SITE SPECIFIC ONTOGENETIC DRIVERS OF MERCURY CONCENTRATIONS IN AMERICAN ALLIGATORS

by

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(Under the Direction of James E. Byers and Benjamin B. Parrott)

ABSTRACT

Mercury (Hg) is a ubiquitous ecological contaminant, with elevated exposure risk stemming from anthropogenic activity. Due to the propensity of certain metals to bioaccumulate over time and biomagnify across trophic levels, long-lived apex predators can carry significant body burdens in affected ecosystems. Yet, how interactions between organismal ontogeny and habitat contribute to variation in exposure is not well resolved. Total Hg, carbon ($\delta^{13}C$), and nitrogen ($\delta^{15}N$) isotopic ratios were assessed in blood samples (n=133) across three distinct habitats in the southeastern US to investigate how size and dietary shifts in the American alligator (*Alligator mississippiensis*) influence Hg accumulation. Collectively, findings demonstrate that whereas Hg concentrations in *A. mississippiensis* are primarily driven by site-level dynamics, diet and size are linked to individual variation within populations, suggesting that organismal ontogeny interacts with site-specific contamination and ecological factors to affect Hg body burdens. Further exploration of patterns associated with individual variation is recommended.

INDEX WORDS: Ecotoxicology, Bioaccumulation, Biomagnification, Contaminant Load, Bioindicator, American Alligator, Apex Predator, Stable Isotopes, Diet, Ontogeny

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DEDICATION

For my Mother, Kendra Lynn Geffert my Grandmother, Marcia Draft, and my sister, Erika who never questioned my love for scaly critters, and were my biggest cheerleaders.

TABLE OF CONTENTS

Page

CHAPTER

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Environmental and Ecological Drivers of Mercury Contamination

Anthropogenic activity alters the distribution of naturally occurring heavy metals, which can lead to elevated exposure risk and consequential impacts to organismal health (Selin 2014, Nilsen et al. 2016, Bjørklund et al. 2017). Mercury (Hg) is one of several widely distributed heavy metals known to have adverse effects on human and wildlife health, including neurological and behavioral disorders, reproductive issues, and in extreme cases, death (Stepanauskas et al. 2005, Magos and Clarkson 2006, Bjørklund et al. 2017). Though a naturally occurring element, most environmental inputs of Hg stem from atmospheric releases of Hg as a waste product of industrial activity (e.g., waste incineration) resulting in contaminated precipitation and/or runoff (Nguyen et al. 2008). Spatial patterns of environmental Hg contamination are driven by proximity to point sources, meteorological patterns of wind and precipitation, as well as regional hydrology (VanArsdale et al. 2005, Biester et al. 2007). This heterogeneity can result in hotspots of contamination, in which Hg interacts with local ecological factors to affect organismal exposures (Evers et al. 2007). Thus, to predict the concentrations of contaminants in wildlife, it is important to determine what factors increase exposure and body burdens in organisms both within and across different ecological systems.

For many species, accumulation of Hg is dependent upon the extent of exposure and the extent of Hg methylation. Although impacts of Hg exposure can be detected in terrestrial systems, organisms inhabiting wetlands are typically at greater risk from Hg exposure as inorganic mercury (Hg0, Hg2+) is converted to its bioavailable form, methylmercury (MeHg) through metabolic processes of aquatic bacteria (Compeau and Bartha 1985, Fleming et al. 2006). MeHg, a potent neurotoxin, is more quickly absorbed through biological membranes, not easily excreted, and can accumulate in high concentrations, especially in upper trophic level species (Zillioux et al. 1993, Mazrui et al. 2016). Saltwater and brackish wetlands have longer retention of MeHg due to interactions with chlorine, resulting in greater bioavailable fractions when compared to freshwater wetlands (Gilmour and Henry 1991, Benoit et al. 2003, Ulus 2021). However, anaerobic, acidic freshwater wetlands across the Southeastern U.S. are also known to experience higher rates of Hg methylation due to increased atmospheric deposition, altered pH, humidity, soil composition, and hydroperiod (Rudd 1995, Porvari and Verta 2003). In particular, the low pH observed within swamps plays a dominant role in altering the speciation of mercury, resulting in higher ratios of bioavailable MeHg (Gilmour and Henry 1991, Branfireun et al. 1999). Once methylated, Hg is integrated from the surrounding environment into biological systems primarily through ingestion and adherence to organic matter, resulting in a high affinity for accumulation in fatty tissue (Nakada and Imura 1983, Ravichandran 2004).

1.2 Bioaccumulation and Biomagnification of Mercury Contaminants

In addition to ecological influences on Hg speciation and bioavailability, Hg accumulation may also be driven by life history patterns and organismal ontogeny (Wiener and Spry 1996, Butler et al. 2022). Studies have repeatedly observed the bioaccumulation of Hg in correlation with age, where older individuals routinely sustain higher body burdens, for example in fishes, birds, and marine mammals (Grieb et al. 1990, McHuron et al. 2016, Kucharska et al.

2019, Bighetti et al. 2021). Furthermore, patterns of biomagnification have been observed in correlation with diet and trophic level, typically resulting in higher concentrations in apex predators (Gnamuš et al. 2000, Teffer et al. 2014, Cardoso et al. 2014). Biomagnification is more readily observed in multi-level food webs where body burdens of organisms occupying lower trophic levels are consumed by organisms at higher trophic levels. Interestingly, many species display changes in feeding ability and habits with age and size, resulting in ontogenetic variation across multiple scales, for example growth, development, metabolic processes, sexual maturation, morphology, physiology, and senescence. Together, these concepts of bioaccumulation and biomagnification suggest that two very important factors of an organism's life history impact their body burdens of toxins: age and diet.

Recently, studies have aimed to identify patterns of Hg transfer using ontogenetic traits in species that can function as bioindicators of ecosystem health (Finger et al. 2016, Lemaire et al. 2021b, Haskins et al. 2021, Moore et al. 2022). Crocodilians are long-lived predators, and ecotoxicological studies in these species have demonstrated the potential of contaminants to both bioaccumulate and biomagnify (Rainwater et al. 2007, Lawson et al. 2020, Lemaire et al. 2021). The geographical range of American alligators (*A. mississippiensis)* extends throughout the Southeastern US from Texas to Florida, and as far north as Virginia (Fig. 1), where individuals inhabit diverse wetland habitats including freshwater lakes, ponds, rivers, blackwater swamps, and coastal impoundments surrounded by seawater. Due to this diversity of habitats and their generalist diets, alligator populations are likely to utilize distinct prey bases depending on available resources. For example, whereas coastal populations have been reported to feed in marine environments (e.g., crabs, saltwater fish, and elasmobranchs) (Rosenblatt et al. 2013, Nifong 2014), populations inhabiting freshwater swamps customarily lack these resources.

