Comparative assessment of blood mercury in American alligators (Alligator mississippiensis) from Coastal North Carolina and Florida

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Abstract
Mercury (Hg) is a widespread and harmful persistent pollutant of aquatic ecosystems. Except for the northern most populations of American alligators (Alligator Mississipiensis) found in North Carolina, the potential adverse health impacts of Hg on ecosystems and humans consuming alligator meat have been studied for over three decades. Now that alligators are being recreationally hunted and consumed across their range, it is especially important to monitor toxic contaminant levels to best understand possible adverse impacts of exposures on alligator populations and human health. In this study, we determined blood Hg concentrations in American alligators from an urbanized site in Wilmington, NC, a nearby site at Lake Waccamaw, NC, and a site on the St Johns River in Florida. Median blood total Hg (tHg) concentrations were particularly high at Lake Waccamaw (526 ng/g, range 152–946 ng/g), resulting in median muscle concentrations (0.48 mg/kg, range 0.13–0.88 mg/kg) well above US EPA screening values for fish consumption. Median concentrations at the Wilmington site (69 ng/g, range 22–336 ng/g) were generally low, and Hg concentrations from the St Johns River site (143 ng/g, range 54–244 ng/g) were comparable to those reported in previous studies. Analysis of relationships between tHg concentrations and a panel of blood chemistry biomarkers found only modest concentration-dependent impact on biomarkers of renal function. The results of this study reveal that local environmental factors greatly impact Hg bioaccumulation in alligators, findings that reaffirm local contaminant biomonitoring in alligator populations will be critical for affective management and determination of guidelines for safe consumption of harvested alligators.

Keywords Bioaccumulation · Biomonitoring · Ecotoxicology · Mercury · Metals · Reptile

Introduction
Mercury (Hg), whether in elemental (Hg0), inorganic (Hg2+), or organic forms is persistent and pervasive in the environment. Natural and anthropogenic mercury is released into the atmosphere and becomes deposited into soils and sediments where it becomes transformed primarily to organic methyl mercury (MeHg) by microbial methylation of Hg2+ (Gilmour and Henry 1991; Graham et al. 2012). Along with the volume of atmospheric wet and dry deposition, the production and bioaccumulation of MeHg in aquatic environments and food webs is dependent on a variety of factors including pH, dissolved organic
matter, and percentage of wetland area in a watershed (Gilmour and Henry 1991; Graham et al. 2012; Guentzel 2009; Miller et al. 2007).

In animals, MeHg is a potent developmental neurotoxicant with a slow elimination half-life due to its high affinity for glutathione and reduced sulphydryl groups (-SH) present in other cellular proteins (ATSDR 2022). As a result, MeHg accumulates in various tissues to cause additional adverse effects on hepatic, renal, and reproductive functions in humans and wildlife (Landrigan et al. 2020; Lemaire et al. 2021; Mergler et al. 2007). Because of its efficient trophic transfer through aquatic food webs, MeHg constitutes >95% of the total organic mercury in biota with concentrations found in large apex predators that may be millions of times higher than concentrations in surface water (Compeau and Bartha 1985; Lavoie et al. 2013; Wagemann et al. 1998, 1997). Such effectual bioaccumulation and biomagnification can result in MeHg accumulating to toxic concentrations in fish and other fish-eating apex predators; the consumption of contaminated fish and wildlife is also a risk for humans, especially exposures occurring during pre- and perinatal periods of development (Jagoe et al. 1998; Landrigan et al. 2020; Lavoie et al. 2013; Lawson et al. 2020; Nilsen et al. 2017a; Yanochko et al. 1997). Based on the results of numerous studies, the Agency for Toxic Substances and Disease Registry (ATSDR) has established a provisional minimal risk level (MRL) for oral exposure to MeHg of 0.1 mg/kg/day (ATSDR 2022), the US Food and Drug Administration has established a legal action level of 1.0 mg/kg Hg in consumable portions of fish, and the US EPA has set screening values of 0.4 mg/kg and 0.049 mg/kg for fish consumed by recreational and subsistence fishers respectively (Table 1 (US EPA 2000)).

