Summer 8-4-2022

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August 2022

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Abstract

Estuaries are transitional environments that play key roles in coastal ecosystem functioning by providing essential habitats to ecologically important plant and animal species. Estuaries are exposed to a number of anthropogenic impacts, including heavy metal contamination from human activities. However, few studies have examined heavy metal distribution within Georgia's estuaries and how intact salt marsh ecosystems could play a role in filtering and sequestering contaminants, such as Mercury (Hg). This thesis examined blood total mercury (THg) concentrations of four coastal shark species and concentrations of five heavy metals (Hg, As, Cr, Cu, and Pb) within the water and sediments of three Georgia estuaries with varying levels of anthropogenic impact (Wassaw Sound-high impact, Ossabaw Sound-medium impact, and Doboy Sound-low impact). Contrary to predictions, Bonnethead (*Sphyrna tiburo*) showed no differences in their blood THg concentrations among estuaries ($p = 0.115$). However, there were species-specific differences, with THg concentrations in Atlantic Sharpnose (*Rhizoprionodon terraenovae*) being significantly higher (85.66 ± 192.49 ppb) than Blacktip (*Carcharhinus limbatus*; 13 ± 18.02 ppb), Bonnethead (12.84 ± 34.42 ppb), and Sandbar (*Carcharhinus plumbeus*; 12.77 ± 15.44 ppb; $p = 0.003$). The concentrations of water and sediment heavy metals were similar among the three estuaries ($p \geq 0.074$), except for arsenic (As), where it was significantly higher in water samples from Doboy Sound with a mean concentration of 2669.17 ng/L (60.3% higher than Wassaw Sound and 75.1% higher than Ossabaw Sound) ($p < 0.001$). This data helps fill gaps in the literature regarding the effectiveness of THg as an indicator of local contamination, where other factors, such as diet,

length, and migratory behavior appear to play larger roles in the accumulation of THg within shark blood.

Introduction

Estuaries are transitional environments where rivers meet the sea, creating an ecosystem that encompasses terrestrial, aquatic, and marine characteristics (Kennish, 2002). This unique ecosystem is among the most productive environments found on earth (Constanza et al., 1997), providing vital ecosystem services to coastal communities, such as water filtration, storm protection, flood mitigation, and soil stabilization (Barbier et al., 2011). Sudden changes in water and sediment quality (e.g., salinity, temperature, dissolved oxygen, turbidity, contaminants), as well as physical stressors (e.g., habitat alteration and loss), can take a physiological toll on plant and animal species over time (Nichols et al., 1986; Kennish, 2002). The biodiversity of estuarine plant and animal species plays a major role in how these ecosystems function (Pinto et al., 2014). Due to anthropogenic stress, impacted areas receive high inputs of nutrients, sediment, and contaminants that can degrade the quality of water and sediment in these ecosystems (Dauer et al., 2000). As a result, stressed estuaries exhibit lower species diversity, less trophic complexity, altered food webs, altered community composition, and reduced habitat diversity (McLusky & Elliot, 2004; Tecchio et al., 2016). Maintaining balance among trophic positions is critical in preserving the structure and productivity of these ecosystems (Lobry et al., 2008).

Estuarine ecosystems are under increasing pressures from anthropogenic activities that can lead to detrimental effects in these essential habitats. Historically, estuaries have attracted

people worldwide due to the accessibility of food and travel (Roman et al., 2000; Birch et al., 2015). However, with human encroachment, stressors can compromise the ecological integrity of these coastal areas, including coastal development, habitat fragmentation, depletion of natural resources and introduction of pollutants (Freeman et al., 2019). Estuaries contribute to the global economy by providing food and jobs, as well as contributing to the tourism economy of local towns and cities (Barbier et al., 2011). In particular, both point and non-point source pollution has increased, which has created several contamination issues for these important transitional ecosystems (e.g., nutrient and carbon-loading, chemical pollution) (Kennish, 1997). In addition, physical impacts from human encroachment have caused habitat loss and alteration in many locations throughout the world, thus increasing the vulnerability of estuarine ecosystems which threatens their productivity (Kennish, 2002). These impacts will continue to cause environmental issues for estuaries worldwide; especially within the United States where the coastal population increased by 84.3% from 1960 to 2008 (USA Census).

Despite conservation efforts, the health and long-term viability of estuarine ecosystems are continuously threatened by numerous anthropogenic pressures (Vasconcelos et al., 2007). These issues can lead to habitat fragmentation that may alter and shift the distribution of certain plant and animal species within estuaries (Eggleston et al., 1999). Altered habitats within estuaries have been recognized to affect not only the health of marine organisms but also the health of the local human population (Kennish, 2002). Since estuaries are home to ecologically and economically important fish species, the health and productivity of these environments are a concern to fisheries (Lellis-Dibble et al., 2008), including coastal Georgia.

Therefore, understanding how humans negatively impact estuaries is critical in developing better strategies to protect these important ecosystems.

Located within the South Atlantic Bight, the Georgia coast is home to several estuary systems that remain relatively pristine and have not been heavily affected by anthropogenic impacts until recent decades (Verity, 2002). The coast of Georgia is home to the second largest salt marsh habitat within the United States and contributes to one-third of all salt marshes found on the East Coast (Salt Marsh Ecology, n.d.), partly due to protection efforts by local, state, and federal government entities, as well as nonprofit organizations. For example, in 1972, Congress passed the Coastal Zone Management Act that supports three national programs, the National Coastal Zone Management Program, the National Estuarine Research Reserve System (NERR), and the Coastal and Estuarine Land Conservation Program that aim to protect and conserve the United States' coastal ecosystems under the National Oceanic and Atmospheric Administration (NOAA, 1972). The coast of Georgia benefits from all three national programs, including being home to one of the thirty NERR sites, which is located at Sapelo Island along the State's central coast.

Contamination of estuarine environments has caused varying effects on animal species inhabiting these brackish waters (Chapman & Wang, 2001), depending on the pollutant(s) released locally from specific human activities or industries. Human activities such as the burning of fossil fuels, shipping traffic, improper disposal of industrial wastes, and runoff from local roadways have created numerous ways that excessive contaminants can enter local estuaries. One class of contaminants are heavy metals, which includes mercury (Hg), arsenic (As), chromium (Cr), copper (Cu), and lead (Pb). Of these, mercury is of great concern due to its

ability to bioaccumulate and biomagnify throughout marine food webs when mercury is in its methylated form (Kidd et al., 2012). Bioaccumulation is the process of a contaminant accumulating within an organism's tissues over time (Matulik et al., 2017), and biomagnification is the process of a contaminant increasing in concentration as it passes from one trophic position to another within an ecosystem through predator consumption of prey (Baeyens et al., 2003).

Mercury is most frequently found in its organic form, methylmercury (MeHg), within estuaries (Kannan et al., 1998). Methylmercury is created from the methylation of inorganic Hg by anerobic bacteria found within aquatic and marine environments (Li & Cai, 2012). Since MeHg is the most toxic and potent form of Hg, it has the ability to cause an array of physiological and neurological issues for aquatic organisms (Scheuhammer & Sandheinrich, 2008). Methylmercury can comprise a majority of total Hg found within fish tissues (> 70%) (Storelli et al., 2002), and because of this, total mercury can be used as a conservative proxy for MeHg levels as total Hg is easier to quantify than MeHg. Total mercury levels in food are, therefore, a concern as Hg is known to cause neurological issues, such as impairment of visual and motor functions, for people who consume large amounts of fish as part of their regular diet (United States Environmental Protection Agency; Fillion et al., 2011).

Typically, top predators accumulate the most Hg within ecosystems due to their large size and long lifespans (Teffer et al., 2014). When predators reside in contaminated environments, bioaccumulation can cause increases in Hg concentrations within body tissues due to their higher relative exposure to contaminated prey. Fish accumulate Hg through both their diet and through their gills via respiration (Korhonen et al., 1995), which makes fish more prone to Hg

accumulation than terrestrial animals. Due to these factors, aquatic predators can be indicators of local environmental Hg exposure and aid in understanding how contaminated ecosystems are functioning.

Degree of human encroachment varies along Georgia's coast and could result in estuary systems having differing mercury inputs due to increases in urban runoff, disposal of toxic wastes, and sewage outfalls, which would be expected to be reflected in the flora and fauna of these ecosystems. Animals may serve as biological proxies for quantifying the amount of Hg present within estuarine ecosystems. Due to their high trophic positioning, "top predators", such as coastal sharks, can be used as sentinels for Georgia estuaries. Sharks receive most of their heavy metal toxicity (e.g., MeHg) from their diet, which is expected to reflect contaminants accumulated locally in the prey species that reside in that specific locality. Methylmercury increases in magnitude at each trophic level which results in predators, like sharks, exhibiting the highest levels of contamination within marine ecosystems (Pinho et al., 2002; Matulik et al., 2017).

Additionally, as MeHg has an affinity to bind to proteins (Clarkson & Magos, 2006), it can be detected in both muscle and blood tissue (Merly et al., 2019). Collecting blood samples via a caudal venipuncture is less invasive than collecting muscle samples via a biopsy punch, and therefore one of the more humane methods of quantifying MeHg levels within shark tissue (Gelsleichter et al., 2020; Matulik et al., 2017). MeHg tends to accumulate in aquatic organisms and can persist for long periods of time as detoxification and removal from the body can be limited (Ren et al., 2019). Therefore, sharks can be used as sentinels of local contamination in Georgia estuaries. Understanding how MeHg is accumulating within these ecosystems is

important because many estuarine animal species (fish, crustaceans, etc.) are consumed by local people and Hg is a well-known neurotoxin for humans (United States Environmental Protection Agency).

There are several small- to medium-bodied shark species that are native to coastal Georgia waters that may serve as candidate models to understand human pollution impacts on Georgia's estuaries, including Bonnethead (*Sphyrna tiburo)*, Atlantic Sharpnose Shark (*Rhizoprionodon terraenovae*; herein "Sharpnose"), Sandbar Shark (*Carcharhinus plumbeus*; herein "Sandbar"), and Blacktip Shark (*Carcharhinus limbatus*; herein "Blacktip") (Dumont, 2011, Belcher and Jennings, 2010). However, because of this rich species diversity, ecological theory of niche differentiation dictates that each shark species may utilize estuarine habitats in differing ways to avoid competing for identical resources (Elston et al., 2020). For example, Bonnethead diet consists mainly of crustaceans with very few small teleost fishes (Kroetz et al., 2017), whereas Blacktip, Sandbar, and Sharpnose sharks primarily feed on teleost fishes with very few crustaceans (Hoffmayer & Parsons, 2003; McElroy et al., 2006; Delorenzo et al., 2015). Therefore, diet may dictate MeHg levels within sharks depending on their prey niche width while in estuaries. Additional metrics, such as length and sex, may also have an effect on MeHg levels within animals' tissues. This could be due to several factors, including the natural process of bioaccumulation that predicts that as a shark's length increases so does its MeHg levels. Therefore, considering the combination of these factors (species identity, length, and sex) inferences can be made on the degree of mercury exposure in estuaries with varying levels of presumed anthropogenic impact.

Quantifying MeHg, using total Hg (herein "THg") as a conservative proxy, within Georgia coastal shark species will be helpful in understanding if Georgia estuaries are experiencing elevated levels of Hg contamination and if so, are juvenile sharks at risk of THg exposure along the coast. The present study used coastal sharks as sentinels for THg contamination within Georgia estuaries, with the objectives of: (1) determine the effects of species, length, and sex on THg concentrations in blood and (2) determine if there is an estuary-specific difference on THg concentrations in blood samples. Examining THg concentrations will give insight into the differences of THg concentration among the three estuaries and if there are estuary-specific differences among the sharks inhabiting the three sampling locations.

