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Trace element biodistribution in the American alligator (*Alligator mississippiensis*)

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HIGHLIGHTS

- Tissue distribution of trace elements in American alligators in Florida.
- Significant correlations among the tissues are described.
- Suggestions for improving biomonitoring efforts are made.
- Data confirms persistence of mercury contamination in Southern Florida alligators.
- The regional contamination of mercury is problematic in this alligator population.

GRAPHICAL ABSTRACT

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ABSTRACT

Routine monitoring of contaminant levels in wildlife is important for understanding chemical exposure and ultimately the link to ecosystem and human health. This is particularly important when the monitored species is recreationally hunted for human consumption. In the southeastern United States, recreational alligator harvesting takes place annually and in locations that are known to be contaminated with environmental pollutants. In this study, we investigated the biodistribution of trace elements in the American alligator (*Alligator mississippiensis*) from five sites in Florida, USA. These sites are locations where annual recreational alligator harvesting is permitted and two of the sites are identified as having high mercury contamination with human consumption advisories in effect. We utilized routinely collected monitoring samples (blood and scute), a commonly consumed tissue (muscle), and a classically analyzed tissue for environmental contaminants (liver) to demonstrate how the trace elements were distributed within the American alligator. We describe elemental tissue compartmentalization in an apex predator and investigate if noninvasive samples (blood and scute) can be used to estimate muscle tissue concentrations for a subset of elements measured. We found significant correlations for Hg, Rb, Se, Zn and Pb between noninvasive samples and consumed tissue and also found that Hg was the only trace metal of concern for this population of alligators. This study fills a gap in trace elemental analysis for

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reptilian apex predators in contaminated environments. Additionally, comprehensive elemental analysis of routinely collected samples can inform biomonitoring efforts and consumption advisories.

1. Introduction

The American alligator (*Alligator mississippiensis)* is a long-lived reptilian apex predator that resides in coastal freshwater and marsh habitats, ranging from the Gulf coast of Texas to the northern region of North Carolina. The foraging and nesting activities of the American alligator serve to create disturbance in the marsh ecosystem and increase biodiversity, making it an ecologically important keystone species (Kushlan, 1974; Kushlan and Kushlan, 1980; Palmer and Mazzotti, 2004). Alligators also serve as an indicator of ecosystem restoration throughout parts of their range, particularly in the Everglades, as the troubled alligator population in this region is associated with anthropogenic impacts on the native hydrology (Mazzotti et al., 2009; Mazzotti and Brandt, 1994). While some alligator populations at the extremes of their geographic range remain sparse, substantial increases in the number of individuals as a whole have been documented since the 1970's as a result of protection afforded by the Endangered Species Act and implementation of the Lacey Act (Hines, 1979; Joanen and McNease, 1987; Dutton et al., 2002). The alligator population has recovered to the point at which annual harvesting of alligators is currently permitted in many states. In Florida alone, over 18,000 alligators were harvested in 2014 as a result of recreational, nuisance and private harvesting, providing a significant contribution to the economy (FFWCC, 2015a; Hord et al., 1990). The recreational harvest removed approximately 7000 alligators with roughly 100 alligators coming from the Everglades region (FFWCC, 2015b). Recreational harvest results in consumption of animals from contaminated areas including the Everglades. This puts the human consumers at risk of exposure to the body burden of contaminants within each animal. In areas that are known to have elevated levels of contamination, such as the Everglades, there are advisories against consuming harvested animals including pig frogs (*Rana grylio*) and American alligators since the muscle Hg concentrations are above the U.S. Food and Drug Administration (FDA) limit of 1.0 mg/kg (FWC, 2006; Heaton-Jones et al., 1997). As regulations are not always followed by the public, routine and updated measurements of contaminants in wildlife tissues consumed by humans is important to environmental and public health (Edwards, 2013).

Recent studies have found that the environmental parameters of the Everglades ecosystem, particularly the hydro-period and soil bacteria composition, make it a hotspot for Hg methylation (DeAngelis et al., 1998; Julian, 2013). The rate of methylation taking place in the soil allows for bioaccumulation and biomagnification of mercury through the food web, with very high levels persisting in top predators, regardless of point source reduction or bioremediation efforts (Frederick et al., 2005; Rumbold et al., 2008; Axelrad et al., 2009). Despite the growing body of work surrounding Hg contamination in the Everglades ecosystem and food web, little is known regarding the biodistribution of mercury and other trace elements in American alligators or other apex predators residing in this ecosystem. Recent studies have shown that there are varying concentrations and correlations of trace elements (cadmium, selenium, chromium, manganese, arsenic, tin and mercury) in alligator hepatic, adipose, dermal and muscle tissue from different sites in Florida (Burger et al., 2000; Horai et al., 2014). However, blood samples were not included in the analysis (Burger et al., 2000). Inclusion of these routinely collected samples in monitoring a broad suite of trace elements will allow a more frequent analysis using less invasive techniques to show consumer exposure in different regions.

This study aims to expand the current knowledge base regarding trace element biodistribution in American alligators by using whole blood, liver, scute, and muscle tissues collected from 37 American alligators from recreational harvest areas across the state of Florida, including the Everglades. This project will assess the body burden of Hg, as it is a known contaminant of concern in this region, and 16 other trace elements to determine if other elements warrant further investigation. We aim to uncover the relationship between routine non-lethally-collected samples and the muscle tissue to improve the opportunity for assessing contaminant exposure for both alligators and the humans who may consume them.

