01]; and 17.90 ppt, 95% CI [15.20, 20.60], respectively; Figure 3). The mean salinity of Baffin Bay was higher than Matagorda Bay, Aransas Bay, or San Antonio Bay, in that respective order (28.90 ppt, 95% CI [28.21, 29.59]; 20.834 ppt, 95% CI [17.88, 23.78]; 18.66 ppt, 95% CI [17.96, 19.35]; and 17.09 ppt, 95% CI [14.01, 20.17], respectively; Figure 3).

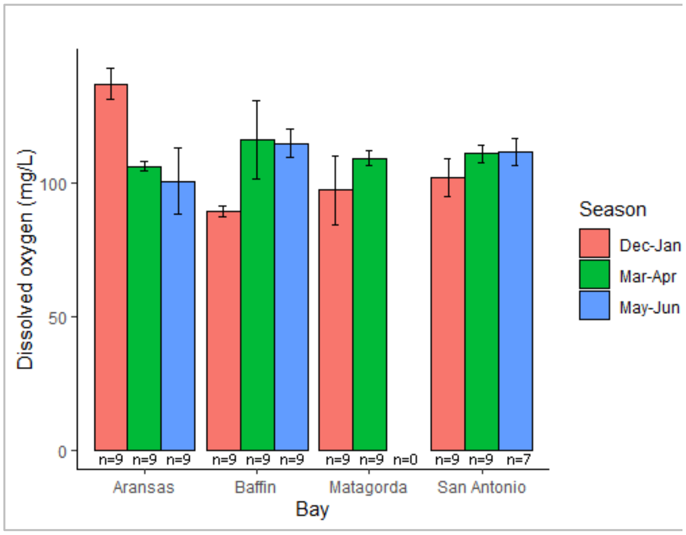

Figure 4. Mean dissolved oxygen content of the waters of each bay at the time of each sampling event (error bars = SE). Values calculated from YSI EXO1 sonde measurements taken at each of three locations within each site of each bay in each sampling season. Nine measurements were missed from Matagorda Bay and two from San Antonio Bay in the May-Jun sampling season.

4.1.3 Dissolved Oxygen

The mean dissolved oxygen (DO) saturation of these bays at the time of each sampling event was 108.62 mg/L (95% CI [103.94, 113.62]). Mean DO was lowest in the Dec-Jan sampling season and highest in the Mar-Apr sampling season, but not to a statistically significant degree, with the May-Jun sampling season falling in the middle (105.71 mg/L, 95% CI [96.36, 115.07]; 110.59 mg/L, 95% CI [103.08, 118.11]; and 109.86 mg/L, 95% CI [102.14, 117.59], respectively; Figure 4). The mean DO saturation was highest in Aransas Bay, second highest in San Antonio Bay, third highest in Baffin Bay, and lowest in Matagorda Bay, but not to a significant degree (114.58 mg/L, 95% CI [103.45, 125.70]; 107.84 mg/L, 95% CI [101.40, 114.28]; 106.66 mg/L, 95% CI [95.30, 118.01]; and 103.20 mg/L, 95% CI [89.42, 117.98], respectively; Figure 4).

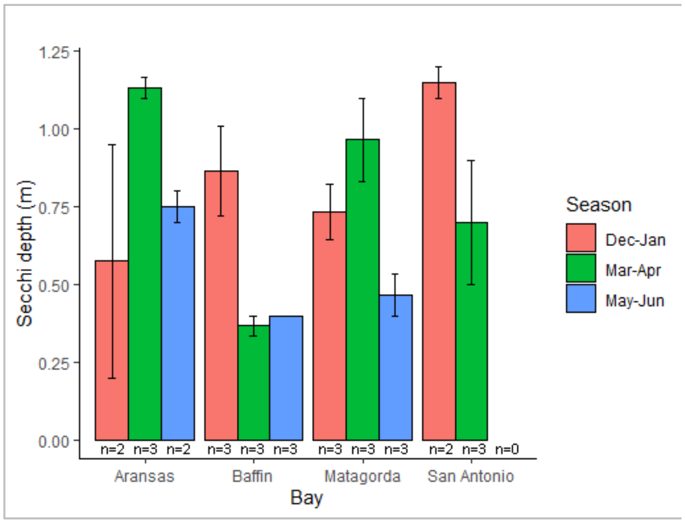

Figure 5. Mean Secchi depth within each bay at the time of each sampling event (error bars = SE). Values calculated from one Secchi depth measurement within each site of each bay in each sampling season. Secchi depth approximates relative turbidity (higher Secchi depth = less turbidity). One measurement from Aransas Bay and one measurement from San Antonio Bay were missed in the Dec – Jan sampling season, and one measurement from Aransas Bay and three measurements from San Antonio Bay were missed in the May – Jun sampling season.

4.1.4 Turbidity

The mean measured Secchi depth (which serves as an approximation of turbidity) was 0.69 m (95% CI [0.58, 0.80]). The turbidity, as approximated by Secchi depth, was highest in the May-Jun sampling season, second highest in the Dec-Jan sampling season, and lowest in the Mar-Apr sampling season, but not to a significant degree (Secchi depth = 0.48 m, 95% CI [0.38, 0.58]; Secchi depth = 0.78 m, 95% CI [0.56, 1.00]; and Secchi depth = 0.79 m, 95% CI [0.57, 1.02], respectively; Figure 5). The mean turbidity, as approximated by Secchi depth, was highest in Baffin Bay, followed by Matagorda Bay, Aransas Bay, and San Antonio Bay, but not to a significant degree (Secchi depth = 0.54 m, 95% CI [0.33, 0.76]; Secchi depth = 0.72 m, 95% CI [0.52, 0.92]; Secchi depth = 0.86 m, 95% CI [0.55, 1.18]; and Secchi depth = 0.88 m, 95% CI [0.45, 1.31], respectively, Figure 5).

4.2 "Suspected Microplastics" in water samples

4.2.1 Quantity of suspected microplastics in the water column of Texas Coastal Bend bays

All samples collected from the water column during this study contained suspected microplastics (100% occurrence rate, average concentration of 1.22 mp/L). Black (blk) was the most common color of suspended suspected microplastic in the Texas Coastal Bend bays examined (mean concentration = 0.57 MP/L, 47% of all suspended microplastics), followed by blue (blu; 0.24 MP/L, 20%), clear (clr; 0.16 MP/L, 11%), red (0.14 MP/L), and tan (0.06 MP/L, 5%) (Figure 6). Fibers (fib) were the most common suspected microplastic type (1.14 MP/L, 93%), followed by particles (prt; 0.06 MP/L, 5%), then films (flm; 0.02 MP/L, 2%) (Figure 6).

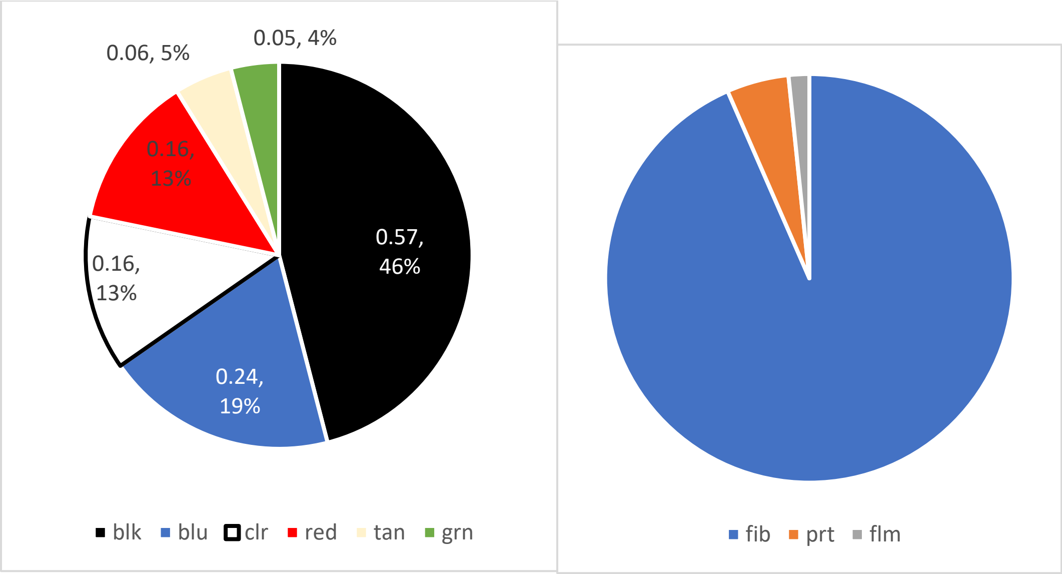

Figure 6. Average suspended "suspected microplastics(SMP)" concentration (SMP/L) and relative contributions by color (left) and by type (right). Blk = black, blu = blue, grn = green, fib = fiber, prt = particle, flm = film.

4.2.2 Spatial variation in suspended suspected microplastic concentration

Aransas Bay contained the highest mean concentration of suspended suspected microplastics (2.57 MP/L), followed by San Antonio Bay (1.00 MP/L), then Baffin Bay (0.74 MP/L), and finally Matagorda Bay (0.55 MP/L). For each bay sampled, the two most frequent colors of suspended suspected microplastics were black and blue, with the exception of Baffin Bay for which black and clear suspected microplastics occurred in the highest concentrations. Black suspected microplastics occurred at a higher concentration than blue suspected microplastics in Aransas and Baffin Bay, whereas blue suspected microplastics occurred in a higher concentration in Matagorda and San Antonio Bay. Clear and red suspected microplastics were also of relatively high concentration in these samples. These two colors were the third and fourth most frequent suspected microplastic colors in each bay with a few exceptions: tan suspected microplastics outnumbered red suspected microplastics in Aransas Bay and clear suspected microplastics were second most abundant in Baffin Bay. Tan and green suspected microplastics additionally occurred in minimal frequency in samples from Aransas Bay, Baffin Bay, and San Antonio Bay (Figure 7).