Methods

Field Methodology

Field Sites

Samples were collected from three Georgia estuaries: Wassaw Sound, Ossabaw Sound, and Doboy Sound (Figure 1a). The sampling locations are located within the Ogeechee River (Wassaw Sound and Ossabaw Sound) and Altamaha River (Doboy Sound) watersheds in Georgia. Wassaw Sound experiences heavy boat traffic from shipping commerce and recreational activities due to its location directly south of the Port of Savannah and bordering Tybee Island. Ossabaw Sound is bordered by protected islands: Wassaw Island (National Wildlife Refuge) to the north and Ossabaw Island (Georgia Heritage Preserve) to the south. Lastly, Doboy Sound is located within a NERR that is under federal protection by NOAA. Both Ossabaw Sound and Doboy Sound experience less boat traffic due to their location within

protected areas, therefore resulting in reduced shipping and recreational use. Six sampling stations within each estuary system were selected that encompass two sound, two river, and two creek locations and all were sampled four times during June to August 2021 (Figure 1).

Sample Collection

Shark Sampling

Mature, juvenile, and young-of-year (YOYs) sharks were sampled in three Georgia estuaries using bottom longline surveys during the months of June, July, and August 2021 coinciding with peak shark occurrence in Georgia (Dumont 2011). The sampling areas included three estuary systems: Wassaw, Ossabaw, and Doboy. The longline consisted of a 333 m mainline made of 9.5 mm braided nylon rope with markers every 6 m for even placement of gangions, anchored at both ends with 4.1 kg Danforth (fluke) anchors and A-2 Polyform buoys as surface floats. Fifty gangions were deployed at each set. Each gangion was composed of one tuna clip attached to 0.5 m of 90.7 kg monofilament and terminating in one 12/0 Mustad circle hook (no offset) with the barb depressed and baited with squid (*Loligo* spp).

Longlines were deployed at each of the six sampling sites once per fishing day. Each longline set was soaked for a total of 30 minutes from the time when the first hook was deployed. Upon capture, sharks less than 1 m were brought onboard for sampling and sharks larger than 1 m remained in the water and were restrained using a tail rope. Morphometrics for each shark were taken, and included species, sex, life stage, mass, girth and three length measurements. Life stage (mature, juvenile, or young of year (YOY)) of each animal was determined by observing the presence of an umbilical scar for YOYs or absence for juvenile and mature

animals. Males were deemed mature if they had fully calcified claspers while length-at-maturity values from the literature were used to assign female maturity status post hoc. Lengths taken included precaudal length (PCL), fork length (FL), and total length (TL). A blood sample was collected from sharks ≥ 500 g using an appropriately sized sterile needle (20-gauge or 18-gauge dependent on shark's TL) and 3 mL syringe and placed in a lithium heparin tube to prevent clotting. Sampled blood volume did not exceed 1% of an animal's total body mass. Blood samples were kept cool on ice packs until processing at the end of the field day. At the lab, blood samples were centrifuged at 4,000 RPM for 5 mins to separate layers. Plasma and packed red blood cells were stored separately and frozen on dry ice while in the field prior to storage at -80℃ at Georgia Aquarium until analysis. In the rare instance of an animal failing to revive after procedures, the animal was sacrificed and placed on ice to be later necropsied for muscle and liver samples. All animal handling and tissue sampling followed a Georgia Aquarium Institutional Animal Care and Use Committee (IACUC) approved protocol (GAI #21-06).

Water and Sediment Collection

In June 2021, prior to shark fishing, paired water and sediment samples were collected at each of the 18 sites for trace and heavy metal analysis. Surface water was collected using 1-liter pre-treated (acid washed) Nalgene water bottles and sediment was collected using an Ekman grab sampler and placed into glass jars. All water and sediment samples were placed on ice and shipped overnight in a Styrofoam cooler containing ice packs to PHYSIS Environmental Laboratory in Anaheim, California, for heavy metal analysis.

Laboratory Analysis

Tissue Mercury Analysis

Total mercury (THg) levels were quantified from shark blood samples using a MA-3000 mercury analyzer (Nippon Instruments, Osaka, Japan), which employs thermal decomposition amalgamation and atomic absorption spectrophotometry to detect mercury. Mercury in blood samples were quantified using the US EPA Method 7473 (https://www.epa.gov/esam/epa-method-7473-sw-846-mercury-solids-and-solutions-thermal-decomposition-amalgamation-and). Prior to analysis, thawed packed red blood cells were quickly vortexed and 10 µL was weighed into individual ceramic boats. All samples were run in duplicate and blanks were run every 10th sample to ensure no mercury carry over from previous samples. A certified reference material (Lake Michigan Trout tissue 1947, National Institute of Standards and Technology) was run every 10 samples to ensure accuracy as part of quality assurance/quality control protocols. Mercury was quantified from the linear portion of the standard curve calibration curve (0.1 ng to 1000 ng Hg, $r^2 = 0.9999$) and the mean obtained from duplicates. Samples were reanalyzed if the coefficient of variation between duplicates was greater than 10%.

Lysozyme Assay

Lysozyme activity was quantified in non-hemolyzed plasma samples using a fluorescence based EnzChek® Lysozyme Assay (Thermo Fisher Scientific, Waltham, MA, Cat. E-22013) following kit protocols by the manufacturer. Kit optimization was performed by analyzing a set of two samples from every species at various dilutions to determine what dilution was appropriate to

run remaining samples. All samples were diluted (1:5) in a reaction buffer provided by the manufacturer into a 96 well plate in triplicate, after the buffer was added samples were protected from light using tin foil and incubated at 37ºC for 30 minutes. Using a microplate fluorescence reader, the fluorescence intensity (~518 nm) of each sample was measured.

Water and Sediment Heavy Metal Analysis

Water and sediment samples were analyzed following US EPA methods EPA 6020 for trace metals, EPA 245.7 for trace mercury, EPA 1640 for total trace metals, and EPA 1631E for total trace mercury at the PHYSIS Environmental Laboratory.

Statistical Analysis

All data was analyzed in IBM SPSS Statistics (Version 28.0.1). All data in this study was tested for normality and homoscedasticity and was log transformed if failed to meet assumptions. For water and sediment samples, one-way ANOVAs were performed to look for differences in heavy metal concentrations among the three estuaries. Additional one-way ANOVAs were performed to look for differences in heavy metal concentration among site type (creek, river, sound) in water and sediment samples. Pearson's Correlations were used to look for correlations between water and sediment samples, as well as correlations for water and sediment samples correlated with distance from the mouth of the sound for each estuary.

Due to uneven sampling among species, both individually and within each individual estuary, sample sizes for all species were not equal and two species (Bull Shark (*Carcharhinus leucas)* and Finetooth Shark (*Carcharhinus isodon*), both n =1) were excluded in statistical analysis. Additionally, one Sharpnose THg sample was not included due to being an extreme outlier in

the data set (Table 4). ANOVAs or Kruskal-Wallis, followed by appropriate post-hoc tests, were performed to determine if there was a length bias among the three estuaries. Student's t-tests were performed to test for differences in sex and length in both juvenile and mature animals, where appropriate.

Overall differences in THg concentrations among species were determined by a Kruskal-Wallis test, which included both juvenile and mature animals. Additionally, differences in juveniles among the species were compared using one-way ANOVAs. The effect of length on THg concentration was evaluated using linear regression, using log-transformed data when appropriate. When length was significant, within-species ANCOVAs were used to test for the effect of estuary or sex on THg concentrations. When length was not a significant factor, the effect of estuary or sex on THg concentrations was evaluated using Student's t-test within species.

Linear regressions were performed for all species to determine if lysozyme activity was significantly related to length. If significant, length was a covariate in ANCOVAs to evaluate the relationship between lysozyme activity and THg within species.

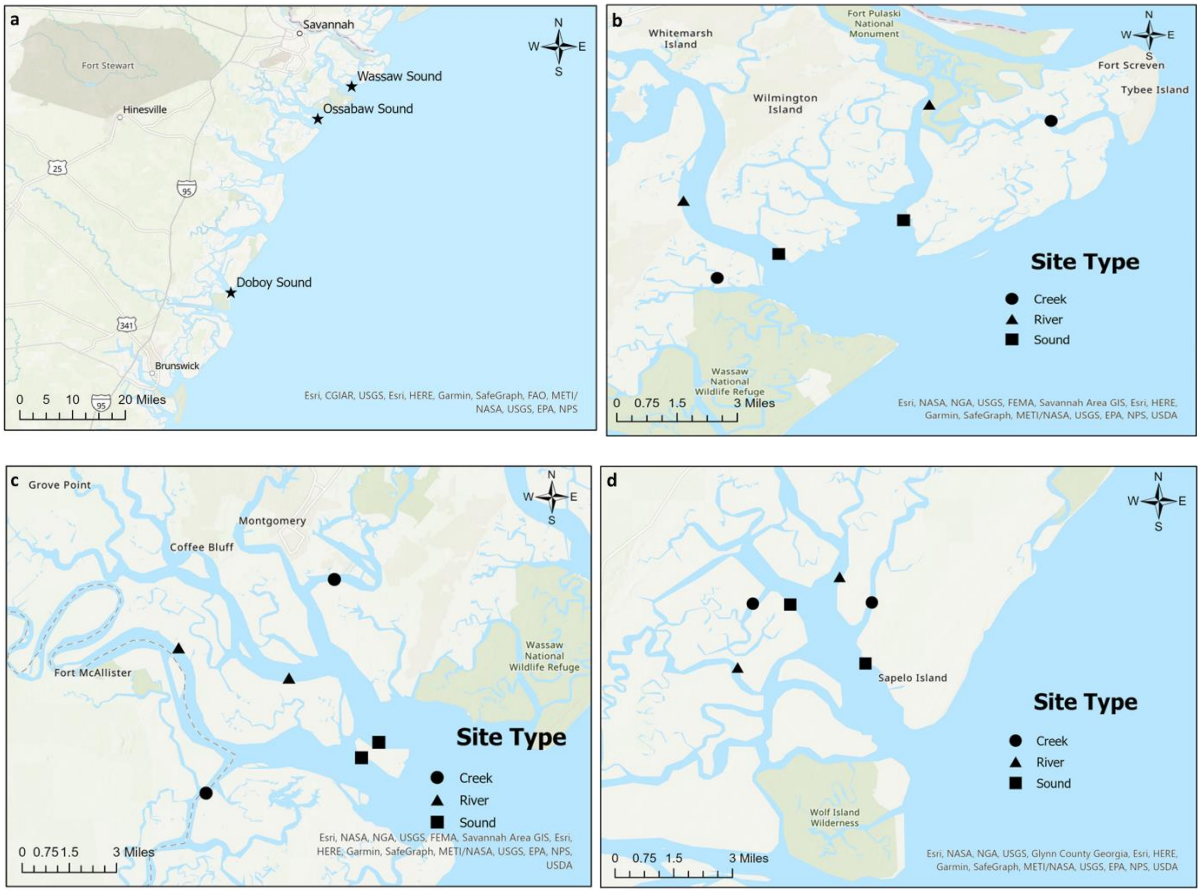

Figure 1. Maps displaying overall study area (a) and each individual estuary: (b) Wassaw Sound, (c) Ossabaw Sound, and (d) Doboy Sound. Estuary maps display distribution of site types: creek = circle, river = triangle, and sound = square.

Results

Environmental Contaminants

Heavy Metal Concentrations in Water

Concentrations of heavy metals from water samples collected from Wassaw Sound (n = 6), Ossabaw Sound (n = 6), Doboy Sound (n = 6) are summarized in Table 1. Across sites, As and Cr consistently had the highest concentration (Mean ± SD: 1465 ± 990 ng/L and 1000 ± 434 ng/L, respectively) and Hg the lowest (2.26 ± 0.8 ng/L), with Cu and Pb an order of magnitude lower than As and Cr (582 ± 130 ng/L and 288 ± 166 ng/L, respectively; Table 1). For all heavy metals examined, except As ($p < 0.001$), estuary system had either a marginal effect (Pb: $p = 0.074$) or was insignificant (Cr: $p = 0.28$, Cu: $p = 0.38$, and Hg: $p = 0.43$). For As, Doboy Sound had the highest mean ($\pm$ SD) concentration (2,669 $\pm$ 655 ng/L), followed by Wassaw Sound (1,062 $\pm$ 379 ng/L) and Ossabaw Sound (665 $\pm$ 237 ng/L). In general, Doboy Sound had elevated mean concentrations for most metals (except Cu, where it was elevated in Wassaw Sound), while Ossabaw usually had lower mean concentrations, except for Hg, where Hg fell mid-range among the estuaries (Figure 2). While not significantly different among estuary systems, Hg concentration was more variable within Doboy Sound, followed by Wassaw Sound and Ossabaw Sound (Figure 3). All heavy metals concentrations examined were much lower than the Environmental Protection Agency's National Recommended limits for aquatic life (EPA).

