BIOACCUMULATION AND MATERNAL TRANSFER OF MERCURY IN SHARKS OFF THE SOUTHEASTERN UNITED STATES AND IN THE NORTHERN GULF OF MEXICO

by

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

ABSTRACT

Mercury (Hg) is a global, pervasive, nonessential trace element that is capable of biomagnifying through marine food webs, bioaccumulating in tissues of large predatory species, and being maternally transferred during embryonic development. Since many shark species occupy high trophic positions, they are at increased risk of Hg exposure through their diet. To better understand Hg concentrations in sharks off the southeastern United States and in the northern Gulf of Mexico, total Hg (THg) concentrations were measured in the muscle, liver, and fin of ten species [spinner shark, *Carcharhinus brevipinna*; bull shark, *Carcharhinus leucas*; blacktip shark, *Carcharhinus limbatus*; sandbar shark, *Carcharhinus plumbeus*; tiger shark, *Galeocerdo cuvier*; lemon shark, *Negaprion brevirostris*; Atlantic sharpnose shark, *Rhizoprionodon terraenovae*; scalloped hammerhead, *Sphyrna lewini*; great hammerhead, *Sphyrna mokarran*; and smooth hammerhead, *Sphyrna zygaena*] using a direct mercury analyzer. Maternal transfer of Hg in embryonic sharks was also investigated by measuring THg concentrations in the muscle, heart, brain, kidney, liver, skin, and fin of bull, blacktip, and sandbar shark embryos. In the adult sharks, both inter- and intraspecies differences in THg concentrations were observed. Overall, the greatest mean THg concentrations (µg/g dry weight) were measured in the liver, followed by the muscle, and fin, with the smooth hammerhead, lemon shark, and blacktip shark containing the greatest mean liver concentrations (98.0, 61.1, and 44.0 µg/g dry weight, respectively) while the smooth, scalloped, and great hammerhead shark contained the greatest mean concentrations in the

muscle (53.2, 13.8, and 11.6 µg/g dry weight, respectively). Significant positive relationships between muscle and liver THg concentration and body length were also observed for bull, blacktip, and sandbar sharks. Except for the Atlantic sharpnose shark, the mean muscle THg concentration for each species exceeded the U.S. Food and Drug Administration (FDA) 1 µg/g wet weight action limit for human consumption, indicating that sharks should be consumed infrequently to limit the risk of Hg exposure in humans. The greatest mean THg concentrations in the bull, blacktip, and sandbar shark embryos were measured in the muscle followed by either the heart or kidney, and the lowest mean concentrations were measured in the fin and liver. Overall, concentrations of THg were greater in bull and blacktip embryos than in sandbar embryos. When compared to the parent muscle and liver THg concentrations, the muscle of these embryos contained a greater percentage of maternal THg than the liver, despite the greater THg concentrations observed in adult liver, with the highest percentages being measured in the blacktip embryos, followed by bull and sandbar embryos. The results of this study suggest that THg is accumulating to high concentrations in the tissues of sharks caught off the southeastern United States and in the northern Gulf of Mexico, and therefore the consumption of these species should be limited to reduce the risk of Hg exposure within humans. In addition, the accumulation of THg in embryos provided further evidence to suggest that maternal transfer represents a significant exposure pathway for THg in sharks that could, in part, account for the elevated THg tissue concentrations previously observed in young-of-year sharks.

1. MERCURY CONCENTRATIONS IN SHARKS OFF THE SOUTHEASTERN UNITED STATES AND IN THE NORTHERN GULF OF MEXICO

1.1 Introduction

1.1.1 Mercury in the Marine Environment

Mercury (Hg) is a global, pervasive, nonessential trace element that is released into the environment through both natural (e.g., volcanic eruptions, erosion of rocks, and forest fires) and anthropogenic sources, primarily as emissions from coal-fired power plants and small artisanal gold mining operations (Varekamp and Buseck, 1986; De Lacerda, 1995; Malm 1998; Ferrara et al., 2000; Pacyna et al., 2001; Nriagu and Becker, 2003; Pacyna and Pacyna, 2005; Veiga et al., 2006; Dabrowski et al., 2008; Witt et al., 2009). In the marine environment Hg can be found as elemental (Hg^0), inorganic (Hg^{2+}), or organic (CH_3Hg^+) mercury (Clarkson, 1997). Organic mercury, also known as methylmercury (MeHg), is the most concerning of the three forms because it is toxic to wildlife and humans at low concentrations and is capable of bioaccumulating within marine organisms and biomagnifying in marine food webs (Bargagli et al., 1998; Bank et al., 2007; Magalhães et al., 2007; Chen et al., 2008; Harding et al., 2018).

After being released into the environment, Hg^0 is capable of spending long periods, ranging from six months to two years, within the atmosphere before it is photooxidized into Hg^{2+} (Morel et al., 1998; Bergan et al., 1999; Schroeder et al., 1998; Ariya et al., 2015; Deng et al., 2015; Gonzales-Raymat et al., 2017). This provides Hg the opportunity to undergo long-range atmospheric transport to remote locations far from its original source such as the Arctic and Antarctica (Muir et al., 1999; Sprovieri et al., 2002; Ariya et al., 2004; Campbell et al., 2005; Scheuhammer et al., 2015; Schuster et al.,

2018). Inorganic Hg then enters marine environments through wet or dry deposition (Lindqvist and Rodhe, 1985; Mason et al., 1994; Wang et al., 2004, Risch et al., 2012; Driscoll et al., 2013). In marine environments, Hg^{2+} can then be converted to MeHg, primarily by sulfate-reducing bacteria within the sediment and overlying water column (Berdicevsky et al., 1979; Compeau and Bartha, 1985; Trevors, 1986; Matilainen, 1995; Fitzgerald et al., 2007; Gilmour et al., 2013). Once methylated, MeHg can then be taken up by organisms at the base of planktonic and benthic food webs and be trophically transferred up to top predators, including sharks (Krabbenhoft and Rickert, 1995; Mason et al., 1994; Lawson and Mason, 1998; Cai et al., 2007; Nfon et al., 2009; Gosnell and Mason, 2015; Lee and Fisher, 2016).

In 2010 it was reported that 7,527 tonnes (Mg) of Hg is emitted into the atmosphere on an annual basis (Pirrone et al., 2010). This amount is likely to continue increasing as it is estimated that the current amount of atmospheric Hg deposition globally is three times greater than what was in preindustrial times (~1850) (Fitzgerald et al., 2007; Lindberg et al., 2007; Drevnick et al., 2012). Of all the anthropogenic sources, fossil-fuel-fired power plants are the number one contributor to the estimated annual amount of Hg emitted each year (Mason et al., 1994; Pirrone et al., 2010; Driscoll et al., 2013). The effects of this continuous influx of Hg have been seen in marine species throughout the world. These emissions have reportedly led to increases in the concentrations of Hg found in the tissues of long-lived predatory species such as yellowfin tuna (*Thunnus albacares*) in the north Pacific Ocean, with concentrations in these fish increasing at a rate of 3.8% per year (Drevnick et al., 2015), which is consistent with current models on the anthropogenic inputs of Hg into the atmosphere from Asia.

Based on this, further increases in anthropogenic Hg emissions will lead to increased concentrations of Hg in marine species. To prevent this, reductions in global atmospheric Hg emissions from point sources need to be made. For example, decreases in overall emission within an area over time have been found to lead to decreases in Hg concentrations within marine species (e.g., Cross et al., 2015). This has been reported in species such as the Atlantic bluefin tuna (*Thunnus thynnus*) and bluefish (*Pomatomus saltatrix*) in the north Atlantic Ocean previously and provides evidence to suggest that emission reduction efforts can benefit vulnerable marine species in the surrounding area (Cross et al., 2015; Lee et al., 2016). However, if emissions are not reduced in the future, concentrations of Hg in commercially important marine species will likely continue to increase.

1.1.2 Mercury in Sharks

Since Hg is known to bioaccumulate in fish (gradual increase in concentration over time, so larger, older individuals contain higher Hg concentrations than smaller, younger individuals of the same species) and biomagnify in marine food webs (increase in concentration with each increase in trophic step), the highest concentrations are commonly found within long-lived predatory species that occupy high trophic levels such as tuna, billfishes, and sharks (Campbell et al., 2005; Cai et al., 2007; García-Hernández et al., 2007; Chen et al., 2008; Chen et al., 2009; Escobar-Sanchez et al., 2010; Ordiano-Flores et al., 2011; Maz-Courrau et al., 2012; Lavoie et al., 2013). These trends have often been attributed to the diet of these organisms as studies utilizing smaller forage fish [e.g., mummichog (*Fundulus heteroclitus*) and Atlantic silverside (*Menidia menidia*)], that are more easily managed and studied in a laboratory setting compared to large

sharks, have found that the uptake of MeHg greatly exceeds its rate of elimination from the body following dietary exposure (assimilation efficiency ≥ 90%, loss rate = 0.8% per day; Dutton and Fisher, 2010, 2011).

Due to the difficulty involved in breeding these large predators and monitoring their health in a laboratory setting, there is a lack of information on the negative effects Hg can have on larger and more mobile species, such as sharks, at different life stages. Despite this, accumulating high levels of Hg has been found to have negative neurotoxic, cardiovascular, morphological, and reproductive effects in both juvenile and adult teleosts. Neurotoxic effects previously reported include metabolic disorders, cytoskeletal assembly dysfunction, oxidative stress, and altered behavior (Webber and Haines, 2003; Wang et al., 2015; Barboza et al., 2018). Mercury exposure can also have cardiovascular effects including damage to blood vessels, hemorrhages, heartbeat irregularities, and an overall reduction of blood cell counts (Heisinger and Green, 1975; Devlin, 2006). Morphological and developmental impairments brought on by Hg toxicity include damaged gills and olfactory organs, reduced liver function and metabolism, and malformations of the craniofacial and skeletal systems such as stunted growth, spinal curvature, and deformation of the eyes ranging from partially fused eyes to cyclopia (Weis and Weis, 1977; Weis et al., 1981; Jagoe et al., 1996; Ribeiro et al., 1996; Ribeiro et al., 2000, Devlin, 2006; Adams et al., 2010). Reproductive effects include reduced spawning and hatching success (Dial, 1978; Latif et al., 2001; Hammerschmidt et al., 2002; Drevnick and Sandheinrich, 2003; Crump and Trudeau, 2009), altered sex ratios (Matta et al., 2001), slowed embryonic development (Perry et al., 1988), and increased mortality during early development (Wiener and Spry, 1996; Samson and Shenker,

2000).

The effects of Hg on sharks have been previously reported in a small number of species. Norris et al. (2021) found evidence of Hg-induced damage in the liver of juvenile blacktip sharks (*Carcharhinus limbatus*) based on increased melanomacrophage density. Of the few other studies attempting to investigate the effects of Hg in sharks, few significant results were found. For example, Ehnert-Russo and Gelsleichter (2020) failed to detect significant levels of Hg within the brain of Atlantic sharpnose sharks (*Rhizoprionodon terranovae*) that might induce any neurological effects, while Walker et al. (2014) found high levels of Hg in the muscle of bonnethead sharks (*Sphyrna tiburo*) but failed to detect any physiological response when utilizing metallothionein levels as a potential biomarker. Because few studies have investigated the potential health effects of Hg in sharks, there is still little information on the dynamics of Hg toxicity within larger sharks that occupy high trophic positions. The threshold for adverse biological effects in fish has been determined to be between 0.5 and 1.0 μg/g wet weight based on Hg concentration in muscle tissue (Scheuhammer et al., 2015); therefore, higher trophic level species could be at greater risk of developing deleterious health effects caused by Hg exposure.

Globally, studies have shown that high trophic level predators accumulate elevated concentrations of Hg (Marcovecchio et al., 1991; Storelli et al., 2002; Hurtado-Banda et al., 2012). As a regional example, shark species caught off the coast of Florida have been found to contain high concentrations of Hg (Table 1). Despite these shark species all containing high concentrations of Hg, there is still a large degree of variability in Hg concentrations among species.

Despite the lack of information available on the effects of Hg on mature sharks, it has been reported that MeHg comprises anywhere from 83-97% of the total Hg (THg) within muscle tissue [83% in small-spotted catshark (*Scyliorhinus canicula*), 88% in kitefin shark (*Dalatias licha*), 88% in smooth hammerhead (*Sphyrna zygaena*), 92% in longnose spurdog (*Squalus blainville*), and 97% in blue shark (*Prionace glauca*) and Atlantic sharpnose shark] (Bloom, 1992; Storelli et al., 2002; Krystek and Ritsema, 2004; Branco et al., 2007; de Carvalho et al., 2014; Ehnert-Russo and Gelsleichter, 2020; Kazama et al., 2020). Because of this, measurements of THg within the muscle tissue can provide adequate estimates on the amount of MeHg that has accumulated within the body (Grieb et al., 1990; Bloom, 1992). However, this also implies that any person that frequently consumes shark muscle will primarily be exposed to MeHg and are at risk of experiencing negative health effects over time.

1.1.3 Human Health Implications

Fish are considered a healthy food source because they are high in protein, selenium (Se), and omega-3 fatty acids, and low in saturated fat (Sidhu, 2003; Domingo et al., 2007; Park and Mozaffarian, 2010; Tacon and Metian, 2013; Khalili Tilami and Sampels, 2018). Despite these benefits, the consumption of seafood is also considered to be the predominant source of Hg exposure for humans (Freire et al., 2010; Silbernagel et al., 2011; Rahbar et al., 2013). Because of this, depending on the species and the frequency at which consumption occurs, people are at risk of being exposed to Hg and the deleterious health effects associated with it when they consume fish.

Exposure in humans can lead to neurological, cardiovascular, and immunological health implications in both adults and children (Mergler et al., 2007; Virtanen et al.,

2007; Guynup and Safina, 2012; Okpala et al., 2018). Methylmercury is a well-known neurotoxin that can cross the blood brain barrier and target the central nervous system (Clarkson et al., 2007; Rice et al. 2014). Because of this, it can cause several neurological health effects including impaired vision, hearing loss, muscle weakness, tremors, and overall mental deterioration (Castoldi et al., 2001; Mergler et al., 2007; Do Nascimento et al., 2008). Methylmercury has also been reported to affect cardiovascular health by increasing the risk of high blood pressure, cardiovascular disease, myocardial infarction, and stroke (Kromhout et al., 1985; Shekelle et al., 1985; Salonen et al., 1995, Virtanen et al., 2007; Choi et al., 2009; Rice et al., 2014). In addition, exposure to MeHg can also result in immunosuppression as observed through an increased frequency of anti-nuclear autoantibodies, changes in serum cytokine levels, and increased risk of malaria infection (Crompton et al., 2002; Gardner et al., 2010; Nyland et al., 2011; Hong et al., 2012).

The maternal transfer of MeHg while *in utero* or during the first year of life can have detrimental effects on the neurological development of children (Trasande et al., 2006; Driscoll et al. 2013). This type of exposure can occur due to the ability of MeHg to cross the placenta while *in utero* or be transferred postpartum from mother to child during breastfeeding (Ask et al., 2002). Once exposed, the child is at increased risk of deleterious health effects including damage to the central nervous system which can cause blindness, deafness, cerebral palsy, reduced IQ, and severe mental retardation (Murata et al., 1999; Castoldi et al., 2001; Fernandes Azevedo et al., 2012)

Because of these potential health risks, federal agencies have set seafood consumption advisories with the aim of decreasing the potential for human exposure to Hg by informing the public about the risks associated with consuming fish contaminated

with Hg. The Food and Drug Administration (FDA) Hg action limit for commercial fisheries advises against consuming fish with Hg concentrations ≥ 1.0 μg/g wet weight, while the Environmental Protection Agency's (EPA) human health criterion for recreational fisheries is set at 0.3 μg/g wet weight. However, the EPA allows each state to set their own Hg advisory level for recreationally caught fishes. For example, the Texas Department of State Health Services (TDSHS) issues a Hg advisory when the MeHg concentration for a given species exceeds the 0.7 μg/g wet weight human health-based standard.

Shark muscle is the most frequently consumed tissue, followed by fins. Due to the vascularization differences in these two tissues and the fact that the turnover time for Hg within the muscle can take over two years (Hesslein et al., 1993; Kwon et al., 2016) these two tissues can have different Hg concentrations. The highest Hg concentrations are typically reported in shark muscle tissue and the lowest reported concentrations are in fins (Escobar-Sánchez et al., 20ho10; Man et al., 2014; O'Bryhim et al 2017; Kim et al., 2016; Barcia et al., 2020; Gelsleichter et al., 2020). Despite fins having the lowest Hg concentrations compared to other tissues, multiple studies have found that shark fins can still contain concentrations of Hg that exceed consumption advisories (Escobar-Sánchez et al, 2010; Man et al., 2014; Nalluri et al., 2014).

Despite shark being less frequently consumed by people in the United States compared to other types of seafood, there were still over 850 metric tons of sharks harvested commercially in the Gulf of Mexico and the Atlantic Ocean in 2019 alone (NOAA NMFS, 2019). With Hg concentrations regularly exceeding the 1.0 μg/g wet weight FDA action, people are at risk of exposure to elevated concentrations of Hg, even

if their consumption of these species is limited.

1.1.4 Stable Isotope Analysis

Carbon and nitrogen stable isotopes can be used as an ecological tracer to understand the foraging habits and trophic ecology of different species within marine environments (Hobson et al., 1996; Davenport and Bax, 2002; Rooker et al., 2006; Jennings et al., 2008; Newsome et al., 2010; Richert et al., 2015). The ratio of nitrogen stable isotopes ($^{15}N/^{14}N$; denoted as $\delta^{15}N$) can be used to infer the trophic position of an organism due to the fractionation caused by an increase in the concentration of the heavier isotope (^{15}N) during the metabolization of nitrogen after ingestion (Minagawa and Wada, 1984). As a result, the $\delta^{15}N$ of an organism will be higher than that of its prey (between 3-4‰ per trophic transfer), meaning organisms with higher $\delta^{15}N$ values will occupy higher trophic positions (Peterson and Fry, 1987). The ratios of carbon stable isotopes ($^{13}C/^{12}C$; denoted as $\delta^{13}C$) are useful in determining sources of primary production within the environment since there are a variety of carbon sources with different $\delta^{13}C$ values (e.g., marine phytoplankton: -24‰ to -18‰, macroalgae: -27‰ to -8‰, and marine seagrasses: -15‰ to -3‰) (DeNiro and Epstein, 1978; Fry and Sherr, 1989). With each increase in trophic step, $\delta^{13}C$ values remain fairly unchanged with differences ranging from only 0-1‰ per trophic transfer. Because these values are relatively conserved at each trophic step, they can serve as indicators for dietary carbon sources and thus be used to distinguish between different feeding patterns of various species across different environments, e.g., feeding in nearshore versus offshore areas (France, 1995; France and Peters, 1997; Cherel and Hobson, 2007).

Stable isotopes have become a standard method in feeding ecology studies

focusing on sharks due to their use in exploring the trophic structure of these species (Domi et al., 2005; MacNeil et al., 2005; Estrada et al., 2006; Kerr et al., 2006; Borrell et al., 2011; Olin et al., 2011). The ability of these values to provide information on the feeding habits of sharks can be applied to Hg bioaccumulation research as well to identify the degree to which biomagnification is occurring at different steps along the food chain (Atwell et al., 1998). In addition to biomagnification, the investigation into the $\delta^{13}C$ values of these species can provide important information on the prey choice of these large predators, which can elucidate which of these prey items are serving as the greatest sources of Hg for sharks.

1.1.5 Research Areas, Species, and Tissues to be Investigated

This study investigated THg concentrations in sharks caught off the southeastern United States and in the northern Gulf of Mexico. Specifically, sharks were collected in the coastal waters of Texas and the Gulf and Atlantic coasts of Florida and North Carolina. Sharks are targeted in these areas in both recreational and commercial fisheries. United States commercial fisheries within the Gulf of Mexico and the northern Atlantic Ocean harvested over 950 tons of shark in 2020 (NOAA NMFS, 2021). Blacktip shark (233.7 metric tons), smoothhound (265.8 metric tons), and large coastal species, including bull shark (*Carcharhinus leucas*), lemon shark (*Negaprion brevirostris*), nurse shark (*Ginglymostoma cirratum*), silky shark (*Carcharhinus falciformis*), tiger shark (*Galeocerdo cuvier*), and spinner shark (*Carcharhinus brevipinna*) sharks (252 metric tons) were the most commonly landed species (NOAA NMFS, 2021).

Although studies have previously investigated THg concentrations in sharks off the southern United States and the northern Gulf of Mexico (Hueter et al., 1995; Adams

and McMichael, 1999; Adams et al., 2003; Evers et al., 2008; Nam et al., 2011; Rumbold et al., 2014; O'Bryhim et al., 2017; Matulik et al., 2017), many were limited regarding the number of species and tissue types investigated. Most studies focused on a small number of species and only measured the THg concentration in the muscle tissue (Hueter et al., 1995; Adams and McMichael, 1999; Adams et al., 2003; Evers et al., 2008; Rumbold et al., 2014; Nam et al., 2011; Matulik et al., 2017), with few investigating the THg concentrations in the liver and fin as well (O'Bryhim et al., 2017). This is likely because shark toxicology research often relies on obtaining samples opportunistically from commercial fisheries observers, federal and state agencies, recreational fishers, and fishing tournaments.

This study was completed in collaboration with National Oceanic and Atmospheric Administration (NOAA) fisheries observers, the NOAA Southeast Fisheries Science Center (Panama City, FL), and fishing tournaments held along the Texas coast. twelve species of elasmobranchs, including eleven shark species [spinner shark, bull shark, blacktip shark, dusky shark (*Carcharhinus obscurus*), sandbar shark (*Carcharhinus plumbeus*), tiger shark, lemon shark, Atlantic sharpnose shark, scalloped hammerhead (*Sphyrna lewini*), great hammerhead (*Sphyrna mokarran*), and smooth hammerhead], and one species of stingray [southern stingray (*Dasyatis americana*)] were included in the study.

Some of these sharks, such as the dusky shark and sandbar shark, are prohibited from being caught in commercial and recreational fisheries while others, like the spinner shark, bull shark, blacktip shark, tiger shark, lemon shark, and all three hammerhead species, have strict limitations in place regarding the size and number of each that can be

retained (NOAA NMFS, 2020). The sandbar shark, in particular, is a prohibited species and cannot normally be retained in any fishery except for NOAA's Shark Research Fishery which allows a set number of commercial fishing vessels to retain a limited quota of sandbar sharks every year. This fishery is meant for NOAA's HMS (highly migratory species) division to monitor the population as it recovers from overfishing, as well as provide scientific samples to further study the species. The inclusion of the sandbar shark into this study provides a unique opportunity to investigate THg concentrations in a species that is rarely allowed to be sampled.

The sharks in this study belong to two families. The spinner shark, bull shark, blacktip shark, dusky shark, sandbar shark, tiger shark, lemon shark, and Atlantic sharpnose are all members of the Carcharhinidae family, also known as the requiem sharks. The hammerhead sharks make up the rest of the species in this study and are members of the Sphyrnidae family. Both families occupy temperate and tropical waters throughout southeastern United States and northern Gulf of Mexico and are all large predatory species [except for the Atlantic sharpnose which is much smaller (maximum body length of 83 cm; Loefer and Sedberry, 2003)]. While many of these species feed primarily on smaller fish and crustaceans, others such as the bull and tiger shark, are more versatile and opportunistic feeders known to eat a variety of prey items including stingrays, sea birds, sea turtles, and even other sharks (Snelson et al., 1984; Simpfendorfer et al., 2001; Estupiñán-Montaño et al., 2017). The hammerheads are apex predators that occupy high trophic positions and feed on teleost fishes and other elasmobranchs (ranging from small rays to large sharks) (Gallagher and Klimley, 2018). This variety in prey choice between species can lead to differences in trophic position

which can, in turn, affect the degree to which THg accumulation occurs within them. More variation can be seen throughout these species in the form of developmental timing. While some species such as the sandbar shark can reach sexual maturity at around 144-183 cm in length, others like the bull shark, do not mature until they reach anywhere from 180-230 cm. Many species have overlapping size ranges for maturity such as the blacktip shark which can reproduce at around 120-190 cm (Compagno, 1984; Compagno et al., 2005). This variation is also notable in terms of total lifespan as bull shark, blacktip shark, and sandbar shark can live up to 25, 12, and 21 years, respectively. Despite some living longer than others, sharks are long-lived species that do not reach sexual maturity for at least several years. Other differences between species include variations in migratory behavior. Many species undergo migrations at different intervals throughout the year to a variety of environments. For example, the Atlantic sharpnose shark undergoes inshore-offshore migrations during the winter months, while blacktip sharks partake in annual migrations along the coast of the southeastern United States (Parsons, 1983; Castro, 1996). These differences in habitat selection are important since they have the potential to cause differences in Hg concentrations between species (Branco et al., 2007).