(Delany and Abercrombie 1986, Delany et al. 1999, Bondavalli and Ulanowicz 1999). In addition, alligators are likely to utilize different prey resources as they grow from hatchlings and juveniles (roughly 12-125 cm total length) to sub adults and adults (>125cm total length) (Delany 1990, Saalfeld et al. 2008, Nifong et al. 2015). For example, American crocodile (*Acutus crocodylus*) hatchlings typically feed on small invertebrates prior to shifting to larger prey items as they grow (Platt et al. 2006). While size can be an indication of alligator age in the early years, evidence of determinate growth has been discovered (Rainwater et al. 2022). Consequently, it can be increasingly difficult to determine the exact age in long-lived adults unless they are part of a long-term study or have been marked for identification. Bioaccumulation with age, therefore, can be slightly more difficult to pinpoint, and requires more exploration. Nevertheless, the extent and relative contributions to which Hg body burdens in alligators reflect variation in age, size, habitat type, and diet are largely unresolved.

Maternal transfer of Hg is also observed in crocodilians (Rainwater et al. 2002, du Preez et al. 2018) and is likely to influence both maternal and offspring body burdens. For example, reproductively active females should theoretically have lower concentrations than males as they offload Hg into eggs (Nilsen et al. 2020). However, some studies report similar Hg concentrations across sexes, suggesting that other factors also contribute to body burdens (Burger et al. 2000, Eggins et al. 2015). In addition, how maternal transfer of Hg and resource provisioning affects offspring body burdens are not well characterized, especially across different habitat types.

1.3 Assessing Diet and Trophic Position using Stable Isotopes

Incorporating carbon and nitrogen isotopes signatures enhances the resolution of diet composition and the trophic position assessments. Ratios of nitrogen stable isotopes ($\delta^{15}N$) of consumers are typically enriched by 3–4‰ relative to their diet, and thus can be used to estimate trophic position of organisms. (Deniro and Epstein 1981, Minagawa and Wada 1984, Peterson and Fry 1987, Vanderklift and Ponsard 2003). Interestingly, the ratios of carbon stable isotopes ($\delta^{13}C$) are not prone to change as they move through food webs, (DeNiro and Epstein 1978, Peterson and Fry 1987) and therefore can be used to infer information on primary sources of carbon (e.g., C3 vs. C4 terrestrial plants, and littoral vs. pelagic algae; (Peterson and Fry 1987, France 1995). Thus, integrating levels of $\delta^{13}C$ and $\delta^{15}N$ isotopes with Hg levels and other organismal traits have the potential to broaden our understanding of the contributions of ontogenetic shifts in diet to body burdens, which might vary across different habitats.

1.4 Objectives

Here, I investigate the relationships between variation in total mercury (THg) concentration in American alligator whole blood, size, and diet in three distinct habitat types across the coastal plain region of the Southeastern U.S. I hypothesize that (1) THg concentrations increase with individual size, as an indicator of age and time of exposure; (2) THg concentrations are positively correlated with $\delta^{15}N$, reflecting increased exposure in individuals feeding at higher trophic positions; and (3) THg concentrations will vary across habitat types, due to spatial differences in environmental contamination and diet. Findings will advance our understanding of how spatial heterogeneity in Hg distribution interacts with habitat-specific ecological dynamics to influence body burdens in long-lived, predatory organisms.

CHAPTER 2

METHODS

2.1 Field Sites

I selected three study sites spanning over 480 km along the coastal plain of GA and SC, USA (Fig. 1). Each site comprises protected alligator populations with ongoing mark-recapture projects. The sites were selected for this study due to habitat differences, predicted variation in environmental Hg levels, and to compare patterns across distinct populations of alligators.

The Okefenokee Swamp (OS) is the largest fresh blackwater swamp in North America, located primarily within southern GA. Mark-recapture of alligators began in 2016. The hydrology of the Okefenokee is dominated by precipitation in addition to small inputs from groundwater and runoff. The swamp is shallow, peat filled, and acidic, dominated by wetland habitats including scrub-shrub thickets, cypress stands, and sedge prairies (Table 1; Hamilton 1982). The habitat and hydrology of the swamp is further detailed by Brook and Hyatt (2013). Alligators sampled in the Okefenokee for this study were captured in the northern Okefenokee Swamp Park and eastern Suwanee Canal entrances.

Jekyll Island State Park (JI) is a barrier island off the coast of GA, with 34 freshwater lagoons, the majority (23) being located on golf courses in the central part of the island. Mark-recatpure of alligators began in 2011. A portion of the island is surrounded by intertidal marsh comprised of cordgrass and a network of intertidal creeks that experience tides of 200-300 cm in amplitude. Most of the marsh is under a fish consumption advisory zone for the Turtle/Brunswick River, implemented by the Georgia Department of Natural Resources

Environmental Protection Division due to PCB and heavy metal contamination from the LCP Chemicals Superfund site (GA EPD, 2018, Bauer, 2012). Jekyll Island uplands consist of maritime oak and pine forest, coastal dune habitat, and high marsh hammocks. For more information and research on the habitat and hydrology of Jekyll Island, see the USGS hydrological report (Gordon and Torak 2016).

The Thomas A. Yawkey Wildlife Center (YW) consists of 4 barrier islands along the SC coast. Mark-recapture of alligators began in 1979. Alligators sampled in this study were captured primarily on South Island and Cat Island, which is surrounded by marine and brackish water habitats with a mean tidal range of 116 cm. The sampling area within YW primarily consists of impounded wetlands managed for waterfowl and contains both emergent vegetation, such as cordgrass and submerged vegetation, such as widgeon grass. Impoundment water levels are typically maintained at an average of 60 cm water depth, apart from a spring draw-down period lasting approximately 5–6 weeks, to promote seed propagation. Water management practices and rainfall influence impoundment water salinity, which ranges from 0 to 35 ppt. For more information on the habitat and hydrology of YW, see publications on the SCDNR website (SC DNR 2022).

