UNF UNIVERSITY *of* NORTH FLORIDA.

UNF Digital Commons

UNF Graduate Theses and Dissertations   Student Scholarship

2017

Mercury Accumulation and Effects in the Brain of Atlantic Sharpnose Sharks (Rhiszoprionodon Terranovae)

Samantha L. Ehnert
University of North Florida

UNF UNIVERSITY *of* NORTH FLORIDA.

MERCURY ACCUMULATION AND EFFECTS IN THE BRAIN OF ATLANTIC
SHARPNOSE SHARKS (*RHIZOPRIONODON TERRAENOVAE*)

By

Samantha Ehnert

A thesis submitted to the Department of Biology
in partial fulfillment of the requirements for the degree of

Master of Science in Biology

UNIVERSITY OF NORTH FLORIDA

COLLEGE OF ARTS AND SCIENCES

March 2017

CERTIFICATE OF APPROVAL

The thesis "Mercury accumulation and effects in the brain of Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*)" submitted by Samantha Ehnert

Approved by the thesis committee: Date

_____   _____

Dr. Jim Gelsleichter
Committee Chair

_____   _____

Dr. Judith Ochrietor

_____   _____

Dr. Kara Yopak

Accepted for the Department of Biology:

_____   _____

Dr. Cliff Ross
Department Chair

Accepted for the College of Arts and Sciences:

_____   _____

Dr. Daniel Moon
Dean

Accepted for the University:

_____   _____

Dr. John Kantner
Dean of the Graduate School

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Content		Page

ABSTRACT

Sharks often bioaccumulate mercury (Hg) concentrations in their muscle to levels that threaten the health of human consumers. However, few published studies have examined if the high Hg levels seen in shark muscle also occur in the shark brain, or if Hg accumulation affects shark neurophysiology. Therefore, this study examined if shark brains accumulate significant levels of Hg, if Hg accumulation occurs in certain subcomponents of the brain, and if Hg accumulation is associated with oxidative stress effects on the shark central nervous system, with special focus on the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*). Sharks were collected along the U.S. Southeastern coast throughout most of the shark's geographical range. Known biomarkers of Hg-induced neurological effects (markers of glial cell damage, S100b, and markers of oxidative stress) in the shark cerebrospinal fluid were examined. Brain Hg levels were correlated with muscle Hg levels, but were significantly lower and did not exceed most known thresholds for neurological effects, suggesting limited potential for such responses. Data on known biomarkers of Hg-induced neurological effects support this premise, because they were not correlated with brain Hg levels. Organic methylmercury did not compose of a high percentage of the total mercury in the brain, indicating demethylation of Hg is occurring in the brain. Higher Hg levels were measured in the forebrain of the shark in comparison with the midbrain and hindbrain, but levels in both were below threshold levels for effects. This study is the first to demonstrate the correlation and significant difference of Hg in the brain and muscle of sharks, and it identifies significantly higher Hg levels in the forebrain; making this study one of the most extensive analysis of Hg in a single shark species.

INTRODUCTION

Mercury (Hg) is a highly toxic, non-essential metal that enters the environment from anthropogenic sources, such as the combustion of Hg-rich coal and waste incineration (Wiener et al., 2003). In aquatic systems, methylation by bacteria in sediment and water can convert inorganic forms of Hg into the metal's most persistent, bioavailable, and toxic form, the organometal methylmercury (CH_3Hg^+, also known as MeHg) (Wiener et al., 2003; Walker et al., 2012). The lipophilic nature of MeHg allows it to be readily absorbed into the body of aquatic organisms, particularly via the digestive system (Wiener et al., 2003). This is problematic because there is slow elimination of MeHg, causing Hg to bioconcentrate in most aquatic taxa (Gelsleichter & Walker, 2010). Hg levels also tend to increase as aquatic organisms grow (bioaccumulate) and with trophic position in the aquatic food webs (biomagnify) (Gelsleichter & Walker, 2010; Wiener et al., 2003).

Sharks (Class: Chondrichthyes) generally have a slow metabolism, lipid-rich livers, and a high trophic position, factors that allow them to bioconcentrate Hg to levels that could threaten the health of human seafood consumers (e.g., the U.S. Environmental Protection Agency's tissue-based criterion of 0.3 ppm Hg wet weight) (Gelsleichter & Walker, 2010; Wiener et al., 2003; U.S. EPA 2001). Because of this, previous studies on Hg accumulation in sharks have largely focused on levels occurring in edible muscle (Gelsleichter & Walker, 2010). In a review of shark toxicology by Gelsleichter and Walker (2010), it was reported that over 75 species of cartilaginous fish have been analyzed for Hg contamination, and 70% of these species exhibited muscle Hg levels that exceed the recommended levels for human consumption.

Although Hg uptake in sharks is well studied with regards to consumer safety, little is known about the health risks that Hg accumulation may pose to shark health and development of the central nervous system. The shark brain consists of certain brain regions that are shared across vertebrates, the forebrain, midbrain, and hindbrain. The following is a brief review of the morphology of the shark brain and proposed function of brain subunits, which is provided in order to inform the reader on structures that are discussed throughout the paper. A more extensive review on the neuroanatomy of the shark brain can be observed in Yopak (2012).

The forebrain of the shark contains the olfactory bulbs, telencephalon, and diencephalon. The olfactory bulbs receive the primary sensory projections from the olfactory nerve (Yopak et al., 2015). The telencephalon is associated with higher cognitive function, receives secondary olfactory information from the olfactory bulbs, and is implicated in multimodal sensory integration, receiving secondary and tertiary projections from the visual and octavolateralis systems (vestibular, auditory, lateral line, and electrosensory systems) (Hofmann and Northcutt, 2012; Northcutt, 1978; Bodznick, 1990; Yopak, 2012; Yopak et al., 2015). The diencephalon is a multisensory relay station to the telencephalon and contains the epithalamus, thalamus, and hypothalamus (Hofmann, 1999; Yopak, 2012). The epithalamus contains the habenula and the light and dark sensitive pineal organ (Yopak, 2012; Hamasaki and Streck,1971; Wilson and Dodd, 1973). The thalamus is a multisensory relay station to the telencephalon, receiving direct input from the retina and optic tectum, and electrosensory and mechanosensory projections from the midbrain, while the hypothalamus regulates the production of

hormones via the endocrine system and other homeostatic behaviors (Smeets, 1982; Yopak, 2012).

Next, the midbrain (mesencephalon) sends multi-sensory information to the diencephalon, and it is composed of the optic tectum and the tegmentum (Hofmann, 1999; Yopak, 2012). The optic tectum receives and integrates input from the optic nerves (axons of the retinal ganglion cells) and it maps the shark's visual space (Bodznick, 1990; Hofmann, 1999; Smeets, 1982; Yopak and Lisney, 2012; Yopak, 2012). Whereas, the tegmentum is critical for controlled locomotion, and it receives input from the spinal cord and tectum, and receives secondary electrosensory, mechanosensory, and auditory projections (Hayle, 1973; Yopak and Lisney, 2012; Yopak, 2012).

Lastly, the hindbrain contains the cerebellum and the medulla oblongata (with cerebellar-like structures) (Montgomery et al., 2012; Hofmann, 1999; Yopak, 2012). The cerebellar-like structures contain the octavolateralis nuclei, which receive electrosensory stimuli from the ampullae of Lorenzini (dorsal octavolateralis nucleus, DON) and the mechanoreceptive stimuli from the lateral line (medial octavolateralis nucleus, MON) (Montgomery et al., 2012; Yopak, 2012). The cerebellum control the shark's movement and reflexes, and corrects self-motion error and self-generated noise (electrosensory) (Paul and Roberts, 1979; Hofmann, 1999; Yopak, 2012). The medulla oblongata relays information to the rest of the brain from the spinal cord, and it receives primary octavolateralis projections (acoustic, vestibular, electroreceptors, lateral line mechanoreceptors) in the cerebellar-like regions (Montgomery et al., 2012; Yopak, 2012).

As stated previously, the knowledge about Hg accumulation in the shark heavily focuses on the shark muscle, and the literature is extremely limited for the brain, as only a few studies have attempted to look at shark brain Hg concentrations (Newman et al., 2011; Nam et al., 2011b; Bergés-Tiznado et al., 2015). This information is important because previous studies have indicated that Hg is primarily a central nervous system toxicant (Krey et al., 2015). Previous studies in other vertebrates have determined that once Hg enters the organism, it is absorbed into the blood and binds with a cysteine, which allows the Hg to be transported into the brain through the blood-brain barrier (a highly selective barrier composed of endothelial cells, pericytes (smooth muscle), and glia cells that separate the blood from the interstitial fluid of the brain) (Zheng et al., 2003; Farina et al., 2011). Once in the brain, Hg can interact with and oxidize portions of several critical proteins involved in the homeostasis and protection of neuronal and glial cells. This can result in oxidative stress; an unfavorable imbalance between the levels of harmful reactive oxygen species (ROS) and the antioxidants (e.g., glutathione) and antioxidant enzymes (e.g., catalase and superoxide dismutase) that are normally produced to counteract their cell-damaging effects (Mieiro et al., 2011; Farina et al., 2011; Mahboob et al., 2001). This can lead to ROS-mediated oxidation of cellular macromolecules, such as membrane lipids (lipid peroxidation), resulting in cell damage or possibly death, and potential impacts on animal behavior or survival (Depew et al., 2012; Farina et al., 2011; Estes et al., 2011; Nam et al., 2011a).

Because of these factors, the overall goal of this study was to examine Hg accumulation and effects in the shark brain. In order to accomplish this, Hg accumulation in both muscle and brain of the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*)

were examined, and brain Hg levels were compared to threshold values for Hg-associated neurological effects from the literature. The percentage of total Hg that was organic methylmercury was determined in subsampled muscle and brain. Additionally, Hg levels in the forebrain was compared to those in the combined midbrain and hindbrain to determine if there are regional differences in the encephalic accumulation of Hg and what possible effects that could have on sharks. This study also determined if Hg levels in the Atlantic sharpnose shark brain was associated with any nervous system damage by measuring the levels of three biomarkers (total glutathione, 8-iso-prostaglandin F2α, and S100b) in the shark cerebrospinal fluid (CSF). The CSF was used in this study because the entire brain was used in Hg analyses, thus histopathology and biomarker concentrations in the brain could not be examined. Nutrient and protein levels in the brain are often reflected in the CSF, as the CSF is produced in the choroid plexus and carries nutrients throughout the ventricles of the brain before it acts as a cushion to the brain in the space surrounding the pia mata and arachnoid membrane (Zheng et al., 2003). The biomarkers used in this study were selected to examine progressive levels of Hg toxicity in the brain. Total glutathione levels were used to demonstrate an initial effect that Hg has on the shark brain, a depletion of the main antioxidant glutathione as it forms a complex with Hg (Farina et al., 2011). The level of 8-iso-prostaglandin F2α, a known indicator of lipid peroxidation, was used to determine if the ROS levels in the brain were high enough to induce lipid oxidation and membrane damage (Greco et al., 1999). Last, S100b, a calcium-binding protein that has been shown to be released into the CSF by astrocytes in response to MeHg-induced cell damage in the rat brain, was used to identify cellular damage (Yoshida et al., 1980; Vicente et al., 2004; Farina et al., 2005).

In all, it was hypothesized that the brain Hg levels would be lower than the Hg in the muscle, and if the Hg concentrations in the brain exceeded threshold values for Hg-associated neurological effects from the literature there would be a decrease in glutathione and an increase in 8-iso-prostaglandin F2α and S100b levels with increasing brain Hg levels. Likewise, if Hg concentrations in the brain did not exceeded threshold values for Hg-associated neurological effects from the literature there would be no change in glutathione, 8-iso-prostaglandin F2α, nor S100b levels with increasing brain Hg levels. Additonally, it was expected that the %MeHg in the muscle and brain would be higher than 90% (as seen in neonate lemon sharks, Nam et al., 2011b).

