MERCURY CONTAMINATION IN FRESHWATER TURTLES OF EASTERN OKLAHOMA: EVALUATION OF NON-DESTRUCTIVE SAMPLING TECHNIQUES

By

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Abstract: Recent studies in Oklahoma have found levels of mercury (Hg) contamination in fish that exceed safe consumption limits in several lakes. This study investigates the degree of Hg pollution in aquatic turtle species of Oklahoma that are used most commonly for human consumption. Turtles have been used as monitors of chemical contaminants in aquatic environments in both freshwater and marine habitats routinely. These studies are often complicated by the requirement to sacrifice long-lived and slowly reproducing species. A need for a nonlethal routine monitoring technique has been recognized due to a decline in turtle populations. Spiny softshell (*Apalone spinifera)*, common snapping turtle (*Chelydra serpentina)*, and red-eared slider (*Trachemys scripta)* are the most commonly harvested turtles in Oklahoma and thus the indicator species chosen for analysis. Multiple tissue types (muscle, liver, claw, and scute) were collected from 72 turtles in eastern Oklahoma during the summers of 2010 and 2011 from 10 water bodies. Softshells had the highest Hg concentrations (0.04-0.72 mg kg^{-1}), followed by snapping turtles (0.03-0.30 mg kg^{-1}) and sliders (0.01-0.20 mg kg^{-1}). Based on the USEPA's food consumption guidelines, seven of the ten sites had average Hg concentrations warranting consumption limits for at least one species. Average muscle Hg concentrations among sites were significantly different ($p<0.01$). No significant relationships were found between Hg burden and size, sex, or age. Liver/muscle ratios indicated current contamination. In addition, claw and scute were removed from each turtle to test the validity of using non-destructive (external) tissues as an alternative to lethal/destructive sampling of muscle and liver. Claw was the best overall predictor for muscle Hg burdens when comparing across species ($R^2=0.79$) with similar slopes between hard and softshell turtles (slopes=0.087 and 0.099). Scute was not as reliable when all species were combined ($R^2=0.41$). However, when turtles were separated between hard and softshelled species, relationships between Hg concentrations in scute correlated well with concentrations found in muscle ($R^2=0.84$ and 0.83). Continuous monitoring programs are recommended to further protect human health and to track changes in contamination levels. These programs can be completed using the non-destructive tissue techniques and the corresponding linear regression models formulated here.

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CHAPTER I

MERCURY CONTAMINATION IN FRESHWATER TURTLES OF EASTERN OKLAHOMA: EVALUATION OF NON-DESTRUCTIVE SAMPLING TECHNIQUES

Mercury (Hg) is persistent, bioaccumulative and is known to biomagnify through aquatic food chains, causing deleterious environmental effects. Due to the volatile nature of mercury, it is distributed primarily through atmospheric transport (Krabbenhoft 2013). Hg is released into the environment in the form of elemental mercury (Hg^0) from anthropogenic sources such as fossil-fuel fired power plants, metals manufacturing facilities, cement plants, chemical production facilities, and medical incinerators. Elemental Hg can be oxidized into the mercuric form (Hg II) and further reduced to the toxic form of methylmercury (MeHg) by methylating bacteria in sediments, allowing bioaccumulation to occur (Wiener and Spry 2006; Sprovieri 2009; Pirrone 2010). Release of mercury into the atmosphere also originates from natural sources (Rasmussen 1994) such as soil and the ocean, which receive Hg from other natural sources (e.g., volcanoes and geothermal activity) but also re-emit previously deposited anthropogenic Hg (Mason 2009).

Since the primary mode of transportation is through atmospheric deposition, mercury is widespread throughout many parts of the world and has an estimated global emission of nearly 7527 Mg per year (Pirrone et al. 2010). The ubiquitous distribution of Hg is likely caused by the long atmospheric residence time of Hg^0 with an average half-life of approximately one year (Hall 1995). In fact, Hg has been detected and found to bioaccumulate even in remote places where no anthropogenic influences or point sources exist (Fitzgerald 1998; AMAP 2005; Huang et al. 2012; Kirk et al. 2012; Riget et al. 2011).

Toxicokinetic studies show nearly 100% of MeHg from fish consumption is absorbed in the gastrointestinal tract of humans (Mergler 2007). The consumption of high levels of mercury can lead to liver, kidney, and nervous system damage. Mercury consumption is most damaging to fetuses and small children, adversely affecting the developing nervous system. Passage across the blood-brain barrier occurs in most vertebrates and MeHg accumulates in the cerebellum and cerebral cortex, causing damage in the central nervous system (Holland and Turekian 2004). Children exposed to MeHg in utero have lower performance on cognitive tests (i.e., attention, memory, motor functions, and language) and poorer development and neurologic function in general (Crump 1998). Adults subjected to even low levels of Hg can exhibit symptoms such as marked negative effects in fine motor function, memory, and attention (Zahir 2005). Chronic exposure to even lower levels of Hg contamination through ingestion can cause long-term distal paresthesias in the arms, legs, and lips (Ekino et al. 2007).

Due to the ubiquity of contamination, mercury monitoring has been conducted throughout all 7 continents, leading to food consumption advisories on aquatic vertebrates with an emphasis on higher trophic level fish (Allen-Gil 1995; Chumchal et al. 2011; Lake

2009). Within our study area, the Oklahoma Department of Environmental Quality (OKDEQ) found Hg contamination in fish throughout Oklahoma water bodies, with at least 16 lakes exceeding safe consumption levels (OKDEQ 2010). Despite the focus on fish, mercury is known to accumulate in other aquatic vertebrates including aquatic turtles (Eisler 1987; Golet and Haines; 2001; Green et al. 2010; Lake 2009; Schneider 2010; Turnquist et al. 2011). The three most common Hg sinks are surface soils, water column, and benthic sediment (UNEP 2002). Aquatic turtles are exposed to at least one of these sinks during each stage of their life cycle. The level of mercury in aquatic biota is determined by several factors. These factors include the amount entering the system, the system's efficiency to convert inorganic mercury to methylmercury, and the size and interactions of the food web (Krabbenhoft 2013). Methylmercury concentrations can increase by a factor of 10 between each successive trophic level (Alpers and Hunerlach 2000). According to Morel et al. (1998), the percent of total mercury comprised by MeHg is approximately 10% in the water column, 15% in phytoplankton, 30% in zooplankton, and >95% in fish, indicating the increased affinity of MeHg to biomagnify to a greater extent than other forms of mercury. Turtles are commonly found in backwater habitats that have anoxic sediments and many are piscivorous, both of which are factors that could lead to higher bioaccumulation potential (Chumchal 2011). Further, methylation typically occurs at the interface of oxic and anoxic conditions, most notably near the top of the sediment where the oxygen gradient is steep (Nordberg et al. 2007). Bergeron et al. (2007) measured both MeHg and total Hg (THg) in the blood of four turtle species. MeHg ranged from 70-100 % of the THg in all species studied and across all sampled sites (reference and contaminated).

In Oklahoma and throughout many regions of the world, people use turtles as a food source. In fact, turtles are the most widely exploited reptiles as a human food source

(Klemens and Thorbjarnarson 1994). Turtle harvesting in Oklahoma and several other states within the US has been so extensive that commercial turtle harvest has been banned on public lands and waters due to exploitation (e.g., export to Asian markets) (Miller 2008). The majority of mercury studies have focused on fish due to the high level of human consumption and easy access to tissue samples, even though many other taxa that occupy surface waters can accumulate mercury. Turtles are not routinely monitored for contaminants, despite their predominately aquatic life style, prominent presence in most water bodies, higher trophic position, and thus relative potential to be as contaminated as fish. Several studies have found Hg concentrations in turtle tissue exceeding safe consumption levels (Golet and Haines 2001; Green et al. 2010; Schneider et al. 2010). For example, Golet and Haines (2001) analyzed muscle tissue from 26 adult snapping turtles with tissue concentrations ranging between 0.05 and 0.50 $\mu g\ g^{-1}$ Hg. The USEPA supports a 0.3 $\mu g\ g^{-1}$ Hg screening value for fish consumption, with higher values warranting a level of concern, or direct action (U.S. Environmental Protection Agency 2006).

Turtle monitoring can be problematic due to the extensive efforts required to collect turtle tissue as compared to fish, along with the ethical concerns of sacrificing long-lived, slow-reproducing vertebrate animals that are facing population declines worldwide. Thus, non-lethal means of mercury determination have been suggested. A few studies have used scute scrapings as a means of nonlethal sampling for Hg analysis (Day 2005; Golet and Haines 2001; Lake 2009; Turnquist 2011). Golet and Haines (2001) recorded a strong relationship between scute and front shoulder muscle (R^2=0.71) in common snapping turtles. Day (2005) examined loggerhead sea turtles and determined that scute tissue accurately reflected Hg concentrations in liver and muscle tissues (R^2=0.956 and 0.983, respectively). In

addition, Lake (2009) found a positive relationship of Hg in claw clippings and tail muscle (R^2=0.70) of common snapping turtles. Green (2010) however, found no strong correlations between non-lethally sampled external tissues (i.e., scute and claw) and internal, edible tissues (i.e., muscle, liver, and kidney). Most of these previous studies evaluated only the relationship of one external tissue within a group of individuals, lacked both softshell and hardshell turtle species comparisons, evaluated a limited number of individuals and species, or were within a specific region or an unknown water body. Recent research by Hopkins et al. (2013; 2013a) has demonstrated the validity of using claw as a non-lethal approach to predicting internal Hg contamination as well as contamination levels in eggs. Hopkins et al. (2013) refer to these techniques as non-destructive sampling.

The objectives of this study were to build on previous studies by first determining the mercury levels in turtles of eastern Oklahoma to further ascertain whether mercury-monitoring programs should be instituted for food consumption guidelines. Our second objective was to evaluate if external, non-destructive sampling techniques for Hg determination were of value for estimating concentrations in internal, edible tissues. We expanded upon previous studies by including three turtle species (one softshell and two hardshell spp.), including two types of external tissue (scute and claw), and working in a region that has not been previously studied (Oklahoma, USA). The three species tested are native to eastern OK and were chosen based on consumption preference and availability. The common snapping turtle (*Chelydra serpentina)* and the spiny softshell (*Apalone spinifera)* are commonly harvested species, whereas the red-eared slider (*Trachemys scripta)* is an abundant, yet less harvested species. According to Johansen (2011), these three species are the most commonly harvested species in the state of Oklahoma. Our approach was to determine mercury concentrations in muscle tissue, the primary edible tissue, and scute and

claw, which are easily obtainable keratinized material likely high in mercury (Golet and Haines 2001; Day et al. 2005; Green 2010; Hopkins et al. 2013). In addition, we measured mercury concentrations in liver, a possible edible tissue, but this tissue was chosen more for its indicative properties. Finally, we also compared liver/muscle ratios at different geographic sites as bioindicators of current Hg uptake. Havelkova et al. (2008) found that fish downstream of an Hg contamination site had higher liver/muscle Hg ratios than fish upstream of the contamination at a reference site. There is presently an insufficient amount of literature regarding liver/muscle ratios in turtles.

METHODS

Materials

Each acid and reagent used was either trace metal or mercury grade and was purchased from VWR Scientific (Radnor, PA). Reagent grade water was obtained from a Milli-Q filtration system. All tissues were removed with stainless steel dissection utensils that were acid washed with 10 % HNO_3 followed by a reagent grade water rinse between each tissue type and each turtle to avoid cross contamination. All other lab materials (e.g., forceps, scissors, dissection pan, and storage containers) were acid washed and surfaces were cleaned with ammonium hydroxide (i.e., Windex®). Windex® (S.C. Johnson and Sons, Inc., Racine, WI,) is formulated without Hg and also neutralizes weak acid residues that could cause contamination or become hazardous.

Site Selection

A total of ten sites were selected across eastern Oklahoma for turtle collection (Figure 1). The majority of these sites were chosen in conjunction with a broader study focused on

surveying the freshwater turtle population in the state during the summers of 2010 and 2011 (Johansen 2011). Seven of the ten sites were therefore chosen from 35 pre-determined sites to comprise a wide latitudinal distribution. Additional sites were incorporated to increase sample size and increase our chances of having some turtles with higher mercury concentrations. Boomer Lake was selected based on the results of another study indicating elevated fish Hg concentrations (pers. comm. Randy Parham, Oklahoma Department of Environmental Quality). The two remaining sites were chosen from an unrelated research project monitoring catfish populations in Oklahoma, where turtles were bycatch (Stewart and Long 2012).

