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Mercury Accumulation and Effects in the Brain of the Atlantic Sharpnose Shark (*Rhizoprionodon terraenovae*)

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Abstract
Few published studies have examined whether the elevated concentrations of the nonessential toxic metal mercury (Hg) often observed in shark muscle also occur in the shark brain or whether Hg accumulation affects shark neurophysiology. Therefore, this study examined accumulation and distribution of Hg in the shark brain, as well as effects of Hg on oxidative stress in the shark central nervous system, with particular focus on the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*). Sharks were collected along the southeastern U.S. coast throughout most of this species' U.S. geographical range. Total Hg (THg) concentrations were measured in and compared between shark muscle and brain, whereas known biomarkers of Hg-induced neurological effects, including glutathione depletion, lipid peroxidation, and concentrations of a protein marker of glial cell damage (S100b), were measured in shark cerebrospinal fluid. Brain THg concentrations were correlated with muscle THg levels but were significantly lower and did not exceed most published thresholds for neurological effects, suggesting limited potential for detrimental responses. Biomarker concentrations supported this premise, because these data were not correlated with brain THg levels. Hg speciation also was examined. Unlike muscle, methylmercury (MeHg) did not comprise a high percentage of THg in the brain, suggesting that differential uptake or loss of organic and inorganic Hg and/or demethylation of MeHg may occur in this organ. Although Hg accumulation in the shark brain generally fell below toxicity thresholds, higher THg levels were measured in the shark forebrain compared with the midbrain and hindbrain. Therefore, there is potential for selective effects on certain aspects of shark neurophysiology if brain Hg accumulation is increased.

Mercury (Hg) is a highly toxic, nonessential metal that becomes concentrated in the environment via anthropogenic actions, 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). 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 it to bioconcentrate in most aquatic taxa (Gelsleichter and Walker 2010). Hg levels also tend to increase as aquatic organisms grow (bioaccumulate) and with trophic position in aquatic food webs (biomagnify) (Gelsleichter and 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., levels above the U.S. Environmental Protection Agency's fish tissue-based criterion of 0.3 ppm Hg wet weight, which represents the concentration in fish tissue that should not be exceeded based on a total fish and shellfish consumption-weighted rate of 0.0175 kg fish/day, U.S. EPA 2001) (Gelsleichter and Walker 2010). Because of this, previous studies on Hg accumulation in sharks have largely focused on levels occurring in edible muscle (Gelsleichter and Walker 2010). In a review of shark toxicology by Gelsleichter and Walker (2010), it was reported that > 70% of the approximately 75 species of cartilaginous fish that had been analyzed for Hg contamination as of that date had been found to exhibit muscle Hg levels that exceeded the recommended levels for human consumption. However, while Hg uptake in sharks has been well studied in muscle, much less

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is known about its accumulation in more specific targets of Hg toxicity or its potential effects on shark health and population ecology. These are important questions to address, because a number of threatened shark species have been shown to accumulate toxicologically relevant levels of Hg, at least based on muscle Hg measurements.

Arguably the most important target organs for Hg toxicity in vertebrates are the brain and other components of the central nervous system (Krey et al. 2015). Previous studies in mammals have determined that once Hg enters an individual, it is taken up into the blood and binds with thiol-containing molecules such as cysteine, allowing it to be actively transported into the brain through amino acid transporters in the blood–brain barrier (BBB), a highly selective barrier composed of endothelial cells, smooth muscle cells, and glial cells that separates blood from the interstitial fluid of the brain (Zheng et al. 2003; Farina et al. 2011). Transfer across the BBB through this mechanism has been shown to be greater for organic Hg compared with inorganic forms (Bridges and Zalups 2010; Lohren et al. 2016), although some studies have demonstrated that inorganic Hg in the form of mercurial salts can still enter the brain perhaps indirectly through structural damage to the barrier. Once in the brain, Hg can interact with and oxidize portions of several critical proteins involved in the homeostasis and protection of neurons 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 potentially cell-damaging effects (Mahboob et al. 2001; Farina et al. 2011; Mieiro et al. 2011). This can lead to ROS-mediated oxidation of cellular macromolecules, such as membrane lipids (lipid peroxidation), DNA, or proteins, resulting in cell damage or possibly cell death, and potential impact on animal behavior or survival (Estes et al. 2011; Farina et al. 2011; Nam et al. 2011b; Depew et al. 2012). However, despite these potential risks, knowledge about Hg accumulation in the shark brain is limited; only a few studies have attempted to address this topic (Nam et al. 2011a; Newman et al. 2011; Bergés-Tiznado et al. 2015). Furthermore, to the best of the authors' knowledge, no published studies have examined whether Hg exposure results in oxidative stress in the shark central nervous system.

With these points in mind, the overall goal of this study was to examine Hg accumulation and effects in the shark brain. To accomplish this, Hg accumulation in both muscle and brain of the Atlantic sharpnose shark (*R. terraenovae*) were examined, and brain Hg levels were compared to threshold values for Hg-associated neurological effects reported in the literature. Hg speciation also was determined and compared in subsamples of shark muscle and brain. Additionally, Hg levels in the forebrain were compared with those in the combined midbrain and hindbrain to determine whether there are regional differences in the accumulation of Hg in the shark brain—an observation that has been made in other vertebrates and may have toxicological relevance (Charbonneau et al. 1976). This study also determined if Hg levels in the Atlantic sharpnose shark brain were associated with any nervous system effects by measuring the levels of three biomarkers of oxidative stress. This included concentrations of the antioxidant glutathione, which can become depleted in response to Hg exposure (Farina et al. 2011); levels of 8-iso-prostaglandin $F2\alpha$, a known indicator of oxidative stress-induced lipid peroxidation (Greco et al. 1999); and concentrations of the calcium-binding protein S100b, which has been shown to be released into the CSF by astrocytes in response to MeHg-induced cell damage in the rat brain (Yoshida et al. 1980; Vicente et al. 2004; Farina et al. 2005).

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 tissue-residue criterion (U.S. EPA 2001) for human consumption (Adams and McMichael 1999; Evers et al. 2008; Rumbold et al. 2014). However, none of the past studies on *R. terranovae* examined Hg accumulation in the brain. Like other members of the family Carcharhinidae, Atlantic sharpnose sharks tend to have larger brains in comparison to most other sharks, perhaps providing greater potential for brain Hg uptake if there are differences in nutrient uptake mechanisms (e.g., amino acid transport) that may be related to metabolic demand (Yopak 2012). Furthermore, this species is known to frequent nearshore areas that are often polluted, which makes them a good candidate for studying coastal pollution (Loefer and Sedberry 2003). The Atlantic sharpnose shark is one of the most common sharks occurring throughout the southeastern U.S. coast, but previous studies have only examined muscle Hg uptake in individuals collected from the Florida coast (Adams and McMichael 1999; Evers et al. 2008; Rumbold et al. 2014). In contrast, this study examined Hg accumulation in Atlantic sharpnose sharks collected throughout much of their southeastern U.S. range, with samples collected from Virginia to Texas, making this study one of most extensive surveys on Hg accumulation in a single shark species.

