Environmental Toxicology and Chemistry, 2025, **44(6),** 1611–1621
https://doi.org/10.1093/etojnl/vgaf060
Advance access publication: March 7, 2025
Original Article

Environmental Toxicology

Site-specific ontogenetic drivers of mercury concentrations in American alligators

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Abstract

Contaminant body burdens are determined by complex interactions between contaminant inputs into the environment, local ecological dynamics, and organismal ontogeny. Although a naturally occurring element, mercury (Hg) is a potent neurotoxin, commonly assessed in contaminant body burdens. Anthropogenic activity has affected its spatial distribution, resulting in regional "hotspots" with elevated Hg concentrations. Due to the propensity of methylated Hg to bioaccumulate within individuals over time and biomagnify across trophic levels, long-lived apex predators can carry substantial body burdens in affected ecosystems. However, the role of an organism's ontogeny and habitat in shaping individual Hg concentrations, especially within and across species, is not well understood. We assessed total Hg, carbon ($\delta^{13}C$), and nitrogen ($\delta^{15}N$) isotopic ratios in whole blood samples ($n = 133$) across three distinct habitats in the southeastern United States to investigate how size and dietary shifts in the American alligator (*Alligator mississippiensis*) influence Hg accumulation. Mercury concentrations were approximately eightfold higher in alligators inhabiting the Okefenokee Swamp, Georgia (mean = 0.62 mg/kg) compared with those inhabiting coastal habitats (Jekyll Island, Georgia and Yawkey Wildlife Center, South Carolina). Whereas individual size and nitrogen isotope signatures generally displayed positive relationships with Hg concentrations, model selection approaches revealed these relationships varied across populations, likely in response to site-specific differences in environmental Hg concentrations and life history attributes of the alligators. Collectively, our findings demonstrate that although Hg concentrations in *A. mississippiensis* are highly influenced by differences between sites, diet and body size can sometimes additionally affect individual variation within populations, suggesting that organismal ontogeny interacts with site-specific contamination and ecological factors to affect Hg body burdens.

Keywords: bioaccumulation, bioindicators, biomagnification, diet, ecotoxicology

Introduction

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 as it both bioaccumulates within exposed individuals and biomagnifies across trophic levels (Stepanauskas et al., 2005, Magos and Clarkson 2006, Chumchal et al., 2011, Bjørklund et al., 2017). Although a naturally occurring element, most environmental inputs of Hg stem from atmospheric releases of Hg as a byproduct of industrial activity (e.g., waste incineration), resulting in contaminated precipitation and/or runoff (Nguyen et al., 2008, Pirrone et al., 1996). Spatial patterns of environmental Hg contamination are driven by proximity to point sources, meteorological patterns of wind and precipitation, and regional hydrology (VanArsdale et al., 2005, Biester et al., 2007). This heterogeneity can result in hotspots of contamination; however, how spatial variation in Hg contamination interacts with local ecological factors and organismal ontogenetic dynamics to further affect body burdens is less well understood (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 on 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 (Hg^0, Hg^{2+}) is converted to its bioavailable form, methylmercury (MeHg) through metabolic processes of aquatic bacteria (Compeau and Bartha 1985, Fleming et al., 2006). Methylmercury, 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 with freshwater wetlands

Received: August 26, 2024. **Revised:** February 18, 2025. **Accepted:** March 03, 2025

(Gilmour and Henry 1991, Benoit et al., 2003, Ulus 2021). However, anaerobic, acidic freshwater wetlands across the southeastern United States 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 addition to ecological influences on Hg methylation 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 fish, birds, and marine mammals (Grieb et al., 1990, McHuron et al., 2016, Kucharska et al., 2019, Bighetti et al., 2021; but see Lawson et al., 2020). 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). Interestingly, many species display changes in feeding ability and habits with age and size, resulting in ontogenetic variation across multiple scales, including growth, development, metabolic processes, sexual maturation, morphology, physiology, and senescence. Given the ontogenetic variability in these processes, two primary life history traits may significantly affect an organism's body burden of contaminants: 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 (Burger et al., 2000, Rainwater et al., 2007, Lawson et al., 2020, Lemaire et al., 2021a). The geographical range of American alligators (*Alligator mississippiensis*) extends throughout the southeastern United States from Texas to Florida and as far north as northeastern North Carolina (Figure 1), where individuals inhabit diverse wetland habitats, including freshwater lakes, ponds, rivers, blackwater swamps, and coastal impoundments. 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 on prey occurring in estuarine and marine environments (e.g., crabs, saltwater fish, and elasmobranchs), populations inhabiting freshwater swamps lack these resources and must exploit different prey (Delany and Abercrombie 1986, Bondavalli and Ulanowicz 1999, Delany et al., 1999, Rosenblatt et al., 2013, Nifong 2014). In addition, alligators are likely to utilize different prey resources as they grow from hatchlings and juveniles (approximately 15–120 cm total length [TL]) to subadults and adults (>120 cm TL; Delany 1990, Saalfeld et al., 2008, Nifong et al., 2015). Such ontogenetic shifts in diet have also been observed in other crocodilians, including Australian freshwater crocodiles, (*Crocodylus johnstoni*), Morelet's crocodiles (*C. moreletii*; Platt et al., 2006), Nile crocodiles (*C. niloticus*; Tucker et al., 1996, Wallace and Leslie 2008), and American crocodiles (*Crocodylus acutus*), with hatchlings typically feeding on small invertebrates prior to shifting to larger prey items as they grow (Platt et al., 2013). Because size and age do not always correlate well in alligators (Wilkinson et al., 2016, Rainwater et al., 2022), bioaccumulation with age can be more difficult to track. 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.

