FROM FACTORY TO TABLE: ASSESSMENT OF COMBINED INDUSTRIAL POLLUTION ON THREE ECONOMICALLY RELEVANT FISHES WITHIN THE MATAGORDA BAY SYSTEM OF TEXAS: *S. OCELLATUS*, *C. NEBULOSUS*, AND *P. LETHOSTIGMA*

A Thesis

by

JESSICA T. MYERS

BS, Coastal Carolina University, 2019

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

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ABSTRACT

Mercury ranks as one of the top three toxic substances. Aquatic wildlife and humans face adverse health effects when exposed to it in the form of methylmercury. The mass output of hazardous waste has resulted in an EPA Superfund Site for mercury on the edge of Lavaca Bay, the northernmost subset of the Matagorda Bay system. Additionally, heavy release of pre-production resin pellets (nurdles) was discharged into the bay within the last two decades. Due to their chemical properties, there is potential for mercury to bind to the discharged pellets as well as typical debris seen in all environments. Sorption to plastic may allow for mercury to travel greater distances throughout the bay, resulting in higher mercury concentrations in the fish within this semi-closed system. A sample of 178 fish (*Sciaenops ocellatus*, n = 47; *Cynoscion nebulosus*, n = 85; and *Paralichthys lethostigma,* n = 46) was opportunistically collected from anglers fishing within the Matagorda Bay System. Muscle and liver tissue samples, as well as digestive tracts, were removed from each fish. Digestive tracts were dissected, removing any suspected plastic visible to the human eye (>1mm). Mercury concentrations (wet weight; $\mu g\ g^{-1}$) of each tissue type and plastic found within the gut were measured in efforts to observe any trends in plastic consumed and mercury concentrations of the fish. No plastic debris (>1mm) was observed in the sampled digestive tracts, inhibiting the analysis of one of the objectives. The study determined mercury concentrations of three economically relevant fish, categorize them by FDA Consumer Advisory levels, and assess a factor of health via hepatosomatic index (HSI). Data from this study will be used to inform the public affected by the pollution in this area and can inform policy and regulations regarding safe consumption limits and reporting efforts.

DEDICATION

This thesis is dedicated to those who want to use accurate science to educate and inspire. To my parents, Dana and Joseph, for their unending support through this wild ride. Without you both, I would not be where and, more importantly, *who* I am today. Lastly, this work is dedicated to my grandmother, Mary Bristol, who passed before the completion of the project. She was a force of nature who gave me my tenacious spirit, which I will forever carry.

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Dr. Jessica Dutton of Texas State University analyzed the mercury in our samples and answered every single question I threw her way. Diane Wilson was integral with her vast knowledge of the Matagorda Bay system. I thank the anglers who let us sample from their catch, namely Larry Sr. and Jr. for going the extra mile to help us get our fish.

I could not have completed this project without the help of Jacob Oster, Jacob Doty, and my lab mates, especially Maddie, Stephanie, Casey, Dusty, Rita, and Quynh for their endless support in the field and lab. To my little family at home, thank you to Robert for the love and support (especially the food), and our crazy cat, Nurdle. Though we were states apart, thank you to my friends and family, namely my parents, Aunt Mare, grandparents, Marissa, and Elise for their long-distance support. Thank you to Katie, Robbie, Kenzie, Will, Lauren, and Anthony for making Texas feel like home. The hosts of my favorite podcasts made hours in the lab fly by with their wealth of information. These past three years of field and lab days, endless writing, and results from this research all happened because of these people.

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1. INTRODUCTION

Coastal waters are the foundation of many communities that rely on fisheries as the base of their economy and diet. Golden *et al.* (2016) estimated that the food intake of 1.39 billion people consisted of >20% fish. Additionally, the Food and Agriculture Organization (FAO 2016) reported that per capita fish consumption has tripled from 9.0kg in 1961 to 20.2kg in 2015 and is expected to reach 21.8kg by 2025. While fish serve as a nutritious protein, mercury exposure is a hazard associated with consuming long-lived species from upper trophic levels, such as tuna and halibut (Hammerschmidt and Fitzgerald 2006). Mercury naturally occurs in the environment through volcanic eruptions and the weathering of rocks (Mason 2009), where it is cycled through various environmental media. The risk to seafood consumers is elevated due to additional mercury releases to aquatic systems from industrial sources. This risk has been a public concern since a mass poisoning event in the 1950s where the Chisso Corporation released mercury-contaminated wastewater into Minamata Bay, Japan (Jobin 2005; Zillioux 2015).

Global anthropogenic mercury emissions are estimated at 2,220 metric tons annually (Pirrone *et al.* 2010), nearly triple the background levels from natural cycling (Clifton 2007). The top mercury-emitting industries are burning fossil fuels, cement production, and refining non-ferrous metals, such as aluminum (AMAP/UNEP 2008). The Australian Aluminum Council (AAC 2014) estimated that bauxite, the material refined for aluminum ore, has a concentration of 0.02-1.50μg g^{-1} of total mercury (THg; all species of mercury in no specific ratio). During refining, a portion of elemental mercury (Hg^0) is released into the air at high temperatures, while the remaining mercury cools within the plant, bonding with atmospheric oxygen or chloride to form inorganic species of mercury (IHg) (Puchakayala 2010). Despite being a heavy molecule (density of 7.55-7.70g cm^3), elemental mercury can travel great distances in the atmosphere before precipitating to the ground or surface waters, where it is eventually settled into sediment due to its hydrophobicity (ATSDR 2022; Gonzalez-Raymat *et al.* 2017). In reducing conditions typical in sediment, bacteria convert inorganic mercury to the more toxic methylmercury (MeHg) (ESTCP 2016).

Methylmercury is a well-known geno- and neurotoxin (Crespo-Lopez *et al.* 2009). Fish exposed to MeHg in laboratory and in-situ studies at different life stages exhibited health impacts and behavioral changes that negatively affect survival, such as reduced prey capture rates (Weis 2009; Weis & Khan 1990; Smith & Weis 1997) or delayed or reduced predator aversion (Alvarez *et al.* 2006; Webber & Haines 2003). Documented effects on fish include impaired reproductive processes, e.g., reduced gamete and reproductive hormone production (Wester 1991; Drevnick & Sandheinrich 2003), and impaired spawning (Hammerschmidt *et al.* 2002). These impacts echo throughout the food web due to the high bioavailability of methylmercury. The fat-soluble compound binds to fish protein (Rice *et al.* 2014), resulting in trophic transfer and bioconcentration from prey to predator (Boszke *et al.* 2003). This yields the greatest mercury concentrations in long-lived species at higher trophic levels (Hall *et al.* 2020). Studies have found that >90% of THg within fish tissues is methylmercury (Bloom 1992; Davidson *et al.* 1998; Hammerschmidt & Fitzgerald 2006). While it is not the only contaminant detrimental to fish health and behavior (e.g., PCBs and PAHs), the ability for MeHg to be retained by the body and bioconcentrate in higher trophic levels is concerning (Hammerschmidt & Fitzgerald 2006).

Fish consumption is the primary route for mercury exposure in humans (Shimshack *et al.* 2007). Like in fish, methylmercury is a neurotoxin and, at high enough concentrations, can disrupt the central nervous system in healthy adults (Shimshack *et al.* 2007; Choi *et al.* 1978). Japanese researchers observing victims of the Minamata Bay release saw early effects of mercury poisoning for hair concentrations of 50 ppm (Foulke 1994). However, developing fetuses and children were affected at concentrations as low as 10μg g^{-1} (Burbacher *et al.* 1990; Choi *et al.* 1978; Cox *et al.* 1989), a level easily achievable in those who consume fish regularly (Airey 1983; U.S. Environmental Protection Agency (USEPA) 1994).

Mercury does not act alone – before it settles into the sediment, the similar hydrophobic nature of plastic polymers makes for an ideal surface for sorption, the physical bonding of chemicals (Duckworth 2008; Turner & Holmes 2015). Many variables influence sorption, such as surface area, hydrophobicity, polymer chemistry, etc. (Bond *et al.* 2018; Fotopoulou and Karapanagioti 2012). As plastics decrease in size, their surface area to volume ratio increases, resulting in higher relative contaminate concentrations on smaller plastics (Teuten *et al.* 2007). Furthermore, hydrophobic contaminant concentrations on plastics can be orders of magnitude higher than the surrounding water (Carbery *et al.* 2018). Because of these properties, some scientists have called for plastic waste to be considered and handled as hazardous waste (Rochman *et al.* 2013). The contaminants on plastic debris can potentially transfer to organisms when consumed directly or indirectly through prey items (Welden *et al.* 2018), potentially leading to food web and human exposure (Teuten *et al.* 2007; 2009). Detrimental effects throughout trophic levels have been observed in estuarine species having consumed plastics with sorbed contaminants such as dichlorodiphenyltrichloroethane (DDT) (Athey *et al.* 2020).

Since their introduction in the late 19th century, plastics have swarmed store shelves (Law 2017). Low production costs and versatility as a material led to a booming industry, with annual global production reaching 367 metric tons in 2020 (Plastics Europe 2021). A century after their creation (Oliveira & Almeida 2019), they found their way all around the globe, from farmland (Zhang *et al.* 2020) to the deepest ocean trenches (Jamieson *et al.* 2019; Kane *et al.* 2020), and even areas virtually untouched by humans (Lavers & Bond 2017). Increased demand for plastic products drives skyrocketing production. Mass production, paired with unregulated industry and waste regulation, leads to high input of plastic debris into the aquatic systems. It is estimated that annual plastic waste input into the oceans will increase from 9-23 million metric tons (MT) in 2016 to 53 million MT by 2030 (Borrelle 2020).

As methods and technology develop, the growing plastic research field has reached standard definitions for some size classes of plastics, a paramount component for comparing research. There are currently four generally accepted plastic size categories: macro-, meso-, micro-, and nanoplastics. The National Oceanic and Atmospheric Administration (NOAA) reports that plastics are considered microplastics once their longest dimension reaches 5 mm (Arthur *et al.* 2009). This is the only agreed-upon size classification. Nanoplastics are typically described as 1-100nm, micro- (1μm-5mm), meso- (5-25mm), and anything > 25mm are known as microplastics (Schwaferts *et al.* 2019). Size affects the bioavailability of the plastic in terms of how it can enter the body (e.g., intake through the gills, active or passive ingestion) (Boyle *et al.* 2020; Wang *et al.* 2020). Additionally, the density of the material will determine its place in the water column. The most abundant plastic, polyethylene terephthalate (PET), unaltered, will float on the surface (density = 1.33-1.39g mL^{-1}) (Naidu *et al.* 2021; Pabortsava & Lampitt 2020). Denser plastics include polyethylene terephthalate (1.38-1.39g mL^{-1}), polyvinyl chloride (1.16-1.35g mL^{-1}), and polystyrene (1.05-1.07g mL^{-1}). These properties will affect the distribution of plastic pollution throughout an ecosystem and their impacts on specific fauna.