Turtle Collection

All turtles were trapped during the summers of 2010 and 2011 according to previously approved protocols described in the broader turtle population survey (Johansen 2011). *A. spinifera, C. serpentina,* and *T. scripta* were all sampled using hoop-nets baited with local fish, cheese bait, and/or chicken liver. Pre-determined sites were baited each evening and checked the following morning for three consecutive trap nights. However, the sample of bycatch turtles from the catfish study were baited and checked every three days. Standard measurements were taken in the field and then again in the laboratory including mass, maximum carapace length, maximum plastron length, and also sex determination. Sex determination was completed visually based on distance of cloaca from the tail and length of front claws (pers. comm. Eric Johansen). Age determination based on quantifying scute annuli is not reliable (Powders, 1978). Therefore, ages were categorized as juvenile or adult based on plastron length of < 100 mm or >100 mm, respectively. Day et al. (2005) found correlations with these physical characteristics and Hg tissue burden in *Caretta caretta*.

The targeted sample size for each site was six individuals of each species to provide sample sizes large enough for statistical analyses. However, due to varying habitats, a decline in population (Johansen 2011), and variation in trapping methods, most sites had fewer turtles collected, especially for *C. serpentina* and *A. spinifera*. All live turtles were returned to the laboratory within 48 hours in an ice bath to achieve a metabolically depressed state (i.e., torpor). To reduce any additional Hg detoxification before analysis, a state of torpor was desired and thus offered a more accurate food consumption guideline. In torpor, turtles can lower their metabolic activity as much as 90%, causing a drastic reduction in cardiac activity (Stecyk, 2004). Upon arrival, turtles were sacrificed and dissected promptly. All turtles (n=73) were euthanized following guidelines approved by Oklahoma State University's Animal Care and Use Protocol (ACUP) No: AS-09-4. Tissues removed for analysis included femoral muscle, liver, carapacial scute, and claw clippings. To remove scute in *C. serpentina* and *T. scripta,* a notch was removed from the dorsolateral edge of the shell and the scute was scraped from the bony shell with a scalpel. Scute was very carefully scraped from the same region in *A. spinifera* using a scalpel. Extra caution was taken to remove only keratinized material from *A. spinifera*, as this layer is very thin. Tissue samples were either immediately digested and analyzed or vacuum-sealed in plastic bags and placed in -20° C storage until further analysis. All tissues were analyzed by January 2012.

Mercury Analysis

EPA Method 245.6 Revision 2.3, *Determination of mercury in tissues by cold vapor atomic absorption spectrometry* (U.S. Environmental Protection Agency 1991), was the method used for all turtle tissue acid digestion and analysis. Total mercury was measured and only slight modifications (i.e., increased aqua regia ratio and sample weight) were made to increase sensitivity and optimize results (Florida Department of Environmental Quality

2012). All results are reported as total Hg. Approximately 0.25 g of muscle tissue and liver from each turtle was analyzed to determine consumption guidelines. However, scute and claw were not always available in this quantity and ranged between 0.05 and 0.25 g. Each analytical run consisted of 20 samples or less and contained at least one laboratory blank, laboratory spike consisting of reagents fortified with 5 μg L^{-1} $HgCl_2$, five calibration solutions and a standard reference material (SRM), 1946 Lake Superior Fish Tissue, for quality control. All samples were digested with a 5:2 H_2SO_4 : HNO_3 aqua regia for 45 minutes at 90° C in a hot water bath to attain an aqueous state. After cooling, a 10-mL aliquot of 5% potassium permanganate ($KMNO_4$) and a 4-mL aliquot of 5% potassium persulfate ($K_2S_2O_8$) solution were added to each sample. Additional 1-mL aliquots of $KMNO_4$ were added if a state of oxidation (i.e., indicated by a purple aqueous solution) was not maintained (i.e., solution became clear) for 15 minutes upon initial application. The samples were returned to the hot water bath for 90 minutes at 90° C. Hydroxylamine hydrochloride (NH_2 • HCl; 6 ml of 12%), a strong reducing agent, was added at the end of acid digestion to reduce any excess $KMNO_4$, which could cause interferences in absorbance. Samples were cooled, brought to a final volume of 50 mL with reagent grade water and an immediate analysis was conducted. Samples were analyzed by cold vapor atomic absorption spectrometry (CVAAS) using a QuickTrace™ M-6100 Mercury Analyzer (CETAC, Omaha, NE). During analysis, each sample was reduced with 10% stannous chloride ($SnCl_2$) in a 7 % HCl solution before absorbance was measured at 243.7 nm with a single wavelength to detect total mercury.

Daily calibrations were strongly linear ($R^2 \geq 0.98$). Continuing calibration standards were always within 15% of expected values. Laboratory spikes and standard reference material were always within 15% of expected values.

Statistical Analysis

All statistical analyses were performed using AnalystSoft Inc., StatPlus:mac software. Mean Hg burden of all tissues with associated standard error are provided for each species. One-way Analysis of Variance (ANOVA) followed by a Tukey post hoc was used to determine significant differences in contaminant levels ($p<0.05$).

Food consumption advisories were determined by inserting our calculated mean muscle concentration from each site into the fish consumption guidelines equations provided by the USEPA (U.S. Environmental Protection Agency 2012). These consumption recommendations are based on an average human body weight of 70 kg and an average meal size of 8 oz.

To determine if average muscle Hg burdens were different among sites, a one-way Analysis of Variance (ANOVA) was used followed by a Tukey post hoc test. Secondarily, average muscle Hg concentration of only *T. scripta* was tested for significant differences among sites. *T. scripta* alone was viewed to remove species biases since this species dominated the number of turtles sampled from most sites and provided consistent sample numbers at those sites. Sample numbers and presence of the *A. spinifera* and *C. serpentina* species were variable at each site.

To test which external tissues were the best predictor of the Hg burden in internal tissues, the following linear models were fitted. Each external tissue was compared to each internal tissue with a linear regression. These regressions were calculated by species, not by site. For each linear model the sites were combined to represent the population. Secondarily,

we separated the sample into softshell and hardshell categories and further into each species. Linear models with R^2 values ≥0.50 and p-values ≤0.05 were considered reliable.

Linear models were used to test whether mass and maximum plastron length (MPL) predicted Hg burden for each species. R^2 values ≥0.50 with p-values ≤0.05 were considered reliable.

An ANOVA was used to determine if there were differences between average muscle Hg concentrations among the sexes. A Tukey-Kramer post hoc test was performed to determine significance among subgroups.

The liver/muscle ratio was also calculated for each site using the geometric mean, to reduce the effect of outliers, of each tissue type (Kruzikova et al. 2013). Only *T. scripta* were included in this analysis. Sites where no *T. scripta* were collected were removed from this section.

RESULTS

Mercury Concentrations in Edible Tissues

Detectable levels of mercury were found within muscle and liver tissues (i.e., edible tissues) in all turtles from all sites. The overall average muscle Hg concentration was 0.09 mg kg^{-1}. The mean muscle values for each species were 0.23 in *A. spinifera*, 0.11 in *C. serpentina*, and 0.05 mg kg^{-1} in *T. scripta*. *A. spinifera* had more than twice the mercury concentration in muscle compared to *C. serpentina* and *T. scripta,* which represented a

significant difference from both ($p=0.02$ and $p<<0.001$; Figure 2). Although *C. serpentina* tended to have higher Hg concentrations in muscle compared to *T. scripta*, the difference was not statistically significant ($p=0.15$).

The average muscle Hg concentration for each species was below the USEPA action level of 0.3 mg kg^{-1}. However, for individual turtles at some sites, concentrations exceeded this level. Multiple individual turtles (43%) of *A. spinifera* had contamination levels in muscle tissues exceeding safe consumption limits with a maximum of 0.72 mg kg^{-1}. Maximum Hg concentrations in *C. serpentina* (0.30 mg kg^{-1}) and *T. scripta* (0.23 mg kg^{-1}) were at or below USEPA action levels (Table 1).

When data from all species were pooled, the average level of contamination was significantly different among all sites ($p<0.01$). Greenleaf Lake had the highest average Hg level in muscle and exceeded the USEPA action level at 0.34 mg kg-^{1}. The Greenleaf Lake average was nearly 20 times greater than levels found in turtles from Sequoyah NWR, at 0.02 mg kg^{-1} (Figure 1). None of the sites examined had high enough Hg levels in muscle tissue to recommend a total consumption ban, but limited consumption is recommended at several (Table 2). However, the *T. scripta* sample size was over twice that of the other two species, potentially reducing overall Hg muscle averages (Figure 2). To avoid causing species biases at sites, only *T. scripta* was analyzed to provide a more cohesive comparison of Hg levels across sites ($p<0.0001$) (Figure 3). Greenleaf Lake and Grand River were not included, as no *T. scripta* were collected from those sites. *T. scripta* collected from the Little River had the highest Hg concentration in muscle of all sites sampled at 0.096 mg kg^{-1}. This average is nearly 10 times higher than the site with the lowest (0.010 mg kg^{-1}) average muscle Hg concentration, Sequoyah National Wildlife Refuge.

Mercury concentrations were higher in the liver than the muscle in all three species (Figure 2). The average liver Hg concentration found was almost twice the USEPA action level (0.30 mg kg^{-1}). The average Hg concentration in liver for each species did not follow the same trend as muscle in regard to species. *C. serpentina* had the highest Hg concentration in liver (1.25 mg kg^{-1}), which was significantly different from *T. scripta* (0.24 mg kg^{-1}) ($p<0.001$), but not significantly different from *A. spinifera* (0.70 mg kg^{-1}) ($p=0.23$). No significant differences in liver Hg levels were found between *A. spinifera* and *T. scripta* ($p=0.21$) (Figure 2). Pooling species, liver Hg levels were over 20 times greater at Greenleaf Lake (3.07 mg kg^{-1}) than at the least contaminated site, Sequoyah National Wildlife Refuge (0.13 mg kg^{-1}).

Mercury Concentrations in Inedible Tissues

Mercury concentrations in easily harvested dermal tissue including marginal carapacial scute (external shell material) and claw were higher than muscle, but not liver. Multiple individual turtles had claw and scute Hg levels an order of magnitude greater than in muscle tissues. The highest Hg levels in scute at any one site was found in Greenleaf Lake at 2.54 mg kg^{-1}, while the lowest scute Hg levels were found in the northeasterly located Spring River at 0.199 mg kg^{-1}. *C. serpentina* had significantly higher Hg levels in scute than both *A. spinifera* and *T. scripta* (Figure 2).

Pooling all species, claw had higher average Hg levels (0.70 mg kg^{-1}) than the similarly keratinized scute tissue (0.63 mg kg^{-1}). As with other tissue levels, Greenleaf Lake had the highest average level of Hg in claw (3.80 mg kg^{-1}) and the lowest was found at Sequoyah National Wildlife Refuge (0.19 mg kg^{-1}). The degree of contamination in claw was significantly different among species ($p<0.001$). While there were no differences between *A.*

spinifera and *C. serpentina* (p=0.4613), both *A. spinifera* and *C. serpentina* were significantly different from *T. scripta,* (p<0.001) and (p=0.03), respectively (Figure 2).

Correlation between Non-destructive Sampling Techniques and Muscle

There was a positive linear relationship between muscle and claw Hg burdens in all species (R^2=0.79; Table 3). However, when hardshells and softshells were separately analyzed, the R^2 increased for hardshells but decreased for softshells (R^2=0.85 and R^2=0.73, respectively; Figure 4). The regressions exhibited similar slopes for all three species (Table 3).

Hg levels in scute also had a strong linear relationship with muscle tissue when hardshells (R^2=0.84) and softshells (R^2=0.83) were separated; however, when all species were combined in the regression, the relationship became much weaker (R^2=0.41). Slopes were very similar for both *C. serpentina* and *T. scripta*, but different for *A. spinifera* (Table 3 and Figure 5).