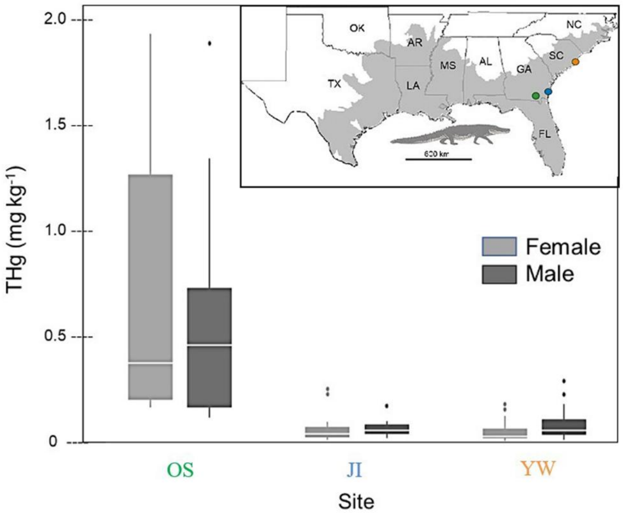

Figure 1. Box plot of the distribution of absolute THg values (mg kg^{-1}) by site and sex. Box extends from the 25th percentile to the 75th percentile with the horizontal line inside the box marking the median. Whiskers extend to the last point that is within 1.5 * interquartile range from the ends of the box, and the points outside are outliers. Inset shows the distribution of the American alligator in the southeastern United States in grey and our three study sites (from south to north): Okefenokee Swamp (OS, green), Jekyll Island (JI, blue), and Yawkey Wildlife Center (YW, orange).

Here, we investigate the relationships between total mercury (THg) concentrations in American alligator whole blood, individual size, and diet in three distinct habitat types across the coastal plain region of the southeastern United States. We test the hypotheses that (1) THg concentrations increase with individual size, as an indicator of age and duration 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. Our study quantifies how spatial heterogeneity in Hg distribution interacts with habitat-specific ecological factors and life history attributes to influence body burdens in a long-lived apex predator.

Materials and methods

Study sites

We sampled alligators at three study sites spanning > 480 km along the coastal plain of Georgia (GA) and South Carolina (SC), USA (Figure 1). Sites were selected for this study due to habitat differences, predicted variation in environmental Hg concentrations, 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. A mark-recapture program on the alligators there began in 2017. 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 (1985). Alligators sampled in the Okefenokee for this study were captured in the northern Okefenokee Swamp Park (Waycross, Ware County) and eastern Suwanee Canal entrances (Folkston, Charlton County).

Jekyll Island State Park (JI) is a barrier island in GA, with 34 freshwater lagoons, the majority (23) being located on golf

Table 1. Site characteristics for the three 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 (TW)
Location	South Georgia, USA	Coastal Georgia, USA	Coastal South Carolina, USA
Square kilometers	1,772.5	55.1	60.3
Managed by	U.S. Fish and Wildlife Service	Jekyll Island Authority	South Carolina Department of Natural Resources
Water type	Fresh tannic blackwater	Fresh and brackish impoundments (golf course)	Fresh and brackish impoundments (former 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	Tidal wetlands, maritime and pine forests, ocean beaches, and managed tidal impounded ponds
Hunting history	No hunting within refuge; is permitted directly adjacent to the swamp	Alligators >1.83 m removed prior to 2011, No current hunting or removal	Hunting not permitted since early 1900s

courses in the central part of the island. A mark-recapture program on the alligators there began in 2011. A portion of the island is surrounded by intertidal marsh composed of cordgrass and a network of intertidal creeks that experience tides of 200–300 cm in amplitude. Much of the marsh at the time of this study was under a fish consumption advisory zone for the Turtle/Brunswick River implemented by the Georgia Department of Natural Resources, Environmental Protection Division (EPD) due to polychlorinated biphenyls and heavy metal (including Hg) contamination from the LCP Chemicals Superfund site (Georgia EPD 2018, Bauer 2019, Levine 2022). 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 JI, see Gordon and Torak (2016).

The Thomas A. Yawkey Wildlife Center (YW) consists of four barrier islands along the SC coast. A mark-recapture program on the alligators there began in 1979. Alligators sampled in this study were captured primarily on South Island and Cat Island, which are 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, which contain both emergent and submergent vegetation. Impoundment water depths are typically maintained at an average of 60 cm, 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 0–35 ppt. Yawkey uplands consist of maritime oak and pine forest and ocean beach systems. See Wilkinson et al. (2016) and South Carolina Department of Natural Resources (SC DNR; 2022) for more information on the habitat and hydrology of YW.