In crocodilian species the bioaccumulation of anthropogenic toxic metals including Hg has been most well studied in the American alligator (Alligator mississippiensis) of southeastern and south-central United States (Burger et al. 2000; Delany et al. 1988; Elsey et al. 1999; Jagoe et al. 1998; Khan and Tansel 2000; Lawson et al. 2020; Nilsen et al. 2019, 2017b; Rumbold et al. 2002). Along with being a large and long-lived apex and keystone species across its range, American alligators often share rural and urban environments with humans and are important non-migratory sentinels of local adverse impacts from environmental pollutants (Somaweera et al. 2020). Further, wild-caught alligator meat is also consumed extensively by humans throughout most of its range in the southeastern United States where annual recreational hunts and nuisance alligator programs exist. During 2020 in Florida, for example, more than 208,000 pounds (94,530 kg) of alligator meat were produced through farming, and more than 17,000 additional alligators were reported as harvested on private lands and through recreational hunting and nuisance programs (FWC 2022). The potential public health effects resulting from consumption of alligator meat contaminated with toxic metals and other pollutants is unclear. Previous analysis in South Carolina were at total of 253 alligators were harvested in 2020 found that alligator hunters were at potential risk from mixtures of perfluorooalkyl substances (PFAS) and other environmental contaminants in alligator meat (SCDNR, 2022; Tipton et al. 2017a, 2017b).

With the exception of the northern most populations of American alligators found in North Carolina, Hg concentrations and potential adverse health impacts of MeHg exposure has been periodically assessed in American alligators (Burger et al. 2000; Delany et al. 1988; Horai et al. 2014; Jagoe et al. 1998; Khan and Tansel 2000; Lawson et al. 2020; Nilsen et al. 2020, 2019, 2017a, 2017b; Rumbold et al. 2002; Yanochko et al. 1997). As is the case elsewhere, Hg is a pervasive pollutant in North Carolina that has resulted in state-wide fish consumption advisories for largemouth bass based upon the presence of unsafe concentrations of Hg. Additional advisories are in place for bluegill, bowfin, and channel catfish from the Cape Fear River for elevated concentrations of Hg and other toxic metals (NC DPH, 2022). In light of the elevated concentrations of Hg found in these prey species of coastal North Carolina alligators, we sought to evaluate blood tHg concentrations in alligators from an urbanized site along the Cape Fear River, and from more rural sites surrounding Lake Waccamaw, NC which is in the adjoining Lumber River watershed. To better contextualize our findings from the NC alligator populations, we also

| Compound          | CAS ID       | Duration | Sensitive Endpoint | MRL       |
|-------------------|--------------|----------|--------------------|-----------|
| Methyl Mercury    | 22967-92-6   | Chronic  | Developmental      | 0.1 μg/kg/day |
| Mercury           | FDA Action Level | EPA SV Recreational | EPA SV Subsistence |
|                   | 1.0 mg/kg    | 0.4 mg/kg | 0.049 mg/kg        |

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ahttps://www.cdc.gov/TSP/MRLS/mrlslisting.aspx

bhttps://www.epa.gov/fish-tech/epa-guidance-developing-fish-advisories

MRL minimal risk level, SV screening value

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Table 1: Minimal risk levels for mercury exposure<sup>a</sup>, and FDA action and US EPA screening values for fish for consumption<sup>b</sup>
sampled alligators from the St John’s River in Florida. To evaluate potential adverse health impacts related to exposures we also assessed the relationships between tHg concentrations in blood with a panel of health-related blood chemistry biomarkers.

**Methods**

**Animal procedures**

All animal procedures were performed with approval by the North Carolina State University Institutional Animal Care and Use Committee (protocol #18-085-O) and under approved permits issued by NC Wildlife Resource Commission (21-ES00535) and the Florida Fish and Wildlife Conservation Commission (SPGS-20-49). Alligators were sampled using active capture methods that employed snatch hooks from Greenfield Lake (34.2107388211135, −77.93707241283391) Wilmington, NC near the Cape Fear River, around Lake Waccamaw, NC in the adjacent Lumber River basin (34.28726947673369, −78.50965117663293), and from the St. John’s River in Brevard and Orange Counties Florida (between 28.371883105432342, −80.89630680361354) and from the St. John’s River in Brevard and Orange Counties Florida (between 28.371883105432342, −80.89630680361354). Sampling in NC occurred between February 29 to October 29, 2021, and in FL from February 6 to February 8, 2021.