2.2 Sample Collection

All alligators sampled were part of concurrent, long-term mark-recapture studies at the three sites (see dates above). Alligators of all size classes were captured intermittently from April – October. For each individual, sex was determined through cloacal examination and a visual health exam was conducted to identify any physical abnormalities, injuries, or symptoms of underlying conditions. Individuals were uniquely marked, and recaptures did occur, but only one sample from each individual was included in the analysis (N=133). Morphometric measurements (total length, snout-vent length (SVL), right hind foot length, body girth, and tail girth) were collected from all individuals using a soft measuring tape to the nearest 1 mm. Head measurements (head length, head width, eyes to snout, and distance between eyes) were collected using calipers to the nearest 1 mm. Body condition indices, such as Fulton’s K, can be valuable in exploring relationships between individual health, growth rates, and contaminant levels (Zweig et al. 2014, Brandt et al. 2016). Fulton’s K index can be calculated by comparing the ratio of one skeletal size measurement (commonly total length or snout-vent length) to mass (Zweig et al. 2014, Brandt et al. 2016). However, mass can be difficult to obtain in the field, especially for larger individuals. Accordingly, tail girth has been shown to be a suitable substitute for mass in Fulton’s K calculations (Zweig et al. 2014). We calculated Fulton’s K for captured alligators by comparing tail girth with head length (TG/HL), which is reported to be the most accurate proxy of body condition in alligators due to decreased human error in these measurements (Zweig et al. 2014).

Immediately following each capture, 0.5-35 ml of blood was drawn from the supraoccipital sinus using a 27-20-gauge, 0.5-1.5 in. needle and a 3-12 ml heparinized syringe, dependent upon the size of the individual. Blood samples were transferred to heparinized Vacutainer tubes and placed on wet ice in the field (< 5 hr) before being stored at −20°C until contaminant and isotope analysis could be performed. Following marking, measurements, and blood collection, all alligators were released at their capture sites. Samples were obtained under collection permits from the Georgia Department of Natural Resources, South Carolina Department of Natural Resources, and the study was approved by the University of Georgia’s Institutional Animal Care and Use Committee.

A total of 201 alligator blood samples were collected during April-October of 2019 (87 OS, 38 JI, 76 YW). We had initially included hatchlings to cover the full size-range of alligators, however, recently hatched individuals < 19 cm SVL (n=56) displayed elevated concentrations of THg. Because the concentrations in these hatchlings were likely due to a dominant effect of maternal transfer rather than diet (Rainwater et al. 2002, Nilsen et al. 2020), we excluded these individuals from the primary analyses (Fig. 5). In addition, 11 samples representing recaptured individuals, and 1individual observed with major health problems presumed to impact feeding ability and metabolism (e.g., broken jaw) were excluded from analyses, yielding a final dataset including 133 individual alligators (29 OS, 34 JI, 70 YW - Table 2).

2.3 Quantifying Total Mercury

Total mercury (THg) encompasses all species of mercury, including but not limited to MeHg and all bioavailable forms. THg concentrations in whole blood samples were assessed using a Direct Mercury Analyzer (DMA-80: Milestone, Shelton, CT, USA, hereafter DMA-80) at the University of Georgia's Marine Extension and Georgia Sea Grant Brunswick office. Blood samples were thawed at room temperature and placed on a Vortex homogenizer for 30s, and a 50 µl aliquot was transferred to individual quartz weigh boats (Milestone, Shelton, CT, USA). The DMA-80 analyzes mercury by thermally decomposing samples, using carrier gas to transport the mercury and combustion products into a catalyst chamber. A gold amalgamator captures the mercury before releasing it into the spectrophotometer's optical path. Both a system blank and a standard (DORM-3 Fish Protein; National Research Council Canada, Ottawa, Canada) were run between every 10 samples to ensure proper calibration of the machine. Mercury levels are reported as means ± 1 standard deviation, and all concentrations are reported as mg kg^{-1} wet weight.

2.4 Stable Isotope Analysis

The relative abundances of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopes were determined on either a Thermo Scientific Flash2000 Organic Elemental Analyzer or a Carlo Erba NA1500 Elemental Analyzer, coupled with a Thermo Finnigan DeltaPlus Mass Spectrometer or Thermo Finnigan Delta Plus XP Isotope Ratio Mass Spectrometer at the Stable Isotope Ecology Lab at UGA's Center for Applied Isotopes. Occasionally, it is necessary to extract lipids prior to Nitrogen analysis. However, 10 whole blood samples were tested for lipids, and it was determined that lipid extraction was not necessary. Samples were freeze-dried, ground to a fine powder, and aliquots of 0.4 mg-1.5 mg were placed in tin capsules. Spinach (NIST 1570a, 6.06% ± 0.20 N) and Bovine (NIST 1577c, 10.30% ± 0.34N) NIST standard reference materials were used for both Carbon and Nitrogen. Stable carbon and nitrogen isotope ratios are expressed as:

$$\delta^{13}C \text{ or } \delta^{15}N = [(Rsample/Rstandard)-1]x1000, \text{ where R is } ^{13}C/^{12}C \text{ or } ^{15}N/^{14}N$$

2.5 Modeling and Data Analysis

To analyze the relationships between site, alligator morphometrics, stable isotope ratios, and THg concentrations, I ran a generalized linear model (GLM) in JMP Pro® 16 (JMP®Pro Version 16. SAS Institute Inc., Cary, NC). In this model, main effects included Snout-Vent-Length (SVL), Sex, Body Condition Index (BCI), and the two isotopic values $\delta^{13}C$ and $\delta^{15}N$ which reflect diet. Because model residuals were not normal, I used the natural log (ln) of THg in these analyses. Standardization using z-scores of all continuous numerical variables was used to enable comparison between the multiple independent variables measured at different scales (i.e., standardized ß’s). Site as a variable was overwhelmingly influential in this initial analysis.

Due to the propensity of Hg levels and individual diet and ontogeny to vary by site, it was hypothesized that relationships between the model variables might differ by site. To account for these differences, subsequent modeling was performed separately for each site using the same variables. To explore associations between the alligator trait data, a Pearson’s correlation coefficient test was run to estimate collinearity among the continuous numerical variables within each site (sex was excluded because it is a categorical variable). Several of the variables are hypothesized to be strongly correlated: SVL and BCI are expected to increase with age, and $\delta^{15}N$ and $\delta^{13}C$ values are expected to shift across size and age classes, although these shifts could vary within sites. Despite the presence of collinearity, variables were retained in the stepwise models for continuity and site comparison because the AICc selection method handicaps highly collinear variables from being selected in the same model.