Heavy Metal Concentrations in Sediment

Concentrations of heavy metals from sediment samples collected from Wassaw Sound (n = 6), Ossabaw Sound (n = 6), Doboy Sound (n = 6) are summarized in Table 2. For all sediment heavy

metals examined there was no significant difference found among estuary systems ($p \geq 0.56$) and there was high within-estuary variation. Generally, mean concentrations of Cr were elevated compared to other heavy metals (Mean ± SD: 29.0 ± 21.0 µg/dry g), followed by Pb (14.94 ± 33.53 µg/dry g) with Hg being the lowest (22.43 ± 16.05 ng/dry g) (Table 2). This is in contrast to the paired water samples, where As had the highest concentrations (see above). Overall, the three estuaries had comparable mean sediment heavy metal concentrations, except for Pb where Wassaw Sound had over four times the amount of Pb found in either Ossabaw Sound or Doboy Sound (Table 2). One sample taken at Wassaw Sound was an outlier (148 µg/dry g) and was removed from data analysis (Table 2).

Across all sites, Pb was the only contaminant with correlated concentrations in water and sediment ($r(15) = -0.482$, $p = 0.05$). Additionally, water and sediment heavy metal concentrations were not related to distance from the mouth of the sound (all estuaries combined; $p \geq 0.095$). Lastly, for all water and sediment heavy metal samples, there was no significant difference found between site type (Creek, River, Sound) (One-way ANOVA; $p \geq 0.148$).

Tissue Hg Concentrations

Sample Distribution

A total of 125 sharks, comprised of juvenile (n = 67) and mature (n = 58) animals, were sampled from Wassaw Sound (n = 46), Ossabaw Sound (n = 40), and Doboy Sound (n = 39) between June and August 2022 (Table 3). Six species of sharks were captured, with Bonnethead being the

most numerous (n = 75), followed by Blacktip (n = 21), Sandbar (n = 16), Sharpnose (n = 11), Bull Shark (n = 1), and Finetooth Shark (n = 1). All age classes from juveniles to adults were represented in Bonnethead (for both males and females) and Sharpnose, although only males were represented in the adult category for the latter species. For Blacktip, Sandbar, Finetooth, and Bull Sharks, only juveniles were captured.

Animal length varied across estuary and among species. Mean Bonnethead length was significantly influenced by estuary (ANOVA, $p < 0.001$). Bonnethead captured in Ossabaw Sound (mean ± SD, 92.4 ± 12.81 cm) were marginally longer than Wassaw Sound (83.2 ± 15.46 cm; $p = 0.044$) and significantly longer than Doboy Sound (75.6 ± 8.23 cm; $p < 0.001$), with the latter two not being different from each other ($p = 0.109$). Mature female Bonnethead were significantly longer than mature male Bonnethead (T-test: $p < 0.001$; Figure 6). There was no difference in length between male and female juvenile Bonnethead (T-test: $p = 0.201$). Captures of Sharpnose were biased towards Wassaw Sound (n = 8), where animals ranged in size from 35 to 94.2 cm total length. One adult was captured in Doboy (93.3 cm, male) and two adults in Ossabaw Sound (63.5 cm and 94.5 cm, both males). Blacktip (all juvenile) were similar in size in Wassaw Sound (66.8 ± 7.57 cm) and Ossabaw Sound (68 ± 11.59 cm) (T Test, $p = 0.269$). Doboy Sound only had three samples, two juvenile females at 64 cm each and one juvenile male at 84 cm and were not included in further analysis. Additionally, no differences in length were found for Sandbar (all juvenile) between Doboy Sound (73 ± 16.17 cm) and Ossabaw Sound (70.6 ± 19.28 cm) (Mann-Whitney, $p = 0.859$). Wassaw Sound had one juvenile Sandbar capture with a length of 86 cm and was not included in further analyses. One juvenile

Finetooth Shark (60.3 cm) was sampled in Wassaw Sound and one juvenile Bull Shark (152.2 cm) in Doboy Sound.

Blood THg Concentrations

Blood THg levels for all species combined ranged from 1.08 to 663.89 part per billion (ppb) wet weight (median = 13.87 ppb) (Figure 7). Overall, Sharpnose had the highest THg concentrations (median, range: 62.14 ppb, 16.9 - 266.42 ppb), with Sandbar (12.77 ppb, 2.22 - 52.14 ppb), Blacktip (13 ppb, 1.08 – 59.32 ppb) and Bonnethead (12.84 ppb, 1.58 - 191.72 ppb) all being similar to each other (Kruskal-Wallis, $p = 0.003$). One mature male Sharpnose from Ossabaw Sound was not included in the analysis due to its elevated concentration (663.89 ppb; Table 4).

Considering that presence of adults may bias overall species comparisons, only juveniles were subsequently examined. Even when excluding mature animals, Sharpnose remained the species with the highest median concentrations (median, range: 21.66 ppb, 18.17 - 38.61 ppb); however, because there were only four individuals, they could not be statistically tested against the other species. Among the other species, no differences were found in blood THg concentrations (Bonnethead: 9.53 ppb, 1.58 - 39.44 ppb; Sandbar: 12.77 ppb, 2.22 – 52.14 ppb; Blacktip: 13 ppb, 1.08 – 59.32 ppb; Kruskal-Wallis, $p = 0.110$). The juvenile Bull shark had a concentration similar to the median value of an adult Sharpnose (200.86 ppb vs 210.36 ppb, respectively), while the juvenile Finetooth was most comparable to a juvenile Sandbar with the lowest THg concentration (2.28 ppb vs. 2.22 ppb).

Generally, blood THg concentrations appeared to be unaffected by estuary location (Figure 8); however, only in Bonnethead could all three estuaries be statistically compared together

because of uneven sampling for the other species. With length as a covariate, no effect of estuary was found to influence blood THg in Bonnethead (ANCOVA, $p = 0.110$; Figure 9a). Blood THg concentration significantly increased with length in both Bonnethead and Sharpnose data sets (Linear Regression, $p \leq 0.001$, all estuaries combined). However, the effect of estuary for Sharpnose could not be evaluated due to low sample sizes at Ossabaw Sound ($n = 2$) and Doboy Sound ($n = 1$). Nevertheless, THg was similar among the three sites (Table 4).

For Blacktip and Sandbar no relationship was found between length and THg ($p \geq 0.063$), and thus length was not included in the evaluation of estuary effect. Among Blacktip, THg was not different between Wassaw Sound and Ossabaw Sound (T-Test: $p = 0.889$). Doboy Sound was excluded as only 3 individuals were sampled. For Sandbar, THg was not different between Ossabaw Sound and Doboy Sound (T-Test: $p = 0.074$). Wassaw Sound was excluded due to having one sample.

In addition, sex was not a significant factor for any species when including length as a covariate (all $p \geq 0.58$). Sharpnose was not included in the ANCOVA analysis due to only one juvenile female having been sampled. Additionally, blood THg concentration in sharks was unaffected by site type (Creek, River, Sound), regardless of when species were examined separately (ANOVA, $p \geq 0.47$ for each species) or together (Kruskal-Wallis, $p = 0.952$).

Lysozyme Assay

Linear regressions were used to test if length had an effect on lysozyme activity in non-hemolyzed plasma of blood samples in the four species with largest sample sizes (Bonnethead: n = 35, Blacktip: n = 11, Sandbar: n = 7, and Sharpnose: n = 8) (Figure 10). Only in Bonnethead was there a positive relationship between lysozyme activity and length ($p = 0.002$; Figure 10b) and was therefore included as a covariate in subsequent analysis; all other species did not display a significant effect between length and lysozyme activity ($p \geq 0.168$).

No significant relationship found between lysozyme activity and blood THg concentrations for any species (Linear Regression, $p \geq 0.114$; Figure 11). Note that the outlier Sharpnose with the highest THg was not included in analyses. In addition, lysozyme activity was similar among Bonnethead (mean = 82.89 U/mL), Sharpnose (99.99 U/mL), Sandbar (108.01 U/mL), and Blacktip (152.22 U/mL) (ANOVA, $p = 0.561$; Figure 12).

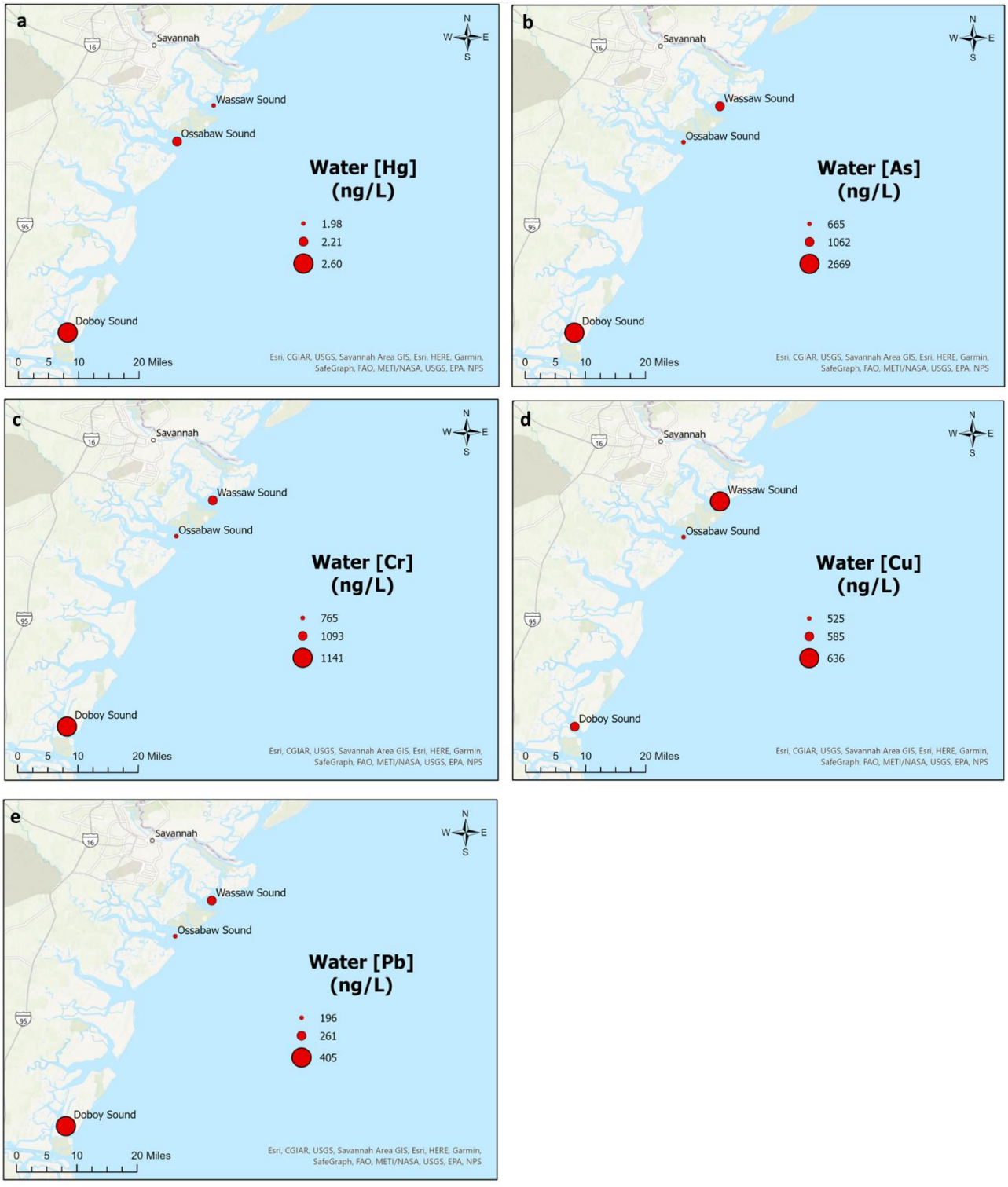

Figure 2. Graduated symbol maps of mean water heavy metal concentrations between estuaries (a) Mercury (Hg), (b) Arsenic (As), (c) Chromium (Cr), (d) Copper (Cu), and (e) Lead (Pb). Note that only As concentrations were significantly different among sites.