Claw also had a strong linear relationship with liver Hg concentration in *A. spinifera* and *C. serpentina,* but not *T. scripta* (R^2= 0. 93, 0.77, and 0.22, respectively; Figure 6). Pooling data from all species resulted in a weak relationship between the two tissue types (R^2= 0.61). Similarly, scute had a strong linear relationship with liver Hg concentration in *A. spinifera* (R^2=0.85), and *C. serpentina* (R^2= 0.79), but not *T. scripta* (R^2=0.13). Pooled data for all species also yielded a weak linear relationship with liver (R^2=0.67).

Hg Concentration and Turtle Characteristics

The turtles sampled had a wide range of mass and shell size (Table 4). For the combined species data, no significant linear relationships were found between turtle mass or shell size and level of Hg contamination in muscle. When each species was evaluated separately across all sites, most linear relationships were weak. The relationship between Hg concentration in muscle and mass in *A. spinifera* was significant and strongest (R^2=0.53, p=0.02). There was a weaker, insignificant relationship between Hg concentration in muscle and shell size (R^2=0.30, p=0.10). *C. serpentina* had contrasting results with an insignificant relationship between Hg concentrations in muscle and mass (R^2=0.22, p=0.17) and a stronger, significant relationship between muscle and shell size (R^2=0.51, p=0.02). There were no significant relationships between Hg concentrations in muscle with either turtle mass or shell size (R^2=0.02, p=0.39 and R^2=0.01, p=0.51, respectively) in *T. scripta*. Since there was a much larger number of *T. scripta* analyzed from each site, data only for *T. scripta* were analyzed for linear relationships of mass and shell size with Hg concentrations in muscle for each site. Relationships were still insignificant (p>0.05). To further observe site-specific influences, data for *T. scripta* and *A. spinifera* were examined from Lake McMurtry to show a species comparison with similar sample sizes from one site (Figures 8 and 9). This site was chosen since it had similar sample sizes of more than one species. Neither species showed that mass or shell length was significantly correlated with mercury concentration, although *A. spinifera* had a relationship between mercury and mass that approached significance (R^2=0.83, p=0.08).

Additionally, there was no relationship between levels of Hg in muscle or other tissues between the sexes (p>0.05). All turtles collected had an average plastron length

greater than 100 mm and were therefore considered adults. As such, no age-based comparisons related to tissue mercury levels were conducted.

Liver/muscle ratio

Liver to muscle Hg ratios greater than one were observed in turtles from all sites. Lake McMurtry had the lowest ratio (1.35), with ratios ranging as high as 15.71 at Oxbow Lake (Figure 10). Separately, the linear relationship between muscle and liver Hg concentrations with all species combined was weak (R^2=0.37) and became weaker when each species was evaluated separately. *C. serpentina* had the strongest linear regression (R^2=0.33), with *T. scripta* (R^2=0.03) and *A. spinifera,* (R^2=0.001) showing no relationship.

DISCUSSION

Public Health Implications

Oklahoma has not historically been recognized as a state with mercury-impaired water bodies. However, the Oklahoma Department of Environmental Quality has begun regular monitoring of Hg levels in fish and results indicate the state's Hg contamination was previously underestimated (Oklahoma Department of Environmental Quality 2010). Hg is transferred primarily through atmospheric deposition (Arctic Monitoring and Assessment Programme 2005; Pirrone et al. 2010). Point-source emissions from outside the state, along with sources within the state, may have an effect on Oklahoma biota as well. All of the sites sampled in this study are presently contaminated with Hg and at some locations the levels of contamination in muscle and liver are high enough to warrant concern for human consumption of turtles. While *Trachemys scripta* are the safest turtles to consume overall, the

other two species examined here are more likely to be used for human consumption (Gardner 2006). The average muscle Hg concentration for individual sites was all below the 0.3 mg kg^{-1} USEPA action level, except at Greenleaf Lake. Both site and species should be considered before consumption. For example, at least half of the sites with spiny softshells had consumption guidelines recommending <5 meals per month based on the Hg concentrations found in this study.

Food consumption guidelines for sensitive populations (i.e., children, pregnant women, and women of childbearing age) are more restrictive than the general population guidelines used here, recommending only two meals per month or less if the Hg burden is 0.5 mg kg^{-1} or more (Oklahoma Department of Environmental Quality 2010). Albeit the average muscle Hg concentrations were lower than the USEPA action level overall, the majority of sites contained average liver Hg levels well above the action level. Ingestion of potentially contaminated liver can be particularly dangerous to sensitive populations (e.g., developing fetus). High liver Hg levels should elicit concern since this organ has been known to be consumed by humans, primarily as soup (Aguirre et al. 2006), and can also be mistaken as meat.

Toxicological effects of Hg on turtles at environmentally relevant exposure levels

The effects of Hg on turtles at exposure levels in a field setting have received little attention, and the few studies that have been conducted focus on the sublethal effects on reproduction. For example, female snapping turtles transfer MeHg to their offspring and when inhabiting contaminated sites can have a decreased level of fertility and produce eggs with higher mortality rates and thus lower hatchling success (Hopkins et al. 2013a). Reduced reproduction for this slowly reproducing species could attribute to the overall decline

documented in turtles, yet the degree to which this can be extrapolated to another species is unclear. For example, common loons (*Gavia immer*) are a species of concern, similar to some turtle species studied here, due to their primarily aquatic nature, high trophic status, and longevity. Furthermore, Hg bioaccumulation is reported to similarly affect egg production and development (Barr 1986; Meyer et al. 1998). However, Hg levels found to negatively affect common loons are lower than those found to affect reproduction in *C. serpentina* (Hopkins et al. 2013a), and they encompass the range of Hg levels found in the present study. One can conclude that each species may have varying levels of sensitivity to Hg. *C. serpentina* showed higher resilience to relative maternal transfer of Hg to eggs (3.00 mg kg^{-1}) than other hardshelled turtle species (Hopkins et al. 2013a). The effects of Hg on reproduction in *A. spinifera* have not been studied even though Hg levels are similar to those found in *C. serpentina*. The aforementioned study emphasizes the use of *C. serpentina* as an indicator species and highlights its resilience, yet also raises a concern for lowered reproductive output. There is a need to further investigate the reproductive effect on turtles in eastern Oklahoma. While *C. serpentina*, and turtles in general, may be more resilient to Hg loads than other wildlife, high levels of Hg in turtles can still cause negative effects to other wildlife exposed, namely predators and scavengers.

A number of studies have examined the effects of environmentally relevant concentrations of mercury on non-reptilian species and have found sublethal effects at low levels (Beyer and Meador 2011). Lowered reproductive success is the primary effect of MeHg in fish at environmentally relevant exposure levels (Crump and Trudeau 2009), likely due to its negative effects as an endocrine disrupter (Tan et al. 2009). Other groups of vertebrates, including other amniotes, have been studied more extensively than turtles. Lowered reproductive success due to mercury exposure has been found in multiple bird

species with an associated liver Hg concentration constituting a wide range (2-52 mg kg^{-1}), although the effect depended on species (Beyer and Meador, 2011). *C. serpentina* and *A. spinifera* in the present study had liver Hg levels near the bottom of the above range.

Use of non-destructive tissue techniques as a predictor for internal tissues

Turtle populations are on the decline worldwide partly due to anthropogenic effects. Gibbons et al. (2000) identified six predominant causes in which environmental pollution and unsustainable use are indicated for turtle population decline. The use of non-destructive tissue techniques to predict internal muscle and liver Hg concentrations is an important step in conservation-minded sampling techniques (Hopkins et al. 2013). This procedure can allow calculation of the risk for human consumption without disturbing a population that could be suffering from harvesting pressures and/or anthropogenic influences. Calculations of food consumption guidelines can be demonstrated through analyzing Hg concentrations in claw or scute and incorporating this value into a linear model. Measurement of blood Hg levels shows only recent exposure and does not correlate as well with muscle Hg in species such as *C. serpentina* (e.g., R^2=0.32,p=0.004; Golet and Haines 2001). In contrast, claw and scute can provide an estimate of Hg levels over time and as demonstrated in the current study, provide a reliable measure of internal mercury body burdens. This non-destructive sampling technique of external tissue is more cost and time efficient without the technical expertise needed to conduct biopsy sampling of live animals. Some of the benefits of non-destructive sampling techniques include a larger sample size along more locations, without detriment, and the added ability to repeatedly sample the same individual over time if desired.

Claw Hg levels were higher than other tissues analyzed, similar to previous studies (Hopkins et al. 2013; Lake et al. 2009). Green et al. (2010) found higher levels of Hg in scute

than claw. Like Hopkins et al. (2013), this study showed that claw could effectively predict Hg muscle concentrations. Lake et al. (2009) also found claw to be useful in determining muscle Hg concentrations (R^2=0.70) in *C. serpentina*. Most studies investigating usefulness of claw have only looked at one species such as *C. serpentina* or osprey (*Pandion haliaetus),* or have focused on the relationship with blood (Hopkins et al. 2007). In this study, when all species were included in the model, claw continued to be a valid predictor, albeit the predictive power increased when species were analyzed separately.

Predictive models may be best suited for a specific area. For example, when the predictive equation developed using *C. serpentina* by Hopkins et al. (2013b) was applied to the present samples, they overestimated Hg concentrations by over 50%. Additionally, when estimating Hg levels in *C. serpentina* only the overestimation was nearly 100%. However, incorporating more variables into the model, such as landscape characteristics and proximity to point sources, could strengthen the predictability of the model across regions (Turnquist et al. 2011).

Turnquist et al. (2011) found that scute Hg concentrations in *C. serpentina* ranged from 0.47-7.43 mg kg^{-1}, with associated muscle Hg concentrations of 0.041-1.50 mg kg^{-1}. These values are higher than Hg levels found here, 0.23-3.40 and 0.03-0.30 mg kg^{-1}, respectively. If data from *C. serpentina* and *T. scripta* are combined to formulate a predictive equation for Oklahoma hardshell turtles, claw can be used as an effective predictor of Hg in liver. Scute was also useful as a predictor for liver Hg concentrations. However, the linear model for *T. scripta* alone for both claw and scute, in regards to liver, is not useful. Studies on differences in sequestration between *T. scripta* and other species could provide a clearer description on why these differences occur. Allocation of excess Hg to scutes instead of liver

could lend to the resilience of Hg bioaccumulation in some turtles. Softshell turtles do not have thick layers of scute, and could thus sequester Hg in scute to a lesser extent or to a different tissue/organ. As red-eared sliders are one of the most abundant turtle species in many areas, their ability to remove toxicants more efficiently could relate to their success as a species.

Again, separating turtle species into hard/softshell categories can provide a more effective way to use claw to estimate Hg concentration in internal tissues, but even more so for scute as a non-destructive technique. Scute was more useful for predicting Hg levels in liver of softshells than of liver in hardshell species. Furthermore, Day et al. (2005) found the most accurate predictor of Hg in liver was with scute (R^2=0.95). The previous study also stated scute to be a good predictor of Hg in muscle in *Caretta caretta* (R^2=0.82). Since keratin may vary in thickness based on the location that the carapace is sampled, it can potentially provide temporal variation in Hg levels if sampling is not consistent. This also could be pertinent with claw sampling; however, research to determine temporal variation based on exposure time and growth rate in Hg levels of claw has not been conducted.

The more abundant *T. scripta* was a hopeful indicator species for Hg levels in other freshwater turtles. However, this species is not a good predictor of the other two turtle species, and did not elicit linear relationships as useful as did the other two species for non-destructive analysis or turtle characteristics. Based on the linear models formulated, the best predictor was instead determined using an integrative categorical approach wherein hardshell and softshell spp. were separated when using a non-destructive sampling technique to estimate internal tissue concentrations. Further predictions of Hg concentrations in

consumable turtle tissues should be formulated from tissues based on hardshell or softshell equations provided in the appropriate region.

Composition of a turtle's shell may provide valuable information for non-destructive sampling technique analyses. A hardshell turtle species has a shell that is a bony extension of the ribs and vertebrae, consisting of bone and a thin layer of keratinized scute covering. Ernst and Lovich 2009). Softshells are quite different because they lack dermal bones with hard scutes, and they have layers of collagen bundles instead. The keratinized covering over the shell is often referred to as leathery skin or shell epidermis, not scute (Alibardi and Toni 2006).