The Atlantic sharpnose shark was selected for this study because previous studies have shown that the muscle Hg concentrations in this species can exceed the 0.3 ppm U.S. EPA recommended threshold for human consumption (Adams and McMichael, 1999; Evers et al., 2008; Rumbold et al., 2014). However, none of these studies examined Hg in the brain of these sharks. Additionally Atlantic sharpnose sharks are in the family *Carcharhinidae*, which are primarily viviparous and active predators, and this family tends to have larger brains in comparison to other chondrichthyans, allowing this family of shark to be agile, active predators (Loefer and Sedberry, 2003; Yopak, 2012). Furthermore, this small shark species frequents nearshore areas that are often polluted, which makes them a good candidate for studying coastal pollution (Loefer and Sedberry, 2003). Last, this shark species is the most common shark throughout the southeast coast of the United States, but previous studies have only examined Atlantic sharpnose sharks collected from the Florida coast (Loefer and Sedberry, 2003; Adams and McMichael, 1999; Evers et al., 2008; Rumbold et al., 2014). In contrast, this study has examined

Atlantic sharpnose sharks throughout the majority of their range, with samples collected from Virginia to Texas, making this study one of most extensive surveys on Hg accumulation in a single shark species.

METHODOLOGY

Sample Collection

Atlantic sharpnose sharks were collected along the United States southeastern coast from Virginia to Texas (Figure 1) using the bottom longline technique as part of several fishery-independent surveys. Following sex identification, maturity assessment, and measurement of total length (TL), muscle, brain, and cerebrospinal fluid (CSF) were collected. Muscle was collected from a 2 in. x 2 in. skinned site on the left lateral side of the shark below the first dorsal fin. After the brain was exposed, the arachnoid membrane was punctured with a syringe (18 G x 1 ½ in. length) to obtain approximately 1-2 mL of CSF. Next, the cartilage around the brain was cut away with a scalpel, the optic nerves snipped, and a blunt cut was made with scissors at the posterior boundary of the brain, at the level of the first cervical spinal nerve. A subsample of brains had the forebrain separated from the midbrain and hindbrain by a planar cut between the tegmentum of the midbrain and the caudal pole of infundibulum on the diencephalon of the forebrain as determined by Northcutt (1978) (Figure 2). All samples of muscle and brain were wrapped in aluminum foil and frozen at -80°C until Hg analysis could be completed. Samples of CSF were transferred to cryovials and frozen at -80°C until used for biomarker analysis. Although most samples of adult sharks were male because of sex-associated segregation in this species, some pregnant females containing embryos were
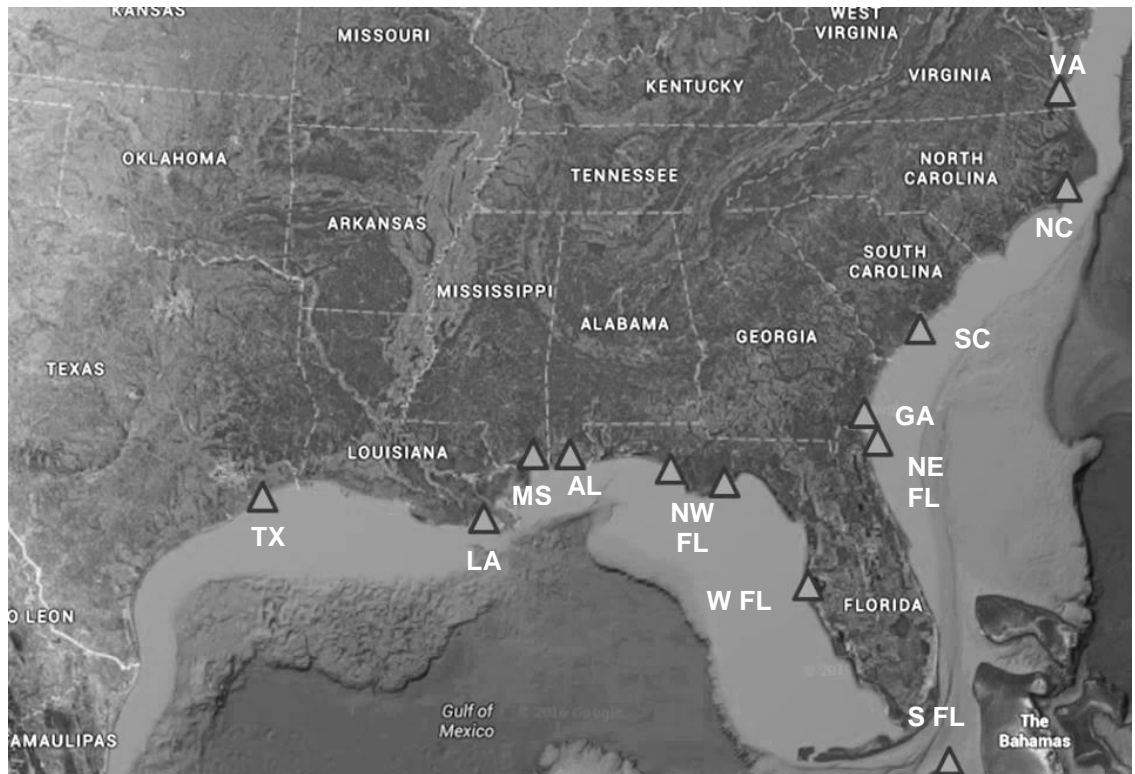
Figure 1. Map of collection sites for Atlantic sharpnose sharks used in the present study (VA = Virginia, NC = North Carolina, SC = South Carolina, GA = Georgia, NE FL = Northeastern Florida, S FL = Southern Florida, W FL = Western Florida, NW FL = Northwestern Florida, AL = Alabama, MS = Mississippi, LA = Louisiana, TX = Texas).

collected. Due to their small size, whole embryos were processed for Hg analysis to provide additional data on maternal transfer of Hg and ontogenetic changes in Hg accumulation.

While collecting Atlantic sharpnose sharks during the independent sampling surveys, several bonnethead sharks (*Sphyrna tiburo*), blacktip sharks (*Carcharhinus limbatus*), and a blacknose shark (*Carcharhinus acronotus*) were additionally sampled and used to examine species-specific differences in brain Hg concentration. Samples of muscle and brain were collected from these individuals and frozen until used for Hg analysis.

Total Mercury Analysis

Total mercury (THg) in shark muscle and brain, as well as in whole embryos, was determined using a Direct Mercury Analyzer (DMA-80 Milestone Inc.) following EPA Method 7473. Samples were weighed and dried at 60°C for 48 - 60 hours (or until there was no further change in sample weight). Once the tissue was dried, it was re-weighed and then crushed using a mortar and pestle. Approximately 0.05 g of the sample was loaded into the DMA-80 and analyzed for THg following protocols established by Nam et al. (2011b). THg concentrations were converted from dry weight (d.w.) to wet weight (w.w.) for comparisons with literature reference values and past studies, and were expressed as means ± standard deviation (SD) in mg/kg w.w.

Muscle and brain samples were categorized into three size classes for data analysis: adults (> 75 cm TL; calcified claspers), juveniles (37-75 cm TL), and embryos
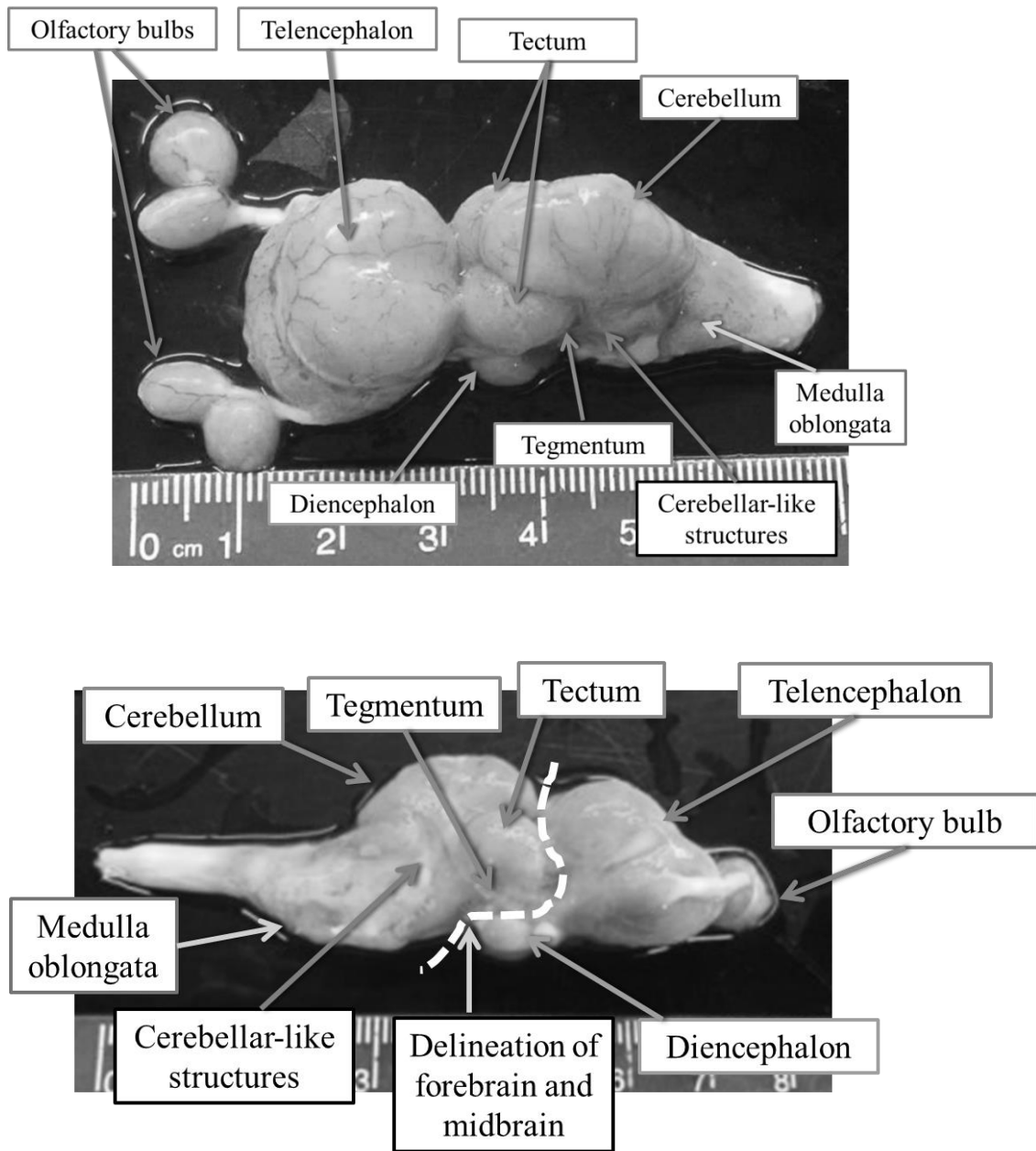
Figure 2. The subcomponents (Olfactory bulbs, Telencephalon, Diencephalon, Tectum, Tegmentum, Cerebellum, Medulla) of the Atlantic sharpnose shark brain

(whole embryo THg was categorized with muscle). Adults were separated from the juvenile samples because most sharks caught ranged from 76 to 105 cm TL; therefore, the primary focus was to compare data from adults of this size range to reduce variance associated with size. However, the overall data set presented includes sharks from the entire size range and was reported to characterize the association between THg accumulation and TL, as well as to identify correlations between THg concentrations in muscle and brain.

Data were analyzed using a Wilcoxon signed rank test to determine if there were any significant differences between THg concentrations in the brain and the muscle, and between the THg concentrations in the forebrain and midbrain/hindbrain. Spearman's rank order correlation coefficient test was used to determine if there were correlations between TL and THg concentrations in shark muscle and brain, as well as correlations between THg concentrations in shark brain and muscle. THg concentrations in adult shark muscle and brain were grouped by location of capture and analyzed using a Quade's Rank analysis of covariance with TL as a covariate, followed by a Tukey's post-hoc test, to determine if they differed by site. All statistics were run using IBM SPSS v 22, and $p < 0.05$ was considered statistically significant.