In the next GLM model, I ran step-wise model selection in JMP® Pro 16 for each site and differences in Akaike information criterion (AICc) values ($\Delta_i(AICc) = AICc_i - minAICc$) were used to determine the most parsimonious model. To determine the order of the next best fitting models, all models with < 2 ΔAICc are presented. The relative likelihood of each model ($n = 31$ models generated per site) was then calculated using the following formula: $x = \exp(-0.5 * \Delta AICc)$. The weight of each model was calculated using the relative likelihood value (x) of each model is then divided by the sum of all (x). Linear regressions were then used to explore the most significant relationships identified by the top models between predictor variables and Hg at each site. Additionally, linear regressions of $\delta^{15}N$ v. SVL and $\delta^{13}C$ v. SVL were used to examine potential relationships between size and diet shifts.

CHAPTER 3

RESULTS

THg levels at OS were 6-8 fold higher than those observed at either JI or YW, with the lowest THg concentrations in alligators at OS comparable to the higher values at both JI and YW (Table 2; Fig. 2). The full generalized linear model showed that site is a highly significant factor (G^2 = (2, N = 133) = 50.276, $p < 0.0001$; (Table 3).

For site-specific analyses, the top model explained 69% of variation in THg concentrations in OS alligators, which indicated SVL (ß = 0.419) was the most influential positively correlated predictor of THg, followed closely by δ^{15}N (ß = 0.376) and BCI (ß = 0.271; Table 4). The second model mirrored the first except that it did not contain BCI as a predictor variable (R^2 = 0.65) and had a heightened influence of SVL (ß =0.62). In the top models the Pearson's correlation coefficients (r) for all predictor variables at OS indicate moderate to high positive correlations between δ^{13}C and SVL (r = 0.79), δ^{13}C and δ^{15}N (r = 0.65), and BCI and SVL (r = 0.62; Table 5).

Within Jekyll Island (JI), the null model was the best fit to the data (Table 4). The next three best supported models all had low weights (w_i <0.121) and included only single predictor variables, ^{15}N (ß = -0.229), δ^{13}C (ß = -0.223), or Sex (ß = -0.131; Table 4). Interestingly, trendlines for JI were negative, in comparison with positive correlations seen at the other sites. All R^2 values for the models were low (<0.063) and the predictor variables were not significant.

The Pearson's correlation coefficients (r) for the predictor variables were also small, ranging from r = 0.21-0.51 (Table 5).

The best supported model predicting THg concentrations in alligators at YW included $\delta^{15}N$ (ß = 0.806), $\delta^{13}C$ (ß = -0.347), and Sex (ß = -0.231; Table 4). The second model included SVL in addition to the same three predictor variables: $\delta^{15}N$ (ß = 0.676), $\delta^{13}C$ (ß = -0.370), Sex (ß = -0.245), and SVL (ß = 0.170). The weight of these two models combined was 0.68. The R^2 values for both models were 0.50 and 0.51, respectively, and all p-values for the predictor variables were significant, with the exception of SVL in model 2. The four predictor variables at YW were all moderately to strongly positively correlated with one another, ranging from r = 0.62 to 0.85 (Table 5).

The linear regressions between $\delta^{15}N$ and SVL showed that the two variables were positively correlated at all three sites, although OS and JI had weaker correlations compared to YW (OS: $R^2 = 0.21$, $p = 0.0132$; JI: $R^2 = 0.17$, $p = 0.0162$; YW: $R^2 = 0.71$, $p < 0.0001$) (Fig. 4). Likewise, linear regression between $\delta^{13}C$ and SVL showed that the two variables were also positively correlated at both OS and YW (OS $R^2 = 0.62$, $p <0.0001$, JI $R^2 = 0.07$, $p = 0.127$, YW $R^2 = 0.39$, $p<.0001$).

CHAPTER 4

DISCUSSION

Overall, body burdens of THg were highly variable across sites. Of the three sites, alligators inhabiting the OS had the highest concentrations of THg, an average of 6-8 times higher than the other study sites at JI and YW. This is likely due to a combination of environmental factors that contribute to increased bioavailability of the heavy metal at OS. For instance, precipitation is the dominant source of environmental mercury deposition, and the hydrology of OS is dominated by precipitation 70-90 % (Brook and Hyatt 2013) with an average annual rainfall of 132.23 cm (ONWR, 1945-2021, unpublished data). The OS is also not far from several industrialized power plants, which are a major contributor to atmospheric Hg (Porter 2000, Sherman et al. 2012). In addition, OS has an acidic pH of 3.5-4 and is dominated by tannic waters and peat, both of which contribute to increased levels of Hg methylation and subsequent bioavailability of Hg(Hamilton 1982, Gilmour and Henry 1991, Branfireun et al. 1999). Finally, patterns of wetting and drying allow for the resuspension and methylation of Hg, which is a commonly observed at OS both within and across years (Winger and Lasier 1997).

Positive relationships between THg body burdens and animal size are consistently reported for crocodilians (Buenfil-Rojas et al. 2015, Nilsen et al. 2020, Lawson et al. 2020, Lemaire et al. 2021b, Buenfil-Rojas et al. 2022, Belcher et al. 2022). Findings from this study largely corroborate this trend but also suggest diet as an influence, both within and across sites. Alligators inhabiting both JI and YW displayed lower THg concentrations overall when compared to OS. Due to the increased methylation of Hg, presence of chlorine, and current advisories against fish consumption in adjacent tidal creeks surrounding JI, elevated environmental Hg was expected to be observed in the surrounding marshes and tidal ponds where saltwater intrusion occurs. Previously, spatial ecology studies analyzing movements and home ranges of adult alligators on JI determined that most alligators use freshwater habitats, while select individuals of larger sizes use the marsh ecosystem (Skupien et al. 2016). The alligators I sampled at JI in 2019 consisted primarily of smaller size classes, less than 7ft in total length (SVL ~ 100cm), and it is possible that larger individuals might have higher concentrations of THg. Interestingly, on Jekyll, smaller alligators appear to reach “peak” body burdens of THg earlier in life, with little variation compared to larger size classes. This could explain why the models for JI do not show significant THg variation with size. One hypothesis could be that several size classes feed at similar trophic levels throughout their lifetime, with little variation in prey availability (or equal dispersion across trophic levels). Additional explanations could be that THg levels have 1) increased environmentally and within prey in recent years, and we are just beginning to see this show up in body burdens of young individuals, or 2) maternal transfer is high enough to impact THg burdens for several years.