Industrial mercury pollution within the Matagorda Bay system began in 1966 (EPA 2016). The Alcoa Corporation aluminum refinery is located on the eastern edge of Lavaca Bay (Fig. 1) but is now decommissioned. Mercury-contaminated sediment was initially kept on Dredge Island, 420 acres of land near the plant, but would discharge into Lavaca Bay after rain events (EPA 2016). Due to high mercury values in the sediment and biota in the system, the location was designated as a Superfund Site (Fig. 1) in 1994, prohibiting the collection of finned fish and shellfish. Mercury pollution in the sediment and waters around the plant affects the biota in the surrounding areas to this day. Red drum (*Sciaenops ocellatus*, L. 1766) captured within the closed area during the most recent EPA survey (2016) had concentrations of 1.32μg g^{-1} THg within their tissues, whereas those caught within adjacent open areas had an average of 0.42μg g^{-1} THg. Sediment collected in open areas during the survey ranged from 0.05 to 0.46μg g^{-1} THg, with an average of 0.25μg g^{-1}. The consent decree for the Clean Water Act violation requires sediment levels < 0.05μg g^{-1} dried weight in the closed area for two years before the area can be reopened (EPA 2016).

Mercury is not the only over-abundant contaminant in the Matagorda Bay system, specifically Lavaca Bay. In addition to typical anthropogenic litter, this bay system has received steady discharges of pre-production plastic from a large and growing manufacturing facility. In 2018, the Formosa Plastics Corporation of Point Comfort, Texas (Fig. 1) was sued by members of the surrounding community under the Clean Water Act (Bradley 2019). This plant's main product is pre-production resin pellets, also known as nurdles, and powders of various polymer types. The Texas Commission on Environmental Quality (TCEQ) reported that pellets and powder were released at concentrations greater than trace amounts, polluting Cox Creek and Lavaca Bay. The size of the plastic released makes it more bioavailable to wildlife, and its small, round shape can resemble desirable prey items like eggs (Azzarello & Van Vleet 1987). Pellets are not the only actively consumed plastics. Studies have found that plastics with congruent color and form to prey items can result in active uptake by predatory fish or be indirectly consumed during natural predation (Ory *et al.* 2017; Tabb & Manning 1961).

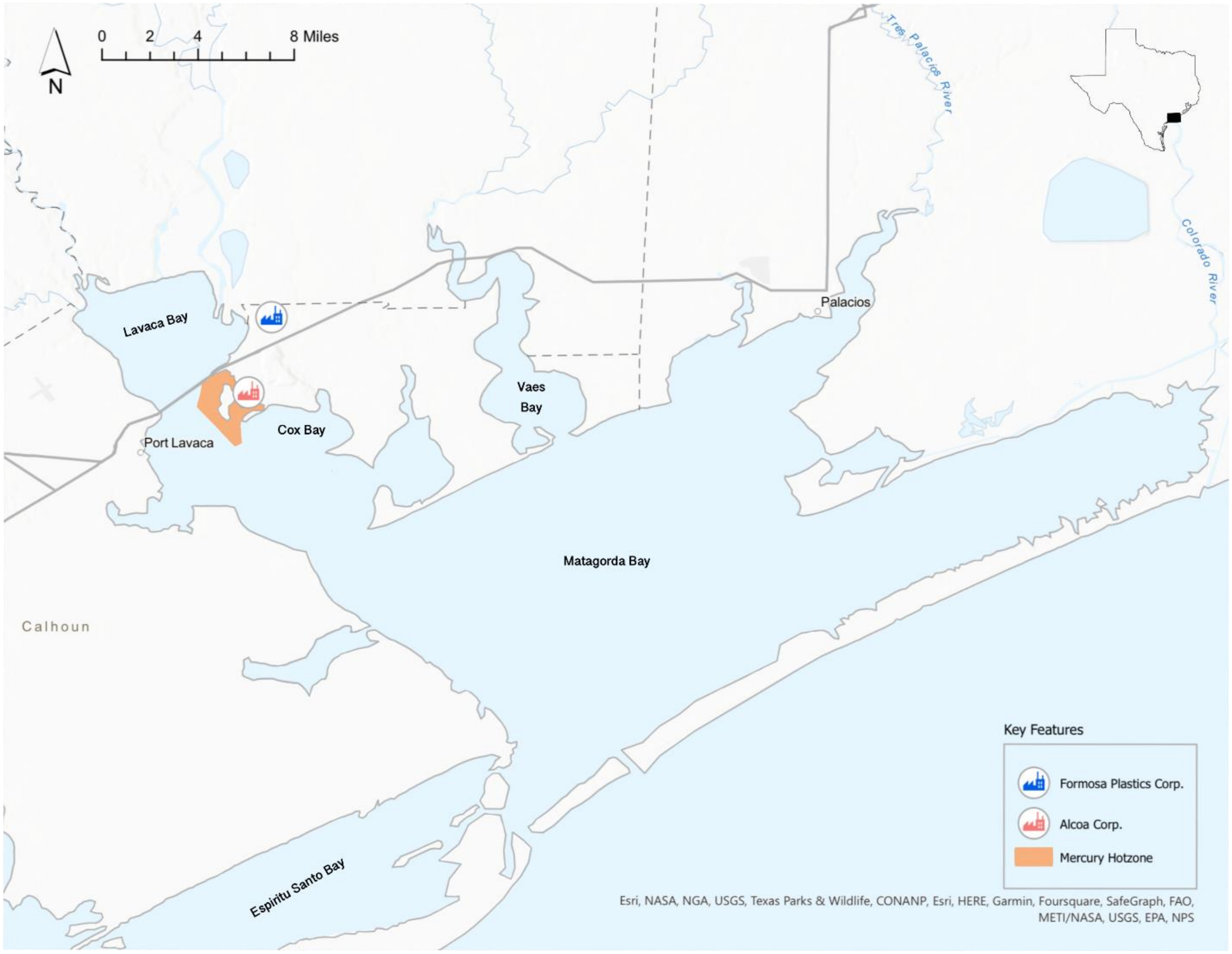

Figure 1: Map of the Matagorda Bay System, Texas, USA (28.624761ºN 96.584759ºW) showing the area of capture for the sampled fish (*C. nebulosus*, *P. lethostigma, S. ocellatus*). Bays: Matagorda Bay, Lavaca Bay, Cox Bay, Keller Bay, Vaes Bay, and Espiritu Santo Bay.

Physiological issues due to the consumption of plastic have been observed in numerous species, from direct lesions (Ahrendt *et al.* 2020) to a less visible disruption of physiological processes (Koelmans *et al.* 2014; Rochman 2015). Certain condition factors can measure fish health. One method looks at the hepatosomatic index (HSI), a ratio of liver weight to the dressed (gutted) weight of the fish (Nikolsky & Birkett 1963). The purpose of calculating the hepatosomatic index is to assess energy reserves and relative health across species through a comparison of liver weight and dressed weight of the fish itself. In general, lower relative values when compared between species or treatments indicate the distribution of energy reserves elsewhere in the body (e.g., in preparation for gonadal development or energy expended during detoxification) (Rizzo & Bazzoli 2020; Nikolsky & Birkett 1963).

Due to the organ's function, the liver is important to observe when studying contaminants in the body. Because of the harmful effects of methylmercury on the consumer, the Food and Drug Administration (2022) have developed advisory levels for the concentration of THg in fish tissues which are summarized in Table 1. The advisories highlight how often a fillet (4oz, uncooked) can be consumed depending on the concentration of mercury using categorical rankings: best, [better], good, and avoid.

Table 1: Fish consumption advisories adapted from the Food and Drug Administration (2022) data. Categories include maximum number of recommended servings per week, total mercury concentrations (μg g^{-1}), and quality of choice deemed by the FDA. Serving sizes refer to a 4oz, uncooked fish fillet. Note: the source considers both middle categories as "Good Choices," but the third category was adjusted to "Better Choices" to differentiate between categories.

Weekly fish servings	**Screening value (μg g^{-1})**	**Chart Category**
0	> 0.46	Choices to Avoid
1	≤ 0.46	Good Choices
2	≤ 0.23	Better Choices
3	≤ 0.15	Best Choices

Three species of fish were assessed for mercury concentrations and ingested plastics in this study: red drum (*S. ocellatus*), southern flounder (*Paralichthys lethostigma;* Jordan & Gilbert 1884), and the speckled or spotted seatrout (*Cynoscion nebulosus*; Cuvier 1830). These fish were selected due to their varying life history traits and environmental relevancy. All three species remain in the same estuary throughout their life (Pattillo 1997) and are of the most popular recreational fish frequently sought after and consumed by anglers in the Matagorda Bay system (Alcoa 1998; Ropicki *et al.* 2016).

Red drum are found throughout shallow waters of the western Atlantic and Gulf of Mexico (Lee *et al.* 1980; Simmons 1962; Yokel 1966). They are typically found schooling in estuaries until they mature (year 3), transitioning into solitary behavior in shallow offshore waters (Simmons 1962). However, all life stages of red drum are commonly found in Matagorda Bay (Pattillo 1997). They are fast-growing and long-lived fish, reaching 630mm (TL) within the first three years (Simmons 1962) and an average of 800-850mm in adulthood (Pearson 1929; Miles 1949). The Texas Parks & Wildlife (TPWD) (2022) defines allowable catch limits for fish between 508-711mm, though anglers can keep one individual above this range each license year as long as a proper tag is retained. Their average lifespan is 37 years (Murphy & Taylor 1990; US Fish and Wildlife & Johnson 1978). Red drum are carnivorous at all life stages, with crustaceans dominating their diet both in volume and fish in number consumed (Pattillo 1997). Dominance toward crustaceans occurs in the summer and the beginning of fall. They feed throughout the water column and along the bottom of their habitat, particularly in seagrass beds (Malinowski *et al.* 2019). Seagrass, sand, and other detritus have been observed in their stomachs but are assumed to be accidentally consumed when lunging toward prey (Pattillo 1997). Prey is consumed through rapid expansion of the jaw to either bite large prey or suck in smaller prey (Matlock 1990). This feeding strategy makes red drum susceptible to passive capture of plastics.

Speckled trout are a top predator in estuaries and have a distribution and niche similar to that of red drum (Killam *et al.* 1992; Pattillo 1997). All life stages can be found in the Matagorda Bay system (Pattillo 1997). With a much shorter lifespan (~7-9 years), they mature by 300mm (years 1-2) (Brown-Peterson *et al.* 1988; Lassuy 1983; Pearson 1929; Simons *et al.* 2015). Fish between 432-584mm may be retained by anglers when fishing within southern Texas (TPWD 2022). They are mainly visual, piscivorous hunters that feed in surface and midwaters above seagrass beds, though juveniles will consume small crustaceans, bivalves, and gastropods (Darnell 1958; Stewart 1961; Hettler 1989; McMichael & Peters 1989). Some studies have observed a shift to shrimp in the warmer months (Pearson 1929). Seagrass and shells have been observed in stomach contents, suggesting incidental ingestion of ambient particles (potentially plastics) when feeding (Tabb & Manning 1961).

Southern flounder are demersal predators found along the western Atlantic from North Carolina to Florida, and in the Gulf of Mexico from Florida to northern Mexico (Hoese & Moore 1977; Manooch 1984). Adults are abundant and juveniles are commonly found in the Matagorda Bay system, however, fish leave to spawn in the neritic zone (Pattillo 1997; Sabins & Truesdale 1974). Though age and length relationships vary greatly (Fitzhugh *et al.* 1996), studies have observed maturity between 243-560mm TL (Shepard 1986; Stokes 1977). Anglers are prohibited from keeping any flounder under 381mm and have no maximum limit (TPWD 2022). Their average lifespan ranges from 4-8 years (Pattillo 1997). Juvenile flounder predate on brown shrimp in Texas estuaries as well as other crustaceans and benthic fishes (Gilbert 1986; Minello *et al.* 1989). Once adults, flounder shift into a mainly piscivorous diet, relying less on crustaceans (Darnell 1958; Overstreet & Heard 1982). Their predation style consists of an upward lunge from the sandy bottom and vacuum suction from the mouth to ingest prey quickly (Malinowski *et al.* 2019). Chronic interaction with sediment in a polluted system and the ability to ingest plastic passively through prey makes for great risk of exposure to both contaminants in this system.