Percent Methylmercury (%MeHg)

A subsample of the collected muscle and brains were analyzed for the percentage of total mercury that was methylmercury by Johanna L. Imhoff, PhD Student, Florida State University Department of Biological Science. The subsampled muscle and brains were weighed, freeze-dried, reweighed, and ground into a fine powder. The mercury was

extracted by mixing the dried, ground sample with nitric acid (6M HNO$_3$) and heating in

an oven at 70°C for 8 hours. The samples were then centrifuged at 7,000 × g for ten

minutes, and the supernatant was diluted with DI water. Mercury in extracted and diluted

samples, blanks, extraction replicates (one per 15 samples), mercury standards, and

certified reference materials were derivatized using tetraethylborate (1% NaBEt$_4$) and

analyzed on a Tekran 2700 mercury analyzer at the National High Magnetic Field

Laboratory. Mercury standards were used to generate calibration curves for MeHg and

inorganic mercury (IHg), and these curves were used to calculate the percentage of MeHg

and IHg of the total mercury (MeHg + IHg = THg) in the samples.

Biomarker assays

Concentrations of total glutathione, 8-iso-Prostaglandin F2α, and S100b were

measured in shark CSF to determine if Hg exposure was associated with nervous system

damage. Total glutathione was measured in shark CSF using a commercially available

assay (OxiSelect™ Total Glutathione (GSSG/GSH) Assay Kit, Cell Biolabs, Inc.)

following the manufacturer's protocol. The molecule 8-iso-prostaglandin F2α was

measured in 1/4 diluted shark CSF using a commercially available ELISA (OxiSelect™

8-iso-Prostaglandin F2α ELISA Kit, Cell Biolabs, Inc.) following the manufacturer's

protocol. Concentrations of S100b were measured in 1/5 diluted shark CSF using a

commercially available ELISA (Human S100B ELISA, EMD Millipore Corporation)

following the manufacturer's protocol. Biomarker data were analyzed by Spearman's

rank order correlation coefficient test to determine if there were correlations between

THg concentrations in the brain and concentrations of each biomarker in shark CSF.

RESULTS

THg and %MeHg concentrations

      THg levels in all sharks examined (n = 179) ranged from 0.040 to 3.091 mg/kg wet weight (w.w.) (mean ± SD = 1.153 ± 0.659 mg/kg w.w., median = 1.286 mg/kg w.w.) in muscle, and 0.005 to 1.107 mg/kg w.w. (mean ± SD = 0.202 ± 0.222 mg/kg w.w., median = 0.126 mg/kg w.w.) in brain. Only 31 of the 179 individuals were females; therefore, differences between sexes were not examined. Measurements of TL could not be obtained for 6 samples due to a large portion of the shark's body being scavenged upon by other sharks; therefore, these samples were not used for examining correlations between TL and Hg accumulation in the brain or muscle. Brains from two samples, 1 adult and 1 juvenile, could not be processed due to poor condition.

      THg levels in adult sharks, which represented 84% of all samples examined (n = 151), ranged from 0.207 to 3.091 mg/kg wet weight (w.w.) (mean ± SD = 1.330 ± 0.551 mg/kg w.w., median = 1.412 mg/kg w.w.) in muscle, and 0.005 to 1.107 mg/kg w.w. (mean ± SD = 0.234 ± 0.227 mg/kg w.w., median = 0.166 mg/kg w.w.) in brain (Table 1). Of all muscle samples analyzed in adult individuals, 97.4% were found to have muscle THg concentrations above the U.S. EPA recommended level of human consumption (0.3 ppm) (Table 1).

      THg levels in the juvenile individuals (n = 28) ranged from 0.040 to 0.755 mg/kg w.w. (mean ± SD = 0.193 ± 0.206 mg/kg w.w., median = 0.097 mg/kg w.w.) in muscle, and 0.009 to 0.055 mg/kg w.w. (mean ± SD = 0.024 ± 0.012 mg/kg w.w., median = 0.023 mg/kg w.w.) in brain (Table 1). Of all the muscle samples analyzed in juvenile

individuals, 21.4% were found to have THg concentrations above the U.S. EPA recommended level of human consumption (0.3 ppm) (Table 1).

The %MeHg of the THg (MeHg + IHg = THg) in shark muscle (n = 10) ranged from 95.69 to 97.57% (mean ± SD = 96.63 ± 0.60%) (Figure 3). Whereas, the %MeHg in the shark brain (n = 8) was significantly lower with high variance, and ranged from 31.56 to 66.49% (mean ± SD = 50.73 ± 11.69%) (Figure 3) (Wilcoxon signed rank test: $Z = -2.521$, n = 8, $p < 0.05$).

The THg levels of embryos from the six pregnant females (n = 20) ranged from 0.020 to 0.151 mg/kg w.w (mean ± SD = 0.059 ± 0.033 mg/kg w.w., median = 0.057 mg/kg w.w.). On average, THg concentrations measured in whole embryos was approximately 4.8% of the THg found in the mother's muscle (Table 2). Strong positive correlations were observed between TL and THg concentrations in shark muscle (Spearman's Rank Order Correlation, r = 0.744, n = 193, $p < 0.05$), and brain (Spearman's Rank Order Correlation, r = 0.554, n = 171, $p < 0.05$). Shark TL and muscle THg exhibited an exponential relationship (Figure 4; $y = 0.022e^{0.044x}$, $R^2 = 0.811$). Additionally, THg concentrations in shark muscle were significantly correlated with those in brain (Figure 5; Spearman's Rank Order Correlation, r = 0.842, n = 177, $p < 0.05$).

THg levels in the brain and muscle of the other shark species examined were consistent with the relationship determined for the Atlantic sharpnose shark (Figure 5). THg concentrations in bonnethead sharks (n = 5) ranged from 0.501 to 0.877 mg/kg w.w. (mean ± SD = 0.761 ± 0.150 mg/kg w.w., median = 0.821 mg/kg w.w.) in the muscle, and 0.041 to 0.128 mg/kg w.w. (mean ± SD = 0.075 ± 0.039 mg/kg w.w., median = 0.055

Table 1. Range and mean ± SD of total mercury (THg) concentrations in muscle and brain of adult (>75 cm total length) and juvenile (≤75 cm total length) Atlantic sharpnose sharks reported in mg/kg wet weight (w.w.). The number of individuals in which muscle THg concentrations exceeded US EPA's threshold for human consumption at 0.3 ppm Hg w.w. (U.S. EPA 2001) is represented as a percentage. Adults were sampled from 12 coastal locations throughout the Southeastern United States (VA = Virginia, NC = North Carolina, SC = South Carolina, GA = Georgia, NE FL = Northeastern Florida, S FL = Southern Florida, W FL = Western Florida, NW FL = Northwestern Florida, AL = Alabama, MS = Mississippi, LA = Louisiana, TX = Texas).

| | | | Muscle | | Brain | | % > EPA LIMIT |
		n	THg Range (mg/kg w.w.)	THg Mean ± SD (mg/kg w.w.)	THg Range (mg/kg w.w.)	THg Mean ± SD (mg/kg w.w.)	
Adults		151	**0.207 – 3.091**	**1.330 ± 0.551**	**0.005 – 1.107**	**0.234 ± 0.227**	**97.4**
	Area						
	VA	10	0.880 – 2.264	1.637 ± 0.438	0.027 – 0.372	0.187 ± 0.103	100
	NC	11	0.893 – 1.886	1.468 ± 0.287	0.037 – 0.356	0.130 ± 0.108	100
	SC	10	0.971 – 1.497	1.304 ± 0.195	0.019 – 0.651	0.376 ± 0.168	100
	GA	15	0.441 – 2.360	1.358 ± 0.549	0.005 – 0.579	0.159 ± 0.165	100
	NE FL	29	0.842 – 2.427	1.679 ± 0.369	0.030 – 0.845	0.246 ± 0.160	100
	S FL	10	0.840 – 2.524	1.432 ± 0.525	0.051 – 0.901	0.334 ± 0.292	100
	W FL	17	0.920 – 3.091	1.550 ± 0.529	0.102 – 1.107	0.481 ± 0.315	100
	NW FL	11	0.982 – 2.077	1.542 ± 0.355	0.088 – 0.803	0.446 ± 0.234	100
	AL	13	0.328 – 1.035	0.642 ± 0.251	0.022 – 0.163	0.070 ± 0.044	100
	MS	12	0.207 – 0.945	0.406 ± 0.221	0.014 – 0.052	0.029 ± 0.011	66.7
	LA	3	1.176 – 1.473	1.312 ± 0.150	0.053 – 0.164	0.093 ± 0.062	100
	TX	10	0.302 – 1.675	1.147 ± 0.491	0.015 – 0.167	0.088 ± 0.054	100
Juveniles		28	**0.040 – 0.755**	**0.193 ± 0.206**	**0.009 – 0.055**	**0.084 ± 0.012**	**21.4**
Total		179	**0.040 – 3.091**	**1.153 ± 0.659**	**0.005 – 1.107**	**0.202 ± 0.222**	**85.5**
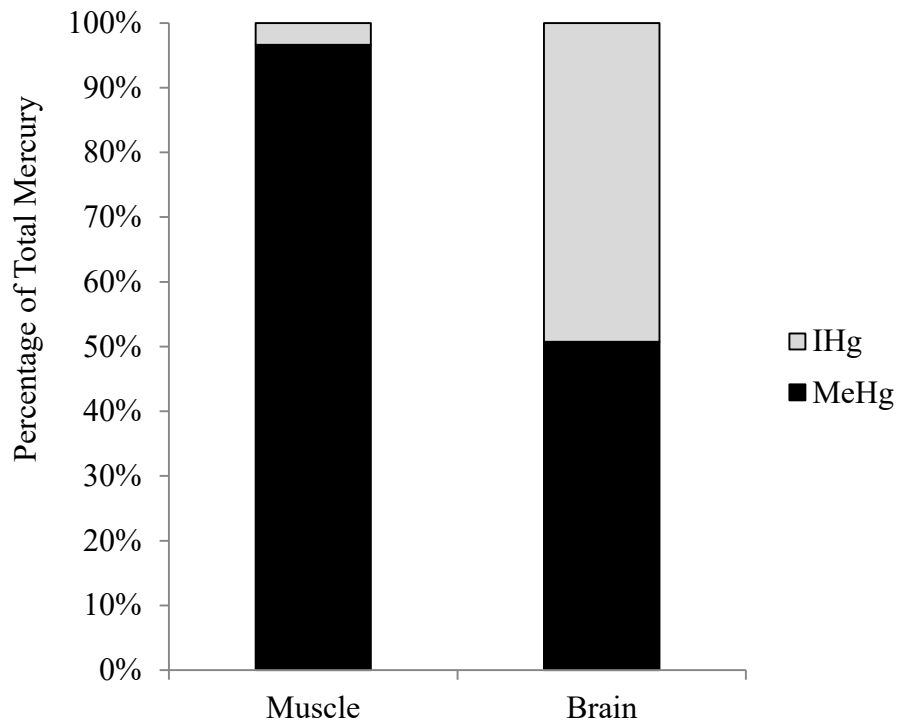
Figure 3. The percentage of methylmercury (MeHg) and inorganic mercury (IHg) observed in a subsample of Atlantic sharpnose shark muscle (n = 10) and brain (n = 8). The total mercury in shark muscle and brain primarily consisted of MeHg (muscle = 96.63% MeHg, brain = 50.73% MeHg).

Table 2. Range and mean ± SD of total mercury (THg) concentrations in whole embryos of six pregnant female Atlantic sharpnose sharks. THg concentrations in maternal muscle and the percentage of embryo to maternal THg concentrations is presented.