Based on linear patterns of bioaccumulation, the highest mercury concentrations would be predicted in the oldest individuals. However, in *A. mississippiensis*, growth has been shown to reach an asymptote in long-lived adults, who also appear to exhibit reduced THg concentrations in their later years (Lawson et al. 2020, Rainwater et al. 2022). Therefore, age may be a better predictor of total mercury than size, but this requires long-term data. Given the cessation of growth documented in alligators at YW, individuals within the adult size class could range from 15-60+ years of age, unless age is indicated by previous documentation and tagging. Alligators at OS have only been tagged since 2016, and within JI since 2011, offering limited data on the actual ages of individuals captured in those populations. When comparing body size (SVL) to THg across OS and YW, we see linear increases in body burdens, as expected with bioaccumulation. In the larger size classes, there appear to be a handful of individuals with lesser body burdens, resembling those of mid-sized individuals. Overall, few samples were available for the largest size classes, and ages of the adults were unknown for all 3 sites, thus limiting my ability to fully interpret the THg data when considering bioaccumulation over time. General hypotheses for the observed reduction in THg include age-related changes in metabolism, foraging behaviors, THg excretion, the influence of early Hg exposure on growth rates (e.g., biodilution), and individual variation in bioaccumulation tendencies (Lawson et al. 2020). Therefore, it appears that bioaccumulation of Hg within crocodilians may have its limits, and long-term, longitudinal monitoring studies are crucial to our understanding and efforts.

Both $\delta^{15}N$ and $\delta^{13}C$ metrics give insight into the diet of the alligators. Many of the same site level variation and behavioral factors mentioned above support evidence of biomagnification as $\delta^{15}N$ increases in relation to THg values and size class (SVL), and indicator of elevated trophic level feeding. These patterns are significantly positively correlated at all three sites, although YW had the strongest correlation. (Fig 2, Fig 3). Carbon values imply which primary producers are contributing to an organism’s diet, but exact matching to species level requires intensive reference values of the site-specific $\delta^{13}C$ values. Because these values can vary greatly with space and time, and reference values for each site do not exist, I was not able to determine which primary producers were contributing most to the diet of alligators. However, trends in $\delta^{13}C$ were shown to be significantly positively correlated with THg (Fig 2) and size at both OS and YW (Fig 3) indicating that there are shifts in diet as individuals grow. Additionally, $\delta^{13}C$ values were expressed over a higher range at both JI and YW compared to OS (mean $\delta^{13}C > 4$

units (Table 2, Fig 3). This is likely attributed to the availability of species closely associated with salt water and coastal habitats at both JI and YW. Many of the impoundments sampled at YW were subjected to regular drawdowns of water, resulting in temporal variation of fresh and brackish water for seed propagation and waterfowl management. Similarly, several golf-course impoundments at JI were in flux with tidal creeks. Sulfur isotope analysis was not available to me at the time of this project, but I would recommend seeking this analysis in coastal ecosystems in future projects to allow for more detailed results regarding diet as it relates to marine species.

Findings from this study as well as other reports suggest that maternal transfer of THg results in elevated concentrations in crocodilian offspring (Fig. 5), which has the potential to carry consequences for reproductive success, and overall health of young alligators (Rainwater et al. 2002, du Preez et al. 2018, Nilsen et al. 2020, Lemaire et al. 2021a). However, the implications of heavy metal burdens in eggs and hatchlings are understudied; yet findings in the current study raise the possibility of negative long-term impacts. For example, Hg exposure in most avian species is associated with decreased survival (Heinz et al. 2009). It is also not well understood why or how THg concentrations decrease as hatchlings grow. There may be a dilution effect as they begin to feed on their own, combined with a higher metabolism for fast-paced growth (up to 2X length/year) the first years of their lives. Crocodilians are also ectotherms, known to be more efficient than endotherms at converting dietary resources into growth. Endothermic species exhibiting higher metabolic conversion rates typically require higher rates of consumption and are therefore prone to acquiring higher body burdens of Hg (Hallanger et al. 2011, Lavoie et al. 2013).

Important caveats of using stable isotope analysis to establish trophic level and food webs have been raised, especially in terms of using large ectothermic taxa. Trophic discrimination factors (TDF) (change in isotope value between a tissue and a food source) and turnover rates (rates at which stable isotope values are incorporated into tissues) have been well established for several endothermic species. However, these values are not well studied or established for all taxa, especially in large ectothermic species and those who may experience several shifts in size and trophic position. Rosenblatt and Heithaus (2013) and Villamarin et al. (2018) have highlighted the need for a change in the way that researchers estimate trophic position when turnover rates and TDF values are unavailable. Much like the variation in $\delta^{13}C$ across sites, there are not great reference values available for these types of analyses with all taxa. Further investigation into the patterns associated with these rates could enhance the understanding of isotopes in species like the American alligator.

Implications for elevated levels of Hg can impact hunting and fishing advisories for game species adjacent to and downstream of any site. The OS, where THg levels were the highest, is the headwaters of two major southeastern rivers, with 85% outflow into the Suwannee River through Florida into the Gulf of Mexico, and 15% into the St Mary's River through Georgia into the Atlantic Ocean (Rykiel, 1977). Currently, the FDA and EPA have released advisories that consuming fish with levels >0.15-0.46 mg kg^{-1} 1-3 times per week, respectively, can cause health implications in humans, particularly for pregnant women and young children (Nutrition 2022). According to consumption risk evaluations comparing blood and muscle tissue in American alligators, some of the THg values found would warrant moderated consumption with these advisory levels in mind (Moore et al. 2022, Kojima et al. 2023). Additionally, when we place the observed alligator THg values in the context of other taxa with lower tolerance for contaminants, we must consider how adverse and widespread the effects could be. For example, avian species are known to have severe health implications, with lethal concentrations (LC50) starting as low as 0.25 mg kg-1 for common species (Heintz et al., 2009.) The highest levels of THg observed in the whole blood of alligators in the OS were seven-fold higher than this amount, leading us to believe that other species in this area are likely sustaining harmful effects. These factors incentivize expanding our studies of the ecological dynamics that drive variation in THg levels in crocodilians and other taxa.

As we continue to assess trends in bioaccumulation and magnification both within and food webs and across habitats, it is important to consider the multitude of factors that are at play. Variables such as specific diet, age, and extent of exposure appear influential to understanding the variation in contaminant exposure. Studies aimed to address these factors in species that can serve as bioindicators of their local ecosystems and food webs will be especially valuable as we move forward in the field of ecotoxicology.