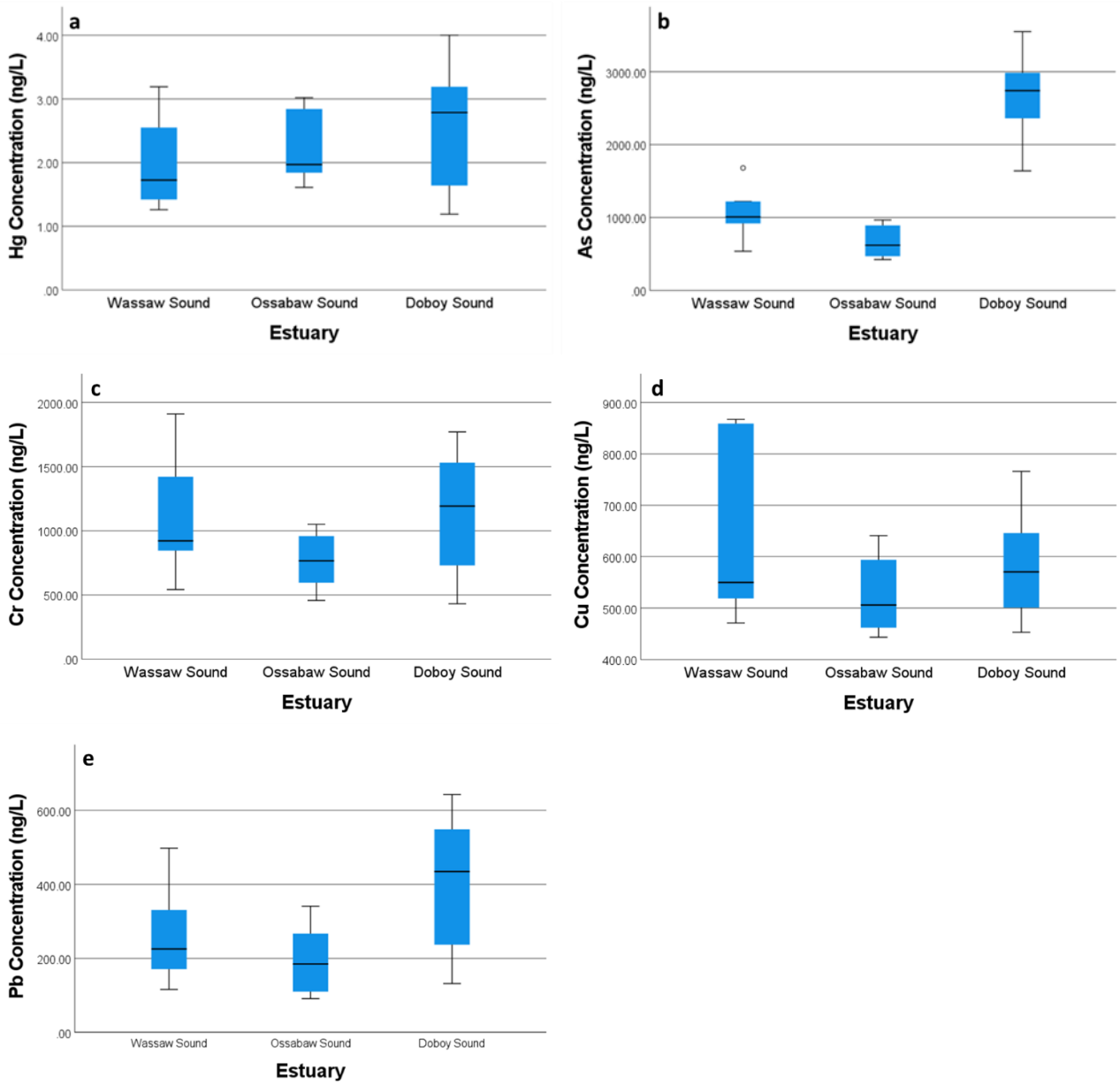

Figure 3. Boxplots of water heavy metal concentrations at each of the three estuary systems (n = 6 sites per estuary): (a) Mercury (Hg), (b) Arsenic (As), (c) Chromium (Cr), (d) Copper (Cu), and (e) Lead (Pb). Lower and upper box boundaries represent the 25th and 75th percentiles, respectively, the line within each box is the median, lower and upper error bars represent 10th and 90th percentiles, respectively. Arsenic was significantly higher in Doboy Sound.

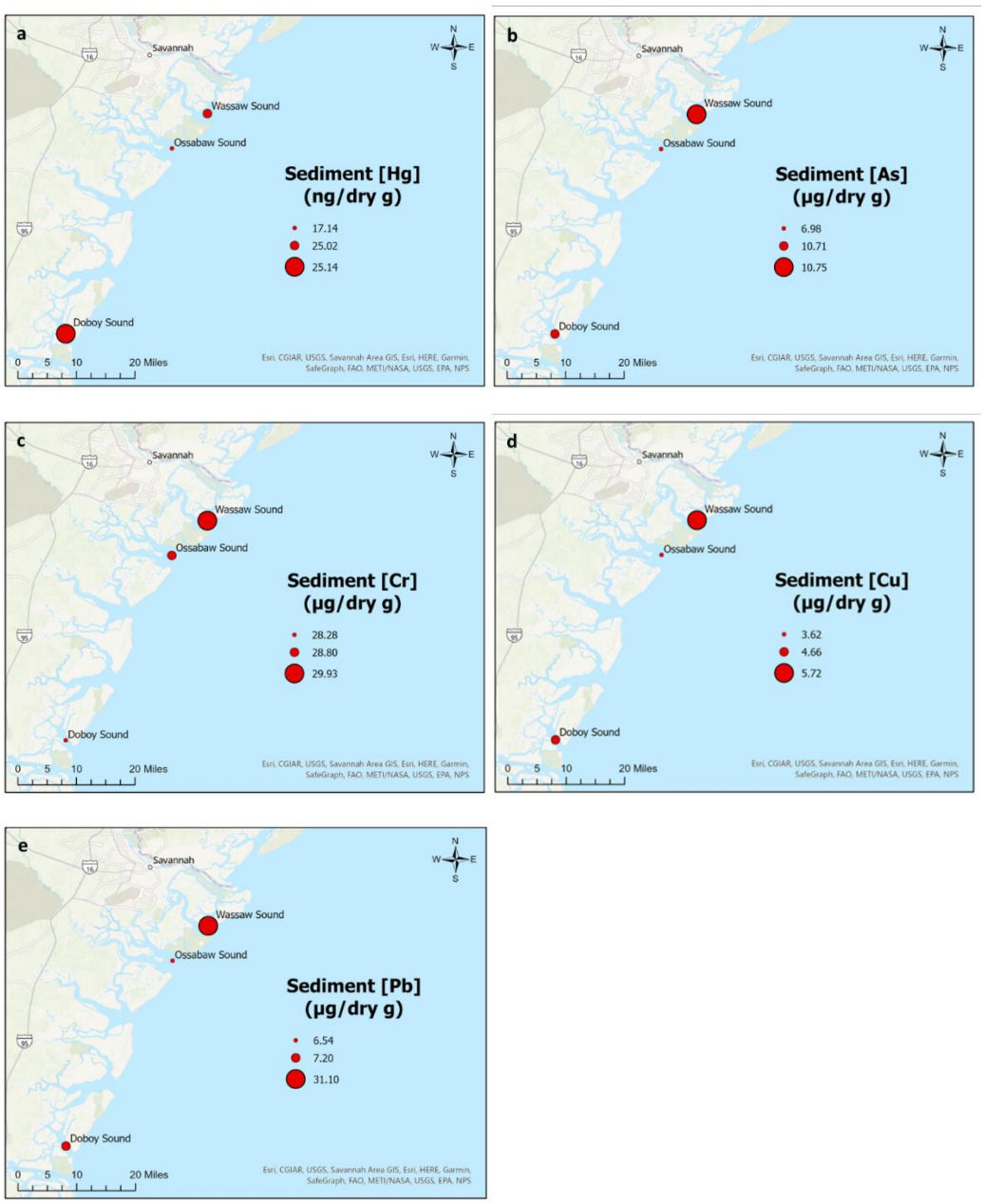

Figure 4. Graduated symbol maps comparing mean sediment heavy metal concentrations between estuaries: (a) Mercury (Hg), (b) Arsenic (As), (c) Chromium (Cr), (d) Copper (Cu), and (e) Lead (Pb). Note Hg is given in ng/g dry weight due to low concentration. No statistical difference was found among estuaries.

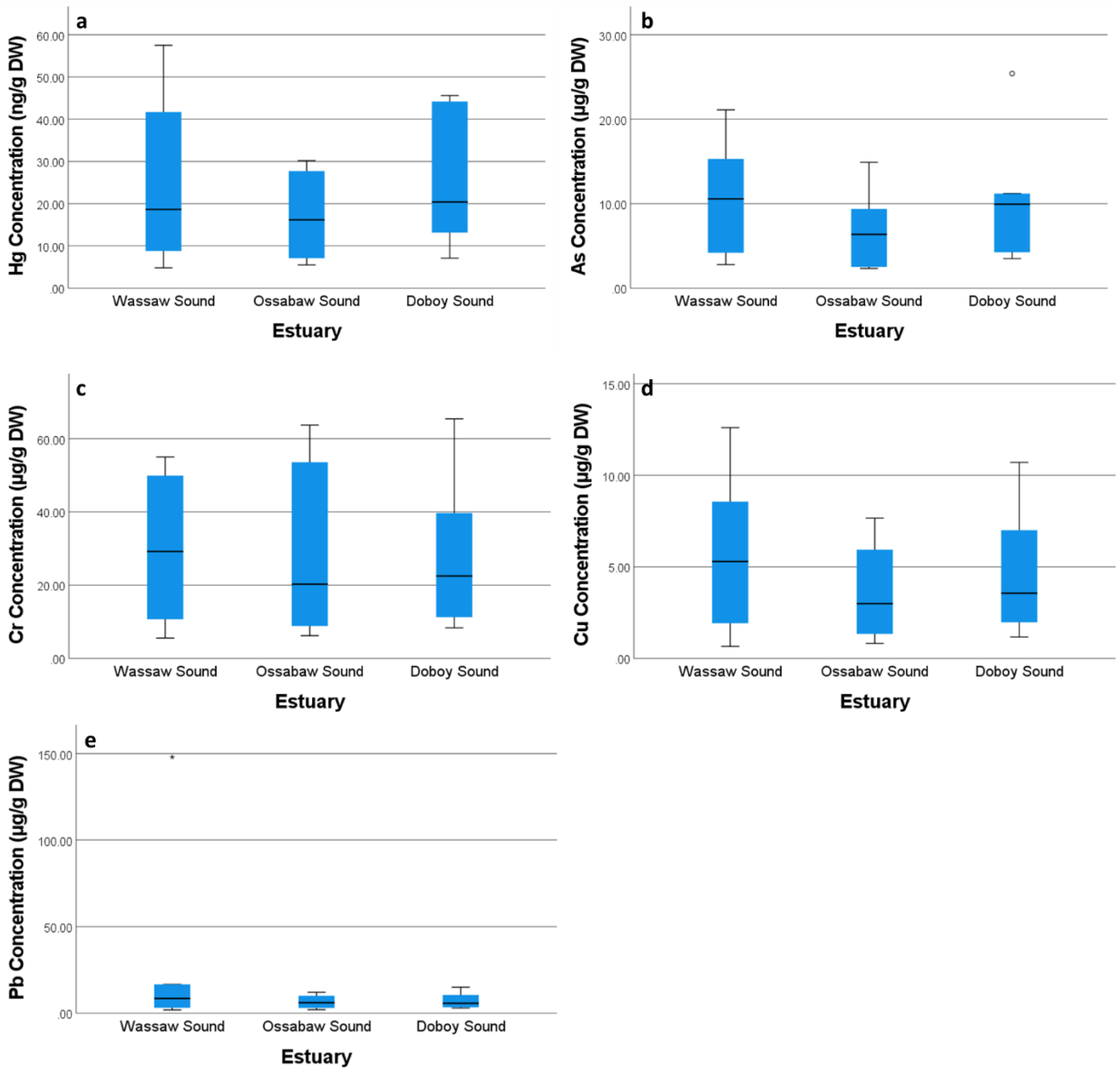

Figure 5. Boxplot of sediment heavy metal concentrations at each of three estuary systems (n = 6 per estuary): (a) Mercury (Hg), (b) Arsenic (As), (c) Chromium (Cr), (d) Copper (Cu), and (e) Lead (Pb). Lower and upper box boundaries represent the 25th and 75th percentiles, respectively, the line within each box is the median, lower and upper error bars represent 10th and 90th percentiles, respectively. No statistical difference was found among estuaries.