This study investigated the concentrations of THg in the muscle, liver, and fin. The muscle is regularly used when investigating Hg concentrations in sharks for two reasons. Firstly, the muscle typically contains the highest concentrations of Hg and is most frequently consumed by humans (Pethybridge et al., 2010; Delshad et al., 2012; Hurtado-Banda et al., 2012). Secondly, muscle Hg concentrations regularly exceed the threshold level for adverse biological effects in fish and can provide information on

which species are at greatest risk. The liver serves as a detoxification organ, energy storage site, and provides energy reserves required during reproduction (Rossouw, 1987; Hurtado-Banda et al., 2012; Lyons and Lowe, 2013). Because of the liver's importance in the overall health of individual sharks, it is often included in Hg studies (Branco et al., 2007; Le Bourg et al., 2014; Endo et al., 2015). Lastly, although fins typically contain the lowest Hg concentrations of the three tissues, they are utilized for human consumption in the form of shark fin soup and medicinal pills, and therefore present another pathway for human exposure (Man et al, 2014). Although the practice of shark finning is an ecological threat in its own right, the potential for Hg toxicity from consuming shark fins is an issue that requires further investigation (Clarke et al., 2006, Man et al., 2014; Nalluri et al., 2014, Barcia et al., 2020).

1.1.6 Objectives of the Study

The goal of this study was to determine the concentration and tissue distribution of THg in eleven species of elasmobranchs (10 sharks and 1 stingray) caught off the southeastern United States and the northern Gulf of Mexico. This can be broken down into four objectives:

1. Determine the intra- and interspecies variability of THg concentrations in the muscle, liver, and fin of each species with the prediction that a) within a species, THg concentration in each tissue will increase with body length, and b) species with a higher trophic position, as inferred by $\delta^{15}N$ values, will have a higher concentration of THg.

2. For each species, determine the percentage of individuals that have a muscle and fin THg concentration that exceeds the 0.3 μg/g wet weight EPA human health criterion and 1.0 μg/g wet weight FDA action limit and at what body length each advisory level is surpassed. It is predicted that trophically higher species will have a greater percentage of individuals that exceed the advisories and that within a species, individuals of greater length will more likely exceed both advisories.

3. Determine the $\delta^{13}C$ and $\delta^{15}N$ values in muscle tissue for each species and investigate the relationship between $\delta^{13}C$ and $\delta^{15}N$ values and THg concentration, with the prediction that species with a higher $\delta^{15}N$ will have higher THg concentrations.

4. Determine whether muscle and fin THg concentrations can be used to predict the THg concentrations in other tissues, with the prediction that muscle will be a suitable predictor.

1.2. Methods

1.2.1 Sample Collection

Muscle, liver, and fin samples were collected by NOAA fishery observers working on commercial shark fishing vessels off North Carolina and the eastern and western coasts of Florida during November and December 2017. Tissue samples were also collected from fishing tournaments along the Texas coast (Port Aransas, Port Isabel, and Port O'Connor) in July and August 2016, and August 2019. The species sampled along with their corresponding sample sizes and collection locations are shown in Figure 1 and listed in Table 2.

For each individual sampled, the fork length (wingspan for the southern stingray) was recorded prior to tissue sampling (Table 3). Muscle, liver, and fin samples were obtained from each specimen and stored individually in labeled Ziploc bags, shipped to Texas State University, and held at -20°C until further processing. Sampling of each shark and stingray was opportunistic, therefore, all three tissues could not be sampled from every individual, e.g., a commercial vessel wanted to retain all the fins, and fishers at tournaments did not want their catch cut open to take a liver sample. The sample sizes for each tissue type by species are shown in Table 4. Muscle tissue was collected from below the anterior portion of the first dorsal fin, liver samples were taken from the tip of the left lobe, and an entire fin (either first dorsal, pectoral, or pelvic, depending on availability) was removed.

1.2.2 Sample Preparation

All muscle, liver, and fin samples were thawed, and the edges of the muscle and liver trimmed using a ceramic knife to remove any exogenous contamination. All

samples were then cut into small pieces and placed into 50 ml trace metal clean tubes. The wet weight of each sample was recorded before being freeze-dried (Labconco FreeZone2.5; Labconco, Kansas City, MO) at -54°C for 48 hours. After drying, the dry weight was recorded allowing moisture content percentages to be calculated (Table 4) and used for the conversion of dry weight to wet weight THg concentration. Muscle and liver samples were then homogenized into a powder and stored in trace metal clean tubes. The skin was removed from the fin samples using a ceramic knife or scalpel and the fins were then cut into small pieces (≤ 5 mm).

1.2.3 Mercury Analysis

Total Hg concentrations in muscle, liver, and fin were determined by analyzing a subsample of each tissue [mean ± standard deviation, minimum and maximum weight in parentheses; muscle = 14.2 ± 3.23 mg (5.30 - 22.1 mg); liver = 11.8 ± 2.45 mg (5.90 - 22.9 mg); fin = 46.89 ± 5.83 mg (33.4 - 51.8 mg)] in a Direct Mercury Analyzer (DMA-80; Milestone Inc., Shelton, CT) which uses thermal decomposition, gold amalgamation, and atomic absorption spectrometry, as described in EPA Method 7473 (U.S. EPA, 2007). The DMA was calibrated using certified reference materials [CRM; MESS-4 marine sediment, 0.08 µg/g THg; TORT-3 lobster hepatopancreas, 0.292 µg/g THg; and PACS-3 marine sediment, 2.98 µg/g THg; National Research Council Canada (NRCC)] as needed. Blanks (empty quartz boat: n = 125), CRMs [DORM-4 fish protein, 0.412 µg/g THg, NRCC (n = 62); DOLT-5 dogfish liver, 0.440 µg/g THg, NRCC (n = 21); and ERM-CE464 fish protein, 5.24 µg/g THg, European Reference Materials (n = 49)], and duplicate samples (n = 144) were used for quality control. Blank samples had a THg concentration ≤ 0.0001 µg/g. The CRM percentage recovery values (mean ± standard

deviation) were 97.2 ± 3.04% for DORM-4, 92.8 ± 4.81% for DOLT-5, and 97.1 ± 3.57% for ERM-CE464. The relative percentage difference in THg concentration in duplicate samples (mean ± standard deviation) was 1.42 ± 0.961% for the muscle (n = 28), 8.41 ± 7.16% for the liver (n = 20), and 15.6 ± 14.7% for the fin (n = 12).

1.2.4 Stable Isotope Analysis

A subset of 52 muscle samples (6 spinner shark, 8 bull shark, 8 blacktip shark, 3 dusky shark, 8 sandbar shark, 2 tiger shark, 8 lemon shark, 3 Atlantic sharpnose shark, 1 scalloped hammerhead, 4 great hammerhead, and 1 smooth hammerhead) were used for $\delta^{13}C$ and $\delta^{15}N$ analysis. Both lipids and urea were extracted prior to analysis following the protocol described in Li et al. (2015) which utilizes the rinsing of samples in a chloroform/methanol solution followed by rinses in deionized water. The process of removing lipids, which are deplete in $\delta^{13}C$, reduces variation when measuring carbon isotopes. Similarly, removing urea, which is $\delta^{15}N$ deplete, helps to remove variation when measuring nitrogen isotopes. For each sample, approximately 1.0 mg of muscle tissue was packaged into 3.5 x 5 mm tin capsules and shipped to the UC Davis Stable Isotope Facility (Davis, CA) for dual abundance $\delta^{13}C$ and $\delta^{15}N$ analysis using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Three randomly selected duplicate samples were also analyzed for quality control with relative percent differences between samples (mean ± standard deviation) being 1.84 ± 2.52% for $\delta^{13}C$ and 1.21 ± 1.83% for $\delta^{15}N$.

1.2.5 Statistical Analysis

All data was analyzed in R (R Core Team; Vienna, Austria-version 3.5.1) and SigmaPlot version 14 (Systat Software, San Jose, CA) at a 95% confidence level. Since sampling was opportunistic, sample sizes for all species and tissue types were not equal, therefore, species with limited sample sizes (Table 4) were excluded from statistical analyses. All data was tested for normality using the Shapiro-Wilk test and homoscedasticity using the Levene's test prior to analysis and was natural log-transformed if they did not meet the assumptions.

The sandbar and bull were the only two species that were sampled off both the southeastern United States and in the northern Gulf of Mexico that had adequate sample sizes for all tissues ($n > 5$). To determine if there were any significant differences in tissue THg concentrations based on sampling location an analysis of covariance (ANCOVA) utilizing fork length as the covariate was performed. For the sandbar shark, there was no significant difference in THg concentration for samples from the Gulf of Mexico and the Atlantic Ocean when using length as a covariate for muscle, $F_{(1,56)} = 1.57$, $p = 0.216$, liver, $F_{(1,55)} = 0.487$, $p = 0.331$, or fin, $F_{(1,9)} = 0.275$, $p = 0.612$. Similarly, no significant differences in THg concentrations between the two areas were detected for the bull shark for the muscle, $F_{(1,25)} = 3.50$, $p = 0.073$, liver $F_{(1,22)} = 0.614$, $p = 0.442$, or fin, $F_{(1,15)} = 0.545$, $p = 0.472$, when using length as a covariate. Since there were no significant differences in tissue THg concentrations for either species based on location, samples from the Gulf of Mexico and the Atlantic Ocean were incorporated together for all statistical analyses.

The relationship between the THg concentrations of each tissue and fork length

was examined using a linear regression analysis for bull, blacktip, and sandbar sharks. A linear regression analysis was also used to examine whether muscle and fin THg concentrations of these species can be used to predict the THg concentration in other tissues.

A one-way analysis of variance (ANOVA) and Tukey post-hoc test was used to examine whether there was a significant difference in THg concentrations among tissues within a species and within a tissue among species. If the assumptions of normality and homoscedasticity were not met a Kruskal-Wallis ANOVA on Ranks and Dunn's pairwise comparison was performed.

An ANOVA with Tukey post-hoc test was used to determine whether there was a significant difference in $\delta^{13}C$ and $\delta^{15}N$ values among species; only species with a $n \geq 5$ were examined. A linear regression analysis was used to determine if there was a relationship between $\delta^{13}C$ and $\delta^{15}N$ values, $\delta^{13}C$ values and THg concentrations, and $\delta^{15}N$ values and THg concentrations for all species combined.

1.3. Results

1.3.1 Interspecies Variability in Tissue THg Concentrations

The dry weight and wet weight muscle, liver, and fin THg concentrations in the twelve species sampled in this study are shown in Table 5. Overall, the highest THg concentrations were measured in the liver, followed by muscle, and then fin. The greatest mean THg concentrations were measured in the liver of the smooth hammerhead, lemon shark, and blacktip shark which contained mean THg concentrations (± SD) of 98.0, 61.1 ± 56.7, and 44.0 ± 39.4 µg/g dry weight, respectively. The lowest liver THg concentrations were measured in great hammerhead, dusky shark, and tiger shark which contained mean THg concentrations (± SD) of 2.02 ± 0.714, 1.55 ± 1.04, and 1.40 ± 0.285 µg/g dry weight, respectively. On average the muscle contained the second greatest THg concentration with the highest concentrations found in the smooth hammerhead, scalloped hammerhead, and great hammerhead which had mean concentrations (± SD) of 53.2, 13.8 ± 20.9, and 11.6 ± 1.43 µg/g dry weight, respectively. Tiger sharks, spinner sharks, and Atlantic sharpnose sharks contained the lowest muscle THg concentrations with mean concentrations (± SD) of 4.30 ± 0.637, 3.64 ± 2.30, and 2.26 ± 1.47 µg/g dry weight, respectively. Overall, the lowest mean THg concentrations were found within the fin with the bull shark, smooth hammerhead, and lemon shark having the greatest mean THg concentrations (2.08 ± 2.03, 1.25, and 1.23 ± 0.822 µg/g dry weight, respectively), while the dusky shark, great hammerhead, and Atlantic sharpnose had the lowest mean THg concentrations (0.167 ± 0.122, 0.113 ± 0.085, and 0.086 ± 0.097 µg/g dry weight, respectively).

Most species had small sample sizes, but the most abundant species were the bull

shark, blacktip shark, and sandbar shark (Table 2). When evaluating these three species, the mean THg concentrations were greatest in the liver, followed by muscle and fin. The greatest mean liver THg concentration (± SD) (dry weight) was measured in blacktip sharks (44.0 ± 39.4 µg/g), followed by bull sharks (34.6 ± 18.3 µg/g) and sandbar sharks (14.0 ± 10.8 µg/g). The bull sharks contained the greatest mean dry weight concentration of THg (± SD) in the muscle (9.05 ± 3.30 µg/g), followed by blacktip (7.74 ± 1.89 µg/g) and sandbar (5.76 ± 1.26 µg/g) sharks. The lowest concentrations of THg were detected within the fins of these three species with the bull sharks containing the greatest mean dry weight concentration (2.08 ± 2.03 µg/g) followed by the sandbar (0.409 ± 0.285 µg/g) and blacktip sharks (0.314 ± 0.270 µg/g).

Significant interspecies differences in the mean THg concentration of the muscle and liver were observed for species with a $n \geq 5$ [one-way ANOVA; muscle: $F_{(6,151)} = 12.0$, $p < 0.0001$; liver: $F_{(3,123)} = 12.1$, $p < 0.001$] as well as for the mean THg concentration of the fins of bull, blacktip, and sandbar sharks [one-way ANOVA: $F_{(2,38)} = 8.37$, $p < 0.002$] (Figure 2). The Tukey's post-hoc test determined that bull sharks and Atlantic sharpnose contained the greatest and lowest muscle THg concentrations of all species and were responsible for the overall one-way ANOVA result with the bull shark having a significantly higher THg concentration than all species ($p < 0.02$) and the Atlantic sharpnose having a significantly lower THg concentration than the bull, blacktip, and sandbar sharks ($p \leq 0.003$). The Tukey's post-hoc test for the liver determined that the sandbar shark was responsible for the overall one-way ANOVA result as it contained a significantly lower concentration of THg when compared to the bull, blacktip, and lemon shark ($p < 0.01$). Lastly, the Tukey's post-hoc test for the fin determined that the

mean THg concentration of the bull shark was significantly greater than that of the blacktip and sandbar shark ($p \leq 0.006$).

1.3.2 Relationship between tissue THg concentrations and body length in Bull, Blacktip, and Sandbar Sharks

The relationship between muscle, liver, and fin THg concentration and body length in bull, blacktip, and sandbar sharks is shown in Figure 3. Significant positive relationships were observed between body length and the THg concentration in both muscle ($p < 0.001$) and liver ($p < 0.01$) for all three species. The bull shark was the only species that had a positive relationship between body length and the THg concentration in the fin ($p < 0.001$). The relationship between THg concentration in muscle, liver, and fin, and body length was not investigated for the other species due to the small sample size.

1.3.3 Human Health Implications

The percentage of muscle and fin THg concentrations from each investigated species that exceeded the U.S. EPA Hg human health criterion (0.3 µg/g wet weight) and the FDA Hg action limit (1.0 µg/g wet weight) regarding human consumption and the body length at which they start to be exceeded are shown in Table 6. All species had individuals that contained THg concentrations within the muscle that exceeded both the EPA and FDA advisories. Blacktip shark and Atlantic sharpnose were the only species that had individuals with muscle THg concentrations lower than the EPA's recommended Hg human health criterion. Despite this, both species still had most individuals surpass the advisory with 93.2% of the blacktips and 62.5% of the Atlantic sharpnose exceeding 0.3 µg/g wet weight. The FDA Hg action limit was also regularly surpassed with nine species (bull shark, blacktip shark, dusky shark, sandbar shark, lemon shark, scalloped

hammerhead, great hammerhead, smooth hammerhead, and southern stingray) having more than 50% of individuals exceed 1.0 µg/g wet weight. The bull shark and hammerhead sharks exceeded this limit most commonly with 96.4% of bull sharks and all three species of hammerheads containing >1.0 µg/g wet weight THg in the muscle.

The fins sampled in this study failed to surpass the advisories as frequently as the muscle, with five species exceeding the EPA limit (bull shark, blacktip shark, sandbar shark, lemon shark, and smooth hammerhead) and only one species surpassing the FDA limit (bull shark). The greatest occurrence of the advisories being exceeded occurred in the bull sharks with 77.8% exceeding 0.3 µg/g wet weight and 33.3% exceeding 1.0 µg/g wet weight. The sandbar and blacktips also had some individuals exceed the EPA limit, albeit to a lesser degree, with 8.33% and 9.09% containing > 0.3 µg/g wet weight THg in the fin, respectively.

The minimum body lengths at which both the EPA and FDA advisories are exceeded for each species are also listed in Table 6. Within the muscle of many of the species analyzed, fewer individuals exceeded the FDA action limit than the EPA human health criterion. However, in most cases, those that surpassed the FDA limit did so at greater body lengths than those that surpassed the EPA limit. For the fins, the few species that managed to surpass the EPA limit did so at body lengths greater than the minimum body length that was required to surpass the limit within the muscle. For example, the minimum body length that the blacktip sharks surpassed the 0.3 µg/g wet weight limit within the fins was 154 cm, but for the muscle, individuals were able to surpass 1.0 µg/g wet weight at a smaller body length of only 107 cm.

1.3.4 Stable Isotope Analysis of Muscle Tissue

$\delta^{13}C$ and $\delta^{15}N$ values for each species are displayed in Figure 4 and Table 7. $\delta^{13}C$ ranged from -16.89 to -14.33‰ across all species. The great hammerhead was the most enriched in C of all species (mean $\delta^{13}C$ = -15.04‰) while the spinner shark was the most deplete (mean $\delta^{13}C$ = -16.42‰). Significant differences in $\delta^{13}C$ values between species were detected using a one-way ANOVA [$F_{(5,36)} = 6.81$, $p < 0.001$] on species with a $n \geq 5$. A Tukey's post-hoc test determined that significant differences were detected between spinner and bull ($p = 0.047$), spinner and blacktip ($p = 0.008$), spinner and lemon ($p = 0.002$), blacktip and sandbar ($p = 0.009$), and sandbar and lemon shark ($p < 0.001$).

$\delta^{15}N$ values ranged from 12.57 to 17.33‰. The greatest $\delta^{15}N$ values were measured in the Atlantic sharpnose which had a mean value of 16.99‰. The blacktips were the most deplete amongst all species with 13.46‰. Significant differences in $\delta^{15}N$ values were detected between many of the species by utilizing a one-way ANOVA [$F_{(5,36)} = 11.2$, $p < 0.001$]. A Tukey's post-hoc test found significant differences between the blacktip and spinner ($p < 0.001$), spinner and lemon ($p < 0.001$), bull and blacktip (p = 0.002), bull and lemon ($p = 0.005$), sandbar and blacktip ($p = 0.007$), Atlantic sharpnose and blacktip ($p <0.001$), Atlantic sharpnose and sandbar ($p = 0.046$), and Atlantic sharpnose and lemon shark ($p < 0.001$).

A significant negative relationship was observed between $\delta^{15}N$ and $\delta^{13}C$ values ($p < 0.001$) for all species combined (Figure 5). A significant relationship was detected between muscle THg concentration and $\delta^{13}C$ ($p = 0.03$) for all species combined; however, the model fails to significantly explain the variation within the dataset ($R^2 = 0.087$) (Figure 6A). There was no relationship detected between muscle THg

concentration and $\delta^{15}N$ values for the species analyzed in this study ($p = 0.056$; Figure 6B).

1.3.5 Muscle and Fin THg Concentrations as a Predictor of Other Tissue Concentrations in Bull, Blacktip, and Sandbar Sharks

Relationships between tissue THg concentration were analyzed for bull, blacktip, and sandbar sharks using the THg within the muscle and fin to predict the THg concentration in one another as well THg concentrations in the liver (Figures 7 and 8). Significant positive relationships were found for the bull shark when using the muscle as a predictor for THg concentrations in the liver ($R^2 = 0.56$; $p < 0.001$) and fin ($R^2 = 0.35$; $p = 0.01$) as well as when using the fin as a predictor for the THg concentrations in the liver ($R^2 = 0.72$; $p < 0.001$) and muscle ($R^2 = 0.35$; $p = 0.01$). Despite the significant relationships detected, the low R^2 values for the relationship between the THg in the muscle and fin indicate that these models are weak and may be inadequate for predicting THg concentrations in these tissues. However, the relationships between the THg concentrations in the muscle and liver and the fin and liver of bull sharks are stronger and explain more of the variation in the data. The blacktip and sandbar sharks had significant positive relationships between the THg concentrations in the muscle and liver ($R^2 = 0.84$; $p < 0.001$ and $R^2 = 0.45$; $p < 0.001$, respectively) but no significant relationships were found for either species when using the muscle to predict concentrations in the fin ($p > 0.05$) or when using the fin as a predictor for concentrations in the muscle and liver ($p > 0.05$). Once again, while the R^2 for the relationship between THg concentrations in the muscle and liver is strong enough to explain the variation in the data for the blacktip shark, the low R^2 value for the same tissues in the sandbar shark was too weak to

adequately explain the variation in the model.

1.4. Discussion

This study provided new and updated information on the concentrations of THg present in shark species off the southeastern United States and the northern Gulf of Mexico and how factors such as species and size can contribute to the increased bioaccumulation of THg in their tissues. In addition, this study provided evidence of significant differences in THg concentrations between the examined species as well as between the different tissue types within a species. Further evidence which emphasizes the importance and necessity of food safety practices concerning Hg exposure are also provided, based on the large number of sharks in this study that contained elevated concentrations within tissues that are consumed by humans. Lastly, the effectiveness of utilizing the THg concentrations from one tissue to predict those within another was also investigated with results varying based on species and tissue type.

1.4.1 Interspecific Variability in Tissue THg Concentrations

Although elasmobranchs are known to accumulate high levels of Hg, the concentrations within different species can vary greatly. The age, location, and diet of a species represent several factors that can affect their rate of Hg intake and have been used previously to explain the differences in concentrations between them (Adams and McMichael, 1999; Szczebak and Taylor, 2011). In the present study, concentrations of THg varied by both species and tissue type. Significant interspecies differences were detected when comparing THg concentrations within the muscle, liver, and fin. Of the three tissues analyzed the highest concentrations were detected within the liver, followed by the muscle and the fin. This was the case for the bull, blacktip, sandbar, lemon,

Atlantic sharpnose, and smooth hammerhead shark. Although similar findings on the tissue distribution of Hg have been previously reported within teleost fish (Mieiro et al., 2009; Adams et al., 2010), concentrations within sharks do not typically follow this trend. Past studies have routinely found that elasmobranchs contain higher levels of Hg in muscle tissue compared to the liver (Pethybridge et al., 2010, Delshad et al., 2012, Hurtado-Banda et al., 2012; Mull et al., 2012; O'Bryhim et al, 2017). The high concentrations normally detected within the muscle are usually accredited to Hg's affinity for the sulfhydryl groups associated with the thiol-containing amino acids found within the protein-rich muscle tissue (Bloom, 1992). On the other hand, the lower Hg levels commonly reported within the liver are often explained due to its ability to serve as an agent for contaminant detoxification within the body. Despite the liver typically containing lower concentrations of THg than the muscle, there are a small number of findings like those reported here, in which the THg was greater in the liver when compared to the muscle in blacktip (Boush and Thieleke, 1983), bull (Ruelas-Inzunza and Páez-Osuna, 2005; Branco et al., 2008), and sandbar sharks (Branco et al., 2008) throughout the Pacific Ocean.

The greatest liver THg concentrations were found in the smooth hammerhead, lemon, and blacktip shark. Information on the THg concentration in the liver of the smooth hammerhead has not been reported previously in this area, as the individual sample analyzed in this study was the first verified record of the species within the northern Gulf of Mexico (Deacy et al., 2020). Total Hg concentrations in the liver of lemon (0.111 µg/g wet weight) (Nam et al., 2011) and blacktip sharks (0.868 µg/g wet weight) (Reistad et al., 2021) have been reported previously off the Atlantic Coast of

Florida, however, those studies focused on juveniles rather than adults and therefore reported concentrations that were much lower compared the adults sampled in this study (lemon = 43.9 µg/g wet weight; blacktip = 18.1 µg/g wet weight).