Fibers were by far the most frequent type of suspected microplastics sampled from the water column, making up an overwhelming majority of suspected microplastics in every bay. Suspected microplastic particles also occurred at low concentrations in each bay, and suspected microplastic films occurred at low concentrations in Baffin Bay, Matagorda Bay, and San Antonio Bay.

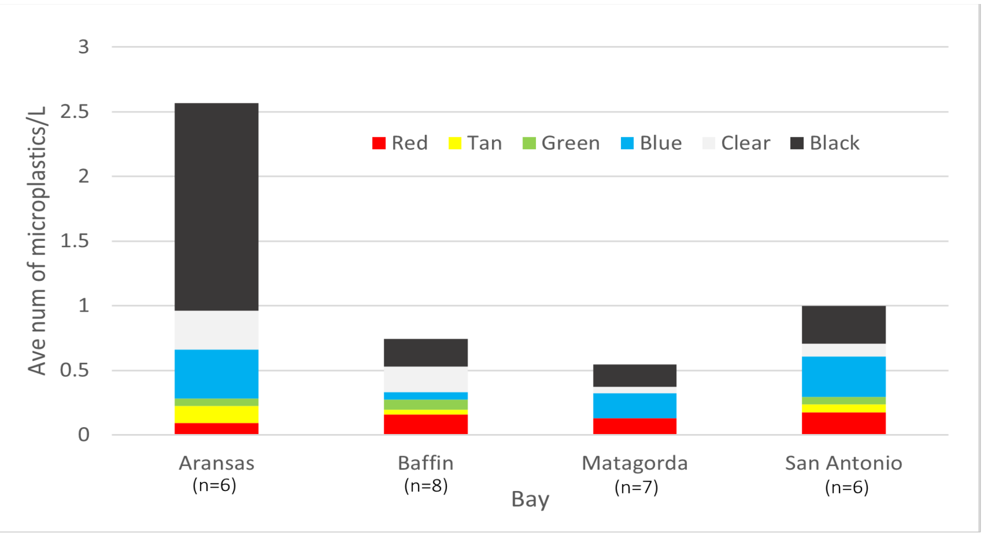

Figure 7. Mean number of "suspected microplastics" of each color category per liter (L) of seawater sampled by plankton net in each bay. The "red" category includes any shade of red, orange, or pink; the "tan" category includes any warm non-red color, including beige- and brown-leaning neutrals and yellows; the "green" category includes all shades of green; the "blue" category includes all shades of non-green cool colors like blue and purple; the "black" category includes all non-white gray-scale shades; and the "clear" category includes white and unpigmented translucent.

4.2.3 Seasonal variation in suspended suspected microplastics concentration

Suspended suspected microplastics concentrations were highest in the Mar-Apr sampling season (1.18 MP/L), followed by May-Jun (0.64 MP/L), then Dec-Jan (0.42 MP/L).

The mean color composition of suspended suspected microplastics also varied between sampling seasons (Figure 8). In the Dec-Jan sampling season, blue suspected microplastics were most abundant, followed by red, black, and clear. In both the Mar-Apr and the May-Jun sampling seasons, black suspected microplastics occurred in the highest concentration, followed by blue, clear, and red. Tan and green suspected microplastics additionally occurred in minimal concentrations in Mar-Apr and May-Jun.

Fibers were also the most frequent type of suspected microplastics sampled from the water column in every sampling season (Appendix 8). Particles occurred in minimal concentrations during the Mar-Apr and May-Jun sampling seasons, and films occurred in minimal concentrations in the Mar-Apr sampling season.

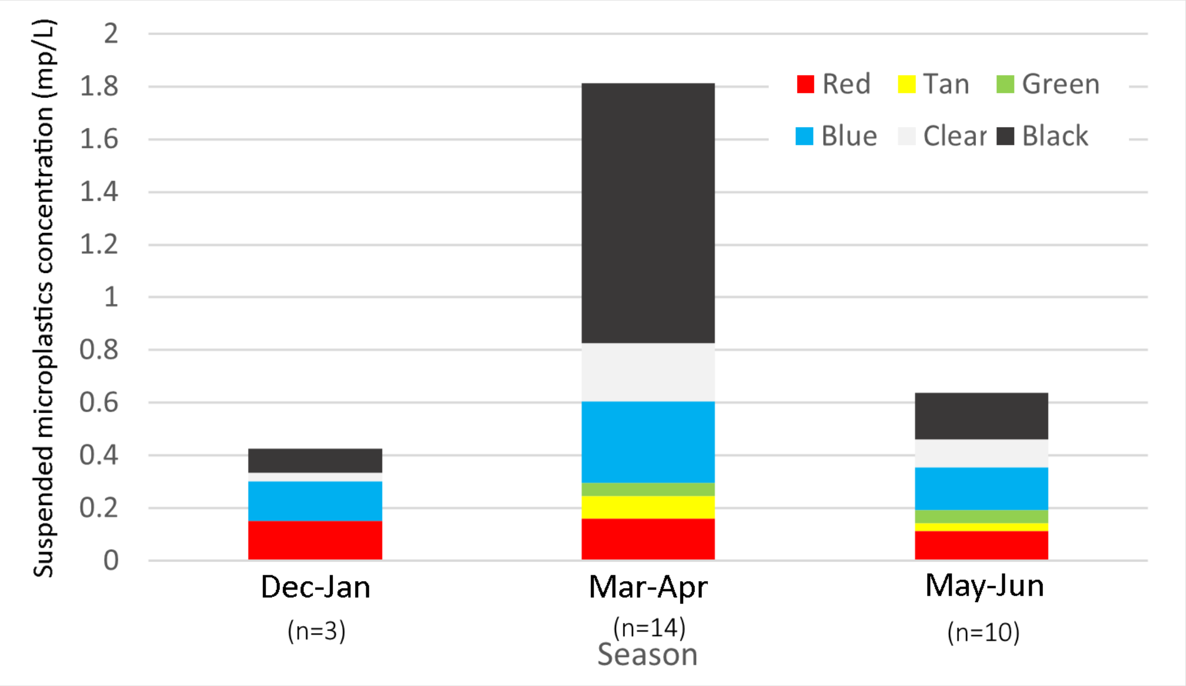

Figure 8. Mean number of suspected microplastics of each color category per liter (L) of seawater sampled by plankton net in each season. The "red" category includes any shade of red, orange, or pink; the "tan" category includes any warm non-red color, including beige- and brown-leaning neutrals and yellows; the "green" category includes all shades of green; the "blue" category includes all shades of non-green cool colors like blue and purple; the "black" category includes all non-white gray-scale shades; and the "clear" category includes white and unpigmented translucent.

4.3 "Suspected Microplastic" (SMP) Ingested by Fish

Digestive tracts of 735 juvenile fish were analyzed for this study, of which 261 fish belonged to the taxonomic group "Silversides", 251 fish belonged to the taxonomic group "Sciaenidae" and 223 fish belonged to the group "other species". Silversides and Sciaenidae were present at most stations in each bay and season (Table 7) and were therefore chosen for comparisons between bay systems and seasons. A higher species richness was only encountered at some stations and for those additional species were analyzed summarized under the "other species" category (Table 7) that were included in the interspecific/intertaxa comparisons.

Table 7 Numbers of Juvenile Fish Digestive Tracts Analyzed for each of the three Taxonomic Groups by Sampling Season (Winter, Spring, Summer), Bay (MB= Matagorda Bay, SB = San Antonio Bay, AB = Aransas Bay, BB = Baffin Bay) and sampling station

Season	**Winter**											
Bay	**MB**			**SB**			**AB**			**BB**		
Station	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Silversides	8	8	8	8	6	7	8	7	6	8	8	8
Sciaenidae	15	8	10	8	8	6	2	8	10	2	8	8
Other Species				21		11	24			22		
Grand Total	**23**	**16**	**18**	**37**	**14**	**24**	**34**	**15**	**16**	**32**	**16**	**16**

Season	**Spring**											
Bay	**MB**			**SB**			**AB**			**BB**		
Station	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Silversides	7	8	7	8	8	7	8	8	7	7	6	3
Sciaenidae		15		13	8	3	13	15	11	8		8
Other Species	1			20		19	19			16		
Grand Total	**8**	**23**	**7**	**41**	**16**	**29**	**40**	**23**	**18**	**31**	**6**	**11**

Season	**Summer**											
Bay	**MB**			**SB**			**AB**			**BB**		
Station	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Silversides	7	8	8	8	8	8	8	8	8	8	8	8
Sciaenidae	15	8	6	6			2	9	8		2	
Other Species			3	16		25	18			8		
Grand Total	**22**	**16**	**17**	**30**	**8**	**33**	**28**	**17**	**16**	**16**	**10**	**8**

In total 1997 "*suspected microplastic (SMP)*" were identified visually from the analyzed 735 digestive tracts, of which the vast majority was fibers (1772), followed by particles (224) and only 1 film. In terms of color composition, "clear" ranked first, followed by blue (Figure 9). For Fibers, color composition was more diverse than for particles in which clear dominated (Fig. 9).

At least one *"SMP"* was found in the digestive tracts of 77% of the analyzed fish (564 of 735) after correcting for airborne contamination. Silversides showed the highest ingestion incidence with 91% (244 of 269) followed by Sciaenidae with 74% (179 of 243) and the other species group with 63% (141 of 223).