Alligators were visually examined for general health, and external injuries were noted and photographed. Sex was determined by cloacal examination with total length, snout to vent length, tail girth, head/snout, and rear foot measurements recorded. Following data collection, passive integrated transponder (PIT) tags were implanted intramuscularly into the right base of the tail of alligators in NC. In Florida, PIT tags were inserted into the right jowl and sampled alligators were also identified by a numbered toe web tag. All alligators were released at the site of capture. A typical time from first contact to release of animal was dependent on animal size with the average time to release being <15 min.

**Blood sampling procedures**

Immediately following capture and prior to collection of any additional data, a 10–15 mL whole venous blood sample was collected from the post-occipital spinal sinus using a sterile 20 g or 18 g needle and a 30 mL syringe (Myburgh et al. 2014). Whole blood samples were transferred to blue caped EDTA whole blood tubes for metals analysis, and green capped lithium heparin containing tubes (Vacutainer, BD Biosciences, San Jose, CA). Samples were mixed by gentle inversion and immediately stored on ice until the end of each field sampling days. At the end of each field collection day, plasma and packed blood cells in the lithium heparin tubes were separated by centrifugation (1800 x g for 10 min), transferred to Teflon-free cryovials, and then frozen. Whole blood samples in EDTA tubes were frozen until thawed for Hg analysis. All samples were archived at −80° C until analysis.

**Total mercury analysis**

Mercury analysis was performed beginning 11/22/2021 and completed 01/04/2022. Total Hg in whole blood samples was quantified by thermal decomposition amalgamation, and atomic absorption spectrophotometry using a MA-3000 mercury analyzer (Nippon Instruments, Osaka, Japan) following guidance outlined in US EPA methods 7473 (US EPA 1998). Each control and experimental whole blood sample was thawed, vortexed, and approximately 10 µl aliquots were weighed at room temperature in a ceramic sample boat that was previously heated for a minimum of 30 min in a muffle furnace at 800°C. Total Hg concentrations in each sample was determined in duplicate using a linear equation derived from a calibration curve that ranged from 0.1 ng to 1000 ng (r2 = 0.9999) Hg. The aqueous HgCl2 standards were made by serial dilution of a certified HgCl2 standard in 2% HCl (1000 ± 5 µg/ml; lot # 1923928, AGS Scientific, Bryan, Tx). Matrix-matched quality control samples were prepared by combining aliquots from a randomly selected subset of analyzed blood samples. National Institute of Standards and Technology Standard Reference Material (SRM) 955d Toxic Metals and Metabolites in Frozen Human Blood level 3 (55.3 ng/g, SD = 0.8) was used to validate measurement of tHg in blood. Replicate measurements of SRM 955d had a percent relative standard deviation of 7.3% (M = 51.3 ng/g, CV% = 1.09, n = 10). The concentration of total Hg in the pooled blood was determined and used as reference material (M = 204.94 ± 5.14 ng/g, CV% = 2.51, n = 23), with pooled blood samples included every 10 sample measurements. Additional quality control samples on each day of the analysis included method and field blanks, and aqueous standards of known Hg concentration (0.1, 1.0, and 10 ng; percent relative standard deviation from expected was <4.2%). Aliquots of the pooled alligator blood were spiked with 1 ng/µl of the HgCl2 standard and used to calculate recoveries. The mean percent recovery of Hg in 10 µl of the pooled blood was 96.92 ± 2.89% (n = 3) and the limit of detection defined as [LOD = meanblanks + 3(SDblanks)] was 0.01 ng (n = 20).

**Analysis of blood chemistry biomarkers**

Blood chemistry values for 11 parameters (listed in supplemental table S1) were obtained for a subset of samples
with sufficient volume and quality of serum for analysis (Cape Fear River: \(n = 11\), Lake Waccamaw: \(n = 30\); St Johns River, \(n = 24\)) using a VetScan VS2 Whole Blood chemistry analyzer with Avian/Reptilian Profile Plus rotors (# 500–7131; Abaxis, Union City, CA). Samples not meeting individual assay QC assessments were excluded from the analysis. All procedures followed manufacturer’s protocols using 100 \(\mu\)L of serum. Bile acids are included on the blood chemistry panel, but values were not reported because none were above the limit of assay detection (<35 \(\mu\)mol/L). A similar finding was observed in other alligator populations (Hamilton et al. 2016). For the other analytes, values below the linear range of detection were interpolated (values = LOD/\(\sqrt{2}\)) and reported values above the maximum of the linear range were substituted with the maximum value for the specific assay.

