Relationships of mercury concentrations across tissue types, muscle regions and fins for two shark species

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Running title: Mercury concentrations in two species of shark

Abstract

Mercury (Hg) exposure poses a threat to both fish and human health. Sharks are known to bioaccumulate Hg, however, little is known regarding how Hg is distributed between different tissue groups (e.g. muscle regions, organs). Here we evaluated total mercury (THg) concentrations from eight muscle regions, four fins (first dorsal, left and right pectorals, caudal- from both the inner core and trailing margin of each fin), and five internal organs (liver, kidney, spleen, heart, epigonal organ) from two different shark species, bonnethead (*Sphyrna tiburo*) and silky shark (*Carcharhinus falciformis*) to determine the relationships of THg concentrations between and within tissue groups. Total Hg concentrations were highest in the eight muscle regions with no significant differences in THg concentrations between the different muscle regions and muscle types (red and white). Results from tissue collected from any muscle region would be representative of all muscle sample locations. Total Hg concentrations were lowest in samples taken from the fin inner core of the first dorsal, pectoral, and caudal (lower lobe) fins. Mercury concentrations for samples taken from the trailing margin of the dorsal, pectoral, and caudal fins (upper and lower lobe) were also not significantly different from each other for both species. Significant relationships were found between THg concentrations in dorsal axial muscle tissue and the fin inner core, liver, kidney, spleen and heart for both species as well as the THg concentrations between the dorsal fin trailing margin and the heart for the silky shark and all other sampled tissue types for the bonnethead shark. Our results suggest that biopsy sampling of dorsal muscle can provide data that can effectively estimate THg concentrations in specific organs without using more invasive, or lethal methods.

Capsule: Muscle tissue had the highest THg concentrations in two shark species with no significant difference between muscle regions and muscle biopsies, and direct relationships allow reliable prediction of THg in other tissues.

Keywords: Non-invasive Techniques, Shark Health, Human Health, Total Mercury

Introduction

Mercury (Hg) pollution poses a potential threat to the health of organisms in the marine environment. In fish, Hg accumulates principally from dietary sources (Trudel & Rasmussen 2001) and causes numerous sublethal effects including impaired reproduction (Matta *et al.* 2001; Hammerschmidt *et al.* 2002; Drevnick & Sandheinrich 2003; Klaper *et al.* 2006; Crump & Trudeau 2009), reduced liver function and metabolism (Adams *et al.* 2010), and altered behavior (Webber & Haines 2003). Mercury can also lead to neurological damage (Basu *et al.* 2007, 2009; Scheuhammer *et al.* 2008), damage to gills and olfactory organs (Jagoe *et al.* 1996; Oliveira Ribeiro *et al.* 1996, 2000) and mortality (Wiener & Spry 1996). Much attention has been paid to methylmercury (MeHg) contamination in high trophic level fishes such as tuna, swordfish and sharks because of the health risks to humans from consumption of these organisms (Rice *et al.* 2000; Escobar-Sánchez *et al.* 2010; Burger & Gochfeld 2011; Lopez *et al.* 2013). Many shark species are long lived and occupy high trophic positions and thus accumulate substantial concentrations of Hg (Lyle 1984; Adams 2004; Branco *et al.* 2004). The human demand for shark products including fins has increased dramatically since the 1950's (Dulvy *et al.* 2014; Dent & Clarke 2015). It is thus critical to examine the potential Hg exposure levels that result from the consumption of shark meat and fins (Swain *et al.* 2007) to better understand associated human health implications.

Mercury concentrations in sharks vary within and among species. Differences in Hg concentrations (Hueter *et al.* 1995; Storelli *et al.* 2002; McMeans *et al.* 2007; Endo *et al.* 2008) are likely due to variations in prey type. At the individual level differences in length (age) (Walker 1976; Hornug *et al.* 1993; Hueter *et al.* 1995; Lacerda *et al.* 2000; Endo *et al.* 2008), sex (Pethybridge *et al.* 2010), season (Escobar-Sánchez *et al.* 2010), habitat type and location (Hornug *et al.* 1993; McMeans *et al.* 2007) all contribute to variation in Hg uptake. Mercury concentrations in sharks also vary among tissue types (Pethybridge *et al.* 2010; Nam *et al.* 2011; Delshad *et al.* 2012; Hurtado-Banda *et al.* 2012; Lopez *et al.* 2013). Typically, the highest concentrations of total mercury (THg) are found in muscle tissue, followed by internal organs (e.g. kidney, liver), fins, and skin (Pethybridge *et al.* 2010; Nam *et al.* 2011; Delshad *et al.* 2012; Hurtado-Banda *et al.* 2012).

The levels of Hg in shark tissues are of concern to both human (Swain *et al.* 2007) and shark health (Nam *et al.* 2011). Currently, there are insufficient data on Hg contamination in shark organs and the potential influence of these concentrations on shark health. Collecting samples from internal organs of sharks typically involves lethal sampling methods. Transition from lethal to primarily non-lethal sampling is important given that 25% of all chondrichthyan fishes (sharks, skates, rays, and chimeras) are currently threatened with extinction (Dulvy *et al.* 2014) and some are classified as protected species where collection is restricted or not possible. Non-lethal techniques that utilize a subset of tissues to estimate Hg concentrations throughout a shark's body (i.e. organs) would provide the ability to expand our understanding of how Hg is distributed throughout shark tissues, and reduce the need for lethal sampling. Previous studies on amphibians (Todd *et al.* 2012), birds (Eagles-Smith *et al.* 2008), and teleost fish (Gremillion *et al.* 2005; Schmitt & Brumbaugh 2007; Piraino & Taylor 2013) have shown that THg

concentrations in one tissue can be used to accurately predict relationships among other tissues. Yet, to our knowledge, no previous studies have looked at the relationship of Hg concentrations among a full array of shark tissue types and organs.