Many animals secrete an ectodermal protective covering such as keratin, which specifically has a strong chelation property to heavy metals such as lead and mercury (Coello and Khan 1998). Few studies have looked at the chelation properties of these keratinized secretions in turtle scutes. It is, however, thought that MeHg binds strongly to keratin in turtle scutes (Crewther 1965). Alibardi and Toni (2006) looked at the composition of soft and hard-shelled turtle skin structure and cornification proteins. They found that softshell turtles have a much lower composition of beta-keratin in both the soft epidermis (e.g., neck and legs) and in the shell epidermis (i.e., scute), especially compared to typical chelonians. In most chelonians, scute is composed primarily of beta-packets, while softshell scute lack beta-packets and contain few beta-keratins among alpha-keratin filaments. To increase the waterproof barrier, there is a high production of mucus and lipid that fills the extracellular space among corneocytes in shell epidermis unlike most chelonians where corneocytes are filled with densely packed beta-packets. This may suggest that softshell scute has a lower protein composition compared to chelonians with a hard shell and could potentially influence

Hg sequestration in softshells. The rate of beta-keratin deposition is relative to seasonal growth and varies upon position in the scute (Alibardi 2002). Shell growth is thought to occur near the edges of the scute, which is where our samples were taken.

All species of turtles have claws with structural makeup very similar to the composition of hardshell scutes (i.e., high levels of beta keratin; Valle et al. 2009). This ubiquitous composition among species could explain the close predictive abilities of claw when combining all turtle species (R^2=0.79), as opposed to scute (R^2=0.41). There could also be more potential for error in sampling the thin layer of scute on softshells. Turtle claw Hg levels could serve as a predictor not only for internal turtle Hg burdens but also other species in the area that could be more difficult to sample or are protected (e.g., endangered status). Similar use of keratinized tissues for non-destructive analysis has been used in other taxa. Mammalian hair contains a high amount of cytesine residues in the keratin proteins that account for the binding affinity and storage ability of Hg in hair (Clarkson et al. 2007), hence the historical use of hair as an indicator for Hg in humans. One study found the addition of hair or scales to a heavy metal solution reduced the Hg toxicity to water fleas (*Daphnia magna)* and zebra mussels (*Dreissena polymorpha)* (Coello and Khan 1998). The former study primarily used alpha-keratins (i.e., hair), but also found beta-keratin forms (i.e., scute and feather) to have equal chelation properties. Shell, along with other keratinized tissues, is an important component in sequestration and in preventing further bioaccumulation in the food web.

Variation in turtle Hg levels among sites

In this investigation, there was high variability in average Hg levels in turtles among sites. Other studies have found significant site differences when comparing average muscle Hg levels, even in sites in close proximity (Golet and Haines 2001). Turnquist et al. (2011) concluded that Hg levels in *C. serpentina*, and likely other species of turtles, are spatially variable. Specifically, muscle Hg levels were positively correlated with sulfate levels in water, along with the maximum elevation of the watershed. They reported that Hg levels in turtle carapace were also correlated with elevation of the watershed, along with several other abiotic variables (e.g., the water's acid neutralizing capacity and lake/watershed size ratio).

Significant differences were found among turtles at separate sites ($p<0.001$), but due to unequal samples sizes of different species of turtles, it is difficult to discern the influence of significance solely based on site location. The average muscle Hg levels ranged over an order of magnitude (0.02-0.34 mg kg^{-1}) among the different sites. This appreciable range was likely due to both unequal samples sizes of each species and also from the different study sites' characteristics. Moreover, sites where *A. spinifera* and *C. serpentina* were sampled, had higher average Hg levels than those where only *T. scripta* were sampled ($p<0.001$). For example, Greenleaf Lake had the highest average Hg level, but there were no *T. scripta* sampled there. This can be expected since *T. scripta* consistently had the lowest average Hg concentration. Both *A. spinifera* and *C. serpentina* are considered more carnivorous than *T. scripta*, increasing their potential level of exposure.

In addition to species differences at sites, proximity to point sources could also enhance the significant difference among sites. Six of the top 50 US mercury-emitting coal-fired power plants are located in Oklahoma (Environmental Integrity Project 2010). In this

study, several of our sampling sites were within approximately 80 km of one of these six coal-fired power plants. Prevailing winds are a major factor in the influence of location of power plants to these sites. The three most contaminated sample sites were each in relatively close proximity to one of these power plants. The sampling site with the highest level of Hg contamination in muscle is located 35 km southeast of the Georgia-Pacific Coal Power Plant in Muskogee County, OK. Furthermore, both Boomer and McMurtry Lakes are within 50 km, south and southwest respectively, of Sooner Power Plant in Noble County, OK. The site with the fourth highest Hg burden, Little River, was located 80 km due east of the coal-fired Hugo Power Plant in Choctaw County, OK. Each of the coal-fired power plants mentioned above produced over 100 lbs of Hg emission per year on average (U.S. Environmental Protection Agency 2013). There are other pertinent variables that contribute to bioaccumulation of Hg in aquatic food chains beyond proximity to coal-fired power plants (e.g., landscape characteristics, size of watershed, pH, levels of DOC, proximity to wetlands, and length of the food chain). However, recognizing point sources (e.g., coal-powered power plants, waste incinerators, and chlor-alkalai plants) and non-point sources (i.e., atmospheric deposition) plays a key role in future prevention of contamination to aquatic wildlife and public awareness of turtles as a food source.

Liver/muscle ratio

According to previous studies that have compared liver/muscle ratios (Cizdziel et al. (2003; Havelkova et al. 2008; Kruzikova et al. 2013), our results indicate there is current uptake of Hg at all sites analyzed. The liver plays a crucial role in the detoxification, storage, redistribution, and transformation of heavy metals (Newman and Unger 1998; Havelkova et al. 2008). Liver/muscle ratios have not been a focus of research for reptiles. Radula et al. (2007) measured mercury levels in two species of fish both upstream and downstream of a

chlor-alkali factory and found liver/muscle ratios were significantly higher downstream from the plant. Higher liver/muscle ratio implies Hg contamination is present and detoxification is occurring. Theoretically, after increased prolonged exposure to MeHg, the Hg liver levels will quickly have an initial increase; whereas, Hg exposure that is decreased over prolonged periods will cause a slower decrease in muscle Hg levels (Olsson 1976). Fish downstream had higher Hg levels in all tissues tested and histopathological analyses revealed severe pathologies in livers with the highest Hg levels. Specifically, the target organ in lightly contaminated sites is muscle, with a ratio of less than one, but in heavily contaminated sites, or those with a ratio greater than one, liver is considered the target organ (Havelkova 2008; Kruzikova et al. 2013). This is due to the organism's ability to store and detoxify Hg when exposed. An elevated liver Hg content compared to muscle Hg content indicates active uptake of Hg in turtles; and further, that the water bodies analyzed in the present study are presently contaminated sites, with all sites having ratios greater than one. Implications of the higher liver/muscle Hg ratio include concerns that Hg will continue to be present in muscle tissue even after ratios decrease as current uptake subsides. Redistribution of Hg from the more visceral organs (i.e., kidney and liver) to muscle for storage can occur following a decrease in MeHg from diet (Oliveira Ribeiro et al. 1999); further, Hg can also be eliminated from the liver via bile (Kade 2012). Hg can be stored in the liver for a few weeks, but redistributes in muscle several weeks to months after exposure (Oliveira Ribeiro et al. 1999). This may explain why linear regression models comparing muscle to liver have lower R^2 values because these tissues are not temporally linear. Further, decreases in site contamination can still warrant consumption risks for humans and wildlife for an extended time period.

Caution should be taken in drawing conclusion of liver/muscle ratios since only THg was measured in each tissue and inorganic Hg has the potential to accumulate in liver tissues. Berzas Nevado et al. (2011) did, however, measure both MeHg and THg in liver and found MeHg to compose 7-59% of THg. To further investigate the ratio in turtles, multiple types of organic and inorganic Hg should be measured in both the liver and muscle for comparison. Very high inorganic levels in the liver of turtles could potentially influence the large liver/muscle ratios. Snapping turtles and spiny softshells should be examined independently, whereas data presented here (Figure 10) included *T. scripta* only. *T. scripta* was highlighted because interspecies variability has resulted when comparing liver/muscle ratios to different fish species from the same water body (Berzas Nevado et al. 2011).

Turtles as useful bioindicators

Turtles are potential bioindicators of Hg accumulation in aquatic environments due to many inherent traits, including a high trophic position, potential for continual exposure to contamination due to sedentary life styles, and greater longevity than most aquatic biota. Several turtle species been used to investigate aquatic mercury contamination, including *C. serpentina* (Helwig and Hora 1983; Meyers-Schone et al. 1993; Golet and Haines 2001; Green et al. 2010; Turnquist et al. 2011), *T. scripta* (Hays et al. 2007; Meyers-Schone and Walton 1994), and *A. spinifera* (Hopkins et al. 2012). Traditionally, an organism's level of bioaccumulation of Hg is based on its trophic position along with the age, size and amount of exposure in the system (Rincon-Leon et al. 1993). Significant relationships between turtle demographic variables such as size and sex and Hg levels in muscle were not found consistently throughout this study. When species were separated and size was compared, no significant relationship was found in our larger *T. scripta* sample (R^2=0.02), yet *A. spinifera* and *C. serpentina* did exhibit some linear relationships with different size demographics.

Hopkins et al. (2013a) found no differences between size and Hg levels at reference sites, but did find a significant increase in Hg levels with carapace size at their contaminated site. Studies have found mixed results when comparing size and sex with Hg levels. Turnquist et al. (2011) found no significant relationship with Hg levels in tissue and biometric variables such as mass or length. Neither sex nor size characteristics were found to be significant variables in *C. serpentina* according to Golet and Haines (2001).

Meyers-Schone et al. (1993) compared *T. scripta* and *C. serpentina* as bioindicators for Hg pollution at both a contaminated and a reference site and determined both to be useful. The latter species had significantly higher Hg levels and was thought to be a better indicator because of its more prominent omnivorous diet. Rincon-Leon et al. (1994) compared piscivorous and omnivorous fish species as bioindicators of Hg contamination and also found omnivorous species provided a more confident level of estimation compared to other species in the system. *A. spinifera* has also been determined to be an adequate bioindicator for contamination studies based on diet and bottom-dwelling nature, exposing them to anoxic sediments where methylation of inorganic Hg is highest (Gardner 2006; Fu et al. 2010; Green et al. 2010). Results here support these conclusions. Overall in the current analysis, *A. spinifera* had significantly higher average Hg concentrations in muscle than both *C. serpentina* and *T. scripta*. *A. spinifera* is predominately carnivorous (Webb 1962; Ernst et al. 1994), but has also been considered omnivorous (Gardner 2006; Green et al. 2010). Fu et al. (2010) determined that MeHg levels in muscle tissue were positively correlated with trophic level in rivers and further determined Chinese softshell turtles were in the highest trophic level. Previous studies measuring Hg in softshells have been quantified using blood or scute samples, or have focused on a different softshell species (Green et al. 2010; Hopkins et al.

2013), yet have not focused specifically on the relationship of non-destructive sampling techniques and Hg accumulation in muscle and liver and not in Oklahoma.

Most studies using turtles as indicators of Hg contamination have focused on *C. serpentina*. Mercury concentrations found in the present study in *C. serpentina* are similar to those found by Golet and Haines (2001), with a range of 0.05 to 0.50 mg kg^{-1}. Helwig and Hora (1983) found values closer to those presented here at 0.05 to 0.30 mg kg^{-1}. This species has a wide latitudinal distribution in North America from Canada to Mexico (Ernst and Lovich 2009) and is ubiquitous throughout these waterways, making it an ideal sentinel species. Both *A. spinifera* and *C. serpentina* are widely exploited for consumption (Webb 1962).

T. scripta is known to inhabit highly contaminated sites (Gardner 2006). This species is also omnivorous, and although *C. serpentina* is listed at a higher trophic level than *T. scripta* (Meyers-Schone et al. 1994), both share a similar scavenging behavior. In this study, *T. scripta* had the lowest average Hg concentration in muscle compared to all other tissues analyzed; yet no significant difference was found compared to *C. serpentina* Hg concentrations in muscle. The average muscle Hg concentration of *T. scripta* in the current analysis falls within the range Meyers-Schone et al. (1993) reported: 0.002-0.48 mg kg^{-1}. Further, *T. scripta* is more of a basking species than the often bottom-dwelling *A. spinifera* and *C. serpentina,* potentially lowering the level of exposure to MeHg since this toxic form of Hg is methylated by sulfate-reducing bacteria that occur in sediment (Morel et al. 1998). Additionally, based on an ontogenetic shift in diet towards greater herbivory (Meyers-Schone et al. 1994*),* one could expect *T. scripta* to bioaccumulate less Hg than *C. serpentina* and *A. spinifera*.