Female	Female THg (mg/kg w.w.)	# of Embryos	Embryo THg Range (mg/kg w.w.)	Embryo THg Mean ± SD (mg/kg w.w.)	% Maternal offloading
1	1.473	5	0.069 – 0.091	0.080 ±0.009	5.42
2	1.286	3	0.020 – 0.043	0.028 ±0.012	2.21
3	0.840	3	0.040 – 0.074	0.053 ±0.018	6.33
4	0.886	3	0.020 – 0.025	0.022 ±0.003	2.49
5	1.412	3	0.044 – 0.151	0.088 ±0.056	6.25
6	1.207	4	0.042 – 0.095	0.071 ±0.022	5.72
Total	**1.184 ± 0.266**	**20**	**0.020 – 0.151**	**0.059 ±0.033**	**4.8**
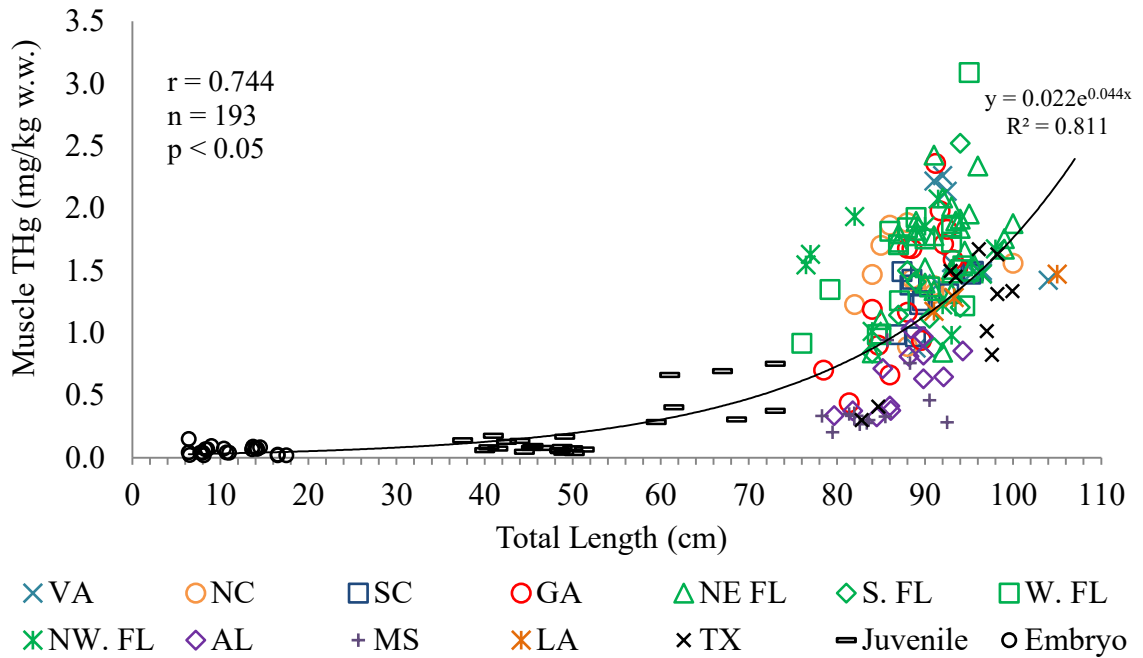
Figure 4. Total mercury (THg) concentrations (mg/kg wet weight [w.w.]) and total length in Atlantic sharpnose shark muscle (n = 173) and whole embryos (n = 20) collected from 12 coastal locations throughout the Southeastern United States (VA = Virginia, NC = North Carolina, SC = South Carolina, GA = Georgia, NE FL = Northeastern Florida, S FL = Southern Florida, W FL = Western Florida, NW FL = Northwestern Florida, AL = Alabama, MS = Mississippi, LA = Louisiana, TX = Texas). A significant positive correlation between length and THg was observed (Spearman's Rank Order Correlation, r = 0.744, n = 193, p < 0.05). The line represents the exponential relationship between the muscle THg and the TL (y = 0.022e0.044x, R² = 0.811, p < 0.05).
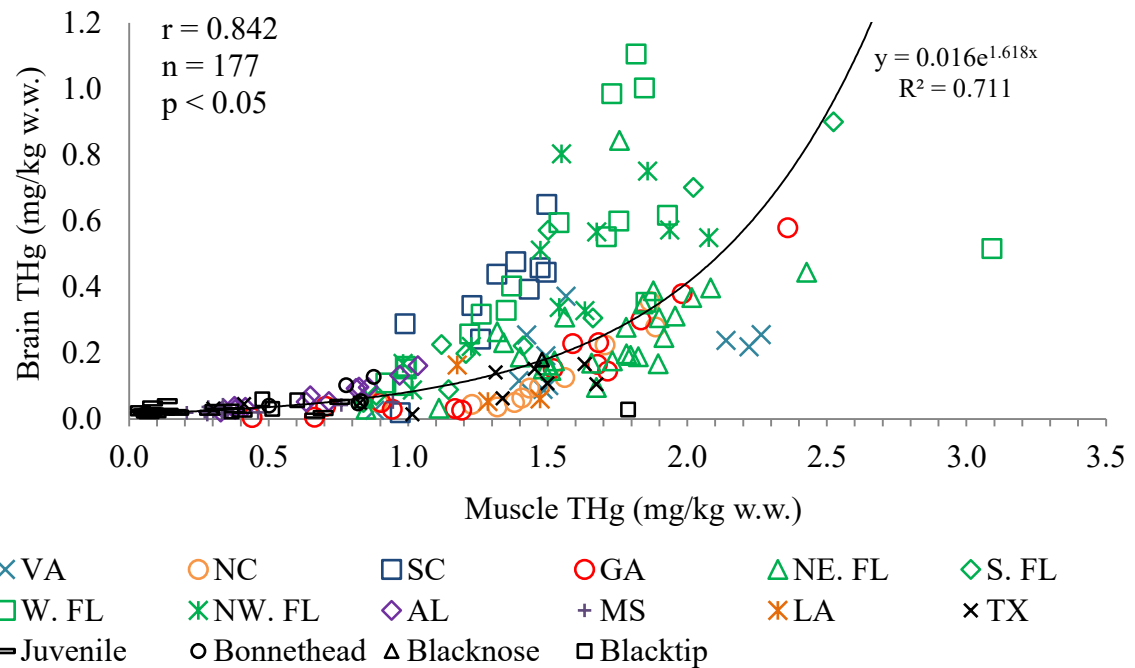
Figure 5. Total mercury (THg) (mg/kg wet weight [w.w.]) in muscle and brain of Atlantic sharpnose sharks (n = 177) collected from 12 coastal locations throughout the Southeastern United States (VA = Virginia, NC = North Carolina, SC = South Carolina, GA = Georgia, NE FL = Northeastern Florida, S FL = Southern Florida, W FL = Western Florida, NW FL = Northwestern Florida, AL = Alabama, MS = Mississippi, LA = Louisiana, TX = Texas). A significant positive correlation between THg in muscle and brain was observed (Spearman's Rank Order Correlation r = 0.842, n = 177, p < 0.05). The line represents the exponential relationship between the muscle THg and the brain THg ($y = 0.016e^{1.618x}$, $R^2 = 0.711$, p < 0.05). Muscle and brain THg concentrations in individuals from three other shark species, bonnethead sharks (n = 5), blacktip sharks (n = 6), and blacknose sharks (n = 1) were consistent with the relationship observed for Atlantic sharpnose sharks.

mg/kg w.w.) in the brain. THg concentrations in blacktip sharks (n = 6) ranged from 0.367 to 5.185 mg/kg w.w. (mean ± SD = 1.489 ± 0.558 mg/kg w.w., median = 1.885 mg/kg w.w.) in the muscle, and 0.023 to 0.587 mg/kg w.w. (mean ± SD = 0.132 ± 0.044 mg/kg w.w., median = 0.223 mg/kg w.w.) in the brain. Last, THg concentrations in blacknose shark (n = 1) was 1.480 mg/kg w.w. in the muscle, and 0.181 mg/kg w.w. in the brain.

THg concentrations were significantly higher in the muscle than in the brain of adult sharpnose sharks (Figure 6; Wilcoxon signed rank test: Z = -11.538, n = 177, p < 0.05). In addition, significant differences in both muscle and brain THg were observed by the geographical location of capture (Figure 6; Quade's Rank analysis of covariance with TL as a covariate: Muscle: F = 8.739 on 11 d.f., p < 0.05; Brain: F = 13.619 on 11 d.f., p < 0.05). In general, adult individuals from northeast, west, and northwest Florida had significantly higher muscle THg levels than those from Alabama and Mississippi, with several intermediate groups in between. Likewise, adult individuals from west and northwest Florida had significantly higher brain THg levels than those from Mississippi and Texas, with several intermediate groups in between (Figure 6).

Concerning regional differences in THg concentrations in the brain, the forebrain contained significantly higher THg concentrations than those observed in the hindbrain and midbrain (n = 89) in every geographical location observed (Figure 7; Wilcoxon signed rank test: Z = -6.266 on 88 d.f., p < 0.05).
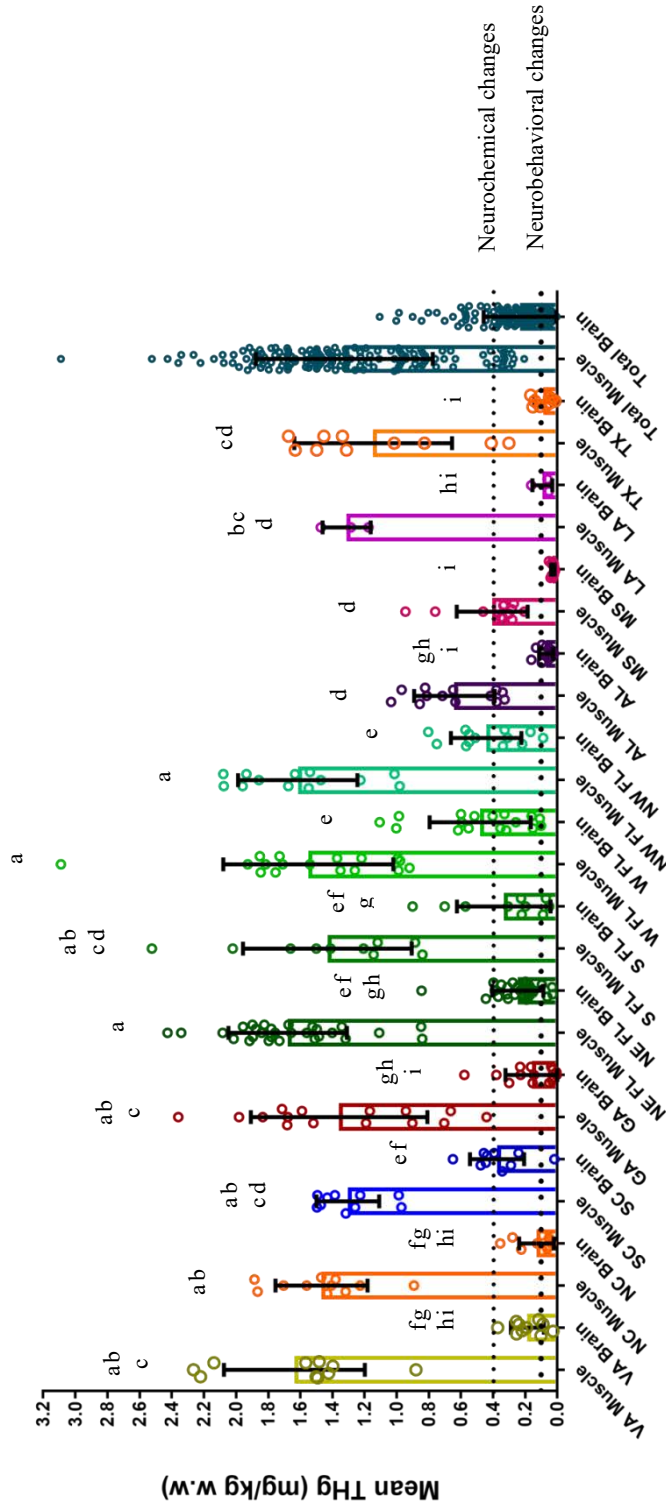
Figure 6. Total mercury (THg) (mg/kg wet weight [w.w.] in muscle and brain of adult Atlantic sharpnose sharks (n = 151) collected from 12 coastal locations throughout the Southeastern United States (VA = Virginia, NC = North Carolina, SC = South Carolina, GA = Georgia, NE FL = Northeastern Florida, S FL = Southern Florida, W FL = Western Florida, NW FL = Northwestern Florida, AL = Alabama, MS = Mississippi, LA = Louisiana, TX = Texas). Bars represent means ±SD. Sample sizes for each location are provided in Table 1. Brain THg concentrations were significantly lower than the muscle THg concentrations (Wilcoxon signed rank test: Z = -11.538, n = 177, p < 0.05). Significant differences in THg were observed by site of capture (Quade's Rank analysis of covariance with TL as a covariate: Muscle: F = 8.739 on 11 d.f., p < 0.05; Brain: F = 13.619 on 11 d.f., p < 0.05). Significantly different groups are represented by different lowercase letters. Individuals from NE FL, W FL, and

30
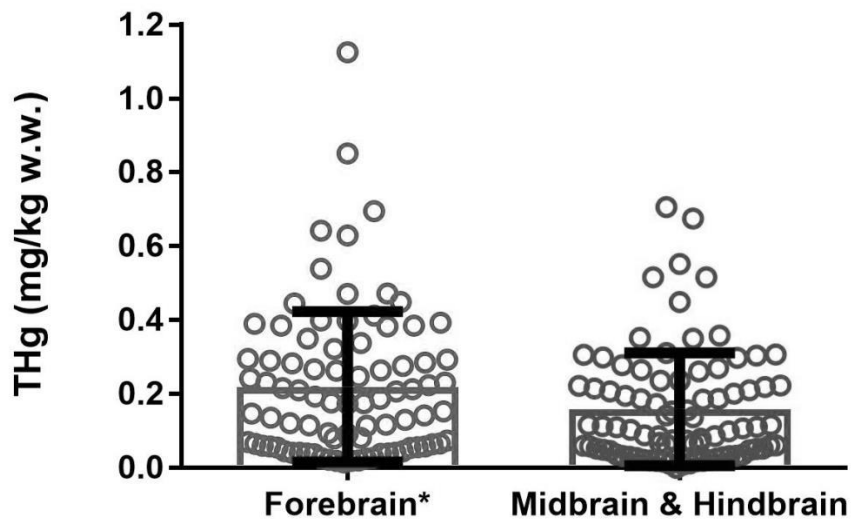
Figure 7. Total mercury (THg) (mg/kg wet weight [w.w.]) in Atlantic sharpnose shark forebrain and hindbrain/midbrain (n = 89). Bars represent means ±SD. The forebrain was significantly higher in THg than the hindbrain/midbrain (Wilcoxon signed rank test: Z = -6.266 on 88 d.f., p < 0.05).