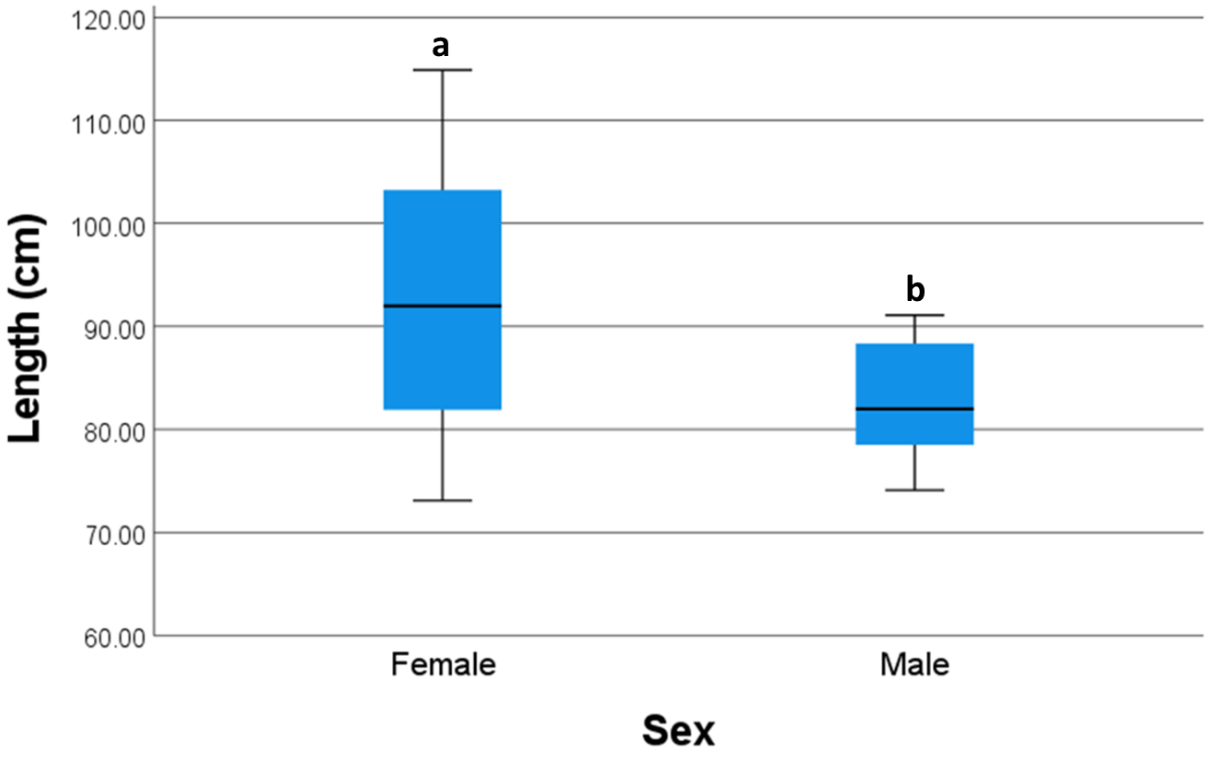

Figure 6. Boxplot displaying differences in length by sex for mature Bonnetheads. Lower and upper box boundaries represent the 25th and 75th percentiles, respectively, the line within each box is the median, lower and upper error bars represent 10th and 90th percentiles, respectively. Different letters indicate significant differences between groups.

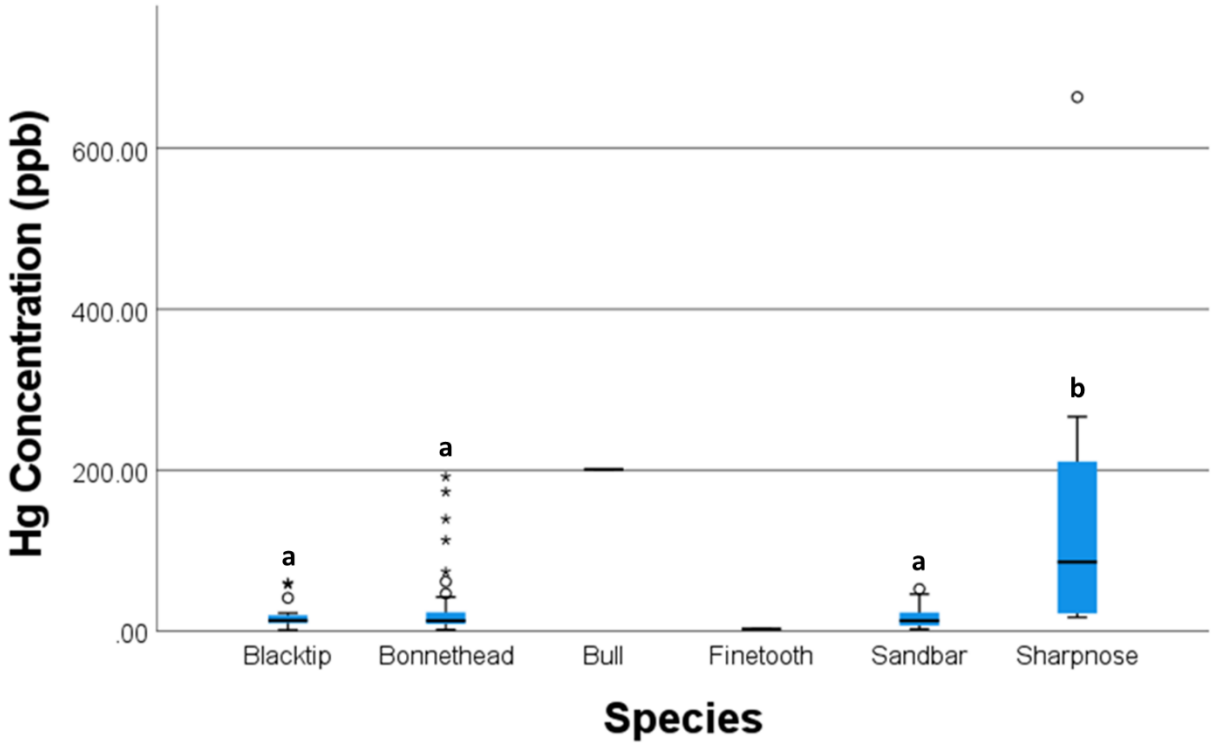

Figure 7. Boxplot of blood [THg] across all species; Bull and Finetooth Sharks are included for graphical purposes only and were not included in analyses. Lower and upper box boundaries represent the 25th and 75th percentiles, respectively, the line within each box is the median, lower and upper error bars represent 10th and 90th percentiles, respectively, open circles are outliers in data, and asterisks indicate extreme values. Different letters indicate significant differences between groups.

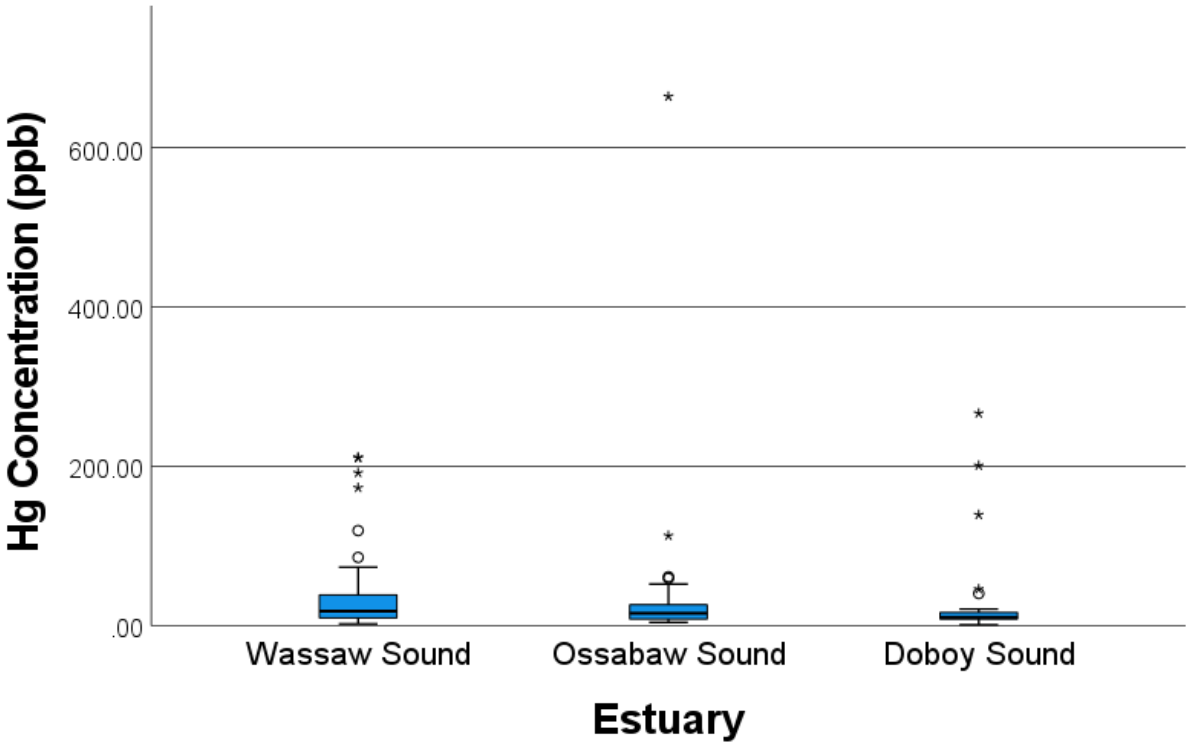

Figure 8. Boxplot displaying [THg] in each of the three sampling locations. Lower and upper box boundaries represent the 25th and 75th percentiles, respectively, the line within each box is the median, lower and upper error bars represent 10th and 90th percentiles, respectively, open circles are outliers in data, and asterisks indicate extreme values.