2. Methods

2.1. Study area & sample collection

Thirty-seven American alligators were sampled in April of 2012 at five sites in Florida, representing areas of high and low Hg contamination based on previously measured biota (Burger et al., 2000; Rumbold et al., 2002). St. Johns River ($n = 3$) and Lochloosa ($n = 2$) have low Hg contamination; Lake Trafford has moderate contamination ($n = 18$); and the Everglades sites (WCA2A $n = 5$ and WCA3A $n = 9$) have high Hg contamination (Fig. 1) (Lange et al., 2000; Nilsen et al., 2016). The size, sex and location of capture for each animal are listed in Table S1. While these five sites are diverse in their levels of historic Hg contamination, it should be noted that they are also similar in many aspects. These sites are all within a narrow area of the alligators natural range, and are similar wetland ecosystems with similar trophic structures (Axelrad et al., 2009).

Alligators were handled by researchers from the Florida Fish and Wildlife Conservation Commission using methods approved by the American Society of Ichthyologists and Herpetologists (ASIH, 2004). Animals were classified as adults or subadults based on size, which was determined using the snout to vent length (SVL) (Table S1); sub-adults had an SVL $\geq$ 45 cm and <90 cm and adults had an SVL $\geq$ 90 cm, cutoffs that were established in a previous report in Florida pertaining to reproductive maturity per Woodward et al. (1992).

Whole blood samples were collected following the method described by Myburgh et al. (2014) and detailed in the Supplemental Information. Briefly, the blood samples were collected at the time of capture with Luer lock syringes and transferred to lithium-heparin blood collection tubes (BD), before being frozen at -80 °C until analysis. Blood was used in this analysis as it is a commonly collected sample, is non-invasive, and can be repeatedly collected for monitoring efforts.

Tissue samples, scute, muscle and liver, were collected and processed using the detailed method provided in the Supplemental Information and chosen based on the experiments described below. Briefly, all samples were placed on dry ice upon removal from the animal and subsequently stored at -80 °C until being

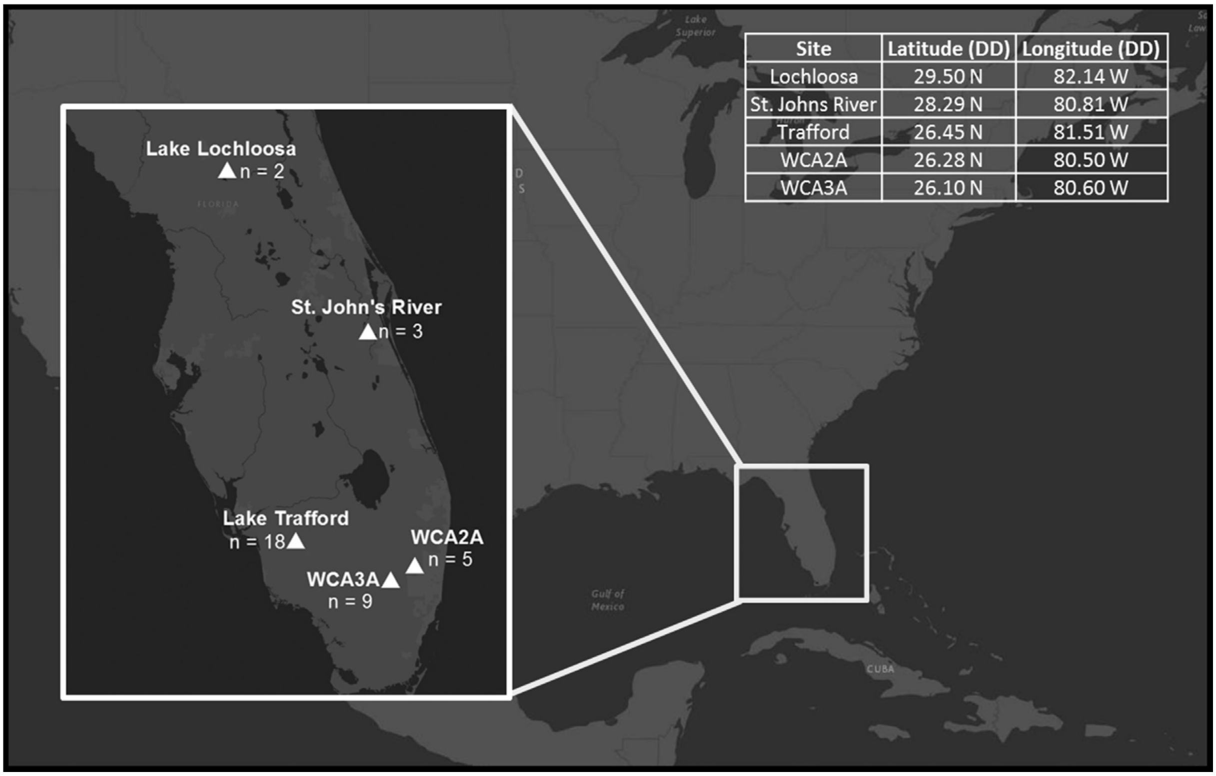

Fig. 1. Map and geographical coordinates of the five sites sampled for American alligators (*Alligator mississippiensis*).

cryohomogenized into fine powders prior to analysis (method detailed in the Supplemental Information).