Although there was high interspecies variability in THg concentrations in the liver among species, only four species (bull, blacktip, sandbar, and lemon shark) had large enough sample sizes for adequate statistical comparisons. Of those four species analyzed, all contained similar mean THg concentrations except for the sandbar shark which had a mean concentration that was significantly lower than the others. Despite having a lower concentration than the other three species, the sandbar still contained a mean THg concentration of 14.0 µg/g dry weight which was higher than the mean muscle THg found in all species in this study excluding the three *Sphyrnids*. There are several explanations for why Hg is accumulating to such a high degree within the livers of the sharks in this study with most relating to the liver's capability for detoxifying contaminants. These include strong protein-binding occurring within the liver in a matter similar to what has been found to happen with cadmium (Cd) (Lucis et al., 1970; Boush and Thieleke, 1983), potentially insufficient concentrations of Se available within the liver to counteract the high levels of Hg being accumulated (i.e. Se:Hg molar ratios < 1), or recent exposure to Hg took place before sampling and was concentrated within the liver soon after. Another factor that could have led to increased THg concentrations in the liver involves the speciation of Hg throughout the body. As mentioned previously, the majority of THg in the muscle, is present as MeHg while the majority in the liver is made up of inorganic Hg (Storelli et al., 2002; Branco et al., 2007; Nam et al., 2011). This is due to the process of demethylation of MeHg that is known to occur within the liver, in

which MeHg is converted into inorganic Hg. While this has been known to serve as an elimination pathway for Hg in the body, the high concentrations of THg in the sharks in this study may indicate a decreasing demethylation efficiency in their livers as they age (Wagemann et al., 1998; Storelli et al., 2002). If these sharks are not able to demethylate and remove MeHg from the liver effectively, it is likely to build up to high concentrations such as those observed in this study.

Mercury not stored in the liver or eliminated in the feces is then distributed throughout the body and cumulatively stored within the muscle where it can accumulate to high concentrations (Branco et al., 2007; Yang et al., 2008). Although concentrations in this study were highest overall within the liver, high levels of THg were also found within the muscle of each species. The highest concentrations of muscle were detected within the three species of hammerhead sharks with each containing >10 μg/g THg dry weight. These concentrations, along with those of the other species reported in this study, are greater than or equal to what has previously been reported for these species in other locations such as the Caribbean and the northern coast of Australia (Lyle, 1984; Maz-Courrau et al., 2012; Rumbold et al., 2014; Mohammed and Mohammed, 2017; Ruelas-Inzunza et al., 2020). Of the three hammerhead species investigated in this study, THg concentrations for these species in the southeastern coast of the United States have only been reported in the muscle of the great hammerhead (1.54 μg/g wet weight) (Rumbold et al., 2014). While concentrations of THg in the scalloped hammerhead have been reported off the northern coast of Trinidad (0.074 – 1.90 μg/g wet weight), concentrations for the smooth hammerhead have not been reported in the Atlantic Ocean previously. They have been throughout parts of the Pacific Ocean, however. The highest mean concentration

reported in the smooth hammerhead was 8.25 µg/g wet weight in the Gulf of California (García-Hernández et al., 2007), while other studies found much lower mean concentrations of 2.27 µg/g dry weight off the eastern coast of South Africa (McKinney et al., 2016) and 0.73 µg/g wet weight from the Mexican Pacific Ocean (Escobar-Sánchez et al., 2010). Mean THg concentrations for the scalloped hammerhead were reported to be 1.56 µg/g dry weight off the eastern coast of South Africa (McKinney et al., 2016). The concentrations reported in this study were higher than those reported previously throughout the Pacific and the Atlantic Ocean for each of the three species analyzed.

Although most species contained high THg concentrations within the muscle, not all species contained similar amounts. This provides further evidence to suggest that various factors are playing a role in the accumulation of THg within these sharks. The diet and age of these organisms are the likely explanation for why these differences are occurring. The significant differences in the muscle THg concentration between species were mainly centered around the Atlantic sharpnose and bull sharks which contained the lowest and highest mean THg levels amongst those analyzed, respectively. In the case of these two species, both their diets and age ranges differ greatly, which likely explains the large differences in THg levels found between them. For instance, since the Atlantic sharpnose is a much smaller species its diet is limited to smaller prey items which are less likely to contain high concentrations of Hg compared to the larger prey of species such as the bull shark.

Overall, the fins had the lowest sample sizes and contained the lowest concentrations of THg amongst all tissues. Collecting fin samples can be difficult since they are one of the most highly traded shark tissues globally and most commercial fishing

operations are reluctant to give them up due to the potential profits that can be made from selling them. Because of this, few studies have investigated Hg concentrations within shark fins previously. Many of those that have, have only done so on a limited number of species or used fins that have already been dried and/or processed for human consumption (Escobar-Sánchez et al., 2010; Delshad et al., 2012; Man et al., 2014; Nalluri et al., 2014; Barcia et al., 2020; Gelsleichter et al., 2020). Of the sharks analyzed in this study, the greatest fin THg concentrations (μg/g dry weight) were measured in the bull (2.08), smooth hammerhead (1.25), and lemon shark (1.23). These findings are consistent with other studies that have found low THg concentrations in the fin compared to other tissue types (Escobar-Sánchez et al., 2010; Delshad et al., 2012; O'Bryhim et al., 2017; Gelsleichter et al., 2020). This is likely due to the internal composition of the fins. The cartilaginous ceratotrichia that make up the internal structure of the fins are less likely to accumulate Hg to the degree that other tissues are capable of due to the minimal amount of vascularization within them (Nalluri et al., 2014).

The concentrations of THg reported in this study either fall within or exceed the estimated range of concentrations in which biochemical changes, cell and tissue damage, and reduced reproductive success are thought to occur within teleost fish (0.5 – 1.0 μg/g wet weight) (Sandheinrich and Wiener, 2011). If the negative effects associated with Hg in teleost fish occur similarly within sharks, they are likely already dealing with these complications based on the high concentrations observed in their tissues. While these impacts are important at the individual level, they also have the potential to have profound effects on the overall population by potentially altering the reproductive success of the individual and thus decreasing the overall fitness of the species. Based on the

elevated concentrations of THg in the tissues of sharks in this study, and the information available regarding the toxicological effects of Hg in teleost fish, future studies are warranted in order to investigate the potentially toxic effects that Hg may have on shark species throughout the northern Gulf of Mexico and off the southeastern United States.

1.4.2 Relationship between Tissue THg Concentrations and Body Length in Bull, Blacktip, and Sandbar Sharks

The positive relationship found between THg concentration and body length of the three most sampled species in this study further suggests that larger, presumably older individuals, contained greater amounts of Hg within their tissues. Overall, based on the strength of the R^2 values from the regression analyses, the strongest relationships were detected within the livers of the blacktip and sandbar sharks, followed by the muscle of bull and blacktip sharks. Positive relationships similar to these have been recorded previously in bull and blacktip sharks off the coast of Florida (Adams and McMichael, 1999; Rumbold, 2014; Matulik et al., 2017). This relationship has also been observed in multiple species of sharks in marine environments around the world including those such as the common thresher and shortfin mako in the northeast Pacific Ocean (Suk and Smith, 2009), tiger sharks from the coast of Japan (Endo et al., 2008), blacknose sharks (*Carcharhinus acronotus*) off the coast of Florida (Matulik et al., 2017), and the narrownose smoothhound (*Mustelus schimitti*) off the coast of Brazil (Marcovecchio et al., 1991). The fins of the blacktip and sandbar sharks were the only tissues that did not have a significant relationship with fork length. As mentioned previously, the cartilaginous ceratotrichia that make up the internal structure of the fins are less likely to accumulate Hg than other more vascularized tissues such as the muscle. The findings of

this analysis indicate that the amount of THg within the various tissues of these species is increasing as they grow. The accumulation of large concentrations across multiple tissues indicates that the methods of detoxification and elimination used by these species become increasingly ineffective as they increase in size.

1.4.3 Human Health Implications

The THg assessment done in this study also provided information on the potential risks humans face when they choose to consume shark. Since many of the species sampled in this study are readily targeted in both commercial and recreational fisheries in the United States for human consumption it is important to emphasize the threats that people face when they eat them. As mentioned previously, MeHg accounts for the majority (83-97%) of THg found in muscle tissue in sharks (Storelli et al., 2002; Krystek and Ritsema, 2004; Branco et al., 2007; de Carvalho et al., 2014; Ehnert-Russo and Gelsleichter, 2020; Kazama et al., 2020). Because of this, people who consume shark muscle face an increased risk of being exposed to large amounts of MeHg based on the high concentrations of THg that were reported in this study. Almost every individual shark in this study had a THg concentration that surpassed the advisory set forth by the EPA (0.3 μg/g wet weight). More concerning, however, is the fact that most also surpassed the FDA advisory (1.0 μg/g wet weight) with all but three species (spinner, tiger, and Atlantic sharpnose) having more than 60% of individuals exceeding this limit. Because of this, the consumption of nearly every species in this study could pose a health risk to anyone who chooses to consume them.

Unlike muscle, the number of species that had THg concentrations in the fin that exceeded the advisories was much lower. Only three of the nine species analyzed for fin

THg concentrations had ≥ 50% of their samples exceed the EPA Hg human health criterion of 0.3 μg/g wet weight [smooth hammerhead (100%), bull (77.8%), and lemon shark (50.0%)]. The bull shark was also the only species to have individuals with THg concentrations in the fin exceeding the FDA Hg action limit of 1.0 μg/g wet weight. Despite the limited number of species that managed to exceed the advisories for the fin, those that did still raise cause for concern. Within the fins, MeHg has been found to make up around 62% of the THg on average (Nalluri et al., 2014). Although this percentage is lower than that of the muscle, it still means that the majority of Hg that someone is exposed to when they consume shark fins is toxic MeHg. However, with nearly 78% of bull shark fins exceeding the EPA human health criterion for Hg and 33% surpassing the FDA Hg Action limit, the consumption of the fins from this species can still pose a risk for humans. In addition to this, even though many of the species failed to surpass these limits, fins used for dishes like shark fin soup have been found to originate from a variety of species, which are usually unknown to both those who both serve and consume them (Cardeñosa et al., 2020). Therefore, when one chooses to eat dishes containing shark fins there is little way of knowing whether the species being consumed is one that is known to contain high levels of Hg.

The minimum lengths at which these thresholds were surpassed were also determined. The species that did exceed the EPA Hg human health criterion did so well before their recorded size ranges of maturity. This indicates that these species are capable of accumulating harmful levels of Hg at early life stages. On the other hand, those that managed to surpass the FDA Hg action limit did so within their reported size ranges at maturity (Compagno, 1984; Compagno et al., 2005). These results, along with those from

the intraspecies variation portion of this study which found significant correlations between body length and THg concentration, provide evidence to suggest that concentrations of THg in these sharks accumulate rapidly and will continue to do so throughout their lifespans. With most of these species exceeding advisory levels, it is clear that the consumption of shark muscle should be limited, if not completely avoided.

1.4.4 Stable Isotope Analysis of Muscle Tissue

Although the isotopic values reported in this study add to the existing information available and provide information on the stable isotope values of sharks that have previously been unreported in the southeastern United States and the northern Gulf of Mexico, there was a large degree of variability in both $\delta^{13}C$ and $\delta^{15}N$ values amongst those analyzed. Since samples were collected from different locations, the spatial variation in baseline carbon and nitrogen values could explain the wide range of isotopic values that were observed both within a species and between species. Because of this, it is difficult to make conclusions based on this data without first gaining a better understanding of the baseline isotopic values in the different samples areas and how they may differ.

In regards to $\delta^{13}C$, nearly all species contained a narrow range of values (~ 1‰) which may suggest that these species are feeding on localized sets of prey items. Despite this, the significant differences found between many of the species analyzed suggest that there is variability in the carbon pools that make up their diets. These differences may be a product of the variability in $\delta^{13}C$ values found in the various environments from which these species are feeding or could be caused by differences in feeding based size and age of the analyzed species. For instance, the waters of pelagic environments are deplete in

$\delta^{13}C$, while coastal environments are typically more enriched (Hussey et al., 2010; Kiszka et al., 2015). Therefore, since the species analyzed in this study contained intermediate $\delta^{13}C$ values on average, it is likely that there is varied use of both pelagic and coastal waters across species (Rumbold et al., 2014). This is also evident from the significant negative relationship found between the $\delta^{13}C$ and $\delta^{15}N$ values of each species. Based upon this relationship, as $\delta^{13}C$ levels become more enriched, the $\delta^{15}N$ values of these sharks should become more deplete, which may suggest that trophic position decreases as species shift from the carbon pools of pelagic environments to coastal ones. Despite the significant relationship observed, the $\delta^{13}C$ values reported here should be interpreted with caution as many of these species, such as the sandbar shark and blacktip shark, are highly migratory and could therefore have $\delta^{13}C$ values representative of different environments (Castro, 1996; Grubbs et al., 2007; Shiffman et al., 2014).

When compared to previous studies the $\delta^{13}C$ values reported here were more deplete. Rumbold et al. (2014) found more enriched $\delta^{13}C$ values in species sampled off the southwest coast of Florida including the bull (-13.2‰), blacktip (-13.3‰), tiger (-12.7‰), lemon (-12.6‰), Atlantic sharpnose (-13.6‰), and great hammerhead shark (-12.3‰). Both that study as well as this one were limited in sample sizes for many of the species analyzed when it came to stable isotope analysis.

Interspecific variability in $\delta^{15}N$ values suggests that differences in trophic position may be present for many of the species analyzed. Overall, the species with the most enriched $\delta^{15}N$ values were the Atlantic sharpnose, followed by the scalloped hammerhead and spinner sharks. Atlantic sharpnose values reported here (17.0‰) are much higher than what has been reported for this species in the Gulf of Mexico previously (14.1‰;

Drymon et al., 2012). It is difficult to determine why these values are so much higher than what is normally recorded. It is possible that these values could have been skewed by the input of an anthropogenic nitrogen signature that would have caused artificial enrichment, but it is difficult to know if this was the case. One natural explanation for these high values could be due to locational variability caused by their seasonal migrations. Atlantic sharpnose sharks are known to spend the winter in offshore waters in the Gulf of Mexico where they alter their diet to feed on prey at higher trophic positions. This behavior has been previously reported in other predatory marine species within the Gulf of Mexico, such as the bottlenose dolphin (Barros et al., 2010). Drymon et al. (2012) reported intraspecific differences in $\delta^{15}N$ values within Atlantic Sharpnose off the coasts of Mississippi and Alabama due to seasonal shifts (14.2‰ in the spring, 13.7‰ in the summer, and 12.3‰ in the fall), however, even the most enriched $\delta^{15}N$ values they reported were still lower than what was observed in this study. The most deplete $\delta^{15}N$ values amongst all species were found in the blacktip, tiger, and lemon sharks. This agrees with the order of assigned general trophic positions given to these species in the past (Cortés, 1999). However, since trophic position will change based upon location, other factors may be influencing these values. For example, each of these species contained relatively similar $\delta^{13}C$ values (blacktip = -15.7; tiger = -15.6; lemon = -15.5) indicating the possibility of shared resource usage between them which may explain why $\delta^{15}N$ values were low in each of these species. In addition to this, the low $\delta^{15}N$ values of the tiger shark could be explained by the significant ontogenetic shifts in feeding patterns that occur after a certain size range (> 200 cm) (Lowe et al., 1996). These shifts are thought to occur due to their ability to catch larger and more agile prey and therefore

place them at higher trophic positions (Cortés, 1999). However, while one of the individuals in this study surpassed this size with a fork length of 235 cm, it was more deplete in $\delta^{15}N$ (12.8‰) than the smaller individual that had a fork length of only 199 cm (14.28‰).

Like $\delta^{13}C$, consideration for spatial differences in samples should be used when interpreting the $\delta^{15}N$ values of these sharks. Various factors, such as differing amounts of anthropogenic nitrogen input, have been found to affect $\delta^{15}N$ values in marine environments throughout the Gulf of Mexico previously and could potentially explain some of the variability in the isotopic values reported in this study (Barile, 2004; Kwon et al., 2018). Because of this, future studies involving the use of stable isotopes in sharks should include stomach content analyses, if possible, to better understand and explain the variations in diet such as those seen in this study. In addition to this, food webs could potentially be constructed by obtaining the isotopic values of prey items which may reduce bias caused by spatiotemporal variation in baseline nitrogen values, thus allowing for a more direct comparison between species.

Overall, the $\delta^{15}N$ values for all species in this study were higher than what has been recorded for sharks off the Gulf Coast of Florida previously (Rumbold et al., 2014; Matulik et al., 2017). The methods used for lipid extraction in this study included a second step for the removal of urea from the muscle tissue. Urea is a soluble nitrogenous compound that has been found to artificially lower $\delta^{15}N$ values of shark tissue by an average of 1.2 ± 0.6‰ (Li et al., 2016). Because of this, removing it from the samples analyzed in this study may explain why the values reported here are more enriched when compared to other studies which used different tissues and did not remove urea in

addition to lipid extraction.

A weak significant linear relationship ($R^2 = 0.087$, $p = 0.03$) was found between THg concentration in the muscle and the $\delta^{13}C$ of each species. Typically, a positive relationship is seen between THg and $\delta^{15}N$ since increased levels of Hg are expected as trophic levels increase thus indicating the occurrence of biomagnification through the food web. However, no relationship was observed for the sharks in this study, likely due to the intraspecies variability of $\delta^{15}N$ across most of the species analyzed. Although the lack of a relationship between $\delta^{15}N$ and THg concentration may indicate the possibility of lower rates of biomagnification in these environments, the large concentrations of THg detected within each species along with the significant relationships found between THg and length indicate that Hg is biomagnifying through marine food webs.

1.4.5 Muscle and Fin THg Concentrations as a Predictor of Other Tissue Concentrations in Bull, Blacktip, and Sandbar Sharks

Another objective of this study was to determine the effectiveness of using the THg concentrations of one tissue (muscle or fin) to estimate the concentration of the other tissues. When looking at the regression analyses for the three most abundant species, the muscle was the most effective tissue to use in order to predict the THg concentrations within the liver. Significant relationships were found for all three species when comparing those two tissues. Of the three species analyzed, this method of estimation was strongest for the blacktip ($R^2 = 0.84$), with the bull ($R^2 = 0.56$) and sandbar ($R^2 = 0.45$) models being less effective. The only other tissues that contained positive relationships were the fin and liver and the fin and muscle from the bull shark. This is notable since the bull shark was one of the only species to contain elevated

concentrations of THg within the fins. The higher concentrations within them may have increased their effectiveness in estimating the concentrations within other tissues such as the liver. Although there was a significant relationship detected between the fin and the muscle, the model fails to reliably explain the variation among individual samples (R^2 = 0.35). Based on these results, of the investigated tissues, the muscle is likely the only one that could be used to effectively predict the THg concentration in other tissues. The effectiveness of the muscle to predict the concentrations within the liver indicates that muscle biopsies may present a reliable option for studying Hg concentrations within the tissues of elasmobranchs. This method can help to ensure minimal harm and reduce the use of lethal sampling practices on species that are already threatened or endangered. In addition to his, future studies should investigate the practicality of using THg concentrations within shark blood as it may provide an even less invasive method of sampling compared to muscle biopsies. One factor that would need to be considered, however, would be the turnover time for Hg within the blood since it would be more representative of recent exposures to Hg compared to the muscle which provides a more long-term account of Hg accumulation in the body (Rumbold et al., 2014).

1.4.6 Conclusions

Based on the results of this study, shark species off the southeastern United States and in the northern Gulf of Mexico are accumulating high concentrations of THg in their muscle, liver, and fin. The variability in THg concentrations between species and tissue type suggests that differences in diet, age, and metabolic processes are occurring. Based on the interpretations of the stable isotope data presented, it is evident that there is variability in the feeding patterns between species despite each of them feeding from a

narrow range of $\delta^{13}C$ values. The data presented also provides evidence that muscle biopsies taken from bull, blacktip, and sandbar sharks can be used to estimate the concentrations of THg within the liver of these species to reduce the need for lethal sampling practices. Lastly, based on the high concentrations of THg found within the muscle and fin in the sharks in this study, the human consumption of shark tissues, especially by pregnant women and children, should be avoided to reduce the risk of exposure to Hg and the negative health effects associated with it.

1.4.7 Future Research

Although THg concentrations in sharks have been better studied in recent years, there is still a lot that is not known about the effects it can have on the health of these species. The difficulty in researching these large marine predators has led to limited sample sizes in both species and tissue types in this study as well as those done previously. Future Hg studies utilizing increased sample sizes are important to gain a more accurate understanding of the concentrations of Hg within these species and others throughout the southeastern United States and northern Gulf of Mexico. For instance, there is still little known about the THg concentrations in the spinner, dusky, and hammerhead sharks which were analyzed in a limited fashion in this study. Increased sample sizes would allow for more reliable statistical analyses to be performed and allow for a better understanding of how Hg is accumulating within these species.

Despite being studied in other taxa like teleost fish, there is still little understanding of the health effects of Hg in large predatory sharks. Although difficult to study due to their large size and the inability to monitor them in a laboratory setting, future research needs to be performed to understand how sharks are being affected by the

large concentrations of Hg that they have been found to accumulate. Despite Hg being found to have harmful impacts on multiple tissue types within mammals, birds, and smaller teleost fish, little information is available on the potential effects it can have on the organs of large shark species. If these deleterious health effects are present within sharks, future research is required to identify what tissues are being affected and how. One factor that could help identify the potential for Hg to be affecting these sharks is its interaction with selenium (Se). Selenium has been found to have an antagonistic relationship with Hg and has the potential to mitigate the toxic effects it can cause within the body by forming toxicologically inert Hg-Se complexes (Endo et al., 2005; Ralston and Raymond, 2010). However, Se is only capable of serving a protective role against Hg if it is in molar excess. Because of this, Se:Hg molar ratios provide information on the capability of Se to protect against Hg toxicity with ratios > 1 suggesting that there is potential for Se to play a protective role against Hg while ratios < 1 indicate that there are inadequate amounts of Se available. The use of Se:Hg molar ratios has become common in Hg studies involving sharks (Lyle, 1986; Escobar-Sánchez et al., 2011; Nam et al., 2011; Bergés-Tiznado et al., 2015; Dutton and Venuti, 2019; Pancaldi et al., 2019) and should be included in future research to better understand the relationship between these two elements in sharks and to provide insight into why these sharks are capable of surviving despite accumulating such high concentrations of Hg in their bodies.

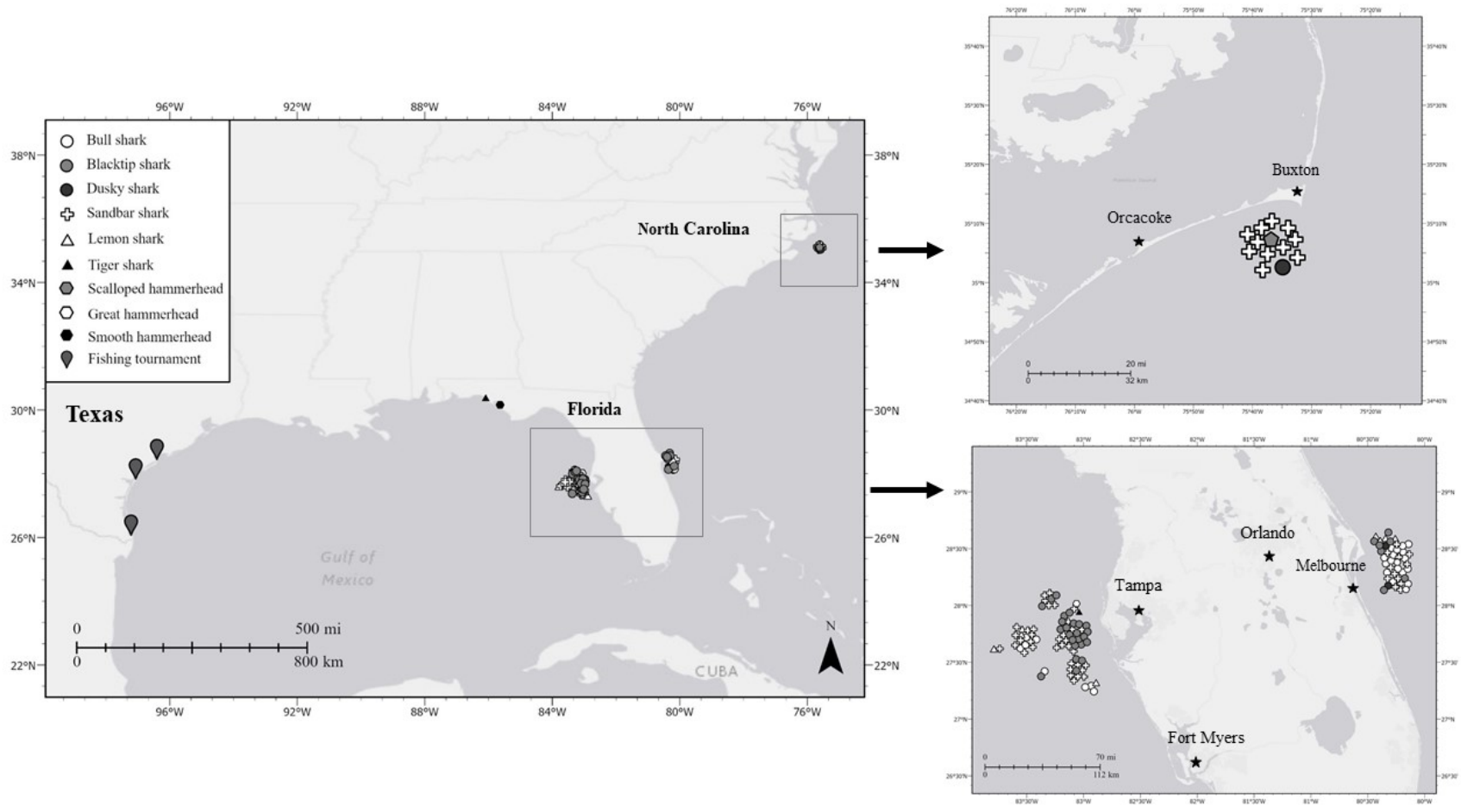

Figure 1**.** Collection locations for each species. All Atlantic sharpnose, spinner shark, and southern stingray samples were collected from fishing tournaments on the Texas coast along with additional blacktip, bull, tiger, great hammerhead, and scalloped hammerhead samples.