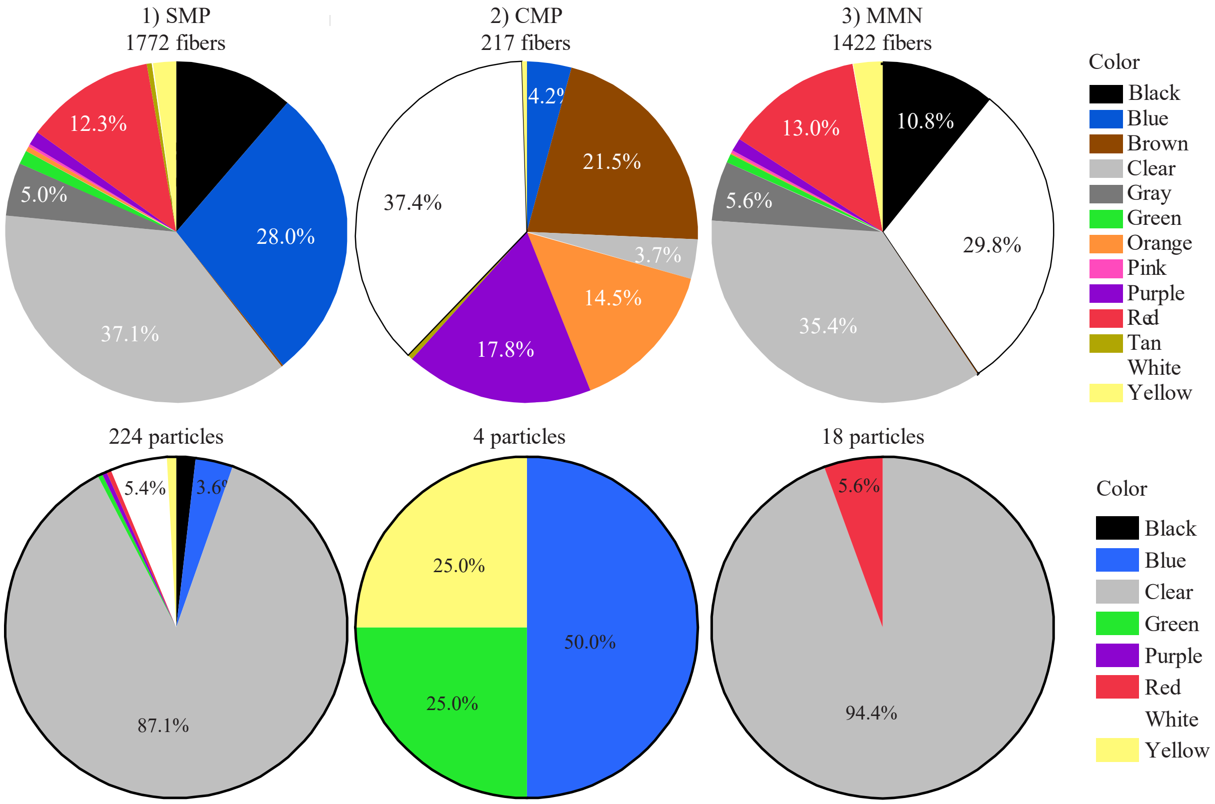

Figure 9. Total numbers and color proportions of "suspected microplastic"(SMP), "confirmed microplastic" (CMP) and "Man-Made Natural" fibers and particles found in digestive tracts of 735 juvenile fish.

4.4. From "Suspected" to "Confirmed Microplastic" and "Man-Made Natural fibers" – FTIR analyses results

Of the 2048 "*SMP*" found, 259 (12.6%) were analyzed by the FTIR method to confirm their identity and identify their chemical make-up, of which 232 were fibers and 27 particles. Of those 220 showed a match of > 60% to a FTIR profile of known material (with 213 showing a match of >70%) in at least one of the duplicate reads. They could be associated to one of the following categories: *"Confirmed Microplastic (CMP)"* (FTIR database match with either: Acrylic, Alkyd, Epoxy, Polyester, Polyethylene, Polypropylene, Polyurethane, PVA, Resin), *"Man-Made Natural (MMN)"* (FTIR database match with either: Linen, Rayon, Cellophane or Polysaccharide film), *"Natural"* (FTIR database match with either: Cellulose or Organic categories), and "Mineral" (FTIR database match with Mineral category). For each color approximately 10% were analyzed.

The overall positive rate of "*CMP*" was 17% (38 of 220 *"suspected microplastics"* matched), with a higher positive rate for the taxa "Sciaenidae" (22%, 20 of 92) than for the taxon "Silversides" (10%, 13 of 129) and "Other Species" (13%, (5 of 38). The dominant material in the category *"Confirmed*

Microplastic" was Polyester (71%, 27 of 38). The positivity rate of *"CMP fibers"* was 18% overall (34 of 193 analyzed fibers), 13% for "Silversides" only (12 of 93), 21% for "Sciaenidae" (17 of 80), and 21% for the "other species" group (5 of 24). For the four most abundant colors, black and clear fibers had positivity rates of 21% (5 of 24) and 18% (12 of 67) respectively, whereas blue and red, the other two dominant colors, showed lower positivity rates pf 12% (6 of 51) and 14% (3 of 22), respectively. Positivity rate of "*CMP particles*" was 17% (4 of 23 analyzed particles), with "Silversides" at 5% (1 of 20) but "Sciaenidae" at 100% (3 of 3).

At least one *"CMP"* was found in the digestive tracts of 24% of the analyzed fish (177 of 735) after correcting for airborne contamination and FTIR confirmation. Silversides showed the highest ingestion incidence with 33% (90 of 269) followed by Sciaenidae with 28% (67 of 243) and the other species group with 8% (20 of 223). Overall, 217 CMP fibers, but only 4 particles were found in the digestive tracts (Fig. 9). The predominant colors of these fibers were white, brown, purple or orange (Fig 9).

A second material group identified by the FTIR analyses that we termed *"man-made natural fibers"* stemming from processed natural fibers dominated the in the *"SMP"* making up 78% of the analyzed fibers (153 of 197). This is a similar category to "semi-synthetics" used by Hajovsky (2019). Of the 153 fibers falling into this category, 63% were identified as Rayon and 35% as Linen. Positivity rates ranged from 74% and 75% for "Sciaenidae" (59 of 80 fibers analyzed) and "other species" (18 of 24) to 82% for "Silversides" (76 of 93). For the four most abundant colors (black, blue, clear, red), positivity rates ranged from 73% (49 of 67) for clear, 79% (19 of 24) for black, 82% (18 of 22) for red, to 84% (43 of 51) for blue.

At least one *"MMN fiber"* was found in the digestive tracts of 71% of the analyzed fish (521 of 735) after correcting for airborne contamination and FTIR confirmation. Silversides showed the highest ingestion incidence with 88% (238 of 269) followed by Sciaenidae with 66% (160 of 243) and the other species group with 55% (123 of 223). Overall, 1422 MMN fibers, but only 18 particles were found in the digestive tracts (Fig. 9). The predominant colors of these fibers were clear, white, red and black (Fig 9).

4.5 Bay Comparison

Including data from Sciaenidae and Silversides, ingested mean numbers were between 3 and 4 SMP for each of the four bays (Table 8). Ingested mean numbers of CMP were much lower ranging from 0.37 to 0.41 for each of the four bays, and ingested mean numbers of MMN fibers ranged from 2.18 to 2.60 for each of the four bays (Table 8). Neither SMP, CMP or MMN fiber numbers differed significantly between the four bays.

Table 8 Comparison of means between Bay Systems of ingested i) Suspected Microplastic, ii) confirmed Microplastic (CMP) and iii) Man-Made Natural fibers (MMN) based on Silverside and Sciaenidae digestive tracts (MB= Matagorda Bay, SB = San Antonio Bay, AB = Aransas Bay, BB = Baffin Bay). StdDev = Standard Deviation, Std Err = Standard Error.

	Bay	Fish Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
SMP	1) MB	146	3.84	5.24	0.43	2.98	4.69
	2) SB	120	3.05	3.18	0.29	2.48	3.62
	3) AB	146	3.43	4.65	0.38	2.67	4.19
	4) BB	100	3.19	4.77	0.48	2.24	4.14
CMP	1) MB	146	0.41	0.66	0.05	0.30	0.52
	2) SB	120	0.37	0.56	0.05	0.26	0.47
	3) AB	146	0.38	0.74	0.06	0.26	0.50
	4) BB	100	0.41	0.84	0.08	0.24	0.58
MMN Fibers	1) MB	146	2.26	2.74	0.23	1.81	2.71
	2) SB	120	2.18	2.28	0.21	1.76	2.59
	3) AB	146	2.60	3.71	0.31	2.00	3.21
	4) BB	100	2.53	3.74	0.37	1.79	3.27

4.6 Seasonal Comparison\

Including data from Sciaenidae and Silversides, ingested mean numbers ranged from 2.44 to 4.61 SMP between the three sampled seasons (Table 9). Ingested mean numbers of CMP were much lower ranging from 0.26 to 0.51 CMP, and ingested mean numbers of MMN fibers ranged from 1.73 to 2.92 between the seasons (Table 9). All three parameters, ingested SMP, CMP or MMN fiber numbers differed significantly between the three sampled seasons (Fig. 10, Table 10). Nonparametric comparisons using

Table 9 Comparison of means between Seasons of ingested i) Suspected Microplastic, ii) confirmed Microplastic (CMP) and iii) Man-Made Natural fibers (MMN) based on Silverside and Sciaenidae digestive tracts (MB= Matagorda Bay, SB = San Antonio Bay, AB = Aransas Bay, BB = Baffin Bay). StdDev = Standard Deviation, Std Err = Standard Error.

	Season	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
SMP	1) Winter	183	4.61	5.36	0.40	3.82	5.39
	2) Spring	178	3.00	4.33	0.32	2.36	3.64
	3) Summer	151	2.44	3.29	0.27	1.92	2.97
CMP	1) Winter	183	0.51	0.80	0.06	0.39	0.63
	2) Spring	178	0.38	0.69	0.05	0.28	0.48
	3) Summer	151	0.26	0.54	0.04	0.18	0.35
MMN Fibers	1) Winter	183	2.92	3.48	0.26	2.41	3.43
	2) Spring	178	2.41	3.63	0.27	1.87	2.95
	3) Summer	151	1.73	1.76	0.14	1.45	2.01

the Steel-Dwass method identified significant differences between Winter and both Spring and Summer for SMP (p = 0.0026 and <0.0001, respectively), and only Winter and Summer for both CMP and MMN (p = 0.0040 and 0.0047, respectively.