This study aims to assess the impacts of combined exposure to two contaminants: mercury and plastic on body conditions of three estuarine fish within the Texas Matagorda Bay system. We predict that, of the three fish species, red drum will have the highest mercury concentrations within both tissue types and subsequently, the lowest health (as determined by HSI). Given that this species has the widest range of diet and feeds throughout the entire water column, it is expected that their gut contents will contain more plastic than those of the speckled trout and southern flounder. The objectives of the study are as follows:

Objective 1: Determine THg concentrations in liver and muscle tissues of three fish species in the Matagorda Bay system, Texas

Objective 2: Categorize individuals based on FDA consumption advisories

Objective 3: Determine if the presence of plastic in gut content correlates with Hg concentrations in fish tissues

- $H_{0,1}$: There is no relationship between THg and the presence of plastic in fish digestive tracts
- $H_{A,1}$: There is a significant relationship between THg and presence of plastic in the digestive tract
- $H_{0,2}$: There is no relationship between feeding style and consumed polymer type
- $H_{A,2}$: Speckled trout will have a higher frequency of less dense plastics in digestive tracts, flounder will have higher density plastics, and red drum will have a mixture of polymers with varied densities, associated with feeding throughout the water column

Objective 4: Assess the relative impact of THg on the hepatosomatic index of each species

- $H_{0,1}$: There is no relationship between THg and hepatosomatic index
- $H_{A,1}$: There is an inverse relationship between THg and hepatosomatic index

2. METHODS

2.1. Study Site

Matagorda and Lavaca Bay, TX: The Matagorda system (Fig. 1) (28.624761ºN 96.584759ºW) is comprised of a collection of shallow water bays (average depth ~2m) covering about 1100km^2 including East Matagorda Bay, Karankawa Bay, Tres Palacios Bay, and Lavaca Bay, as well as some smaller reservoirs (Moseley & Copeland 1973). This system has little freshwater input, most of which comes from the Colorado River, and has limited output to the ocean due to a barrier island parallel to the coast (Ward & Armstrong 1980). The average salinity of the bay is 19psu, however during sampling for this study, heavy rainfall resulted in higher freshwater input with salinity ranging from 0-13psu throughout the various bays. This system is home to an EPA mercury Superfund Site (EPA 2016) as well as a plastic manufacturer that was sued under the Clean Water Act for illegally discharging millions of kilograms of polyethylene and polypropylene pellets and powder (Bradley 2019). While conducting preliminary research for this case, our lab (Coastal Health & Water Quality Lab at Texas A&M University-Corpus Christi), in collaboration with Dr. Jessica Dutton at Texas State University (TXST), found mercury on plastic pellets (3.9 to 13.3µg kg^{-1} dry weight plastic) and various pieces of plastic litter throughout the bay system (Conkle *et al.* 2018). The highest concentrations were found on expanded polystyrene, at 224.1-257.9µg kg^{-1} dry-weight plastic. Samples collected ~30 miles from the plastics manufacturer and superfund site also contained Hg indicating the potential for mercury transport on plastics and throughout the food web.

2.2. Field

Three species of bony fish, red drum (*Sciaenops ocellatus;* n = 47), speckled trout (*Cynoscion nebulosus*; n = 85), and southern flounder (*Paralichthys lethostigma;* n = 46) were opportunistically collected from fish cleaning stations in Port O'Connor, Texas from May to August 2021. To qualify for sampling, fish must have been caught within the Matagorda Bay system. The total length (mm) and sex (if gonads were present) of each fish were recorded. The livers, digestive tract, and ~2g of muscle tissue (white muscle tissue; skin retained in field collection) were removed from each individual after the fish were filleted. In standard sampling methods, tissue is removed from the same area on the specimen each time (typically below the anterior end of the dorsal fin) (Simons *et al.* 2015), however, a consistent site for tissue removal was not possible due to sampling post-fillet to increase angler involvement. Samples were placed in sealable polyethylene bags and stored on ice until they could be frozen. Samples were stored in a freezer at –20ºC at Texas A&M University-Corpus Christi (TAMUCC) until processing.

2.3. Laboratory

Digestive Tract Dissection: Digestive tracts were thawed and sliced open to reveal contents. All utensils used in this process were cleaned in a 1% nitric acid bath to reduce trace-metal contamination between samples. The contents of the stomach, including both debris and natural prey, were recorded, noting presence and count across the following prey groups: fish, shrimp, crab, seagrass, and anthropogenic debris (hooks, plastic, or non-plastic manmade debris). If little digested material was present, the chyme was washed away with deionized (DI) water. Any foreign particles visible to the naked eye (>1mm) were removed (Jantz *et al.* 2013), cleaned with DI water, and stored at 4ºC for later analysis via Fourier Transform Infrared (FTIR) spectroscopy to determine polymer type. Any particles matching a plastic polymer spectrum when scanned with the FTIR were included in the analysis. Spectrum matches were validated when Pearson correlation coefficient values ≥0.6 and spectra shared similar peak characteristics and relative heights (Cowger *et al.* 2021). Once polymer type was determined, samples were sent to TXST for THg analysis.

Tissue Processing: Tissues were fully thawed before processing. All outer edges of the tissue sample (including skin when present) were trimmed with a trace metal cleaned (1% nitric acid) ceramic knife to reduce trace-metal contamination from the original fillet knife. The mass of the trimmed tissue was taken (~1g), diced into small fragments, and placed in a trace-metal clean vial. Vials were placed in a -70 ˚C freezer for at least 24 hours. Afterward, the sample was freeze-dried at -54ºC (McCormack *et al.* 2020b) for 24 hours. Once completely freeze-dried, the sample was ground into a fine, homogenous powder using a ceramic mortar and pestle (McCormack *et al.* 2020b). The mortar and pestle were lined with a polyethylene bag to reduce contamination between samples. A portion of the powder (~0.05-0.10g) was transferred to a trace metal cleaned vial and stored at room temperature until further processing.

Liver Water Loss Determination: To assess water content lost from freezing, 30 livers from each species (n=90) were collected in June of 2022. Livers were removed at the cleaning station and kept on ice until immediate processing at the TAMUCC lab. The wet weight (g) of each liver was recorded. Livers were stored in a freezer at –20ºC. After at least 24 hours, the samples were removed, fully thawed at 4ºC, blotted dry, and reweighed. The average water content lost for each species was used to adjust liver wet weights to pre-frozen masses when calculating hepatosomatic index. The following equation was used to calculate thawing loss; where m_1 represents mass before and m_2 represents after thawing (Zhou & Xie 2021):

$$Thawing\ loss\ (\%) = \frac{m_1 - m_2}{m_1}\ x\ 100\%$$

The two equations below were used to determine the dressed weight of the fish, where whole and dressed (gutted) weight (g) of the fish are represented as WW and DW, respectively and total length (mm) is represented by TL. The a and b coefficients are species specific and can be found in Harrington (1979).

$$logWW = log(10^{a_1}) + b_1 \, logTL$$

$$DW = WW - a_2 b_2$$

Wet weight of the livers was calculated by taking the thawing loss, representing overall water content, and adding the proportion of water to the original dry weight. The hepatosomatic index was calculated using liver weight (adjusted with thawing loss, LW) and dressed weight (DW) of each fish in the following equation (Rizzo & Bazzoli 2020):

$$HSI = \frac{LW}{DW} \times 100\%$$

Sample Analysis: Ground tissue samples were sorted by species and increasing length of individual and sent to the Department of Biology at Texas State University. A Direct Mercury Analyzer (DMA-80; Milestone Inc., Shelton, CT) was used to determine THg concentrations (μg g^{-1}) within muscle and liver tissues. Blanks (acid with no sample; n = 39), certified reference materials (DORM-4, fish protein (n = 31), CE-464 (n = 7), and DOLT-5, dogfish liver (n = 11) from the National Research Council Canada), and duplicate samples (n = 39) to ensure quality control during mercury analysis (Table 2) (McCormack *et al.* 2020a). The mean percent recovery of THg in both tissue types across all species for DORM-4 was between 97 and 105%, for CE-464 was between 92 and 98%, and for DOLT-5 was between 93 and 99%. The mean relative percent difference in THg between duplicate samples for all tissue types and species was ≤1%.

Table 2: Number of quality control standards (n) (including DORM-5, CE-464, and DOLT-5) applied across varying species and tissue types and their respective averaged percent recoveries and differences when compared.

	Red Drum	**Speckled Seatrout**	**Southern Flounder**
Muscle			
Blank (n)	5	10	5
DORM-4 (n)	5	10	5
Average % Recovery	99%	101%	99%
CE-464 (n)	1	4	0
Average % Recovery	93%	93%	-
Duplicates (n)	5	10	5
Average % Difference	0.77	1.11	0.99
Liver			
Blank (n)	5	10	4
DORM-4 (n)	3	5	4
Average % Recovery	101%	100%	99%
DOLT-5 (n)	3	5	3
Average % Recovery	97%	94%	94%
CE-464 (n)	1	1	0
Average % Recovery	92%	98%	-
Duplicates (n)	5	9	5
Average % Difference	0.91	0.66	1.38

2.4. Statistical analysis:

The data was analyzed at the population (each species individually) and community levels (all species collectively). The following comparisons were made using a one-way analysis of variance (ANOVA): total length of fish to mercury concentrations, mercury concentrations between tissue types, individual tissue types to stomach contents, and number of prey in stomach contents to total mercury concentrations. Generalized linear hypothesis testing (GLHT) was performed on mercury concentrations between tissue types and HSI to tissue liver THg. Lastly, a linear model was generated to compare HSI to liver THg.

3. RESULTS

From July to August 2021, 178 recreationally caught fish were sampled from the Matagorda Bay System. This sampling method did not allow for the recording of specific catch locations. Number of individuals retained and sexed, size ranges (TL), THg concentrations, and hepatosomatic indexes for each species collected are summarized below in Table 3. There were not enough fish with gonads present, limiting analysis of factors influenced by sex. All reported THg concentrations are in wet weight. Length and THg concentrations for individuals are available in the supplementary data appendix.

Table 3: Number of individuals sampled (n), sex, total length [mean ± standard deviation and range], total mercury concentrations [mean ± standard deviation and range], and hepatosomatic index [mean ± standard deviation and range] of three species recreationally caught within the Matagorda Bay System in the summer of 2021.