Materials and Methods

Sample Collection

Atlantic sharpnose sharks were collected along the southeastern U.S. coast from Virginia to Texas (Fig. 1) using bottom longline fishing, as part of several fishery-independent surveys. Collections occurred between September

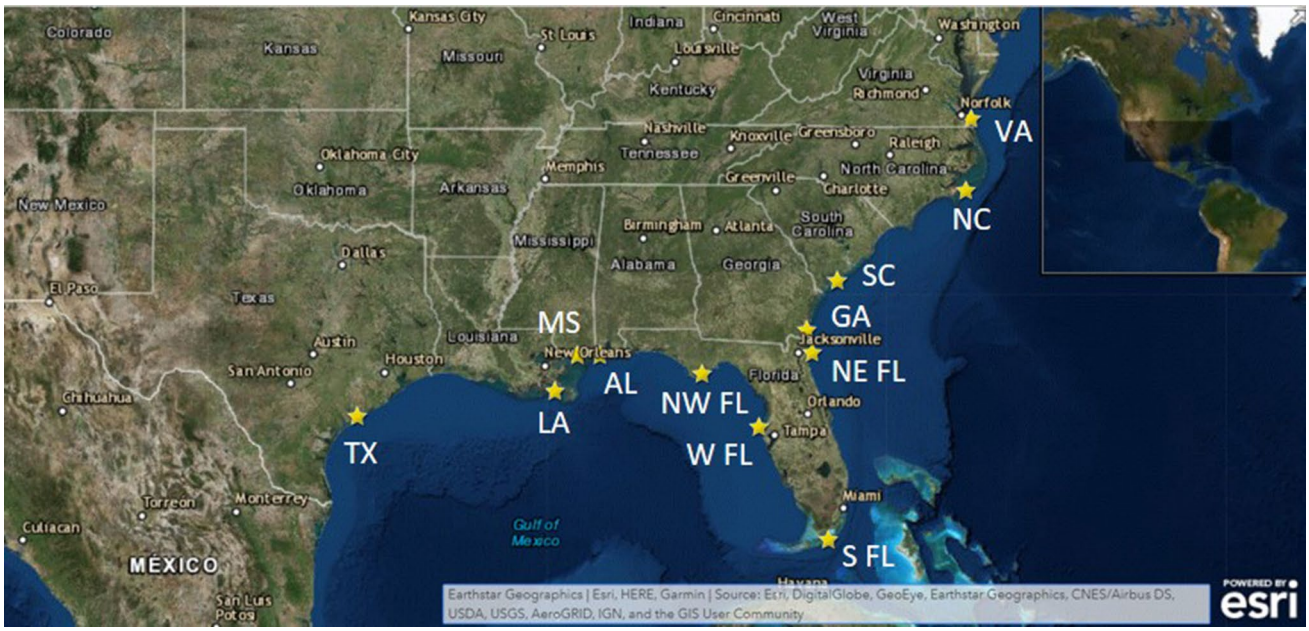

Fig. 1 Map of collection sites for Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*) used in the present study. *VA* Virginia, *NC* North Carolina, *SC* South Carolina, *GA* Georgia, *NE FL* Northeastern Florida, *S FL* Southern Florida, *W FL* Western Florida, *NW FL* Northwestern Florida, *AL* Alabama, *MS* Mississippi, *LA* Louisiana, *TX* Texas

2014 and September 2017. Following sex identification and measurement of total length (TL), sharks were kept on ice for up to 6 h, after which they were immediately dissected or frozen whole for dissection at a later time. Muscle and brain samples were collected from all individuals, whereas samples of cerebrospinal fluid (CSF) were obtained only from non-frozen individuals dissected personally by the primary author to ensure the high quality of these samples. New sampling tools (i.e., sterile scalpel blades and syringes) were used for every specimen, whereas accessory tools (e.g., scalpel handles, forceps, scissors) were cleaned, rinsed with deionized water, and dried prior to re-use. White muscle was obtained from a ~ 5-cm^2 skinned site on the left lateral side of the shark below the first dorsal fin. When collected, CSF was obtained by exposing the brain and puncturing the arachnoid membrane with a sterile syringe and 18 g × 1½-in. needle. Following this, 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) and shown in Fig. 2. The reader is referred to Yopak (2012) for an extensive review on the neuroanatomy of the shark brain, if needed. All samples of muscle and brain were wrapped in aluminum foil and frozen at − 80 °C until Hg analysis was conducted. Samples of CSF were transferred to cryovials and frozen at − 80 °C until used for biomarker analysis.

Most samples used in this study were taken from adult male sharpnose sharks because of limited capture of females in most surveys; this is due to extreme sex-associated segregation in this species that has been well-described in past studies (i.e., adult males greatly outnumber adult females in shallow nearshore sites, whereas adult females greatly outnumber adult males in deeper offshore locations; Parsons and Hoffmayer 2005; Drymon et al. 2010). However, some juvenile male and female sharks, as well as a small number of mature pregnant females bearing embryos, were collected and analyzed for obtaining additional data on maternal transfer of Hg and ontogenetic changes in Hg accumulation. Due to their small size, whole embryos were dried and crushed for Hg analysis.

A small number of bonnethead sharks (*Sphyrna tiburo*) and blacktip sharks (*Carcharhinus limbatus*) also were sampled from northeast Florida waters and used to examine species–specific differences in muscle and brain Hg concentrations. Samples of muscle and brain were collected from these individuals as described above, 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 via thermal

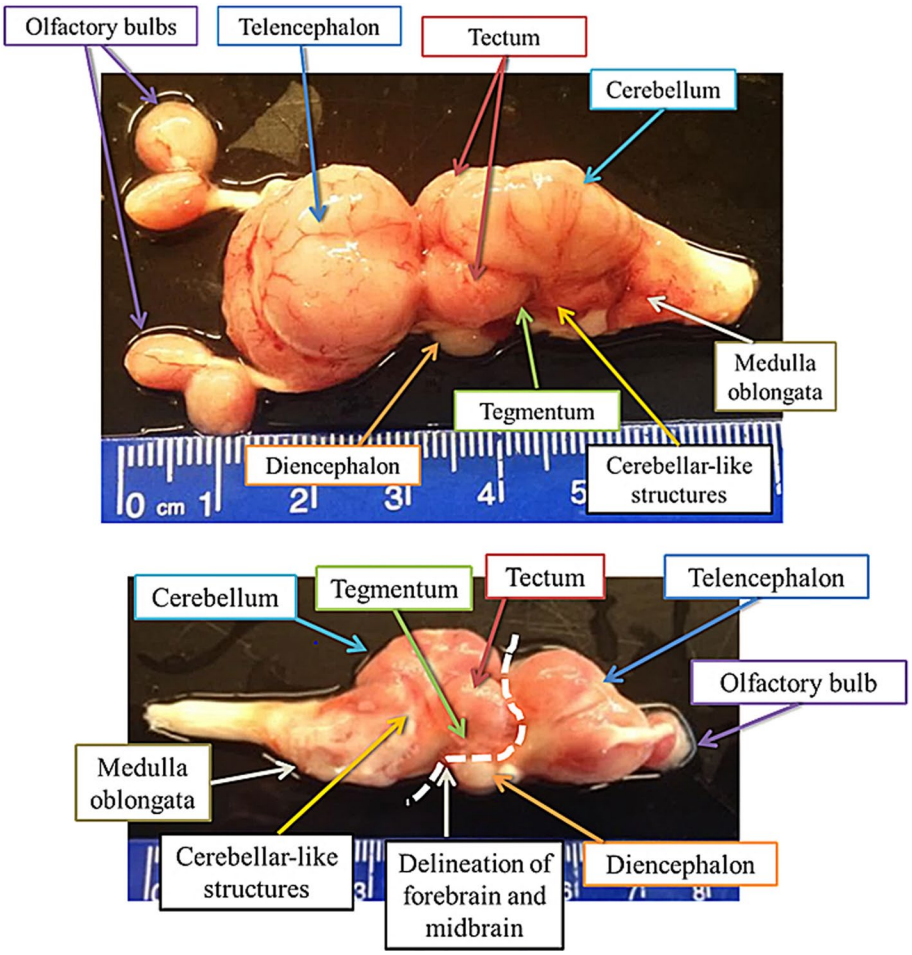

Fig. 2 Dorsal (top) and lateral (bottom) view of the subcomponents (Olfactory bulbs, Telencephalon, Diencephalon, Tectum, Tegmentum, Cerebellum, Medulla) of the brain of the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*). The dashed line in the lateral view demonstrates the site at which the forebrain and midbrain/hindbrain were separated