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TABLES AND FIGURES

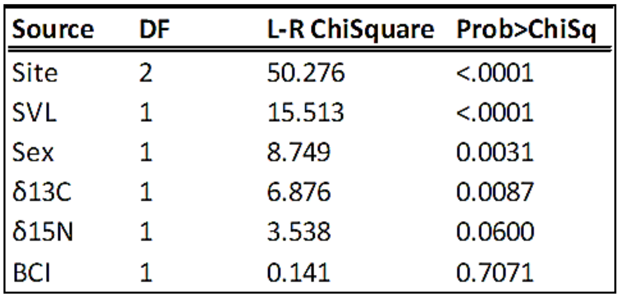
Table 1. Site characteristics for the 3 distinct habitat types sampled; Okefenokee Swamp, Jekyll Island, and Tom Yawkey Wildlife Center.

Site Characteristics	**Okefenokee Swamp (OS)**	**Jekyll Island (JI)**	**Tom Yawkey Wildlife Center (YW)**
Location	South Georgia, US	Coastal Georgia, US	Coastal South Carolina, US
Square Kilometers	1772.52	22.38	60.33
Managed By	Okefenokee National Wildlife Refuge	Jekyll Island Authority	South Carolina Department of Natural Resources
Water Type	Fresh, Tanic Blackwater	Fresh and Brackish Impoundments (golf course)	Fresh and Brackish Impoundments (rice fields)
Average pH	3.5 - 4.5	7.0 - 8.5	7.0 - 8.5
Dominant Habitat Type	Scrub-shrub thickets, cypress stands, sedge prairies, bordered by pine savannah	brackish tidal creeks, fresh and brackish impoundments, maritime oak and pine forest, coastal dune habitat, high marsh hammocks	pine savannah, tidal & non-tidal wetland, mudflats,hardwood forest, shrub-scrub, grassland, cultivated field, bald cypress-tupelo gum swamp
Hunting History	No hunting within refuge, is permitted directly adjacent to the swamp	Alligators >6ft removed prior to 2011, No current hunting or removal	Hunting not permitted since early 1900s

Table 2. Summary of site-specific data for Okefenokee, Jekyll, and Yawkey including total number of individual alligators sampled, number male, number female, minimum and maximum snout-vent length (SVL) in cm, minimum and maximum total mercury (THg) wet weight in mg kg^{-1}, mean total mercury (THg) wet weight in mg/kg^{-1} ± standard deviation, minimum and maximum $\delta^{13}C$, mean $\delta^{13}C$ ± standard deviation, minimum and maximum $\delta^{15}N$, and mean $\delta^{15}N$ ± standard deviation.

Site Name:	**Okefenokee**	**Jekyll Island**	**Yawkey Widlife**
N Alligators	29	34	70
N Male: N Female	19 : 10	18 : 16	43 : 27
SVL (cm) Min ; Max	25.8 ; 182.2	22 ; 116	19.3 ; 143
THg (mg/kg) Min ; Max	0.125 ; 1.961	0.022 ; 0.257	0.018 - 0.302
THg (mg/kg) Mean ± SD	0.62 ± 0.535	0.074 ± 2.816	0.074 ± 0.055
δ13C Min ; Max	-30.27 ; -22.42	-28.92 ; -15.63	-29.51 ; -18.1
δ13C Mean ± SD	-28.036 ± 1.724	-24.017 ± 2.186	-23.639 ± 2.573
δ15N Min ; Max	4.70 ; 10.05	4.09 ; 11.46	4.19 ; 9.99
δ15N Mean ± SD	6.822 ± 1.412	7.251 ± 1.826	6.557 ± 1.614

Table 3. Likelihood Ratio Chi-Squared statistic results from the full generalized linear model (GLM) analyzing the effects of Site, Snout-Vent-Length (SVL), Sex, δ^{13}C, δ^{15}N and Body Condition Index (BCI) on total Hg concentration in whole blood (ln-transformed). N = 133.



Source	DF	L-R ChiSquare	Prob>ChiSq
Site	2	50.276	<.0001
SVL	1	15.513	<.0001
Sex	1	8.749	0.0031
δ13C	1	6.876	0.0087
δ15N	1	3.538	0.0600
BCI	1	0.141	0.7071

Table 4. Generalized linear models conducted at the site-level on the effects of Snout-Vent-Length (SVL), Sex, δ^{13}C, δ^{15}N and Body Condition Index (BCI) on whole blood HG concentration. Stepwise model selection was run separately for each of the three sites (Okefenokee, Jekyll, and Yawkey). Only the null model, the best model (i.e., with ΔAICc =0), and models within 2ΔAICc units of the best model are shown. Independent variables in the model column are in order of highest to lowest standardized ß-estimate. Model wights (W_i) were calculated from the full set of models (n=31 per site).

OKEFENOKEE SWAMP (OS)						**Model Variables:** ß-estimate, (*p-value*)				
Model	**# Variables**	**R2**	**AIC$_c$**	**ΔAIC$_c$**	**w$_i$**	**δ15N**	**δ13C**	**SVL**	**BCI**	**Sex {F-M}**
Null	0	0.000	85.742	25.735	0.000					
SVL, δ15N, BCI	3	0.689	60.007	0.000	0.285	0.376, (*.008*)		0.419, (*.019*)	0.271, (*.081*)	
SVL, δ15N	2	0.648	60.663	0.656	0.205	0.303, (*.028*)		0.620, (*<.001*)		
JEKYLL ISLAND (JI)						**Model Variables:** ß-estimate, *(p-value)*				
Model	**# Variables**	**R2**	**AIC$_c$**	**ΔAIC$_c$**	**w$_i$**	**δ15N**	**δ13C**	**SVL**	**BCI**	**Sex {F-M}**
Null	0	0.000	99.860	0.000	0.161					
δ15N	1	0.052	100.442	0.582	0.121	-0.229, (*.192*)				
δ13C	1	0.050	100.530	0.670	0.115		-0.223, (*.204*)			
Sex	1	0.018	101.664	1.804	0.065					-0.131, (*.453*)
TOM YAWKEY WILDLIFE CENTER (YW)						**Model Variables:** ß-estimate, (*p-value*)				
Model	**# Variables**	**R2**	**AIC$_c$**	**ΔAIC$_c$**	**w$_i$**	**δ15N**	**δ13C**	**SVL**	**BCI**	**Sex {F-M}**
Null	0	0.000	201.823	41.758	0.000					
δ15N, δ13C, Sex	3	0.500	160.065	0.000	0.446	0.806, *(<.001)*	-0.347, *(.003)*			-0.231, *(.010)*
δ15N, δ13C, Sex, SVL	4	0.508	161.374	1.309	0.232	0.676, *(<.001)*	-0.370, *(.002)*	0.170, *(.317)*		-0.245, *(.007)*

Table 5. Pearson's correlation coefficients (r) between all continuous independent variables included in the stepwise model selection for all three sites. [** indicates a strong positive relationship (~0.8), * indicates a moderately positive relationship (0.6).]