Sample collection

Alligators of all size classes were captured intermittently from April–October 2019 under collection permits from the Georgia Department of Natural Resources (#1000771776 [OS], #1001047434 [JI]), SC DNR (#SC-08-2019 [YW]), and U.S. Fish and Wildlife Service Okefenokee National Wildlife Refuge (41590 20-02 [OS]). The study was approved by the University of Georgia's (UGA's) Institutional Animal Care and Use Committee (AUP A2016 07-004-Y3-A4 and A2016 10-011-Y3-A2). Immediately following each capture, 0.5–30 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 on 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. For each individual, sex was determined through cloacal examination and total length (TL), snout-vent length (SVL), right hind foot length, body girth, and tail girth were measured using a soft measuring tape to the nearest 1 mm. Head measurements (head length, head width, eyes to snout length, and distance between eyes) were collected using calipers to the nearest 1 mm. Body condition indices (BCIs) can be valuable in exploring relationships between individual health, growth rates, and contaminant concentrations (Zweig et al., 2014, Brandt et al., 2016). In situations where measuring mass is not feasible (e.g., large animals in the field), ratios of tail girth with head length (TG/HL) are reported to be the most accurate proxy of BCI in alligators due to decreased human error in these measurements; thus, we used the TG/HL ratio to examine BCI in this study (Zweig et al., 2014). Following measurements and blood collection, all alligators were uniquely marked for future identification to avoid resampling individuals and released at their capture sites.

A total of 196 alligator blood samples were collected (87 OS, 38 JI, 71 YW). Blood collection is less invasive than muscle tissue collection, and Hg concentrations in blood have been compared with other tissues in American alligators (Moore et al., 2022, Eggins et al., 2015, Kojima et al., 2023). Blood also has a relatively short turnover time that represents recent diet and consumption of Hg as well as redistribution from internal tissues (Rosenblatt & Heithaus 2013). We initially included hatchlings to cover the full size-range of alligators; however, recently hatched individuals known to be less than 2 years of age, that is, < 19 cm SVL ($n = 51$), displayed elevated concentrations of THg (see *Results* section). Because we had no individuals sampled that were <19 cm at YW, in 2020 we sampled nine hatchlings at this site to include in our hatchling comparisons. Because the concentrations in hatchlings were likely due to a dominant effect of maternal transfer rather than diet, we excluded these individuals from the primary analyses to be analyzed separately (Rainwater et al., 2002, Nilsen et al., 2020, Johnson et al., 2023). In addition, 11 samples representing recaptured individuals and one individual observed with major health problems presumed to affect feeding ability and metabolism (e.g., broken jaw) were excluded from analyses. Thus, our final dataset for formal statistical analysis included 133 individual alligators above hatching size (Table 2).

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

Site	Okefenokee	Jekyll Island	Yawkey
Alligators, *n*	29	34	70
Male, *n*	19	18	43
Female, *n*	10	16	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 ± 0.055	0.074 ± 0.056
δ13C min; max	−30.27; −22.42	−28.92; −15.63	−29.51; −18.1
δ13C mean ± SD	−28.036 ± 1.724	−24.017 ± 2.858	−23.639 ± 2.591
δ15N min; max	4.70; 10.05	4.09; 11.46	4.19; 9.99
δ15N mean ± SD	6.92 ± 1.44	7.251 ± 1.853	6.557 ± 1.614

Mercury analysis

Total Hg concentrations in blood can encompass both inorganic forms (InHg) and MeHg; however in this study, we did not assess their relative proportions. Total mercury concentrations in whole blood samples were assessed using a Direct Mercury Analyzer (DMA-80: Milestone, Shelton, CT, USA, hereafter DMA-80) at the UGA Marine Extension and Georgia Sea Grant Brunswick laboratory. Blood samples were thawed at room temperature and placed on a Vortex homogenizer for 30 s, and a 50 μl aliquot was transferred to individual quartz weigh boats (Milestone, Shelton, CT, USA). 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 instrument. Mercury concentrations are reported as mean ± 1 SD, and all concentrations are reported as mg kg^{-1} wet weight. Additionally, a subset of blood samples from 20 OS individuals was independently analyzed at the Savannah River Ecology Laboratory in Aiken, SC to validate the initial measurements. These samples represented the higher concentrations of THg in this study and, given the low concentration of THg in our initial standard reference material (DORM-3), we also included a reference material (PACS-3) in this analysis with higher THg concentrations. After excluding a potential outlier sample, concentrations from this validation correlated strongly with our initial values ($R^2 = 0.94$) and were on average 11% higher.

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 Laboratory at UGA's Center for Applied Isotopes. Under some circumstances, it is necessary to extract lipids prior to nitrogen isotope analysis or apply a carbon-based interference formula. However, we tested 10 whole blood samples, and bulk C-N ratios were low enough to not warrant lipid extraction or correction (Logan et al., 2008, Pilecky et al., 2023). Samples were freeze-dried, ground to a fine powder, and aliquots of 0.4 mg–1.5 mg were placed in tin capsules. Spinach (National Institute of Standards and Technology [NIST] 1570a, 6.06% ± 0.20 N) and Bovine (NIST 1577c, 10.30% ± 0.34 N) NIST standard reference materials were used for both carbon and nitrogen. Stable carbon and nitrogen isotope ratios are expressed as

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000, \text{ where R is } {}^{13}C/{}^{12}C \text{ or} {}^{15}N/{}^{14}N$$