decomposition (combustion), amalgamation, and atomic absorption spectrometry using a DMA-80 Direct Mercury Analyzer (Milestone Inc., Shelton, CT) calibrated with Hg standard liquid solution, following EPA Method 7473 (U.S. EPA 2007). Samples were weighed and dried at 60 °C for 48–60 h or until there was no further change in sample weight. Once the tissue was dried, it was reweighed to determine percent moisture 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 the U.S. EPA (2007) and implemented in previous mercury studies on sharks (Nam et al. 2011a; Newman et al. 2011; Rumbold et al. 2014). Quality control procedures included analysis of laboratory method blanks, duplicate tissue samples, and certified reference materials (DORM-2, NIST) for each group of 10 samples analyzed. All QC procedures fell within accepted ranges for this procedure. THg concentrations were converted from dry weight (d.w.) to wet weight (w.w.) measurements using moisture data for comparisons with literature reference values and past studies (which are largely reported in w.w., Gelsleichter and Walker 2010) and were expressed as mean ± standard deviation (SD) in mg/kg w.w.

Hg Speciation

A subset of the muscle and brain samples were analyzed to determine Hg speciation—the percentage of total mercury that comprised MeHg and inorganic Hg (IHg). Samples were weighed, freeze-dried, reweighed, and ground into a fine powder. Hg was extracted by mixing ~ 0.2 g of the dried, ground sample with nitric acid (6 M HNO_3) and heating the mixture in an oven at 70 °C for 8 h. The samples were then centrifuged at 7000 × *g* for 10 min, and the supernatant was diluted with DI water. Hg in extracted and diluted samples, blanks, extraction replicates (1 per 15 samples), Hg standards, and certified reference materials were derivatized using tetraethylborate (1% $NaBEt_4$) and analyzed on a Tekran 2700 mercury analyzer at the Florida State University National High Magnetic Field Laboratory (Tallahassee, FL) using a modified version of EPA method 1631, as described in Mickle (2016). Hg standards were used to

generate calibration curves for MeHg and IHg, and these curves were used to calculate the percentage of MeHg and IHg of THg (MeHg + IHg = THg) in the samples.

Biomarker Assays

CSF was used for measuring biomarkers of Hg-induced oxidative stress in the central nervous system, because the entire brain was previously dried for Hg analyses; therefore, histopathology and/or biomarker concentrations in the brain could not be examined. The biochemical composition of the CSF is essentially the same as that of the brain extracellular fluid, because it is produced in the choroid plexus and carries nutrients throughout the ventricles of the brain along with acting as a cushion to the brain in the space surrounding the pia mata and arachnoid membrane (Zheng et al. 2003). As previously mentioned, biomarker concentrations were measured only in a subset of "high-quality" CSF samples that were collected via dissection and frozen within 6 h following animal capture—conditions not possible for all samples due to the broad range of sampling locations and animal collectors.

The biomarkers used in this study were selected to examine progressive levels of Hg toxicity in the brain. Total glutathione levels were used to examine an initial effect that Hg has on the vertebrate brain—the depletion of the main antioxidant glutathione. Concentrations of 8-iso-prostaglandin $F2\alpha$ were measured to determine if levels of oxidative stress were high enough to induce lipid peroxidation and membrane damage. Concentrations of S100b were measured to determine if cell damage in the brain resulted in increased release of this protein in CSF.

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 $F2\alpha$ was measured in 1/4 diluted shark CSF using a commercially available ELISA (OxiSelect™ 8-iso-Prostaglandin $F2\alpha$ 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. Previous studies have demonstrated the presence of S100b-like proteins in the elasmobranch brain, along with its cross-reactivity with antibodies against mammalian S100b (Chiba 2000). All biomarker assays were run in triplicate.

Data Analysis

Muscle and brain samples from Atlantic sharpnose sharks were categorized into three size classes for data analysis: adults (> 75 cm TL; confirmed based on calcification of the male intromittent organs, the claspers), juveniles (37-75 cm TL), and embryos (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 included 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 non-normal, and attempts to normalize data using various transformations were unsuccessful. Data on Hg concentrations were analyzed using the Wilcoxon signed-rank test to determine if there were any significant differences between THg concentrations in the brain and the muscle, between the THg concentrations in the forebrain and midbrain/hindbrain, and between the % MeHg in the brain and muscle. 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 Quade's Rank analysis of covariance with TL as a covariate, followed by Tukey's post hoc test, to determine if they differed by site. Biomarker data were analyzed by Spearman's rank order correlation coefficient test to determine if there were correlations between brain THg concentrations and concentrations of each biomarker in shark CSF. All statistics were run using IBM SPSS v22, and $p < 0.05$ was considered statistically significant.

Results

THg Concentrations and Hg Speciation

THg levels in all Atlantic sharpnose sharks examined ($n = 191$) ranged from 0.040 to 3.091 mg/kg wet weight (w.w.) (mean ± SD = 1.152 ± 0.641 mg/kg w.w.) in muscle and 0.005 to 1.107 mg/kg w.w. (0.198 ± 0.216 mg/kg w.w.) in brain. Measurements of TL could not be obtained for six 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, one adult and one juvenile, could not be processed due to poor condition.

THg levels in adult sharks, which represented 85.3% of all samples examined ($n = 163$), ranged from 0.207 to 3.091 mg/kg w.w. (1.317 ± 0.538 mg/kg w.w.) in muscle and 0.005 to 1.107 mg/kg w.w. (0.227 ± 0.221 mg/kg w.w.) in

Table 1 Range and mean ± SD of total mercury (THg) concentrations in muscle and brain of adult and juvenile Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*)

		N	Muscle		Brain		% > EPA limit	TL (cm) Range
			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		163	0.207–3.091	1.317 ± 0.538	0.005–1.107	0.227 ± 0.221	97.5	76–105
	Area							
	VA	10	0.880–2.264	1.637 ± 0.438	0.027–0.372	0.187 ± 0.103	100	89–104
	NC	11	0.893–1.886	1.468 ± 0.287	0.037–0.356	0.130 ± 0.108	100	82–100
	SC	10	0.971–1.497	1.304 ± 0.195	0.019–0.651	0.376 ± 0.168	100	86.7–95.5
	GA	15	0.441–2.360	1.358 ± 0.549	0.005–0.579	0.159 ± 0.165	100	78.5–93.7
	NE FL	29	0.842–2.427	1.679 ± 0.369	0.030–0.845	0.246 ± 0.160	100	84–100
	S FL	10	0.840–2.524	1.432 ± 0.525	0.051–0.901	0.334 ± 0.292	100	84–94
	W FL	17	0.920–3.091	1.550 ± 0.529	0.102–1.107	0.481 ± 0.315	100	76–95
	NW FL	11	0.982–2.077	1.542 ± 0.355	0.088–0.803	0.446 ± 0.234	100	76.5–98
	AL	13	0.328–1.035	0.642 ± 0.251	0.022–0.163	0.070 ± 0.044	100	79.7–94.3
	MS	12	0.207–0.945	0.406 ± 0.221	0.014–0.052	0.029 ± 0.011	66.7	78.3–92.5
	LA	15	0.593–1.799	1.182 ± 0.274	0.029–0.295	0.126 ± 0.076	100	91–105
	TX	10	0.302–1.675	1.147 ± 0.491	0.015–0.167	0.088 ± 0.054	100	82.8–99.9
Juveniles		28	0.040–0.755	0.193 ± 0.206	0.009–0.055	0.024 ± 0.012	21.4	37.5–73
Total		191	0.040–3.091	1.152 ± 0.641	0.005–1.107	0.198 ± 0.216	86.4	37.5–105