Table 10 Results of Wilcoxon / Kruskal Wallis tests of rank sum comparisons between the three sampled seasons for i) Suspected Microplastic (SMP), ii) confirmed Microplastic (CMP) and iii) Man-Made Natural fibers (MMN) based on Silverside and Sciaenidae digestive tracts. DF = degrees of freedom

	ChiSquare	**DF**	**Prob>ChiSq**
SMP	19.9224	2	<.0001
CMP	10.3461	2	0.0057
MMN	10.3208	2	0.0057

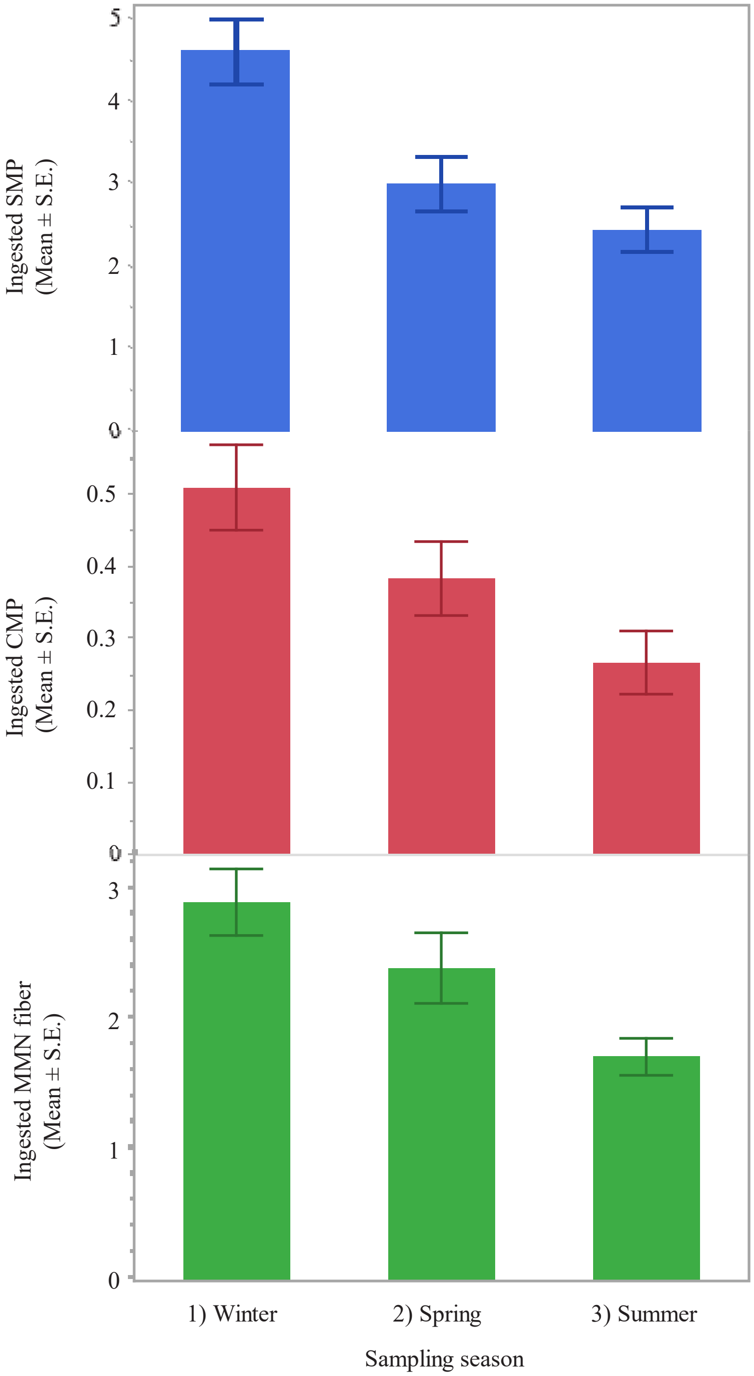

Figure 10 Mean ingested i) Suspected Microplastic, ii) confirmed Microplastic (CMP) and iii) Man-Made Natural fibers (MMN) between seasons based on Silverside and Sciaenidae digestive tracts. Shown as means and standard errors.

4.7 Multitaxa/species comparison

Ingested mean numbers ranged from 0.33 to 4.83 SMP between the 13 species/taxa including data from all analyzed fish per taxon (Table 11). Ingested mean numbers of CMP were much lower ranging from 0.00 to 0.67 CMP, and ingested mean numbers of MMN fibers ranged from 0.69 to 3.37 between the taxa/species (Table 11). All three parameters, ingested SMP, CMP or MMN fiber numbers differed significantly between the 13 taxa/species (Table 12). Nonparametric comparisons of ingested SMP using the Steel-Dwass method identified significant differences between Silversides vs. Silver Perch, Spot Croaker, Atlantic Croaker, Red Drum, Sheepshead Minnow, Gobies, Pinfish, Mullet, and Anchovies ($p < 0.001$ for all pairs). In addition, Red Drum differed significantly from Sheepshead Minnow and Anchovies ($p = 0.0132$ and 0.0135, respectively), and Atlantic Croaker differed from Sheepshead Minnows ($p = 0.037$).

Table 12 Results of Wilcoxon / Kruskal Wallis tests of rank sum comparisons between species/taxa for i) Suspected Microplastic (SMP), ii) confirmed Microplastic (CMP) and iii) Man-Made Natural fibers (MMN) based on all analyzed fish respectively. DF = degrees of freedom

	ChiSquare	DF	Prob>ChiSq
SMP	190.15	12	<.0001
CMP	59.85	12	<.0001
MMN	174.13	12	<.0001

For CMP, nonparametric comparisons using the Steel-Dwass method identified significant differences between Mullets vs. Silversides, Seatrouts, Sheepshead, Red Drum and Atlantic Croaker ($p = 0.0006$, 0.0011, 0.0136, 0.0026, 0.0016, respectively); Goby vs. Silversides, Seatrouts, Red Drum and Atlantic Croaker ($p = 0.0226$, 0.0297, 0.0475, 0.0349 respectively); Anchovies vs. Red Drum, Atlantic Croaker and Silversides ($p = 0.0493$, 0.0211, 0.0056 respectively).

For MMN fibers, nonparametric comparisons using the Steel-Dwass method identified significant differences between Silversides vs. Silver Perch, Spot Croaker, Atlantic Croaker, Red Drum, Sheepshead Minnow, Gobies, Pinfish, Mullet, and Anchovies ($p < 0.001$ for all pairs except for Red Drum at $p = 0.0002$).

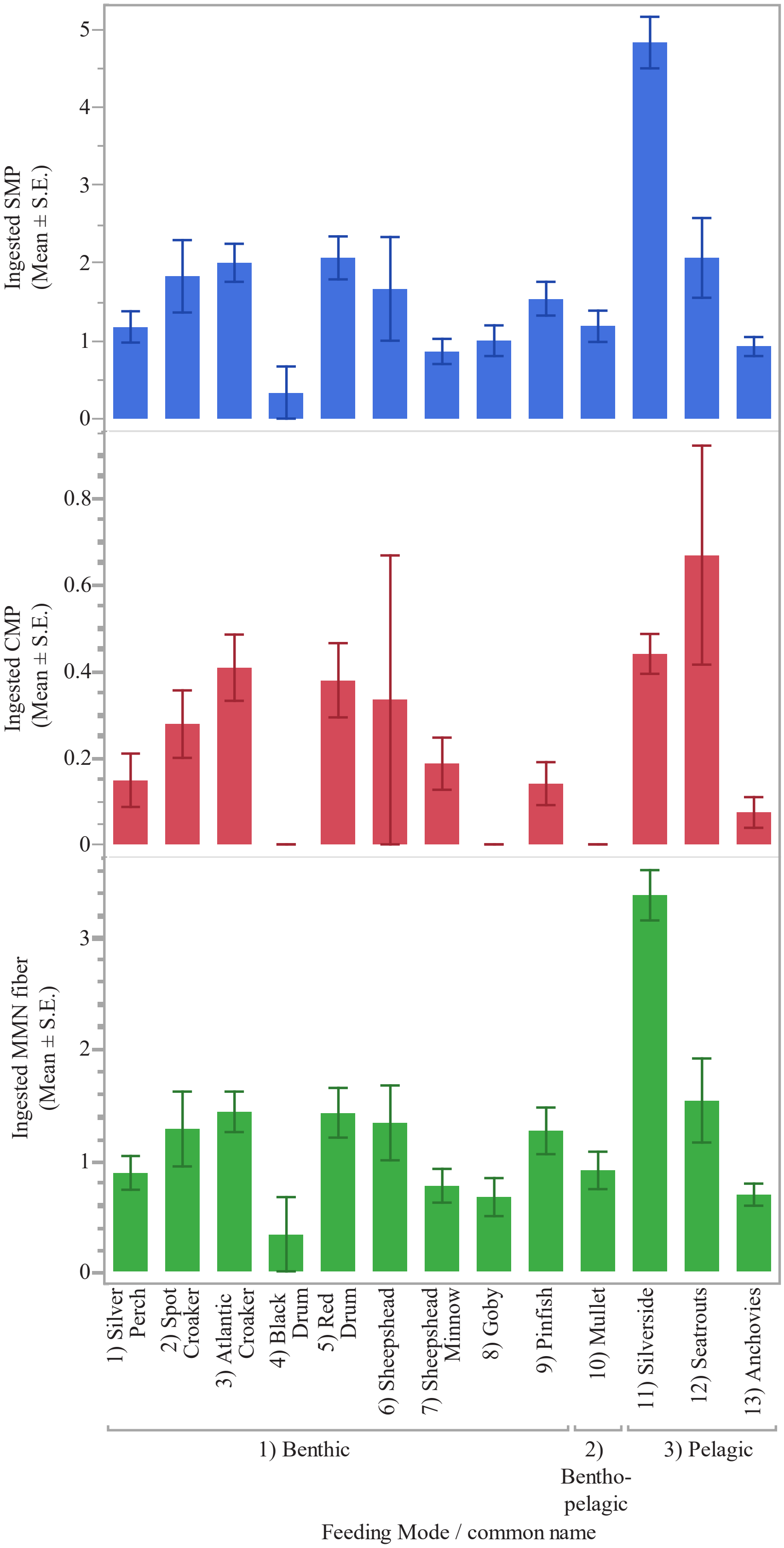

Figure 11 Comparison of mean ingested i) Suspected Microplastic (SMP), ii) confirmed Microplastic (CMP) and iii) Man-Made Natural fibers (MMN) between species/taxa grouped by feeding guild. Shown as means and standard errors.