Statistical analysis

Relationships between site, alligator morphometrics (SVL and BCI), sex, stable isotope ratios, and THg concentrations were assessed using a generalized linear model in JMP Pro 16 (JMP Pro Ver. 16. SAS Institute Inc., Cary, NC). Due to the propensity of Hg concentrations, individual diet, and ontogeny to vary strongly by site, subsequent modeling was performed separately for each site using the same variables but excluding site. In these models, the main effects included SVL, sex, BCI, and the two isotopic values $\delta^{13}C$ and $\delta^{15}N$. Because model residuals were not normal, THg values were transformed using the natural log (ln). Standardization using z-scores of all continuous independent variables was used to enable comparison between the multiple independent variables measured at different scales (i.e., standardized βs). To explore associations between the alligator trait data (SVL, BCI), a Pearson's correlation test was run to estimate collinearity among the continuous variables (sex excluded) within each site (see online supplementary material Table S1). Despite the potential of collinearity among alligator trait measures, variables were retained in the stepwise models for continuity and site comparison because the corrected Akaike information criterion (AICc) selection method handicaps highly collinear variables from being selected in the same model. Stepwise model selection was performed in JMP Pro 16 for each site, and differences in AICc values (Δ_iAICc) 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 was then calculated using the following formula: x = exp(-0.5 * ΔAICc). The weight of each model was calculated using the relative likelihood value (x) of each model, which was then divided by the sum of all (x). Linear regressions were then used to explore the relationships at each site of ln (THg) with SVL, $\delta^{15}N$, and $\delta^{13}C$, which in many cases were the top-fitting variables identified by the top models. 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 at each site.

Results

Total mercury concentrations in alligators from OS were eightfold higher than those observed at either JI or YW. In fact, the lowest measured THg concentrations at OS were comparable to the highest values measured at JI and YW (OS: 0.125–1.916 mg/kg, JI: 0.022–0.257 mg/kg, YK: 0.018–0.302 mg/kg, Table 2; Figure 1). The full generalized linear model showed that site is a strong determinant of THg concentrations ($\chi^2 = 50.3$, $n = 133$, $p < 0.0001$; Table 3).

For subsequent site-specific models, the top model for OS explained 69% of variation in ln (THg) concentrations in alligators. SVL (β = 0.337) and $\delta^{15}N$ (β = 0.375) were the most influential positively correlated predictors of ln (THg), followed closely by BCI (β = 0.279; Table 4). The second-best supported model ($R^2 = 0.65$) mirrored the first except that it did not contain BCI as a predictor variable and had a heightened influence of SVL (β = 0.498). At OS, univariate linear regressions showed that ln (THg) concentrations were positively related to SVL ($R^2 = 0.58$, $p < 0.0001$), to $\delta^{15}N$ ($R^2 = 0.34$, $p = 0.0008$), and to $\delta^{13}C$ ($R^2 = 0.43$, $p = 0.0001$; Figure 2).

The best supported model predicting ln (THg) concentrations in alligators at YW included $\delta^{15}N$ ($\beta = 0.551$), $\delta^{13}C$ ($\beta = -0.274$), and sex ($\beta = 0.315$) and explained 50% of the variation (Table 4). The positive coefficients for sex indicate that males contain higher THg concentrations than females (female-male). The second-best supported model included SVL in addition to the same three predictor variables: $\delta^{15}N$ ($\beta = 0.462$), $\delta^{13}C$ ($\beta = -0.292$), Sex ($\beta = 0.334$), plus SVL ($\beta = 0.111$). All *p*-values for the predictor variables were significant, with the exception of SVL in the second model. At YW, ln (THg) concentrations were positively related to SVL ($R^2 = 0.25$, $p < 0.0001$), and to $\delta^{15}N$ ($R^2 = 0.36$, $p < 0.0001$), but not to $\delta^{13}C$ ($R^2 = 0.03$, $p = 0.18$; Figure 2).

Within JI, the null model was the best fit to the data (Table 4). The next three best supported models of ln (THg) concentrations all had low weights ($w_i < 0.121$) and included only single predictor variables, ^{15}N ($\beta = -0.124$), $\delta^{13}C$ ($\beta = -0.145$), or sex ($\beta = 0.159$; Table 4). At JI, ln (THg) was not related to SVL ($R^2 = 0.00$, $p = 0.79$), $\delta^{15}N$ ($R^2 = 0.05$, $p = 0.19$), nor $\delta^{13}C$ ($R^2 = 0.05$, $p = 0.20$; Figure 2).

Table 3. Likelihood ratio chi-squared statistic results from the full generalized linear model analyzing the effects of site, snout-vent-length (SVL), sex, $\delta^{13}C$, $\delta^{15}N$ and body condition index (BCI) on total Hg concentration in whole blood (ln-transformed).

Source	DF	χ^2	*p*
Site	2	50.28	<0.0001
SVL	1	15.51	<0.0001
Sex	1	8.75	0.003
$\delta^{13}C$	1	6.88	0.009
$\delta^{15}N$	1	3.54	0.060
BCI	1	0.14	0.71

Note. $n = 133$. DF = degrees of freedom.

Linear regressions revealed positive correlations between $\delta^{15}N$ and SVL at all three sites, although these relationships were weaker at OS and JI compared with YW (OS: $R^2 = 0.21$, $p = 0.013$; JI: $R^2 = 0.17$, $p = 0.016$; YW: $R^2 = 0.71$, $p < 0.0001$; Figure 3). Linear regressions between $\delta^{13}C$ and SVL showed that the two variables were positively correlated at both OS and YW but not JI (OS: $R^2 = 0.62$, $p < 0.0001$; YW: $R^2 = 0.39$, $p < 0.0001$; JI: $R^2 = 0.07$, $p = 0.13$).

Individuals with SVL < 19 cm were considered hatchlings and generally displayed greater THg concentrations than expected based on the relationships between SVL and THg (Figure 4). Mean THg concentrations (± SD) in hatchlings were 0.89 mg/kg (± 0.48) at OS and 0.25 mg/kg (± 0.04) at JI. The YW hatchlings, which were sampled in 2020, had THg concentrations of 0.25 mg/kg (± 0.12).