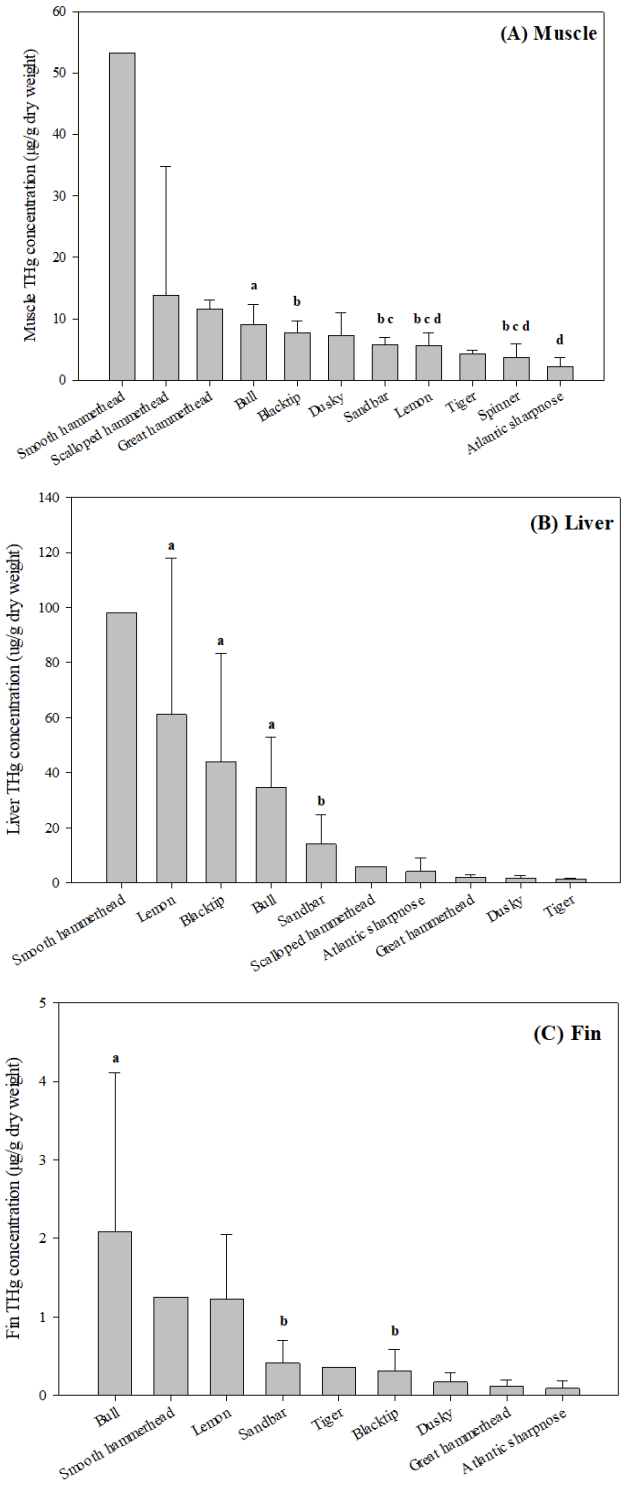

Figure 2. Mean (± standard deviation) muscle (A), liver (B), and fin (C) THg concentrations in the twelve species investigated in this study. Lowercase letters represent species grouped by similar tissue THg concentrations (one-way ANOVA). If the tissue sample size for a given species was small (≤ 5) it was not included in the statistical analysis.

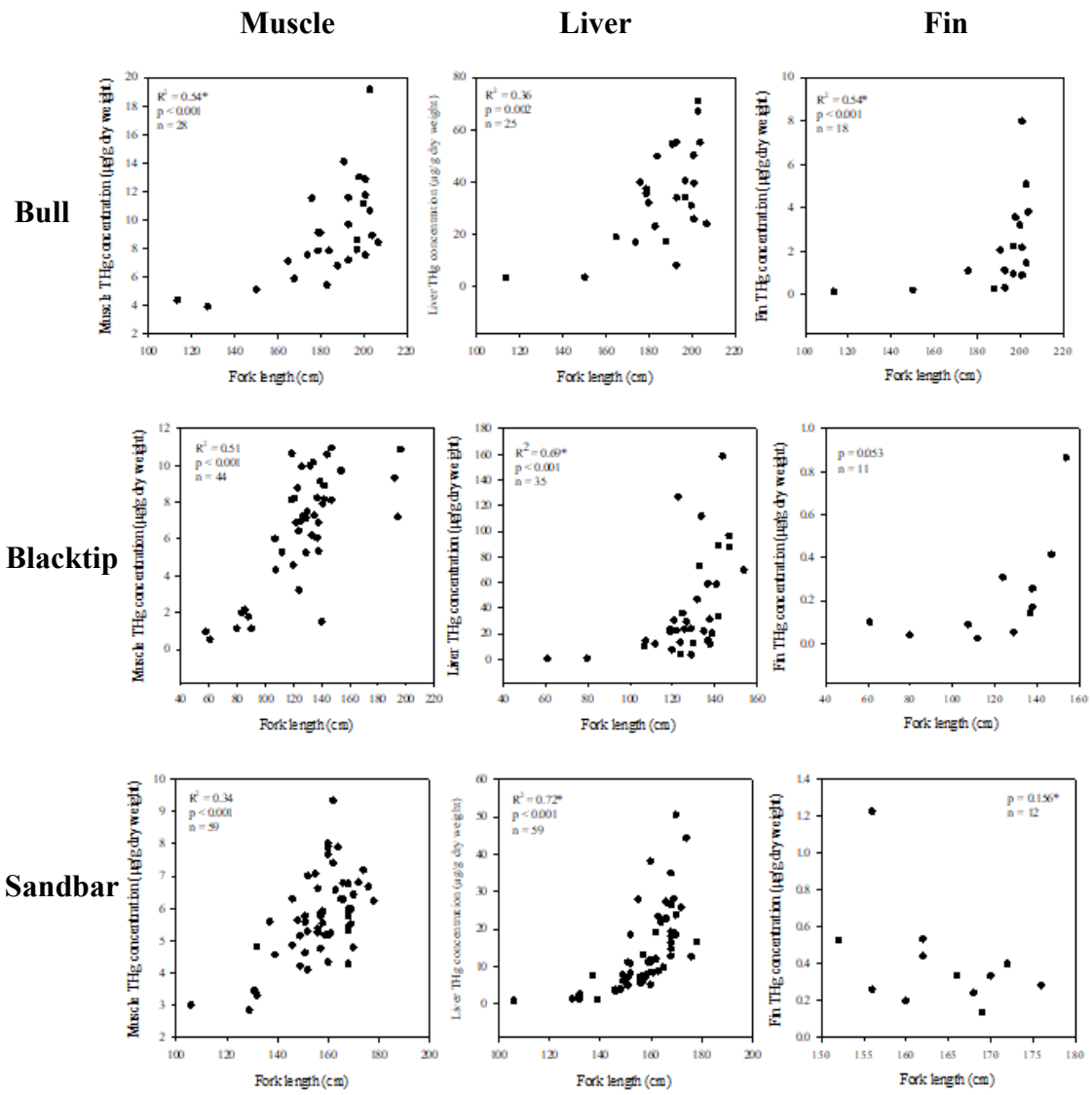

Figure 3. Relationship between THg concentration and body length in muscle (left column), liver (middle column), and fin (right column) from bull (top row), blacktip (middle row), and sandbar (bottom row) sharks. Relationships that failed to meet assumptions of normality were natural log-transformed prior to linear regression analysis (R^2*).

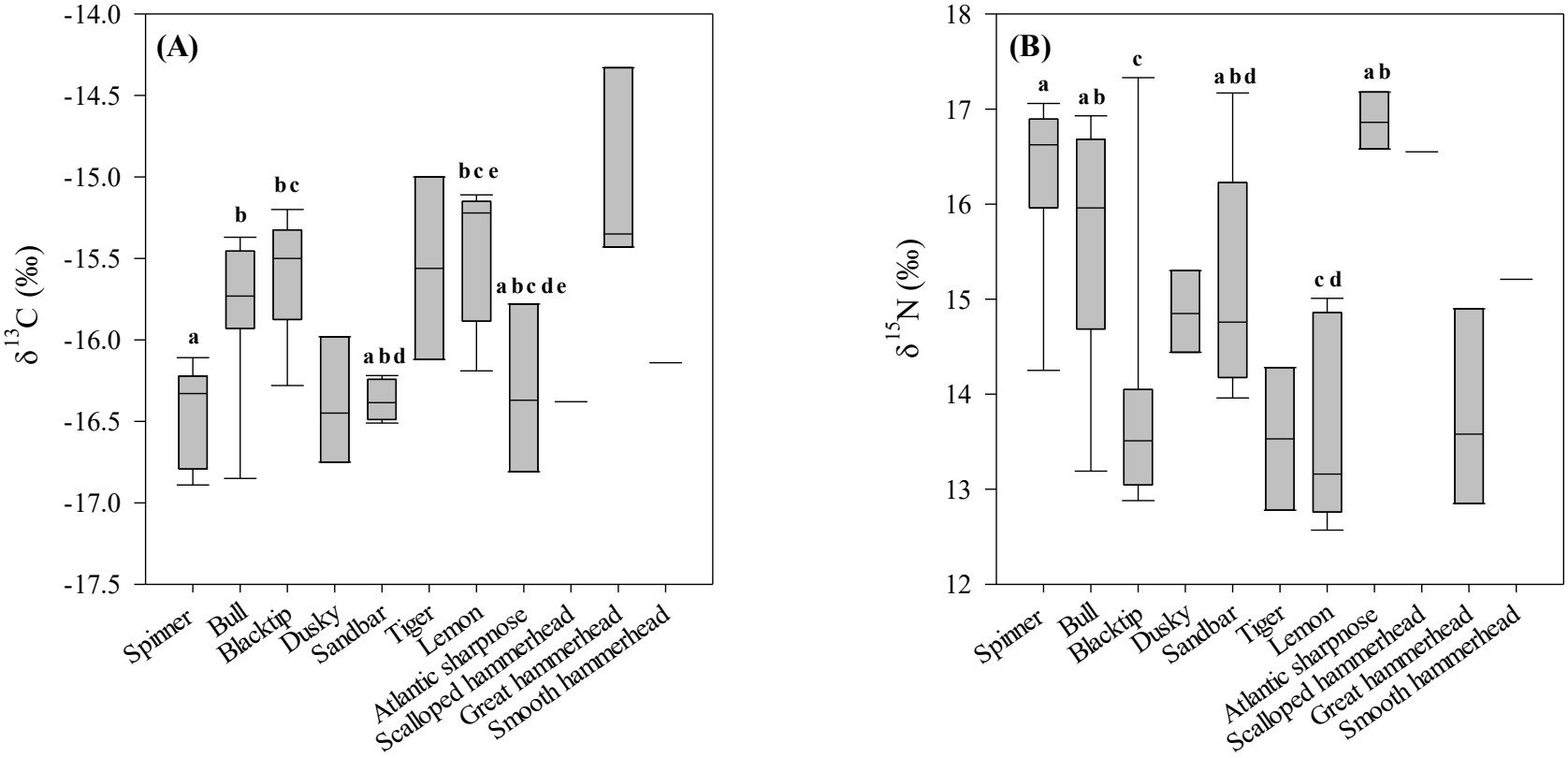

Figure 4. Box and whisker plot displaying the $\delta^{13}C$ (A) and $\delta^{15}N$ (B) values for each species. The upper and lower boxes represent the quartiles, the whiskers represent the complete range, and the horizontal line represents the median. Lowercase letters represent species grouped by similar tissue THg concentrations (one-way ANOVA). If the sample size for a given species was small (≤ 5) it was not included in the statistical analysis.

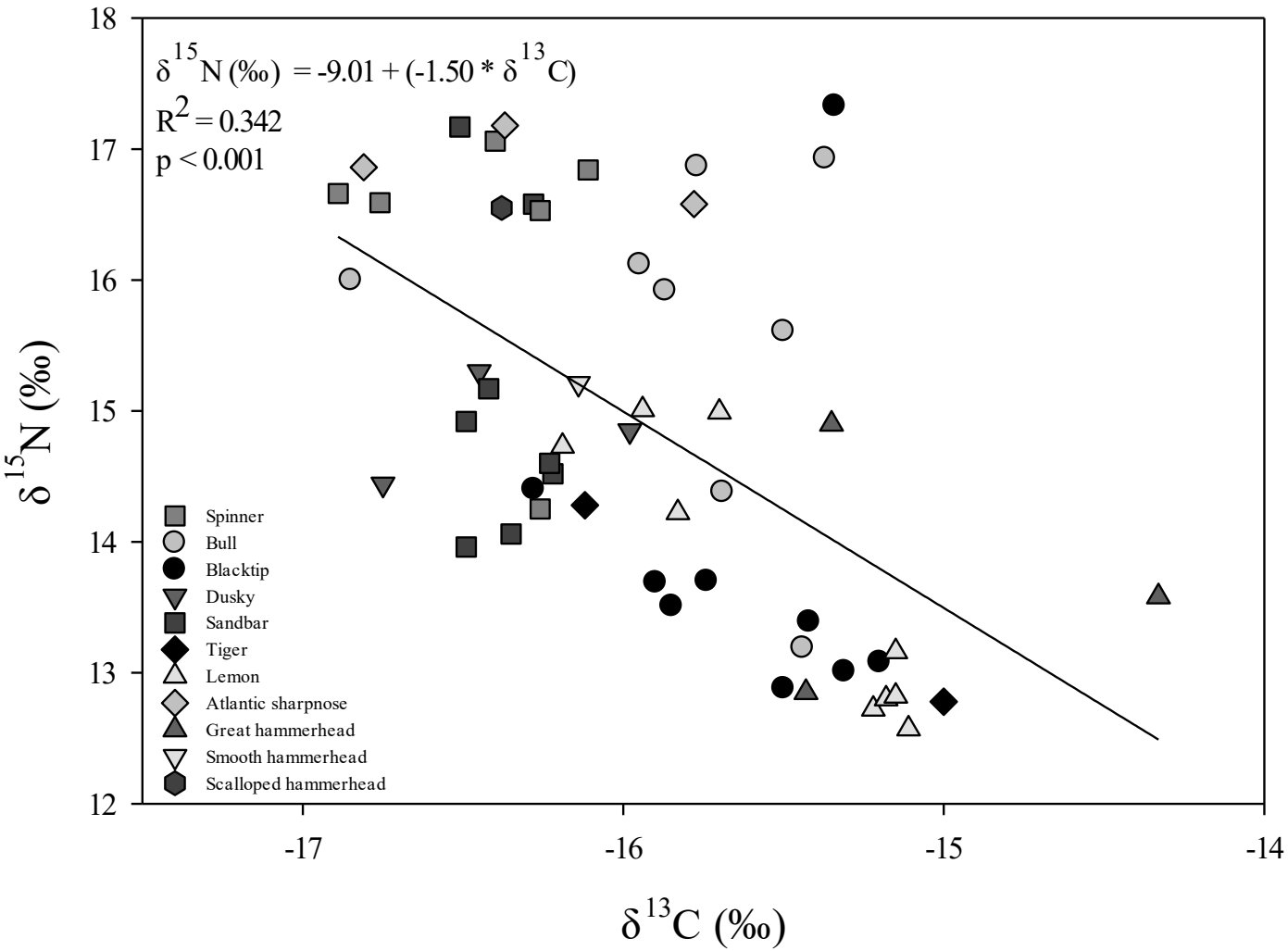

Figure 5. Relationship between $\delta^{15}N$ ‰ and $\delta^{13}C$ ‰ for all species combined.

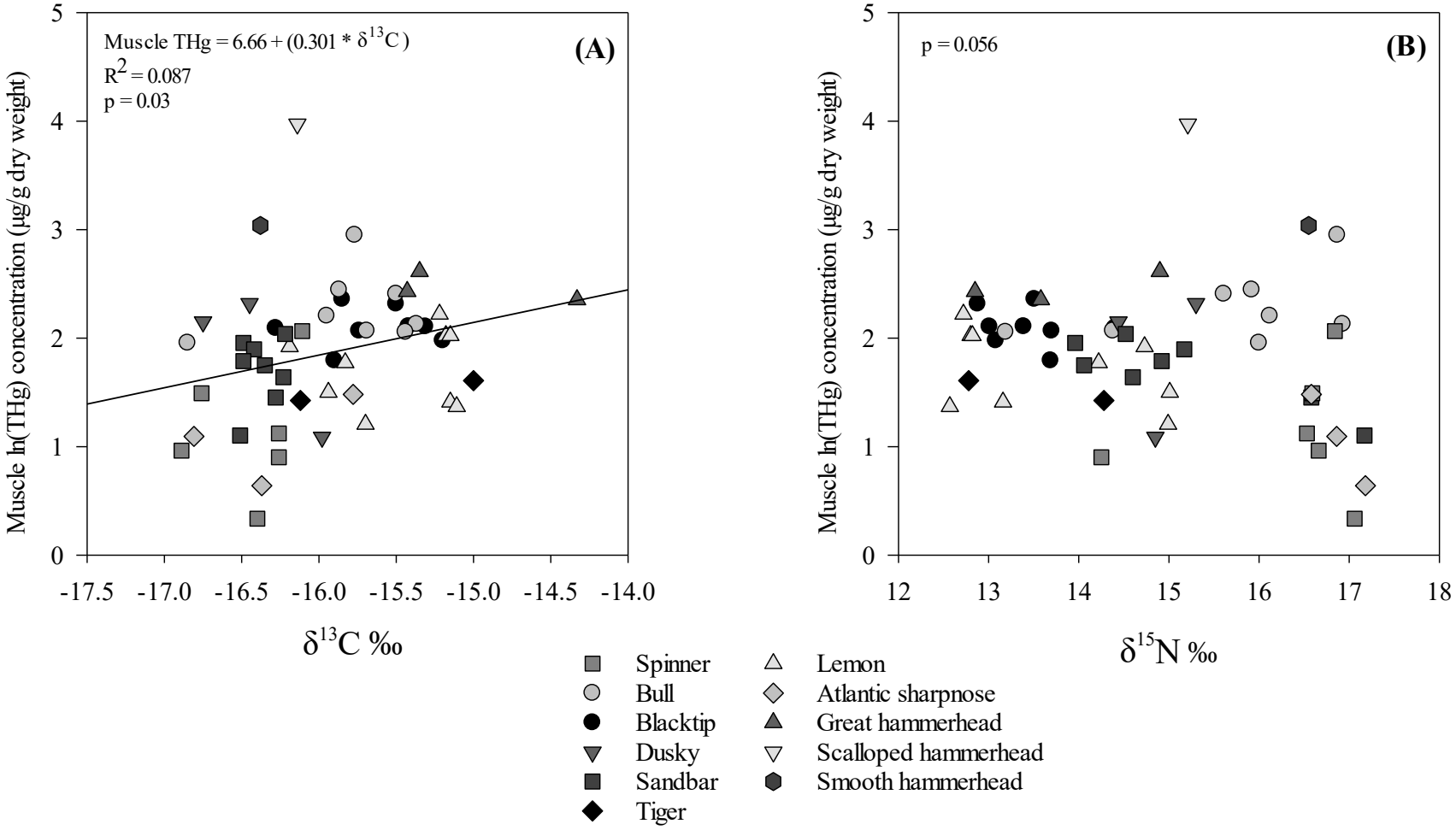

Figure 6. Relationships between THg concentration and $\delta^{13}C$ (A) and THg concentration and $\delta^{15}N$ (B) for all investigated species

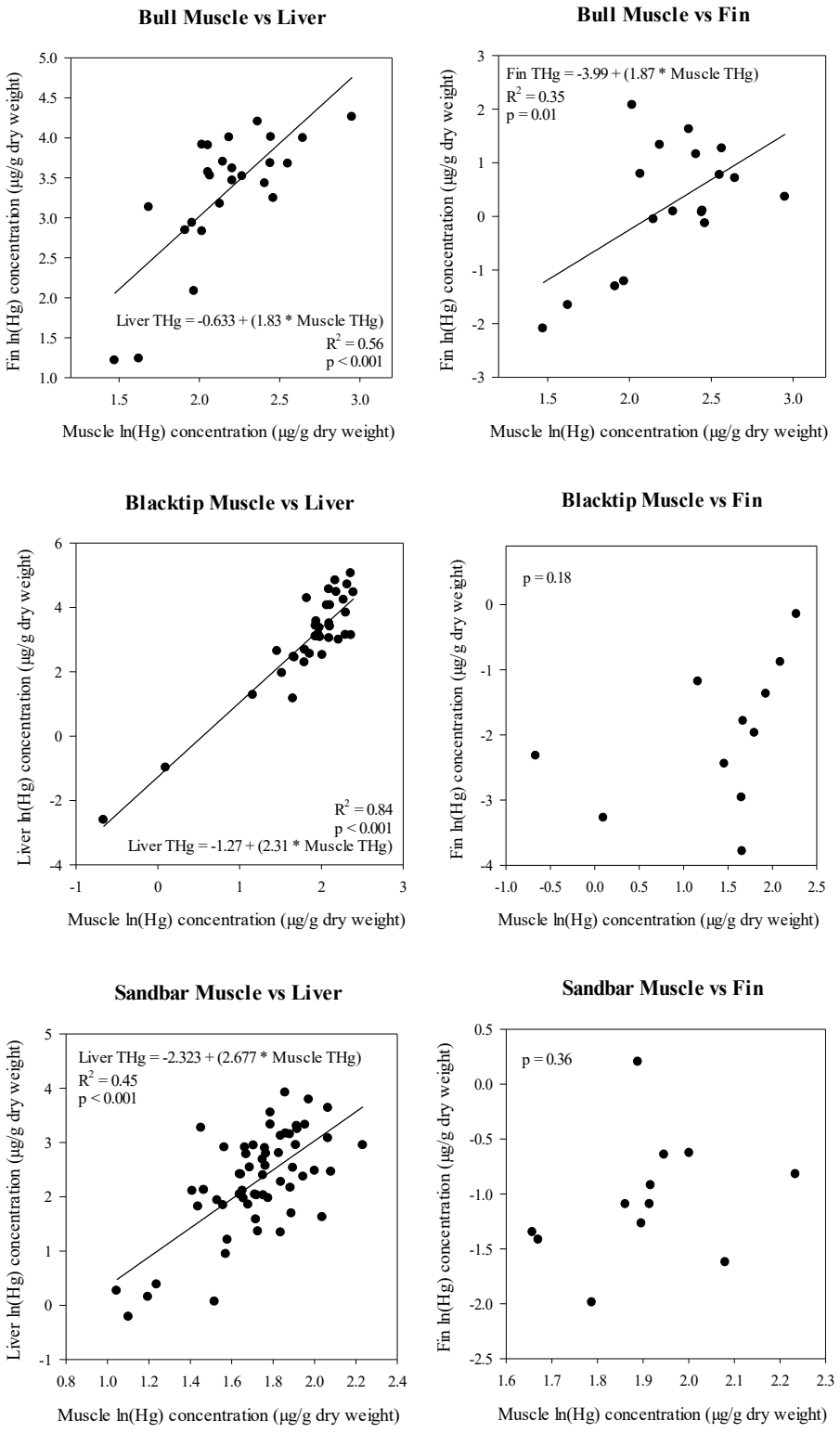

Figure 7. Relationship between tissue types using the muscle THg concentration (µg/g dry weight) as a predictor of liver and fin THg concentrations (µg/g dry weight)

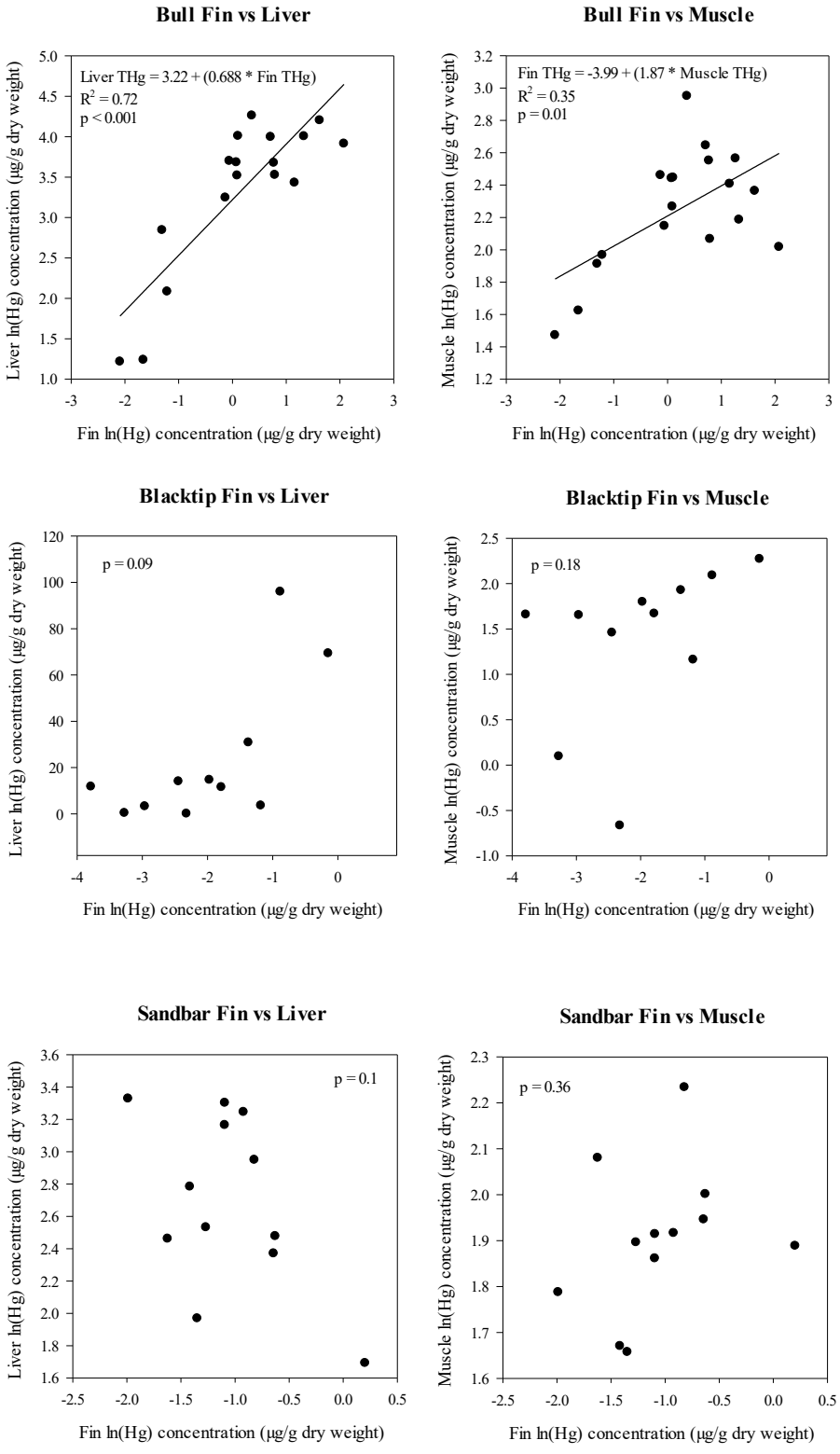

Figure 8. Relationship between tissue types using the fin THg concentration (µg/g dry weight) as a predictor for muscle and liver THg concentrations (µg/g dry weight).