To illustrate the usefulness of turtles as bioindicators, the level of Hg contamination in muscle tissues in multiple species of fish and turtles sampled from the same three lakes in Oklahoma is presented in Figure 11. All fish data references and their corresponding Hg analyses were provided by the OKDEQ (Oklahoma Department of Environmental Quality 2010). None of the fish or turtle species at these three sites in eastern OK had Hg levels low enough to recommend unlimited consumption. Each site had at least one species, usually largemouth bass (*Micropterus salmoides)*, with Hg levels high enough for sensitive population consumption restriction of <2 meals per month. Largemouth bass Hg burdens were greater than all other fish and turtle species tested at each site. In general, *A. spinifera* and *C. serpentina* had higher levels than all other species apart from largemouth bass, further illustrating importance of trophic position in relationship to bioaccumulation. Comparisons of fish and turtle data indicate turtles do provide useful information about their habitat and are adequate bioindicators relative to other species at a similar trophic level. Specifically, *C. serpentina* and *A. spinifera* had Hg concentrations within the range of contamination in fish, and contamination levels in largemouth bass were generally parallel with *A. spinifera*. However, *T. scripta* provided Hg levels below that of all the fish analyzed, reducing its utility as a bioindicator for higher trophic fish. Furthermore, persons that choose to consume turtle species such as *C. serpentina* or *A. spinifera* in eastern Oklahoma could potentially use consumption guidelines calculated for higher trophic fish species such as bass, and could gain the nutritional benefits available in lower trophic level fish and turtles without the adverse effects of Hg.

Similar to the fish species referenced above, all turtle species in this investigation are secondary consumers at minimum, but exhibit differences in feeding ecology. Van Dyke et al. (2013) compared trophic levels of these three turtle species using claw tissue, but focused

on Se bioaccumulation; results indicated that all species were feeding at multiple trophic levels, although *C. serpentina* was feeding at the highest level, with *A. spinifera* feeding mostly on invertebrates and *T. scripta* proving to be the most opportunistic species with the lowest $\delta^{15}N$ values. While *A. spinifera* and *C. serpentina* are recognized to be exploited as a food source in North America, *T. scripta* is primarily exploited as such in Asia and is also harvested for the pet trade worldwide (Gardner 2006). All three species are, at one geographical location or another, used as a food source.

In summary, differences in diet and thus trophic level seem to be a contributing factor in variation of Hg levels among species. *A. spinifera* and *C. serpentina* typically had higher levels of Hg, paralleling trophic status. However, our results did not consistently find other variables often thought to influence Hg bioaccumulation in other species to be significant (i.e., size and sex). The differences in Hg levels of each species could be affecting the reproductive success of these slowly reproducing vertebrates. Environmentally relevant exposure levels of contamination found here may have toxicological significance in contributing to the decline of *A. spinifera* and *C. serpentina* in Oklahoma. These interspecific differences in Hg contamination should be strongly considered by the public when consuming turtles in this region. Similarly, sites in close proximity can drastically differ in level of contamination and should also be considered. Sensitive populations are especially at risk for adverse health effects if consumption limits are not advised.

Conclusions

Turtles in Oklahoma are contaminated and the contamination suggests potential hazard to humans through harvesting. The Hg burdens obtained also suggest a potential reproductive hazard for turtles. Turtle monitoring should continue in Oklahoma because of

these hazards. The liver/muscle ratios indicate all water bodies examined are presently contaminated with Hg, and to varying degrees. Monitoring should be conducted using Hg concentrations in scute and claw as non-destructive measures for muscle. Although this measure could provide slightly under or overestimated values, accuracy and precision would be sufficient to evaluate trends in Hg levels without adding more stress to the populations. Finally, these surrogate mercury measurements are simple and nondestructive. Since turtles are useful biomonitoring species, these measurements could be used as preliminary mercury screening at sites of interest.

Future directions

Useful information was gathered here regarding the use of claw and scute as non-destructive sampling techniques for prediction of Hg in internal tissue. Yet, a more complete analysis needs to be conducted with larger, more even sample sizes of each species from multiple sites. This could allow for a more reliable model for predicting internal tissue concentrations where influences of each site and species can be better understood. A more consistent method of population sampling could have benefited our study. Larger samples of each species were obtained at sites where sampling nets remained for three consecutive days. With larger samples, the degree of contamination at each site can be better established and predictive models made more valid.

Future non-destructive sampling technique studies could also benefit from a focus on the composition of claw and scute samples. Percentage of MeHg in THg analysis of scutes is thought to be similar to birds, wherein 100% of THg is MeHg in feathers (Day et al. 2005). Approximately 90% of total Hg in terrapin scutes was MeHg, according to Blanvillian (2007). No research has examined the level of MeHg in turtle claw; only THg levels have

been evaluated. Understanding the rates at which MeHg accumulates in the claw could be applicable to predictive models. It is known that Hg is allocated to the scute and also claw tissues, but the rate and relative proportion distributed are unknown. These variables may help distinguish the temporal variation of contaminants in turtles through non-destructive sampling techniques, and further help determine associations between Hg levels and how they vary with abiotic variables (e.g., seasonal variation or flooding events) or biotic influences (e.g., algal blooms). Studies assessing the affinity for Hg of keratinized tissue (i.e., feathers) found that this contaminant was stable in this tissue regardless of exposure to UV radiation, and other natural degradation processes (Day et al. 2005). The stability of Hg in tissues sampled non-destructively is promising for both field procedures and analysis of deceased individuals collected opportunistically. Resources needed to preserve more time-sensitive tissues for analysis can be preserved.

Non-destructive sampling techniques in turtles may be helpful in determining Hg levels in other aquatic biota or birds near water with high site fidelity, especially those in similar trophic levels. Researchers could gain valuable information regarding Hg levels in threatened or endangered species without unnecessary detriment to those species. Furthermore, if turtle claw and scute are predictive of Hg in fish, for example, food consumption guidelines can be formulated for each water body studied without sacrificing the fish or using resources needed for fish sampling (e.g., watercraft or electroshocking packs). State departments, and most importantly human consumers, could benefit from these models. Ancillary data such as Hg levels in surface waters, pH levels, and DOC levels could add to the predictability of these models.

The turtle species most commonly consumed in Oklahoma include *C. serpentina* and *A. spinifera*. These species are, however, not as prevalent as *T. scripta*. Further research in the difference in Hg demethylation between *T. scripta* and other species could bring insight into the decline of *C. serpentina* and *A. spinifera*. A difference is believed to occur based on the lack of correlation between external tissues and liver in *T. scripta,* unlike the other two species analyzed. Many other anthropogenic pressures on turtles exist besides Hg contamination and are likely having an effect on their decline also. Other contaminants that accumulate in keratinized tissues could be studied using these same non-destructive sampling techniques in turtles.

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Tables

Table 1. The range of Hg concentrations found in each tissue for each species sampled from sites in eastern Oklahoma during the summers of 2010 and 2011.

Species	Tissues (mg kg^{-1})			
	Muscle	Liver	Claw	Scute
Apalone spinifera	0.04-0.72	0.03-2.58	0.11-6.21	0.06-1.86
Chelydra serpentina	0.03-0.30	0.13-5.33	0.36-3.64	0.23-3.40
Trachemys scripta	0.003-0.23	0.01-1.29	0.02-1.95	0.04-2.19

Table 2. Food consumption guidelines: Average mercury concentration in the leg muscle of turtles sampled from sites in eastern Oklahoma during the summers of 2010 and 2011. One standard error is shown in parenthesis, if no standard error is listed, only one individual was captured at the site. Tissue concentrations are based on analyses of total Hg (THg). Food consumption guideline values for allowable meals per month were calculated based on a reference dose of 0.1 ng/g d, 72 kg body mass, and a serving of 8 oz (172 g). Reference dose was obtained from US EPA (http://www.epa.gov/iris/subst/0073.htm). "--" indicates that no turtles of that species were captured from the site "UR" indicates unrestricted consumption levels.

Species		Sites									
		Spring River	Neosho River	Little River	Verdigris River	Grand River	Oxbow Lake	Boomer Lake	Sequoyah NWR	Greenleaf Lake	McMurtry Lake
Chelydra serpentina	THg, mg kg^{-1}	.039 (.009)	--	--	--	.076 (.038)	.059 (.036)	.200	.047 (.008)	.250 (.070)	.071
	Allowable meals per month	UR	--	--	--	14	UR	5.1	UR	4.1	14
Apalone serpentina	THg, mg kg^{-1}	--	--	--	--	--	.067	.290 (.300)	--	.519	.160 (.170)
	Allowable meals per month	--	--	--	--	--	15	3.6	--	2.0	6.7
Trachemys scripta	THg, mg kg^{-1}	.026 (.006)	.033 (.030)	.096 (.070)	.067 (.037)	--	.022 (.018)	.035 (.018)	.010 (.002)	--	.095 (.055)
	Allowable meals per month	UR	UR	11	15	--	UR	UR	UR	--	11

Table 3. Summaries of regression analyses of predictive equations formulated with non-destructive sampling techniques for predicting internal tissue samples, muscle (a) and liver (b). Linear regression models used with p-values based on alpha level of 0.05.

(a)	Claw		
	R^2	P-Value	Equation
All species	0.79	<.0001	y = 0.0167 + 0.102x
Hardshells	0.85	<.0001	y = 0.0160 + 0.087x
Chelydra serpentina	0.85	<.0001	y = 0.0218 + 0.077x
Trachemys scripta	0.87	<.0001	y = 0.0069 + 0.115x
Softshell (*Apalone spinifera*)	0.73	<.0001	y = 0.0718 + 0.099x
	Scute		
All species	0.41	<.0001	y = 0.0176 + 0.103x
Hardshells	0.84	<.0001	y = 0.0098 + 0.081x
Chelydra serpentina	0.79	<.0001	y = 0.2008 + 0.073x
Trachemys scripta	0.87	<.0001	y = 0.0033 + 0.094x
Softshell (*Apalone spinifera*)	0.83	0.0002	y = 0.0176 + 0.347x

(b)	Claw		
	R^2	P-Value	Equation
All species	0.61	<.0001	y = 0.080 + 0.576x
Hardshells	0.71	<.0001	y =-0.522 + 0.962x
Chelydra serpentina	0.77	0.0189	y = 0.201 + 0.469x
Trachemys scripta	0.21	0.0024	y =-0.197 + 1.094x
Softshell (*Apalone spinifera*)	0.93	<.0001	y = 0.030 + 0.423x
	Scute		
All species	0.67	<.0001	y = 0.2908 + 0.630x
Hardshells	0.66	<.0001	y =-0.1559 + 1.037x
Chelydra serpentina	0.79	<.0001	y = 0.3506 - 1.314x
Trachemys scripta	0.13	0.0135	y = 0.1363 + 0.248x
Softshell (*Apalone spinifera*)	0.85	0.0001	y = 0.1041 - 1.369x

Table 4. Size parameters of all species of turtles sampled from sites in eastern Oklahoma during the summers of 2010 and 2011.