Discussion

Overall, body burdens of THg were highly variable across sites, with concentrations in alligators at OS approximately 8 times higher than those measured at JI and YW (OS: 0.125–1.916 mg/kg, JI 0.022–0.257 mg/kg, YK 0.018–0.302 mg/kg). 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 in other systems, and the hydrology of OS is dominated by precipitation and runoff with an average annual rainfall of 132.23 cm (Brook and Hyatt 1985, Wang et al., 2019, Okefenokee National Wildlife Refuge 1945–2021). Okefenokee is also in close proximity to several industrialized power plants, which have the potential to contribute to atmospheric Hg deposition (Porter 2000, Sherman et al., 2012). In addition, OS is acidic (pH of 3.5–4) and dominated by tannic waters and peat, both of which have been shown to contribute to increased concentrations of Hg methylation and subsequent bioavailability of Hg in

Table 4. Generalized linear models conducted at the site-level on the effects of snout-vent-length (SVL), sex, $\delta^{13}C$, $\delta^{15}N$ and body condition index (BCI) on whole blood hemoglobin concentration (ln-transformed).

Okefenokee Swamp (OS)						**Model variables: β-estimate, (*p*-value)**				
Model	**Variables, *n***	**R^2**	**AIC_c**	**ΔAIC_c**	**w_i**	**$\delta^{15}N$**	**$\delta^{13}C$**	**SVL**	**BCI**	**Sex [F-M]**
Null	0	0.00	77.01	25.73	0.00					
SVL, $\delta^{15}N$, BCI	3	0.689	51.28	0.00	0.285	0.375 (0.008)		0.337 (0.019)	0.279 (0.081)	
SVL, $\delta^{15}N$	2	0.648	51.93	0.65	0.205	0.302 (0.028)		0.498 (<0.001)		

Jekyll Island (JI)						**Model variables: β-estimate, (*p*-value)**				
Model	**Variables, *n***	**R^2**	**AIC_c**	**ΔAIC_c**	**w_i**	**$\delta^{15}N$**	**$\delta^{13}C$**	**SVL**	**BCI**	**Sex [F-M]**
Null	0	0.00	65.61	0.00	0.161					
$\delta^{15}N$	1	0.052	66.19	0.58	0.121	−0.124 (0.193)				
$\delta^{13}C$	1	0.050	66.28	0.67	0.115		−0.145 (0.204)			
Sex	1	0.018	67.42	1.81	0.065					0.159 (0.453)

Tom Yawkey Wildlife Center (YW)						**Model variables: β-estimate, (*p*-value)**				
Model	**Variables, *n***	**R^2**	**AIC_c**	**ΔAIC_c**	**w_i**	**$\delta^{15}N$**	**$\delta^{13}C$**	**SVL**	**BCI**	**Sex [F-M]**
Null	0	0.00	145.34	41.76	0.000					
$\delta^{15}N$, $\delta^{13}C$, sex	3	0.50	103.58	0.00	0.446	0.551 (<0.001)	−0.274 (0.003)			0.315 (0.010)
$\delta^{15}N$, $\delta^{13}C$, sex, SVL	4	0.508	104.89	1.31	0.232	0.462 (<0.001)	−0.292 (0.002)	0.111 (0.317)		0.334 (0.007)

Note. Stepwise model selection was run separately for each of the three sites (Okefenokee Swamp, Jekyll Island, and Yawkey Wildlife Center). Only the null model, the best model (i.e., with differences in corrected Akaike information criterion values [$\Delta AICc = 0$]), and models within $2\Delta AICc$ units of the best model are shown. Note that the null model is the best model at Jekyll Island. Model weights (w_i) were calculated across the full set of models ($n = 31$ per site). Independent variables in the model column show standardized β-estimates (*p*-value). Sex was the only categorical variable, and positive coefficients for sex indicate that males contain higher total mercury concentrations than females (Female-Male).