	Okefenokee Swamp				**Jekyll Island**				**Yawkey Wildlife Center**			
	δ15N	**δ13C**	**SVL**	**BCI**	**δ15N**	**δ13C**	**SVL**	**BCI**	**δ15N**	**δ13C**	**SVL**	**BCI**
δ15N	1				1				1			
δ13C	0.65*	1			0.51	1			0.64*	1		
SVL	0.45	0.79**	1		0.41	0.27	1		0.85**	0.62*	1	
BCI	0.07	0.45	0.62*	1	0.21	0.3	0.5	1	0.70*	0.62*	0.82**	1

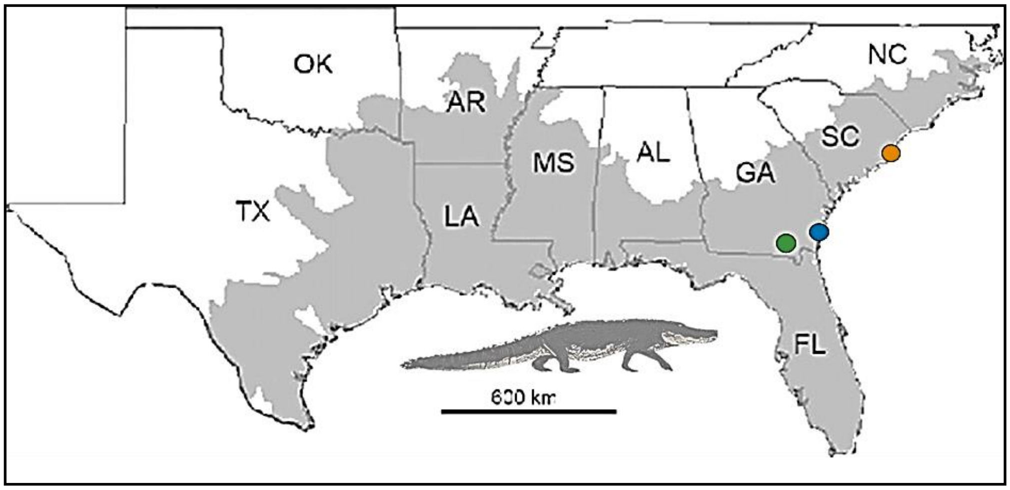


Figure 1. American Alligator *(Alligator mississippiensis)* geographicacl range in the US (grey), field sites Okefenokee Swamp (green), Jekyll Island (blue), and Tom Yawkey Wildlife Center (orange).

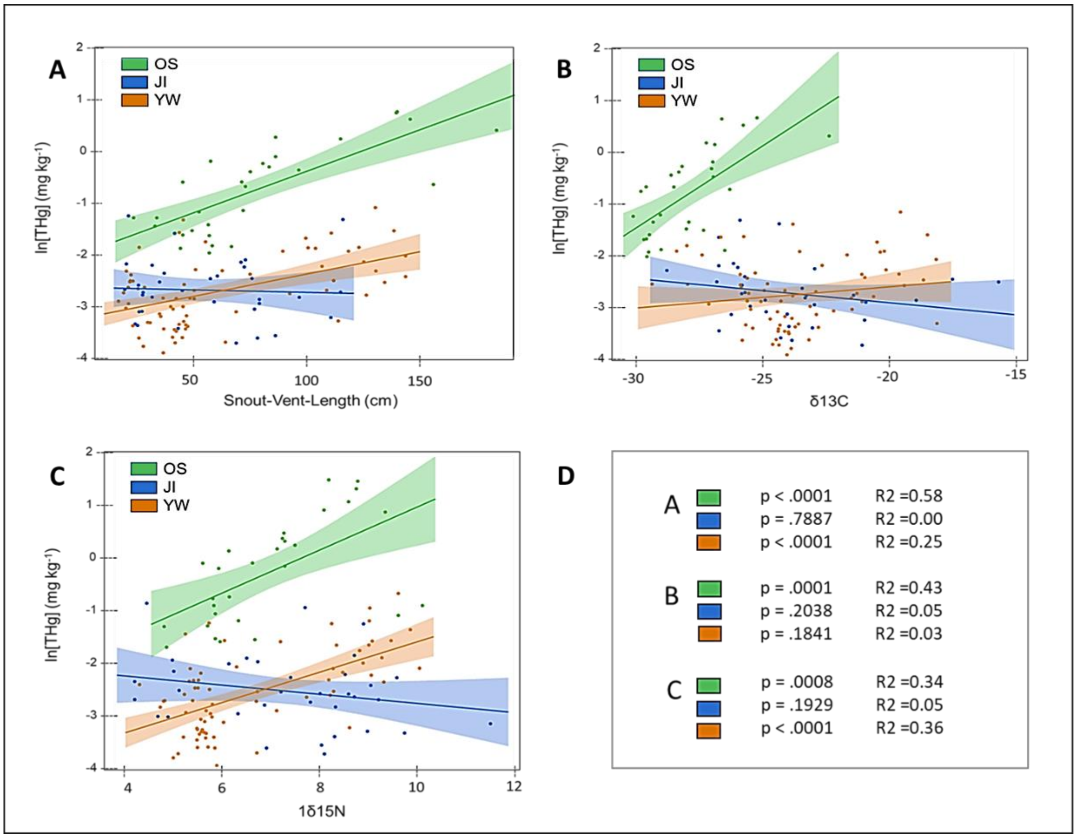


Figure 2. : Linear regressions of total mercury concentration ln transformed in whole blood ln[THg] (mg kg-1) against Snout-Vent-Length (SVL) (A), δ13C (B), and δ15N (C). Panel D shows all p-values and R2 values for the associated regression lines in panels A, B, and C at each site; Okefenokee (green), Jekyll Island (blue), and Yawkey (orange)