Species	Mass (g)		Maximum Carapace Length (mm)		Maximum Plastron Length (mm)	
	Average	Range	Average	Range	Average	Range
Chelydra serpentina	5360.91	890-9500	283.36	161-367	218.82	114-310
Apalone spinifera	1690.36	540-3800	283.18	140-405	188.91	60-313
Trachemys scripta	976.30	335-2500	195.96	133-258	176.11	121-230
All *spp*.	1826.08	335-9500	224.21	133-405	185.09	60-313

Figures

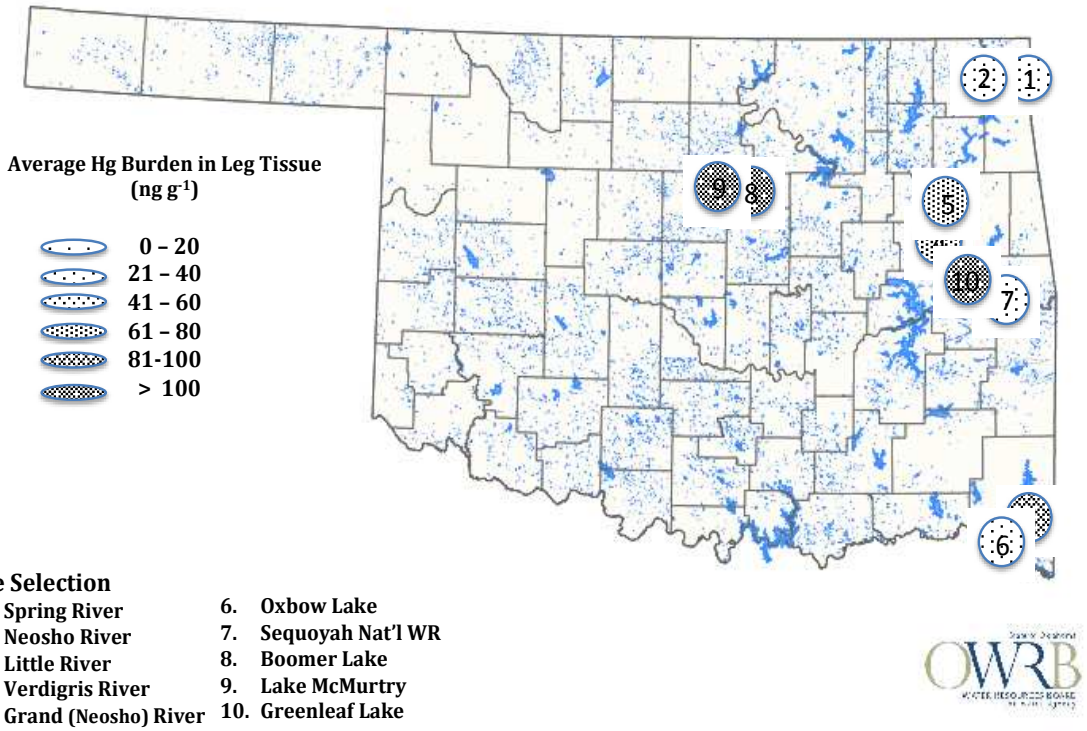

Figure 1. Map of lakes sampled in eastern Oklahoma during the summers of 2010 and 2011, illustrating the average Hg concentration found in leg muscle of all turtles collected from each site.

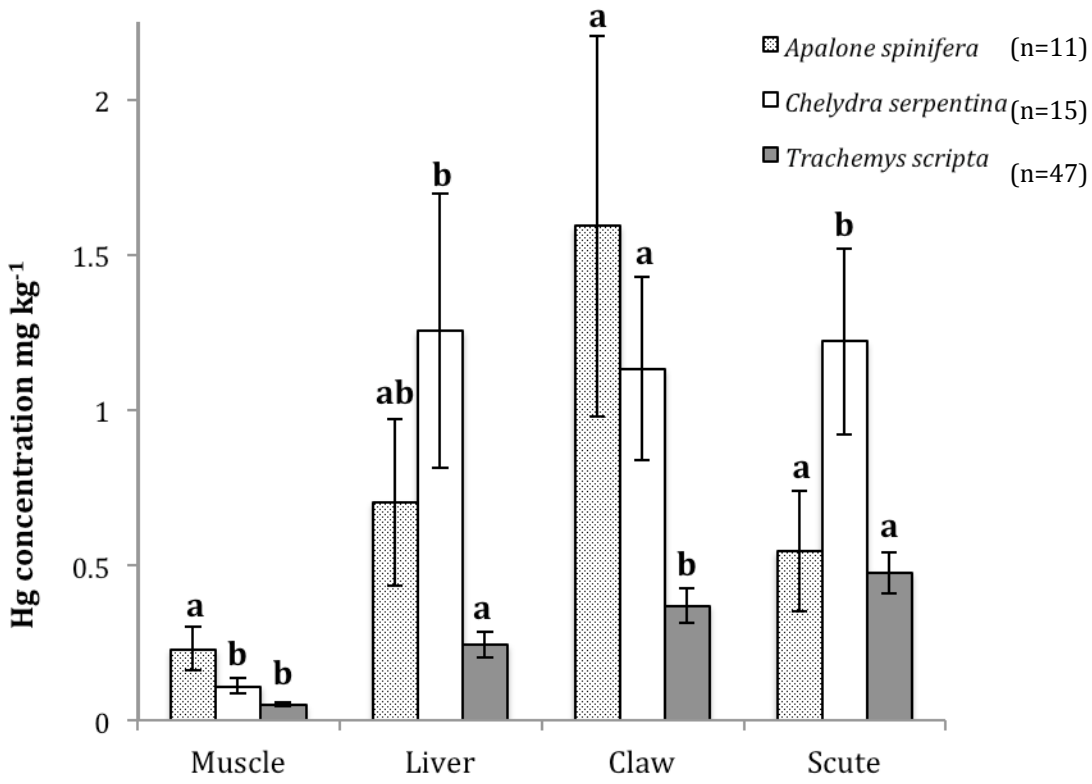

Figure 2. Average Hg concentrations in each turtle tissue type examined. The values are derived from data combined across all sites sampled in eastern Oklahoma during the summers of 2010 and 2011. Error bars represent ±1 standard error and bars with different letters represent values that are significantly different ($p<0.05$) among species within tissues.

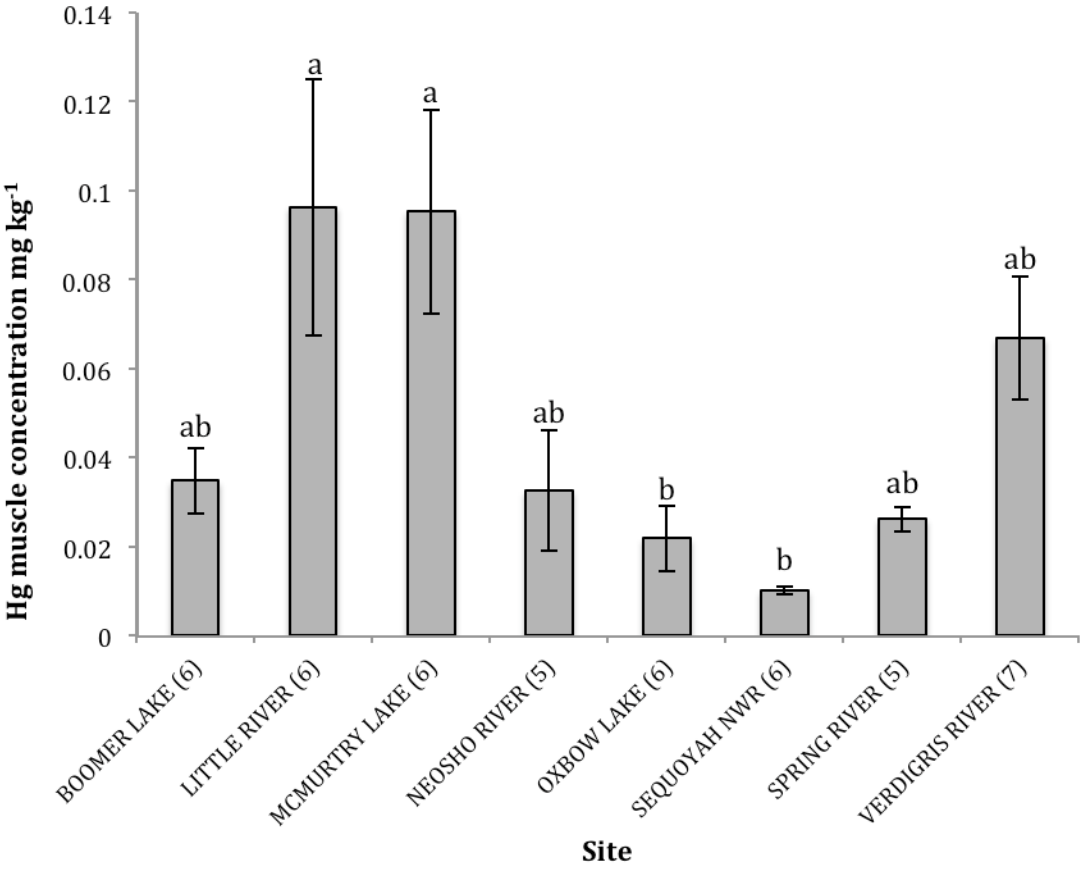

Figure 3. Average Hg concentrations (mg kg^{-1}) of only *Trachemys scripta* from 8 sites in eastern Oklahoma sampled during the summers of 2010 and 2011. Error bars represent ±1 standard error and bars with different letters represent sites that are significantly different ($p<0.05$).

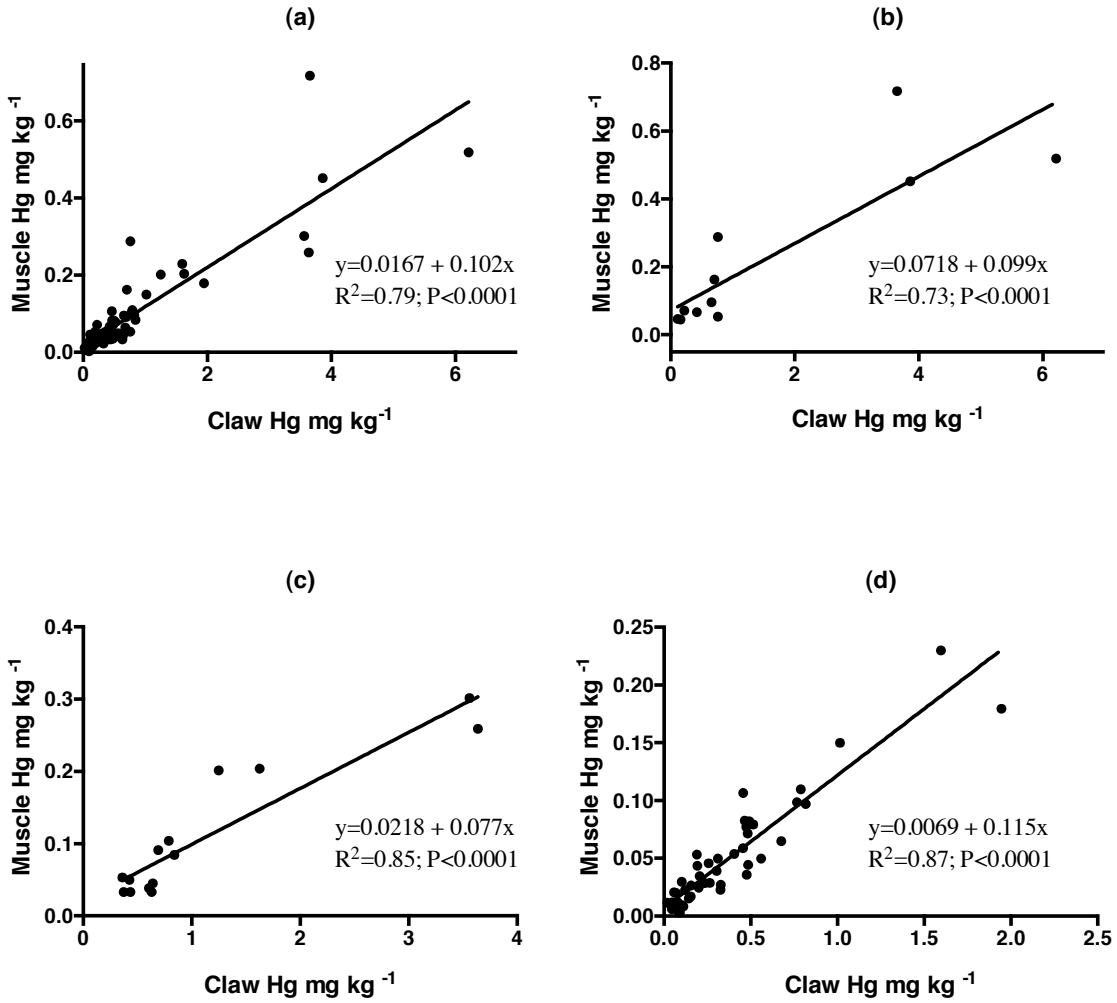

Figure 4. Linear regressions and associated equations illustrating relationship between Hg concentration in femoral leg muscle and claw clippings as a means of measuring non-destructive sampling technique efficiency. Linear regressions are separated by a) all species, b) *Apalone spinifera*, c) *Chelydra serpentina*, and d) *Trachemys scripta* sampled from sites in eastern Oklahoma during the summers of 2010 and 2011.