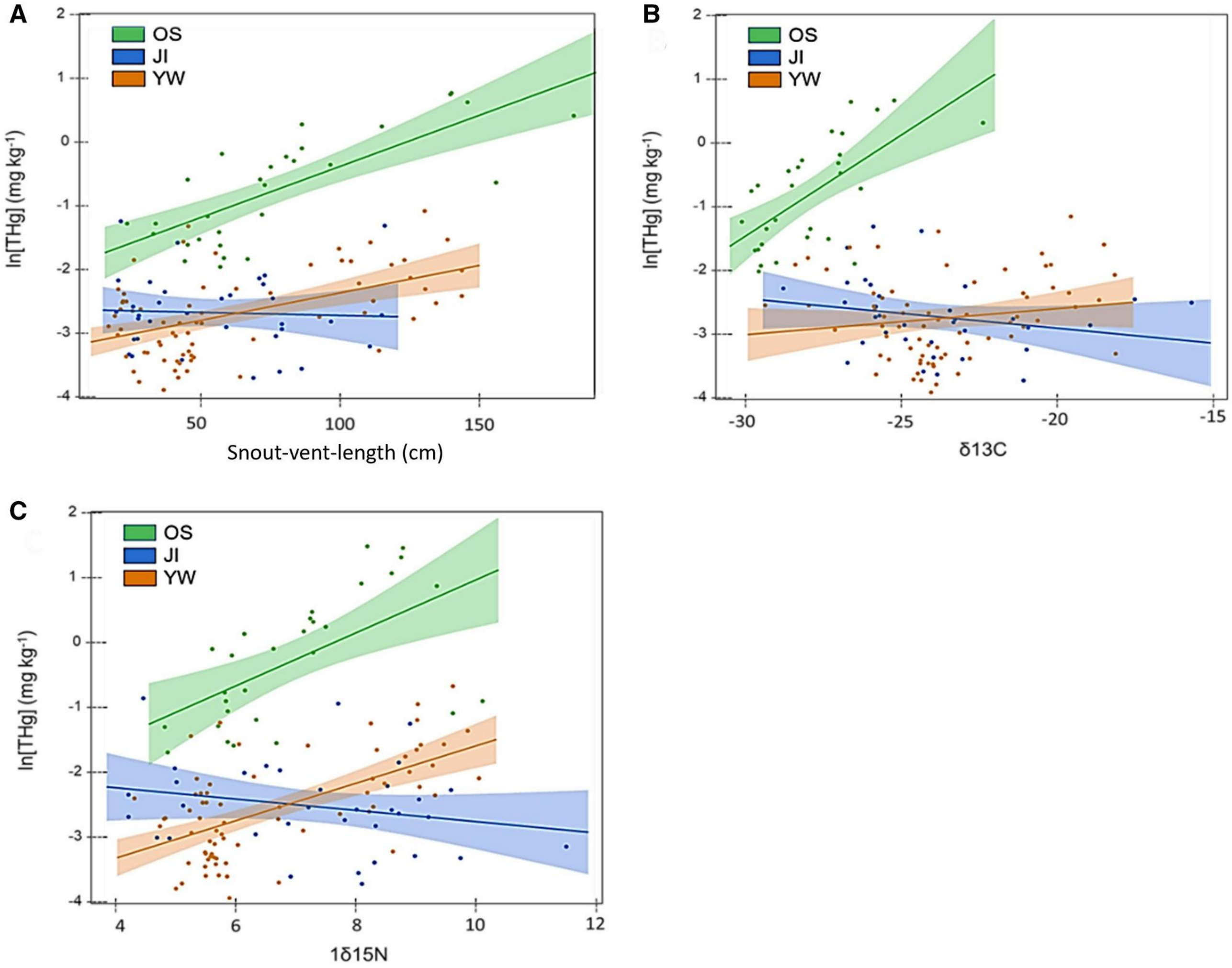

Figure 2. Linear regressions of total Hg concentration (ln transformed) in whole blood (mg kg^{-1}) against (A) snout-vent-length (SVL); (B) $\delta^{13}C$; and (C) $\delta^{15}N$. Sites are Okefenokee Swamp (OS, green, top-most line), Jekyll Island (JI, blue), and Yawkey Wildlife Center (YW, orange, line with the lowest intercept). Shaded regions around each line represent the 95% confidence interval.

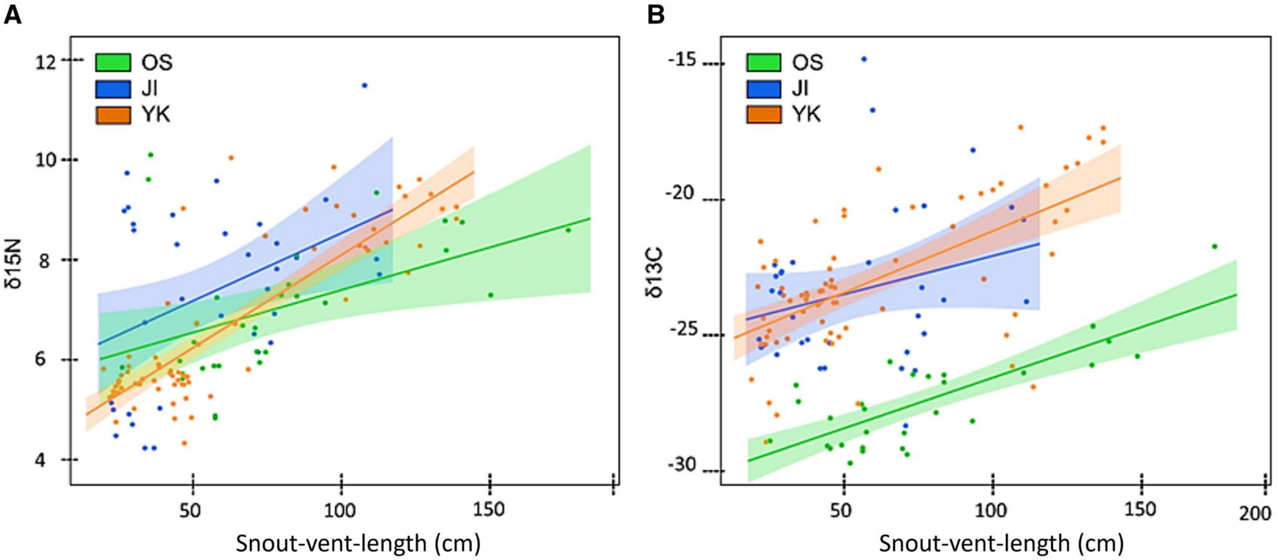

Figure 3. (A) Linear regression between snout-vent-length (SVL) and $\delta^{15}N$ for each of the three sites: Okefenokee (green, $p = 0.013$, $R^2 = 0.21$), Jekyll Island (blue, $p = 0.016$, $R^2 = 0.17$) and Yawkey (orange, $p < 0.001$, $R^2 = 0.71$). **B)** Linear regression between SVL and $\delta^{13}C$ for each of the three sites: Okefenokee Swamp (green, $p < 0.001$, $R^2 = 0.62$), Jekyll Island (blue, $p = 0.128$, $R^2 = 0.07$) and Yawkey Wildlife Center (orange, $p < 0.001$, $R^2 = 0.39$). Shaded regions around each line represent the 95% confidence interval.