The objectives of this study were to: 1) examine THg concentrations in tissues from two species of shark, with varying life histories: bonnethead (*Sphyrna tiburo*) and silky shark (*Carcharhinus falciformis*); 2) examine the relationships of THg concentrations across a wide range of tissues including eight muscle regions (dorsal axial, interdorsal, caudal peduncle white muscle, caudal peduncle red muscle, mid-flank, mid-abdominal, cranial, pre-pectoral), fin cores and fin trailing margins from four different fins (first dorsal, left and right pectoral, caudal), and five organs (liver, heart, kidney, spleen, epigonal) and 3) determine if measurements of THg via less invasive fin clips (fin trailing margins) or muscle biopsy punches can be used to effectively estimate THg concentrations in other tissue types.

Methods

Study Species

The bonnethead shark is a small coastal shark, reaching a maximum total length (TL) of 150cm (Castro 2011). Females mature between 6 to 7 years of age (~81.9cm) and can live to nearly 18 years, while males mature between 3 to 9 years (~61.9cm) and can live to 16 years (Frazier *et al.* 2014). The estuarine waters of the southeastern U.S. coast are important seasonal feeding grounds for this species (Driggers *et al.* 2014). Bonnethead sharks specialize on hard-shelled prey and feed primarily on crustaceans, particularly blue crabs (*Callinectes sapidus*) (Cortés *et al.* 1996; Lessa & Almeida 1998; Wilga & Motta 2000; Bethea *et al.* 2007; Gurshin 2007) that can make up 99% of their diet (Ulrich *et al.* 2007). The silky shark is a semi-pelagic species that frequents both coastal and open waters in tropical oceans around the world (Bonfil

2008). They are most commonly found on the edge of continental and insular shelves, particularly around seamounts, at depths averaging 200m (Ebert *et al.* 2013; Bonfil 2008) or near offshore reef habitats or related natural and artificial structure (D.H. Adams, pers. observation). The silky shark is relatively long-lived (to 22+ years), with a maximum observed TL of ~330cm (Ebert *et al.* 2013; Bonfil 2008). In general females reach maturity at 6 to 10 years (>180cm) and males after 5-10 years (>180cm) (Branstetter 1987; Bonfil 1993; Hoyos 2003; Oshitani *et al.* 2003). The silky shark feeds primarily on fish including marine catfish (*Ariidae*), mullets (*Mugilidae*), mackerel (*Scombridae*), yellowfin tuna (*Thunnus albacares*), albacore tuna (*T. alalunga*), porcupine fish (*Diodontidae*), and is also known to prey on a variety of cephalopods (Bonfil 1993; Ebert *et al.* 2013).

Sample Collection

A total of 42 bonnethead and 13 silky sharks were examined. All bonnethead and five silky sharks were collected from 2012 to 2013 in estuarine and coastal areas of the southeastern United States (St. Catherine's Island, GA and the Indian River Lagoon, FL and adjacent nearshore waters) using gill nets, haul seines and hook and line gear. Eight silky sharks caught in the Red Sea were collected during market surveys in Jeddah, Saudi Arabia, in 2013. We recorded precaudal length (PCL), fork length (FL), total length (TL), total weight, and sex for each specimen. To assess maturity state, we used a combination of TL and macroscopic examination of female reproductive tract or male clasper length and calcification.

We sampled four tissue groups (muscle, fin inner core, fin trailing margin, organ) and within each group sampled multiple regions (Figure 1). For the muscle group, we sampled eight regions: dorsal axial, interdorsal, caudal peduncle white muscle, caudal peduncle red muscle,

mid-flank, mid-abdominal, cranial, and pre-pectoral. For the two fin groupings, we sampled ten regions: fin cores and fin trailing margins from the first dorsal fin, left and right pectoral fins, and the upper and lower caudal fin lobes. For the organ grouping we sampled five regions: liver, heart, kidney, spleen, and epigonal organ. Caudal peduncle red muscle was only sampled for bonnethead sharks.

For all samples, we used a clean stainless-steel knife or scalpel to initially remove whole fins and sections of muscles and organs. Care was taken during dissection to ensure that muscle samples did not come into contact with shark epidermal or dermal layers, or surrounding surfaces (Adams *et al.* 2003). Tissue and fin samples were immediately placed in new sterile polyethylene containers and frozen at -20°C or -80°C before transfer to the University of Georgia's Savannah River Ecology Laboratory. To sub-sample tissues for Hg analysis we allowed samples to thaw at room temperature. To sub-sample the muscle regions and organs we used sterile forceps and scissors to remove $\sim 1\text{ g}$ samples. We used 8mm disposable biopsy punches (Premier Medical, Plymouth Meeting, PA) to take two samples from each of the fins: fin trailing margin and inner core (Figure 1). We determined the fin inner core sample location by measuring the halfway point on the leading edge of the respective fin and then measuring to the center point between the leading and trailing edge (Figure 1). We then took fin trailing margin samples from the trailing edge of the fin on the same bisecting line as the fin inner core sample. We treated the upper and lower lobes of the caudal fin as two separate fins for sub-sampling, resulting in a fin trailing margin and inner core sample for both the upper and lower lobe. However, lower lobe caudal fin samples were only obtained for the bonnethead shark ($N = 14$). We stored all sub-samples in individual 29 ml clean polyethylene plastic sample bags.