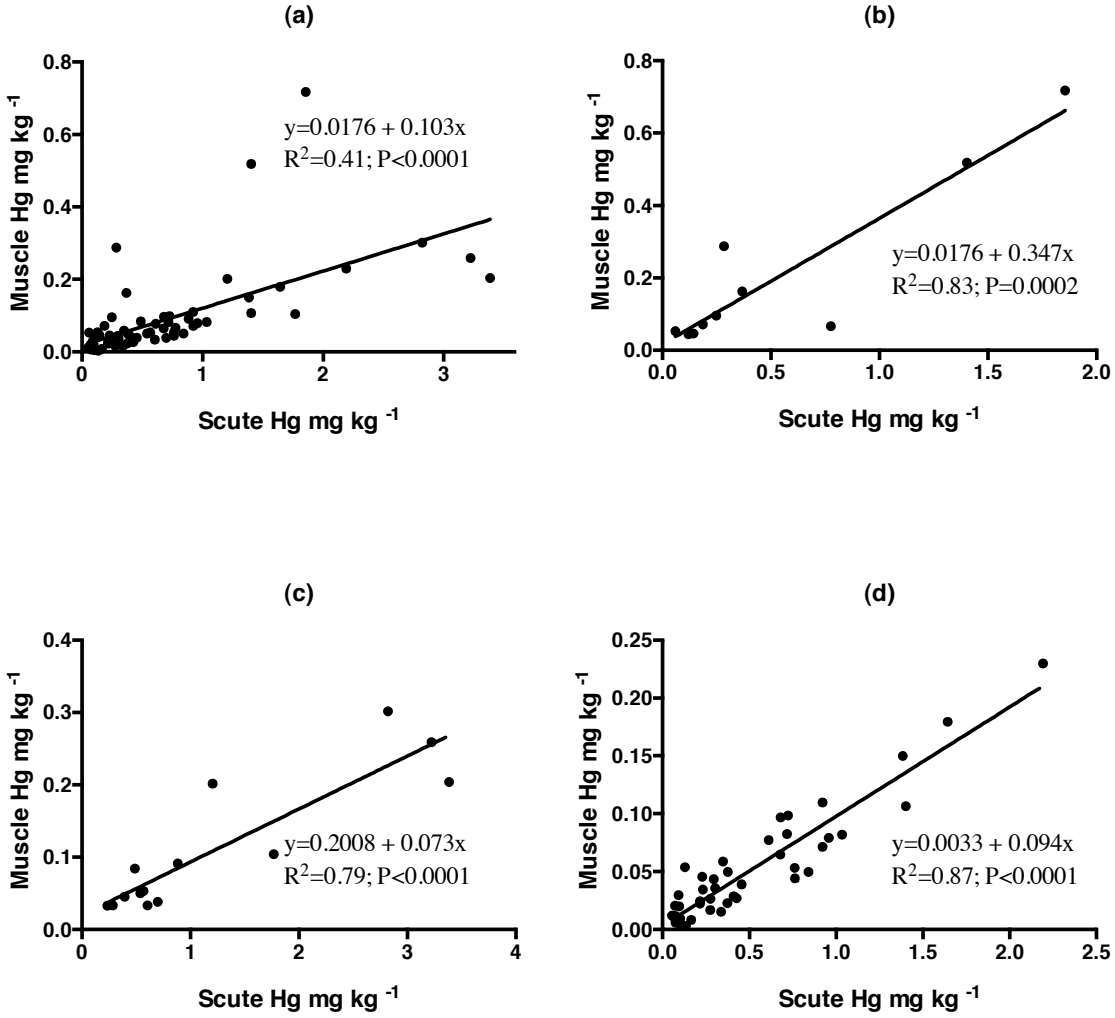

Figure 5. Linear regressions and associated equations illustrating relationship between Hg concentration in femoral leg muscle and scute as a means of measuring non-destructive sampling technique efficiency. Linear regressions are separated by a) all species, b) *Apalone spinifera*, c) *Chelydra serpentina*, and d) *Trachemys scripta* sampled from sites in eastern Oklahoma during the summers of 2010 and 2011.

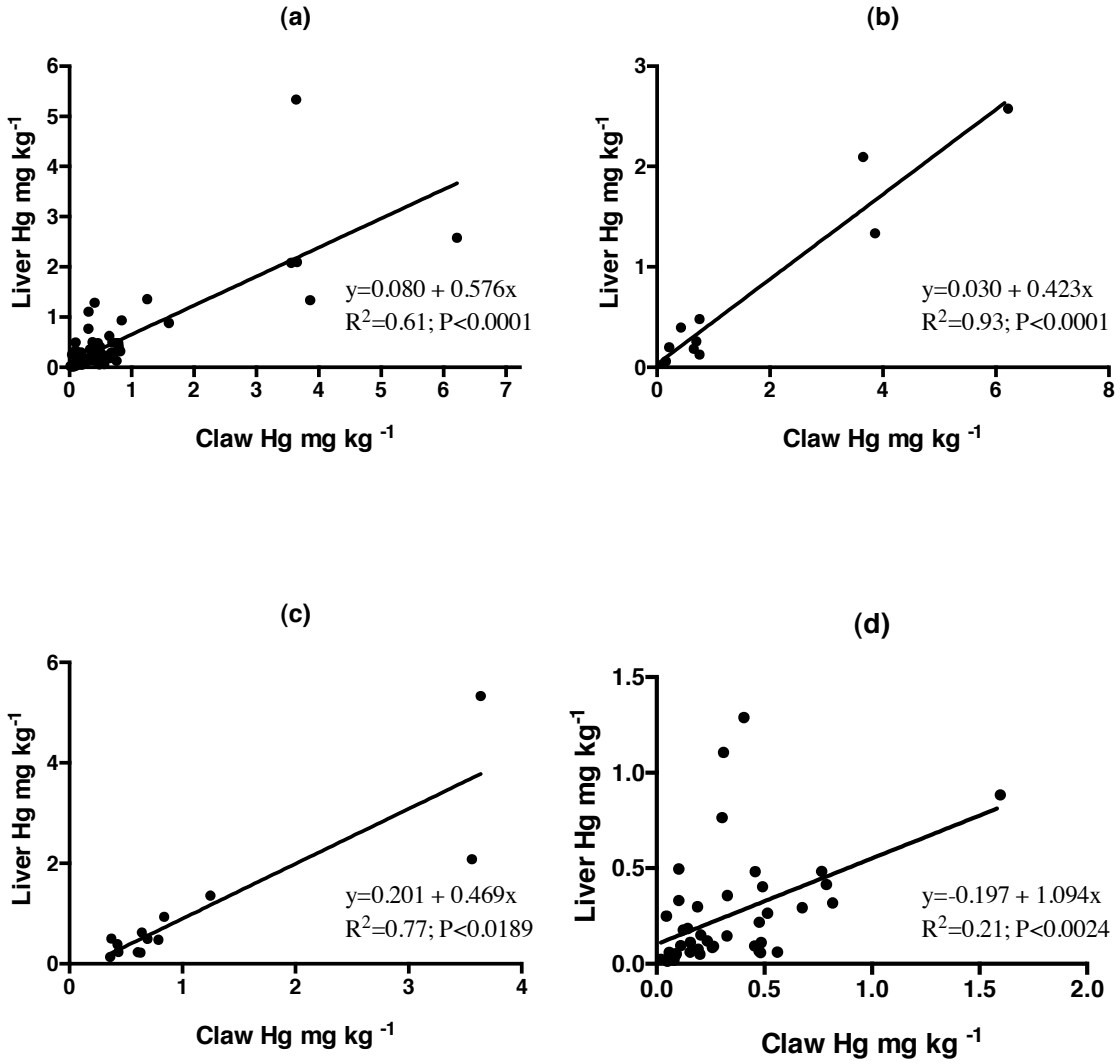

Figure 6. Linear regressions and associated equations illustrating relationship between Hg concentration in liver and claw clippings as a means of measuring non-destructive sampling technique efficiency. Linear regressions are separated by a) all species, b) *Apalone spinifera*, c) *Chelydra serpentina*, and d) *Trachemys scripta* sampled from sites in eastern Oklahoma during the summers of 2010 and 2011.

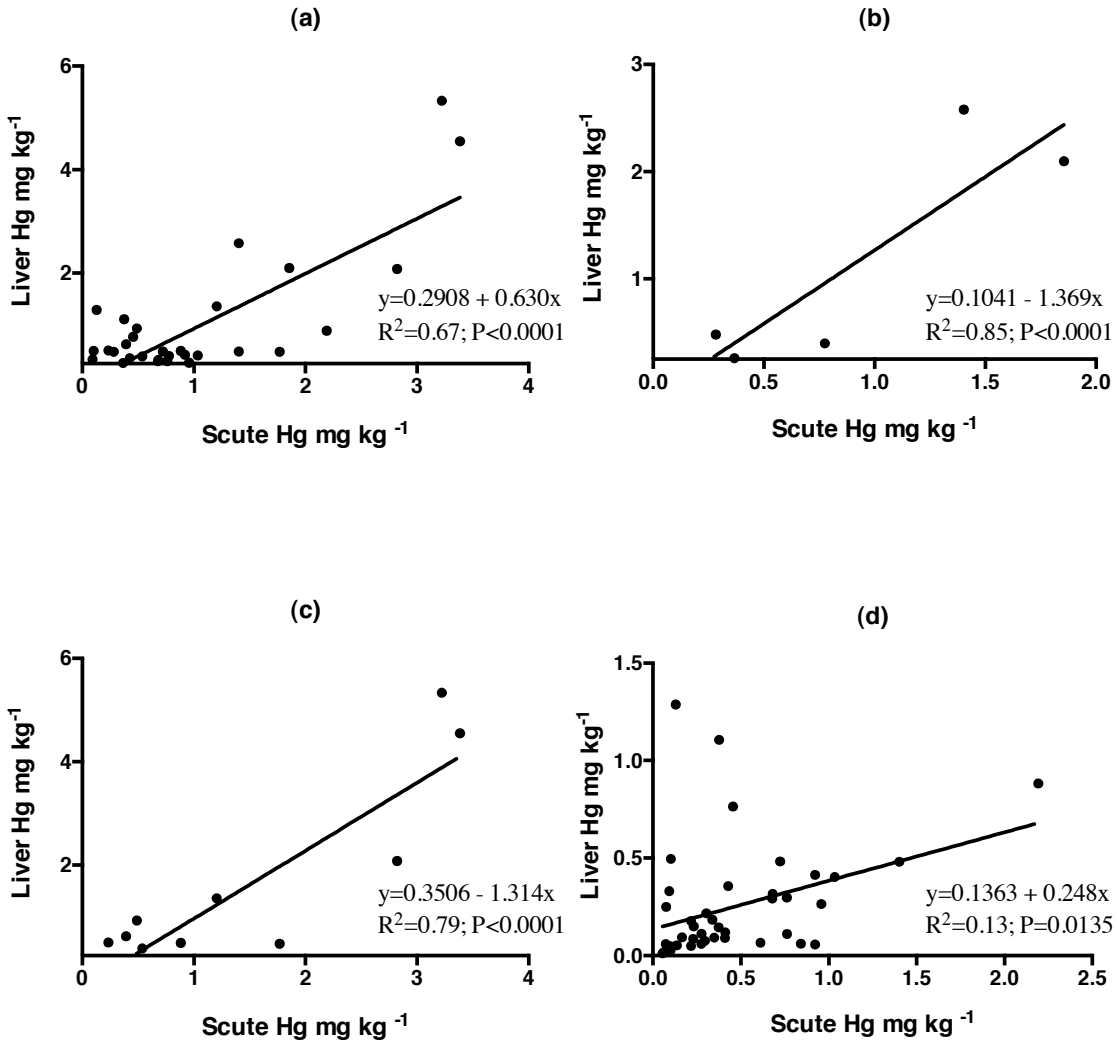

Figure 7. Linear regressions and associated equations illustrating relationship between Hg concentration in liver and scute as a means of measuring non-destructive sampling technique efficiency. Linear regressions are separated by a) all species, b) *Apalone spinifera*, c) *Chelydra serpentina*, and d) *Trachemys scripta* sampled from sites in eastern Oklahoma during the summers of 2010 and 2011.