2.2. Sample selection

To determine the most appropriate samples for trace element analysis, two experiments were conducted using total Hg as the model trace element. The first experiment investigated the homogeneity of Hg across the surface of the three commonly collected scutes from American alligators (Figs. S1, S2). The second experiment included all target organs for Hg, consisting of central nervous system (brain and/or spinal cord), heart, lung, liver, muscle, scute and whole blood, to determine the most effective tissues to analyze for this study (Table S2). See Supplemental Information for more detailed information.

Whole blood, muscle, liver and scute (three caudal scutes (2 cm section) to be pooled) samples were collected from each animal for a total of 37 samples of each tissue, except scute, which only had 30. There were less scutes available for this experiment as some animals did not have all 3 caudal scutes present, either due to previous marking or loss in the environment. Scutes are commonly removed from alligator as a means of identifying individuals; muscle is commonly consumed by people in this region; and liver is a commonly monitored tissue as it sequesters the contaminants that the body cannot detoxify and can provide valuable information about the health status of the animal.

2.3. Sample preparation for ICP-MS

Whole blood samples were thawed to room temperature, gently mixed prior to analysis. Tissue powders (scute, muscle and liver) were kept frozen, using liquid nitrogen, while a sub-sample was taken. Approximately 0.5 g of sample (both, blood and tissue powder) was weighed into a pre-cleaned Teflon CEM microwave digestion vessel (CEM Corporation, Matthews, NC, USA).

Blood and tissue samples were treated identically in the digestion procedure, where internal standards consisting of Scandium (Sc), Yttrium (Y), and Ruthenium (Ru) (250 ng) were gravimetrically added to the digestion vessel, followed by 3.5 mL of nitric acid. The samples were digested using a CEM Microwave Accelerate Reaction System (MARS) Xpress (Matthews, NC). The microwave program was set to ramp for 10 min to 125 °C, hold for 5 min, then ramp for 5 min to 210 °C with a hold of 15 min, followed by a 15 min cool down. The resulting digest was transferred to a pre-cleaned 50 mL centrifuge tube and diluted to approximately 50 g with the addition of 1.5 mL hydrochloric acid for element stabilization. The control materials, NIST SRM 1577c Bovine Liver and Seronorm Trace Elements Whole Blood L-3 reference material replicates, were weighed, digested and analyzed under the same conditions.

2.4. Reference materials

National Institute of Standards and Technology (NIST) 3100 Standard Reference Materials (SRM) (Gaithersburg, MD, USA) series primary standard solutions were utilized in inductively coupled plasma mass spectrometric (ICP-MS) analysis. Control materials utilized included SRM 1577c Bovine Liver (NIST), SRM 955c Toxic Metals in Caprine Blood (NIST), and Seronorm Trace Elements in Whole Blood L-3 obtained from Sero AS (Billingstad, Norway; Lot #1112691). Nitric acid Optima Grade (67–70%) and hydrochloric acid Optima Grade (32–35%) were purchased from Fisher Scientific (Waltham, MA, USA). Millipore 18 MΩ water (Millipore EMD, Darmstadt, Germany) was used in all experiments.

2.5. Elemental analyses

All samples were analyzed on a Thermo X2 quadrupole inductively coupled plasma mass spectrometer (ICP-MS) system (Thermo

Fisher Scientific, Waltham, MA, USA), equipped with a standard introduction system and an ESI SC4 autosampler (Elemental Scientific, Omaha, NE, USA). The ICP-MS was operated in kinetic energy discrimination mode with a collision gas of 1% ammonia in balance helium, operating at 3.0 mL/min or 8% H_2 in balance helium, operating at 4.0 mL/min. The addition of collision gas serves to reduce plasma and matrix based interferences as well as decreases the transmission of low kinetic energy polyatomic ions. The method utilized peak jumping, with each of five replicate runs consisting of 50 sweeps and a dwell time of 25 ms. The instrument monitored the signal from aluminum (Al), vanadium (V), chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), rubidium (Rb), strontium (Sr), molybdenum (Mo), cadmium (Cd), tin (Sn), lead (Pb), and the internal standards, scandium (Sc), yttrium (Y), and, ruthenium (Ru).

The working calibration stock solutions were prepared by gravimetric dilution and external ten-point calibration curves were constructed ranging from 0.5 ng/g to 1000 ng/g. Individual elemental curves were defined by the data set, resulting in a five-point calibration curve within the sample range. Analytical signals were corrected for blank contributions. A first order fit was applied to the data, where the slope and intercept from the calibration curves were based on the measured isotopic responses from SRM calibration solutions and utilized to calculate the mass fraction of trace elements in the samples.

The mass fraction of Hg was determined with a direct mercury analyzer DMA-80 (Milestone Scientific, Shelton, CT). One aliquot of approximately 100 mg of alligator whole blood or tissue powder was analyzed with the direct combustion atomic absorption spectroscopy. NIST SRM 3133, Mercury Standard Solution was utilized for external calibration and SRM 955c (Level 3) was used as a control material for the Hg determinations (Table S3).