	Red Drum	**Speckled Seatrout**	**Southern Flounder**
Individuals Retained (n)	47	85	46
Male	10	13	1
Female	6	38	43
Lacking Gonads	31	34	2
Total Length (mm)	625 ± 125	457 ± 47	404 ± 31
Range (mm)	251 - 954	362 - 600	346 - 520
Muscle THg (µg g^{-1})			
Average	0.14 ± 0.21	0.16 ± 0.30	0.07 ± 0.05
Range	0.03 - 0.74	0.03 - 0.46	0.04 - 0.13
Liver THg (µg g^{-1})			
Average	0.40 ± 1.30	0.15 ± 0.11	0.04 ± 0.01
Range	0.06 - 0.77	0.04 - 0.87	0.03 - 0.08
Average HSI	0.71 ± 0.43	0.63 ± 0.32	1.10 ± 0.49
HSI Range	0.13 - 2.08	0.08 - 2.03	0.30 - 2.54

3.1. Objective 1: Mercury Content

Flounder had significantly less THg in their tissues than red drum or speckled trout, 42% and 35%, respectively (GLHT, $F_{2,347} = 41.73$, $p < 0.0001$). For both flounder and speckled trout tissues, muscle contained a greater amount of THg (ANOVA $F_{2,348} = 21.06$, $p < 0.0001$) than liver (Fig. 2 & 3). The average total mercury concentration in the muscle tissue was 0.14μg g^{-1} for red drum, 0.16μg g^{-1} for speckled trout, and 0.07μg g^{-1} for southern flounder. The maximum detection in muscle THg was 0.74μg g^{-1} for red drum, 0.46μg g^{-1} for speckled trout, and 0.31μg g^{-1} for southern flounder. THg in liver was 0.40μg g^{-1} for red drum, 0.1μg g^{-1} for speckled trout, and 0.04μg g^{-1} for southern flounder (Table 3; Fig. 3). There was no significant difference between tissue THg concentrations within red drum ($p = 0.58$). Both speckled trout and southern flounder had significantly more THg in their muscles (ANOVA $F_{1,166} = 19.76$, $p < 0.05$; $F_{1,89} = 497.7$, $p < 0.05$, respectively) (Fig. 3). At the community level, there was a significant relationship between muscle THg and liver THg (Linear model $F_{1,170} = 136.1$, $p < 0.05$, $r^2 = 0.44$). At the population level, there was a significant relationship for all species. THg concentrations in muscle tissue were influenced by liver tissue concentrations in all three species: red drum (Linear model $F_{1,43} = 13.95$, $p < 0.001$, $r^2 = 0.23$), speckled trout (Linear model $F_{1,42} = 13.47$, $p < 0.001$, $r^2 = 0.22$), and flounder (Linear model $F_{1,81} = 108.4$, $p < 0.001$, $r^2 = 0.57$) (Fig. 4). An ANOVA analysis showed a significant difference between overall health advisory rankings of flounder and the other species (comparison with red drum: $F_{2,210} = 2.036$, $p < 0.05$; speckled trout $F_{5,210} = 2.036$, $p < 0.001$) (Fig. 5).

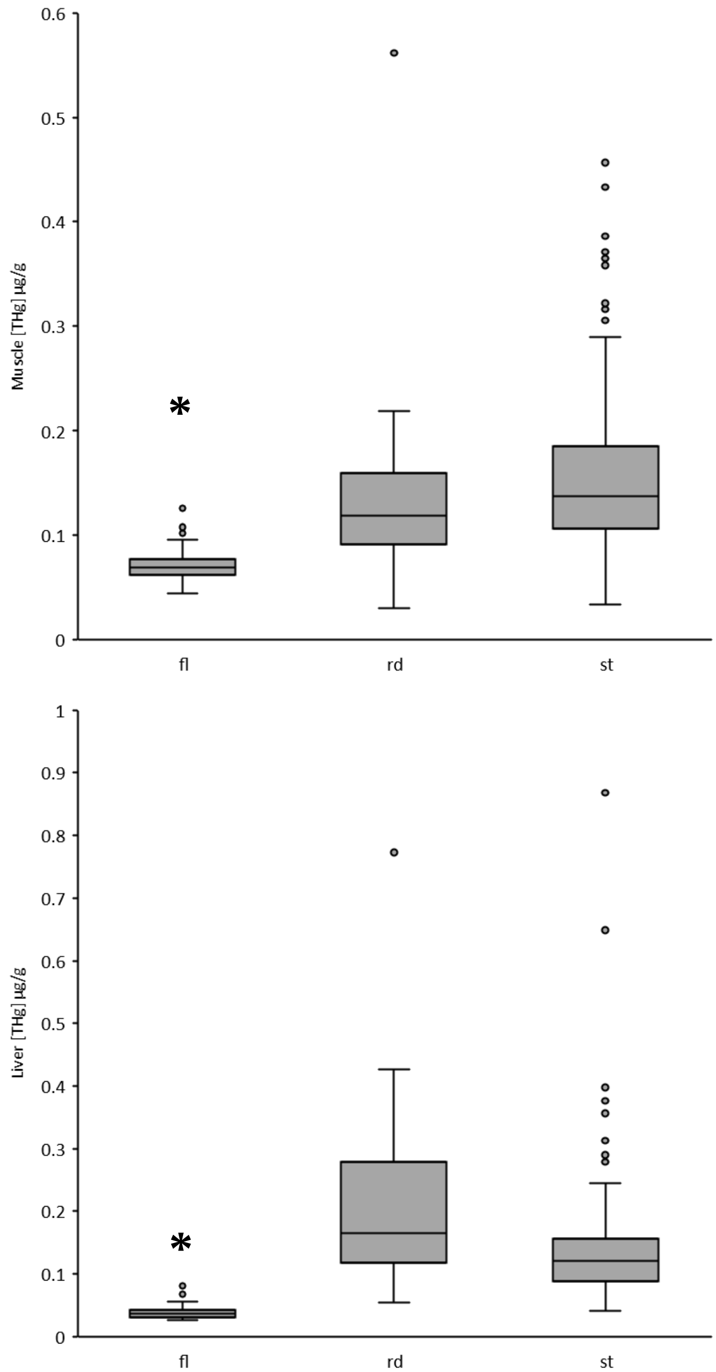

Figure 2: THg concentration of muscle and liver tissue between three species, red drum (RD), speckled trout (ST), and southern flounder (FL). Significance is represented by * on the figure.

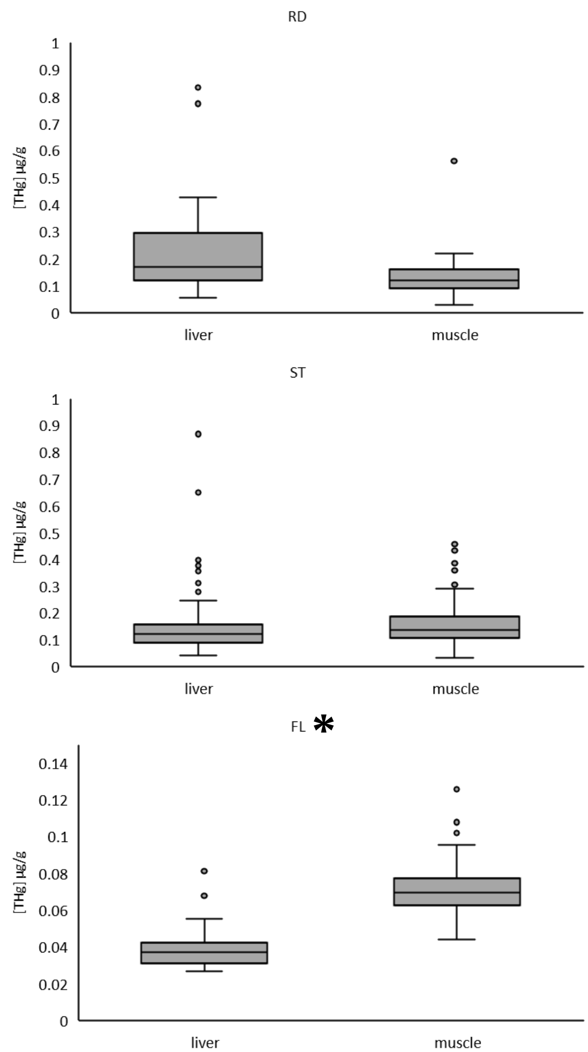

Figure 3: Total mercury (THg) concentrations between tissue types for each species, red drum (RD), speckled trout (ST), and southern flounder (FL).

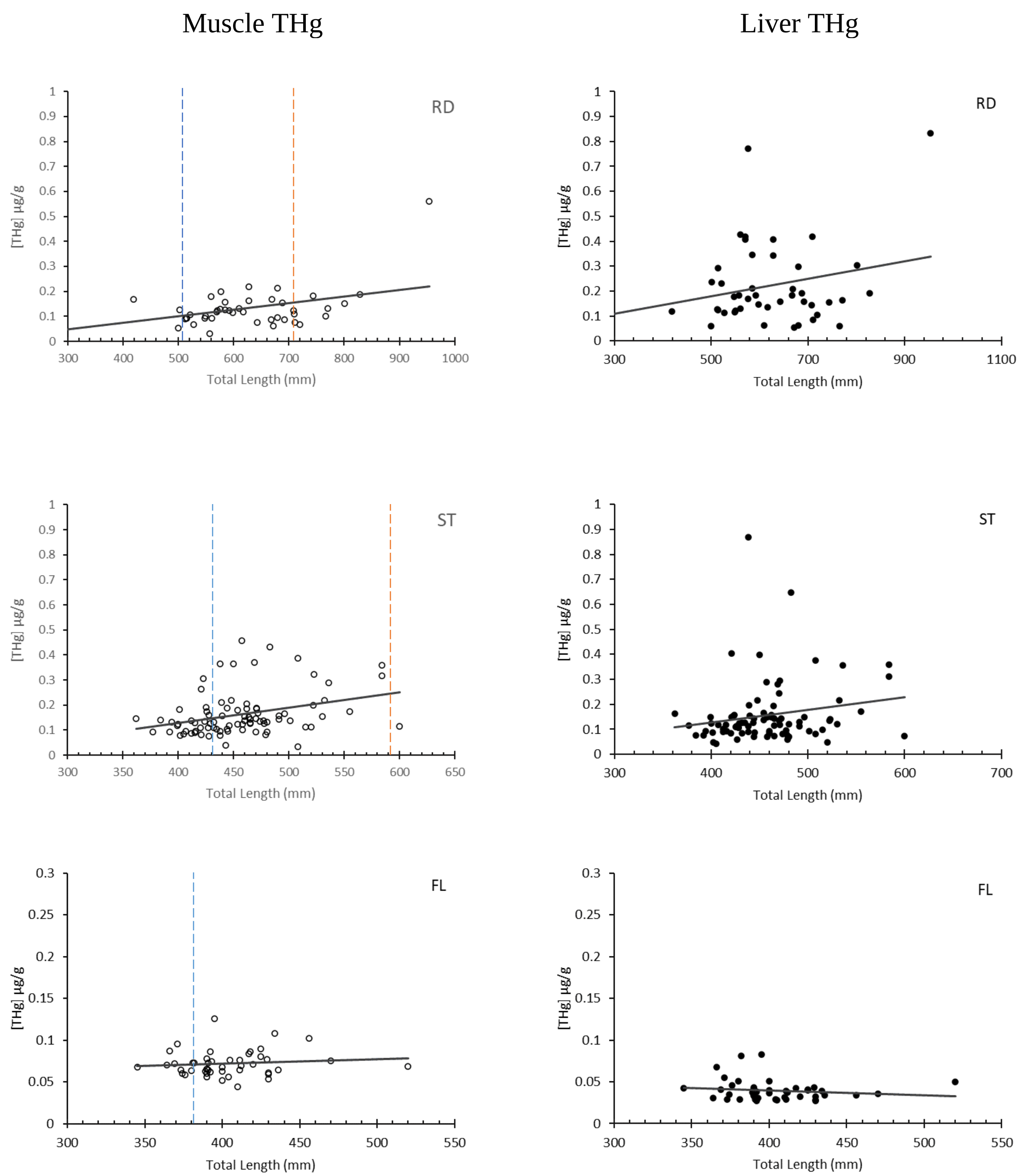

Figure 4: Length of species (TL, mm) compared to muscle (open points) and liver (shaded points) THg concentrations across three species: red drum (RD), speckled trout (ST), and southern flounder (FL). Dashed lines on the muscle graphs denote the max (orange) and minimum (blue) catch limit sizes defined by the Texas Parks & Wildlife Department (2022).