**Data and statistical analysis**

Each animal/blood sample was assigned a randomized numeric code at the time of collection. Samples were decoded for site and morphometric measures/sex only after measurement/analysis and biological marker analysis were completed. Descriptive data analysis was performed in Excel (Microsoft) with statistical comparisons and data visualization done using Prism (version 9.3, GraphPad La Jolla, CA). Gravimetric measurements were made on Model MS105DU analytical balance with a sensitivity of 0.01 mg (Mettler-Toledo, Greifensee, Switzerland). Total Hg concentration data is reported as wet weight, with the central tendencies of tHg concentrations (mean or median) and ranges determined for each site. A D’Agostino & Pearson test was used to assess normality of data; tHg concentration data was log10 transformed, morphometric and serum biomarker data was analyzed using non-parametric statistics. Linear regression and Spearman’s rank correlation coefficient were used to calculate relationships between tHg concentrations, SVL, and serum biomarkers. Multivariant analysis was first used to evaluate whether differences between capture site, month, log10 tHg concentration, SVL, and serum biomarkers had significantly affected the analysis models. ANOVA and Tukey’s multiple comparison was used to evaluate differences in SVL between sites, and a Kruskal-Willis test and Dunn’s multiple comparison’s test was used to evaluate differences in individual blood biochemistry endpoints between sites. Estimated tHg concentrations in muscle (mg \(kg^{-1}\)) were calculated using the formula \(\frac{[\text{muscle}]}{[\text{blood}]} = 18.701, 0.9475 \times \frac{1}{1000}\) and body mass index (BMI) was estimated using the formula \(\text{BMI} = \frac{\text{Tail Girth}}{(\text{SVL} \times 2)}\) (Lawson et al. 2020; Nilsen et al. 2017b, 2017a). A minimal level of statistical significance for differences in values among or between groups was considered \(p \leq 0.05\).

**Results and discussion**

**Study sites**

During spring and summer of 2021, we collected blood samples from a total of 68 immature and sexually mature alligators at two sites in NC, and from a site in FL where blood Hg concentrations had previously been assessed (Fig. 1A). The Cape Fear River site was chosen because of the relatively high density of alligators located in an urban location. This site has direct access to the Cape Fear River which has well defined upstream industrial sources of pollution from nearby coal-fly ash storage ponds and fluorochemical use and production (Aly et al. 2021; McCord and Strynar 2019). Lake Waccamaw, the largest of the Bay Lakes found on the North Carolina coastal plain, is located 50 kilometers northwest of the Cape Fear River site in the adjacent Lumbar River basin. This location has a similarly high density of American alligators that inhabit the canals and extensive wetlands surrounding the lake. We further selected this site for comparison because mercury deposition in precipitation at Lake Waccamaw has been tracked weekly since 1996 by the National Atmospheric Deposition Program. The availability of that data allowed us to compare mean weekly Hg deposition at Lake Waccamaw with Hg deposition at two sites in Florida with similar durations of long-term tracking (Fig. 1A, B; NADP, 2022). Between 1996 and 2020 mean weekly Hg deposition (\(\mu g/m^2\)) at Lake Waccamaw (NC05; \(M = 10.89, SD = 2.42\)) was significantly lower (\(F(2, 69) = 58.35, p < 0.0001, \eta^2 = 0.628\)) than deposition at sites in the Everglades (FL11; \(M = 19.62, SD = 3.52; p < 0.0001\)) or Chassahowitzka National Wildlife Refuge (FL05; \(M = 15.91, SD = 2.87; p < 0.0001\)).

**Study population characteristics**

There was a marked male bias in the sex of animals sampled from all 3 sites with males accounting for 84.8% of the entire study population (Table 2). A male sex bias in alligator populations is frequently reported in scientific sampling studies and in annual alligator harvests suggesting that some populations of American alligators maybe male biased (FWC 2022; Lance et al. 2000). We believe the notable sex bias could also have resulted from numerous factors related to the opportunistic sampling approach that we used, differences in behavior between sexes, and our repeated annual sampling at the North Carolina sites. Because the study is underpowered for determination of sex related differences, males and females were combined and analyzed in aggregate.