Chemical Analysis

All tissue samples were lyophilized. In addition, muscle and organ samples were homogenized by crushing the bagged samples in a mortar, insuring no direct contact with the sample to reduce cross contamination. Fin tissue samples were either analyzed whole or they were cut in half using clean and sterile scissors (when duplicates of particular fins needed to be run). We analyzed THg content by thermal decomposition, catalytic conversion, amalgamation, and atomic absorption spectrophotometry (DMA 80; Milestone, Monroe, CT, USA), according to U.S. Environmental Protection Agency (EPA) method 7473 (U.S. E.P.A. 2007).

Concentrations of THg for all samples were analyzed on a dry weight basis to standardize comparisons and avoid the confounding factor of variable moisture loss among samples.

For quality-assurance measures we included the analysis of two certified reference materials (dogfish liver DOLT-4, and lobster hepatopancreas TORT-2; National Research Council of Canada, Ottawa, ON) and system and method blanks. We initially ran three system and method blanks followed by the reference materials to verify system calibration. Then for every 10-15 samples we ran a single duplicate of the last sample run, followed by a system and method blank, the two reference materials, and finally by two more system and method blanks. Our recoveries averaged $101.9\% \pm 7.1\%$ ($n = 149$) and $99.6\% \pm 6\%$ ($n = 67$) for the certified reference materials and calibration checks respectively. We found the absolute relative percentage difference (RPD) for duplicates in all tissues combined averaged to be $11.1\% \pm 13.3\%$.

Statistical Analyses

We used R statistical software (3.1.2) for all statistical analyses (R Development Core Team 2014). We tested for normality using the Shapiro-Wilk test (Shapiro & Wilk 1965) and

homogeneity of variance using a Bartlett test (Bartlett 1937) for all tissue groups (muscles, organs, fin inner cores, fin trailing margins). To better meet the assumptions of standard parametric statistical tests and to normalize residuals we natural-log transformed all THg data. After transformation, each of the tissue groups met at least one of the assumptions of an analysis of variance (ANOVA), but generally not both assumptions. However, ANOVA is fairly robust to violations of a single assumption, especially if sample sizes are relatively large (Glass *et al.* 1972).

To determine if THg concentrations were significantly different between tissue groupings (muscle, organs, fin inner core, fin trailing margin) or within a tissue group (i.e. kidney, heart, liver, spleen, epigonal organ) we used an ANOVA. When sampling regions within a tissue group were significantly different we used a Tukey's multiple comparison of means to determine which sample types had significantly different THg concentrations. We used correlation analyses to further compare each of the tissue regions within the various tissue groups. Paired sample t-test were used to determine if relationships of THg concentrations between different tissues remained the same throughout a species ontogeny. We used an independent samples t-test to compare THg concentrations found in muscle, liver, kidney, heart, spleen, epigonal organ, IUCL, fin inner cores, and fin margins between the two species.

To examine the potential to use fin trailing margins or muscle biopsies to predict THg concentrations in other tissue groups and regions we used linear regressions. The goal was to use non-lethal fin trailing margins (fin clips) and muscle samples to predict Hg concentrations in tissues that are typically collected via lethal sampling. In the first set of regressions, we used the fin trailing margin as the independent variable and performed separate regressions for the following dependent variables: muscle (dorsal axial), fin inner core (first dorsal, inner core of the

upper caudal lobe (IUCL), liver, kidney, spleen, heart, and epigonal organ. In the second set of regressions, we used muscle (dorsal axial) as the independent variable. Within the muscle tissue group there was no significant difference in THg and thus we chose a representative variable, dorsal axial muscle, for further analyses and ran separate regressions for the following dependent variables: fin inner core (first dorsal), IUCL, liver, kidney, spleen, heart, and the epigonal organ. From the regressions, we developed equations for predicting THg concentrations in tissues based on the concentration found in either the fin trailing margin or muscle tissue.

Four of the five silky sharks from the southeastern U.S., and 28 of the 42 bonnethead sharks only had dorsal axial muscle and liver samples collected. Therefore, these individuals were only included in the following analyses: the ANOVA comparing the different tissue groups for each species; the paired samples t-test determining if the relationship of THg concentrations between muscle and liver remained constant throughout ontogeny; the independent samples t-tests comparing THg concentrations in muscle and liver between species; and the regression analysis determining if THg concentrations in muscle could predict THg concentrations in the liver.

Results

Overall we measured THg in 577 samples from 42 bonnethead and 13 silky sharks. Bonnethead sharks in this study sampled from nearshore and estuarine waters of the Southeastern US ranged from 36.6 to 120.1cm TL, encompassing adult (n= 33) and juvenile (n= 9) life-history stages. Silky sharks included juvenile males and females (n= 9) and adult male (n= 4) sharks ranging from 86.6 to 220 cm TL. The data include THg concentrations from 200 muscle, 142 organ, 118 fin inner core, and 177 fin trailing margin samples (Table 1). The lowest

THg levels (0.02 ± 0.01) were in the fin inner cores of silky sharks and the highest (3.1 ± 2.12) in the pre-pectoral muscle of bonnethead sharks. Of all tissue types sampled, only the total Hg in the IUCL ($t = -2.94$, $p = 0.05$) and fin trailing margins ($t = 4.08$, $p < 0.001$) were significantly different between the two species.