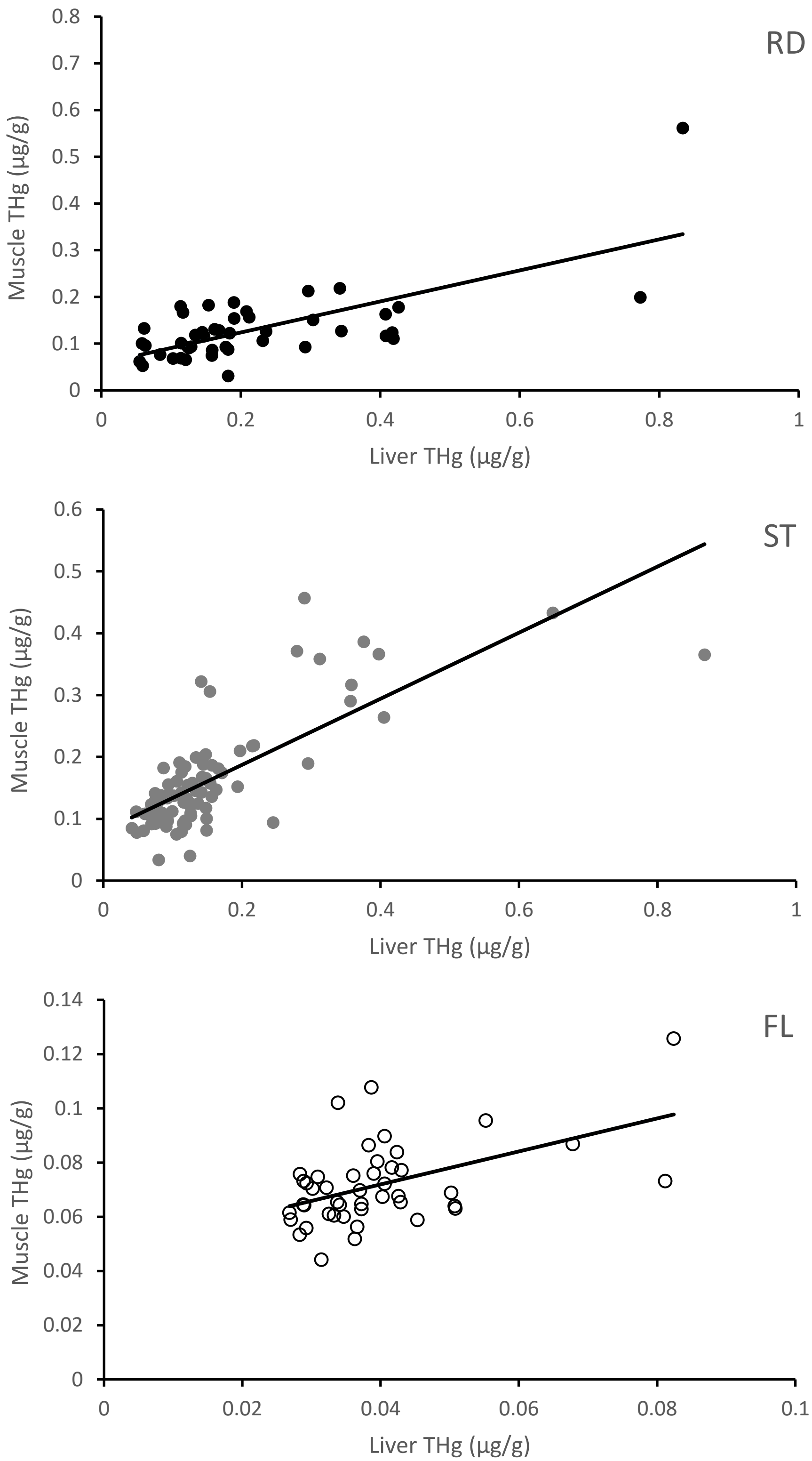

Figure 5: Comparison of THg concentrations between muscle and liver tissues in three species collected in Matagorda Bay, Texas.

3.2. Objective 2: FDA Consumption Advisories

The distribution of sampled fish according to FDA screening values (Table 1) can be found in Figure 6. US FDA (2022) screening values are based on the number of 4oz (raw weight) servings consumed per week. Of the 178 fish collected, 72% fell into the "best choices" category (≤ 0.15μg g^{-1}) allowing for 3 meals weekly, 4oz servings per week, while 20% could be consumed twice weekly, and roughly 7% of the collected fish could be consumed once weekly. Three individuals had THg muscle concentrations that should be avoided (>0.46μg g^{-1}). Note that the 2 weekly meals category has been adjusted from "Good Choices" to "Better Choices," as both middle categories use the same categorical terminology in the source. When broken down by species (Fig. 7) all "choices to avoid" individuals (n = 3) ranked were red drum. The remaining 20% and 75% ranked as "better" and "best" choices, respectively. Speckled trout were the only species with "good choice" ranked individuals (n = 14). Trout had a similar ratio of "better" and "best" choices as the red drum. Lastly, all southern flounder collected ranked in the "best choice" category.

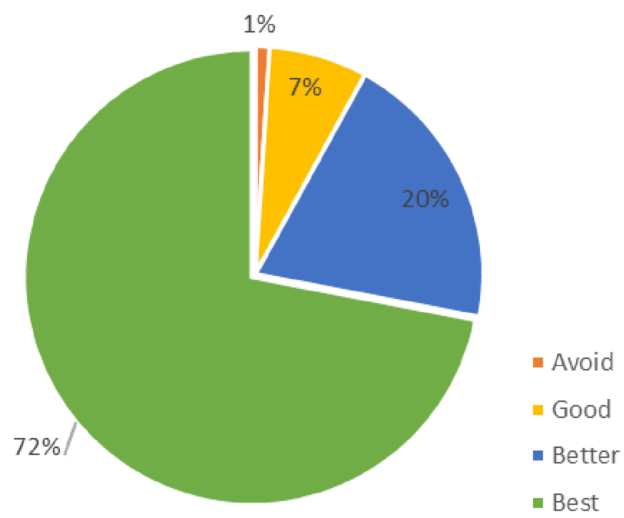

Figure 6: Portion of fish collected by FDA consumption guidelines ranks. Categories are based on 4oz, uncooked servings as follows: Avoid (>0.46μg g^{-1}, not to be consumed); Good (≤ 0.46μg g^{-1}, to be consumed no more than once weekly); Better (≤ 0.26μg g^{-1}, to be consumed up to twice weekly); Best (≤ 0.15μg g^{-1}, 3 servings weekly is allowable).

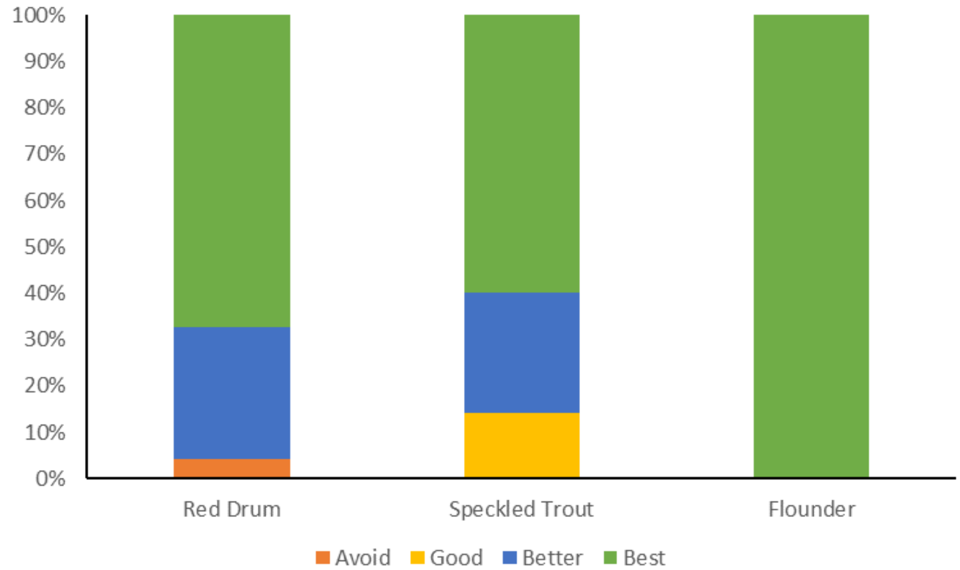

Figure 7: Breakdown of each species of interest by FDA consumption guidelines. Categories are based on 4oz, uncooked servings as follows: Avoid (>0.46µg g^{-1}, not to be consumed); Good (≤ 0.46µg g^{-1}, to be consumed no more than once weekly); Better (≤ 0.26µg g^{-1}, to be consumed up to twice weekly); Best (≤ 0.15µg g^{-1}, 3 servings weekly is allowable).

3.3. Objective 3: Gut Content Analysis

No plastic debris was observed with the naked eye within the gut contents of any of the three species. However, a fishing line and lure were recovered from two red drum stomachs (Fig. 8a-b). This material was not categorized as debris as they were cast into the environment from recreational fishing and were thus not litter. Despite the lack of plastic debris, general gut content was analyzed. Gut contents were divided into 6 categories, crab (*Brachyura*), shrimp (*Penaeidae*), fish (*Osteichthyes*), seagrass, anthropogenic material (the fishing lures, line, and hooks), and empty. Detailed taxonomy of the gut contents was not determined due to heavy digestion. All prey items (crab, fish, shrimp, and seagrass) were observed within all fish species. Red drum were the only species with any anthropogenic debris (plastic or otherwise) within their stomachs, i.e., fishing lures, line, and hooks (note that anthropogenic material were metal hooks with no plastic attached) (Fig. 8a-b). There was no significant difference in muscle THg between stomach content categories (ANOVA, $F_{5,210} = 2.036$, $p = 0.07$; Fig. 9). There was no significant relationship between number of prey items consumed and muscle THg (ANOVA, $F_{1,132} = 3.111$, $p = 0.08$; Fig. H). However, there was a significant relationship between items consumed and liver THg (ANOVA, $F_{5,199} = 2.546$, $p = 0.02$; Fig. 9). In addition to seagrass, one sea trout had consumed some notable prey, a stingray pup (*Dasyatidae*), disc width: ~50mm; total length: 160mm (Fig. 8c-d).

Figure 8: Notable items from fish gut content analysis. (A) A hook with a soft plastic lure found in a red drum. (B) Hook with plastic fishing line attached found in the stomach of a red drum. (C) Heavily digested stingray pup and (D) its barb found in the stomach of a speckled trout.