The population of alligators at the St Johns River was composed almost entirely of mature alligators where we
observed a higher density of large alligators with few immature alligators found in accessible portions of the river. Unlike the NC sites, this site is largely devoid of overhanging tree and shrub growth along its banks that serves as protective habitat for immature alligators. We were able to observe and more readily sample a variety of size classes of alligators that ranged from juveniles to mature adult alligators at both NC sites. Those observations were supported by our analysis of variance that found a main effect of sampling site on snout-vent length (SVL), $F(2, 65) = 4.59, p = 0.014, \eta^2 = 0.124$. Post-test analysis using Tukey’s multiple comparisons test indicated that the mean SVL of alligators from the St Johns River was significantly greater than the Lake Waccamaw alligators $p = 0.018$. BMI body mass index, $F$ female, $M$ male

### Table 2 Sample size, sex, snout to vent length, and BMI for each population

| Location                  | $N$ | Sex (F/M) | Snout-Vent Length (cm) | BMI       |
|---------------------------|-----|-----------|------------------------|-----------|
|                           |     |           | Mean ($\pm$ SD) | Min-Max | Mean ($\pm$ SD) | Min-Max |
| Cape Fear River, NC       | 13  | 0/13      | 94.7 ± 37.2       | 50.4–190.8| 0.230 ± 0.013   | 0.215–0.254|
| Lake Waccamaw, NC         | 31  | 7/24      | 95.4 ± 27.5       | 57.6–148.9| 0.226 ± 0.014   | 0.202–0.263|
| St. Johns River, FL       | 24  | 4/20      | 119.1 ± 31.9$^a$ | 50.9–163.1| 0.226 ± 0.015   | 0.178–0.260|

$^a$ANOVA found mean SVL of alligators from the St Johns River was significantly greater than the Lake Waccamaw alligators $p = 0.018$. BMI body mass index, $F$ female, $M$ male

**Fig. 1** Location of sampling sites, mercury deposition, and mercury blood concentrations. A Location of alligator sampling sites in North Carolina are indicated with an orange-colored circle, and the site of sampling on the St Johns River is indicated with a blue circle. Location of the Mercury Deposition Network sampling sites are indicated with yellow icons. B Shown is the average weekly mercury deposition in precipitation recorded from the Mercury Deposition Network site NC08 at Lake Waccamaw (orange), and Florida Everglades site FL11 (blue) and Chassahowitzka National Wildlife Refuge site FL05 (dark gray) C Mean total mercury blood concentrations (ng/g) from alligators sampled from the Cape Fear River ($n = 13$), Lake Waccamaw ($n = 31$), and the St Johns River ($n = 24$). Shown are the results from Kruskal-Wallis test and Dunn’s multiple comparison test of log10 transformed concentration data from each site
mean SVL for alligators sampled from the St Johns River was significantly greater than those from both NC sites (Table 2). Using a Kruskal-Wallis test no significant differences in calculated BMI were found across the sampling sites suggesting sampled alligators were similar in body condition (Table 2; p = 0.551).

**Total Hg analysis**

Our analysis of whole blood tHg found a mean concentration of 148.3 ng/g (SD = 48.9) for alligators sampled along the St Johns River in Florida (Table 3). Excluding the 3 immature alligators (SVL < 90 cm) did not significantly change the mean concentration of tHg (M = 151.0 ng/g, SD = 47.5), therefore we included these animals in our comparisons with the other sites. The tHg concentrations we observed at the St Johns River site were similar to concentrations previously reported for adult (M = 177.7 ng/g, SD = 84.9) and subadult alligators (M = 152.9 ng/g, SD = 84.9) from the St Johns River (Nilsen et al. 2016).