Table 11 Comparison of means between species/taxa of ingested i) Suspected Microplastic, ii) confirmed Microplastic (CMP) and iii) Man-Made Natural fibers (MMN) based on all respective fish analyzed. Fish taxa 1-9 belong to benthic feeding guild, taxon 10 is considered bentho-pelagic, and taxa 11-13 pelagic feeders. StdDev = Standard Deviation, Std Err = Standard Error.

		SMP					CMP					MMN				
Species/Taxon	**Number**	**Mean**	**Std Dev**	**Std Err Mean**	**Lower 95%**	**Upper 95%**	**Mean**	**Std Dev**	**Std Err Mean**	**Lower 95%**	**Upper 95%**	**Mean**	**Std Dev**	**Std Err Mean**	**Lower 95%**	**Upper 95%**
1) Silver Perch	34	1.18	1.17	0.20	0.77	1.58	0.15	0.36	0.06	0.02	0.27	0.88	0.88	0.15	0.58	1.19
2) Spot Croaker	65	1.83	3.74	0.46	0.90	2.76	0.28	0.63	0.08	0.12	0.43	1.28	2.69	0.33	0.61	1.94
3) Atlantic Croaker	81	2.00	2.21	0.25	1.51	2.49	0.41	0.69	0.08	0.26	0.56	1.43	1.62	0.18	1.07	1.79
4) Black Drum	3	0.33	0.58	0.33	-1.10	1.77	0.00	0.00	0.00	0.00	0.00	0.33	0.58	0.33	-1.10	1.77
5) Red Drum	45	2.07	1.85	0.28	1.51	2.62	0.38	0.58	0.09	0.20	0.55	1.42	1.48	0.22	0.98	1.87
6) Sheepshead	3	1.67	1.15	0.67	-1.20	4.54	0.33	0.58	0.33	-1.10	1.77	1.33	0.58	0.33	-0.10	2.77
7) Sheepshead Minnow	43	0.86	1.06	0.16	0.53	1.19	0.19	0.39	0.06	0.06	0.31	0.77	1.00	0.15	0.46	1.07
8) Goby	27	1.00	1.04	0.20	0.59	1.41	0.00	0.00	0.00	0.00	0.00	0.67	0.88	0.17	0.32	1.01
9) Pinfish	50	1.54	1.53	0.22	1.11	1.97	0.14	0.35	0.05	0.04	0.24	1.26	1.47	0.21	0.84	1.68
10) Mullet	43	1.19	1.33	0.20	0.78	1.60	0.00	0.00	0.00	0.00	0.00	0.91	1.11	0.17	0.57	1.25
11) Silverside	269	4.83	5.43	0.33	4.18	5.48	0.44	0.75	0.05	0.35	0.53	3.37	3.73	0.23	2.92	3.82
12) Seatrouts	15	2.07	1.98	0.51	0.97	3.16	0.67	0.98	0.25	0.13	1.21	1.53	1.46	0.38	0.73	2.34
13) Anchovies	55	0.93	0.92	0.12	0.68	1.18	0.07	0.26	0.04	0.00	0.14	0.69	0.74	0.10	0.49	0.89

4.8 Microplastic Ingestion and Nutritional Condition Indices

Mean values of Fulton's nutritional condition and hepatosomatic indices did not show correlations with ingested number of "CMP" or "MMP" fibers. However, for Fulton's condition index variance narrowed with increasing "CMP" and "MMN" ingested tapering down to the lower end of the range of Fulton's index values (Fig. 12).

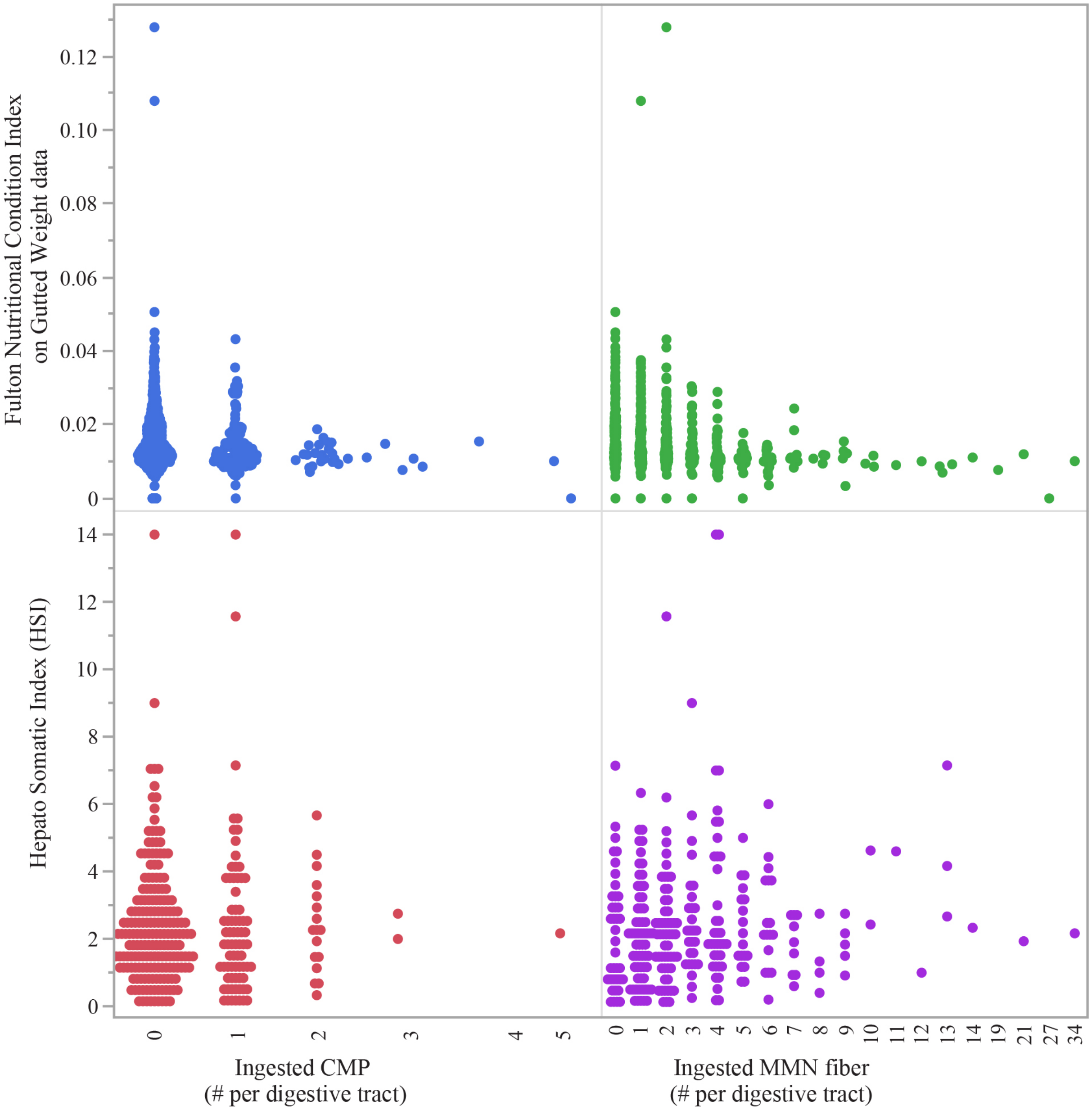

Figure 12 Scatter-Violinplot showing Fulton's Nutritional condition and Hepatosomatic Indices of individual fish in relation to ingested "Confirmed Microplastic" (CMP) and Ingested "Man Made Natural" (MMN) fibers counts. X-Axis is not to scale.

5. Discussion

The majority of "suspected" and "confirmed microplastic", as well as "Man Made Natural" were in the form of fibers, with less particles and rare films, which reflected the order of importance found for "suspected microplastic" in the parallel water samples. Fibers dominating the ingested suspected and confirmed microplastic has been widely observed in fish studies around the world (Abbasi et al., 2018; Alomar and Deudero, 2017; Campbell et al., 2017; Garcia and Cardozo, 2020; Jabeen et al., 2017; Kumar et al., 2018; Lusher et al., 2016; McGoran et al., 2017; Naidoo et al., 2020; Pazos et al., 2017), including a study from Galveston Bay, Texas (Peters et al., 2017) the pre-cursor study in Corpus Christi Bay and the Upper Laguna Madre, Texas (Hajovsky, 2019).