The percentage of individuals in which muscle THg concentrations exceeded the U.S. EPA fish tissue-based criterion for human consumption (0.3 ppm Hg w.w., U.S. EPA 2001) is presented. Adults were sampled from 12 coastal locations throughout the Southeastern United States

Abbreviations for site of capture are identified in Fig. 1

TL total length

brain (Table 1). Of all muscle samples analyzed in adult individuals, 97.5% were found to have muscle THg concentrations above the U.S. EPA fish tissue-based criterion of 0.3 ppm (Table 1). Only 31 of the 191 individuals were females. Although it was not a primary focus of this study, both muscle and brain THg were compared between sexes and were not found to differ significantly (Wilcoxon rank-sum test, $W = 2238$, $p = 0.5024$ for muscle; $W = 2021$, $p = 0.8773$ for brain).

THg levels in the juvenile individuals ($n = 28$) ranged from 0.040 to 0.755 mg/kg w.w. (0.193 ± 0.206 mg/kg w.w.) in muscle and 0.009 to 0.055 mg/kg w.w. (0.024 ± 0.012 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 fish tissue-based criterion of 0.3 ppm (Table 1).

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

The THg levels of embryos from the six pregnant females ($n = 20$) ranged from 0.020 to 0.151 mg/kg w.w. (0.059 ± 0.033 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).

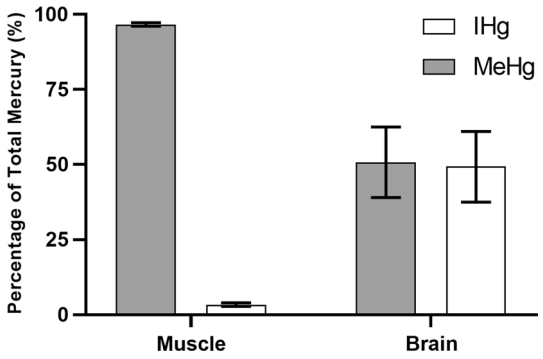

Fig. 3 The percentage of methylmercury (MeHg) and inorganic mercury (IHg) observed in a subsample of Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) muscle ($n = 10$) and brain ($n = 8$). The %MeHg was significantly higher in the muscle (Wilcoxon signed-rank test: $Z = -2.521$, $n = 8$, $p < 0.05$). Values are mean ± SD

Significant correlations were observed between TL and THg concentrations in shark muscle (Spearman's rank-order correlation, $r = 0.704$, $n = 205$, $p < 0.05$) and brain (Spearman's rank-order correlation, $r = 0.518$, $n = 183$, $p < 0.05$). Shark TL and muscle THg exhibited an exponential

Table 2 Range and mean ± SD of total mercury (THg) concentrations in whole embryos of six pregnant female Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*)

Female	Female THg (mg/kg w.w.)	No. 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

THg concentrations in maternal muscle and the percentage of embryo to maternal THg concentrations are presented

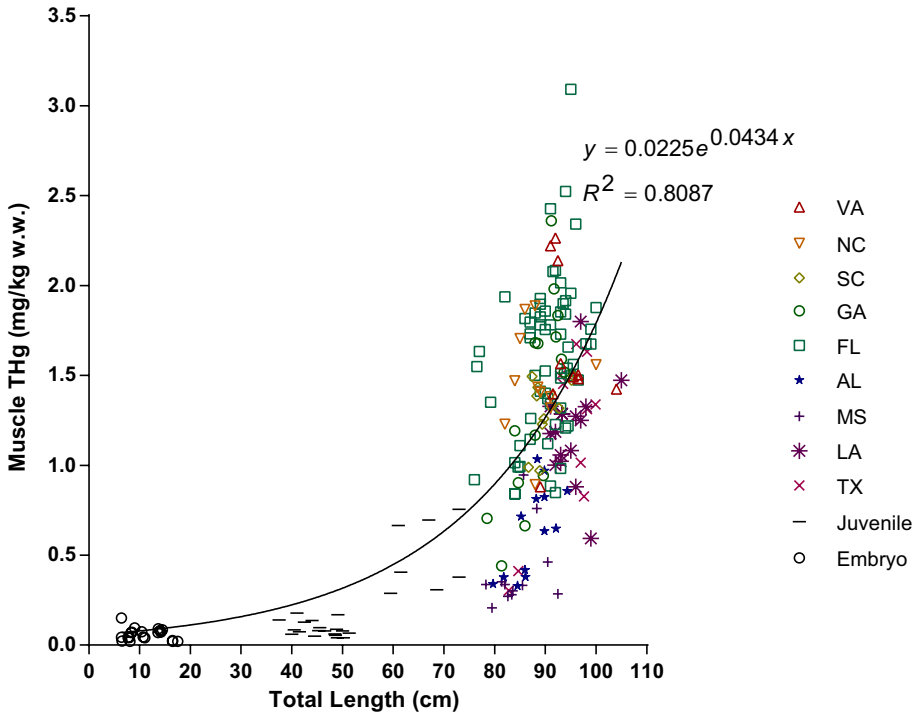

Fig. 4 Total mercury (THg) concentrations (mg/kg wet weight [w.w.]) and total length in Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) muscle ($n = 185$) and whole embryos ($n = 20$) collected from 9 states in the Southeastern United States (VA = Virginia, NC = North Carolina, SC = South Carolina, GA = Georgia, FL = Florida, AL = Alabama, MS = Mississippi, LA = Louisiana, TX = Texas). A significant positive correlation between length and THg was observed (Spearman's Rank Order Correlation, $r = 0.704$, $n = 205$, $p < 0.05$). The line represents the exponential relationship between the muscle THg and the TL ($y = 0.0225e^{0.0434x}$, $R^2 = 0.8$, $p < 0.05$)

relationship (Fig. 4; $y = 0.0225e^{0.0434x}$, $R^2 = 0.8087$). Additionally, THg concentrations in shark muscle were significantly correlated with those in brain (Spearman's Rank Order Correlation, $r = 0.845$, $n = 189$, $p < 0.05$), also exhibiting an exponential relationship ($y = 0.0165e^{1.6095x}$, $R^2 = 0.7$) (Fig. 5). The significance of correlations between muscle THg and TL varied by location (Table 3). Muscle THg and brain THg was significantly correlated in most sampling locations (Table 4).

THg levels in the brain and muscle of the other shark species examined were consistent with the differences observed in Hg accumulation in these tissues in the Atlantic sharpnose shark. THg concentrations in bonnethead sharks ($n = 5$) ranged from 0.501 to 0.877 mg/kg w.w. (0.761 ± 0.150 mg/kg w.w.) in the muscle and 0.041 to 0.128 mg/kg w.w. (0.075 ± 0.039 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. (1.489 ± 0.558 mg/kg w.w.) in the muscle and 0.023 to 0.587 mg/kg w.w. (0.132 ± 0.044 mg/kg w.w.) in the brain.