2.6. Statistical analysis

Means were calculated for each individual element. The statistical analysis was carried out using JMP (Cary, NC, USA). The elements that were found to be above the limit of detection did not demonstrate a normal distribution or equal variances across the population of alligators sampled. A log10 transformation was used as it was the most appropriate for this data structure, but it did not improve either assumption for all elements. Since the assumptions of parametric statistics were not met, non-parametric analyses were used. The tissue data were compared using the Spearman Correlation. Because sample sizes were uneven for age class, sex and location, statistical analyses were only completed on the entire sample set. The element and tissue rankings provided in Table 1 were completed by comparing the means and ordering them from low to high. The correlation data was analyzed without using values that were below the LOD. For this experiment, we elected to remove the values rather than keep them or substitute them with an LOD value calculation, as an incorrect low number or a substituted number than is similar to all other replaced values for that element would change the underlying data structure and increase the likelihood of obtaining a false result from the analysis.

3. Results & discussion

3.1. Quality assurance/quality control

Seventeen trace elements were analyzed for each tissue type, the data represented herein include data that were found to be above the limit of detection and the appropriate control material was within acceptable margin of error provided in the product certification (RSD $\leq$ 20%) (Table S3). The elements that were removed from analysis were as follows – for whole blood: V, Cr, Mg, Co, Mo, Cd and Sn; for liver: Ni, As and Sr; for scute: Mg, Ni, As, and Sr; and for muscle: Ni, As, Sr, Mg, Mo and Cd.

3.2. Analysis of elemental concentrations

Trace element concentrations for all muscle (n = 37), liver (n = 37), blood (n = 37) and scute (n = 30) analyses from the American alligator are presented in Supplemental Table 4. It is important to note that many elements are necessary for biological function, including some of the trace metals reported here. Eight trace elements measured in these samples are known to be essential in low concentrations to a variety of physiological processes for humans, most notably V, Cr, Mn, Co, Cu, Zn, Mo, and Se (Fraga, 2005). Therefore, these elements will always be present in organisms, regardless of environment and it is the elevated concentration, not the presence of these elements, that can lead to toxicity (Fraga, 2005). The necessary concentration for proper biological function of these elements in humans is 100 mg/day or less, which is far less than the daily requirement for other elements, such as potassium (K), which is 4700 mg/day (Fraga, 2005). Other trace elements, such as the toxic heavy metals, Pb, As, Cd and Hg, are derived from the environment and are biologically detrimental as they have no physiological purpose (Davison et al., 1974; Salomons, 1995).

Blood samples are a non-invasive and routinely collected sample for many monitoring efforts. The elements measured in this tissue were Zn, Rb, Cu, Hg, Se, Sr, Pb, As, Al and Ni (ordered from greatest to lowest concentration). Of these ten elements, only three are toxic (Hg, Pb and As). Both Hg and Pb are above the U.S. Center for Disease Control and Prevention's (CDC) toxic level of blood concentrations, indicating that alligators in Florida may be susceptible to the delirious effects of these metals (10 ng/g and 100 ng/g, respectively). Nickel, As, and Sr were only detected in the blood samples and in no other tissue, suggesting that these elements are in low enough concentrations that can be metabolized and excreted.

Scute samples were analyzed as they are commonly removed from alligators for individual identification and genetic analysis. The elements measured within the scutes were Al, V, Cr, Co, Cu, Zn, Se, Rb, Pb and Hg (Table S4). Zinc and Rb were in the greatest concentrations in this tissue as well. Chromium, Al, and Co were found in greatest concentration in the scute samples compared to the other tissue types (Table 1 & Table S4). These data suggest that Cr, Co and Al are potentially stored and eliminated via keratin, which has been demonstrated in Antarctic leopard (*Hydrurga leptonyx*), Baikal (*Pusa sibirica*) and Caspian (*Pusa capica*) seals during molting (Gray et al., 2008; Ikemoto et al., 2004).

Muscle tissue was analyzed as this is the compartment that humans consume and these measurements can directly inform human health monitoring efforts. The elements measured in this tissue were Al, Cr, Co, Cu, Zn, Se, Rb, Pb and Hg. Vanadium was only found in the muscle of animals from the Everglades, at concentrations that are much lower than have been shown to cause biochemical problems in rodent muscle which suggesting that it is not at high enough concentrations to be of concern (Table S4) (Fürnsinn et al., 1996). Lead and Se were lowest in concentration in the muscle (Table 1). Selenium's low concentration is of particular interest, since it has detoxifying effects on Hg when the molar ratio of the two elements is below 1 (Ralston and Raymond, 2010; Raymond and Ralston, 2004). Mercury was measured to be three times the concentration of Se and the molar ratio is 1.2 for all muscle samples, which is problematic for consumers (Ralston and Raymond, 2010).

Liver samples are a commonly measured tissue for

Table 1
The median tissue concentration, minimum and maximum (ng/g, w/w), and average ranking for the six trace elements with statistically significant correlations in American alligator (*Alligator mississippiensis*) samples.