Table 1. Mercury concentrations (µg/g wet weight, mean ± standard deviation) in muscle tissue from shark species caught off the coast of Florida. Sample sizes are in parentheses. (a - Atlantic sharpnose caught in the Indian River Lagoon; b - Determined Hg as MeHg; c - Tiger sharks caught off northeast Florida)

Species	Charlotte Harbor (Adams et al., 2003)	East central Florida (Adams and McMichael, 1999)	Florida Bay (Evers et al., 2008)	St. Petersburg – Jacksonville (Hueter et al (1995)	Lee County (Rumbold et al., 2014)
Spinner shark				0.59 ± 0.53 (*n* = 4)[b]	
Bull shark	0.97 (*n* = 3)	0.77 ± 0.32 (*n* = 53)		1.03 ± 0.42 (*n* = 29)[b]	1.47 ± 1.20 (*n* =7)
Blacktip shark	0.79 (*n* = 12)	0.77 ± 0.71 (*n* = 21)	3.31 ± 0.60 (*n* = 4)	1.30 ± 0.83 (*n* = 7)[b]	2.65 ± 0.90 (*n* = 28)
Dusky shark				1.47 ± 0.13 (*n* = 2)[b]	
Sandbar shark				0.77 ± 0.40 (*n* = 67)[b]	
Tiger shark				0.24 ± 0.14 (*n* = 4)[bc]	0.37 ± 0.30 (*n* = 8)
Lemon shark	0.70 (*n* = 3)		0.6 ± 0.35 (*n* = 8)		1.67 (*n* = 2)
Atlantic sharpnose	1.06 (*n* = 81)[a]	1.06 ± 0.63 (*n* = 81)	0.56 ± 0.52 (*n* = 36)		1.99 ± 0.60 (*n* = 7)
Great hammerhead					1.54 ± 0.50 (*n* = 4)

Table 2. Species sampled for this study along with the total sample size and sample size broken down by location.

Common name	Scientific name	Total number of sharks sampled	Number sampled off the southeastern USA	Number sampled in the northern Gulf of Mexico
Spinner shark	*Carcharhinus brevipinna*	6	0	6
Bull shark	*Carcharhinus leucas*	28	16	12
Blacktip shark	*Carcharhinus limbatus*	44	7	37
Dusky shark	*Carcharhinus obscurus*	3	3	0
Sandbar shark	*Carcharhinus plumbeus*	59	23	36
Tiger shark	*Galeocerdo cuvier*	3	0	3
Lemon shark	*Negaprion brevirostris*	8	4	4
Atlantic sharpnose	*Rhizoprionodon terraenovae*	8	0	8
Scalloped hammerhead	*Sphyrna lewini*	2	1	1
Great hammerhead	*Sphyrna mokarran*	4	2	2
Smooth hammerhead	*Sphyrna zygaena*	1	0	1
Southern stingray	*Dasyatis americana*	5	0	5

Table 3. Mean, standard deviation, minimum and maximum body lengths (cm) for each investigated species. Length measurements were based on fork length for all sharks and wingspan for the southern stingray. ND = not determined due to small sample size.

Common Name	Mean	Standard deviation	Minimum	Maximum
Spinner shark	148	10.6	130	160
Bull shark	184	22.6	113	207
Blacktip shark	132	10.3	107	154
Dusky shark	173	59.5	104	209
Sandbar shark	157	13.3	106	178
Tiger shark	215	18.2	199	235
Lemon shark	210	12.7	184	226
Atlantic sharpnose	77.6	6.77	71	91
Scalloped hammerhead	170	ND	140	200
Great hammerhead	239	67.7	176	328
Smooth hammerhead	215	ND	ND	ND
Southern stingray	120	12.6	99	130

Table 4. Sample size (n) for each tissue type and investigated species along with the corresponding percentage moisture content (mean ± standard deviation; minimum and maximum percentages in parentheses).

Common Name	Muscle n	% moisture content	Liver n	% moisture content	Fin n	% moisture content
Spinner shark	6	72.5 ± 1.8 (69.9 - 73.8)	0	--	0	--
Bull shark	28	76.1 ± 1.3 (72.9 - 78.8)	25	30.1 ± 2.9 (24.6 - 35.2)	18	57.9 ± 1.0 (56.1 - 59.8)
Blacktip shark	44	73.8 ± 1.5 (69.8 - 76.3)	35	58.2 ± 18.6 (30.1 - 79.5)	11	60.8 ± 6.5 (39.6 - 69.8)
Dusky shark	3	75.4 ± 1.4 (73.8 - 76.3)	3	42.6 ± 11.4 (30.3 - 52.8)	3	62.7 ± 0.7 (62.0 - 63.4)
Sandbar shark	59	76.4 ± 1.1 (74.2 - 79.4)	59	35.9 ± 6.0 (24.7 - 53.5)	12	61.3 ± 1.8 (57.9 - 64.4)
Tiger shark	3	79.8 ± 1.9 (77.9 - 81.7)	2	33.9 ± 5.01 (30.4 - 37.5)	2	63.2 ± 1.2 (62.4 - 64.0)
Lemon shark	8	75.7 ± 1.1 (74.2 - 78.0)	8	33.19 ± 6.7 (25.2 - 46.5)	4	61.6 ± 1.6 (59.3 - 63.0)
Atlantic sharpnose	8	73.5 ± 0.9 (71.7 - 74.7)	2	43.8 ± 11.8 (35.4 - 52.1)	7	64.7 ± 2.2 (61.1 - 67.5)
Scalloped hammerhead	2	73.4 ± 3.0 (71.4 - 75.5)	1	36.6	0	--
Great hammerhead	4	74.8 ± 1.0 (73.3 - 75.4)	3	39.6 ± 13.2 (31.1 - 54.7)	3	59.3 ± 1.6 (57.5 - 60.4)
Smooth hammerhead	1	73.6	1	75.4	1	53.6
Southern stingray	5	76.8 ± 0.6	0	--	0	--

Table 5. Dry weight and wet weight THg concentrations for each investigated species. ND = not determined due to small sample size

Species	Tissue	THg concentration (µg/g dry weight)					THg concentration (µg/g wet weight)				
		Median	Mean	Standard Deviation	Minimum	Maximum	Median	Mean	Standard Deviation	Minimum	Maximum
Spinner	Muscle	2.84	3.64	2.30	1.40	7.87	0.773	1.02	0.691	0.365	2.32
Bull shark	Muscle	8.48	9.05	3.30	3.89	19.1	1.95	2.14	0.711	0.939	4.46
	Liver	34.0	34.6	18.3	3.38	70.9	0.797	1.02	0.653	0.132	3.16
	Fin	1.28	2.08	2.03	0.124	7.97	0.549	0.954	0.923	0.052	3.49
Blacktip shark	Muscle	7.90	7.74	1.89	3.20	10.9	2.03	1.98	0.479	0.802	2.84
	Liver	29.0	44.0	39.4	3.23	158	9.92	18.1	20.6	1.68	83.8
	Fin	0.254	0.314	0.270	0.052	0.864	0.107	0.129	0.105	0.031	0.344
Dusky shark	Muscle	8.59	7.24	3.79	2.96	10.2	2.04	1.75	0.868	0.777	2.44
	Liver	2.00	1.55	1.04	0.364	2.29	1.08	0.892	0.619	0.202	1.40
	Fin	0.170	0.167	0.122	0.044	0.287	0.064	0.063	0.047	0.016	0.109
Sandbar shark	Muscle	5.76	5.76	1.26	2.84	9.34	1.35	1.36	0.289	0.680	1.97
	Liver	11.1	14.0	10.8	0.810	50.4	6.93	9.38	7.72	0.544	34.3
	Fin	0.335	0.409	0.285	0.137	1.22	0.128	0.159	0.113	0.054	0.481
Tiger shark	Muscle	4.16	4.30	0.637	3.74	4.99	0.826	0.866	0.129	0.761	1.01
	Liver	1.40	1.40	0.285	1.20	1.61	0.934	0.934	0.259	0.751	1.12
	Fin	ND	0.350	ND	0.162	0.538	0.058	0.058	ND	0.058	0.058
Lemon shark	Muscle	5.18	5.66	2.07	3.34	9.22	1.18	1.37	0.504	0.825	2.28
	Liver	39.0	61.1	56.7	6.35	165	43.3	43.9	35.6	4.31	108
	Fin	1.11	1.23	0.822	0.517	2.19	0.442	0.473	0.312	0.199	0.809
Atlantic sharpnose	Muscle	2.22	2.26	1.47	0.435	4.40	0.580	0.597	0.385	0.118	1.14
	Liver	4.09	4.09	4.92	0.613	7.57	2.60	2.59	3.25	0.293	4.89
	Fin	0.086	0.086	0.097	0.017	0.154	0.032	0.032	0.035	0.007	0.057

Table 5. Continued

Species	Tissue	THg concentration (µg/g dry weight)					THg concentration (µg/g wet weight)				
		Median	Mean	Standard Deviation	Minimum	Maximum	Median	Mean	Standard Deviation	Minimum	Maximum
Scalloped hammerhead	Muscle	13.8	13.8	20.9	6.80	20.9	3.53	3.53	2.24	1.95	5.11
	Liver	ND	5.88	ND	ND	ND	ND	3.72	ND	ND	ND
Great hammerhead	Muscle	11.1	11.6	1.43	10.5	13.7	2.86	2.92	0.323	2.60	3.37
	Liver	1.73	2.02	0.714	1.50	2.84	1.20	1.16	0.141	1.01	1.28
	Fin	0.082	0.113	0.085	0.048	0.209	0.033	0.047	0.037	0.019	0.089
Smooth hammerhead	Muscle	ND	53.2	ND	ND	ND	ND	14.0	ND	ND	ND
	Liver	ND	98.0	ND	ND	ND	ND	24.1	ND	ND	ND
	Fin	ND	1.25	ND	ND	ND	ND	0.579	ND	ND	ND
Southern stingray	Muscle	4.25	4.50	2.51	1.60	8.48	1.02	1.04	0.578	0.375	1.96

Table 6. Percentage of muscle and fin samples from each investigated species that exceeded the U.S. EPA Hg human health criterion (0.3 µg/g wet weight) and FDA action limit (1.0 µg/g wet weight) and the body length at which each advisory began to be exceeded. Sample sizes for each tissue (muscle and fin) are included.

Species	Muscle					Fin				
	N	% exceeding EPA	Body length	% exceeding FDA	Body length	N	% exceeding EPA	Body length	% exceeding FDA	Body length
Spinner	6	100	149	33.3	160	0	--	--	--	--
Bull	28	100	114	96.4	114	18	77.8	176	33.3	191
Blacktip	44	93.2	80	79.5	107	11	9.09	154	0	--
Dusky	3	100	104	66.7	205	3	0	--	0	--
Sandbar	59	100	106	89.8	132	12	8.33	156	0	--
Tiger	3	100	199	33.3	235	2	0	--	0	--
Lemon	8	100	184	87.5	184	4	50	210	0	--
Atlantic sharpnose	8	62.5	71	25.0	76.2	7	0	--	0	--
Scalloped hammerhead	2	100	140	100	140	0	--	--	--	--
Great hammerhead	4	100	176	100	176	3	0	--	0	--
Smooth hammerhead	1	100	215	100	215	1	100	215	0	--
Southern stingray	5	100	99	60.0	117	0	--	--	--	--

Table 7. $\delta^{13}C$ and $\delta^{15}N$ values for each investigated species.

Species	Sample Size	Median	Mean	Standard Deviation	Minimum	Maximum
			$\delta^{13}C$			
Spinner shark	6	-16.40	-16.42	0.362	-16.89	-15.89
Bull shark	8	-15.73	-15.80	0.470	-16.85	-15.98
Blacktip shark	8	-15.62	-15.65	0.358	-16.28	-15.20
Dusky shark	3	-16.45	-16.39	0.388	-16.75	-15.98
Sandbar shark	8	-16.42	-16.39	0.124	-16.56	-16.22
Tiger shark	2	-15.56	-15.56	0.793	-16.12	-15.00
Lemon shark	8	-15.22	-15.50	0.419	-16.19	-15.11
Atlantic sharpnose	3	-16.08	-16.08	0.647	-16.81	-15.34
Scalloped hammerhead	1	-16.38	-16.38	N/A	-16.38	-16.38
Great hammerhead	4	-15.35	-15.04	0.615	-15.43	-14.33
Smooth hammerhead	1	-16.14	-16.14	N/A	-16.14	-16.14
			$\delta^{15}N$			
Spinner shark	6	16.66	16.35	0.957	14.25	17.06
Bull shark	8	15.96	15.63	1.27	13.19	16.93
Blacktip shark	8	13.45	13.46	0.491	12.88	14.40
Dusky shark	3	14.85	14.87	0.429	14.44	15.30
Sandbar shark	8	16.66	15.35	1.28	13.96	17.18
Tiger shark	2	13.53	13.53	1.06	12.78	14.28
Lemon shark	8	13.16	13.67	1.05	12.57	15.01
Atlantic sharpnose	3	17.02	16.99	0.335	16.58	17.33
Scalloped hammerhead	1	16.55	16.55	N/A	16.55	16.55
Great hammerhead	4	13.58	13.78	1.04	12.85	14.90
Smooth hammerhead	1	15.21	15.21	N/A	15.21	15.21

2. MATERNAL TRANSFER OF MERCURY IN THREE PLACENTAL VIVIPAROUS SHARK SPECIES (*CARCHARHINUS LEUCAS*, *C. LIMBATUS*, AND *C. PLUMBEUS*)

2.1 Introduction

2.1.1 Maternal Transfer of Mercury in Sharks

Although the diet is the primary source of mercury (Hg) exposure in sharks, maternal transfer represents another significant, and often overlooked, exposure pathway (Hall et al., 1997; Adams and McMichael, 1999; Sackett et al., 2013). Maternal transfer, also known as maternal offloading, is the process by which females transfer a portion of their contaminant load to their embryos during development. This process has been reported in a wide variety of taxa including mammals (Borrell et al., 1995; Knott et al., 2012), amphibians (Hopkins et al., 2006), and birds (Varian-Ramos et al., 2014; Ackerman et al, 2016), and has also been documented in several elasmobranch species including the common thresher shark (*Alopias vulpunis*), bull shark (*Carcharhinus leucas*), tiger shark (*Galeocerdo cuvier*), blacknose shark (*Carcharhinus acronotus*), blacktip shark (*Carcharhinus limbatus*), bonnethead (*Sphyrna tiburo*), Atlantic sharpnose (*Rhizoprionodon terraenovae*), Pacific sharpnose (*Rhizoprionodon longurio*), spinner shark (*Carcharhinus brevipinna*), scalloped hammerhead (*Sphyrna lewini*), white shark (*Carcharodon carcharias*), shortfin mako (*Isurus oxyrinchus*), salmon shark (*Lamna ditropis*), Pacific angel shark (*Squatina californica),* shortnose spurdog (*Squalus megalops*), smallfin gulper shark (*Centrophorus moluccenis*), leopard shark (*Triakis semifasciata*), and the thornback guitarfish (*Platyrhinoidis triseriata*) (Adams and McMichael, 1999; Lyons et al., 2013; Lyons and Lowe, 2013; Mull et al., 2013; Le bourg

et al., 2014; Frías-Espericueta et al., 2015; Lyons and Adams, 2015; Lyons and Lowe, 2015; Weijs et al., 2015; van Hees and Ebert, 2017; Marler et al., 2018; Dutton and Venuti, 2019; Ehnert-Russo and Gelsleichter, 2020; Chynel et al., 2021). Sharks can transfer several different contaminants using this method including Hg, polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), organochlorine pesticides, and flame retardants [polybrominated diphenyl ethers (PBDEs)] (e.g., Lyons et al., 2013; Lyons and Lowe, 2013; Mull et al., 2013; Le Bourg et al., 2014; Weijs et al., 2015; Marler et al., 2018). While this process serves as a means of reducing a mother's contaminant load, it also has the potential to expose embryos to high concentrations of those contaminants during early development.

Previous studies have found that young-of-year (YOY; age-class 0) shortfin mako and white sharks have greater muscle concentrations of Hg, DDT, and PCBs than expected for their young age (Lyons et al., 2013; Mull et al., 2013). Also, YOY sharks must spend the first few weeks after birth developing foraging skills and learning to feed on their own; therefore, they utilize stored energy reserves in the liver during this period and will not be accumulating much Hg through the diet. In addition, sharks grow rapidly after birth so any Hg uptake through the diet will be offset by growth dilution. It has therefore been hypothesized that the elevated body burden of Hg reported in YOY sharks is a result of exposure while *in utero*.

Several factors can impact the amount of Hg that will be maternally transferred in sharks. The most important is reproductive strategy. In placental viviparous species, such as blacktip, blue (*Prionace glauca*), and hammerhead sharks, nutrients are directly transferred from the mother to the embryos via a pseudoplacenta (Wourms, 1981; Weijs

et al., 2015). Because of this, these species have been found to offload the highest amounts of Hg to their offspring when compared to species that utilize other reproductive modes (Pethybridge et al., 2010; Escobar-Sánchez et al., 2011; Lyons and Lowe, 2013). The lowest transfer of Hg occurs in ovoviviparous species (aplacental viviparous; e.g., spiny dogfish, *Squalus acanthias*), where nutrients are transferred via an external yolk-sac that is absorbed into the body while *in utero* (Le Bourg et al., 2014; Dutton and Gioia, 2019; Duton and Venuti, 2019). Ovoviviparous species that also partake in oophagy (consume unfertilized eggs while *in utero*; e.g., common thresher shark) transfer more Hg than ovoviviparous species that do not partake in oophagy (Lyons and Lowe, 2013; Dutton and Venuti, 2019).

Sharks have long gestation times ranging from four to five months (e.g., bonnethead) to two years (spiny dogfish) (Parsons, 1993; Jones and Ugland, 2001). Because of this, maternal offloading can occur across a range of different time periods depending on the species. However, since reproductive strategy is thought to be the main factor in determining the extent of maternal transfer, aplacental viviparous species, such as spiny dogfish, contain concentrations of Hg that are much lower than placental viviparous species like blacktip sharks, whose gestation period lasts around 12 months (Childs et al., 1973; Castro, 1996)

The mother's ability to offload Hg can also be influenced by her trophic position, age at sexual maturity, and the number of previous pregnancies she has undergone in her lifetime (Lyons et al. 2013; van Hees and Ebert, 2017). Since Hg is known to biomagnify through food webs, concentrations in pregnant sharks vary depending on trophic position, with those that are higher up on the food chain having more Hg to offload to their

embryos than those that feed lower down and have lower body burdens. The age at which sharks reach sexual maturity also affects their ability to transfer Hg. Species that take longer to reach reproductive age have more time to accumulate Hg within their tissues compared to those that mature earlier on; therefore, once mature they typically have more Hg to transfer to their embryos. In addition to this, a shark's first reproductive event often provides the greatest opportunity for Hg offloading to occur. Since the uptake rate of Hg greatly exceeds its loss from the body, the first pregnancy provides an opportunity for sharks to offload the highest amount of Hg with subsequent litters receiving less Hg (Borrell et al., 1995; Lyons et al., 2013; Lyons and Lowe, 2013; van Hees and Ebert, 2017). Although it is hard to investigate the effects of Hg firsthand in sharks due to the difficulty in holding them in a laboratory setting, it is likely that since many species are found at high trophic levels and are often known to accumulate large concentrations of Hg, their embryos may experience an increased risk of facing deleterious health effects like those seen in teleost species during early life stages (Weis and Weis, 1977; Jezierska et al., 2009; Zaera and Johnsen, 2011; summarized in Chapter 1).

Previous studies investigating the maternal transfer of Hg in sharks have focused primarily on Hg concentrations in embryonic muscle and liver tissues (Adams and McMichael, 1999; Lyons and Lowe, 2013; Frias-Espericueta et al., 2014; Le Bourg et al, 2014; Dutton and Venuti, 2019). Muscle tissue is highly vascularized and can accumulate Hg to high concentrations due to its long turnover time (over two years; Hesslein et al., 1993; Kwon et al., 2016); as a result, the muscle often has the highest reported Hg concentration in the body (Domi et al., 2005; Le Bourg et al., 2014). The liver is an energy storage and detoxification organ and provides the necessary energy needed during

pregnancy. Unlike other contaminants, which are primarily found in high concentrations in the liver (e.g., DDT and PCBs in white sharks), Hg is redistributed around the body via the bloodstream and can accumulate in various tissues throughout the body (Mull et al., 2012; Lyons and Lowe, 2013). While these two tissues are important sites for Hg accumulation, Hg concentrations in other tissues such as the brain and heart are rarely reported (Nam et al., 2011; Ehnert-Russo and Gelsleichter, 2020). Since Hg is a well-known neurotoxin and cardiovascular toxin in wildlife and humans (Crump and Trudeau, 2009; Fernandes Azevedo et al., 2012; Scheuhammer et al., 2015; Dong et al., 2016) it is critical to measure the concentration of Hg in these tissues in shark embryos because Hg could negatively impact embryonic development.