THg concentrations were significantly higher in the muscle (Fig. 6a) than in the brain (Fig. 6b) of adult sharpnose sharks (Wilcoxon signed-rank test: $Z = -11.922$, $n = 189$, $p < 0.05$). In addition, significant differences in both muscle (Fig. 6a) and brain (Fig. 6b) THg were observed by the geographical location of capture (Quade's rank analysis of covariance with TL as a covariate: Muscle: $F = 11.348$ on 11 d.f., $p < 0.05$; Brain: $F = 14.716$ on 11 d.f., $p < 0.05$). In general, adult individuals from North Carolina and northeast, west, and northwest Florida had significantly higher muscle THg levels than those from Mississippi, with several intermediate groups in between. Likewise, adult individuals from South Carolina, west Florida, and northwest Florida

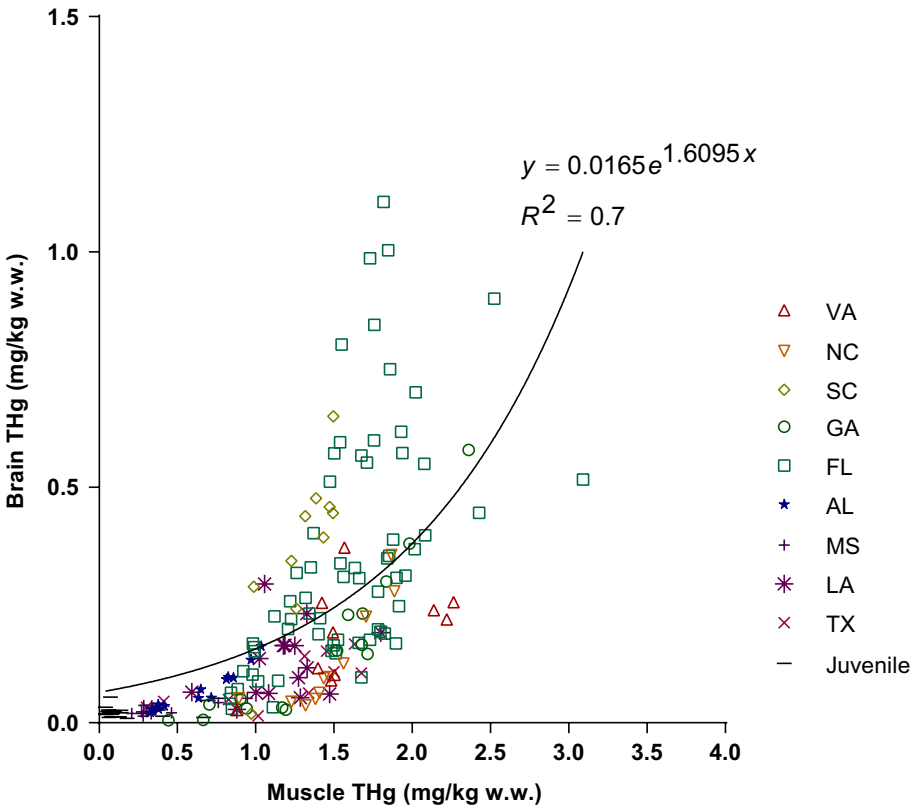

Fig. 5 Total mercury (THg) (mg/kg wet weight [w.w.]) in muscle and brain of Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*) ($n = 189$) collected from 9 states in the Southeastern United States (VA = Virginia, NC = North Carolina, SC = South Carolina, GA = Georgia, FL = Florida, AL = Alabama, MS = Mississippi, LA = Louisiana, TX = Texas). A significant positive correlation between THg in muscle and brain was observed (Spearman's rank order correlation, $r = 0.845$, $n = 189$, $p < 0.05$). The line represents the exponential relationship between the muscle THg and the brain THg ($y = 0.0165e^{1.6095x}$, $R^2 = 0.7$, $p < 0.05$)

Table 3 Results of Spearman's rank-order correlation tests between total mercury (THg) concentrations (mg/kg wet weight [w.w.]) in muscle and total length (TL) of adult, juvenile, and embryo Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*) by site of capture

		N	*r*	*p* value	Range	
					TL (cm)	Muscle THg (mg/kg w.w.)
Adults		159	0.388	< 0.05*	76–105	0.207–3.091
	Area					
	VA	10	−0.067	0.857	89–104	0.880–2.264
	NC	11	−0.096	0.780	82–100	0.893–1.886
	SC	10	0.321	0.368	86.7–95.5	0.971–1.497
	GA	15	0.681	< 0.05*	78.5–93.7	0.441–2.360
	NE FL	29	0.269	0.159	84–100	0.842–2.427
	S FL	8	0.395	0.332	84–94	0.840–2.524
	W FL	16	0.524	< 0.05*	76–95	0.920–3.091
	NW FL	10	−0.188	0.607	76.5–98	0.982–2.077
	AL	13	0.713	< 0.05*	79.7–94.3	0.328–1.035
	MS	12	0.315	0.319	78.3–92.5	0.207–0.945
	LA	15	0.194	0.469	91–105	0.593–1.799
	TX	10	0.346	0.324	82.8–99.9	0.302–1.675
Juvenile		26	0.449	< 0.05*	37.5–73	0.040–0.755
Embryo		20	−0.071	0.767	6.4–17.5	0.020–0.151
Total		205	0.704	< 0.05*	6.4–105	0.020–3.091

Abbreviations for site of capture are identified in Fig. 1

*Denote significant correlations

Table 4 Results of Spearman's rank-order correlation tests between total mercury (THg) concentrations (mg/kg wet weight [w.w.]) in muscle and brain of adult and juvenile Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*) by site of capture

		N	*r*	*p* value	THg (mg/kg w.w.) Range	
					Muscle	Brain
Adults		159	0.785	< 0.05*	0.207–3.091	0.005–1.107
	Area					
	VA	10	0.624	0.053	0.880–2.264	0.027–0.372
	NC	11	0.927	< 0.05*	0.893–1.886	0.037–0.356
	SC	10	0.855	< 0.05*	0.971–1.497	0.019–0.651
	GA	15	0.896	< 0.05*	0.441–2.360	0.005–0.579
	NE FL	28	0.691	< 0.05*	0.842–2.427	0.030–0.845
	S FL	10	0.915	< 0.05*	0.840–2.524	0.051–0.901
	W FL	17	0.820	< 0.05*	0.920–3.091	0.102–1.107
	NW FL	11	0.729	< 0.05*	0.982–2.077	0.088–0.803
	AL	13	0.962	< 0.05*	0.328–1.035	0.022–0.163
	MS	12	0.601	< 0.05*	0.207–0.945	0.014–0.052
	LA	15	0.315	0.253	0.593–1.799	0.029–0.295
	TX	10	0.733	< 0.05*	0.302–1.675	0.015–0.167
Juvenile		27	0.0623	0.758	0.040–0.755	0.009–0.055
Total		189	0.845	< 0.05*	0.020–3.091	0.005–1.107

Abbreviations for site of capture are identified in Fig. 1

*Denote significant correlations

had significantly higher brain THg levels than those from Mississippi, with several intermediate groups in between.

Concerning regional differences in THg concentrations in the brain, the forebrain contained significantly higher THg concentrations than those observed in the combined hindbrain and midbrain ($n = 91$) (Fig. 7; Wilcoxon signed-rank test: $Z = -6.273$ on 90 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 (Fig. 8; Spearman's rank order correlation coefficient, $r = -0.292$, $n = 41$, $p = 0.064$). Concentrations of 8-iso-prostaglandin $F2\alpha$ (Fig. 9; Spearman's rank order correlation coefficient, $r = -0.02$, $n = 35$, $p = 0.907$) and S100b (Fig. 10; Spearman's rank order correlation coefficient, $r = 0.039$, $n = 33$, $p = 0.830$) also were not significantly correlated with brain THg concentrations.