Element	Blood	Muscle	Liver	Scute	Tissue Rank
Se	179.4 (95.7–193.7)	80.9 (18.5–177.5)	1738.8 (899.7–4616)	153.9 (59.4–291.4)	M < S < B < L
Hg	193.7 (56.4–1380)	243.1 (45.3–1183)	3594.1 (566.8–14293.0)	318.5 (62.2–1965.9)	B < M < S < L
Rb	525.8 (389.4–1251.9)	2893.8 (1203.1–8812.3)	3760.6 (2063.0–10507.3)	750.8 (463.9–1841.2)	B < S < M < L
Zn	781.1 (379.3–1227.5)	10443.1 (5180.5–30640.7)	20145.1 (12396.8–33759.5)	4839.8 (3247.5–6428.9)	B < S < M < L
Pb	126.5 (33.3–4470.4)	9.1 (0.2–123.6)	75.2 (4.8–12050.2)	29.6 (0.8–415.6)	M < S < B < L
Al	40.7 (0.8–108.0)	163.1 (8.5–1744.2)	1604.5 (129.3–12064.1)	2015.5 (480.7–21525.8)	B < M < L < S
Elemental Rank	Al < Se < Hg < Pb < Rb < Zn	Pb < Se < Al < Hg < Rb < Zn	Pb < Se < Al < Hg < Rb < Zn	Pb < Se < Hg < Rb < Al < Zn	

environmental contaminants, since the liver is a sequestration site for contaminants the body attempts to detoxify (Gray et al., 2008; Ikemoto et al., 2004; Wayland et al., 2001). More elements were measured in this tissue than any other and included Al, V, Cr, Mn, Co, Cu, Zn, Se, Rb, Mo, Cd, Sn, Pb and Hg (Table S4). Mercury concentrations were highest in the liver, compared to the other tissues (Table S4). The concentrations of Hg provided herein demonstrate that Hg is persistent across all tissues and sites sampled, with great bioaccumulation potential in this species. While Hg was not the trace element in the highest concentration in these samples, it is important to note that it is the highest in concentration for the toxic heavy metals (including Pb, As, Cd). Elements V, Sn, Cd and Mo were found in concentrations less than 200 ng/g in liver samples, which are low concentrations for these elements. The concentrations measured here for the essential trace elements (Cu, Mn, Mo, Zn) are similar to those measured by Almli et al. (2005) in hepatic tissue of another crocodilian apex predator, the Nile crocodile (*Crocodylus niloticus*) in Zambia. The pattern of elemental concentrations is consistent with two other studies in American alligators; however, apparent differences in the studies (i.e. laboratory based, age class, sex, tissue, and dry weight measurements) make direct comparisons difficult (Lance et al., 1983; Tuberville et al., 2016).

In the present study, Hg was found in higher concentrations than Pb in all tissues, except blood samples, which appear to be skewed by a few abnormally high concentrations ($n = 3$) across all animals sampled (Table S4). An individual alligator liver had a Pb concentration that was an order of magnitude greater than the other samples. This value was removed from the medians discussed here, but reported in Table S4. The overall median for both Pb and Hg in scute was 29.7 ng/g and 243 ng/g; in blood was 127 ng/g and 194 ng/g; in muscle was 9.10 ng/g and 328 ng/g; and in liver was 71.3 ng/g and 3590 ng/g, respectively. The concentrations of Pb observed in this study are above the U.S. CDC toxicity limit of 100 ng/g in blood at 129 ng/g, but less than those reported in the liver of ringed seals (*Phoca hispida*) in the Arctic, and the muscle concentrations are similar to what has been observed in canned tuna (Dietz et al., 1996; Voegborlo et al., 1999). The Hg concentrations however are of a greater concern as the muscle concentrations are similar to the concentrations found in Arctic cod (*Arctogadus glacialis*) and canned tuna, all of which are approaching the 0.5 mg/kg mark of infrequent consumption by the U.S. FDA, and are over the 0.3 mg/kg limit for the Danish food standards (Dietz et al., 1996; Voegborlo et al., 1999).

In terms of environmental contamination concerns, Almli et al. (2005) reported Hg concentrations in hepatic tissue of the Nile crocodile in Zambia that were approximately 1000 ng/g lower than the alligators from Florida, which suggests that Hg is a greater contamination problem in Florida as described in the introduction (Table S4). Lead concentrations, however, were three to eight times greater in hepatic tissue in Zambia Nile crocodiles than the values observed here in Florida American alligators, suggesting that Pb is of greater concern in Zambia than Hg, where hunting ammunition runoff is a larger problem. These studies demonstrate the environmental effect of heavy metal contamination on endogenous wildlife species (Almli et al., 2005; Heaton-Jones et al., 1997; Hord et al., 1990; Nilsen et al., 2016; Rumbold et al., 2002, 2008; Yanochko et al., 1997).

3.3. Tissue correlations

As many studies do not incorporate more than one tissue, and historic studies use almost exclusively liver tissue, determining if a relationship exists between these tissues for trace elements of concern will enable to inter study comparisons, as well as benefit monitoring efforts. Correlations of elemental concentrations among the tissues were tested using a Spearman correlation with statistical significance defined as a $p < 0.05$. This analysis found six elements to have statistically significant correlations in the alligator samples, Al, Rb, Zn, Se, Hg, and Pb (Table 1, Table S5). While concentrations are dependent on the element and the tissue, concentration rankings were established, as shown in Table 1. Selenium (Se) and Pb had the lowest concentrations in the muscle tissue, whereas the blood contained the lowest for Hg, Se, and Al. These data suggest that Se and Pb are not bioaccumulated in the muscle tissue, even with the higher concentrations that were found in the blood. Previous studies of Pb in alligator tissue found similar results with the lowest concentrations noted in muscle (Burger et al., 2000). Predictively, the lowest concentrations of most elements were determined in the blood, as observed with other contaminants, since blood is a dynamic biofluid reflective of circulating contaminants and thus has a low accumulation period comparatively (Björkman et al., 2007; Keller et al., 2004). The highest concentrations of Hg, Rb, Zn, Pb and Se were obtained from the liver tissues, further supporting previously reported high Se and Hg concentrations in the liver (Burger et al., 2000). Zinc was found to be the element of highest concentration across all four tissues, followed by Rb in blood, muscle and liver, but by Al in scute (Table 1).