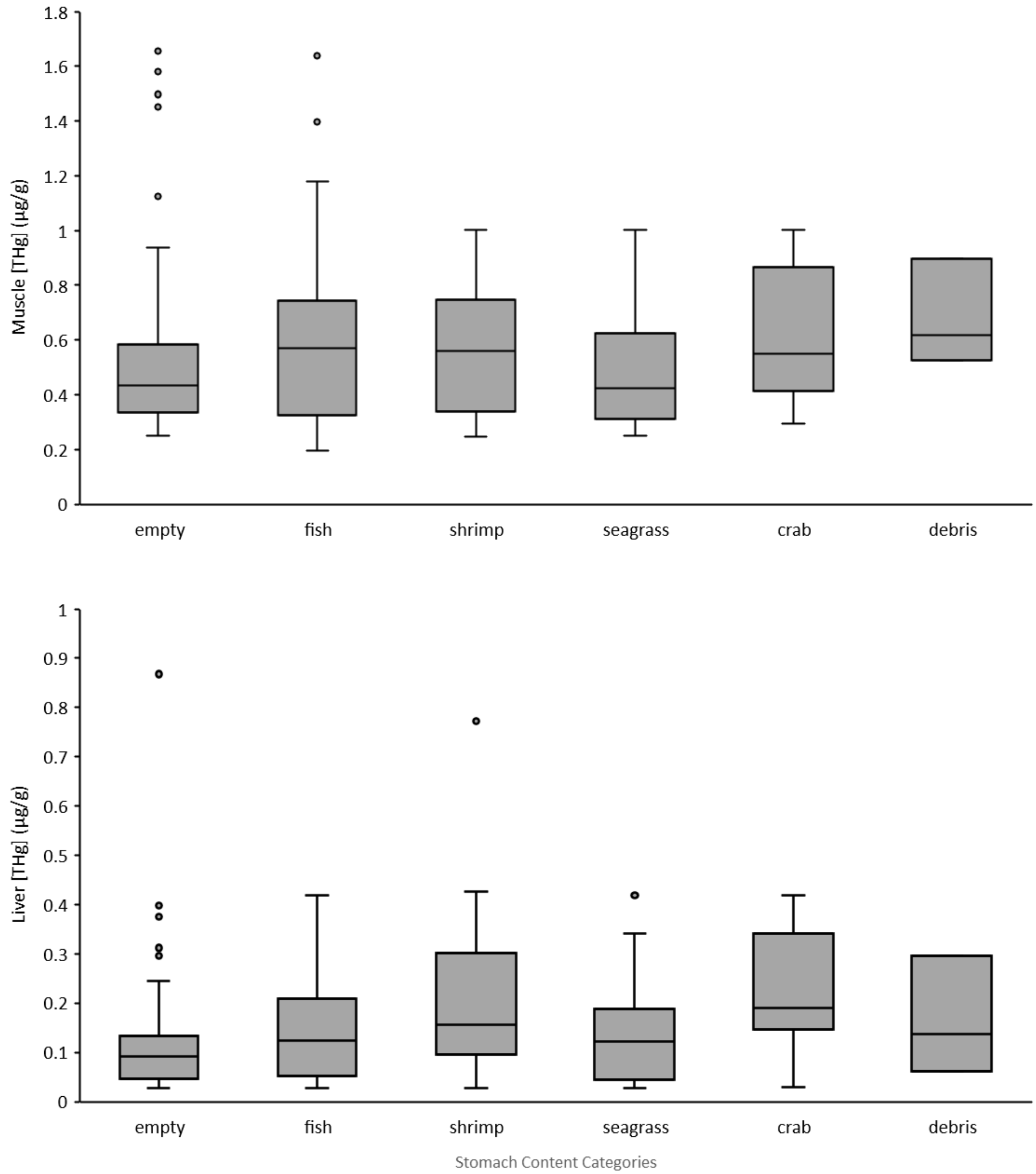

Figure 9: Muscle and liver THg concentrations by prey type for the population.

3.4. Objective 4: Hepatosomatic Index

The average HSI from highest to lowest were 1.10 ± 0.49, 0.71 ± 0.43, and 0.63 ± 0.32 for southern flounder, red drum, and speckled trout, respectively (Table 3). In all species, HSI decreased as liver THg increased, though this relationship was only significant for speckled trout ($p=0.0095$). Flounder had a significantly lower HSI than the other two species (Fig. 10) (GLHT $F_{2,167} = 22.31$, $p < 0.001$). Hepatosomatic indices were 42% and 47% lower in southern flounder than red drum and speckled trout, respectively.

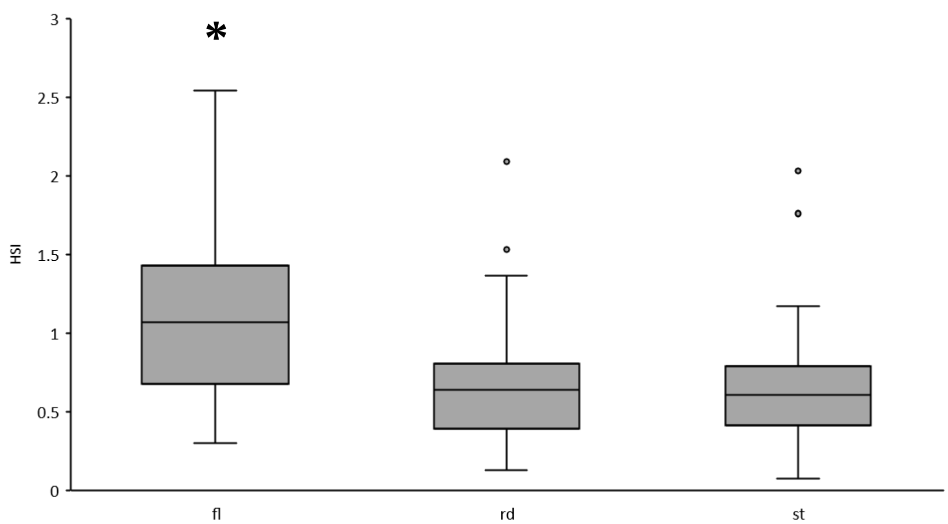

Figure 10: Comparison of Hepatosomatic indices across three species red drum (RD, speckled trout (ST), and southern flounder (FL). Significance is denoted by *.

4. DISCUSSION

Industrial release of contaminants into aquatic environments are a threat to fisheries and the communities reliant on them. Despite health concerns after the 1956 Minamata Bay mercury poisoning event, mercury levels were not heavily monitored until the early 1960s (D'itra 1991). It has since been a federal concern, with research aiding food safety decisions (Zillioux 2015).

The Matagorda Bay System in coastal Texas is home to a mercury Superfund site and a pre-production plastics manufacturer, while also experiencing typical amounts of anthropogenic litter. With the potential for mercury to sorb to plastic in aquatic environments, it is important to study plastic presence and interactions and impacts on fauna, especially in situations that lead to exposure to humans. This study sought insight into the implications of combined contaminant exposure on economically important fishes via industrial pollution. Due to the reliance on voluntary sampling from recreational anglers, specific locations of fish within the bay system could not be determined. While this collection method may deviate from measuring impacts of the point source pollution, it is important to consider that the sampled fish and subsequent dataset represent fillets that were likely consumed by anglers and their families.

4.1. Objective 1: Mercury Content

While species of mercury were not identified in our study, a previous study found that >90% of Hg in fish tissues is the most toxic form: methylmercury (Bloom 1992). Simons *et al.* (2015) measured muscle mercury concentrations of red drum, black drum *(Pogonias cromis)*, speckled trout, and various prey items across three adjacent bays: Lavaca, Nueces, and San Antonio. The highest concentrations of THg were in fish from Lavaca Bay. MeHg concentrations of those fish were >88% of total mercury. In addition to mercury, gut content and N-isotope analysis were conducted to observe trends within the food web. Similar to the results of our study

(Fig. 4), THg and length of red drum and speckled trout had a positive relationship, though it was not significant. A significant positive relationship between THg concentrations and fish length was expected for all species, as fish are assumed to increase in length as they age and advisories suggest avoiding long-lived species (FDA & USEPA 2022). However, studies show that length decouples with age over time, as growth is influenced by multiple factors (e.g., diet, genetics, etc.) (Andrews *et al.* 2016; Choat & Robertson 2002; Newman *et al.* 2015). In a system with industrial input such as Matagorda Bay, there may be additional factors supporting the weak relationship between size and mercury concentrations in this study. Studies on the relationship between mercury levels in fish and their surrounding water and sediment find a strong influence from these environmental factors (Calta & Canpolat 2006; Blevins & Pancorbo 1986). Due to the point-source pollution causing nonhomogeneous mercury levels throughout the bay system, and fish having the ability to swim throughout the system despite their size, mercury intake is expected to be less influenced by size and age.

The lack of significance between THg within each tissue type (Fig. 3) may be a result of the physiology and makeup of the liver itself, as well as fish age. Southern flounder had significantly lower THg in their livers than their tissues. This could be explained by a yearly winter migration out of the bay (Pattillo 1997). The composition and function of the liver in this species may also explain the large difference, where flounder livers may be more effective when processing mercury. Southern flounder are more sedentary in their demersal lifestyle; the metabolic difference between species may explain these results. More research is needed to assess differences in mercury concentrations by liver composition, as it may impact how livers process the contaminant. Similar to speckled trout, flounder also have a shorter lifespan compared to red drum. Livers of older fish may be less effective detoxifiers as they are damaged over the years

(Farmer *et al.* 2010). In especially polluted systems, it is expected that fish livers and muscle tissues would have similar concentrations of mercury due to a higher rate of input into the body and an adequate rate of detoxification from the liver.

It was expected that red drum would have the highest THg concentrations due to their longer lifespan, however, their levels were similar to that of speckled trout (Table 3; Fig.2). Our results contrast with that of Simons *et al.* (2015): between red drum and speckled trout collected from Lavaca Bay during a study in 2015, speckled trout contained THg (~1.5μg g^{-1}) of all sampled species and bays. Speckled trout are not as long-lived as red drum, having a lifespan approximately one-third that of the red drum, and therefore have less time to accumulate mercury (Pattillo 1997). Simons *et al.* (2015) and further evidence from Olson *et al.* (1973) conclude that their comparatively thin skin could act as a vector for mercury in addition to diet and absorption through gills, resulting in high mercury levels for a fish of that size. THg concentrations observed by Simons *et al.* (2015) are roughly one order of magnitude greater than our results. This could be a result of focus on Lavaca Bay as opposed to the entire Matagorda System or the fact that a decrease in ambient concentrations and subsequently the affected biota are expected after a decade since the main source of the contaminant has ceased (Boszke *et al.* 2003; USEPA 1994).

In 2001 the USEPA studied THg levels within various economically relevant fish species collected within and outside of the mercury Superfund Site hot zone. The past study observed average THg levels in red drum at 0.40μg g^{-1} with a maximum detection of 1.30μg g^{-1}, 35% and 57% higher than levels observed in this study, respectively. Speckled trout measured 0.31μg g^{-1} on average and a 0.88 μg g^{-1} maximum detection value; a 52% decrease observed for both average and maximum detection values when compared to the current study (Table 3). In southern flounder, the average THg was 0.14μg g^{-1} and the maximum detection was 0.32μg g^{-1}; a 50% and

41% decrease respectively. Between the 20 years between the USEPA observations and this current study (Table 3), there was an overall THg decrease in red drum, speckled trout, and southern flounder in both average THg and maximum detection levels (USEPA 2001). Additionally, relative levels between species were similar between studies, where southern flounder had significantly less mercury than red drum and speckled trout (Fig. 2).