Discussion

Muscle THg concentrations in Atlantic sharpnose sharks examined in the present study were similar to those observed in the same species in prior investigations (Adams and McMichael 1999; Evers et al. 2008; Rumbold et al. 2014). Adams and McMichael (1999) reported muscle THg concentrations ranging from 0.11 to 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). 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 (0.840–2.524, 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. fish tissue-based criterion for human dietary consumption (U.S. EPA 2001).

Levels of THg in the muscle correlated with the size of the shark. These data are congruent with other studies that have examined muscle THg concentrations in the Atlantic sharpnose shark (Adams and McMichael 1999; Evers et al. 2008; Rumbold et al. 2014). Adams and McMichael (1999) found a significant linear correlation between muscle THg and size of the Atlantic sharpnose shark. 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

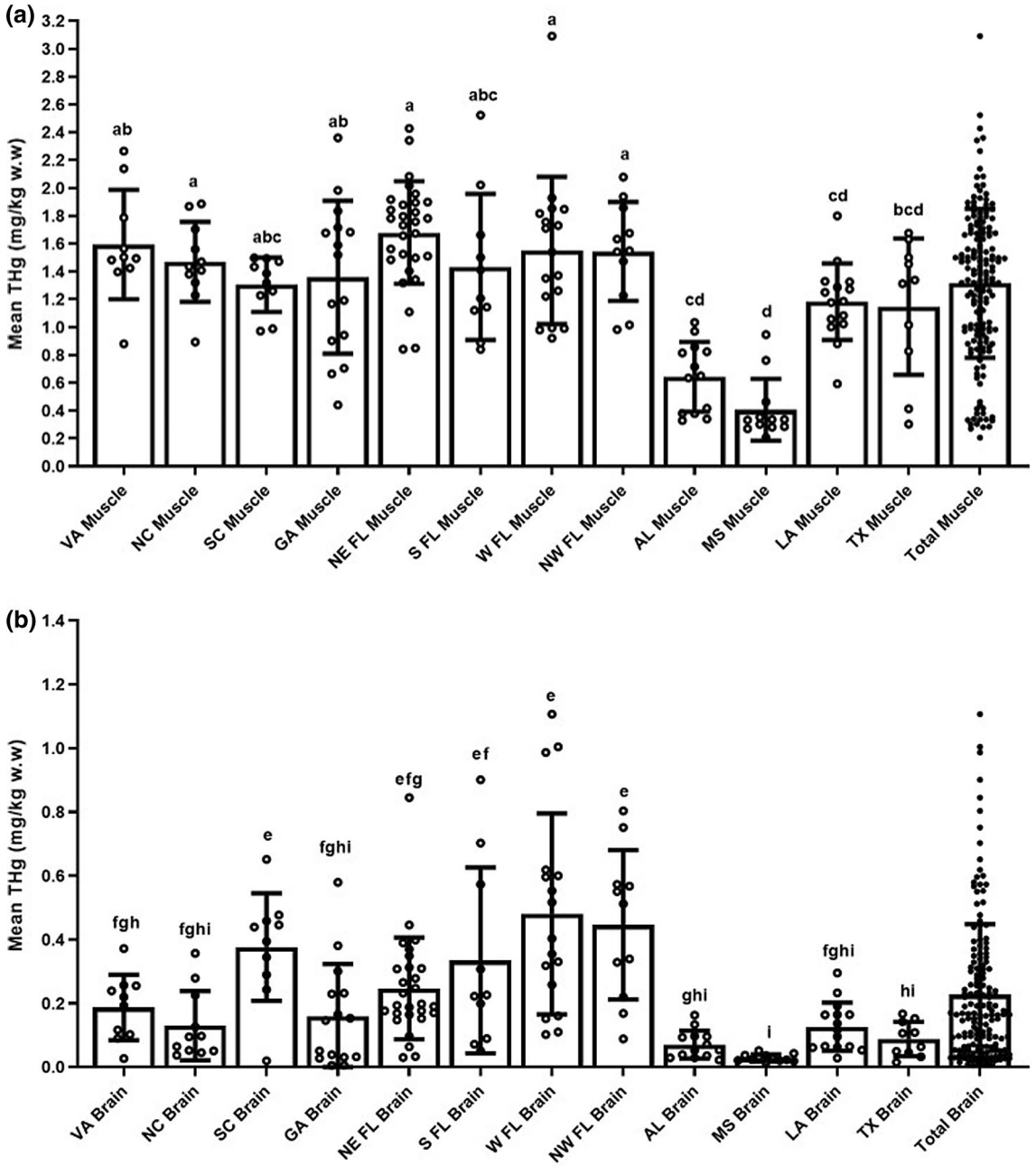

Fig. 6 Total mercury (THg) (mg/kg wet weight [w.w.]) in muscle (**a**) and brain (**b**) of adult, Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*) ($n = 163$) collected from 12 coastal locations throughout the Southeastern United States. Bars represent mean ± standard deviation. Sample sizes and location codes for each site are provided in Table 1. Brain THg concentrations were significantly lower than the muscle THg concentrations (Wilcoxon signed-rank test: $Z = -11.922$, $n = 189$, $p < 0.05$). Significant differences in THg were observed by site of capture (Quade's rank analysis of covariance with TL as a covariate: muscle: $F = 11.348$ on 11 d.f., $p < 0.05$; brain: $F = 14.716$ on 11 d.f., $p < 0.05$). Significantly different groups are represented by different lowercase letters

relationship. In contrast, Rumbold et al. (2014) did not find a significant correlation between muscle THg and Atlantic sharpnose shark size. 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

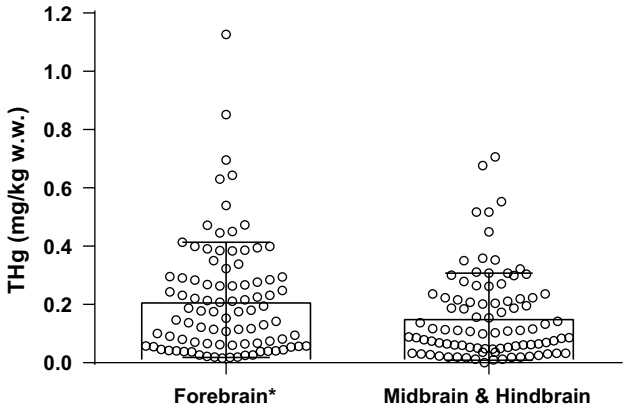

Fig. 7 Total mercury (THg) (mg/kg wet weight [w.w.]) in Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) forebrain and hindbrain/midbrain ($n=91$). Bars represent mean ± standard deviation. The forebrain was significantly higher in THg than the hindbrain/midbrain (Wilcoxon signed-rank test: $Z=-6.273$ on 90 d.f., $p<0.05$)