2.1.2 Stable Isotope Values in Shark Embryos Compared to their Parent

In addition to investigating the diet and trophic position of sharks, stable isotopes ($\delta^{13}C$ and $\delta^{15}N$, respectively) have also been used to investigate the transfer of nutrients between mothers and their embryos (Vaudo et al., 2010; Le Bourg et al., 2014; Olin et al., 2018). Like many species, the diet of sharks changes greatly during their first year of life. During development they rely solely on maternally derived energy sources (e.g., external yolk sac or pseudoplacenta) until they are born and must switch over to independent foraging behaviors (Compagno, 1990). During this transition, in which YOY sharks must learn to develop and rely on their own foraging skills, they utilize the energy provided through the maternal investment they received during the gestation period (Belicka et al., 2012). The liver is thought to be the main site of these energy reserves as it has been found to decrease in size during the first few weeks of life, as newborns utilize them while learning to forage for their own prey (Hussey et al., 2010). Because they are

primarily using energy from these maternally derived reserves, newborn sharks have been found to exhibit stable isotope values reflecting maternal provisioning and therefore have nitrogen isotopic values that are enriched relative to their mother (McMeans et al., 2009; Vaudo et al., 2010). This can lead to the misinterpretation of these values at early life stages as older individuals who have partaken in exogenous feeding and can acquire their own resources will have isotopic values that represent their own diets rather than that of their mother's. The fractionation of isotopic values between mothers and embryos has been investigated previously in elasmobranchs (McMeans et al., 2009; Vaudo et al., 2010; Olin et al., 2018; Osgood et al., 2020); however, it has only been performed on a limited number of species [shortfin spurdog, smallfin gulper shark, bonnethead, smalleye smooth-hound (*Mustelus higmani*), bluntnose sixgill (*Hexanchus griseus*), Pacific sharpnose shark, and speckled guitarfish (*Psuedobatos glaucostigmus*)] and the dynamics of maternal provisioning are still not well understood (Le Bourg et al., 2013; Olin et al., 2018; Baró-Camarasa et al., 2020; de Souza-Araujo et al., 2020).

Since Hg is known to biomagnify through marine food webs, it can be used as a complementary dietary tracer in addition to $\delta^{13}C$ and $\delta^{15}N$ values. Both $\delta^{15}N$ values and Hg concentrations have been found to increase with trophic position. Because of this, positive relationships have been previously reported between the two within a variety of shark species including the blue shark, shortfin mako, oceanic whitetip (*Carcharhinus longimanus),* spiny dogfish, tope shark (*Galeorhinus galeus)*, black-mouthed catshark (*Galeus melastomus)*, starry smoothhound (*Mustelus asterias),* and the lesser-spotted dogfish (*Scyliorhinus canicula*) (Domi et al., 2005; Kiszka et al., 2015). Since $\delta^{15}N$ provides information on the trophic position of sharks, it can be used to predict which

species should contain the highest concentrations of Hg. In addition to estimating trophic position, stable isotopes can also be used when investigating potential differences in Hg concentrations based on where sharks are feeding. Since organisms in pelagic and coastal environments consume prey that feed in different carbon pools, $\delta^{13}C$ values can help provide insight into the roles that foraging habitats might play in the bioaccumulation of Hg in sharks (Kiszka et al., 2015; Le Crozier et al., 2019). This technique has been used for shark species in the past, such as the shortfin mako and blue shark, to identify geographic variation in Hg concentrations (Kiska et al., 2015). Overall, the combined use of both $\delta^{13}C$ and $\delta^{15}N$ values provides additional information on the role that diet and trophic position might have on Hg concentrations in sharks. However, even though stable isotopes have been used previously to investigate these factors in adult sharks, few studies have investigated both stable isotope fractionation and the maternal transfer of Hg to shark embryos (Le Bourg et al., 2014). Because of this, further studies are needed to better understand stable isotope fractionation in parental tissues in comparison to tissues in the corresponding embryos.

2.1.3 Species and Tissues to be Investigated

Of the sharks sampled in Chapter 1 of this thesis, seven sandbar, seven blacktip, and three bull sharks were pregnant providing a total of 108 embryos. All three species are placental viviparous, and their reproductive information is listed in Table 8.

The availability of these samples allowed for an assessment of the concentration of THg in several embryonic tissues, how THg concentrations in embryonic tissues compared to the corresponding THg concentrations in maternal tissues, and comparison of the $\delta^{13}C$ and $\delta^{15}N$ values between mothers and their corresponding embryos in these

species, addressing some of the knowledge gaps that have previously been discussed.

2.1.4 Objectives of the Study

The goal of this study was to investigate total Hg (THg) concentrations in various tissues in bull, blacktip, and sandbar shark embryos and their mothers along with differences in their stable carbon and nitrogen isotopes to determine the importance of maternal transfer of Hg in these species. This can be broken down into three objectives:

1. Investigate the inter- and intraspecies variability in THg concentrations in seven embryonic tissues (muscle, heart, brain, kidney, liver, skin, and fin), with the prediction that concentrations will be highest in the muscle and heart.

2. Compare the concentration of THg in muscle and liver of each embryo to the muscle and liver THg concentration of their corresponding parent with the prediction that species that take longer to sexually mature and have longer gestation times will transfer higher concentrations of Hg to their embryos.

3. Compare muscle stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) and THg concentrations between the mother and their embryos with the prediction that embryos will be more enriched relative to their mothers and that species with more enriched $\delta^{15}N$ values will have higher THg concentrations.

2.2 Methods

2.2.1 Sample Collection

Seven pregnant blacktip and three pregnant bull sharks were caught off the Atlantic and Gulf coasts of Florida (Figure 9) by commercial fishermen and sampled by fisheries observers in collaboration with the NOAA Southeast Fisheries Science Center (Panama City, FL). Seven pregnant sandbars were caught off the Atlantic and Gulf coasts of Florida (Figure 9) through the Shark Research Fishery since sandbar sharks are a prohibited species and cannot be retained in any other fishery.

The embryos were collected from their corresponding mothers, sorted by left and right uterus, placed in plastic bags, and shipped whole to Texas State University. The litter and total sample sizes of embryos from each species used in this study are shown in Table 9. Muscle and liver samples were also collected from the corresponding parent and shipped frozen to Texas State. All embryos and maternal tissue samples were held at -20°C until further processing.

2.2.2 Sample Preparation

Embryos were thawed and the fork length, weight, and uterus (left or right) were recorded prior to dissection. The entire dorsal fin of each embryo was removed, and an axial muscle sample was collected from below the first dorsal fin and separated from the skin. Each embryo was then dissected to remove the liver, kidney, heart, and brain. All samples were then weighed, freeze-dried, and homogenized following the methods described in Chapter 1. The moisture content for each embryonic tissue is shown in Table 10. Maternal muscle and liver tissue samples were processed and analyzed for THg in Chapter 1 of this thesis.

2.2.3 Mercury Analysis of Embryonic Tissues

Total Hg concentrations in embryonic tissues were determined using the methods described in Chapter 1. Blanks (empty quartz boat; *n* =87), CRMs [DORM-4 fish protein, 0.412 µg/g THg, NRCC (*n* =60); DOLT-5 dogfish liver, 0.44 µg/g THg, NRCC (*n* =12); and ERM-CE464 fish protein, 5.24 µg/g THg, European Reference Materials (*n* =12)], and duplicate samples (*n* = 91) were used for quality control. Blank samples had a THg concentration ≤ 0.0001 µg/g. Recovery values [mean ± standard deviation (SD)] for the CRMs was 97.4 ± 3.1% for DORM-4, 90.4 ± 4.8% for DOLT-5, and 96.5 ± 4.4% for ERM-CE464. Mean relative percentage differences (± SD) in THg concentration in duplicate samples from each tissue were as follows: muscle = 1.60 ± 0.96 (*n* = 15); liver = 10.1 ± 7.2 (*n* = 16); fin = 18.9 ± 20.1 (*n* = 12); skin = 12.5 ± 17.5 (*n* = 12); heart = 4.06 ± 3.84 (*n* = 12); kidney = 4.06 ± 3.58 (*n* = 12); brain = 1.84 ± 1.48 (*n* = 12).

2.2.4 Stable Isotope Analysis

Maternal (*n* = 3 bull shark, 4 blacktip shark, and 4 sandbar shark) and embryonic (*n* = 12 bull shark, 15 blacktip shark, and 16 sandbar shark) muscle tissue was lipid and urea extracted following the methods described in Chapter 1. Approximately 1.0 mg of each muscle sample was packaged into 3.5 x 5 mm tin capsules and shipped to the UC Davis Stable Isotope Facility (Davis, CA) to determine $\delta^{13}C$ and $\delta^{15}N$ values using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Two randomly selected duplicate samples were also sent for quality control.

2.2.5 Statistical Analysis

All statistical analysis was performed in R (version 3.5.1) and SigmaPlot version 14 (Systat Software, San Jose, CA) at a 95% confidence level. Data was tested for normality using the Shapiro-Wilk test and for homoscedasticity using the Levene's test prior to analysis and THg concentrations were natural log-transformed if assumptions were not met.

A one-way analysis of variance (ANOVA) and Tukey post-hoc test was used to determine whether there was a significant difference in THg concentrations in embryonic tissues within a species and each embryonic tissue among species. If the data failed to meet the assumptions of normality or homoscedasticity, a Kruskal-Wallis was performed followed by a Dunn's pairwise comparison.

A Welch's t-test was used to determine whether there was a significant difference in $\delta^{13}C$ and $\delta^{15}N$ values in maternal and embryonic muscle tissue for each species. To determine if there were any significant differences in stable isotopes between embryo species an ANOVA and Tukey post-hoc test were used. The relationship between $\delta^{13}C$ and $\delta^{15}N$ values for each embryo species as well as the relationship between both stable isotopes and THg concentration in muscle tissue were investigated using simple linear relationships. Regressions were performed for each species individually as well as for all species combined.

2.3 Results

2.3.1 Biological Data

The fork lengths of the mothers and embryos for each species are shown in Table 11. All adult sharks were within a narrow size range with only a 10 cm, 23 cm, and 13 cm difference between the minimum and maximum body length for bull, blacktip, and sandbar sharks, respectively. Size ranges for the embryos were also limited with bull and blacktip embryos having a difference of 3.9 cm and 6.5 cm, respectively between the minimum and maximum body length, whereas sandbar embryos had a 12.9 cm difference between the minimum and maximum reported body length. As a result of these narrow size ranges, the relationship between tissue THg concentrations and body length in the mothers and embryos were not investigated in this study.

2.3.2 THg Concentrations in Embryonic Tissues

The mean concentration of THg (μg/g dry weight) within various tissues of each species are shown in Figure 10 and Table 12 and the THg concentrations in litters from each pregnant shark are shown in Supplemental Tables A, B, and C. Within each species, mean THg concentrations were highest within the muscle followed by either the heart (bull, sandbar) or kidney (blacktip). The lowest THg concentrations were reported in the fin and liver for each species. Significant differences were detected in all species when comparing the THg concentration in the seven different tissue types. For all three species there was an overall significant difference in THg concentrations among embryonic tissues [Kruskal-Wallis; bull: $H(6) = 97.5$, $p < 0.001$; blacktip: $H(6) = 159$, $p < 0.001$; sandbar: $H(6) = 378$, $p < 0.001$]. However, when comparing THg concentrations among tissues using a Tukey's post-hoc test the following nonsignificant relationships were

found: bull = muscle-kidney $p = 0.513$, muscle-heart $p = 0.704$, heart-kidney $p = 1.00$, kidney-brain $p = 0.071$, brain-fin $p = 0.811$, brain-skin $p = 1.00$, skin-fin $p = 0.946$, fin-liver $p = 0.406$; blacktip = muscle-heart $p = 0.201$, muscle-kidney p = 0.345, kidney-heart $p = 1.00$, skin-fin $p = 0.130$, skin-brain $p = 0.561$, brain-liver $p = 0.179$, brain-fin $p = 0.984$, fin-liver $p = 0.655$; and sandbar = muscle-heart $p = 0.865$, heart-kidney $p = 0.154$, skin-brain $p = 0.390$, brain-fin $p = 0.686$, fin-liver $p = 0.051$.

When comparing tissue THg concentrations (μg/g dry weight) in the three species, blacktip embryos had the highest THg concentration in the muscle (1.92), liver (0.235), kidney (1.22), and skin (0.0613), while bull shark embryos had the highest THg concentration in the heart (1.25), brain (0.634), and fin (0.442). Sandbar shark embryos had the lowest THg concentrations in all examined tissues. For each tissue there was a significant overall difference in THg concentration among the three species [one-way ANOVA; muscle: $F_{(2,105)} = 150$, $p < 0.001$; heart: $F_{(2,105)} = 106$, $p < 0.001$; brain: $F_{(2,105)} = 122$, $p < 0.001$; kidney: $F_{(2,105)} = 130$, $p < 0.001$; liver: $F_{(2,105)} = 122$, $p < 0.001$; skin: $F_{(2,105)} = 99.2$, $p < 0.001$; and fin: $F_{(2,105)} = 83.4$, $p < 0.001$]. The Tukey's post-hoc test determined that all three species had significantly different THg concentrations in the brain and liver ($p < 0.05$), whereas lower THg concentrations in sandbar muscle, heart, kidney, skin, and fin were responsible for the significant overall one-way ANOVA result, however, there was no significant difference in THg concentrations in these tissues between bull and blacktip shark embryos ($p > 0.05$).

2.3.3 Comparison of Parental and Embryonic Muscle and Liver THg Concentrations

Muscle and liver THg concentrations were higher in the mother compared to the embryos for each species (Figure 10; Table 12). On average, the THg concentration in

blacktip embryo muscle and liver tissue was 24.4% and 0.68% of the mother's muscle and liver THg concentration, respectively; for both tissues, this was the greatest percentage when comparing among species (bull: muscle = 19.2%, liver = 0.53%; sandbar: muscle = 12.2%, liver = 0.45%) (Table 13). However, THg concentrations in the mother's liver were more than two times greater than the THg concentration in the muscle; despite this, embryonic muscle tissue had a much greater THg concentration than liver tissue.

2.3.4 Stable Isotope Analysis

Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) in embryonic muscle tissue of each species are shown in Figure 11 and Table 14 along with their corresponding mothers $\delta^{13}C$ and $\delta^{15}N$ values. For $\delta^{13}C$, the most enriched embryos were bull sharks, followed by blacktip and sandbar which had a mean $\delta^{13}C$ value of -14.7, -15.2, and -15.6‰, respectively. For $\delta^{15}N$, bull shark embryos were also the most enriched (16.0‰) followed by sandbar (13.6‰) and blacktip (13.5‰). When comparing $\delta^{13}C$ and $\delta^{15}N$ values of the mothers and their corresponding embryos, significant differences in $\delta^{13}C$ values were found for bull [$t(2) = -8.82$, $p = 0.013$] and sandbar [$t(14) = -10.0$, $p < 0.001$] sharks. Overall, bull, blacktip, and sandbar shark embryos were all $\delta^{13}C$ enriched compared to their mothers. Differences in maternal and embryonic $\delta^{15}N$ values were only detected in the sandbar shark [$t(18) = 2.67$, $p = 0.016$].

Significant interspecies differences in embryonic $\delta^{13}C$ and $\delta^{15}N$ values were observed [one-way ANOVA; $\delta^{13}C$: $F_{(2,40)} = 53.3$, $p < 0.001$; $\delta^{15}N$: $F_{(2,40)} = 31.7$, $p < 0.001$]. For $\delta^{13}C$ the Tukey's post-hoc test found that all three species were significantly different from one another ($p < 0.001$). For $\delta^{15}N$, bull shark embryos were significantly

higher than blacktip and sandbar embryos ($p < 0.001$), however, no significant difference was observed between blacktip and sandbar embryos ($p > 0.05$).

For all species combined, a significant relationship was observed between $\delta^{13}C$ and $\delta^{15}N$ values ($p = 0.02$), however, the R^2 was low (0.103). When investigating the relationship between $\delta^{13}C$ and $\delta^{15}N$ values in each species, both blacktip and sandbar embryos had a negative correlation ($R^2 = 0.88$; $p < 0.001$ and $R^2 = 0.84$; $p < 0.001$, respectively) and no relationship was observed in bull shark embryos ($p > 0.05$) (Figure 12). Relationships between $\delta^{13}C$ and $\delta^{15}N$ values and THg concentration in muscle tissue were also observed. Significant relationships were found between both $\delta^{13}C$ ($R^2 = 0.54$; $p < 0.002$) and $\delta^{15}N$ ($R^2 = 0.62$; $p < 0.001$) and THg for the blacktip embryos. Bull shark embryos only had a significant relationship between $\delta^{13}C$ and THg ($R^2 = 0.84$; $p < 0.001$), while no relationships were detected for either of the isotopic values in the sandbar embryos (Figure 13). When combining all species, a significant relationship was only detected between $\delta^{13}C$ and THg concentration ($R^2 = 0.37$; $p < 0.001$).

2.4 Discussion

The study provided an in-depth analysis of THg concentrations in various tissues of embryonic sharks. Concentrations of THg were found to vary between species as well as between tissue types within a species. While these differences have been reported previously in studies investigating THg concentrations in adult sharks, this study demonstrated that differences in tissue accumulation begin while *in utero* and can be caused by several factors including the diet of the parent and differences in reproduction including gestation time, age at sexual maturity, and age at the time of pregnancy. These were also found to influence the percentage of maternal THg that was observed within the tissues of their embryos with percentages of maternal THg found varying by species. Additionally, this study measured significant differences in $\delta^{13}C$ and $\delta^{15}N$ values between mothers and embryos within a species as well as between embryos of each species.

2.4.1 Tissue THg Concentrations in Embryos

The concentrations of THg detected within the embryos in this study provide evidence that Hg is maternally transferred in each of these species. While this process has been documented in sharks (Lyons et al., 2013; Lyons and Lowe, 2013; Mull et al., 2013; Le Bourg et al., 2014; Frías-Espericueta et al., 2015; Lyons and Adams, 2015; van Hees and Ebert, 2017; Dutton and Venuti; 2019), the distribution of THg in tissues that play a significant role in embryonic development, such as the heart and brain, has not previously been reported. Prior studies have looked at concentrations of THg within reproductively important tissues such as the placenta, umbilical cord, and ova (Lyons and Lowe. 2013; Frías-Espericueta et al., 2015; van Hees and Ebert, 2017) as well as tissues such as the muscle and liver which are regularly investigated in studies involving Hg (Lyons et al.,

2013; Lyons and Lowe, 2013; Dutton and Venuti. 2019). Other tissues, such as the brain and heart, have not been investigated as thoroughly in sharks despite the known deleterious effects Hg has had on these tissues in other species.

Of the seven tissues analyzed in this study the muscle, heart, and kidney contained the highest concentration of THg. These tissues were predictably higher than others for several reasons. The muscle commonly contains the highest concentration of Hg in adult sharks due to its high affinity for the sulfhydryl groups associated with protein in muscle tissue. This combined with the fact that Hg is eliminated from muscle at a slow rate means it can accumulate in high concentrations within it starting at early life stages (Bloom, 1992; Trudel and Rasmussen, 1997). Like the muscle, the heart and kidney are also highly vascularized compared to the tissues in this study that contained low THg concentrations such as the fin and skin which increases their exposure to Hg. The liver represents another important tissue utilized in Hg studies and the concentrations found in the embryos in this study were significantly lower than those reported in the muscle for each species. These findings are consistent with previous studies which have reported THg concentrations of embryonic muscle that were higher than those in the liver (Lyons and Lowe, 2013; Le Bourg et al., 2014; van Hees and Ebert, 2017). Unlike those studies, however, all of the maternal sharks investigated in this study contained higher THg concentrations in the liver than in the muscle. Despite this difference, the tissue THg distribution in the embryos reported here was similar to those that have been found previously. Based on the concentrations in the embryos of this study, and since sharks are known to utilize the energy stores from within their livers to nourish their offspring during development, the liver is likely the tissue from which these species are receiving

most of their maternally derived Hg (Rossouw, 1987; Pethybridge et al., 2011).

For all three species, either the heart or the kidney contained the second-highest concentration of THg amongst the seven tissues. Mercury exposure has been found to result in deleterious health effects in both tissues in fish and humans (Virtanen et al., 2007; Fernandes Azevedo et al., 2012; Monteiro et al., 2013 Houston, 2014). Prior studies have identified the negative effects of Hg on the cardiovascular health of teleost fish species which includes damage to blood vessels and heartbeat irregularities at 0.015 – 0.040 μg/g wet weight (Heisinger and Green, 1975; Weis and Weis, 1977). These effects have been studied in greater detail within humans as Hg toxicity has been found to increase the risk of cardiovascular disease, myocardial infarctions, and stroke (Kromhout et al., 1985; Shekelle et al., 1985; Salonen et al., 1995, Virtanen et al., 2007; Choi et al., 2009; Rice et al., 2014). Prior studies have also shown that after a four-week exposure to dietary MeHg the kidneys of teleost fish displayed negative histomorphological effects in the form of degraded renal tubules and decreased overall function (Ghosh and Mandal, 2012; Lee et al., 2012; Morcillo et al, 2017). While the effects in both tissues have been investigated in teleost fish, there is little information on the effects Hg can have on these same tissues within sharks. Because of this, and the fact that the bull and blacktip shark embryos in this study contained THg concentrations > 0.23 μg/g wet weight in both the heart and kidney, the embryos of these species may be at risk of facing the cardiovascular and kidney impairments seen in other species even before parturition.

The mean THg concentration measured in the brains of all three species were more than 2-times lower than what was detected within the muscle tissue. This supports the finding of the few studies that have previously investigated the bioaccumulation of

Hg in the brains of sharks (Nam et al., 2011; Bergés-Tiznado et al., 2015; Ehnert-Russo and Gelsleichter, 2020). The THg concentrations reported for all species in this study are lower than those that have been found in juvenile lemon sharks (*Negaprion brevirostris)* (0.043 ± 0.023 µg/g wet weight) (Nam et al., 2011), juvenile scalloped hammerhead sharks (0.11 ± 0.01 µg/g wet weight) (Bergés-Tiznado et al., 2015), and juvenile Atlantic sharpnose (0.024 ± 0.012 µg/g wet weight) (Ehnert-Russo and Gelsleichter, 2020). The THg concentrations in embryonic brain tissue reported in this study are below the threshold for clinical neurotoxicity (0.5-1.0 µg/g wet weight) (Sandheinrich and Wiener, 2011). Therefore, it is unlikely that the embryos in this study are at risk of the neurological damages brought on by Hg toxicity. Despite this, little is known about the direct effects it can have on sharks throughout their lifespans, as the concentrations of Hg within their tissues will likely continue to accumulate as they age and therefore increase the risk of neurotoxic implications at later life stages.

Due to the limited information available on overall THg concentrations for the species included in this study, few comparisons could be made to other studies. Adams and McMichael (1999) reported THg concentrations within the muscle of four blacktip shark embryos off the coast of Eastern Florida which ranged from 0.63 – 0.78 µg/g wet weight. Although the sample size for that study was much smaller, the concentrations reported were higher than what was found for the same species in this study (0.490 µg/g wet weight). Additionally, Hueter et al. (1995) reported THg concentrations within the muscle of eight sandbar embryos and found an average of 0.353 ± 0.392 µg/g wet weight which was also higher than what was found in this study. The difference in these values could be explained by both the low sample size in that study and the fact that two of the

embryos included in the study contained much higher concentrations than the others (range of 1.06 μg/g wet weight between embryos). In addition to having much larger sample sizes than the few studies that have investigated these species in this area previously, this study was the first to investigate the levels of THg found in bull shark embryos.

For each species, the fin contained the lowest THg concentration amongst all tissues other than the liver. These results are not in agreement with the findings of previous studies in which adult sharks have been found to contain the highest Hg concentrations in the muscle and liver, followed by the fin (Escobar-Sánchez et al., 2010; Delshad et al., 2012; O'Bryhim et al., 2017). It is important to note, however, that studies that have investigated Hg concentrations in the fins of sharks have only done so for adults. No comparisons have ever been made between the fin and liver of shark embryos. It is unlikely that the distributions of THg found in the tissues of the embryonic sharks in this study will remain the same throughout their lifespans, as the concentrations of THg found within the maternal sharks were highest in the liver for each species. As the embryos continue to grow, the lack of vascularization throughout the fins as well as the increase in the contaminant load within the liver will likely cause the distributions to transition into what is normally seen in adults.

There is also a lack of data on THg concentrations in shark skin and how they compare to those that are found in other embryonic tissues. One of the only studies to investigate Hg concentrations in the skin did so in adult demersal species (longnose velvet dogfish, *Centroscymnus crepidater*; southern dogfish, *Centrophorus zeehaani*; kitefin shark, *Dalatias licha*; New Zealand lantern shark, *Etmopterus baxteri*; plunket

shark, *Proscymnodon plunketi*; spiny dogfish, *Squalus acanthias*; shortnose spurdog, *Squalus megalops*; and shortspine spurdog, *Squalus mitsukurii*) and found that it contained the lowest concentrations when compared to other tissues such as the muscle, liver, and kidney (Pethybridge et al., 2010). The low concentrations found in the skin previously have been accredited to the idea that Hg is taken up through the diet rather than through the environment. However, since the embryos are not exposed to external Hg while in utero, the Hg within their skin must be derived from the diet, which in this case is represented by the nourishment provided via the placental attachment to the mother. As sharks age, however, the dermal denticles that make up their skin continuously grow and are substituted as they are shed and replaced throughout their lifespan (Meyer and Seegers, 2012). This process has been found to cause the skin to have faster isotopic turnover rates in comparison to other tissues as the shedding and replacement of denticles is continuously taking place and essentially resetting these values (Li et al., 2016). Therefore, if this process occurs similarly regarding Hg within shark skin it could explain the higher concentrations of THg found in the skin of these embryos when compared to the other tissues. If this is the case, variation in Hg values in the skin of these sharks will likely become greater as they grow and the turnover rate of Hg in the skin increases as denticles are shed and replaced.