Comparison of THg concentrations within and between tissue groups

Total Hg concentrations between the eight muscle regions sampled were not significantly different from one another for either shark species (bonnethead: $F = 0.185$, $p = 0.988$; silky shark: $F = 0.199$, $p = 0.976$) and were all strongly correlated for both species (Table 2). Total Hg concentrations for the five organs sampled (liver, kidney, spleen, heart, epigonal organ) were also not significantly different from one another ($F = 1.947$, $p = 0.107$), but only the organs of the bonnethead were strongly correlated (Table 3). Thus, we analyzed each organ independently in our regression analyses. Trailing fin margin THg concentrations for each of the sampled fins were not significantly different for either species (bonnethead shark: $F = 0.282$, $p = 0.406$; silky shark: $F = 2.509$, $p = 0.0792$). Overall, the correlation among fin trailing margin samples were strong, the exception is the first dorsal fin of the silky sharks (Table 4). However, since the first dorsal fin represents a commonly sampled region in sharks for multiple types of biological studies with typically easier access during live sampling we used it as a representative “fin” sample for our regression analyses.

Fin inner core samples for each species (excluding the lower caudal lobe for silky sharks, which was not sampled) were initially found to have significantly different THg concentrations (bonnethead shark: $F = 49.25$, $p < 0.001$; silky shark: $F = 66.86$, $p < 0.001$) (Table 1). However, the fin IUCL accounted for much of the variation between these tissues, being significantly

different from each of the other fin inner core samples in both species (Tukey's p -values < 0.001 for each fin inner core compared to the IUCL). When the IUCL is excluded from the analysis, the other fin inner core samples do not have significantly different THg concentrations (bonnethead shark: $F = 0.001$, $p = 1.000$; silky shark: $F = 0.793$, $p = 0.465$) and are highly correlated (Table 4). Consequently, we used the fin inner core THg concentrations of the first dorsal fin to represent fin inner cores from the pectoral and lower caudal lobe (for bonnethead sharks), but analyzed IUCL independently.

In both species, muscle, liver, kidney, heart, spleen, epigonal organ, fin inner core, fin trailing margin, and IUCL had significantly different THg concentrations (bonnethead shark: $F = 208.8$, $p < 0.001$; silky shark: $F = 131.8$, $p < 0.001$). On average, muscle tissue (all sampled regions) had the highest THg concentrations (Table 1). Muscle tissue was also found to have significantly higher THg concentrations compared to liver tissue throughout the ontogeny of both species (bonnethead shark: $t = 7.17$, $p < 0.001$; silky shark: $t = 4.89$, $p < 0.001$). Within bonnethead sharks, THg concentrations were highest in muscle and lowest in fin inner cores with the following pattern: muscle $>$ kidney $>$ liver $>$ heart $>$ spleen $>$ epigonal organ, $>$ IUCL $>$ fin trailing margin $>$ fin inner core (Table 1). For silky sharks, mean THg concentrations were highest in the muscle then liver $>$ epigonal organ $>$ heart $>$ IUCL $>$ spleen $>$ kidney $>$ fin trailing margin $>$ fin inner core (Table 1). Fin trailing margins interestingly had higher THg concentrations than the fin inner cores from the first dorsal, left and right pectoral, and lower lobe of the caudal fin. The IUCL had significantly ($p < 0.001$) higher concentrations of THg compared to all other fin samples.

Fin clips and muscle biopsies as indicators of THg accumulation

Of particular interest to this study was determining whether less invasive sampling methods (e.g. fin clips or muscle biopsy punches) could be used to estimate THg concentrations in a variety of shark tissues. The THg concentrations in the fin trailing margin (first dorsal fin) were significantly related to THg concentrations in all other tissue types for bonnethead sharks (muscle, fin inner core, IUCL, liver, kidney, spleen, heart, epigonal organ) (Table 5; Figure 2). Within silky sharks fin trailing margin tissue was significantly related only to heart tissue, but explained a low percentage of the variation (Table 5). There were also significant relationships between THg concentrations in muscle tissue (dorsal axial muscle) and all of the other tissue types for bonnethead sharks and for all but the epigonal organ for silky sharks (Table 2; Figure 3). For the relationship between muscle tissue and liver THg concentrations the sample size was increased from 22 (total sharks between the two species; individuals with samples taken from all tissue regions) to 55 sharks (33 sharks with only muscle and liver sampled). With the increased sample size, there was a significant relationship between the two tissues for both species (Table 5; Figure 3A).

Discussion

Previous studies on THg concentrations in teleost fish (spotted seatrout (*Cynoscion nebulosus*), Atlantic goliath grouper (*Epinephelus itajara*), golden grey mullet (*Liza aurata*)) found that THg concentrations were greater in the liver and kidneys compared to muscle tissue (Mieiro *et al.* 2009; Adams *et al.* 2010; Adams & Sonne 2013). Likely due to the fact that digestive tissues (i.e., liver, kidneys) are associated with the accumulation, as well as the metabolism and elimination of trace elements, like Hg, leading to increased concentrations of Hg in these tissues (Pethybridge *et al.* 2010). However, for both species analyzed in this study, THg concentrations were highest in muscle tissue, followed by either the liver (silky shark) or kidney