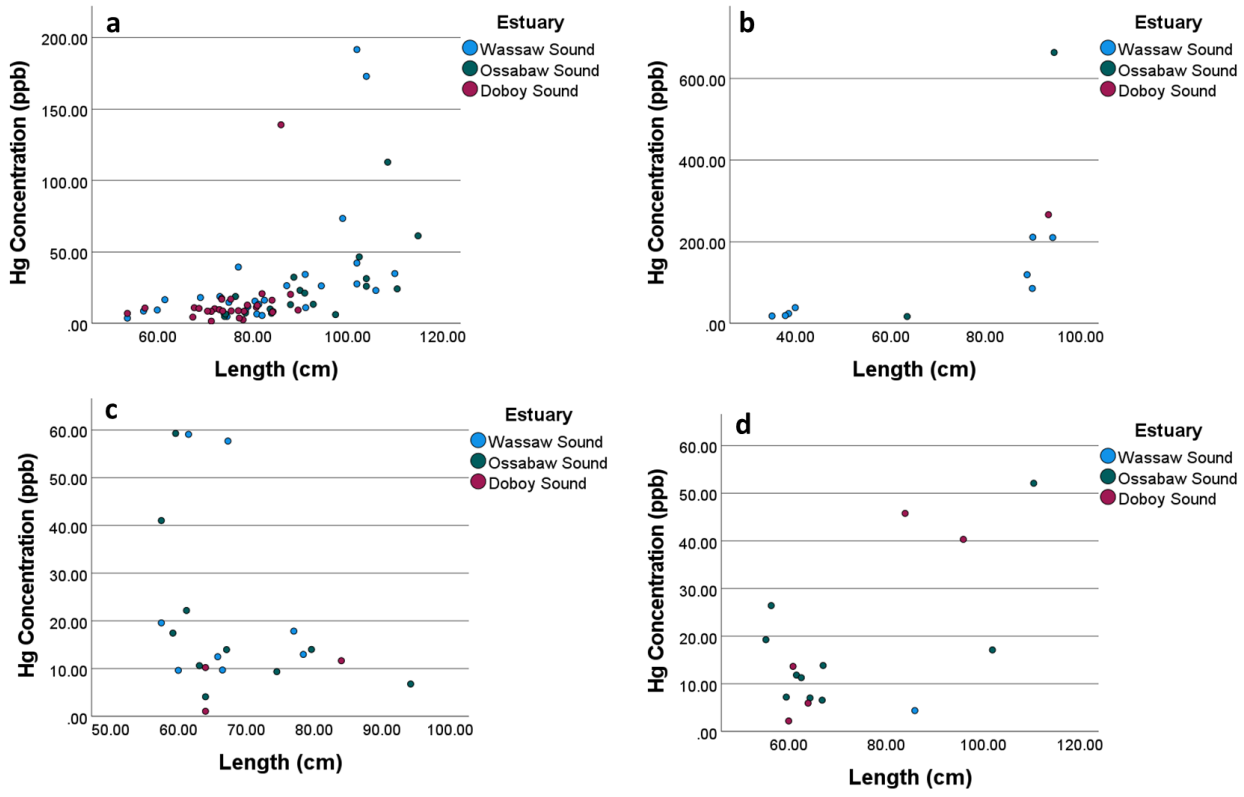

Figure 9. Scatterplots representing THg Concentration (ppb) vs. Length (cm) for (a) Bonnethead, (b) Sharpnose, (c) Blacktip, and (d) Sandbar. Note that both the x- and y-axes are scaled differently among species. Bonnethead showed a significant effect of length on Hg concentration.

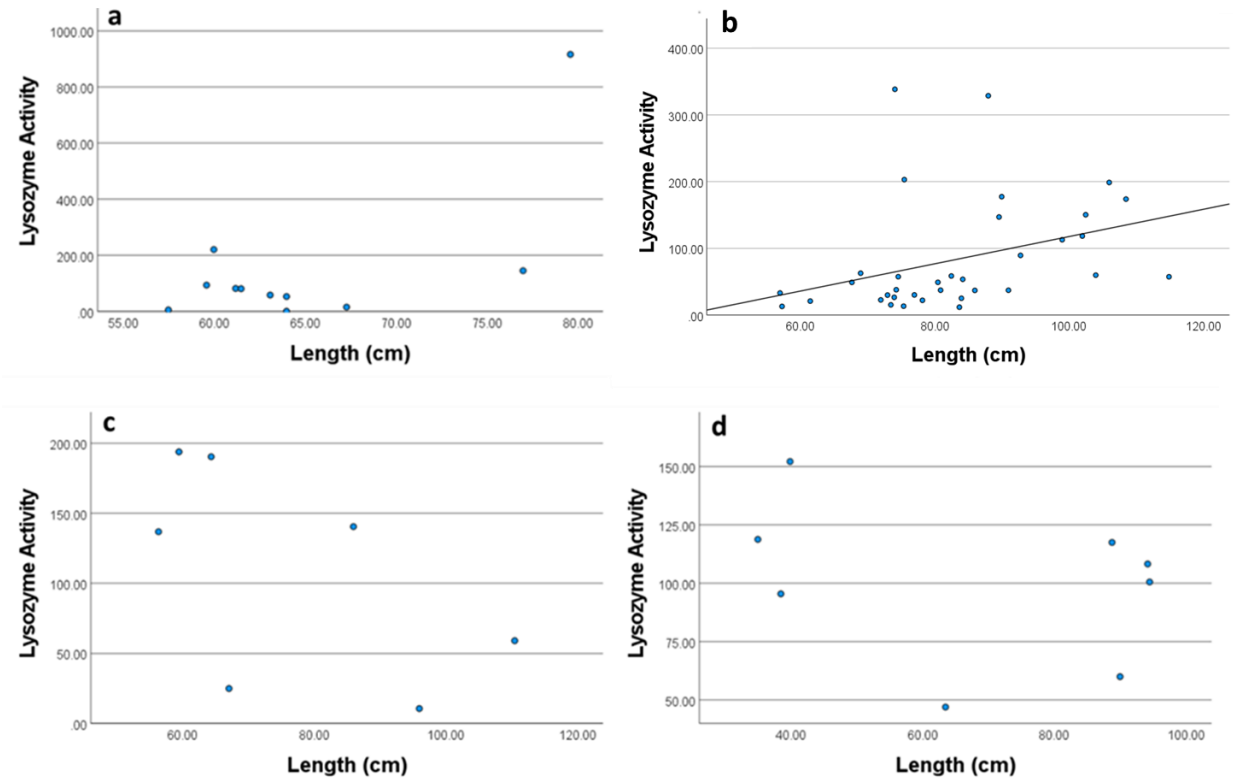

Figure 10. Scatterplots of lysozyme activity against total length (cm) for (a) Blacktip, (b) Bonnethead, (c) Sandbar, and (d) Sharpnose. Note that both the x- and y-axes are scaled differently among species. Length had a significant relationship on lysozyme activity in Bonnetheads ($p = 0.002$) and the relationship is shown as a solid line.

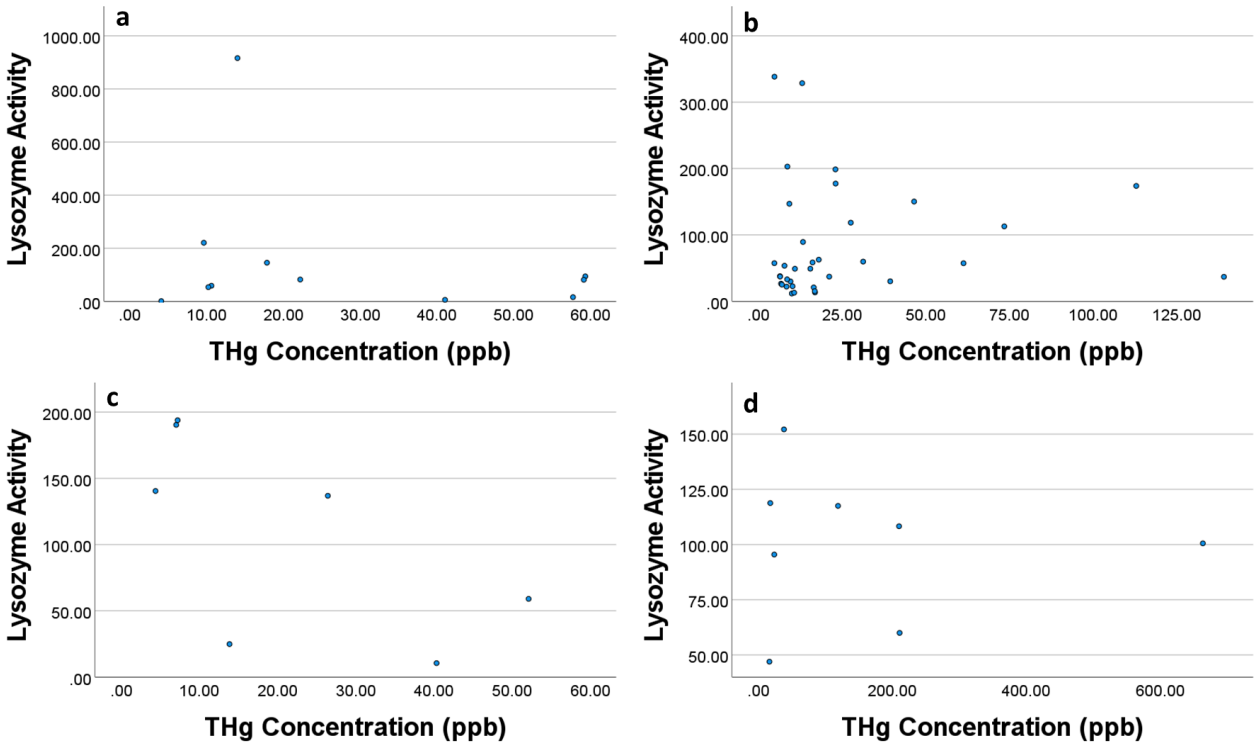

Figure 11. Scatterplots representing THg concentrations against lysozyme activity for (a) Blacktip, (b) Bonnethead, (c) Sandbar, and (d) Sharpnose. Note that both the x- and y-axes are scaled differently among species. There was no statistical relationship between THg and lysozyme activity for any species.

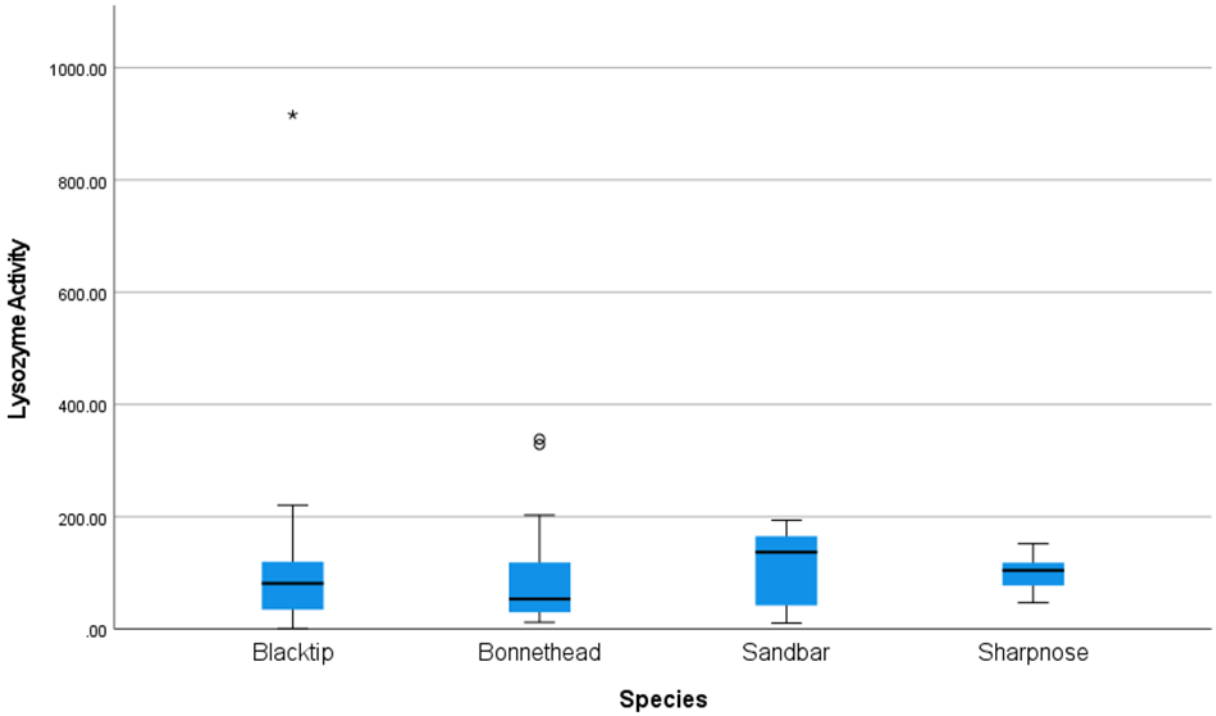

Figure 12. Boxplot of lysozyme activity across species. Lower and upper box boundaries represent the 25th and 75th percentiles, respectively, the line within each box is the median, lower and upper error bars represent 10th and 90th percentiles, respectively, open circles are outliers in data, and asterisks indicate extreme values. There was no significant difference found between species groups.