The rankings for Hg, Rb, Zn, and Al were consistent, suggesting that the biodistribution of these elements does not change, despite the site-specific differences in this data set (Table S4).

The data for all elemental concentrations were log transformed to determine tissue patterns in this specific study (Fig. 2A). Liver tissues generally had the highest concentration for most elements (9 of 17 elements), which was expected as the liver is a detoxification organ and is known to bioaccumulate high concentrations of many elements (Wayland et al., 2001). For the six elements with significant correlations (Fig. 2B), Se and Hg appear to co-vary across all four tissues analyzed, a relationship that has been previously described for many animals (Burger and Gochfeld, 2011; Campbell et al., 2010; Fang et al., 2011; Lance et al., 1983; Smith and Armstrong, 1978; Storelli et al., 1998). Selenium is thought to have a protective effect against the toxicity of mercury, which may explain the covariance observed here (Fig. 2B) (Ralston and

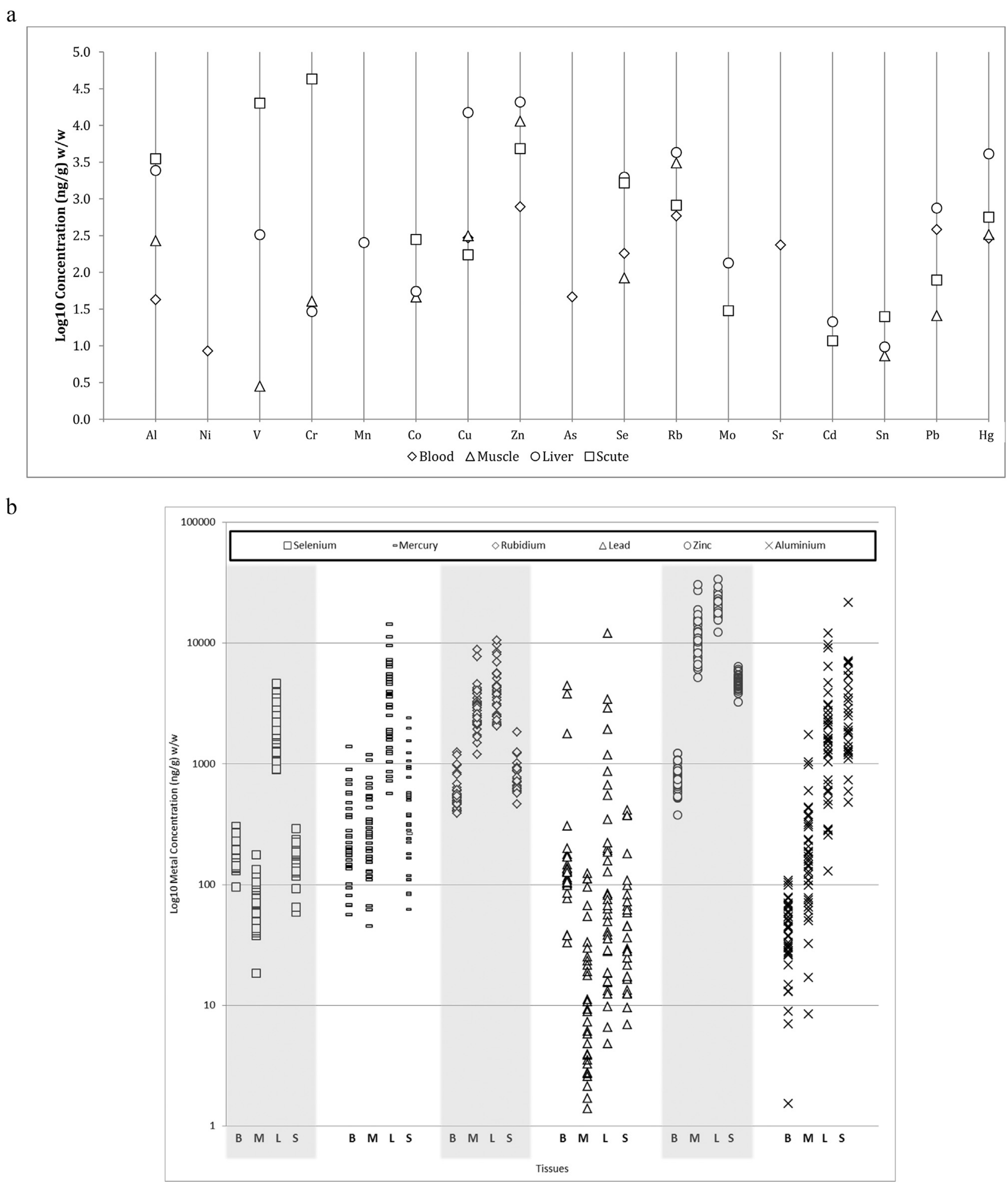

Fig. 2. a. Scatter plot of the log transformed tissue averages of each trace element measured in American alligator (*Alligator mississippiensis*) tissue samples. b. Scatter plot of the statistically significant tissue correlations for trace elements in American alligator (*Alligator mississippiensis*) samples. B, M, L and S represent blood, muscle, liver and scute, respectively.