(bonnethead shark), with fins having the lowest concentrations (excluding IUCL). Despite these findings being counter to observations in teleost fishes, they are in agreement with recent studies on THg concentrations in other shark species (Branco *et al.* 2007; Coelho *et al.* 2010; Pethybridge *et al.* 2010; Nam *et al.* 2011; Delshad *et al.* 2012; Hurtado-Banda *et al.* 2012; Bergés-Tiznado *et al.* 2015). This further supports the hypothesis that THg is preferentially accumulated in muscle tissue of sharks, likely due to its high affinity for sulphhydryl groups associated with the thiol-containing amino acids of the muscles (Lacerda *et al.* 2000; Storelli *et al.* 2002; Wang 2002; Branco *et al.* 2007; Coelho *et al.* 2008; Gelsleichter & Walker 2010; Pethybridge *et al.* 2010). Previous studies have alluded to the possibility that selenium (Se) in the liver of sharks (and other animals) may mitigate the toxic effects of MeHg via demethylation, which would result in organic Hg (i.e. MeHg) being converted into inorganic Hg that is more readily eliminated from the liver (Storelli *et al.* 2002; Branco *et al.* 2007; Nam *et al.* 2011; Branco *et al.* 2012). This could potentially explain why concentrations of THg in the livers were generally lower than those in the muscle tissue. McKim *et al.* (1976) found that following demethylation, inorganic Hg preferentially accumulated in the kidneys of brook trout (*Salvelinus fontinalis*), which could help explain why the kidneys of bonnethead sharks had higher concentrations of THg compared to their livers.

Total Hg concentrations in each of the muscle regions examined were tightly correlated in both bonnethead and silky sharks. This suggests that sampling muscle from any of the eight locations would be representative of all locations. Therefore, when monitoring how consumption of various shark muscle may impact human Hg exposure, particularly MeHg which accounts for ~95% of THg (Adams & McMichael 1999; Storelli *et al.* 2002; Nam *et al.* 2011), any of the shark muscle locations could be used with confidence. Red muscle from the caudal peduncle in

bonnethead sharks showed similar THg concentrations as the other seven white muscle samples. This finding is somewhat contradictory to previous studies demonstrating that red muscle from tilapia (*Oreochromis aureus*), striped bass (*Morone saxatilis*) (Cizdziel et al. 2002), and yellowfin tuna (Bosch et al. 2016) had significantly different concentrations of Hg than white muscle. Given that red muscle uses aerobic respiration (Tyus 2012), and blood is responsible for the distribution of Hg to various tissues (Eagles-Smith et al. 2008), we also expected to find higher THg concentrations in red muscle. In yellowfin tuna, higher concentrations of THg in red muscle compared to white muscle was suggested to be a result of an overabundance of inorganic Hg in this tissue (Bosch et al. 2016). However, the two muscle types did not have significantly different MeHg concentrations (Bosch et al. 2016). The increased levels of inorganic Hg in red muscle of the yellowfin tuna is potentially caused by faster rates of red muscle fiber development compared to white muscle, to sustain their swimming patterns (Bosch et al. 2016). It is possible that either these increased levels of inorganic Hg do not exist within bonnethead sharks, or red muscle fiber development is different in this species. However, further testing of the speciation of Hg in these tissues, ideally including a variety of shark species, is needed to confirm this hypothesis.

Analogous to our findings for muscle tissue, neither species showed a significant difference in THg concentrations among fin inner core samples (excluding the upper caudal lobe), which provides useful information on the exposure of humans to Hg through the consumption of shark fins (i.e., shark fin soup). A recent study showed that MeHg accounts for $62 \pm 22\%$ of THg in dried shark fins (Nalluri et al. 2014). Nalluri et al. (2014) also found that while shark fin soup represents a potentially important source of MeHg in humans, other seafood products (i.e., muscle tissue of top predators) contain much higher concentrations of THg and

could result in higher exposure. The most commonly recorded, and likely consumed, fin positions or types in the Hong Kong fin market (worlds' largest fin market) are the first dorsal, lower caudal lobe and pectoral fins (Clarke *et al.* 2004). Based on our results from fin biopsies, consumption of any of these would provide similar levels of THg exposure. However, the IUCL consistently had higher concentrations of THg, likely due to the comparatively increased amounts of muscle we observed in the inner regions of the caudal fin in relation to other fins. Consumption of IUCL tissue may result in increased Hg exposure compared to the other fins, however the ceratotrichia (i.e., cartilaginous rays) are the main fin component used in shark fin soup compared to muscle and other tissue types found within shark fins (Rose, 1996). The trailing fin margins had proportionately higher concentrations of THg than fin inner core samples for both species. The fin inner cores for the first dorsal fin, pectoral fins, and lower lobe of the caudal fin are composed primarily of ceratotrichia (i.e., cartilaginous fibers) that give structure to the fins, with comparatively less muscle, while the respective fin trailing margins are composed almost entirely of skin and connective tissue. The variation in tissue composition could explain the variations seen in THg concentrations between these two regions of the fin, with skin containing higher concentrations of THg. Total Hg exposure could be reduced by the common practice of removing skin when preparing fins for consumption, although only by a marginal amount (Nalluri *et al.* 2014). Therefore, THg concentrations for fin inner core samples from this study, which still had the skin attached, could be overestimates of possible exposure to people consuming dishes like shark fin soup. With or without skin, our results for the shark species and life stages we tested, support Nalluri *et al.*'s (2014) finding that exposure to Hg from

consumption of fins would be substantially less than from consumption of other shark products (i.e., muscle or liver).

Variations in life-history characteristics (i.e., prey type) generally result in different Hg concentrations between shark species (Storelli *et al.* 2002). Therefore, we expected Hg concentrations in silky sharks to be higher than bonnetheads due to their higher trophic level. However, the majority of tissues examined between the bonnethead and silky sharks in this study exhibited similar THg concentrations. This result may stem from the majority of our samples coming from large adult bonnetheads and juvenile silky sharks. It is likely that these two groups feed at similar trophic levels and thus had similar uptake of Hg.