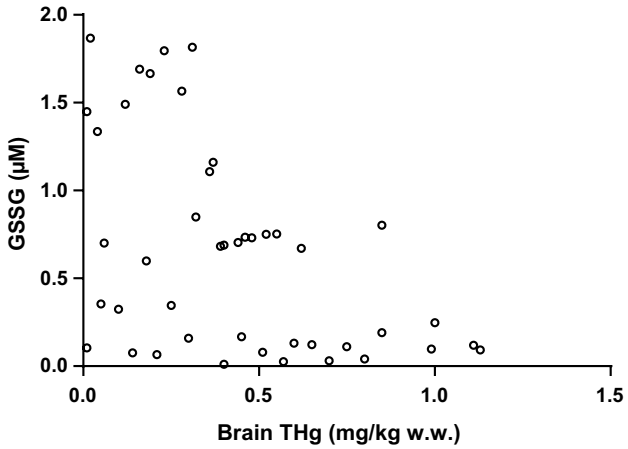

Fig. 8 Concentrations of total glutathione (GSSG) (µM) in cerebrospinal fluid and total mercury (THg) measured in mg/kg wet weight (w.w.) in the brain of Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*). Total glutathione in the CSF was not significantly correlated with brain THg (Spearman's rank order correlation coefficient, $r=-0.292$, $n=41$, $p=0.064$)

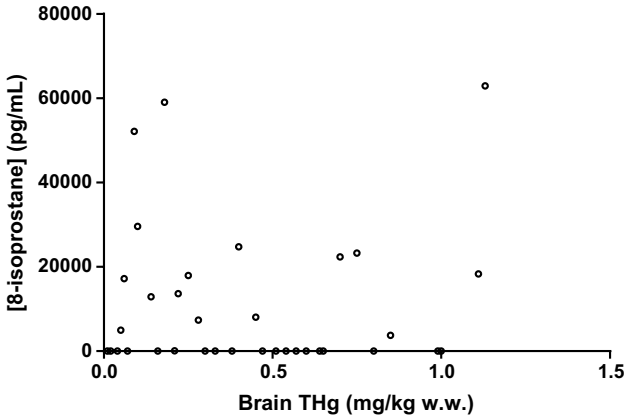

Fig. 9 Concentrations of 8-iso-prostaglandin *F2α* (pg/mL) in cerebrospinal fluid and total mercury (THg) measured in mg/kg wet weight (w.w.) in the brain of Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*). The 8-iso-prostaglandin *F2α* concentrations were not significantly correlated with brain THg (Spearman's rank order correlation coefficient, $r=-0.02$, $n=35$, $p=0.907$)

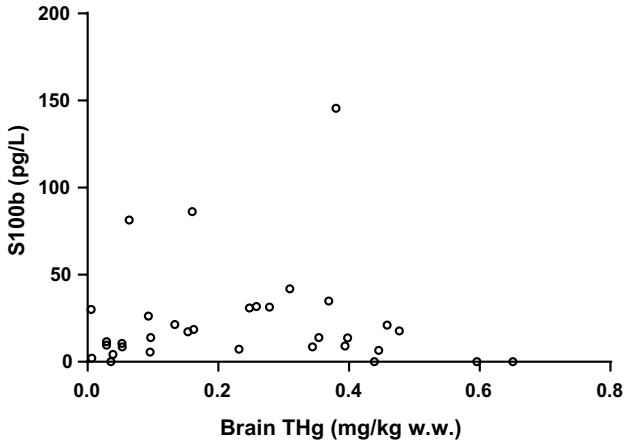

Fig. 10 Concentration of S100b (pg/L) in cerebrospinal fluid and total mercury (THg) measured in mg/kg wet weight (w.w.) in the brain of Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*). S100b concentrations were not significantly correlated with brain THg (Spearman's rank-order correlation coefficient, $r=0.039$, $n=33$, $p=0.830$)

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 to 0.29 ppm; 8.3–15.3% 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 the use of whole embryos in this study, 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 premise is supported by research on other shark species, such as white shark (*Carcharodon carcharias*), mako shark (*Isurus oxyrinchus*), salmon shark (*Lamna ditropis*), and 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 matrotrophic 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. It therefore is plausible that they may be capable of accumulating higher levels of Hg if mechanisms that permit its uptake across the BBB (i.e., the presence of amino acid transporters) differ between species with different degrees of encephalization. This hypothesis could be addressed by follow-up studies on brain Hg uptake in embryonic sharks from species that exhibit different brain morphologies (Yopak et al. 2007; Yopak 2012).

Although muscle THg concentrations in adult Atlantic sharpnose sharks often were elevated, a key finding of this study was that brain THg concentrations were significantly lower in comparison. These data agree with the limited number of studies that have examined Hg accumulation in the shark brain (Nam et al. 2011a; Newman et al. 2011; Bergés-Tiznado et al. 2015). Nam et al. (2011a) found the mean THg concentrations in juvenile lemon shark (*Negaprion brevirostris*) muscle were 0.311 ± 0.152 µg/g w.w., while the mean brain 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). 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 that the mean brain THg concentrations found in the present study (mean ± SD = 0.198 ± 0.216 mg/kg w.w.) were higher than those observed in previous reports. However, these levels still largely fell below most known thresholds associated with severe poisoning and outright mortality (> 10 µg/g w.w.; Wiener et al. 2003) or clinical neurotoxicity (> 1.5 µg/g w.w.; Suzuki 1979) in vertebrates. This suggests limited potential for Hg-induced neurological damage in Atlantic sharpnose sharks on the U.S. east coast. However, future research is needed because previously reported thresholds are largely based on mammalian studies and uptake and effects of Hg in the fish brain has not been well researched (Mieiro et al. 2010).

The conclusion that Hg uptake in the Atlantic sharpnose shark brain is largely below the threshold for clinical neurotoxicity is supported by data on biomarker concentrations, which were not found to be significantly correlated with brain THg levels. The selected biomarkers were examined because past studies 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 glutathione 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 that rats that were exposed to Hg had elevated concentrations of S100b released into the CSF from the brain. Based on the results of the present study, it was concluded that the levels observed in Atlantic sharpnose shark brains were too low to induce significant changes in these biomarkers of oxidative stress.

While average brain THg concentrations were generally low in the present study, it is important to note that individual levels varied considerably, slightly exceeding 1.0 mg/kg w.w. in some cases. Therefore, it is reasonable to consider that there is some, albeit limited, potential for Hg levels in the shark brain to occasionally exceed threshold values for some neurological responses, perhaps such as changes in neurochemistry and/or neurobehavior (~ 0.7–1.2 mg/kg w.w., Dietz et al. 2013). This premise is supported by past studies on brain Hg uptake and neurochemical and/or neurobehavioral responses in various fish species. For example, Shaw et al. (1990) observed inhibition of brain acetylcholinesterase (AChE) activity in three fish species collected from a Hg-contaminated estuary receiving effluent from chlor-alkali plants; maximum brain residual Hg levels in these species was 0.702 ± 0.205 mg/kg w.w. However, Webber and Haines (2003) found no inhibition of brain AChE activity in golden shiner (*Notemigonus crysoleucas*) that were fed a diet containing low or high levels of MeHg and, as a result, exhibited mean brain MeHg concentrations of 0.477 ± 0.148 and 1.118 ± 0.196, respectively. Notwithstanding these results, Webber and Haines (2003) observed alterations in predator avoidance behavior in golden shiner that received the high-MeHg diet, a finding that has been alluded to in other experimental studies but not directly confirmed due to lack of brain Hg measurements and/or quantitative indicators of behavior (Rodgers and Beamish 1982). Adams et al. (2010) also found no difference in brain AChE activity in spotted seatrout (*Cynoscion nebulosus*) that experienced differences in brain Hg uptake (0.11 ± 0.03 and 0.24 ± 0.11 mg/kg w.w. for South Florida and Indian River Lagoon, respectively); however, they did observe significant differences in *N*-methyl-*D*-aspartate (NMDA) receptor levels in these groups. In general, these

studies suggest that there is some potential for at least neurochemical or neurobehavioral effects on fish at brain Hg levels that are on average higher but still overlap with those observed in Atlantic sharpnose sharks in the present study (0.198 ± 0.216 mg/kg w.w.). However, it remains questionable as to what the organism-level responses to such effects would be in sharks if they were to occur.