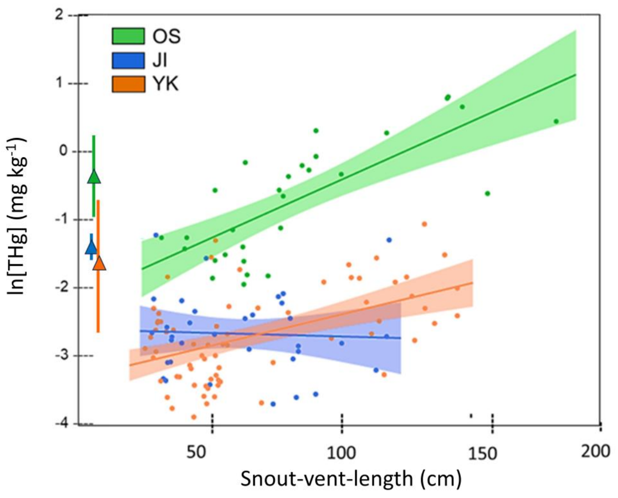

Figure 4. Mean hatchling (ln[THg]) (mg kg^{-1}) concentrations (triangles) plotted alongside linear regressions of the natural log of total mercury (ln[THg]) (mg kg^{-1}) as a function of snout-vent-length (SVL) in juveniles and adults (identical to Figure 2A). Shaded regions around each line represent the 95% confidence interval. Data points with error bars at the far left of the plot display the hatchling alligators (SVL < 19 cm) that were removed from the model analysis and not included in the regression slopes. Data points for hatchlings display site-level mean values (± SD) for multiple hatchlings, most of which are presumably from the same clutch ($n = 40$ OS, $n = 11$ JI, $n = 9$ YW). Note that YW hatchlings were sampled in 2020, 1 year following the collection of the other samples.

other systems (Hamilton 1982, Gilmour and Henry 1991, Branfireun et al., 1999). Finally, studies also show that patterns of wetting and drying allow for the resuspension and methylation of Hg, which is commonly observed within and across years at OS (Winger and Lasier 1997). For comparison, rainfall and runoff play a smaller role in some of the wetlands associated with JI and YK due to the presence of tidal inundation.

Positive relationships between THg body burdens and animal size are consistently reported for crocodilians (Buenfil-Rojas et al., 2015, Lawson et al., 2020, Nilsen et al., 2020, Lemaire et al., 2021b, Buenfil-Rojas et al., 2022, Belcher et al., 2022). Findings from this study add further context to prior studies, as the data largely corroborate these trends, but also suggest diet as an important driver, with δ^{15}N positively correlated with THg concentrations. Greater δ^{15}N values are generally associated with feeding at higher trophic levels. Relationships of THg to δ^{13}C were more variable, being positively correlated at OS and having no relationship at the other two sites (Figure 2B). The δ^{13}C values indicate which lower trophic-level organisms contribute to an organism's diet, yet resolving these relationships to the species level requires intensive reference values of site-specific δ^{13}C values (e.g., Caut et al., 2009). Because these values can vary greatly over space and time, and reference values for each site, both historically and present-day, are not established, we could not determine which lower trophic-level organisms were contributing most to the diet of alligators at each site. Further analysis could examine this level of detail. However, trends in δ^{13}C were shown to be significantly positively correlated with size at OS and YW (and nearly so at JI), indicating clear dietary shifts occurring with organismal growth and age (Figure 3B). Additionally, δ^{13}C values were higher and varied more at both JI and YW compared with OS (Table 2, Figure 2B). These trends are likely attributed to the availability of prey species closely associated with estuarine and marine habitats at both JI and YW that augment available dietary choices. Alligators living in coastal areas are known to leave freshwater impoundments to routinely feed in estuarine and marine environments, with their diet consisting of a variety of crustaceans, fish, mammals, and wading birds (Platt et al., 1990, Nifong 2014, Nifong et al., 2015, Nifong and Lowers 2017). Sulfur isotope analysis can resolve the relative contributions of freshwater, brackish, and marine sources, and thus future studies incorporating sulfur isotopic signatures may better inform the contributions of different biomes to THg burdens in coastal habitats.

Based on patterns of bioaccumulation, the highest Hg concentrations would be predicted in the oldest individuals. Indeed, when comparing body size (SVL) to THg across OS and YW, we see linear increases in body burdens, as expected. However, in the larger size classes, there appear to be a handful of individuals with lesser body burdens, resembling those of midsized individuals. This could stem from environmental variability or perhaps a temporary cessation in eating by these individuals living off fat stores. 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 Hg than size. Given the cessation of growth documented in alligators at YW, large individuals could range from 15 to greater than 60 years of age (Wilkinson, et al., 2016, Rainwater et al., 2022). Alligators at OS have only been marked since 2017, and within JI since 2011, offering limited data on the actual ages of individuals captured in those populations. Overall, few samples were available for the largest size classes, and ages of these animals were unknown for all three sites, thus limiting our ability to fully interpret the THg data when considering bioaccumulation over time. General hypotheses for the observed reduction in THg from middle-aged to older alligators include age-related changes in metabolism, foraging behaviors, THg excretion, survival bias of only the healthy individuals, and individual variation in bioaccumulation tendencies (Lawson et al., 2020).