A primary objective of this study was to determine if THg concentrations in certain tissue samples could accurately predict THg concentrations in other tissues. Based on linear regressions, the use of THg concentrations from the dorsal axial muscle tissue outperformed the dorsal fin trailing margin for estimating THg in the IUCL, liver, kidney, spleen, heart, and epigonal organ for bonnethead sharks. For silky sharks, dorsal axial muscle THg concentrations were better at estimating THg concentrations in the fin inner core, IUCL, liver, kidney, and spleen, compared to the dorsal fin trailing margin. The THg concentrations for the dorsal fin trailing margin yielded slightly more accurate predictions of THg concentrations in fin inner cores (first dorsal, pectoral, lower and upper caudal lobes) within bonnethead sharks. However, the dorsal fin trailing margin THg concentrations of silky sharks could not significantly predict THg concentrations in fin inner cores. Overall, for silky sharks, the fin trailing margins THg concentrations did not explain much of the variation in other tissue samples. Total Hg concentrations from dorsal fin trailing margins were also able to significantly predict THg concentrations in dorsal axial muscle for bonnethead sharks.

To determine THg concentrations throughout a shark's body for either of these species, we recommend collecting muscle biopsy samples. This allows not only for the most accurate values and estimations for a majority of other tissue types, but also causes minimal harm to the animal or its potentially harvestable products. In cases where THg concentrations in the fin inner cores are of primary interest, fin trailing margin samples could be used, as determined in bonnethead sharks. This may be useful in instances where researchers are interested in THg concentrations in shark fins at fish markets, where only fins are available and vendors will not allow them to damage the product (fin) with biopsy punches.

Overall, relationships between either dorsal fin trailing margins or dorsal axial muscle and the other tissue types were stronger for bonnethead compared to silky sharks, which could be attributed to the increased sample size for bonnethead sharks. Dorsal axial muscle THg concentrations were able to significantly predict THg concentrations in liver tissue for both species. Previous studies on teleost fish found that Hg exposure, particularly MeHg, causes disruption of normal morphology within the liver including: degeneration of hepatocytes, alterations in cytoplasmic organization, and necrosis of the liver (Oliveira Ribeiro *et al.* 2002; Raldúa *et al.* 2007). Due to the importance of the liver in eliminating potentially harmful trace elements (i.e. Hg) from the body, THg concentrations in muscle tissue could not only be used to estimate liver THg, but also be an indicator of overall shark health. Additional sampling for both of these species and the inclusion of new shark species would help to verify if this relationship and the others reported here remain constant not only within a species but between them as well.

The potential impacts of Hg on the health of already declining shark populations are not well understood. However, Hg exposure has been shown to result in numerous negative impacts

to other fish species, up to and including mortality (Jagoe *et al.* 1996; Wiener & Spry 1996; Oliveira Ribeiro *et al.* 1996, 2000; Matta *et al.* 2001; Hammerschmidt *et al.* 2002; Drevnick & Sandheinrich 2003; Klaper *et al.* 2006; Basu *et al.* 2007, 2009; Raldúa *et al.* 2007; Scheuhammer *et al.* 2008; Crump & Trudeau 2009; Adams *et al.* 2010). Testing for Hg from muscle biopsy punch samples could provide both baseline estimates for Hg contamination in shark populations and information about Hg contamination within the sharks' ecosystem. The ability to estimate Hg concentrations throughout a shark's body from less invasive samples would also allow for better monitoring of Hg contamination in sharks, a larger variety of their products, and their environment. Mercury concentrations, even within the same species, can vary greatly depending on size (age) (Walker 1976; Hornug *et al.* 1993; Hueter *et al.* 1995; Lacerda *et al.* 2000; Endo *et al.* 2008), sex (Pethybridge *et al.* 2010), season (Escobar-Sánchez *et al.* 2010), habitat type and geographic location (Hornug *et al.* 1993; McMeans *et al.* 2007). However, this sampling method could provide a less invasive mechanism to compare variations in contamination and uptake of Hg between locations for individuals from the same species with similar characteristics (i.e. size, sex, etc.). This could be of importance for both understanding the health of shark populations and for humans that are consuming products of sharks from different locations.

Conclusion

Total mercury concentrations for both shark species analyzed were found to be highest in muscle tissue, followed by either liver (silky shark) or kidney (bonnethead shark) tissue, and lowest in fins. Distribution of THg between muscle regions was not significantly different, indicating that THg concentrations from any muscle location are representative of all muscle regions. Overall, muscle tissue was a better predictor of THg concentrations in other tissues than

fin trailing margins. Our results are not only important for monitoring potential human exposure to Hg contamination via shark products (e.g., meat), but also in estimating Hg exposure in shark populations and the possible health implications.

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455 Table 1. Total mercury (THg) concentrations in muscle regions, organs, and fins of bonnethead
456 and silky sharks with standard deviations.