Interspecies differences observed in THg concentrations in the embryos investigated in this study can best be explained by looking at those found in their mothers. For instance, concentrations of THg varied between each of the three species with both the bull and blacktip embryos containing higher concentrations than the sandbar embryos. In comparison, the concentrations of THg found in maternal bull and

blacktip sharks were both greater than what was found in the maternal sandbar sharks. Based on this, species with higher maternal THg concentrations also had greater THg concentrations in their embryos. Since female contaminant concentration is influencing the maternal transfer of Hg in these sharks, differences in female sharks at the species level, such as trophic position and reproductive history, represent factors that may explain the interspecies variations in embryo THg concentrations seen in this study.

Trophic position is commonly used to explained differences in the maternal offloading of contaminants in sharks (Borgå et al., 2004; Lyons et al., 2013). Since sharks that feed at higher trophic positions typically accumulate increased concentrations of Hg compared to those at lower trophic positions, they should be more capable of transferring larger Hg loads to their offspring during development. Slight differences in the trophic positions of these species have been reported previously with the bull shark feeding at the highest level (4.3) followed by the blacktip shark (4.2) and sandbar shark (4.1) (Cortés, 1999). This difference in feeding ecology could explain the similar order of THg concentrations found within their tissues as well as their embryos.

In addition to trophic position, differences in reproduction represent additional factors that have the potential to influence the degree of maternal transfer. For instance, there are small differences between each of these species when it comes to gestation time and litter size. Sandbar sharks have the lowest estimated gestation time out of all species (8-12 months) and although the difference is only a few months, this still decreases the potential for the continued offloading of Hg when compared to the bull and blacktip which have gestation times of 10 to 11 and 10 to 12 months, respectively. In addition to this, one of the main differences between the species in this study was the average litter

size. Sandbar sharks contained the largest litter size of all species with an average of 9 embryos per mother. The pregnant bull and blacktip sharks on the other hand contained an average of only 5.33 and 4.14 embryos, respectively. Since the litters of the sandbar shark contained roughly twice the number of embryos compared to the other species, they are capable of distributing their Hg load to a greater number of offspring during gestation. This could explain why their embryos contained lower THg concentrations compared to the bull and blacktip embryos.

All three species in this study utilize a placental viviparous reproductive strategy. This method allows for the direct transfer of nutrients from the mother to her developing embryos. However, it also serves as a means of Hg detoxification by allowing her to transfer a portion of her Hg load to her offspring throughout the length of the gestation period (Adams et al., 1999; Pethybridge et al., 2010). This process of maternal offloading has been reported in different placental viviparous shark species in the past. For example, Olin et al. (2014) reported the presence of maternally derived polychlorinated biphenyls (PCBs) in the livers of young-of-year bull sharks, while Weijs et al. (2015) found PCBs being maternally transferred to the livers of blacktip and bonnethead shark embryos. Although studies such as these have investigated the dynamics and occurrences of this process for other contaminants in the past, there is little information available on the maternal transfer of Hg in placental viviparous sharks. Despite this, comparisons can still be made to other species that utilize different forms of reproduction. Since the embryos of placental viviparous sharks receive contaminants directly from the mother throughout the entirety of the gestation period it is not surprising to find that these species contained higher THg concentrations compared to those that utilize yolk-sac viviparity

(ovoviviparous). For example, van Hees and Ebert (2017) found that the embryos of the leopard shark, which receive nutrition via an external yolk-sac, contained a mean muscle THg concentration of 0.015 ± 0.002 μg/g wet weight while Dutton and Venuti (2019) reported a mean Hg concentration of 0.295 ± 0.049 μg/g dry weight in the embryos of the ovoviviparous common thresher shark. The concentrations in both of those species were much lower than what was found in the muscle of the sandbar embryos in this study, which contained the lowest THg concentrations amongst all three species analyzed (0.183 ± 0.064 μg/g wet weight; 0.672 ± 0.190 μg/g dry weight). Because the placental viviparous sharks in this study contained higher concentrations than those that utilize less direct forms of nourishment, it is evident that reproductive strategy plays an important role in the maternal transfer of Hg in sharks.

2.4.2 Comparison of Embryo and Parent THg concentrations

The percent of female THg concentration present in the muscle and liver of embryos was highest in the blacktip, followed by the bull and sandbar sharks. The differences in the percentages between species are likely caused by variation in life-history traits. Although each of these species are placental viviparous, there are slight differences in these traits such as their sizes at sexual maturity and their overall age. Since the maternal bull sharks in this study contained the highest concentrations of THg in the muscle (9.13 μg/g dry weight), one might expect the embryos to contain the highest percentage of maternal THg. However, the blacktip embryos had higher percentages of maternal THg in both the muscle and liver. This could have something to do with the smaller sizes of the maternal blacktips. Since their average size of 135 cm was on the lower end of the species' estimated size range for reproductive maturity (120

– 190 cm) these litters could likely have been one of their first pregnancies. This is important because females have been found to offload the greatest amount of contaminants during their first reproductive event with subsequent litters receiving fewer contaminant loads than previous ones (Borrell et al., 1995; Lyons et al., 2013). If the blacktip embryos in this study were collected from sharks during their first pregnancies, it may explain why a greater percentage of THg was detected within the tissues of their embryos compared to the other sharks in this study which were much larger.

Overall, each species contained a higher percentage of maternal muscle THg than maternal liver THg. This is likely due to the THg concentrations in the livers of the maternal sharks being much higher than those found in their muscle. The THg concentrations in the livers of the female sharks were also much higher than the mean THg values reported in the livers of their corresponding embryos. Therefore, the percentage of maternal THg found in the livers of the embryos was much lower than what was expected. As mentioned previously, females utilize the energy stores within their livers during the reproductive process. Because of this, and the fact that the rate of THg elimination from muscle tissue is slow and decreases with increased body size (Trudel and Rasmussen, 1997), it is likely that the liver represents one of the primary sources of THg offloading in these species. The muscle, which had lower concentrations in adults and higher concentrations in embryos compared to the liver had a greater percentage of the maternal THg found in the embryos. Lyons and Lowe have reported similar findings to the ones reported here in which the muscle of the common thresher shark had a higher percent mercury transfer (8.14%) than the liver (1.47%). While there have been no reports on the percentage of maternal THg concentration found in the

embryos of bull or sandbar sharks previously, Adams and McMichael (1999) reported the percentage of maternal THg in the muscle of blacktip embryos and found percentages comparable to those reported in this study (27.4 - 33.9%).

2.4.3 Stable Isotope Fractionation Between Maternal and Embryonic Sharks

The $\delta^{13}C$ and $\delta^{15}N$ values for the three species in this study are comparable to what has previously been reported in other placentatrophic species. For instance, both the bull and sandbar embryos contained enriched $\delta^{13}C$ values compared to their mothers, which is the same as what has been reported for the scalloped hammerhead (Vaudo et al., 2010), Atlantic sharpnose (McMeans et al., 2010), and bonnethead shark (Olin et al., 2018). Since the expected $\delta^{13}C$ value of an organism is expected to be anywhere from 0-2‰ greater than their diet, it is not surprising to see that these embryos were enriched compared to their mothers. However, the embryos of the blacktip were deplete in $\delta^{13}C$ in comparison to their mother. These findings coincide with what has been found for this species before, with Vaudo et al. (2010) reporting blacktip embryos that were deplete in $\delta^{13}C$ compared to their mothers off the coast of Florida. Those embryos also contained similar carbon values to those found in this study (-15.91 ± 0.09‰). This study introduces another example of variability in the $\delta^{13}C$ values of placentatrophic sharks and further emphasizes the need for further investigation into the sources of this variation in isotopic values.

Contrasting patterns were revealed for the $\delta^{15}N$ values of these species when compared to previous studies. Typically, embryos of placentatrophic sharks, such as the Atlantic sharpnose, scalloped hammerhead, and blacktip, have been shown to have enriched $\delta^{15}N$ values compared to their mothers (McMeans et al., 2010; Vaudo et al.,

2010). In this study, the only species that had embryos that were enriched compared to its mother were those of the bull shark. The $\delta^{15}N$ values reported for the blacktip embryos were comparable to their mother while the sandbar embryos were significantly depleted compared to their mothers. These findings differ from the enriched blacktip embryos reported by Vaudo et al. (2010). While deplete $\delta^{15}N$ values observed in the sandbar could be caused by a shift in the diet of the maternal sharks during the early stages of the pregnancy to feed at a lower trophic position, the isotopic turnover rate for the muscle of sandbar sharks is slow (potentially taking up to two years) likely indicating that these isotopic levels were present before their pregnancy (Logan and Lutcavage, 2010; Broadhurst et al., 2019). Although most stable isotope studies on placentatrophic species find enriched embryos, Olin et al. (2018) reported deplete $\delta^{15}N$ values in the embryos of bonnethead sharks. This further highlights the variability in the patterns of isotopic fractionation and the need for continued research to better understand stable isotope dynamics in placentatrophic sharks.

Although the relationship detected between the $\delta^{13}C$ and $\delta^{15}N$ values for all species was significant, the model fails to accurately explain the data. Because of this, the relationship between the isotopic values of each species should be interpreted individually. Both blacktip and sandbar sharks had significant negative relationships between $\delta^{13}C$ and $\delta^{15}N$ values. Based on these findings, embryos that are more enriched in $\delta^{13}C$ are also more deplete in $\delta^{15}N$. This may suggest that trophic differences seen in embryos could be caused by females feeding from different carbon pools, with those that are enriched in $\delta^{13}C$ feeding in more near-shore areas while those that are deplete feed in more pelagic environments.

The relationship between $\delta^{13}C$ and THg concentration and $\delta^{15}N$ and THg concentration were less apparent. Little information on the relationship between Hg concentration and $\delta^{15}N$ in embryos has been published previously. These relationships have previously been investigated in adults, however, the interpretation of stable isotope data is still uncertain when examining embryonic sharks. For example, similar to this study, Rumbold et al. (2014) found no significant relationship between THg and $\delta^{15}N$ in six species of adult sharks (blacknose shark, bull shark, blacktip shark, great hammerhead, Atlantic sharpnose shark, and tiger shark) but observed significant relationships when investigating $\delta^{13}C$ values and THg. While the blacktip embryos in this study displayed a significant positive relationship between $\delta^{13}C$ and THg, the bull embryos displayed a significant negative relationship. Within adults, increasing $\delta^{13}C$ values and THg concentrations could indicate increased Hg exposure coinciding with increased marine carbon sources (Rumbold et al., 2014). With that in mind, the THg concentrations of bull sharks may be decreasing as they feed from more near-shore carbon pools, however, the range of $\delta^{13}C$ values is small for both the adults and embryos in this study (0.2 and 0.4‰ respectively). Prior studies have also found that negative relationships between $\delta^{13}C$ and $\delta^{15}N$ values and Hg within adult sharks are potentially correlated with increases in Hg accumulation due to growth along with decreases in $\delta^{13}C$ and $\delta^{15}N$ values due to shifts in foraging areas (Endo et al., 2015). Overall, it is difficult to identify the causes of these relationships within embryonic sharks and the results from this study further emphasize that the isotopic values in young-of-year sharks should be interpreted with caution until they can differentiate their values from their mother's by foraging on their own prey items.

2.4.4 Conclusions

Previous studies have reported the maternal transfer of THg in several species utilizing various forms of reproduction and shown that it represents a significant exposure pathway for contaminants in sharks that should not be overlooked. This study built upon this prior research by identifying THg concentrations in species and tissue types that have yet to be reported. The concentrations of THg observed in this study for the embryos of bull, blacktip, and sandbar sharks provide further evidence to suggest that placental viviparous species are capable of offloading larger concentrations of Hg to their offspring when compared to other shark species that utilize less direct forms of reproduction such as an external yolk sac. Overall, these concentrations demonstrate that prior to parturition young-of-year sharks will likely contain high Hg body burdens, in part, due to what they were exposed to while *in utero* via the process of maternal offloading.

2.4.5 Future Research

While this study provided new information on the distribution of THg throughout the tissues of embryonic sharks, including those which have not been previously reported, there are still many unknowns regarding the processes behind maternal transfer and the factors that influence the differences such as those reported here, both between species as well as between the tissues within a species. Further research is needed to identify the mechanics behind the distribution of Hg throughout the various tissues of embryonic sharks to identify why those such as the muscle and heart receive the greatest concentrations of Hg despite those in the liver of the mother containing the highest concentrations in comparison. In addition to this, the effects that Hg has on embryonic tissues is one of the most important pieces of information missing throughout the

literature. Mercury concentrations have been reported for both the adults and embryos of several species yet little to nothing is known about how these large concentrations of Hg are affecting sharks and their overall health. Future research should also include information on selenium (Se):Hg molar ratios and the protective role Se can play within the body by investigating how they vary in shark species that utilize different reproductive strategies. Lastly, any future studies focusing on concentrations of contaminants within embryonic sharks would be beneficial to identify differences between species based on factors such as geographic range, diet, and reproductive strategy and to identify any trends that have yet to be identified for sharks due to the lack of available information on these organisms.

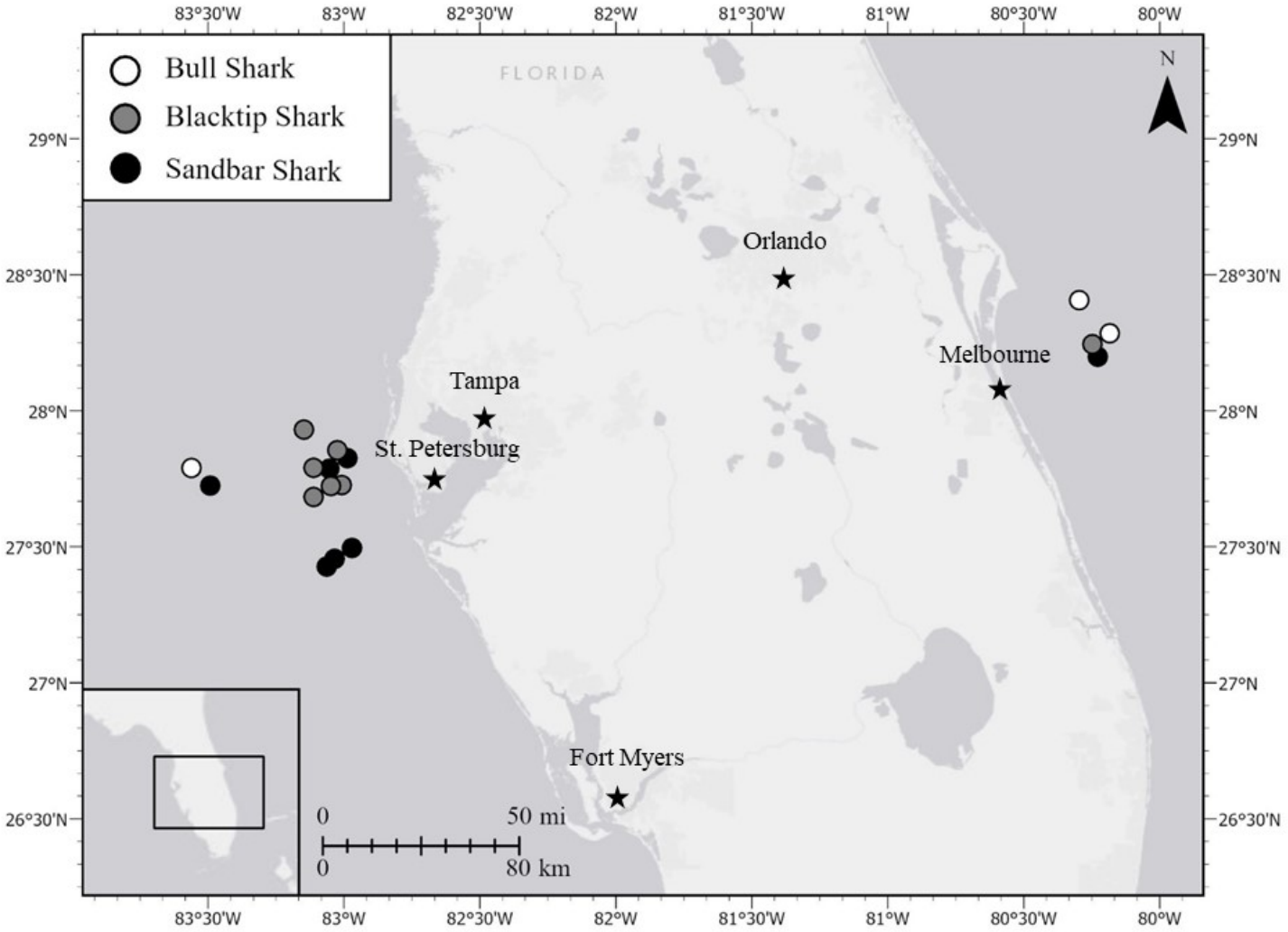

Figure 9. Collection locations of pregnant bull, blacktip, and sandbar sharks off the Gulf and Atlantic coasts of Florida.

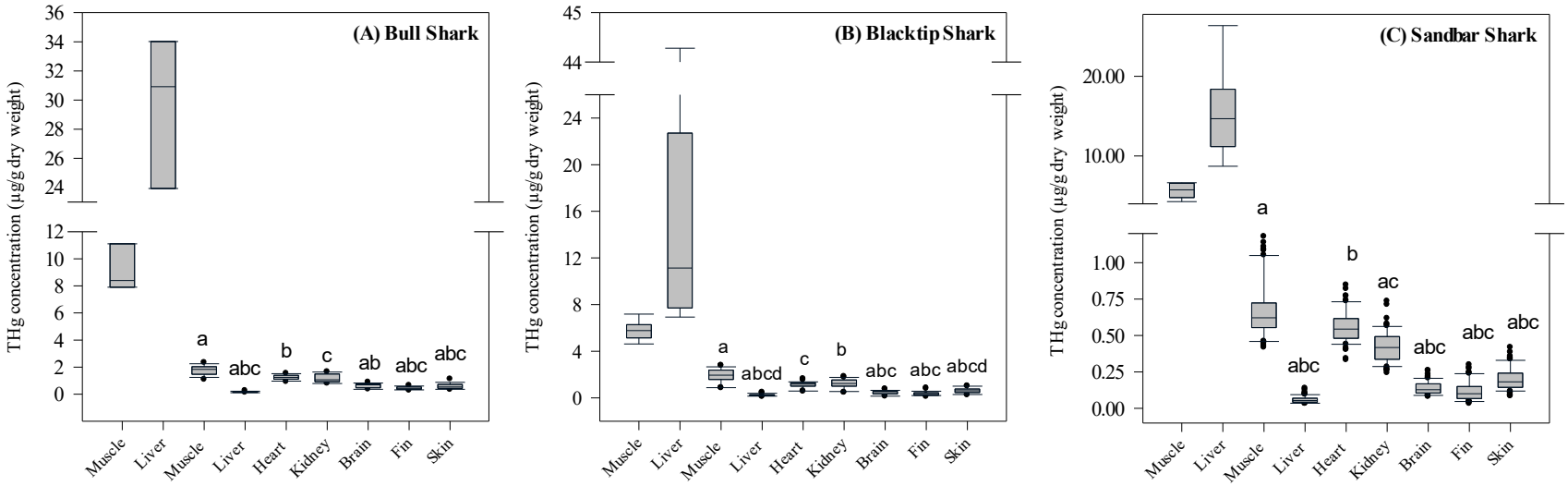

Figure 10. THg concentrations (µg/g dry weight) in maternal and embryonic tissues of bull (A), blacktip (B), and sandbar (C) sharks. Significant differences in THg concentrations between tissue types indicated by similar lettering.

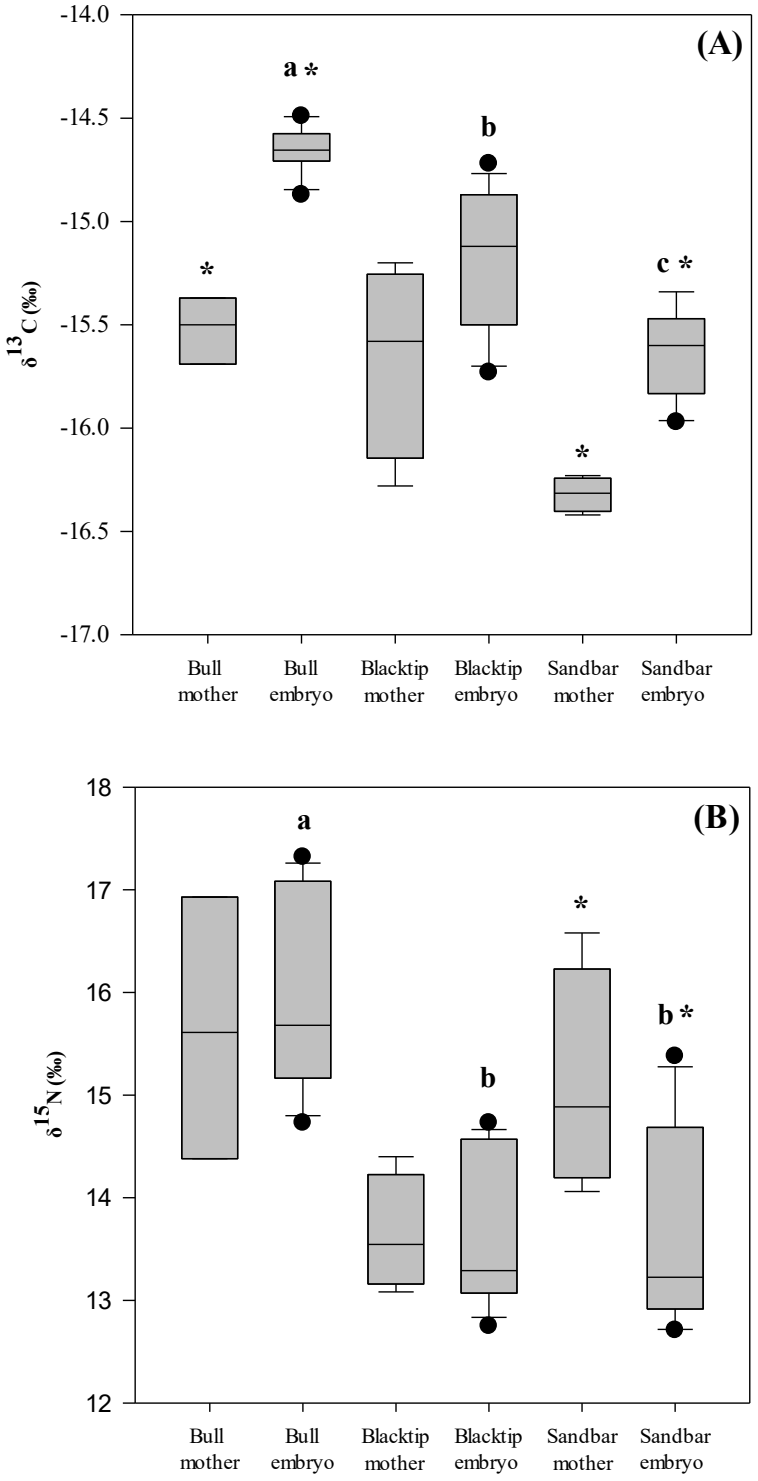

Figure 11. Maternal and embryonic $\delta^{13}C$ (A) and $\delta^{15}N$ (B) values. Significant differences indicated between embryo species by letters and between mothers and embryos by asterisks.