Raymond, 2010; Raymond and Ralston, 2004). Rubidium and Zn also appear to have the same order in biodistribution, highest in liver and muscle and lowest in blood (Fig. 2A and B). This potentially is an effect of both elements being present in the alligator's main prey items at all locations and bioaccumulation within the tissues. Lead and Al do not appear to co-vary with any of the other four elements (Se, Hg, Rb and Zn) that had significant tissue relationships (Fig. 2B). Aluminum was significantly related to the liver and scute tissue (Table S5) but the effect of Al on reptiles is not well documented in the literature. High concentrations of Al in humans

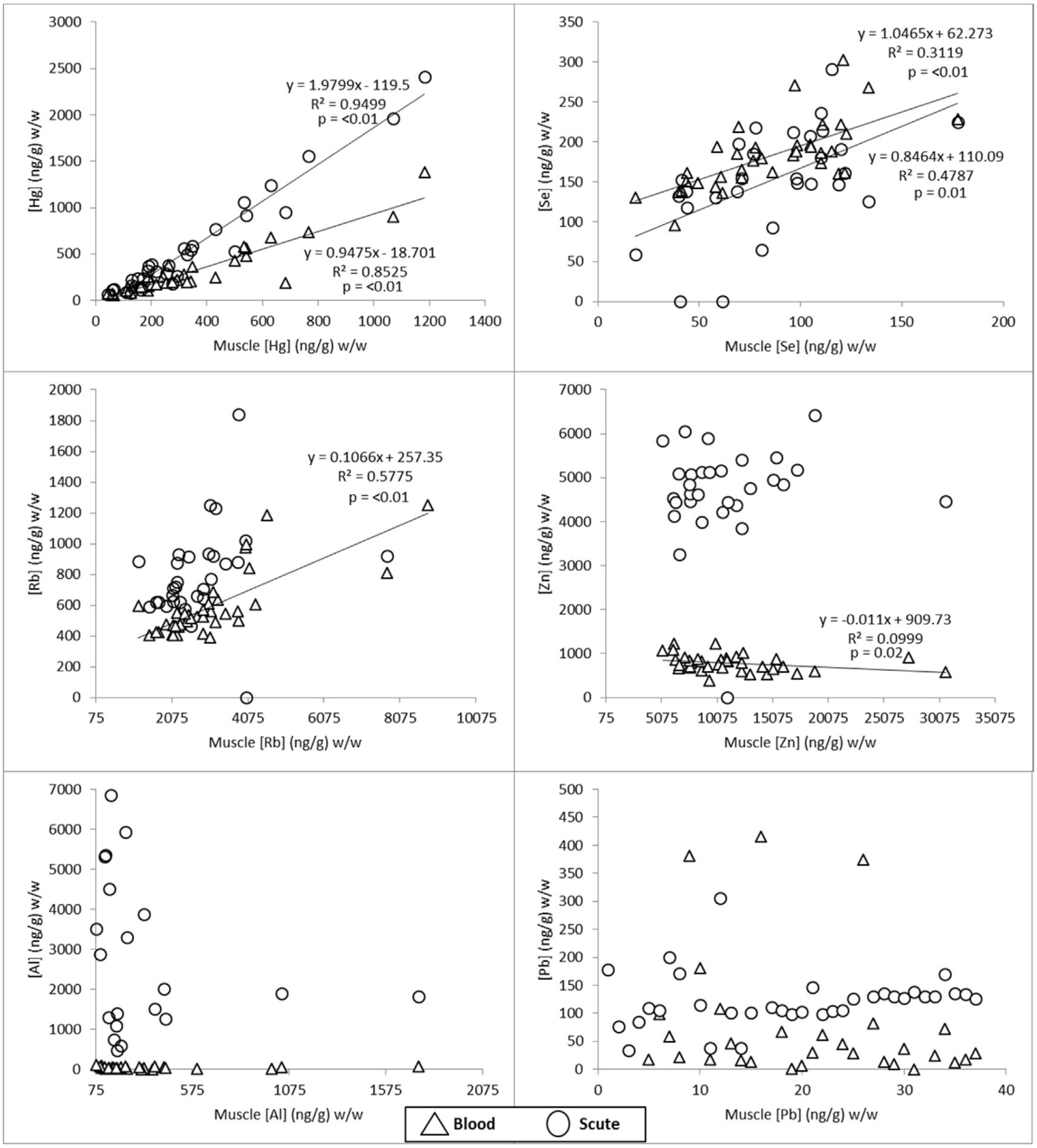

Fig. 3. American alligator (*Alligator mississippiensis*) tissue correlations (whole blood and scute tissue vs. muscle tissue) for trace elements (Hg, Se, Rb, Zn, Al, Pb), where statistically significant relationships were observed (Table S5). Trend lines, equations and R values are given for those tissues that had a statistically significant correlation.

has been associated with certain disease outcomes such as Alzheimer's disease, but the concentrations we detected here are less than those in the human study and are found in different tissues (Mjöberg et al., 1997). Lead concentrations, however, have been consistently lower than other trace elements measured in various reptiles (Hopkins et al., 2001; Loumbourdis, 1997) so our results are not irregular and demonstrate that there is likely no source of Pb contamination in the area of the sampled sites.