		Bonnethead Shark		Silky Shark	
Muscle		n	Mean THg (ppm dry wt)	n	Mean THg (ppm dry wt)
	Dorsal Axial	42	2.37 ± 1.52	13	3.09 ± 2.38
	Interdorsal	14	2.78 ± 1.85	9	2.55 ± 1.61
	Cranial	14	2.95 ± 1.94	9	2.52 ± 1.56
	Mid-abdominal	14	2.42 ± 1.74	9	2.14 ± 1.24
	Mid-flank	14	2.97 ± 2.18	8	2.30 ± 1.65
	Pre-pectoral	14	3.10 ± 2.12	9	2.61 ± 1.46
	Caudal Peduncle (White)	14	3.01 ± 2.02	9	2.67 ± 1.46
	Caudal Peduncle (Red)	14	2.53 ± 1.58	0	NA
Organs					
	Liver	42	1.82 ± 2.94	13	2.10 ± 3.64
	Kidney	14	2.15 ± 2.41	9	0.66 ± 0.44
	Spleen	14	1.43 ± 2.07	9	0.77 ± 0.84
	Heart	14	1.62 ± 1.02	8	1.06 ± 0.52
	Epigonal Organ	14	0.86 ± 0.90	9	1.43 ± 2.23
Fin Inner Cores					
	First Dorsal	18	0.04 ± 0.03	8	0.02 ± 0.01
	Left Pectoral	18	0.04 ± 0.03	8	0.02 ± 0.01
	Right Pectoral	18	0.04 ± 0.02	8	0.02 ± 0.01
	Upper Caudal Lobe (IUCL)	18	0.68 ± 0.50	8	0.98 ± 0.82
	Lower Caudal Lobe	14	0.06 ± 0.08	0	NA
Fin trailing margin					
	First Dorsal	17	0.07 ± 0.06	8	0.05 ± 0.03
	Left Pectoral	18	0.07 ± 0.05	8	0.03 ± 0.01
	Right Pectoral	18	0.07 ± 0.05	8	0.03 ± 0.01
	Upper Caudal Lobe	18	0.10 ± 0.09	8	0.04 ± 0.02
	Lower Caudal Lobe	14	0.08 ± 0.07	0	NA

458 Table 2. Correlations of THg concentrations in muscle tissues for bonnethead and silky sharks.

Muscle		Interdorsal	Cranial	Mid- Abdominal	Mid- Flank	Pre- pectoral	White Caudal	Red Caudal
Dorsal	<i>Bonnethead</i>	0.99*	0.98*	0.93*	0.98*	0.96*	0.95*	0.99*
	<i>Silky</i>	0.98*	0.98*	0.94*	0.98*	0.98*	1*	-
Interdorsal	<i>Bonnethead</i>	-	0.95*	0.91*	0.96*	0.97*	0.96*	0.99*
	<i>Silky</i>	-	0.99*	0.94*	0.99*	0.97*	0.97*	-
Cranial	<i>Bonnethead</i>	-	-	0.95*	0.98*	0.96*	0.92*	0.95*
	<i>Silky</i>	-	-	0.95*	1*	0.98*	0.98*	-
Mid-Abdominal	<i>Bonnethead</i>	-	-	-	0.98*	0.94*	0.95*	0.89*
	<i>Silky</i>	-	-	-	0.97*	0.94*	0.95*	-
Mid-Flank	<i>Bonnethead</i>	-	-	-	-	0.96*	0.96*	0.95*
	<i>Silky</i>	-	-	-	-	0.97*	0.98*	-
Pre-pectoral	<i>Bonnethead</i>	-	-	-	-	-	0.95*	0.97*
	<i>Silky</i>	-	-	-	-	-	0.98*	-
White Caudal	<i>Bonnethead</i>	-	-	-	-	-	-	0.95*
	<i>Silky</i>	-	-	-	-	-	-	-

* Indicates P-value <0.001

460 Table 3. Correlations of THg concentrations in organs for Bonnethead and Silky sharks.

Organ		Kidney	Heart	Spleen	Epigonal Organ
Liver	<i>Bonnethead</i>	0.94**	0.86**	0.95**	0.81**
	<i>Silky</i>	0.57	0.75*	0.51	0.27
Kidney	<i>Bonnethead</i>	-	0.93**	0.93**	0.93**
	<i>Silky</i>	-	0.58	0.54	-0.14
Heart	<i>Bonnethead</i>	-	-	0.82**	0.88**
	<i>Silky</i>	-	-	0.61	0.07
Spleen	<i>Bonnethead</i>	-	-	-	0.89**
	<i>Silky</i>	-	-	-	0.52

* Indicates P-value <0.05, ** Indicates P-value < 0.001

Table 4. Correlations of THg concentrations in fins for bonnethead and silky sharks.

Fin			Right Pectoral	Left Pectoral	Lower Caudal	Upper Caudal
Fin Trailing Margin						
Dorsal	<i>Bonnethead</i>		0.95*	0.94*	0.96*	0.87*
	<i>Silky</i>		0.27	0.24	-	0.24
Right Pectoral	<i>Bonnethead</i>		-	0.96*	0.94*	0.84*
	<i>Silky</i>		-	0.87*	-	0.75*
Left Pectoral	<i>Bonnethead</i>		-	-	0.95*	0.85*
	<i>Silky</i>		-	-	-	0.85*
Lower Caudal	<i>Bonnethead</i>		-	-	-	0.95*
	<i>Silky</i>		-	-	-	-
Fin Inner Core						
Dorsal	<i>Bonnethead</i>		0.95*	0.95*	0.84*	0.74*
	<i>Silky</i>		0.9*	0.79*	-	0.6
Right Pectoral	<i>Bonnethead</i>		-	0.97*	0.74*	0.71*
	<i>Silky</i>		-	0.82*	-	0.56
Left Pectoral	<i>Bonnethead</i>		-	-	0.77*	0.73*
	<i>Silky</i>		-	-	-	0.29
Lower Caudal	<i>Bonnethead</i>		-	-	-	0.44
	<i>Silky</i>		-	-	-	-

* Indicates P-value <0.05

Table 5. Regression results showing the relationships of the fin trailing margin of the first dorsal fin (from the center of the fin) and dorsal axial muscle tissue to the other sampled tissue types for bonnethead and silky sharks.