Biomarker assays

　　Total glutathione concentrations in CSF appeared to have a negative association with brain THg concentrations; in particular, concentrations were lower in sharks in which brain THg was above 0.4 mg/kg w.w.. However, the total amount of glutathione did not significantly correlate with THg concentrations in the shark brain (Figure 8; Spearman's rank order correlation coefficient, $r = -0.292$, n= 41, $p = 0.064$). Concentrations of 8-iso-prostaglandin F2α (Figure 9; Spearman's rank order correlation coefficient, $r = -0.02$, n= 35, $p = 0.907$) and S100b (Figure 10; Spearman's rank order correlation coefficient, $r = 0.039$, n = 33, $p = 0.830$) were also not significantly correlated with brain THg.

r = -0.292
n = 41
p = 0.064
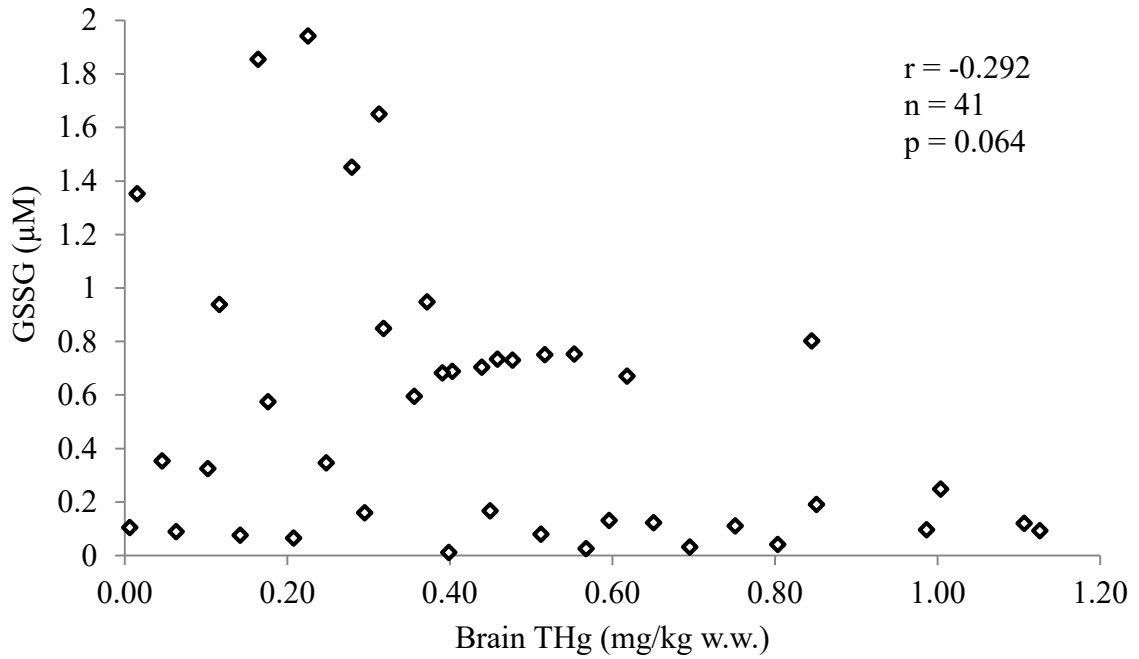
Figure 8. Concentrations of total glutathione (GSSG) (μM) in cerebrospinal fluid and total mercury (THg) measured in mg/kg wet weight (w.w.) in the brain of Atlantic sharpnose sharks. Total glutathione in the CSF was not significantly correlated with brain THg (Spearman's rank order correlation coefficient, r = - 0.292, n= 41, p = 0.064).
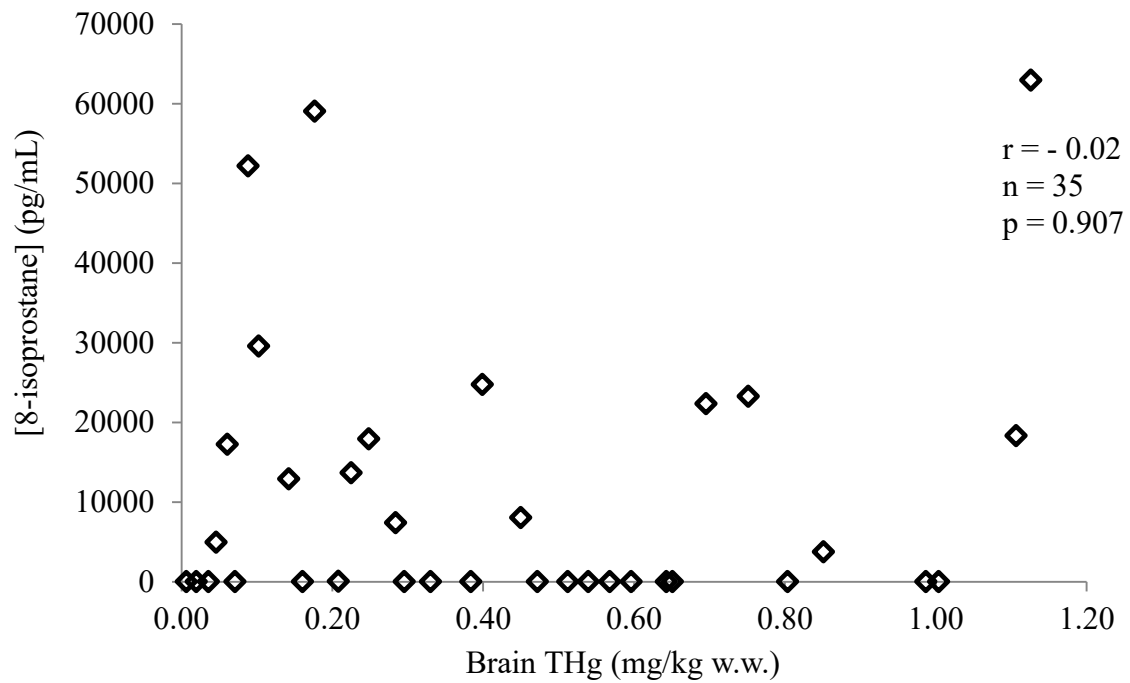
Figure 9. Concentrations of 8-iso-prostaglandin F2α (pg/mL) in cerebrospinal fluid and total mercury (THg) measured in mg/kg wet weight (w.w.) in the brain of Atlantic sharpnose sharks. 8-iso-prostaglandin F2α concentrations were not significantly correlated with brain THg (Spearman's rank order correlation coefficient, r = - 0.02, n= 35, p = 0.907).
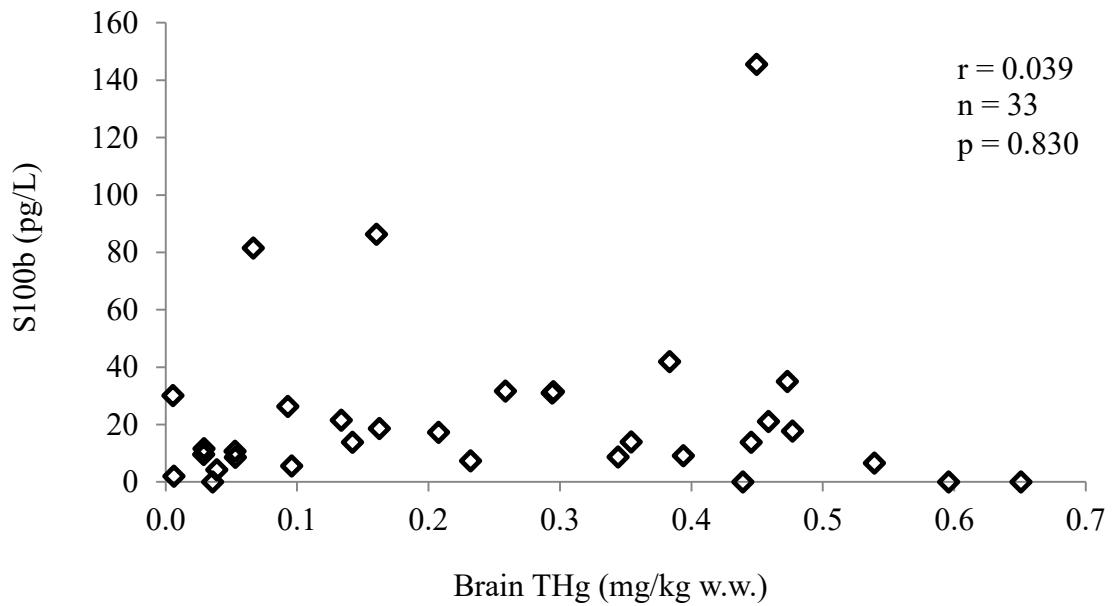
Figure 10. Concentration of S100b (pg/L) in cerebrospinal fluid and total mercury (THg) measured in mg/kg wet weight (w.w.) in the brain of Atlantic sharpnose sharks. S100b concentrations were not significantly correlated with brain THg (Spearman's rank order correlation coefficient, $r = 0.039$, $n = 33$, $p = 0.830$).

DISCUSSION

The goal of this study was to examine Hg accumulation and effects in the brain of the Atlantic sharpnose shark. This was accomplished by examining THg concentrations in the shark muscle and brain, comparing those Hg levels to threshold values for neurological effects, and determining if increasing Hg levels in brain were associated with biomarkers of nervous system damage (expected decrease in glutathione, increase in 8-iso-prostaglandin F2α and S100b). Additionally, the amount of Hg in forebrain was compared to the combined midbrain and hindbrain, and the %MeHg was determined in both the muscle and the brain.

Muscle THg concentrations in Atlantic sharpnose sharks examined in the present study were similar to those of the same species from prior investigations (Adams and McMichael, 1999; Rumbold et al., 2014; Evers et al, 2008). Adams and McMichael (1999) reported muscle THg concentrations ranging from 0.11-2.30 ppm (mean ±SD = 1.06 ±0.71 ppm) in juvenile/adult Atlantic sharpnose sharks ($n = 81$) from the east Florida coast, levels comparable to those observed in northeast Florida samples in the present study (0.842-2.247, 1.679 ±0.369 ppm). Rumbold et al. (2014) found that Atlantic sharpnose sharks from the southwest Florida coast ($n = 7$) exhibited mean muscle THg concentrations of 1.99 ± 0.6 ppm, which are similar to those observed in samples from the west Florida coast in the present study (0.92-3.09, 1.55 ±0.529 ppm). Last, Evers et al. (2008) reported slightly lower mean THg levels in Atlantic sharpnose sharks ($n = 38$) collected from Florida Bay (0.56 ± 0.52 ppm). However, these levels were still consistent with values observed in south Florida sharks examined in this study (0.40-2.524, 1.432 ±0.525 ppm). As observed in the present study, many of the sharks

surveyed in prior studies possessed muscle THg concentrations that exceeded the 0.3 mg/kg w.w. threshold for human dietary consumption (U.S. EPA 2001).