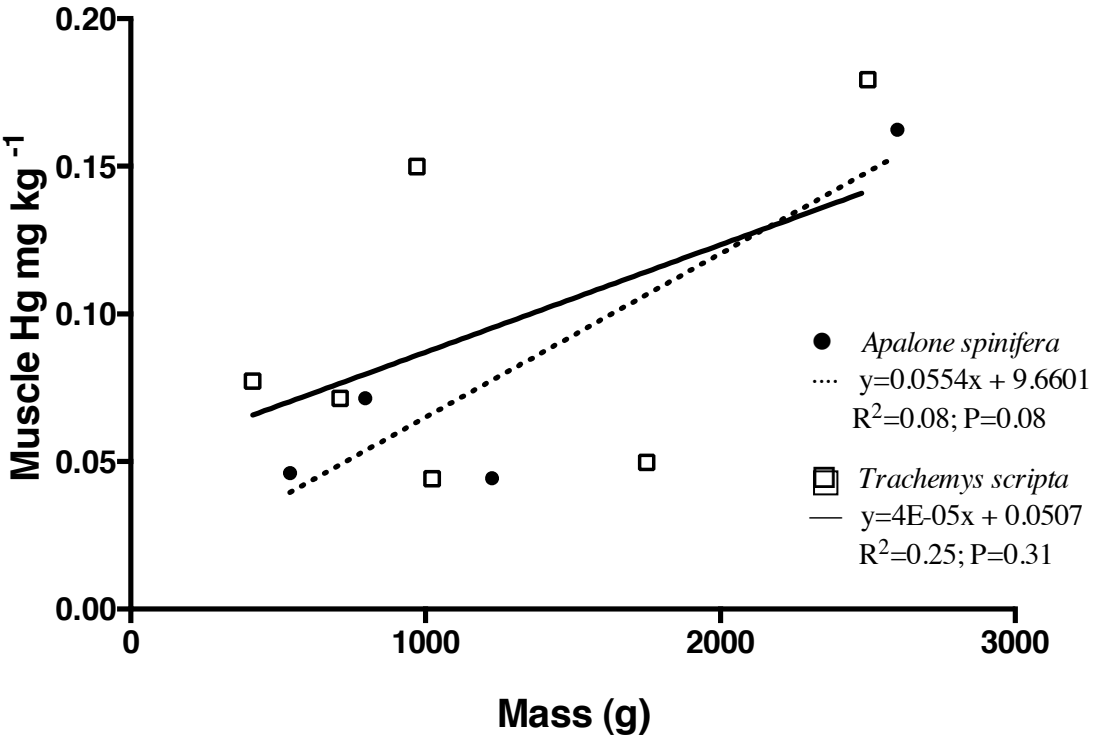

Figure 8. Linear regressions and associated equations illustrating relationship between Hg concentration in femoral leg muscle and mass *in Apalone spinifera* (solid circles and dotted line) and *Trachemys scripta* (squares and solid line) species sampled during the summers of 2010 and 2011 at one site, Lake McMurtry (Payne Co., Oklahoma).

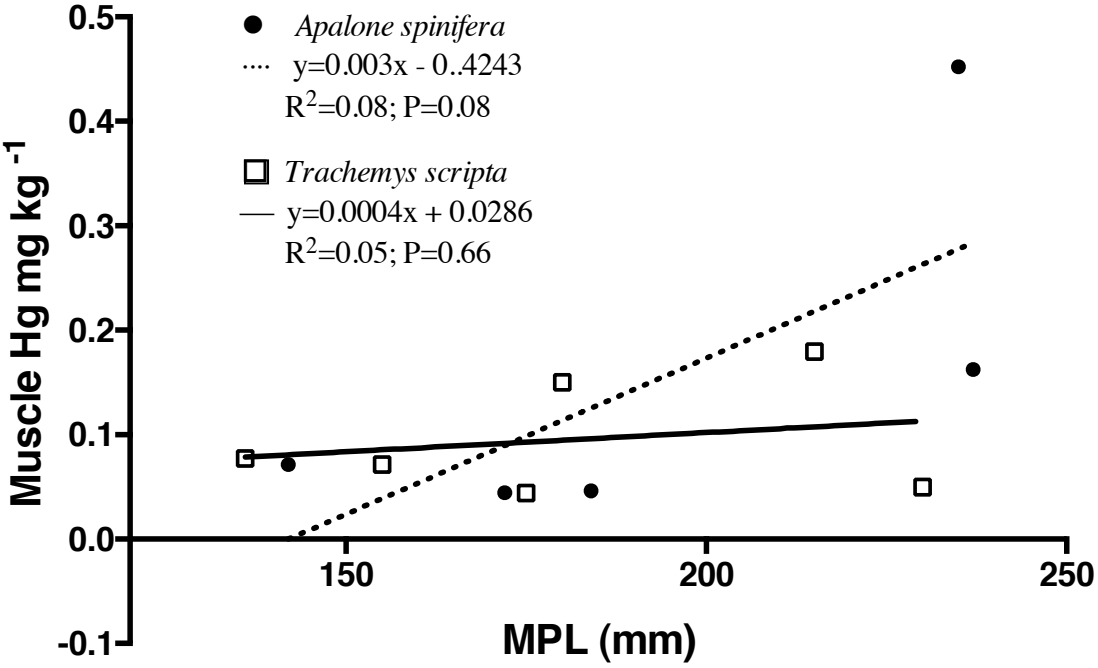

Figure 9. Linear regressions and associated equations illustrating relationship between Hg concentration in femoral leg muscle and size (maximum plastron length, MPL) *in Apalone spinifera* and *Trachemys scripta* species sampled during the summers of 2010 and 2011 at Lake McMurtry (Payne Co., Oklahoma).

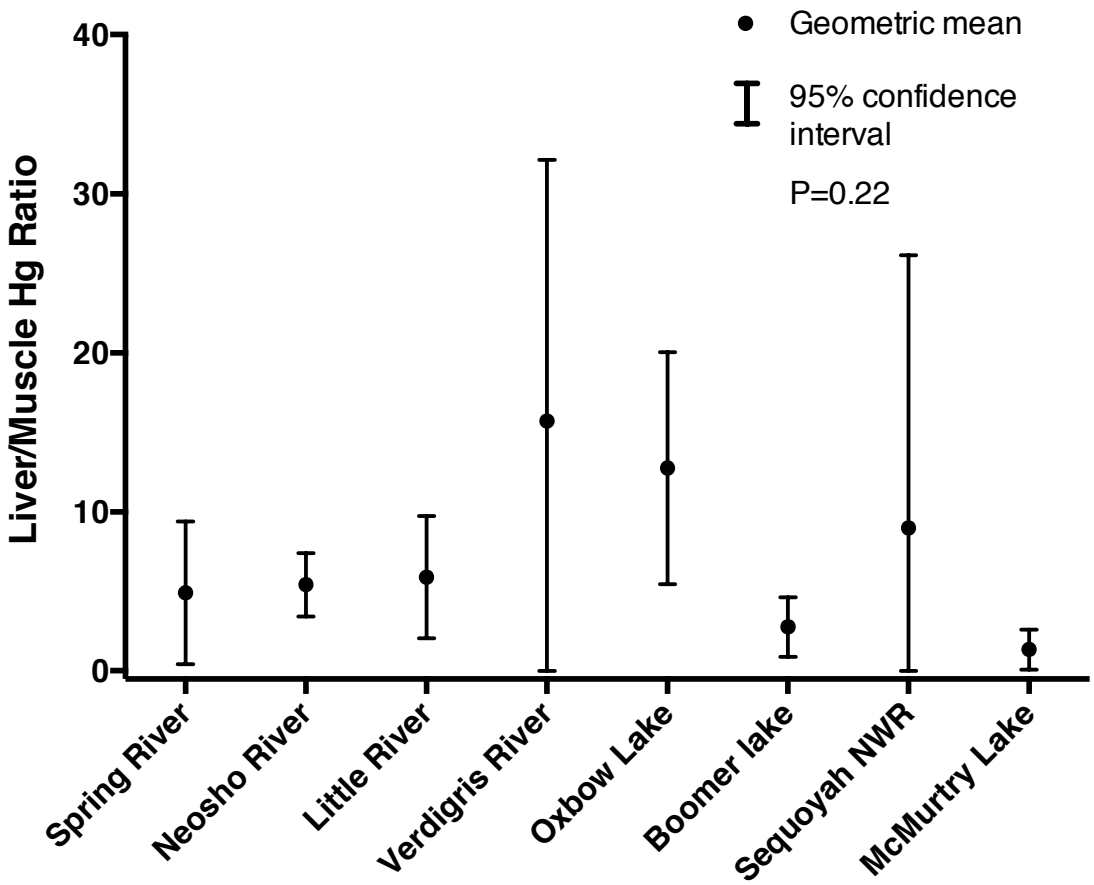

Figure 10. Liver/muscle Hg concentration ratio displayed for each site where *Trachemys scripta* were sampled from sites in eastern Oklahoma during the summers of 2010 and 2011. Bars indicate upper and lower 95% confidence intervals.

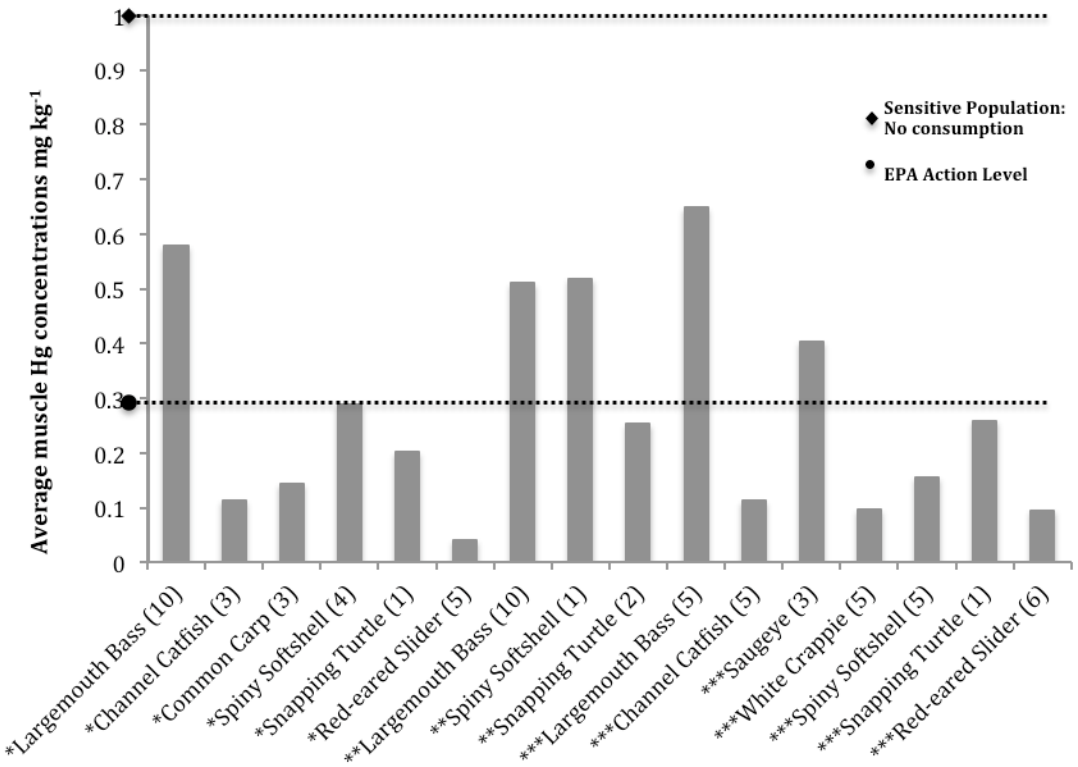

Figure 11. Comparison of fish and turtle Hg concentrations at three Oklahoma lakes and the corresponding consumption guidelines. Fish data provided by the Oklahoma Department of Environmental Quality. "()" indicate number of individuals. *Boomer Lake (Payne Co.), **Greenleaf Lake (Muskogee Co.), ***McMurtry Lake (Payne Co.). (OKDEQ, 2010)

VITA

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