			n	F	p	R ²	log y = log x + b
Fin trailing margin	Muscle	<i>Bonnethead</i>	16	42.18	<0.001*	0.75	y = 0.87x + 3.42
		<i>Silky</i>	8	1.43	0.280	0.19	y = 0.45x + 2.12
	Fin Inner Core	<i>Bonnethead</i>	17	135.20	<0.001*	0.90	y = 0.88x - 0.93
		<i>Silky</i>	8	0.45	0.530	0.07	y = 0.22x - 3.28
	IUCL	<i>Bonnethead</i>	17	24.77	0.001*	0.62	y = 0.71x + 1.49
		<i>Silky</i>	8	2.13	0.200	0.26	y = 0.71x + 1.86
	Liver	<i>Bonnethead</i>	16	32.40	<0.001*	0.70	y = 1.55x + 4.87
		<i>Silky</i>	8	0.74	0.420	0.11	y = 0.59x + 1.74
	Kidney	<i>Bonnethead</i>	13	45.87	<0.001*	0.81	y = 1.50x + 4.76
		<i>Silky</i>	7	0.17	0.690	0.03	y = 0.22x + 0.08
	Spleen	<i>Bonnethead</i>	13	33.24	<0.001*	0.75	y = 1.52x + 4.27
		<i>Silky</i>	8	1.07	0.340	0.15	y = 0.75x + 1.52
	Heart	<i>Bonnethead</i>	13	44.42	<0.001*	0.80	y = 0.75x + 2.66
		<i>Silky</i>	8	7.04	0.05*	0.59	y = 0.74x + 2.30
	Epigonal Organ	<i>Bonnethead</i>	13	60.76	<0.001*	0.85	y = 1.27x + 3.31
		<i>Silky</i>	8	0.00	0.960	0.00	y = 0.04x - 0.67
Muscle	Fin Inner Core	<i>Bonnethead</i>	17	21.82	<0.001*	0.59	y = 0.70x - 4.13
		<i>Silky</i>	8	16.17	0.007*	0.73	y = 0.69x - 4.44
	IUCL	<i>Bonnethead</i>	17	38.78	<0.001*	0.72	y = 0.75x - 1.24
		<i>Silky</i>	8	12.06	0.013*	0.67	y = 1.10x - 1.13
	Liver	<i>Bonnethead</i>	42	99.08	<0.001*	0.71	y = 1.65x - 1.31
		<i>Silky</i>	13	50.54	<0.001*	0.82	y = 1.65x - 1.55
		<i>Species combined</i>	55	144.90	<0.001*	0.73	y = 1.63x - 1.35
	Kidney	<i>Bonnethead</i>	14	191.00	<0.001*	0.94	y = 1.63x - 1.21
		<i>Silky</i>	9	13.52	0.008*	0.66	y = 1.03x - 1.34
	Spleen	<i>Bonnethead</i>	14	96.69	<0.001*	0.89	y = 1.69x - 1.83
		<i>Silky</i>	9	16.73	0.005*	0.71	y = 1.54x - 1.83
	Heart	<i>Bonnethead</i>	14	298.80	<0.001*	0.96	y = 0.86x - 0.39
		<i>Silky</i>	8	7.40	0.035*	0.55	y = 0.80x - 0.52
	Epigonal Organ	<i>Bonnethead</i>	14	107.20	<0.001*	0.90	y = 1.37x - 1.77
		<i>Silky</i>	9	0.53	0.489	0.07	y = 0.58x - 0.96

*Indicates significant relationship between tissue types.

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479 Figure 1. Muscle regions sampled from sharks along with inner core and trailing margin
 480 sampling locations for caudal fin (left insert) and the dorsal and pectoral fins (right insert). Two
 481 types of caudal peduncle muscle were sampled from the same location where one muscle
 482 overlays the other. A) caudal peduncle white and red muscle, B) interdorsal, C) mid-flank, D)
 483 dorsal axial, E) cranial, F) mid-abdominal, G) pre-pectoral.
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A)

B)

C)

D)

Figure 2. Linear relationships of THg concentrations in first dorsal fin trailing margin and A) first dorsal fin inner core, B) dorsal axial muscle, C) liver, and D) kidney of the bonnethead shark.

A)

B)

C)

D)

Figure 3. Linear relationships of THg concentrations in dorsal axial muscle and A) liver, B) first dorsal fin inner core, C) kidney, D) heart for bonnethead($\circ$) and silky($\blacktriangle$) sharks, with 95% confidence intervals (bold regression and confidence interval lines = silky shark).

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812 Figure Legend

813 Figure 1. Muscle sampling regions: (A) caudal peduncle (red and white), (B) interdorsal, (C)
814 mid-flank, (D) dorsal axial target region, (E) cranial, (F) mid-abdominal, (G) pre-pectoral. Fin
815 sampling regions showing sampling locations for the upper and lower lobe of the caudal fin (left)
816 and the dorsal and pectoral fins (right).

817 Figure 2. Linear relationships of THg concentrations in the first dorsal fin trailing margin and the
818 first dorsal fin inner core (a), dorsal axial muscle (b), liver (c), kidney(d) for bonnethead sharks.

819 Figure 3: Linear relationships of THg concentrations in dorsal axial muscle and liver (a), fin
820 inner core (b), kidney (c), and heart (d) tissue for bonnethead (open circle) and silky (closed
821 triangle) sharks, with 95% confidence intervals (bold regression and confidence interval lines =
822 silky shark).

823