Levels of THg in the muscle correlated with the size of the shark. This data is congruent with other studies that have examined muscle THg concentrations in the Atlantic sharpnose shark (Adams and McMichael, 1999; Rumbold et al., 2014; Evers et al., 2008). Adams and McMichael (1999) found a significant linear correlation between muscle THg and size of *R. terranovae* ($R^2 = 0.697$). Additionally, Evers et al. (2008) found the relationship between muscle THg and Atlantic sharpnose shark size to be significant, but with considerable variation leading to a weak overall relationship ($R^2 = 0.24$). In contrast, Rumbold et al. (2014) did not find a significant correlation between muscle THg and Atlantic sharpnose shark size ($R^2 = 0.50$, $p = 0.08$). However, overall sample size was limited ($n = 7$) in the Rumbold et al. (2014) study. The current study expanded upon this knowledge, providing data from every life stage of the Atlantic sharpnose shark (embryo, juvenile, adult). These results demonstrated that an exponential relationship between shark TL and muscle THg concentrations exists, suggesting a rapid rate of Hg uptake in this species. It is probable that the occurrence of an exponential rather than linear relationship between size and Hg accumulation in this species may have complicated earlier efforts to examine this relationship (Evers et al., 2008; Rumbold et al., 2014).

The present study also demonstrated that pregnant Atlantic sharpnose shark females are capable of transferring Hg to their offspring during gestation. This has previously been shown by Adams and McMichael (1999) in a limited sample of Atlantic sharpnose shark embryos ($n = 6$), which exhibited THg levels ranging from 0.17 - 0.29

ppm; 8.3% to 15.3% of maternal THg levels (Adams and McMichael, 1999). The present study observed lower THg concentrations in Atlantic sharpnose shark embryos, ranging from 0.020 to 0.151 mg/kg w.w.; a level only about 4.8% of the THg concentrations found in the maternal muscle. The difference in the percentage of maternal offloading may be due to this study using the whole embryo for analysis, while Adams and McMichael (1999) were able to dissect the muscle from the embryo for analysis. Notwithstanding these differences, these data suggest that maternal offloading can be a source of Hg exposure to sharks during embryogenesis. This fact is also supported by research on other shark species (white shark (*Carcharodon carcharias*); mako shark (*Isurus oxyrinchus*); salmon shark (*Lamna ditropis*); thresher shark (*Alopias vulpinus*)) (Lyons et al., 2013). Lyons et al. (2013) also observed a high degree of variability among these species based on the maternal trophic position, foraging location, age of maturity, and the number of offspring and reproductive events. Even though the amount of maternal Hg offloading is variable, it could pose significant health risks to offspring of ovoviviparous and placental viviparous species, particularly if Hg accumulates in target organs of toxicity. In fact, placental viviparous species such as the Atlantic sharpnose shark may be susceptible to greater effects of maternal Hg offloading because, as Mull et al. (2011) indicated, placental viviparous species tend to have larger brains and are therefore more likely to accumulate higher levels of Hg.

Although muscle THg concentrations in adult Atlantic sharpnose sharks often exceeded thresholds for human consumption, a key finding of this study was that brain THg concentrations were significantly lower in comparison. These data correspond with the limited number of studies that have observed Hg accumulation in the shark brain

(Newman et al., 2011; Nam et al., 2011b; Bergés-Tiznado et al., 2015). Nam et al. (2011b) found the mean THg concentrations in juvenile lemon shark (*Negaprion brevirostris*) muscle (n = 18) were 0.311 ± 0.152 µg/g w.w., while the mean brain (n = 17) THg concentrations were much lower at 0.043 ± 0.023 µg/g w.w. Similarly, Newman et al. (2011) reported mean muscle THg concentrations of 0.92 mg/kg w.w. (95% confidence interval: 0.60-1.24) in Great lantern sharks (*Etmopterus princeps*), compared with mean brain THg concentrations of only 0.14 mg/kg w.w. (95% confidence interval 0.05-0.23). Last, Bergés-Tiznado et al. (2015) found that mean THg concentrations in juvenile scalloped hammerhead shark (*Sphyrna lewini*) were 0.63 ± 0.04 ppm in muscle, but only 0.11 ± 0.01 µg/g w.w. in the brain. It is noteworthy to mention the mean brain THg concentrations found in the present study (mean ± SD = 0.234 ± 0.227 mg/kg w.w.) were higher than those observed in previous reports. However, these levels still largely fell below most known thresholds for clinical symptoms (i.e. loss of motor function, body convulsions, death; > 6.75 mg/kg w.w) and neuropathological signs (i.e. histological changes in the neurons; > 4 mg/kg w.w) associated with Hg toxicity as determined by laboratory studies and field observations on vertebrates (see review by Krey et al., 2015). This premise suggests limited potential for Hg-induced neurological impacts in Atlantic sharpnose sharks on the U.S. east coast; however, future research is needed in order to confirm if sharks have the same thresholds for Hg-induced neurological effects as other vertebrates.

The fact that Hg uptake in the Atlantic sharpnose shark brain is largely below the threshold for neurological effects is supported by data on biomarker concentrations, which though the concentrations were high, they were not found to be significantly

correlated with brain THg levels. This is in contrast to studies that have demonstrated associations between MeHg exposure and/or uptake of MeHg in the brain and indicators of oxidative stress and/or neuron damage in other vertebrates. For example, Stringari et al. (2008) and Franco et al. (2006) found that MeHg exposure reduced the amount of glutathione in the central nervous system of mice, and Kaur et al. (2006) found that GSH concentrations decreased in mammalian neurons that were exposed to MeHg. Furthermore, lipid peroxidation has been shown to be correlated with increased Hg uptake in the brain of Atlantic salmon (*Salmo salar*) (Berntssen et al., 2003) and Forster's tern (*Sterna forsteri*) (Hoffman et al., 2011). Farina et al. (2005) showed rats that were exposed to Hg had elevated concentrations of S100b released into the CSF from the brain. Based on a lack of similar data in the present study, it was concluded that the levels observed in the shark brains surveyed in this study were too low to induce notable increases in these oxidative stress biomarkers. This conclusion is supported by the study by Nam et al. (2010), which found THg concentrations in the brain of juvenile lemon sharks were not significantly correlated with variations in several neurochemical enzymes (cholinesterase, monoamine oxidase) and receptors (muscarinic cholinergic receptor, N-methyl-d-aspartic acid receptor).

While average brain THg concentrations were generally low in the present study, the differences observed in relation to site of capture demonstrate significant variations in Hg exposure and uptake in Atlantic sharpnose sharks within these regions. Therefore, there is some, albeit limited, potential for brain Hg levels to occasionally exceed threshold values for neurological effects in some individuals from certain locations. For example, THg concentrations in brains from some Atlantic sharpnose sharks from

Virginia, North Carolina, South Carolina, Georgia, and Florida exceeded published (Krey et al., 2015) thresholds for possible neurobehavioral (i.e. learning, memory, and attenuation deficits; >0.1 mg/kg w.w.) and neurochemical (i.e. alterations in neurotransmitters; >0.4 mg/kg w.w.) effects. Because of this possible risk, it is still sensible to monitor possible Hg uptake in the shark brain in certain geographical locations, perhaps by using non-lethally obtained muscle biopsies and the relationship between muscle and brain THg concentrations determined in this study. This approach may also be useful for other shark species based on the consistency observed in the relationship between muscle and brain THg levels in the Atlantic sharpnose shark and other species examined in this study (i.e., bonnethead, blacktip shark, blacknose shark).

In cases when brain THg may actually exceed thresholds for neurological effects, there is potential for dissimilar responses in variable portions of the shark brain. This is because brain THg levels were found to be significantly higher in the forebrain than in the rest of the shark brain. Therefore, it is plausible for individuals to expect that individuals will experience THg effects associated with forebrain function, which could include alterations to the integration and perception of the senses, decreased autonomic and neuroendocrine responses to stress, behavioral changes (decreased predator/prey interactions, reproduction, mood, appetite), and uncontrollable voluntary muscle movements (See review, Scott and Sloman, 2004; Pereira et al., 2016). For example, Berlin et al. (1975) observed impaired voluntary coordination, impaired vision, and sensory disturbances in squirrel monkeys with mercury induced cerebral cortical lesions. Fathead minnows (*Pimephales promelas*) exposed to Hg showed a decrease in foraging efficiency, capture speed, reproductive behavior, and the capacity to learn and retain

information regarding habitat characteristics (Grippo and Heath, 2003; Sandheinrich and Miller, 2006). Likewise, MeHg exposure altered the swimming behavior (decreased swimming distance), while IHg induced anxiety-like behaviors (decrease in motivation to swim as determined by the latency to be dragged and to take refuge) in white seabream fish (*Diplodus sargus*) (Puga et al., 2016; Pereira et al., 2016). Additionally, Puga et al. (2016) and Pereira et al. (2016) observed a decrease in the number of brain cells (neurons and glial cells) in the optic tectum (IHg and MeHg exposure), the forebrain's medial pallium (only MeHg exposure), the molecular layer of the cerebellum (only IHg exposure), and the hypothalamus (IHg exposure). As well, there was an increase in cell volume (cell hypertrophy) in the hypothalamic cells (neurons and glial cells) with MeHg exposure, and a decrease in cerebellum cell volume with IHg exposure (Puga et al., 2016; Pereira et al., 2016). Puga et al. (2016) suggested that the behavioral alterations were mediated by cellular dysfunction of the dopaminergic cells in the hypothalamus, but they did propose a mechanism for these effects.

These studies are in comparison to studies that have observed Hg-induced impairment associated with the midbrain and hindbrain function, such as Charbonneau et al. (1976) who found cats with movement disorders associated with Hg neurotoxicity had THg concentrations higher in the cerebellum and lower in the frontal cortex. Additionally, Berntssen et al. (2003) found Hg concentrations in a salmon brain (*Salmo salar*) was highest to lowest in the medulla, cerebellum, ventral regions of the tectum, and cerebrum, and after four months of MeHg exposure vacuolation and astrocyte proliferation spread from the grey-white matter interface in the medulla to the cerebrum. However, when Berntssen et al. (2003) attempted to assess the effects this Hg-induced

cell loss had on salmon, they only found a reduction in active swimming behavior. Berntssen et al. (2003) then suggested the behavior change was due to the alteration of the dopaminergic system within the hypothalamus.

This dissimilar response to the localization of Hg in the brain could be indicative that the forebrain contains more thiol groups (Krey et al., 2015). It could also be due to Hg concentrating in the largest region of the brain that was the last to differentiate from precursor cells as the brain develops in a conserved hindbrain to the forebrain gradient (late equals large principle) (Finlay et al., 2001). In the Atlantic sharpnose shark, 50% of the brain's mass was the forebrain. However, not all shark species have this same pattern of brain organization, though it is hypothesized that the sharks with the same lifestyle characteristics generally have similar patterns of brain organization (termed cerebrotypes) (Yopak, 2012; Yopak et al., 2007). Data has suggested there are associations between: the telencephalon size with the shark's taxon and niche; mesencephalon size with the shark's reliance on vision; the medulla oblongata size with the use of non-visual senses; and the cerebellum's complexity with the shark's habitat and activity levels (Yopak et al., 2007; Yopak and Linsey, 2012; Yopak and Montgomery, 2008; Yopak et al., 2010). Therefore, comparisons should be made with other chondrichthyan species of varying brain cerebrotypes. In particularly, future studies should examine Hg in the brain of sharks with an enlarged medulla oblongata (bathyal, deep sea benthopelagic sharks) or a large, highly foliated cerebellum (reef-associated, oceanic habitats), and determine if Hg accumulates to a greater extent in these enlarged regions.