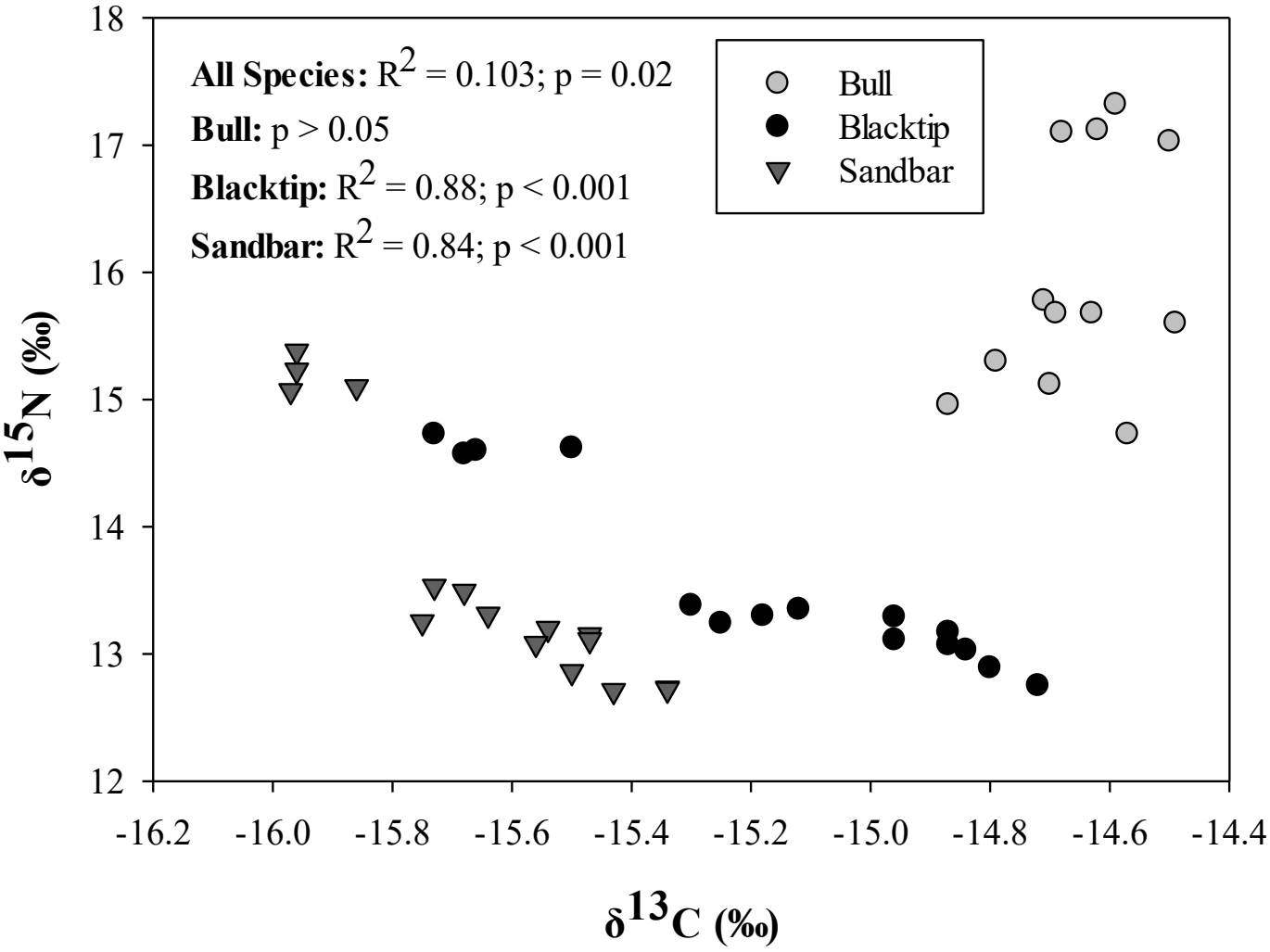

Figure 12. Relationship between $\delta^{13}C$ and $\delta^{15}N$ in bull, blacktip, and sandbar shark embryos.

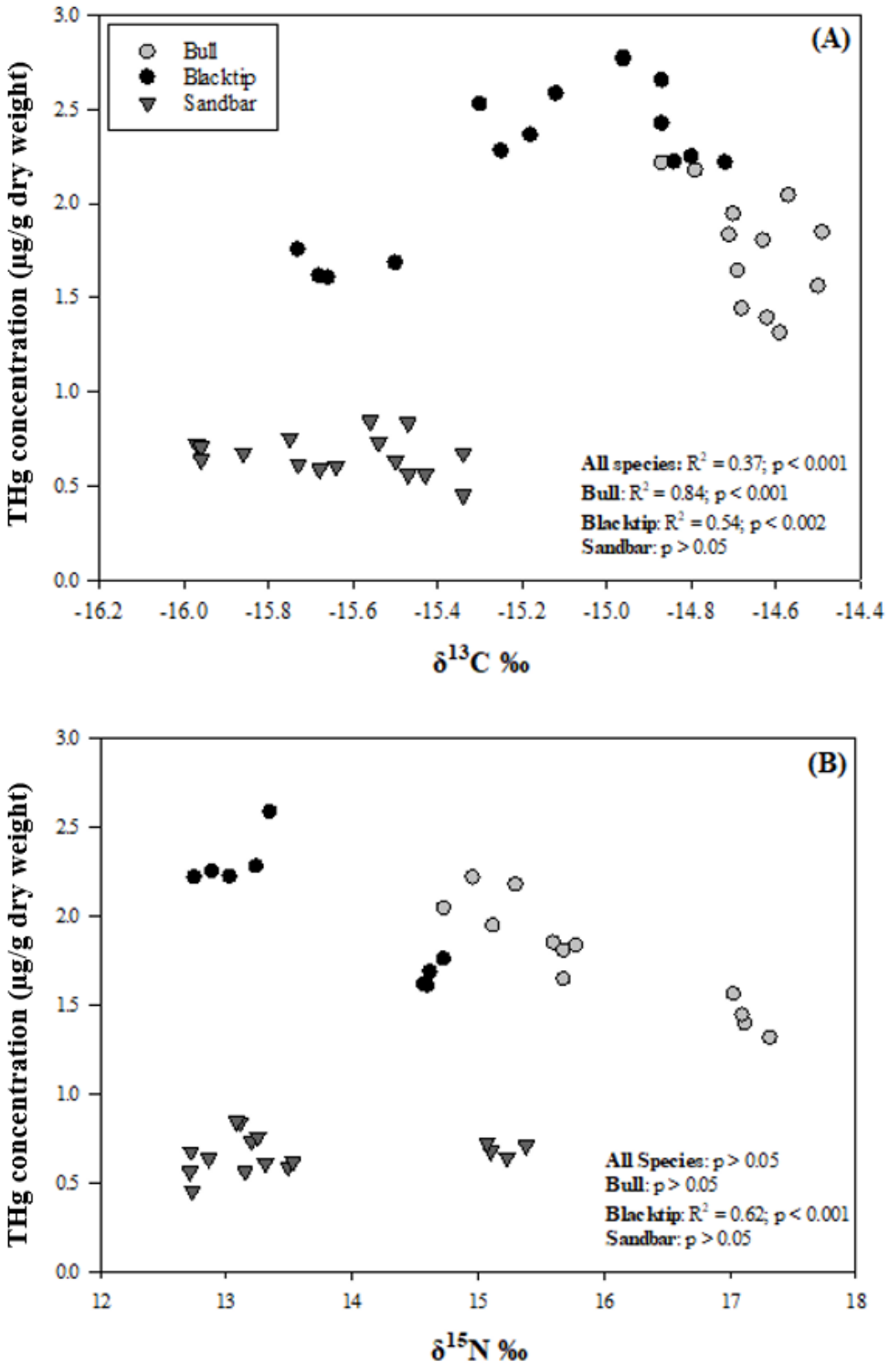

Figure 13. Relationship between $\delta^{13}C$ and THg concentration (A) and $\delta^{15}N$ and THg concentration (B) in muscle tissue of bull, blacktip, and sandbar shark embryos.

Table 8. Reproductive information for bull, blacktip, and sandbar sharks (Campagno, 1984; Branstetter, 1987; Castro, 1996; Baremore and Passerotti, 2013; Klimley, 2013; Natanson et al., 2014).

Species	Fork length at sexual maturity (cm)	Age at sexual maturity (year)	Reproductive frequency	Gestation time (months)	Litter size
Bull	180-230	10	Biennial	10 to 11	1 to 13
Blacktip	150-155	7-8	Biennial	10 to 12	4 to 7
Sandbar	144-183	13	Biennial	8 to 12	5 to 12

Table 9. Species investigated in this study along with the total number of mothers and embryos, and the mean, minimum, and maximum litter sizes.

Species	Mothers	Embryos	Mean litter	Min litter	Max litter
Bull shark	3	16	5.3	4	7
Blacktip shark	7	29	4.1	2	6
Sandbar shark	7	63	9.0	8	10

Table 10. Moisture content (mean ± standard deviation and minimum and maximum percentages) for each embryonic tissue.

Species	Tissue	Mean	Min	Max
Bull	Muscle	76.3 ± 1.2	73.4	77.7
	Liver	33.0 ± 4.4	18.4	38.5
	Fin	68.9 ± 11.0	29.6	83.7
	Skin	64.6 ± 2.6	58.7	68.2
	Heart	80.3 ± 0.73	79.1	81.8
	Brain	85.2 ± 0.41	85.8	87.0
	Kidney	79.5 ± 1.1	77.9	81.4
Blacktip	Muscle	74.0 ± 2.6	67.7	82.5
	Liver	37.5 ± 3.0	27.7	41.4
	Fin	63.5 ± 5.6	40.2	69.4
	Skin	57.9 ± 4.0	50.6	71.3
	Heart	79.1 ± 2.8	76.3	91.7
	Brain	84.5 ± 0.53	83.6	85.7
	Kidney	77.4 ± 2.4	74.6	87.9
Sandbar	Muscle	73.1 ± 2.7	67.3	79.8
	Liver	34.9 ± 8.9	28.1	43.1
	Fin	64.5 ± 3.6	53.6	70.7
	Skin	58.4 ± 2.6	53.1	63.5
	Heart	78.6 ± 1.6	72.4	83.6
	Brain	85.6 ± 0.82	84.2	89.3
	Kidney	76.4 ± 2.5	71.6	89.9

Table 11. Bull, blacktip, and sandbar stahark mother and embryo fork lengths. (Bull: mother $n = 3$, embryo $n = 16$; Blacktip: mother $n = 7$, embryo $n = 29$; Sandbar: mother $n = 7$, embryo $n = 63$)

Species	Median	Mean	Standard Deviation	Minimum	Maximum
Bull mother	200	201	5.13	197	207
Bull embryo	34.7	34.8	1.15	32.8	36.7
Blacktip mother	137	135	8.58	124	147
Blacktip embryo	34.4	34.6	2.1	31.5	38.0
Sandbar mother	168	168	5.09	160	176
Sandbar embryo	33.3	33.1	3.3	26.2	39.1

Table 12. THg concentrations (µg/g dry weight) in the muscle, heart, brain, liver, kidney, skin and fin of bull, blacktip, and sandbar shark embryos and the THg concentrations in the muscle and liver of their mothers.

Species		Tissue	Median	Mean	Standard Deviation	Minimum	Maximum
Bull	Mother	Muscle	8.40	9.13	1.73	7.90	11.1
		Liver	30.9	29.6	5.17	23.9	34.0
	Embryo	Muscle	1.84	1.79	0.355	1.08	2.33
		Heart	1.24	1.25	0.183	0.908	1.53
		Brain	0.687	0.634	0.166	0.355	0.874
		Liver	0.163	0.161	0.040	0.107	0.249
		Kidney	1.07	1.17	0.313	0.775	1.64
		Skin	0.544	0.587	0.200	0.329	1.11
		Fin	0.410	0.442	0.105	0.273	0.640
Blacktip	Mother	Muscle	7.90	7.74	0.795	6.41	8.88
		Liver	58.3	56.3	29.6	12.9	96.0
	Embryo	Muscle	1.94	1.92	0.571	0.838	2.77
		Heart	1.18	1.12	0.270	0.543	1.63
		Brain	0.434	0.427	0.166	0.126	0.745
		Liver	0.218	0.235	0.0880	0.0932	0.465
		Kidney	1.24	1.22	0.388	0.445	1.82
		Skin	0.603	0.613	0.215	0.221	1.01
		Fin	0.348	0.365	0.175	0.0808	0.840

Table 12. Continued

Sandbar	Mother	Muscle	5.75	5.63	0.876	4.27	6.66
		Liver	14.7	15.5	5.78	8.72	26.3
	Embryo	Muscle	0.622	0.672	0.190	0.417	1.18
		Heart	0.543	0.559	0.108	0.331	0.845
		Brain	0.128	0.141	0.0439	0.0791	0.259
		Liver	0.0530	0.0600	0.0229	0.0318	0.138
		Kidney	0.417	0.421	0.109	0.242	0.735
		Skin	0.182	0.201	0.0758	0.0844	0.418
		Fin	0.100	0.112	0.0670	0.0320	0.298

Table 13. Bull, blacktip, and sandbar shark embryo muscle and liver THg concentration as a percentage of the mother's muscle and liver THg concentration.

Species	Tissue	Median	Mean	Standard Deviation	Minimum	Maximum
Bull	Muscle	18.9	19.2	3.30	16.1	22.6
	Liver	0.550	0.530	0.100	4.30	0.620
Blacktip	Muscle	23.8	24.4	7.48	11.6	32.3
	Liver	0.610	0.680	0.540	0.200	1.76
Sandbar	Muscle	10.7	12.1	2.81	9.60	16.4
	Liver	0.330	0.450	0.270	0.270	1.04

Table 14. Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) in maternal and embryonic muscle tissue of each species.

Species	Median	Mean	Standard Deviation	Minimum	Maximum
$\delta^{13}C$					
Bull mother	-15.5	-15.5	0.161	-15.7	-15.4
Bull embryo	-14.6	-14.7	0.110	-14.9	-14.5
Blacktip mother	-15.6	-15.7	0.469	-16.3	-15.2
Blacktip embryo	-15.1	-15.2	0.346	-15.7	-14.7
Sandbar mother	-16.3	-16.3	0.083	-16.4	-16.2
Sandbar embryo	-15.6	-15.6	0.216	-16.0	-15.3
$\delta^{15}N$					
Bull mother	15.6	15.6	1.275	14.4	16.9
Bull embryo	15.7	16.0	0.978	14.7	17.3
Blacktip mother	13.5	13.6	0.564	13.1	14.4
Blacktip embryo	13.3	13.5	0.701	12.7	14.7
Sandbar mother	14.9	15.1	1.084	14.1	16.6
Sandbar embryo	13.2	13.6	0.408	12.7	15.4

APPENDIX SECTION

Supplementary Table A. THg concentrations (μg/g dry weight) in individual pregnant bull sharks and their embryos.

		Tissue	Median	Mean	Standard Deviation	Minimum	Maximum
SBU 5	Mother	Muscle	N/A	8.40	N/A	N/A	N/A
		Liver	N/A	23.9	N/A	N/A	N/A
	Embryos	Muscle	1.39	1.36	0.181	1.07	1.56
		Heart	1.09	1.13	0.117	0.988	1.283
		Brain	0.455	0.464	0.123	0.355	0.665
		Liver	0.115	0.130	0.0276	0.107	0.171
		Kidney	0.842	0.851	0.0674	0.775	0.923
		Skin	0.553	0.552	0.200	0.329	0.766
		Fin	0.354	0.358	0.0573	0.273	0.423
SBU 15	Mother	Muscle	N/A	11.1	N/A	N/A	N/A
		Liver	N/A	30.9	N/A	N/A	N/A
	Embryos	Muscle	2.04	2.10	0.143	1.94	2.33
		Heart	1.42	1.39	0.124	1.20	1.53
		Brain	0.758	0.781	0.0469	0.739	0.874
		Liver	0.188	0.193	0.0309	0.154	0.249
		Kidney	1.55	1.49	0.14	1.33	1.64
		Skin	0.664	0.665	0.241	0.406	1.11
		Fin	0.543	0.534	0.0833	0.384	0.640
SBU 17	Mother	Muscle	N/A	7.90	N/A	N/A	N/A
		Liver	N/A	34.0	N/A	N/A	N/A
	Embryos	Muscle	1.82	1.78	0.094	1.64	1.85
		Heart	1.17	1.15	0.176	0.908	1.33
		Brain	0.592	0.591	0.112	0.490	0.689
		Liver	0.132	0.145	0.026	0.130	0.184
		Kidney	0.985	1.01	0.080	0.948	1.12
		Skin	0.502	0.496	0.0459	0.444	0.537
		Fin	0.377	0.387	0.0262	0.368	0.425

Supplementary Table B. THg concentrations (µg/g dry weight) individual pregnant blacktip sharks and their embryos. ND = not determined due to small litter size.

		Tissue	Median	Mean	Standard Deviation	Minimum	Maximum
SBK 2	Mother	Muscle	N/A	7.48	N/A	N/A	N/A
		Liver	N/A	12.5	N/A	N/A	N/A
	Embryos	Muscle	0.870	0.864	0.0182	0.838	0.879
		Heart	0.580	0.611	0.0879	0.543	0.740
		Brain	0.144	0.143	0.0133	0.126	0.158
		Liver	0.124	0.125	0.0296	0.0932	0.156
		Kidney	0.508	0.515	0.0708	0.445	0.600
		Skin	0.271	0.281	0.0592	0.221	0.359
		Fin	0.189	0.172	0.0641	0.0808	0.230
SBK 12	Mother	Muscle	N/A	6.41	N/A	N/A	N/A
		Liver	N/A	12.9	N/A	N/A	N/A
	Embryos	Muscle	1.52	1.52	ND	1.50	1.55
		Heart	0.903	0.903	ND	0.808	0.999
		Brain	0.352	0.352	ND	0.329	0.375
		Liver	0.228	0.228	ND	0.219	0.237
		Kidney	0.992	0.992	ND	0.925	1.06
		Skin	0.448	0.448	ND	0.371	0.524
		Fin	0.349	0.349	ND	0.318	0.380
SBK 13	Mother	Muscle	N/A	8.88	N/A	N/A	N/A
		Liver	N/A	88.4	N/A	N/A	N/A
	Embryos	Muscle	1.93	1.92	0.0780	1.81	2.04
		Heart	1.25	1.24	0.0674	1.17	1.35
		Brain	0.419	0.421	0.0536	0.359	0.481
		Liver	0.200	0.215	0.0592	0.152	0.327
		Kidney	1.30	1.29	0.131	1.09	1.47
		Skin	0.676	0.684	0.202	0.440	1.01
		Fin	0.370	0.342	0.0813	0.242	0.436

Supplementary Table B. Continued

SBK 17	Mother	Muscle	N/A	8.22	N/A	N/A	N/A
		Liver	N/A	58.6	N/A	N/A	N/A
	Embryos	Muscle	2.71	2.65	0.163	2.42	2.77
		Heart	1.43	1.45	0.156	1.29	1.63
		Brain	0.689	0.675	0.0795	0.579	0.745
		Liver	0.350	0.358	0.0888	0.269	0.465
		Kidney	1.74	1.67	0.206	1.36	1.82
		Skin	0.769	0.823	0.121	0.750	1.00
		Fin	0.671	0.636	0.216	0.360	0.840
SBK 18	Mother	Muscle	N/A	7.90	N/A	N/A	N/A
		Liver	N/A	58.3	N/A	N/A	N/A
	Embryos	Muscle	2.40	2.43	0.124	2.28	2.58
		Heart	1.29	1.24	0.116	1.08	1.37
		Brain	0.571	0.573	0.0187	0.550	0.595
		Liver	0.254	0.284	0.0791	0.205	0.393
		Kidney	1.65	1.61	0.137	1.39	1.75
		Skin	0.726	0.723	0.0925	0.601	0.833
		Fin	0.422	0.395	0.140	0.195	0.570
SBK 20	Mother	Muscle	N/A	7.22	N/A	N/A	N/A
		Liver	N/A	29.0	N/A	N/A	N/A
	Embryos	Muscle	2.22	2.23	0.0175	2.22	2.25
		Heart	1.12	1.10	0.0910	0.998	1.18
		Brain	0.442	0.447	0.0157	0.434	0.464
		Liver	0.245	0.250	0.0400	0.213	0.292
		Kidney	1.21	1.20	0.0326	1.17	1.23
		Skin	0.607	0.711	0.258	0.521	1.01
		Fin	0.518	0.427	0.186	0.213	0.550
SBK 25	Mother	Muscle	N/A	8.10	N/A	N/A	N/A
		Liver	N/A	96.0	N/A	N/A	N/A
	Embryos	Muscle	1.61	1.63	0.0963	1.50	1.76
		Heart	1.11	1.12	0.134	0.914	1.26
		Brain	0.332	0.334	0.0332	0.287	0.374
		Liver	0.178	0.192	0.0478	0.145	0.269
		Kidney	1.02	1.03	0.0752	0.944	1.15
		Skin	0.525	0.521	0.0830	0.444	0.653
		Fin	0.261	0.266	0.0525	0.219	0.348

Supplementary Table C. THg concentrations (µg/g dry weight) individual pregnant sandbar sharks and their embryos.

Litter		Tissue	Median	Mean	Standard Deviation	Minimum	Maximum
SSB 2	Mother	Muscle	N/A	5.85	N/A	N/A	N/A
		Liver	N/A	16.4	N/A	N/A	N/A
	Embryos	Muscle	0.608	0.607	0.0750	0.494	0.721
		Heart	0.0540	0.0557	0.0087	0.0442	0.0698
		Brain	0.0676	0.109	0.0717	0.0516	0.271
		Liver	0.203	0.236	0.0792	0.144	0.356
		Kidney	0.517	0.488	0.0980	0.331	0.632
		Skin	0.358	0.356	0.0637	0.260	0.458
		Fin	0.119	0.121	0.0125	0.100	0.148
SSB 19	Mother	Muscle	N/A	4.78	N/A	N/A	N/A
		Liver	N/A	18.4	N/A	N/A	N/A
	Embryos	Muscle	0.460	0.459	0.0248	0.417	0.500
		Heart	0.0448	0.0494	0.0188	0.0318	0.0937
		Brain	0.0723	0.0734	0.0231	0.0361	0.104
		Liver	0.189	0.192	0.0503	0.135	0.293
		Kidney	0.493	0.515	0.0591	0.439	0.601
		Skin	0.353	0.360	0.0389	0.311	0.419
		Fin	0.0972	0.0985	0.0108	0.0791	0.117
SSB 21	Mother	Muscle	N/A	4.27	N/A	N/A	N/A
		Liver	N/A	26.3	N/A	N/A	N/A
	Embryos	Muscle	0.683	0.677	0.0333	0.622	0.720
		Heart	0.0711	0.0744	0.0105	0.0625	0.0961
		Brain	0.145	0.177	0.0862	0.0710	0.298
		Liver	0.185	0.199	0.0642	0.131	0.319
		Kidney	0.537	0.566	0.0852	0.464	0.717
		Skin	0.502	0.502	0.0529	0.435	0.579
		Fin	0.146	0.152	0.0291	0.114	0.206

Supplementary Table C. Continued

SSB 29	Mother	Muscle	N/A	5.51	N/A	N/A	N/A
		Liver	N/A	11.2	N/A	N/A	N/A
	Embryos	Muscle	0.588	0.591	0.0374	0.539	0.660
		Heart	0.0369	0.0410	0.0091	0.0326	0.0548
		Brain	0.0637	0.0925	0.0657	0.0320	0.199
		Liver	0.159	0.174	0.0716	0.0844	0.280
		Kidney	0.518	0.528	0.0664	0.413	0.629
		Skin	0.410	0.397	0.0275	0.357	0.422
		Fin	0.0886	0.0950	0.0177	0.0817	0.137
SSB 39	Mother	Muscle	N/A	6.66	N/A	N/A	N/A
		Liver	N/A	12.6	N/A	N/A	N/A
	Embryos	Muscle	0.766	0.743	0.108	0.574	0.849
		Heart	0.0589	0.0668	0.0204	0.0473	0.110
		Brain	0.0712	0.0867	0.0464	0.0429	0.175
		Liver	0.214	0.251	0.0983	0.142	0.418
		Kidney	0.604	0.600	0.0984	0.459	0.740
		Skin	0.555	0.570	0.0970	0.427	0.735
		Fin	0.165	0.166	0.0172	0.142	0.191
SSB 45	Mother	Muscle	N/A	6.58	N/A	N/A	N/A
		Liver	N/A	8.72	N/A	N/A	N/A
	Embryos	Muscle	1.09	1.08	0.0648	0.963	1.18
		Heart	0.0779	0.0902	0.0282	0.0649	0.138
		Brain	0.106	0.122	0.0533	0.0765	0.241
		Liver	0.204	0.225	0.0588	0.176	0.346
		Kidney	0.753	0.723	0.0983	0.563	0.845
		Skin	0.478	0.491	0.0353	0.454	0.560
		Fin	0.214	0.223	0.0251	0.193	0.259
SSB 48	Mother	Muscle	N/A	5.75	N/A	N/A	N/A
		Liver	N/A	14.7	N/A	N/A	N/A
	Embryos	Muscle	0.600	0.607	0.0849	0.452	0.724
		Heart	0.0413	0.0475	0.0176	0.0347	0.0952
		Brain	0.166	0.158	0.0592	0.0690	0.239
		Liver	0.130	0.134	0.0405	0.0858	0.215
		Kidney	0.531	0.513	0.0765	0.341	0.598
		Skin	0.291	0.291	0.0347	0.242	0.366
		Fin	0.129	0.136	0.0163	0.119	0.170

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