Though mercury levels within the bay have been declining (US EPA 2001), the system likely still receives mercury from atmospheric sources (incineration and the burning of fossil fuels) albeit at lower concentrations (Gonzalez-Raymat *et al.* 2017). Additionally, the Calhoun County Port Authority is in the planning stages to dredge 26 miles of the bay to expand the Matagorda Ship Channel (Montagna *et al.* 2021). Montagna *et al.* (2021) used models to assess the outcomes of the project and concluded that an expanded ship channel would help boost the local economy by an estimated $6.5 million. The assessment also highlights that dredging resuspends sediments and is expected to reincorporate mercury into the system, possibly elevating ambient THg levels throughout the ecosystem, especially considering the dredge path cuts through the Lavaca Bay Superfund Site. This and other studies before the dredging can be cited as a baseline to assess impacts of mercury resuspension on the ecosystem.

4.2. Objective 2: FDA Consumption Advisories

Consumption advisories at the national level are developed by the Food and Drug Administration (FDA and USEPA 2022) based on a 4oz, uncooked fish fillet. All fish in the avoid category were red drum, with THg ranging from 0.56 - 0.74μg g^{-1}. The individual with the highest THg was removed from analysis as its total length (RD47; 93.5cm) and high mercury concentration deemed it a statistical outlier (Fig. 7; Appendix A). A general look at red drum as a species suggests that the fish was an older individual potentially caught outside of the area of interest. As red drum age and chase bigger prey, they move out of estuaries (Pattillo 1997). With time and consumption of prey in higher trophic levels, greater THg in tissues is expected. However, Matagorda Bay is one of two bay systems described by Pattillo (1997) in which all life stages of this species are seen, meaning that this individual could have been caught within the boundaries of the bay.

In addition to having the lowest mercury concentrations observed in the study (Table 3), all flounder collected fell within the best FDA advisory category, ranging in THg from 0.04-0.13μg g^{-1} (Fig. 7). Similarly, southern flounder had the lowest mercury concentrations (< 0.03μg g^{-1}) between fishes sampled from Northern Gulf of Mexico estuaries (Farmer *et al.* 2010). These findings validate the presence of flounder as a "Best Choice" on the 2021 FDA Advice About Eating Fish infographic (Fig. 7) (FDA and USEPA 2022). However, there is no mention of the other two species of interest (saltwater trout or any drum species).

Other studies highlight the need for individual MeHg testing as opposed to composite testing for a group of fish (Ginsberg & Toal 2000). Bulk testing ignores acute exposure from highly contaminated individuals. Ideally, single-meal testing would be available and accessible to all, especially those in high-risk areas, however, costs and materials make for an unrealistic widescale solution. While only 1% of the fish in our study were above consumption advisory levels, Ginsberg & Toal (2000) highlight that a single meal with THg levels > 2.0μg g^{-1} can raise hair concentrations of the consumer (a measurable reflection of body concentrations) to concentrations that threaten development for 1-2 weeks in those bearing children. This benchmark addresses the single meal alone, if one is consuming other fish (recreational or commercially caught), hair concentrations and subsequent health effects are expected to increase. With developmental effects being so impactful, Ginsberg & Toal (2000) advise regional consumption advisories agencies to report a maximum detect value in addition to average mercury concentrations when producing public data. The maximum detection values for red drum, speckled trout, and southern flounder observed in this study were 0.74, 0.46, and 0.13µg g^{-1}, respectively (Table 3). While the average concentrations observed were within safe consumption limits, the maximum detection of speckled trout meets the avoid category exactly, and the red drum is 1.6% higher than the categorical cutoff. These values are lower than the 2.0µg g^{-1} limit of concern presented by Ginsberg & Toal (2000), but adult anglers of Matagorda Bay are known to have 7-8oz servings of fish at a time with an average of 3-4 meals per month (Alcoa 1998). It should be noted that Alcoa conducted its fish consumption survey ~26 years ago. Considering that annual fish consumption rates have tripled over 50 years (Golden *et al.* 2016), this fishing community likely has higher meal frequencies today.

Mercury concentrations should not be the only concentration considered when estimating consumption safety. Peterson *et al.* (2009) address the need for measuring mercury and selenium ratios (Hg:Se) in fish tissues as selenium has an antagonistic effect that blocks mercury from binding to tissue protein. Selenium is present in most environments and has alleviated neurotoxicological effects of mercury in rats (Pařízek & Ošátdalová 1967; Ohi *et al.* 1980), birds (Ganther *et al.* 1972), whales (Lee *et al.* 2020), and fish (Gerson *et al.* 2021; McCormack *et al.* 2020b) It is imperative to include this ratio in any future studies to gain insight on possible advisory adjustments. However, selenium does not solely combat effects of mercury – as it is toxic to some animals (e.g., mayflies (Gerson *et al.* 2021), fish, and waterfowl (Debruyn & Chapman 2007). A study on the levels of mercury species before and after various cooking methods shows a significant decrease in the bioaccessibility of mercury in fish fillets (40-60% for frying and boiling) (Ouédraogo & Amyot 2011). In the same study, the co-ingestion of ingredients such as green tea and black coffee reduced bioaccessibility of mercury by 50-60%. While the concentrations of mercury did not change before and after these treatments, the adverse effects of consuming them were significantly altered. If consumption advisories reflect concentrations of raw tissues, then cooking a fillet would act as a buffer from high mercury concentrations, though this information may complicate learning outcomes if supplied at the public level.

Researchers have developed a portable fluorogenic probe that measures mercury ions ($MeHg^+$ and Hg^{2+}) in fish to ±0.01μg g^{-1} within 1-2 hours (García-Calvo *et al.* 2016). While induvial ownership of this equipment is not realistic, perhaps grants or city planning could provide high-traffic fish cleaning stations near contaminated areas with probes for the public to test the safety of their catch. Despite the creation of a fast and reliable probe, this is still a lethal testing method for already harvested fish and it is not widely available on the market at this time. If a fish is not safe to eat, this may drive anglers to catch more (within the limits of the relevant Department of Natural Resources (2022)), resulting in greater pressure on the population. Currently, fish size limits are centered around protecting the breeding population, but Cabana & Rasmussen (1994) highlight that this restriction can lead to consumption of fish with higher Hg levels. A slot model avoiding sexually mature and large individuals may better suit a problem such as this for species of greater concern, especially in areas that are threatened with high contamination, however more research is needed with sampling emphasis on a broader range of fish lengths and more individuals with higher tissue concentrations.

4.3. Objective 3: Gut Content Analysis

The two pieces of plastic found in fish digestive tracts were a part of tackle used to land the fish, and depending on level of use, experienced less time in the water than plastic debris moving through the system, decreasing potential to sorb mercury (Fig. 8a-b). Because it would not reflect the original objective of the study, the tackle was not considered as true marine debris that fish would have scavenged, making this objective unattainable within the scope of this study. Though this objective could not be assessed in this study, Dantas *et al.* (2020) observed microplastic within all species collected with no trend regarding trophic guilds. While it was not in the same size categories of plastics of interest, the findings of Dantas *et al.* (2020) support the null hypothesis (1) for this objective. There is evidence that body size, rather than trophic guild, can explain almost 42% of variation in consumed plastic size by various animals, typically a ratio of 1:20 (size of plastic: body length) (Jâms *et al.* 2020).

While the lack of anthropogenic plastic within the sampled fish gut contents is encouraging, it does not indicate that fishes within this system are not consuming plastics. Gut contents reflect a moment in time for individuals, and previous studies report fish intentionally fed plastic experienced no blockage (Hoss & Settle 1990). It should be noted that plastics visible to the human eye (>1mm) were targeted in this analysis, these results do not represent majority of microplastics and smaller particles.

These higher trophic level fishes have an ability to evacuate smaller plastic items before or during capture via regurgitation or fecal matter. It is suggested that stomach contents may be altered as fish may regurgitate a portion or whole during ascent to the surface (Bromley 1994; Lusher *et al.* 2017). Swim bladder inflation with decreased pressure is associated with most regurgitation in these situations (Bowman 1986). With an average depth of 2m in the Matagorda Bay system (Moseley & Copeland 1973), swim bladder inflation is not a likely cause for lack of plastics within the digestive tracts. There are currently no peer reviewed studies on frequency of fecal evacuation upon capture, but this may explain the passing of contents, including plastic debris, during a physiologically stressful event. Additionally, all species considered in this study are primarily visual predators and as such, highly turbid areas of the bay may decrease plastic consumption as they would the consumption of natural prey (Breitburg 1988; De Robertis *et al.* 2003; Holmes 1977).

A study by Jantz *et al.* (2013) on plastic debris in longnose lancetfish (*Alepisaurus ferox*) captured via longline in the North Pacific Ocean successfully observed plastic debris in ~25% of individuals (N = 192). The fish were collected via longline, a potentially less stressful method of capture, as hooked fish do not go through as much physical effort as that of fish captured on rod and reel (Raby *et al.* 2014). The capture methods used by Jantz *et al.* (2013) may have created a more effective environment to avoid loss of stomach contents. Additionally, the debris observed in this study consisted of angular pieces (51.9%), rope (21.3%), or plastic bags (<8%), which can have either sharp edges or are lengthy enough to become entangled in the digestive tract. Lastly, Jantz *et al.* (2013) collected specimens from the middle of an open ocean as opposed to a semi-closed estuarine environment. More studies need to be conducted on the passage rates and potential of various sized plastic within fish.

Results from this study suggest that there is no relationship between prey type and mercury concentration within muscle or liver tissue (Fig. 9), however a more accurate assessment of this analysis should be conducted, as the methods in this study were not designed to prioritize this analysis (as it was adjusted once the original objective was not able to be assessed). The presence of seagrass was an unexpected observation in all stomachs of all three species sampled. While there is no correlation between THg and presence of seagrass in the digestive tract, consumption of seagrass and sediment has been observed in red drum within other systems (Pattillo 1997). If this behavior is common enough across individuals, it has the potential to be another source of mercury in the food web, as the sediment surrounding the plant holds greater mercury concentrations than ambient water (Boszke *et al.* 2003; ESTCP 2016). As a long-lived species

(~40 years) with a small habitat range and wide diet, red drum are subject to high contaminant concentrations. The chronic exposure to contaminated sediments and shorter trophic structure within Matagorda and its secondary bays may be driving higher THg concentrations in red drum. The stingray observed in the stomach of a single speckled trout took up the entirety of the stomach (Fig. 8c-d). Hettler (1989) suggests that speckled seatrout stomach contents reflect availability of prey types within certain estuaries, as they are opportunistic (Darnell 1958). The aforementioned instability of trophic levels in Matagorda Bay along with this abnormal prey item could support limited prey within the system, though more research would need to be conducted.

4.4. Objective 4: Hepatosomatic Index

In a study observing HSI values in response to heavy metal exposure, one of two species of fish (*Cyprinus carpio*) had a significant decrease in HSI (2.756 to 1.930) when exposed to industrial heavy metal (lead (Pb) and cadmium (Cd)) pollution (Dewi & Prabowo 2017). It was theorized that carp had a significant decrease in HSI while the tilapia (*Perca fluviatilis*) did not, as carp are more sensitive to effects in the surrounding water. Slooff *et al.* (1983) also suggests that higher HSI may be a sign of poor liver health as well, for the swelling or hypertrophy of a liver reflects some external stress on the organ. Slooff *et al.* (1983) monitored liver weights of bream (*Abramis brama*) from polluted waters in the Netherlands. The highest HSI (3.5) was observed in the most polluted areas of the river, while the less polluted areas yielded values closer to 1.5, suggesting potential use of this index as an indicator for water pollutants. HSI values in this study were relatively low compared to that of other studies, suggesting that effects from pollution in the bay system and reduced prey availability are significant enough to affect the physiology of the fish. Flounder from Matagorda Bay exhibit the best overall health via highest relative HSI and lowest mercury concentrations of the fish observed in this study (Fig. 10). These results support the negative effects of heavy metals on fish health.