Like the brain, muscle also exhibited significant variations in Hg uptake in Atlantic sharpnose sharks in relation to site of capture. In particular, muscle THg

concentrations in Alabama and Mississippi sharks were generally much lower than those observed in individuals from all other sites; in some cases, there was a greater than two-fold difference in these levels. These regional variations may be due to the differences in dietary habits of Atlantic sharpnose sharks from these sampling locations. Although previous studies have demonstrated that Atlantic sharpnose sharks are largely piscivorous (Gelsleichter et al., 1999), individuals from west of Mobile Bay, Alabama to Mississippi have been found to have a higher contribution of invertebrate prey in their diet in comparison to sharks from east of Mobile Bay to northwest Florida (Drymon et al., 2012; Bethea et al., 2006). Adams et al. (2003) found that fish with more invertebrates in their diet generally have lower THg levels than more piscivorous individuals; therefore, this may explain the comparable differences observed in the present study.

Furthermore, the high percentage of MeHg within the THg (MeHg + IHg) for the muscle is similar to what has been seen in other shark species (Nam et al., 2011b; Storelli et al., 2002; Pethybridge et al., 2010). However, the percentage of MeHg for the brain is drastically below the %MeHg observed by Nam et al. (2011b) (range: 67.6-109%; mean ± SD: 88.8 ± 10.3 %). The study by Nam et al. (2011b) was on neonate lemon sharks, whereas the subsamples from this study were adult individuals. The low percentage of MeHg could indicate the demethylation of organic (MeHg) to inorganic mercury within the brain as the shark ages and that the shark has had a prolonged exposure to Hg. The premise of the demethylation of MeHg to IHg has been observed over time in the brains of monkeys (*Macaca fascicularis*) (Vahter et al., 1995), white seabream fish (*Diplodus sargus*) (Puga et al., 2016; Pereira et al., 2016), and *in vitro* (Shapiro and Chan, 2008; Mailloux et al., 2015). However, this inorganic Hg is still very toxic as it inhibits

neuronal differentiation and increases oxidative stress by alterations of the brain's glutamate and calcium homeostasis (Chan et al., 2017; Pereira et al., 2016).

It is plausible that if mercury is high enough to induce neurotoxic effects that the shark brain would be able to regenerate damaged neurons, because the shark brain, unlike the brain of higher vertebrates, undergoes lifelong neurogenesis (Finger et al., 2008; Ferretti, 2011; Yopak et al., 2010). Finger et al. (2008) demonstrated that the carpet shark's (*Cephaloscyllium isabellum*) brain, as seen in the brain of bony fishes, could undergo lifelong neurogenesis throughout the entire brain. This is in comparison to birds and mammals, with limited adult neurogenesis (Ferretti, 2011). Furthermore, Pereira et al. (2016) and Puga et al. (2016) observed an increase in cells (neurons and glial cells) in the brain of the white seabream fish (*Diplodus sargus*) after exposure to Hg; indicating a potential for the regeneration on Hg-induced neuron damage in sharks.

In conclusion, while previous studies have demonstrated that Atlantic sharpnose shark muscle can accumulate elevated levels of THg that pose potential health risks to human consumers, this study has illustrated that THg concentrations in the sharpnose brain are appreciably lower and generally pose limited risks to shark neurological function. Nonetheless, due to elevated Hg exposure in the brain of Atlantic sharpnose sharks from some locations, there is potential for some individuals to be exposed to levels that could alter neurological function. However, there are suggestions that indicate the shark brain has the potential to regenerate cells damaged by the Hg. Additionally, the brain Hg levels appeared to be highest in the forebrain of the Atlantic sharpnose shark brain, suggesting the possibility of brain region-specific effects on CNS activity. Furthermore, the low percentage of MeHg observed in the brain indicates a prolonged

exposure to Hg and demethylation of the organic MeHg to inorganic mercury. Last, it is also important to note that while the nervous system is generally considered the primary target of Hg toxicity; other organs can be affected by this metal, such as the testes, liver, and kidney (Walker et al., 2012). Therefore, further work on Hg uptake in other potentially sensitive organs is warranted, as well as, determining any sex specific differences in Hg accumulation, and if THg and %MeHg vary in specific brain regions across multiple species.

WORKS CITED

Adams, D. H., & McMichael, R. H. (1999). Mercury levels in four species of sharks from the Atlantic coast of Florida. *Fishery Bulletin, 97*(2), 372-379.

Adams, D. H., McMichael, R. H., & Henderson, G. E. (2003). *Mercury Levels in Marine and Estuarine Fishes of Florida 1989–2001. revised*. Retrieved from St. Petersburg, FL: http://aquaticcommons.org/id/eprint/120

Berges-Tiznado, M. E., Marquez-Farias, F., Lara-Mendoza, R. E., Torres-Rojas, Y. E., Galvan-Magana, F., Bojorquez-Leyva, H., & Paez-Osuna, F. (2015). Mercury and Selenium in Muscle and Target Organs of Scalloped Hammerhead Sharks Sphyrna lewini of the SE Gulf of California: Dietary Intake, Molar Ratios, Loads, and Human Health Risks. *Arch Environ Contam Toxicol, 69*(4), 440-452. doi:10.1007/s00244-015-0226-8

Berlin, M., Grant, C. A., Hellberg, J., Hellstrom, J., & Schultz, A. (1975). Neurotoxicity of methylmercury in squirrel monkeys. Cerebral cortical pathology, interference with scotopic vision, and changes in operant behavior. *Arch Environ Health, 30*(7), 340-348.

Berntssen, M. H., Aatland, A., & Handy, R. D. (2003). Chronic dietary mercury exposure causes oxidative stress, brain lesions, and altered behaviour in Atlantic salmon (Salmo salar) parr. *Aquat Toxicol, 65*(1), 55-72. doi:http://dx.doi.org/10.1016/S0166-445X(03)00104-8

Bethea, D. M., Carlson, J. K., Buckel, J. A., & Satterwhite, M. (2006). Ontogenetic and site-related trends in the diet of the Atlantic sharpnose shark Rhizoprionodon terraenovae from the northeast Gulf of Mexico. *Bulletin of Marine Science, 78*(2), 287-307.

Bodznick, D. (1990). Elasmobranch vision: multimodal integration in the brain. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *256*(S5), pp.108-116.

Chan, M. C., Bautista, E., Alvarado-Cruz, I., Quintanilla-Vega, B., & Segovia, J. (2017). Inorganic mercury prevents the differentiation of SH-SY5Y cells: Amyloid precursor protein, microtubule associated proteins and ROS as potential targets. *J Trace Elem Med Biol*. doi:10.1016/j.jtemb.2017.02.002

Charbonneau, S. M., Munro, I. C., Nera, E. A., Armstrong, F. A., Willes, R. F., Bryce, F., & Nelson, R. F. (1976). Chronic toxicity of methylmercury in the adult cat. Interim report. *Toxicology, 5*(3), 337-349.

Depew, D. C., Basu, N., Burgess, N.M., Campbell, L.M., Devlin, E.W., Drevnick, P.E., Hammerschmidt, C.R., Murphy, C.A., Sandheinrich, M.B., & Wiener, J. G. (2012). Toxicity of dietary methylmercury to fish: derivation of ecologically meaningful threshold concentrations. *Environ Toxicol Chem, 31*(7), 1536-1547. doi:10.1002/etc.1859

Drymon, J. M., Powers, S. P., Carmichael, R. H. (2012). Trophic plasticity in the Atlantic sharpnose shark (Rhizoprionodon terraenovae) from the north central Gulf of Mexico. *Environmental biology of fishes, 95*(1), 21-35.

Estes, J.A., Terborgh, J., Brashares, J.S., Power, M.E., Berger, J., Bond, W.J., Carpenter, S.R., Essington, T.E., Holt, R.D., Jackson, J.B. & Marquis, R.J. (2011). Trophic downgrading of planet Earth. *Science, 333*(6040), 301-306. doi:10.1126/science.1205106

Evers, D. C., Hammerschlag, N., & Die, D. (2008). Mercury levels in Florida sharks: interim Report. *Report BRi, 2*.

Farina, M., Cereser, V., Portela, L.V., Mendez, A., Porciúncula, L.O., Fornaguera, J., Gonçalves, C.A., Wofchuk, S.T., Rocha, J.B.T. & Souza, D.O. (2005). Methylmercury increases S100B content in rat cerebrospinal fluid. *Environ Toxicol Pharmacol, 19*(2), 249-253. doi:10.1016/j.etap.2004.07.008

Farina, M., Rocha, J. B., & Aschner, M. (2011). Mechanisms of methylmercury-induced neurotoxicity: evidence from experimental studies. *Life Sci, 89*(15-16), 555-563. doi:10.1016/j.lfs.2011.05.019

Ferretti, P. (2011). Is there a relationship between adult neurogenesis and neuron generation following injury across evolution? *European Journal of Neuroscience, 34*(6), 951-962.

Finger, J., Kubke, M., Wild, J., & Montgomery, J. (2008). *An examination of the extent of adult neurogenesis in the carpet shark (Cephaloscyllium isabellum).* Paper presented at the 26th International Australasian Winter Conference on Brain Research.

Finlay, B. L., Darlington, R. B., & Nicastro, N. (2001). Developmental structure in brain evolution. *Behavioral and Brain Sciences, 24*(02), 263-278.

Franco, J.L., Teixeira, A., Meotti, F.C., Ribas, C.M., Stringari, J., Pomblum, S.C.G., Moro, Â.M., Bohrer, D., Bairros, A.V., Dafre, A.L. & Santos, A.R. (2006). Cerebellar thiol status and motor deficit after lactational exposure to methylmercury. *Environ Res, 102*(1), 22-28. doi:10.1016/j.envres.2006.02.003

Gelsleichter, J., Musick, J. A., & Nichols, S. (1999). Food habits of the smooth dogfish, Mustelus canis, dusky shark, Carcharhinus obscurus, Atlantic sharpnose shark, Rhizoprionodon terraenovae, and the sand tiger, Carcharias taurus, from the northwest Atlantic Ocean. *Environmental biology of fishes, 54*(2), 205-217.

Gelsleichter, J., & Walker, C. J. (2010). *Pollutant exposure and effects in sharks and their relatives.* Boca Raton: CRC Press.

Greco, A., Minghetti, L., Sette, G., Fieschi, C., & Levi, G. (1999). Cerebrospinal fluid isoprostane shows oxidative stress in patients with multiple sclerosis. *Neurology, 53*(8), 1876-1876.

Grippo, M. A., & Heath, A. G. (2003). The effect of mercury on the feeding behavior of fathead minnows (Pimephales promelas). *Ecotoxicol Environ Saf, 55*(2), 187-198.

Hayle, T.H. (1973). A comparative study of spinal projections to the brain (except cerebellum) in three classes of poikilothermic vertebrates. *J Comp Neurol, 149*(4), pp.463-476.

Hamasaki, D.I. and Streck, P. (1971). Properties of the epiphysis cerebri of the small-spotted dogfish shark, Scyliorhinus caniculus L. *Vision research*, 11(3), pp.189-IN3.

Hofmann, M. H. (1999). Nervous system. In *Sharks, Skates, and Rays: The Biology of Elasmobranch Fishes* (Hamlet, W. C., ed.), pp. 273 – 299. Baltimore, MD: Johns Hopkins University Press.

Hoffman, D. J., Eagles-Smith, C. A., Ackerman, J. T., Adelsbach, T. L., & Stebbins, K. R. (2011). Oxidative stress response of Forster's terns (Sterna forsteri) and Caspian terns (Hydroprogne caspia) to mercury and selenium bioaccumulation in liver, kidney, and brain. *Environ Toxicol Chem, 30*(4), 920-929. doi:10.1002/etc.459

Hofmann, M.H. and Northcutt, R.G. (2012). Forebrain organization in elasmobranchs. *Brain, behavior and evolution*, *80*(2), pp.142-151.

Kaur, P., Aschner, M., & Syversen, T. (2006). Glutathione modulation influences methyl mercury induced neurotoxicity in primary cell cultures of neurons and astrocytes. *NeuroToxicology, 27*(4), 492-500. doi:10.1016/j.neuro.2006.01.010

Krey, A., Ostertag, S. K., & Chan, H. M. (2015). Assessment of neurotoxic effects of mercury in beluga whales (Delphinapterus leucas), ringed seals (Pusa hispida), and polar bears (Ursus maritimus) from the Canadian Arctic. *Sci Total Environ, 509-510*, 237-247. doi:10.1016/j.scitotenv.2014.05.134

Loefer, J. K., & Sedberry, G. R. (2003). Life history of the Atlantic sharpnose shark (Rhizoprionodon terraenovae) (Richardson, 1836) off the southeastern United States. *Fishery Bulletin, 101*(1), 75-88.