Table 1. Heavy metal concentrations from water samples (n = 6 per estuary) collected at the three estuary systems along the Georgia coast. For each estuary, mean ± standard deviation are shown and for each site classification range is given (n = 2 per site type).

Estuary	*Site Type*	*Hg (ng/L)*	*As (ng/L)*	*Cr (ng/L)*	*Cu (ng/L)*	*Pb (ng/L)*
Wassaw Sound	Across sites	1.98 ± 0.75	1,062 ± 379	1,093 ± 490	636 ± 179	261 ± 14
	Creek	1.26 - 1.58	537 - 919	542 - 925	471 - 867	116 - 171
	River	1.42 - 3.19	1,220 - 1,680	918 - 1,910	529 - 859	172 - 498
	Sound	1.87 - 2.55	935 - 1,080	845 - 1,420	519 - 571	279 - 331
Ossabaw Sound	Across sites	2.21 ± 0.58	655 ± 237	765 ± 235	525 ± 77	196 ± 98
	Creek	1.61 - 2.84	423 - 891.5	457 - 637	443.5 - 594	91.3 - 142.5
	River	1.97 - 3.02	470 - 483	595 - 1,050	462 - 641	110 - 341
	Sound	1.84 - 1.97	757 - 966	894 - 958	501 - 511	227 - 267
Doboy Sound	Across sites	2.60 ± 1.03	2,669 ± 655	1,141 ± 495	585 ± 111	405 ± 191
	Creek	1.19 - 2.96	1,640 - 2,970	431 - 1,530	453 - 646	132 - 549
	River	2.61 - 3.19	2,510 - 3,550	1,220 - 1,770	579 - 766	452 - 643
	Sound	1.64 - 4	2,360 - 2,985	730 - 1,165	501 - 562	237 - 417.5

Table 2. Heavy metal concentrations from sediment samples (n = 6 per estuary) collected at the three estuary systems along the Georgia coast. For each estuary, mean ± standard deviation are shown and for each site classification range is given (n = 2 per site type). Note Hg is given in ng/g dry weight due to small concentration.

Estuary	*Site Type*	*Hg (ng/g DW)*	*As (µg/g DW)*	*Cr (µg/g DW)*	*Cu (µg/g DW)*	*Pb (µg/g DW)*
Wassaw Sound	Across sites	25.02 ± 20.61	10.75 ± 7.40	29.93 ± 21.16	5.72 ± 4.56	31.10 ± 57.54
	Creek	14.3 - 23	6.14 - 15	18.1 - 55	3.27 - 7.31	5.29 - 11.7
	River	41.7 - 57.55	15.3 - 21.1	40.3 - 49.9	8.56 - 12.6	16.6 - 148
	Sound	4.8 - 8.86	2.79 - 4.19	5.55 - 10.7	0.65 - 1.92	1.86 - 3.12
Ossabaw Sound	Across sites	17.14 ± 11.30	6.98 ± 4.81	28.80 ± 24.47	3.62 ± 2.84	6.54 ± 4.25
	Creek	23.85 - 30.23	7.91 - 14.9	53.55 - 63.7	5.94 - 7.66	10 - 12.05
	River	8.5 - 27.72	2.52 - 9.38	8.83 - 27.6	1.34 - 4.54	3.02 - 8.79
	Sound	5.5 - 7.16	2.34 - 4.81	6.2 - 12.9	0.82 - 1.45	2.01 - 3.36
Doboy Sound	Across sites	25.14 ± 16.51	10.71 ± 7.89	28.28 ± 21.31	4.66 ± 3.61	7.20 ± 4.72
	Creek	44.2 - 45.64	11.2 - 25.4	39.7 - 65.4	7 - 10.7	10.5 - 15
	River	7.1 - 26.21	3.5 - 9.95	8.35 - 24.5	1.17 - 4.38	2.96 - 6.75
	Sound	13.15 - 14.6	4.26 - 9.93	11.25 - 20.5	1.97 - 2.74	3.37 - 4.62

Table 3. Sample distribution of sharks caught in each estuary by species and size class analyzed for blood mercury concentration.

Estuary	*Species*	*Total Number Sampled*	*Juvenile*	*Mature*
Wassaw Sound	Blacktip	8	8	0
(n = 46)	Bonnethead	28	8	20
	Finetooth	1	1	0
	Sandbar	1	1	0
	Sharpnose	8	4	4
Ossabaw Sound	Blacktip	10	10	0
(n = 40)	Bonnethead	18	0	18
	Sandbar	10	10	0
	Sharpnose	2	0	2
Doboy Sound	Blacktip	3	3	0
(n= 39)	Bonnethead	29	16	13
	Bull	1	1	0
	Sandbar	5	5	0
	Sharpnose	1	0	1

Table 4. Total mercury concentrations (ppb) from shark blood samples collected at the three estuary systems. For each species, mean ± standard deviation are shown and for each estuary, range is given.

Estuary	*Blacktip*	*Bonnethead*	*Bull*	*Finetooth*	*Sandbar*	*Sharpnose*
mean ± SD (estuaries combined)	13 ± 18.02	12.84 ± 34.42			12.77 ± 15.44	85.66 ± 192.49
Wassaw Sound	n = 8	n = 28		n = 1	n = 1	n = 8
	9.66 - 59.13	3.68 - 191.72		2.28	4.4	18.17 - 211.17
Ossabaw Sound	n = 10	n = 18			n = 10	n = 2
	4.12 - 59.32	4.88 - 112.89			6.59 - 52.14	16.9 – 663.89
Doboy Sound	n = 3	n = 29	n = 1		n = 5	n = 1
	1.08 - 11.68	1.58 - 139.06	200.86		2.22 - 45.82	266.42

Discussion

Environmental Variation Among Estuaries

Wassaw, Ossabaw, and Doboy Sound are categorized geologically as tidally dominated, bar-built estuaries which results in vertically, well-mixed systems (New Georgia Encyclopedia). These dynamic ecosystems seasonally change with respect to flooding, droughts, storms, and increasing anthropogenic influences (e.g., human population, coastal development, industry, etc.) (Xia et al., 2020; Elliot & Quintino, 2007). Particularly, coastal development is of great concern to estuaries since development influences heavy metal concentrations and distributions (Sanger et al., 1999). The results show that the concentration of heavy metals in the water and sediment samples vary from site to site within each estuary and may have obscured the ability to make any estuary-wide conclusions on the relationship between proximity to human activity and heavy metal concentration.

Contrary to predictions, the "control" estuary (Doboy Sound) generally had higher concentrations of Hg, As, Cr, and Pb in water samples than predicted. Although four of the five heavy metals were higher in Doboy water samples, only As exhibited a significant difference among the three estuaries. Water heavy metal concentrations in this study were much lower than previously reported in other estuarine systems in Northeastern USA, Spain, and Malaysia (Table 6). However, the estuary with the most anthropogenic influence, Wassaw Sound, generally had higher concentrations of As, Cr, Cu, and Pb in sediment samples as predicted. Additionally, there was no significant difference found among sediment heavy metals among the three estuaries. Again, contrary to predictions, Ossabaw Sound consistently had the lowest

or intermediate heavy metal concentrations. Overall, each estuary had considerably lower Hg levels than the Savannah River estuary, which is known to be extremely contaminated due to excessive amounts of waste products released into the estuary each year (Table 7).

These results do not support our hypothesis of Wassaw Sound being the most polluted estuary, followed by Ossabaw Sound then Doboy Sound. Although Hg, As, Cr, Cu, and Pb are naturally found within the environment, these contaminants are also used within industrial facilities. There are several factors that likely influence these results which include both natural and anthropogenic elements. Water sampling reflects more recent contamination, while sediment sampling represents longer-term contamination. Water contamination is related to seasonal changes in weather (e.g., freshwater inflow differs throughout year). However, sedimentation rates within estuaries influence residency times of heavy metal within the top layers of sediment, where "clean" sediment can cover up older, more contaminated sediment during sedimentation events (Deely & Fergusson, 1994).

The three estuaries sampled exhibit different levels of anthropogenic influence, including localized land uses, that could affect contamination within both water and sediment. Wassaw Sound is surrounded by an uninhabited nature preserve (Little Tybee Island) and a national wildlife refuge (Wassaw Island), however the estuary receives freshwater input from the Wilmington and Bull Rivers, which ultimately receive water from the Savannah River. Due to receiving subsequent freshwater input from one of Georgia's most polluted rivers (Savannah River) and high anthropogenic influence from the local area, Wassaw Sound has likely received more long-term contamination that has built up in its sediments. Ossabaw Sound is surrounded by a national wildlife refuge (Wassaw Island) and a Georgia heritage preserve

(Ossabaw Island) with a lower level of anthropogenic influence than Wassaw Sound. Lastly, Doboy Sound is located within a NERR that is federally managed and protected, and it was predicted to have the lowest level of anthropogenic influence among the three estuaries. Despite these protections, there is clearly some source(s) of heavy metal input, whether natural or anthropogenic, that is affecting heavy metal contamination within the estuary. However, since Doboy Sound is less influenced by anthropogenic activities than the other two estuaries, it is possible that the process of methylation from methylating bacteria is responsible for the higher-than-expected Hg concentrations (Merritt & Amirbahman, 2009). However, other metals concentrations could be due to several factors including both natural and anthropogenic.

Blood THg Accumulation

The differences in THg accumulation in four coastal shark species provide insights into factors influencing THg accumulation in predatory marine fishes within Georgia estuaries. THg accumulation in shark blood samples does not appear to be influenced by estuary, as was initially hypothesized; however, only Bonnethead were statistically compared in all three estuaries due to uneven sampling in the other species. By contrast, it appeared that THg accumulation in blood samples was related to species-specific differences in ecological behavior in how species use the estuary across life stages and physiology (e.g., migration, diet, maternal offloading, etc.). Due to their trophic level positioning, coastal shark species are susceptible to THg accumulation, likely accumulated through diet.

Interspecies Variation

Large variations in THg concentration were found among the four main shark species sampled, which may be related to differences in prey as diet is one of the main ways that sharks accumulate THg (McMeans et al., 2015). Bonnethead primarily feed on crustaceans, particularly Blue Crab (*Callinectes sapidus*) (Kroetz et al., 2017), while Blacktip, Sandbar, and Sharpnose mainly feed on various-sized teleost fishes (Hoffmayer & Parsons, 2003, McElroy et al., 2006, & Delorenzo et al., 2015), which are assumed to be trophically elevated compared to crabs (Mizukawa et al., 2009). Subsequently, sharks that consume prey at higher trophic positions will likely accumulate higher concentrations of THg due to biomagnification (Suk et al., 2009). This study's mean THg blood concentrations were generally lower than previously reported for coastal shark species (Table 5). However, some of the blood samples were similar to neonate and juvenile Blacktip blood samples from Southwest Florida, USA (Reistad et al. 2021; Table 5). In our data set, Sharpnose had significantly higher THg concentrations than Blacktip, Bonnethead, and Sandbar. However, Blacktip, Bonnethead, and Sandbar had similar mean THg concentrations (13, 12.84, and 12.77 ppb, respectively), even though Bonnethead has a different diet than the other two species.

Expected differences in diet (i.e. crab-based diet versus teleost diet) did not clearly predict differences in THg; therefore the similar mean THg concentrations could be attributed to other factors, such as differences in age class. Bonnethead were comprised of juvenile and mature animals, while Blacktip and Sandbar were comprised solely of juveniles (including young-of-year) animals. If the Blacktip and Sandbar data sets included mature animals, it is likely that the

mean THg concentrations would have been higher due to higher trophic level diets and larger size at maturity in adults compared to mature Bonnethead.