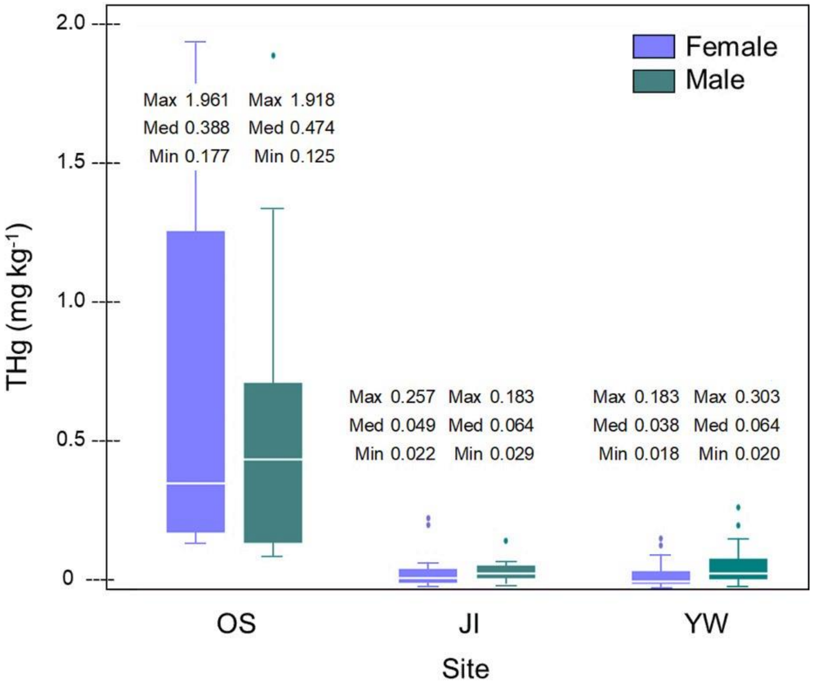


Figure 3. Box plot showing the distribution of absolute THg values (mg kg^{-1}) by site and sex.

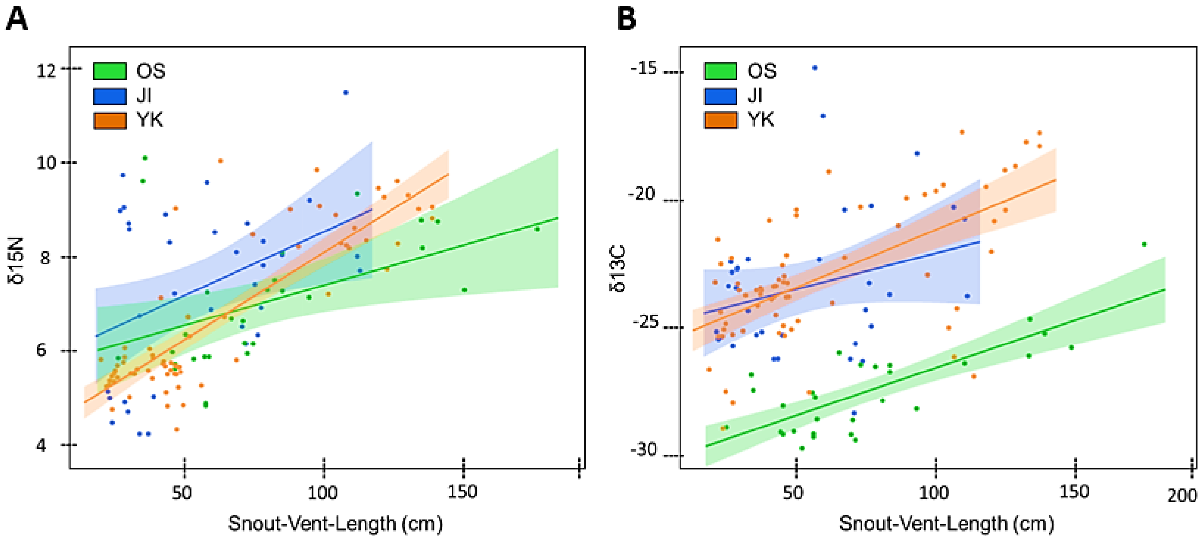


Figure 4. **A)** Linear regression between SVL and δ^{15}N for each of the three sites: Okefenokee (green, p=0.013, R^2=0.21), Jekyll Island (blue, p=0.016, R^2=0.17) and Yawkey (Orange, p<0.001, R^2=0.71). **B)** Linear regression between SVL and δ^{13}C for each of the three sites: Okefenokee (green, p<0.001, R^2=0.62), Jekyll Island (blue, p=0.128, R^2=0.07) and Yawkey (Orange, p<0.001, R^2=0.39).

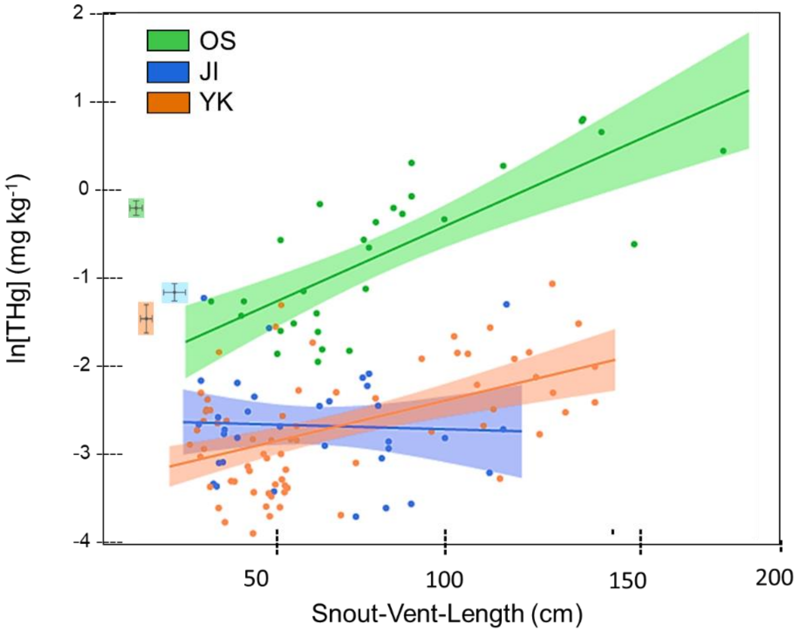


Figure 5. Linear regressions of the natural log of total Mercury ln[THg] (mg kg-1) as a function of snout-vent-length (SVL) (identical to Figure 2A). Data points with error bars display the hatchling and yearling alligators <19cm SVL that were removed from the model analysis. Hatchling data points were not included in the regression slopes. Data points for hatchling and yearling alligators display mean values for multiple hatchlings, most of which are presumably from the same pod (n=40 OS, n=11 JI, n=9 YW). Error bars represent the standard deviation of all points.