Lyons, K., Carlisle, A., Preti, A., Mull, C., Blasius, M., O'Sullivan, J., Winkler, C.& Lowe, C.G. (2013). Effects of trophic ecology and habitat use on maternal transfer of contaminants in four species of young of the year lamniform sharks. *Mar Environ Res, 90*, 27-38. doi:10.1016/j.marenvres.2013.05.009

Mahboob, M., Shireen, K. F., Atkinson, A., & Khan, A. T. (2001). Lipid peroxidation and antioxidant enzyme activity in different organs of mice exposed to low level of mercury. *J Environ Sci Health B, 36*(5), 687-697. doi:10.1081/PFC-100106195

Mailloux, R. J., Yumvihoze, E., & Chan, H. M. (2015). Superoxide anion radical (O2(-)) degrades methylmercury to inorganic mercury in human astrocytoma cell line (CCF-STTG1). *Chem Biol Interact, 239*, 46-55. doi:10.1016/j.cbi.2015.06.028

Mieiro, C. L., Pereira, M. E., Duarte, A. C., & Pacheco, M. (2011). Brain as a critical target of mercury in environmentally exposed fish (Dicentrarchus labrax)--bioaccumulation and oxidative stress profiles. *Aquat Toxicol, 103*(3-4), 233-240. doi:10.1016/j.aquatox.2011.03.006

Montgomery, J.C., Bodznick, D. and Yopak, K.E. (2012). The cerebellum and cerebellum-like structures of cartilaginous fishes. *Brain, behavior and evolution*, *80*(2), pp.152-165.

Mull, C.G., Yopak, K.E. and Dulvy, N.K. (2011). Does more maternal investment mean a larger brain? Evolutionary relationships between reproductive mode and brain size in chondrichthyans. *Marine and Freshwater Research*, *62*(6), pp.567-575.

Nam, D. H., Adams, D. H., Flewelling, L. J., & Basu, N. (2010). Neurochemical alterations in lemon shark (Negaprion brevirostris) brains in association with brevetoxin exposure. *Aquat Toxicol, 99*(3), 351-359. doi:10.1016/j.aquatox.2010.05.014

Nam, D., Head, J., Adams, D., Evers, D. C., Carvan, M., Goetz, F. W., Murphy, C., & Basu, N. (2011a). *Neuroendocrine effects of mercury in several fish species.* Paper presented at the Conference Abstract: NASCE.

Nam, D. H., Adams, D. H., Reyier, E. A., & Basu, N. (2011b). Mercury and selenium levels in lemon sharks (Negaprion brevirostris) in relation to a harmful red tide event. *Environmental Monitoring and Assessment, 176*(1-4), 549-559. doi:10.1007/s10661-010-1603-4

Newman, M. C., Xu, X., Cotton, C. F., & Tom, K. R. (2011). High mercury concentrations reflect trophic ecology of three deep-water chondrichthyans. *Arch Environ Contam Toxicol, 60*(4), 618-625. doi:10.1007/s00244-010-9584-4

Northcutt, R. G. (1978). Brain organization in the cartilaginous fishes. *Sensory biology of sharks, skates, and rays*, 117-193.

Paul, D.H. and Roberts, B.L. (1979). The significance of cerebellar function for a reflex movement of the dogfish. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, *134*(1), pp.69-74.

Pereira, P., Puga, S., Cardoso, V., Pinto-Ribeiro, F., Raimundo, J., Barata, M., Pousão-Ferreira, P., Pacheco, M. & Almeida, A. (2016). Inorganic mercury accumulation in brain following waterborne exposure elicits a deficit on the number of brain cells and impairs swimming behavior in fish (white seabream-Diplodus sargus). *Aquat Toxicol, 170*, 400-412. doi:10.1016/j.aquatox.2015.11.031

Pethybridge, H., Cossa, D., & Butler, E. C. (2010). Mercury in 16 demersal sharks from southeast Australia: Biotic and abiotic sources of variation and consumer health implications. *Mar Environ Res, 69*(1), 18-26. doi:10.1016/j.marenvres.2009.07.006

Puga, S., Pereira, P., Pinto-Ribeiro, F., O'Driscoll, N.J., Mann, E., Barata, M., Pousão-Ferreira, P., Canário, J., Almeida, A. & Pacheco, M. (2016). Unveiling the neurotoxicity of methylmercury in fish (Diplodus sargus) through a regional morphometric analysis of brain and swimming behavior assessment. *Aquatic Toxicology, 180*, 320-333.

Rodriguez-Moldes, I. (2009). A developmental approach to forebrain organization in elasmobranchs: new perspectives on the regionalization of the telencephalon. *Brain Behav Evol, 74*(1), 20-29. doi:10.1159/000229010

Rumbold, D., Wasno, R., Hammerschlag, N., & Volety, A. (2014). Mercury accumulation in sharks from the coastal waters of southwest Florida. *Arch Environ Contam Toxicol, 67*(3), 402-412. doi:10.1007/s00244-014-0050-6

Sandheinrich, M. B., & Miller, K. M. (2006). Effects of dietary methylmercury on reproductive behavior of fathead minnows (Pimephales promelas). *Environ Toxicol Chem, 25*(11), 3053-3057.

Scott, G. R., & Sloman, K. A. (2004). The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. *Aquat Toxicol, 68*(4), 369-392. doi:10.1016/j.aquatox.2004.03.016

Shapiro, A. M., & Chan, H. M. (2008). Characterization of demethylation of methylmercury in cultured astrocytes. *Chemosphere, 74*(1), 112-118. doi:10.1016/j.chemosphere.2008.09.019

Smeets, W.J. (1982). The afferent connections of the tectum mesencephali in two chondrichthyans, the shark Scyliorhinus canicula and the ray Raja clavata. *J Comp Neurol*, *205*(2), pp.139-152.

Storelli, M. M., Giacominelli-Stuffler, R., & Marcotrigiano, G. (2002). Mercury accumulation and speciation in muscle tissue of different species of sharks from Mediterranean Sea, Italy. *Bull Environ Contam Toxicol, 68*(2), 201-210.

Stringari, J., Nunes, A.K., Franco, J.L., Bohrer, D., Garcia, S.C., Dafre, A.L., Milatovic, D., Souza, D.O., Rocha, J.B., Aschner, M. and Farina, M. (2008). Prenatal methylmercury exposure hampers glutathione antioxidant system ontogenesis and causes long-lasting oxidative stress in the mouse brain. *Toxicol Appl Pharmacol, 227*(1), 147-154. doi:10.1016/j.taap.2007.10.010

U.S.EPA. (2001). *Water Quality Criteria for the Protection of Human Health: Methylmercury*. Washington, D.C Retrieved from URL: http://www.epa.gov/waterscience/criteria/methylmercury/criteria.html.

Vahter, M. E., Mottet, N. K., Friberg, L. T., Lind, S. B., Charleston, J. S., & Burbacher, T. M. (1995). Demethylation of methyl mercury in different brain sites of Macaca fascicularis monkeys during long-term subclinical methyl mercury exposure. *Toxicol Appl Pharmacol, 134*(2), 273-284. doi:10.1006/taap.1995.1193

Vicente, É., Boer, M., Leite, M., Silva, M., Tramontina, F., Porciúncula, L., Dalmaz, C. and Gonçalves, C.A. (2004). Cerebrospinal fluid S100B increases reversibly in neonates of methyl mercury-intoxicated pregnant rats. *NeuroToxicology, 25*(5), 771-777. doi:10.1016/j.neuro.2004.03.001

Walker, C. H., Sibly, R. M., Hopkin, S. P., & Peakall, D. B. (2012). *Principles of ecotoxicology.* : CRC Press.

Wiener, J. G., Krabbenhoft, D. P., Heinz, G. H., & Scheuhammer, A. M. (2003). *Ecotoxicology of mercury* (Vol. 2).

Wilson, J.F. and Dodd, J.M. (1973). The role of the pineal complex and lateral eyes in the colour change response of the dogfish, Scyliorhinus canicula L. *J of Endocrinol*, *58*(3), pp.591-NP.

Yopak, K. E. (2012). Neuroecology of cartilaginous fishes: the functional implications of brain scaling. *J Fish Biol, 80*(5), 1968-2023. doi:10.1111/j.1095-8649.2012.03254.x

Yopak, K.E., Lisney, T.J., Collin, S.P. and Montgomery, J.C. (2007). Variation in brain organization and cerebellar foliation in chondrichthyans: sharks and holocephalans. *Brain, behavior and evolution*, *69*(4), pp.280-300.

Yopak, K.E. and Montgomery, J.C. (2008). Brain organization and specialization in deep-sea chondrichthyans. *Brain, Behavior and Evolution*, *71*(4), pp.287-304

Yopak, K. E., Lisney, T. J., Darlington, R. B., Collin, S. P., Montgomery, J. C., & Finlay, B. L. (2010). A conserved pattern of brain scaling from sharks to primates. *Proc Natl Acad Sci U S A, 107*(29), 12946-12951. doi:10.1073/pnas.1002195107

Yopak, K.E. and Lisney, T.J. (2012). Allometric scaling of the optic tectum in cartilaginous fishes. *Brain, behavior and evolution*, *80*(2), pp.108-126.

Yopak, K.E., Lisney, T.J. and Collin, S.P. (2015). Not all sharks are "swimming noses": variation in olfactory bulb size in cartilaginous fishes. *Brain Structure and Function*, 220(2), pp.1127-1143.

Yoshida, M., Shimada, E., Arai, F., & Yamamura, Y. (1980). The Relation between Mercury Levels in Brain and Blood or Cerebrospinal Fluid (Csf) after Mercury Exposure. *The Journal of Toxicological Sciences, 5*(3), 243-250. doi:10.2131/jts.5.243

Zheng, W., Aschner, M., & Ghersi-Egea, J. F. (2003). Brain barrier systems: a new frontier in metal neurotoxicological research. *Toxicol Appl Pharmacol, 192*(1), 1-11.

<div align="center">

VITA

Samantha Ehnert

E-Mail: Samantha.Ehnert@gmail.com

</div>

EDUCATION

B.S in Marine Biology, *Cum Laude* with Departmental Honors
University of North Carolina at Wilmington
Minors: Mathematics and Chemistry
Departmental Honors Thesis: *Gut content analysis of batoid species (skates and rays) off the southeastern coast of North Carolina*

TEACHING EXPERIENCE

University of North Florida Graduate Teaching Assistant
University of North Florida REU Program Graduate Assistant and Mentor

RESEARCH EXPERIENCE

Graduate
University of North Florida Research Assistant, *UNF Shark Biology Lab*
University of North Florida Graduate Student
Advisor: Dr. Gelsleichter

Professional
North Pacific Groundfish Observer
Contractor: Saltwater Inc.

Undergraduate
Research Assistant*, UNCW Benthic Ecology Lab*
Undergraduate Research Assistant
Supervisors: Troy Alphin and Martin Posey, Ph. D.

St. James Plantation Intern, *UNCW Benthic Ecology Lab*
Supervisors: Troy Alphin and Taylor Ryan

Sea Turtle Monitoring Directed Independent Study, *UNCW*
Supervisor: David Webster, Ph. D.

Sea Turtle Intern, *Karen Beasley Sea Turtle Rescue and Rehabilitation Center*
Supervisors: Jean Beasley and Amanda Southwood-Williard, Ph. D. (UNCW)

MAJOR PRESENTATIONS

2016 Oral Presentations
> Society of Environmental Toxicology and Chemistry North America
> Annual Meeting of the American Elasmobranch Society at JMIH

2015 Poster Presentation.
> Annual Meeting of the American Elasmobranch Society at JMIH
> Annual Meeting of the American Fisheries Society Southern Division
> The Statewide Graduate Student Research symposium

2011 Exhibit.
> The St. James Honorary Oyster.

SCIENTIFIC MEMBERSHIP

Society of Environmental Toxicology and Chemistry
American Elasmobranch Society
American Fisheries Society