Findings from this study as well as others suggest that maternal transfer of THg results in elevated concentrations in crocodilian offspring (Figure 4), which may have potential effects on reproductive success and overall health of individuals later in life (Du Preez et al., 2018, Rainwater et al., 2002, Nilsen et al., 2020, Lemaire et al., 2021a). For example, Hg exposure in most avian species is associated with decreased survival (Heinz et al., 2009). A recent study in alligators did not detect a relationship between THg concentrations in hatchlings and subsequent survival, but this study examined only one population (Johnson et al., 2023). The toxicokinetics of Hg in alligators (and other crocodilians), particularly hatchlings and juveniles, are not well understood. However, it is likely that the higher than expected THg concentrations observed in hatchlings in this study is more reflective of maternal resources (e.g., yolk) derived from prey at higher trophic positions; thus, as hatchlings grow using resources acquired at lower trophic levels (and having less THg), THg concentrations become diluted and decrease in response.

Elevated concentrations of Hg can trigger human consumption advisories for game species adjacent to and downstream of any site (Kojima et al., 2023). The headwaters for two major southeastern rivers are located within the OS, where THg concentrations were the highest, with 85% outflow into the Suwannee River, through Florida and into the Gulf of Mexico, and 15% into the St Mary's River, through Georgia and into the Atlantic Ocean (Rykiel, 1977). Currently, the U.S. Food and Drug Administration and U.S. Environmental Protection Agency have released advisories that consuming fish with concentrations

$>0.15–0.46\,\mathrm{mg\,kg^{-1}}$ 1–3 times per week, respectively, can have adverse health implications in humans, particularly for pregnant women and young children (US Department of Agriculture and US Department of Health and Human Services, 2020). Based on consumption risk evaluations comparing blood and muscle tissue in American alligators (Moore et al., 2022, Kojima et al., 2023), some of the THg values found in this study are more than this advisory level and would warrant moderated consumption of alligator. Additionally, when we place the observed alligator THg values in the context of other taxa with lower tolerance for contaminants, adverse and widespread effects in these organisms should also be considered. For example, Hg is known to have severe health effects on some bird species, with lethal concentrations starting as low as $0.25\,\mathrm{mg\,kg^{-1}}$ (Heinz et al., 2009). The highest concentrations of THg observed in the whole blood of alligators in the OS were sevenfold higher than this concentration (OS $0.125–1.916\,\mathrm{mg\,kg^{-1}}$), suggesting that other wildlife species in this area may be susceptible to chronic, and in some cases acute, effects of Hg exposure. Additionally, a previous study assessing THg concentrations across different trophic levels in the OS reported that several fish species (e.g., flier, pickerel, and bowfin) and racoons (*Procyon lotor*) exhibited higher concentrations than those measured in alligators (Arnold 2000). These findings support the expansion of research efforts to assess the ecological dynamics that drive variation in THg concentrations in crocodilians and other taxa.

In summary, our results indicate that Hg accumulation within a species can be highly spatially heterogeneous, likely driven by a combination of spatial variation in contaminant inputs interacting with the organism's life history traits, such as diet, age, and duration of exposure. In our study system, knowledge of organismal ontogeny, life history traits, and habitat characteristics are vital to understand spatial heterogeneity in Hg body burdens in a long-lived species. As we continue to assess trends in Hg bioaccumulation and biomagnification, both within food webs and across habitats, it is important to consider the influence of these various factors and how they vary over time and space. Studies aimed to address these factors will be especially valuable for species that may serve as bioindicators of their local ecosystems and food webs.

Data availability

The data used for this study is publicly available at Dryad. It is entitled: "American Alligator Mercury and Stable Isotope Analysis_Zemaitis2025" and may be accessed at https://doi.org/10.5061/dryad.51c59zwk6

Author contributions

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Funding

This research was supported by the Okefenokee Swamp Park, the Coastal Ecology Lab (University of Georgia Marine Extension and Georgia Sea Grant), and the University of Georgia Odum School of Ecology. In-kind support was provided by the Jekyll Island Authority, the Tom Yawkey Wildlife Center (Clemson University and South Carolina Department of Natural Resources), the Okefenokee National Wildlife Refuge (United States Fish and Wildlife), Okefenokee Adventures, and Okefenokee Swamp Park. Their contributions included the use of boats, vehicles, fuel, field equipment, laboratory equipment, and invaluable time and planning efforts during field seasons.

Conflicts of interest

None declared.

Ethics statement

Our work was conducted under IACUC permits #2374 (Parrott) and #3022 (Andrews) from the University of Georgia.

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Acknowledgments

This article represents technical contribution number 7342 of the Clemson University Experimental Station. Funding came from the Jekyll Island Authority and the Okefenokee Swamp Park. We further thank AmeriCorps, Okefenokee Adventures, and Okefenokee National Wildlife Refuge for in-kind support through access to personnel and boats. We also thank numerous individuals from Jekyll Island Authority and the UGA Coastal Ecology Lab (Marine Extension and Georgia Sea Grant) for assisting with captures.

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Environmental Toxicology and Chemistry, 2025, 44, 1611–1621
https://doi.org/10.1093/etojnl/vgaf060
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