The hepatosomatic index varies with many factors. Increased HSI with the onset of sexual maturity and a later decrease once the onset of sexual hormones takes place has been observed in various species of fish (Ribeiro *et al.* 2007; Santos *et al.* 2004). Variation by sex could not be explored in this study due to a low sample size of each sex (Table 3), but other studies have observed varied patterns in male and females (Rizzo & Bazzoli 2020). Females express greater variations throughout their hormonal cycles, with lower HSI during gonadal formation, while males stay relatively stable on the same times scale (Ribeiro *et al.* 2007). Rizzo & Bazzoli (2020) also highlight that cyclical variations of the HSI can be dampened by liver stress via detoxification. A seasonal overview of fish HSI in this bay system could gain insight into the effects of mercury in the bay. If data reveals that HSI cycles are reduced, it may support that mercury or other contaminants are impacting physiological cycles within the fish. While it is not the most concrete factor for measuring fish health conditions, it is valuable in multi-variable (e.g., gonadosomatic index (GSI), condition factor (K), and stomach repletion index (SRI)) assessments of fish health (Rizzo & Bazzoli; Nikolsky & Birkett 1963).

4.5. Conclusions

Overall, it is important to continuously monitor contaminant levels in highly polluted areas. Southern flounder had the lowest mercury concentrations as well as best relative health observed in our study. While speckled trout and red drum had the highest mercury averages overall, red drum had the highest individual values and was the only species with individuals within the avoid consumption category. Hepatosomatic indices of each species reflect health impacts from higher mercury concentrations. The majority (99%) of the fish were safe to eat according to FDA guidelines, though the realities of consumption rates (larger serving sizes and frequencies) call for reconsideration. The data collected from our study supports additional adjustment to these guidelines via implementation of maximum detection as opposed to averaged batch testing when developing consumption advisories. In addition to adjusted advisory protocols, future studies should incorporate selenium measurements, as its antagonistic nature against the health impacts from mercury are pivotal in guiding safe consumption. Certain Se:Hg levels may even allow for consumption of fish with higher mercury without adverse health effects. This study was unable to determine any relationship between mercury on consumed plastics and in fish tissues, but further steps should be taken to improve monitoring abilities as the consumption of plastic is a prominent issue for aquatic life. Plastic's ability to sorb to contaminants such as mercury, creates more exposure pathways in the environment. This relationship is necessary when assessing the overall impacts of plastic on biota as plastic discharge in the environment continues to grow.

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APPENDIX A: RED DRUM SUPPLEMENTARY DATA

Fish ID	Species	Total Length (mm)	Muscle THg (μg g^{-1})	Liver THg (μg g^{-1})
RD01	red drum	251	0.18	0.11
RD02	red drum	419	0.17	0.12
RD04	red drum	500	0.05	0.06
RD05	red drum	502	0.13	0.24
RD06	red drum	513	0.09	0.13
RD07	red drum	515	0.09	0.29
RD08	red drum	515	0.09	0.12
RD09	red drum	522	0.11	0.23
RD10	red drum	528	0.07	0.11
RD11	red drum	548	0.09	0.18
RD12	red drum	549	0.10	0.11
RD13	red drum	551	0.07	0.12
RD14	red drum	557	0.03	0.18
RD15	red drum	560	0.18	0.43
RD16	red drum	561	0.09	0.13
RD17	red drum	570	0.12	0.41
RD18	red drum	571	0.12	0.42
RD19	red drum	576	0.13	0.17
RD20	red drum	577	0.20	0.77
RD21	red drum	585	0.16	0.21
RD22	red drum	585	0.13	0.34
RD23	red drum	592	0.12	0.18
RD24	red drum	598	0.12	0.15
RD25	red drum	610	0.13	0.06
RD26	red drum	617	0.12	0.13
RD27	red drum	628	0.16	0.41
RD28	red drum	628	0.22	0.34
RD29	red drum	643	0.07	0.16
RD30	red drum	668	0.09	0.18
RD31	red drum	669	0.17	0.21
RD32	red drum	672	0.06	0.06
RD33	red drum	680	0.21	0.30
RD34	red drum	680	0.10	0.06
RD35	red drum	688	0.15	0.19
RD36	red drum	692	0.09	0.16
RD37	red drum	708	0.12	0.14
RD38	red drum	710	0.11	0.42

RD39	red drum	711	0.08	0.08
RD40	red drum	720	0.07	0.10
RD41	red drum	744	0.18	0.15
RD43	red drum	766	0.10	0.06
RD44	red drum	771	0.13	0.16
RD45	red drum	801	0.15	0.30
RD46	red drum	828	0.19	0.19
RD47	red drum	935	0.74	-
RD48	red drum	954	0.56	-

APPENDIX B: SPECKLED TROUT SUPPLEMENTARY DATA

Fish ID	Species	Total Length (mm)	Muscle THg (μg g^{-1})	Liver THg (μg g^{-1})
ST01	speckled trout	362	0.15	0.16
ST02	speckled trout	377	0.09	0.11
ST03	speckled trout	384	0.14	0.07
ST04	speckled trout	392	0.09	0.08
ST05	speckled trout	394	0.13	0.09
ST06	speckled trout	399	0.12	0.15
ST07	speckled trout	400	0.12	0.12
ST08	speckled trout	401	0.18	0.09
ST09	speckled trout	402	0.08	0.05
ST10	speckled trout	405	0.08	0.04
ST11	speckled trout	407	0.10	0.12
ST12	speckled trout	412	0.14	0.10
ST13	speckled trout	412	0.09	0.09
ST14	speckled trout	415	0.13	-
ST15	speckled trout	415	0.09	0.12
ST16	speckled trout	416	0.10	0.09
ST17	speckled trout	420	0.11	0.08
ST18	speckled trout	421	0.08	0.15
ST19	speckled trout	421	0.26	0.40
ST20	speckled trout	423	0.31	0.15
ST21	speckled trout	424	0.14	0.16
ST22	speckled trout	425	0.19	0.11
ST23	speckled trout	426	0.18	0.11
ST24	speckled trout	427	0.11	0.06
ST25	speckled trout	428	0.07	0.11
ST26	speckled trout	428	0.16	0.11
ST27	speckled trout	429	0.13	0.12
ST28	speckled trout	432	0.11	0.13
ST29	speckled trout	432	0.13	0.08
ST30	speckled trout	435	0.10	0.13
ST31	speckled trout	438	0.37	0.87
ST32	speckled trout	438	0.10	0.09
ST33	speckled trout	438	0.08	0.11
ST34	speckled trout	439	0.21	0.20
ST35	speckled trout	440	0.16	0.15
ST36	speckled trout	443	0.04	0.13
ST37	speckled trout	444	0.10	0.07

ST38	speckled trout	444	0.19	0.14
ST39	speckled trout	445	0.09	0.09
ST40	speckled trout	446	0.12	-
ST41	speckled trout	448	0.22	0.22
ST42	speckled trout	450	0.37	0.40
ST43	speckled trout	454	0.12	0.14
ST44	speckled trout	454	0.18	0.17
ST45	speckled trout	458	0.10	0.15
ST46	speckled trout	458	0.12	0.07
ST47	speckled trout	460	0.14	0.08
ST48	speckled trout	460	0.16	0.09
ST49	speckled trout	462	0.19	0.16
ST50	speckled trout	462	0.20	0.15
ST51	speckled trout	464	0.15	0.19
ST52	speckled trout	465	0.13	0.07
ST53	speckled trout	465	0.14	0.14
ST54	speckled trout	465	0.13	0.12
ST55	speckled trout	469	0.37	0.28
ST56	speckled trout	470	0.15	0.13
ST57	speckled trout	470	0.09	0.24
ST58	speckled trout	471	0.19	0.30
ST59	speckled trout	471	0.18	0.12
ST60	speckled trout	472	0.17	0.14
ST61	speckled trout	474	0.13	0.08
ST62	speckled trout	477	0.14	0.10
ST63	speckled trout	478	0.13	0.08
ST64	speckled trout	479	0.08	0.06
ST65	speckled trout	480	0.13	0.12
ST66	speckled trout	480	0.09	0.07
ST67	speckled trout	491	0.14	0.11
ST68	speckled trout	491	0.16	0.13
ST69	speckled trout	496	0.17	0.15
ST70	speckled trout	501	0.14	0.09
ST71	speckled trout	508	0.03	0.08
ST72	speckled trout	508	0.39	0.38
ST73	speckled trout	515	0.11	0.10
ST74	speckled trout	520	0.11	0.05
ST75	speckled trout	522	0.20	0.13
ST76	speckled trout	523	0.32	0.14
ST77	speckled trout	530	0.15	0.12
ST78	speckled trout	532	0.22	0.22

ST79	speckled trout	536	0.29	0.36
ST80	speckled trout	555	0.17	0.17
ST81	speckled trout	584	0.32	0.36
ST82	speckled trout	584	0.36	0.31
ST83	speckled trout	600	0.12	0.07
ST84	speckled trout	457.2	0.46	0.29
ST85	speckled trout	482.6	0.43	0.65

APPENDIX C: SOUTHERN FLOUNDER SUPPLEMENTARY DATA

Fish ID	Species	Total Length (mm)	Muscle THg (μg g^{-1})	Liver THg (μg g^{-1})
FL01	flounder	345	0.07	0.04
FL02	flounder	364	0.07	0.03
FL03	flounder	366	0.09	0.07
FL04	flounder	369	0.07	0.04
FL05	flounder	371	0.10	0.06
FL06	flounder	373	0.06	0.03
FL07	flounder	374	0.06	0.03
FL08	flounder	376	0.06	0.05
FL09	flounder	380	0.06	0.05
FL10	flounder	381	0.07	0.03
FL11	flounder	382	0.07	0.08
FL12	flounder	389	0.06	0.04
FL13	flounder	390	0.06	0.04
FL14	flounder	390	0.08	0.04
FL15	flounder	390	0.06	0.03
FL16	flounder	390	0.07	0.04
FL17	flounder	390	0.07	0.03
FL18	flounder	391	0.07	0.03
FL19	flounder	391	0.06	0.04
FL20	flounder	392	0.09	0.04
FL21	flounder	392	0.06	0.03
FL22	flounder	393	0.07	0.03
FL23	flounder	395	0.13	0.08
FL24	flounder	400	0.06	0.05
FL25	flounder	400	0.05	0.04
FL26	flounder	400	0.07	0.04
FL27	flounder	404	0.06	0.03
FL28	flounder	405	0.08	0.03
FL29	flounder	410	0.04	0.03
FL30	flounder	411	0.08	0.04
FL31	flounder	411	0.06	0.03
FL32	flounder	412	0.07	0.04
FL33	flounder	417	0.08	0.04
FL34	flounder	418	0.09	-
FL35	flounder	420	0.07	0.03
FL36	flounder	425	0.09	0.04
FL37	flounder	425	0.08	0.04
FL38	flounder	429	0.08	0.04
FL39	flounder	430	0.06	0.03
FL40	flounder	430	0.06	0.03

FL41	flounder	430	0.05	0.03
FL42	flounder	434	0.11	0.04
FL43	flounder	436	0.06	0.03
FL44	flounder	456	0.10	0.03
FL45	flounder	470	0.08	0.04
FL46	flounder	520	0.07	0.05