Feeding incidence, the number of juvenile fish that contained at least one item in their digestive tract, was high at 77% and 71% respectively for the categories "suspected microplastic" and "man-made natural fibers", whereas it was only 24% for "confirmed microplastic". This drastic difference is noteworthy to keep in mind when relating it to other studies. The Corpus Christi Bay and Upper Laguna Madre, Texas study (Hajovsky, 2019) reported an even slightly higher feeding incidence of 81% of "suspected microplastics", with the sample analysis to confirm the identification still underway due to several technical obstacles in the past years. The feeding incidence of suspected microplastic is also higher than most other incidences reported in literature. If looking at "confirmed microplastics" instead, the 24% rate appears to fall at the lower end of the range reported in the literature. Comparison with literature data are hampered by different identification and correction methods used which is a limitation for direct comparison of data reported hereafter: At the high end of the range are the 100% incidence for a sciaenid, *Micropogonias furnieri* from Argentina (Arias et al., 2019), and also the majority of snooks (*Centropomus undecimalis* and *Centropomus mexicanus*) in a Brazilian estuary had recently ingested microplastics (Ferreira et al., 2019). Lower incidences at 52% were reported for juvenile fish from the South African coast (Naidoo et al., 2020). In adult and juvenile fish belonging to six coastal species from Galveston Bay to Freeport, Texas, 42.4% of fish had ingested microplastics (Peters et al., 2017). Similar incidence rates (45%) were reported from Bluegill and Longear in the Brazos River Basin, Texas (Peters and Bratton, 2016). In Corpus Christi Bay, 36% of blue crabs contained microplastics (Waddell et al., 2020).

Two studies found even lower values than the 24% incidence for "confirmed microplastic" in our study: 12% of the particles in juvenile blueback herring diets from the Hudson River were microplastics (Ryan et al., 2019), and in several watersheds feeding the Gulf of Mexico, only 8% of freshwater and 10% of marine fishes examined contained microplastics in their digestive tracts (Phillips and Bonner, 2015). Therefore, the 24% incidence rate together with the very low mean of ingested "confirmed microplastic" per fish measured in this study (< 1 CMP / fish) leads us to conclude that microplastic ingestion is present in the four studied Texas Bays, but at a lower rate that reported from many other places in the world. However, the incidence of "Man-

Made Natural Fibers" was high in comparison at >71%. This dominance of man-made natural fibers over microplastic in diets found in our study may have been the case for earlier studies on microplastic ingestion that reached an identification level equivalent to our "suspected microplastics" level. The study of the importance and effect of man-made natural fibers on aquatic food webs has not been addressed by many to date and warrants further study. A current report from Brazil is one of the few to our knowledge that reported a similar pattern, and the authors emphasized the need for research on this category of anthropogenic debris (Macieira et al., 2021).

Clear, white, blue, black and red were the dominant colors in "suspected microplastics" from both water samples and fish digestive tracts as well as from ingested "manmade natural fibers". These colors were also the most abundant found in Corpus Christi Bay and Upper Laguna Madre fish (Hajovsky, 2019) and are commonly found in digestive tracts of fish reported from South America (Dantas et al., 2011; Mizraj et al., 2017), Canada (Hipfner et al., 2018), Europe (Kazour et al., 2018) and Asia (Jabeen et al., 2017). Interestingly, the colors white, brown, purple and orange dominated in the "confirmed microplastic" in our study, with the latter three seldom reported elsewhere. Species and locations in which transparent (clear) or white was the most frequently reported color include pelagic fish of the North Pacific gyre (Boerger et al., 2010), 21 marine species and 6 freshwater species of China (Jabeen et al., 2017), fish of Magdalena Bay on the Pacific Coast of Mexico (Jonathan et al., 2021), Indian Mackerel and Honeycomb Grouper of the Tuticorin coast of India (Kumar et al., 2018), coastal fish of the Guarapari Islands (Macieira et al., 2021), 46 species of the Amazon River estuary (Pegado et al., 2018), five species of the North and Baltic Seas (Rummel et al., 2016), and Japanese Anchovy of Tokyo Bay, Japan (Tanaka and Takada, 2016). Black/gray microplastics were most common in estuaries, rivers and deep see which are all environments with higher turbidity or lower light levels than the neritic, pelagic ocean and studies stem from the Musa Estuary and Persian Gulf (Abbasi et al., 2018), mesopelagic fish of the North Atlantic (Lusher et al., 2016), fish of the River Thames (McGoran et al., 2017), and Brazos River Basin of Texas (Peters and Bratton, 2016). Blue was the most common color ingested by a shark in Mediterranean Sea (Alomar and Deudero, 2017) and fish of the coast of KwaZulu-Natal, South Africa (Naidoo et al., 2020).

There were no differences in microplastic ingestion levels between the four bay systems regardless of confirmation level. This may be explained by their relative similarity of the four bay systems characterized by relatively low population densities along their coastlines, with Aransas Bay being the one with highest exposure compared to the rural environment of the other three bays, but all four bays ranking comparatively low compared to Galveston and Corpus Christi Bays as examples of Texan bays with high human impact.

A strong seasonal difference was found with Silversides and Sciaenidae juvenile fish collected during winter showing a significantly higher amount of ingested "suspected" and "confirmed microplastic" as well as "Manmade Natural fibers" than those collected during summer. One explanation could be higher density of microplastics in the water column during seasons with lower precipitation and water levels in the bays, similar to streams in upstate New York, where suspended microplastic concentrations were higher in the summer during low-flow conditions and higher in the spring during high-flow conditions, likely due to the increased springtime stream discharge effectively diluting the contamination (Mason et al., 2016; Watkins et al., 2019). Precipitation levels during the winter period were indeed lower, which may have led to a higher concentration of microplastic particles in the environment, although the limited and snapshot data from parallel water column "suspected microplastic" did not show higher densities during winter. Different food availability between the seasons may be an alternative explanation to the observed seasonal pattern which may have led to a seasonally higher ingestion of microplastic during winter, but further study would be needed to test this hypothesis.

No general pattern was found for amount or rate of ingestion of "microplastic" or "manmade natural fibers" in relation to feeding guilds. Some previous studies reported differences between pelagic and benthic feeders (Ory et al. 2018; Hajovsky, 2019, McNeish et al. 2018) Vendel et al. 2017), whereas others did not find such differences (Dantas et al., 2020; Filgueiras et al., 2020; Lusher et al., 2013; Phillips and Bonner, 2015; Vendel et al., 2017).

Similar to Hajovsky (2019) we found that single species belonging to different feeding guilds ingested elevated amounts of microplastic. Silversides stood out in terms of "suspected microplastics" and "man-made natural fibers" and was also reported as a taxon of elevated suspected microplastic ingestion for Corpus Christi Bay and the Upper Laguna Madre, previously (Hajovsky, 2019) so that this taxon, (*Menidia* sp.) can be identified as of concern and interest of in-depth studies for the effect of microplastics and also man-made natural fibers on the health of estuarine fish. For "confirmed microplastics", that were ingested at a much lower level throughout, the three sciaenid taxa, Seatrouts, Red Drum, and Atlantic Croaker, joined Silversides as species with higher ingestion numbers. Of those three sciaenid taxa, especially Red Drum and Seatrouts occupy a higher trophic level and are voracious predators in the bay systems as adults which makes them popular sportfish. In their juvenile fish they already make this transition including documented cannibalism when reared in controlled environments (Manley et al, 2015). For these two taxa, biomagnification which is feeding on prey that had microplastics ingested, may have contributed to their elevated position in the interspecific/taxa comparison. Silversides on the other hand feed in the water column and underneath the surface, so their high rank supports the hypothesis that pelagic foraging types are more prone to the ingestion of man-made materials. Less selective feeding and bulk ingestion of prey during filter feeding in opposition to more selectively picking prey from surfaces may have contributed to our results. Interestingly though, the second pelagic, filter-feeding taxon, Anchovies, showed much lower ingestion of man-made items.

Lastly, we did not find any correlation of number of ingested "confirmed microplastic" nor "man-made natural" fibers with one of the two integrative indicators of fish health, the nutritional condition factor and the Hepatosomatic index. This may be interpreted that there is no effect of ingestion load of man-made items on fish health at the observed levels for juvenile fish. However, we noted a decrease in variance and a tapering off to lower condition scores with higher number of both “confirmed microplastic” and “man-made natural fibers”, which may warrant further investigations. This observation matches the inconclusive picture when reviewing previous studies. Microplastic ingestion can negatively affect nutritional condition and survival rates of juvenile fish and shellfish species, resulting in poor stock recruitment and less fishable adults. However, the relationship between microplastic ingestion and body condition or behavior was not always clear. For example, of omnivorous intertidal fish, those that had ingested higher levels of microplastics tended to have lower body condition factors (Mizraji et al., 2017). However, laboratory studies did not show a difference in fish growth, body condition, or behavior as a result of microplastic exposure (Critchell and Hoogenboom, 2018; Dibona et al. 2021).

In conclusion, we found prevalence of “confirmed microplastic” pollution in digestive tracts of fish in all four bay systems with a quarter of fish containing at least one item, but overall load in digestive tracts appeared to be low. In addition, we detected a much higher number of fibers belonging to a newly established category, “man-made natural fibers”, which may warrant more focus potential effects on aquatic food webs and health in the future. Higher ingestion of man-made materials during winter and in Silversides, Red Drum, Seatrout and Atlantic Croaker provide guidelines for species and seasons of concern to investigate further especially in the light of the high economic and ecologic importance of these species.