3.4. Using the relationships for biomonitoring

Blood and scute samples can be routinely collected for monitoring wild populations without sacrificing animals (Lázaro et al., 2015; Myburgh et al., 2014). Only four elemental concentrations within the muscle tissue can be discerned from these routine samples; Hg, Se, Rb and Zn (Table S5). Blood sample concentrations can be used to infer the muscle concentration of Hg, Se, Rb and Zn, while scute sample concentrations can be used to infer the muscle concentration of Hg, Se and Rb (Fig. 3). The association of the concentration between scute and muscle we provide in Fig. 3 are based on statistical significance ($p \leq 0.05$). Thus, the concentration in scute could be used for estimating the muscle burden of each trace element in alligator populations but it should be done with caution, as the correlation for Hg is much more direct than it is for Rb, Se or Zn. These equations are provided to aid in the routine monitoring of alligators but not replace the periodic analysis of the internal tissues to verify the relationship provided here. The linear relationship we see in this study would likely change based on habitat characteristics and prey consumption in different locations.

The biodistribution trends demonstrated with these samples for the six trace elements: Hg, Se, Rb, Zn, Pb and Al are the first reported, specifically using routinely collected non-invasive monitoring samples for American alligators. The correlative relationships observed in this study for Hg are mirrored in other reptilian studies in blood and scute samples from sea turtles (*Caretta caretta*) and with blood, keratin, liver and muscle of the black caiman (*Melanosuchus niger*) from the Amazon (Day et al., 2005; Eggins et al., 2015). A relationship for Hg, Se, and Rb, as well as several other elements, has been observed in blood and skin samples from bottlenose dolphins (*Tursiops truncatus*) (Bryan et al., 2007). Previous studies have measured trace metals (Ca, Zn, Mg, Se, Cu and Fe; As, Se, Cd, Se and V) within alligators; however, they utilized other tissues that are not included in this study (plasma only), or calculate the measurements based on dry weight, so a direct comparison cannot be made (Lance et al., 1983; Tuberville et al., 2016). On the other hand, the trends and values observed previously for Zn and Se, in both liver and blood samples, are reflected in the data reported here. This pattern suggests that these essential trace elements are consistent for reptiles, including the American alligator and Nile crocodile (Almli et al., 2005). Zinc and Se do not appear to fluctuate as a result of local contamination of other metals as shown in a study using painted dragons (*Agama stellio stellio*), since they are tightly regulated (Almli et al., 2005; Lance et al., 1983; Loumbourdis, 1997). Other reptilian studies generally focus on smaller, softer bodied reptiles with shorter lifespans than alligators, which make comparisons difficult (Hopkins et al., 2001; Loumbourdis, 1997). Squamate reptiles have shown a similar correlative relationship (between liver, kidney and gonad) to the relationship between liver and kidney in American alligators shown previously and to those that we highlight in this study (Hopkins et al., 2001; Tuberville et al., 2016). However, the concentrations in the present study are orders of magnitude higher than those reported for the squamate reptiles (Hopkins et al., 2001). The alligator is an apex predator bioaccumulating a lifetime of exposure in a vastly different ecosystem.

4. Study limitations

Unfortunately, due to the group characteristics of this sample set, including uneven age and sex distributions as well as small sample sizes at some locations, group specific analyses were not reasonable. However, there are some emerging patterns in this data set worth mentioning. Some differences in elemental concentrations were observed between the two age classes. However, the majority of the sub-adult animals were collected from the Everglades sites which are known to have higher mercury concentrations in the environment, as well as altered hydrology and increased anthropogenic input (WCA2A and WCA3A, Fig. 1, Table S4) (Cleckner et al., 1998; DeAngelis et al., 1998). In contrast, the adult samples were collected from Lake Trafford where a 'biodilution' of Hg has been observed previously with concentrations decreasing as the trophic level increases (*Rumbold, pers. comm.*;-Ware et al., 1990; Fig. 1). These two sites represent opposite ends of the Hg contamination spectrum in Florida, whereas St John's River and Lochloosa display predictable bioaccumulation of Hg. The differences between the sites observed in this preliminary comparison could also be due to dietary differences at each site. Without sampling stomach contents of alligators from each location, the importance of dietary differences on trace element analysis will remain a significant question, and could be the focus of future studies.

5. Conclusions

The trace elemental analysis reported here provides insight to the trace elemental burden in the American alligator and the biodistribution of the elements across tissues including blood, liver, scute and muscle. Six elements, including Se, Hg, Rb, Zn, Pb and Al were determined to be biodistributed in the samples and non-invasive tissues (blood and scute) can estimate these elemental concentrations in muscle. Importantly, the levels of many elements in the muscle, the primary tissue for human consumption, were found to be below the European Union and U.S. FDA guidelines for human consumption, with the exception of Hg. We recommend future studies investigating the differences in the elemental burden between age class, sex and location to be conducted with larger sample sizes of the Florida alligator population.

Disclaimer

Certain commercial equipment, instruments, or materials are identified in this paper to specify new technological advancements in the field. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology; nor does it imply that the materials or equipment identified are necessarily the best for the purpose.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2017.04.102.

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