Because of occasional risk of high brain Hg exposure, it is still sensible to monitor possible Hg uptake in the shark brain in highly-contaminated locations, perhaps by using nonlethally obtained muscle biopsies and the relationship between muscle and brain THg concentrations determined in this study ($y = 0.0165e^{1.6095x}$). 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).

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 that individuals could experience Hg-related effects associated with forebrain function, which could include alterations in sensory function, decreased autonomic and neuroendocrine responses to stress, behavioral changes (e.g., decreased predator/prey interactions, reproduction, mood, appetite), and uncontrollable voluntary muscle movements (Scott and Sloman 2004; Pereira et al. 2016). As an example, Berlin et al. (1975) observed sensory disturbances and impaired voluntary coordination in squirrel monkeys with Hg-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 (i.e., decreased swimming distance), whereas IHg induced anxiety-like behaviors (i.e., decrease in motivation to swim as determined by the latency to be dragged and to take refuge) in white seabream fish (*Diplodus sargus*) (Pereira et al. 2016; Puga et al. 2016). Puga et al. (2016) suggested that behavioral alterations observed in Hg-exposed seabream may be mediated by dysfunction of the dopaminergic cells in the hypothalamus, but they did not propose a molecular mechanism for these effects. However, both Puga et al. (2016) and Pereira et al. (2016) observed alterations in cell volume and number of neurons and glial cells in various portions of the forebrain (i.e., optic tectum, medial pallium, and the hypothalamus) of white seabream exposed to IHg and/or MeHg, although the cerebellum also appeared to be affected. In contrast, studies that have observed greater levels of Hg accumulation in the midbrain and/or hindbrain compared with the forebrain have generally reported motor disturbances as the primary responses (Charbonneau et al. 1976).

Variations in the accumulation of Hg in different portions of the brain of sharks and some other vertebrates could result from a greater number of thiol groups in the forebrain, as suggested by Krey et al. (2015). However, it also could be due to Hg concentrating in the largest region of the brain that was the last to differentiate from precursor cells, as the brain is believed to develop in a conserved gradient of hindbrain to forebrain (i.e., the "late equals large" principle) (Finlay et al. 2001). In the Atlantic sharpnose shark, the forebrain comprises 50% of the brain's mass. However, not all shark species have this same pattern of brain organization. It has been hypothesized that sharks with similar lifestyle characteristics generally have similar patterns of brain organization, generally termed "cerebrotypes" (Yopak et al. 2007; Yopak 2012). For example, data have suggested there are associations between telencephalon size with the shark's taxon and niche; mesencephalon size with the shark's reliance on vision; medulla oblongata size with the use of nonvisual senses; and the cerebellum's complexity with the shark's habitat and activity levels (Yopak et al. 2007; Yopak and Montgomery 2008; Yopak et al. 2010; Yopak and Lisney 2012). Therefore, comparisons should be made with other chondrichthyan species of varying brain cerebrotypes. In particular, future studies should examine Hg in the brain of sharks with an enlarged medulla oblongata (e.g., bathyal, deep sea benthopelagic sharks) or a large, highly foliated cerebellum (e.g., reef-associated, oceanic habitats) and determine whether Hg accumulates to a greater extent in these enlarged regions.

Both brain and muscle exhibited significant variations in Hg uptake in Atlantic sharpnose sharks in relation to site of capture. In particular, 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 twofold 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 compared with sharks from east of Mobile Bay to northwest Florida (Bethea et al. 2006; Drymon et al. 2012). 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. Site-associated differences also could be due to variations in regional differences in MeHg availability between these locations, as well

as differences in movement patterns of sharks from different subpopulations (Harris et al. 2012). However, more information on spatial differences in environmental Hg concentrations in these areas and variations in fish movements and migratory behavior are needed to address these hypotheses properly.

The high percentage of MeHg within the THg for the muscle is similar to what has been reported in other shark species (Storelli et al. 2002; Pethybridge et al. 2010; Nam et al. 2011b). In contrast, the percentage of MeHg in the Atlantic sharpnose shark brain is notably lower than the %MeHg observed by Nam et al. (2011b) for lemon sharks (range: 67.6–109%; mean ± SD: 88.8 ± 10.3%). However, it is important to note that the study by Nam et al. (2011b) was on neonate lemon sharks, whereas the subsamples from this study were from adult individuals. This may be a relevant point because the lower percentage of MeHg observed in the Atlantic sharpnose shark brain may reflect demethylation of MeHg to IHg within the brain, which would be expected to increase as sharks age. The demethylation of MeHg to IHg in the brain has been observed over time in other vertebrates, including fish (e.g., white seabream; Pereira et al. 2016; Puga et al. 2016) and mammals (e.g., crab-eating macaque *Macaca fascicularis*; Vahter et al. 1995). This hypothesis has been used to explain the lower accumulation of MeHg in the brain of golden grey mullet *Liza aurata* compared with other tissues, such as the eye wall and lens (Pereira et al. 2014). Alternatively, it is possible that variations in Hg speciation observed between Atlantic Sharpnose shark muscle and brain may reflect differences in the uptake of IHg and MeHg in the brain. However, this premise is not consistent with findings from earlier studies, which have observed far greater uptake of organic versus inorganic Hg into the vertebrate brain (Lohren et al. 2016). This is believed to be due to the relative impermeability of the BBB to IHg, although it has reported that IHg may be transferred to the brain via axonal transport following uptake across receptor cells of sensory neurons (Rouleau et al., 1999).

Based on in vitro studies on human neuroblastoma cells, Mailloux et al. (2015) suggested that demethylation occurs by the superoxide anion radical (O_2^-) cleaving the methyl group from MeHg, resulting in formation of IHg. This has been suggested to be a detoxification process, as it may be followed by the formation of insoluble and inert forms of Hg similar to mercury selenide. However, not all studies agree with the premise that demethylation of MeHg in the brain may be beneficial, as the efflux of MeHg from the brain may be greater than that for IHg (Krey et al. 2012). Furthermore, IHg is still very toxic, because it inhibits neuronal differentiation and increases oxidative stress in the brain by altering glutamate and calcium homeostasis (Shapiro and Chan 2008; Pereira et al. 2016; Chan et al. 2017).

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; Yopak et al. 2010; Ferretti 2011). 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 both neurons and glial cells in the brain of the white seabream fish (*Diplodus sargus*) after exposure to Hg, indicating a potential for neuronal regeneration following Hg-induced neuron damage in fish.

Conclusions

Whereas 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 illustrated that THg concentrations in the sharpnose brain are appreciably lower and generally pose limited risks to shark neurological function. Nonetheless, due to occasionally elevated Hg exposure in the brain of some Atlantic sharpnose sharks, there is potential for some individuals to be exposed to levels that could alter neurological function. Additionally, Hg levels appeared to be highest in the forebrain of the Atlantic sharpnose shark brain, suggesting the possibility of brain region-specific effects on central nervous system activity. Furthermore, the low percentage of MeHg observed in the brain indicates a prolonged exposure to Hg and demethylation of organic to inorganic mercury and/or dissimilarities in uptake or loss of organic versus inorganic Hg. It is 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 (Rice et al. 2014). 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.

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