Shark migration likely plays a role in where and how shark species are accumulating THg due to animals potentially being exposed to higher THg concentrations in contaminated prey items and ecosystems (e.g., elevated THg concentrations in water) as they migrate. All shark species examined are migratory species that spend part of the year within estuaries, but most will migrate during winter in search of warmer water temperatures (McEachran, 2004). Sharks utilize estuaries in different ways, depending on their life history stage, and this type of habitat is especially used in early life-stages (e.g., neonate, young-of-year, and juvenile) when young sharks are vulnerable to predation (Heupal et al., 2007). For example, a few months after their birth (while some may stay within estuaries for longer), juvenile Blacktip and Sandbar will begin migrating out of the estuary (Matich et al., 2021; Rechisky & Wetherbee, 2003; Heupal et al., 2007). Mature Blacktip and Sandbar will migrate to lower latitudes in the winter, yet stay relatively close to shore (Kajiura et al., 2016; Baremore & Hale, 2012). Young-of-year Sharpnose migrate from offshore birthing areas to inshore environments for protection and copious food sources (Bethea et al., 2004). Mature Sharpnose will migrate offshore during winter into the deeper waters of the Atlantic Ocean (Parsons, 1938). Therefore, mature Sharpnose spend most of the year in offshore environments, while the other species are in coastal environments. Pregnant female Bonnethead will typically migrate to lower latitudes to give birth then return to their home estuary after giving birth (Driggers et al., 2014). Bonnethead were not significantly different among estuaries, which is interesting since this group represents the only “resident” population of animals in the data set. Bonnethead do not

migrate far and typically stay close to or within their home estuary. This further demonstrates the lack of estuary-specific differences within our study.

Differences in prey items between habitats (e.g., inshore, coastal, offshore) could likely play a role in the variation of THg concentrations among species. For example, Blacktip and Sandbar primarily feed on teleost fishes yet had relatively low THg concentrations within blood samples. However, Sharpnose also feed primarily on teleost fishes, yet exhibited elevated concentrations of THg. This could be contributed to the fact that Blacktip and Sandbar stay relatively close to shore while migrating, while Sharpnose are migrating into deeper, offshore waters. Because THg concentrations in prey items increase as water depth increases (Choy et al.,2009), Sharpnose may have the opportunity to consume higher THg concentrations when feeding due to capturing prey in deeper waters. Additionally, Bonnethead (all life stages) are typically found within inshore, coastal environments. Which could explain why Bonnethead (both juvenile and mature) had similar THg concentrations to Blacktip and Sandbar (all juveniles) that reside in estuaries and feed on teleost fishes yet did not have the influence of mature animals in the dataset.

Pregnant female sharks maternally offload contaminants to embryos through placental exchange (Lyons et al., 2013), which could be another factor contributing to the THg concentrations within our species groups for the youngest of individuals. This exchange of contaminants enables pregnant sharks to reduce their contaminant load, however young-of-year sharks will begin life with an elevated amount of the offloaded contaminants. In our data set, the only mature females were Bonnethead. Mature female Bonnethead had slightly higher THg concentrations than males. However, all were sampled at various points of gestation, and

more data needs to be gathered on the relationship of pregnancy stage to mercury circulating in the blood. It is important to note that age at sexual maturity and gestational period differs between each shark species, however longer gestational periods may give pregnant sharks the opportunity to significantly offload more contaminants to their offspring than shorter gestational periods (Lyons et al., 2013). There is some evidence of maternal offloading within our data set, due to young-of-year animals exhibiting elevated mean concentrations of THg in blood samples (Blacktip: 23.23 ppb, Sandbar: 11.41 ppb, and Sharpnose: 25.02 ppb). The THg concentrations in young-of-year samples were elevated compared to most of the juvenile samples, which suggests maternal offloading within our data set.

Bioaccumulation of THg is often a problem for high trophic level marine fish, such as sharks, due to their long lifespans and large size (e.g., length). Because of their propensity to bioaccumulate, it is common to see higher THg concentrations in sharks as they increase in length (Adams & McMichael, 1999). Of the four shark species examined in this study, only Bonnethead and Sharpnose displayed a significant relationship between THg concentration and length. This is likely due to the fact that both groups included juvenile and mature animals, while only juvenile Blacktip and Sandbar were sampled and showed no significant relationship with length. Although analyzing blood as a proxy for THg accumulation is a non-traditional method, the effect of length can still be studied, whether the THg accumulation is due to bioaccumulation and/or that larger sized animals ingest larger prey items and perhaps feed more frequently.

However, another important factor of bioaccumulation to consider is how fast red blood cell turnover occurs in sharks, which has been found to occur every 60-135 days (Caut et al., 2013).

THg concentrations within muscle is more stable and does not easily detoxify from the body, perhaps staying within muscle tissue for years. This information allows us to assume that THg concentrations within blood samples in juvenile and mature animals are primarily related to where they were feeding 2-5 months prior, which aids in understanding short-term THg exposure. This is likely the reason that we did not see differences among estuaries, as THg concentration within the blood samples may not have accurately reflected each animal's estuary location. Other body tissue samples (e.g., muscle, liver, brain) may be better indicators of long-term THg accumulation within the animals (Heuter et al., 1995; Barrera-Garcia et al., 2013; Ehnert-Russo et al., 2020).

Lysozyme was used as a first attempt to determine if Hg exposure had any negative correlation on lysozyme activity, which measures immune impaction. This appears to be the first study to examine lysozyme activity in Blacktip, Bonnethead, Sandbar, and Sharpnose species. Since lysozyme activity can be used as a blood biomarker of health to look at the immune response in fish (Guillete et al., 2020), it was examined here to investigate for relationships between THg concentration and length in the main four species. No species exhibited a relationship between THg concentration and lysozyme activity. However, Bonnethead did display a significant relationship between lysozyme activity and length, indicating that lysozyme activity may be more related to changes in life history than mercury exposure in blood. It is important to note that sample sizes were low for all species, which could have contributed to the lack of relationship in Blacktip, Sandbar, and Sharpnose. We found no correlation between THg concentration and immune response from our data set and cannot determine whether THg concentrations in shark tissues has an effect on lysozyme activity within blood plasma.

Further Research

A subsequent investigation into the prey of the shark species examined would further uncover the THg inputs sharks receive during feeding. Additional studies into the isotopic composition of prey are needed to gather baseline data on how these species are utilizing the estuaries and if this is influencing their THg concentrations. For example, documenting the types of prey these sharks are feeding on and if they are consuming prey inshore, coastally, or offshore is needed to understand sources of THg. In addition, residency times of shark species inhabiting the Georgia coast is needed to understand when sharks arrive and when they leave to predict what THg exposures they may be susceptible to while using estuarine habitats, specifically in Georgia. Acoustic monitoring would be useful in understanding exactly where animals spend their time and be used as a proxy for where they may be feeding. This information would give insight into possible areas of THg contamination. Lastly, further gathering of environmental data is needed to accurately access the level of contamination that each estuary is experiencing, and if the contamination is disrupting the ecosystem services provided by these invaluable ecosystems. Gathering long-term water and sediment data would aid in understanding environmental patterns along the Georgia coast.

Table 5. Comparison of present study to previously reported THg concentrations within shark blood. All studies shown are reported in ppb wet weight, except Frías-Espericueta et al., 2015 which is reported in ppb dry weight (denoted with an *).

	Present Study	**Pine Island Sound & San Carlos Bay, USA 2016, Reistad et al. (2021)**	**SE Gulf of California, USA 2012, Frías-Espericueta et al. (2015)**	**Eastern Tropical Pacific Ocean, China 2019-2020, Li et al. (2022)**
Blacktip	13 ± 18.02	50 (neonates) 110 (juveniles)		
Bonnethead	12.84 ± 34.42			
Bull	200.86			
Finetooth	2.28			
Sandbar	12.77 ± 15.44			
Atlantic Sharpnose	85.66 ± 192.49			
Pacific Sharpnose			540* (pregnant females)	
Silky				220 (juvenile & mature animals)

Table 6. Comparison of present study water heavy metal concentrations with previously reported heavy metal concentrations among other estuaries. All metals are reported in ng/l.

	Present Study	**Ría de Huelva, Spain 2004-2006, Vicente-Martorell et al. (2009)**	**Plum Island Sound, Massachusetts 2018-2020, Wang & Obrist (2022)**	**Jejawi Estuary, Malaysia 2005, Alkarkhi et al. (2008)**
Hg	Wassaw Sound: 1.98 Ossabaw Sound: 2.21 Doboy Sound: 2.60	---	3.89 (all levels of marsh)	10,000
As	Wassaw Sound: 1,062 Ossabaw Sound: 655 Doboy Sound: 2,669	4,790	---	3,440,000
Cr	Wassaw Sound: 1,093 Ossabaw Sound: 765 Doboy Sound: 1,141	---	---	200,000
Cu	Wassaw Sound: 636 Ossabaw Sound: 525 Doboy Sound: 585	46,380	---	50,000
Pb	Wassaw Sound: 261 Ossabaw Sound: 196 Doboy Sound: 405	6266	---	250,000

Table 7. Comparison of present study sediment heavy metal concentrations with previously reported heavy metal concentrations within estuaries. All metals reported in µg/g DW, except Hg, which is reported in ng/g DW.

	Present Study	**Savannah River, Georgia 2000-2001, Kumar et al. (2008)**	**St. Lucie Estuary, Florida 1996, Zhang et al. (2003)**	**Port Royal Sound, South Carolina 1995, Sanger et al. (1999)**
Hg	Wassaw Sound: 25.02 Ossabaw Sound: 17.14 Doboy Sound: 25.14	1533	---	20
As	Wassaw Sound: 10.75 Ossabaw Sound: 6.98 Doboy Sound: 10.71	32	---	5.4
Cr	Wassaw Sound: 29.93 Ossabaw Sound: 28.80 Doboy Sound: 28.28	0.79	40	25.05
Cu	Wassaw Sound: 5.72 Ossabaw Sound: 3.62 Doboy Sound: 4.66	---	26	3.88
Pb	Wassaw Sound: 31.10 Ossabaw Sound: 6.54 Doboy Sound: 7.20	0.62	10	8.58

Statement of Integration

This thesis integrated the fields of marine biology, hematology, chemistry, environmental science, and geography. Marine biology and hematology were used to analyze the relationship between coastal sharks and mercury accumulation in shark blood along the Georgia coast. Chemistry and environmental science were used to analyze water and sediment heavy metal concentrations to look for patterns in anthropogenic impact within three Georgia estuaries. Lastly, ArcGIS Pro was used to geographically analyze spatial patterns in heavy metal concentrations within the three estuaries.

Acknowledgements

I would like to thank my PIs, Dr. Troy Mutchler and Dr. Thomas McElroy for their continual support, guidance, and patience throughout the past two years. Thank you both for allowing me the opportunity to form my own project. This project turned into something better than I could have imagined, and it would not have been possible without your support. I would also like to thank, Dr. Allen Roberts for his guidance and expertise in GIS. Dr. Roberts gave me the tools and guidance to complete my spatial analysis in ArcGIS Pro, which was a large part of this project. I would like to thank the KSU Office of Research for providing me with a CARET grant, and the College of Math and Sciences for providing me with a Mentor Protégé grant. I would also like to thank Jake Smith for his assistance in the lab. Additionally, I would like to thank undergraduate students: Kayley Agan, Brandi Lofton, Sarah Limer, Angela Raez, and Kayla Schrimsher for their help with field sampling and laboratory analysis.

I would like to thank the Georgia Aquarium for their financial support including stipend, travel, and everything that I needed to successfully complete my thesis project. I would like to thank Dr. Kady Lyons for her continual support, guidance, and mentorship throughout my entire master's project. Dr. Lyons went above and beyond to help me in any way possible. Additionally, I would like to thank Dr. Lisa Hoopes for her support of my master's project, and Georgia Aquarium volunteers: Piper Rackley, Katelyn Herman, and Elaine Alberts for their help with field sampling.

I would like to thank the University of Georgia Marine Extension staff: Devin Dumont, Lisa Kovalanchik, Luke Roberson, Todd Recicar, and Ike Sellers for their help with field sampling.

Thank you to Dr. Scott Belcher and Dr. Kylie Rock at North Carolina State University for opening up their lab to me and teaching me how to analyze my blood samples. Thank you to Dr. Asli Aslan at Georgia Southern University for help with water analysis.

Last but certainly not least, I would like to thank my friends and family for their support during the past two years. I would like to specifically thank my husband, George, who supported me every step of the way.

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