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Appendix 1 Photos of Field Sampling


2019/01/16


MANGO
2019/04/01


2019/01/16

2019/01/16


2018/12/11


2019/05/19

MANGO
2019/04/01

2019/01/16


2018/12/11


2019/01/16


2019/01/16


2018/12/11


2019/01/16

2019/06/26


2019/06/26

STINGER
2019/06/26

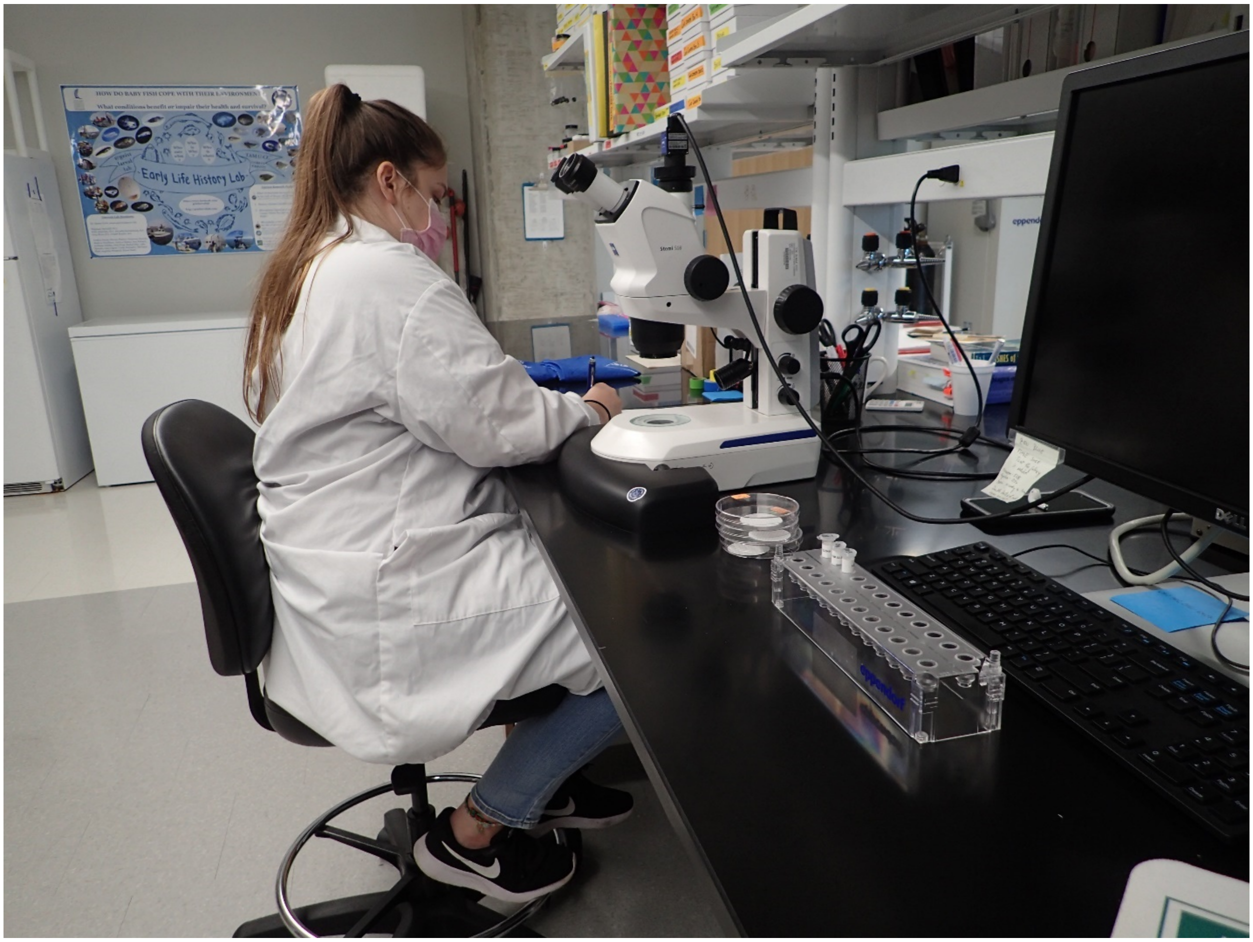

MAKE AMERICA
GAY AGAIN
2019/06/26

Appendix 2 Photos of Laboratory Analyses

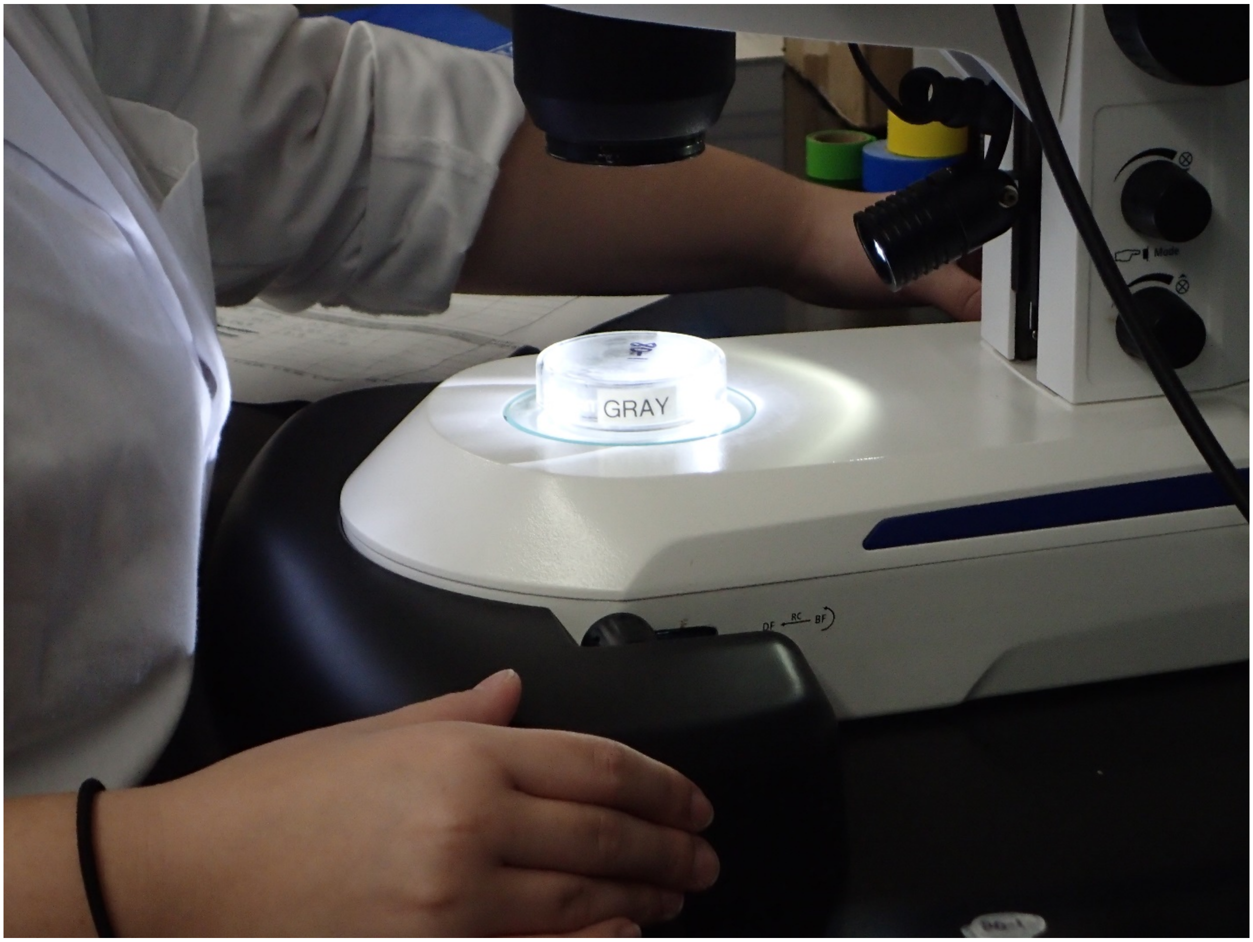
Collected fish in the deep freezer

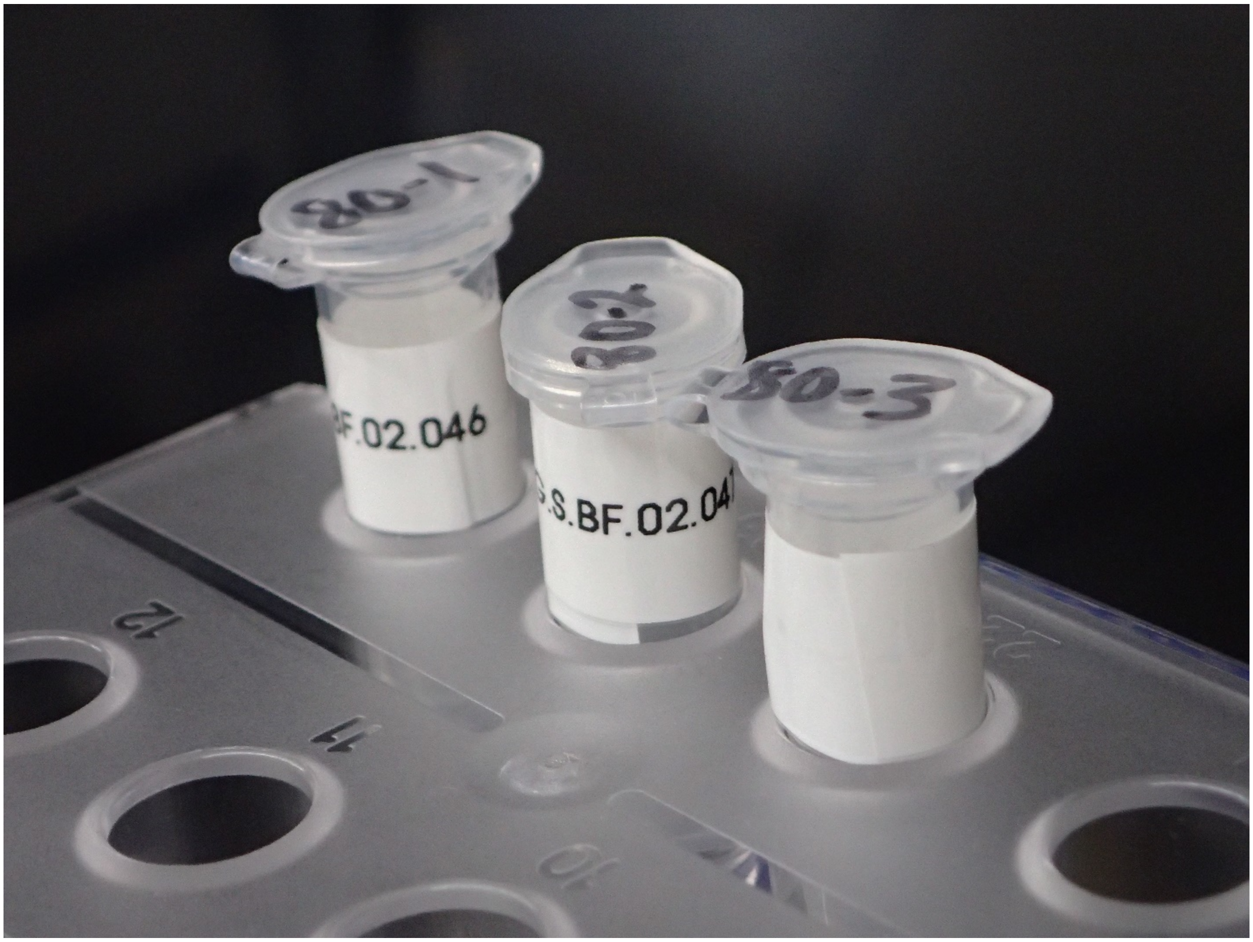
Filtration apparatus under clean hood

Fish Dissection

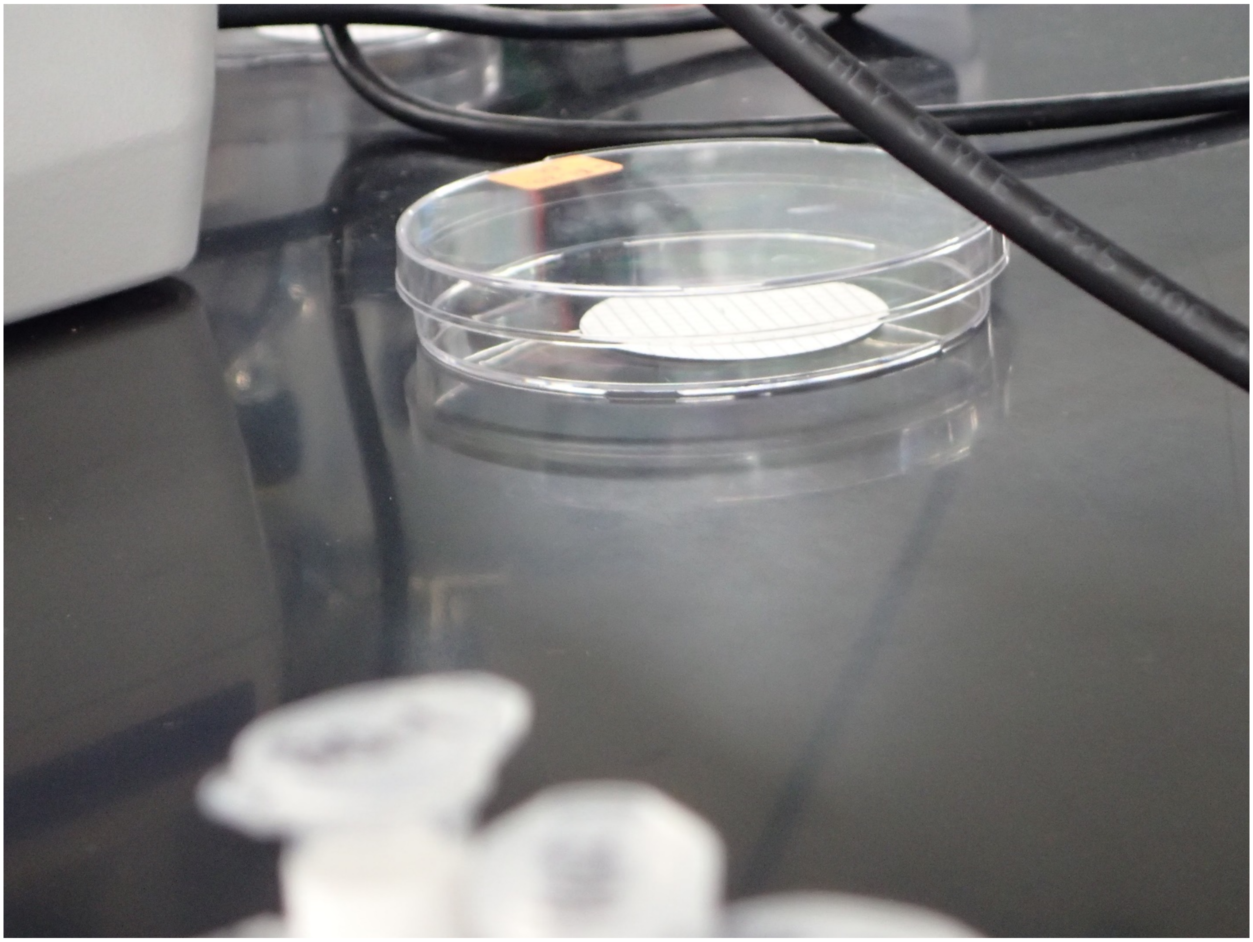


Fish Dissection


GRAY
Mode
DF RC BF



Digestive Tract Digestion Preparation



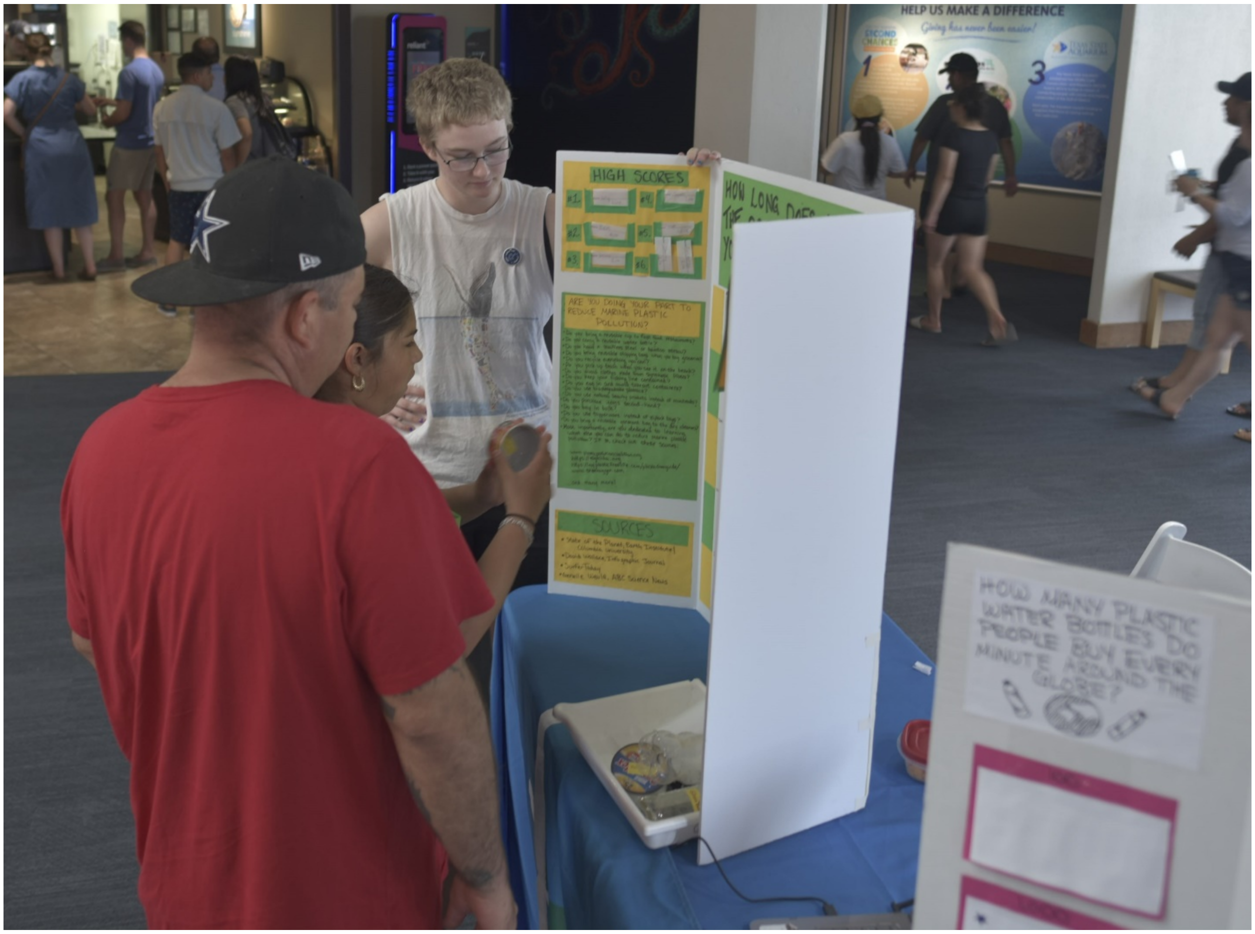
Filter with digested content of digestive tract ready for visual analyses

Appendix 3 Photos of Outreach Events at Texas State Aquarium Stem Café and World Ocean Day 2019


HOW LONG DOES IT TAKE THE OCEAN TO DECOMPOSE YOUR PLASTIC WASTE?
ANSWERS


HOW LONG DOES IT TAKE THE OCEAN TO DECOMPOSE YOUR PLASTIC WASTE?
Dr. Simon Geist Lab
TEXAS A&M UNIVERSITY CORPUS CHRISTI


LEVEL 1
LEVEL 1
W LONG DOES IT TAKE
E OCEAN TO DECOMPOSE
OUR PLASTIC WASTE?


HELP US MAKE A DIFFERENCE
Giving has never been easier!
HIGH SCORES
ARE YOU DOING YOUR PART TO REDUCE MARINE PLASTIC POLLUTION?
SOURCES
HOW LONG DOES
HOW MANY PLASTIC WATER BOTTLES DO PEOPLE BUY EVERY MINUTE AROUND THE GLOBE?


Microplastics can be found in:
50 years
4 weeks
FISH
SEA TU