Whereas Hg deposition is significantly lower at Lake Waccamaw, the mean blood tHg concentration for all alligators sampled at Lake Waccamaw was significantly greater than the concentration of the St Johns River population (Table 3). Concentrations of tHg for adults from Lake Waccamaw (M = 640.4 ng/g, SD = 250.8) were significantly greater (t(29) = 3.18, p = 0.004, d = 1.18) than mean tHg concentrations in juveniles (M = 389.9, SD = 167.4) sampled from the same site. When we compared tHg concentrations in blood of mature or immature alligators at Lake Waccamaw with alligator from the other two sites, a significant main effect of tHg was found (F(2, 49) = 46.08, p < 0.0001, η2 = 0.653) and mean blood concentrations of tHg of each size class from Lake Waccamaw was significantly greater than the St Johns River population (Fig. 1C; p = 0.0001). For alligators from the Cape Fear River we found the mean blood tHg concentrations for this population was significantly lower than alligators from Lake Waccamaw and the St Johns River (Fig. 1C; Table 3). The average concentrations detected in the adults (M = 97.32 ng/g, SD = 94.45) and immature (M = 50.17 ng/g, SD = 28.23) alligators from the Cape Fear River was comparably low and not significantly different (p = 0.603) from one another. The lower concentrations of tHg observed in the Cape Fear River alligators that inhabit this urbanized ecosystem in Wilmington NC were likely the result of altered hydrology, morphology, and water chemistry of the sampling site that resulted in limited conversion of Hg into bioavailable MeHg. Numerous experimental and observational studies have linked habitats with high MeHg concentrations to sulfate- and iron-reducing microbial populations that convert inorganic mercury to methylmercury compounds (Guentzel, 2009; Lavoie et al. 2013). Therefore, we hypothesize that anthropogenic factors at the Cape Fear River have caused a decrease in sulfate- and iron-reducing microbial communities in the ecosystem resulting in decreases in bioavailable organic Hg compounds. Further ecosystem sampling, characterization of microbial populations, and food-web dynamic analysis is needed to understand factors responsible for the differences in Hg concentrations observed at each study site.

Previous analysis by Lawson and coworkers (2020) identified nonlinear relationships between blood tHg concentrations and size/age of alligator in populations from Florida and South Carolina, however the predicted decreases in blood concentrations of tHg for largest animals in our populations do not explain the elevated blood concentrations of tHg found at Lake Waccamaw. The Lake Waccamaw ecosystem itself is remarkable; as a Bay Lake it has an unusually high pH due to calcareous marine formations that buffer the acidic and high tannin blackwater entering the lake from surrounding wetlands (Stager and Cahoon, 1987). However, we anticipate that the large surrounding acidic wetlands, composed of large cypress and gum swamps from which the alligators were sampled, is a main driver of the elevated concentrations of MeHg in the Lake Waccamaw food web and the observed elevation of circulating tHg in alligators from this location.

In 2018 limited Alligator hunting became legal in North Carolina despite the much lower density of animals compared to populations in more southern states

| Location                  | N=  | Total Blood Hg (ng/g) Mean (± SD) (Min-Max) | Calculated Muscle Hg (mg/kg) Mean (± SD) (Min-Max) |
|---------------------------|-----|-------------------------------------------|---------------------------------------------------|
| Cape Fear River, NC       | 13  | 79.2 ± 79.6*                             | 0.56 ± 0.75                                      |
| Lake Waccamaw, NC         | 31  | 511.1 ± 246.1b                           | 0.47 ± 0.23                                      |
| St. Johns River, FL       | 24  | 148.3 ± 48.9                             | 0.12 ± 0.05                                      |

*ANOVA found mean blood [tHg] was significantly lower in alligators from the Cape Fear River compared to those from Lake Waccamaw (p < 0.0001) and St Johns River (p = 0.0005)

blood [tHg] was significantly higher in alligators from Lake Waccamaw compared to those from both the Cape Fear River and St Johns River (p < 0.0001). F female, M male
(NCWRC, 2018). To evaluate whether consumption of alligator meat represents a potential threat to consumers we estimated muscle concentrations using previously developed conversion factors that estimate muscle concentrations of tHg in alligator muscle/meat (Lawson et al. 2020; Nilsen et al. 2017b, 2017a). The calculated average tHg concentrations for the alligators from Lake Waccamaw (Lawson et al. 2018; Nilsen et al. 2017b, 2017a). The calculated average tHg concentrations for the alligators from Lake Waccamaw (M = 0.47 mg/kg, SD = 0.23) were found to be well above the US EPA advisory levels for Hg in fish (Tables 1 and 3 (US EPA 2022)). Highlighting the need to understand local influences in Hg cycling and concentrations in fish and wildlife consumed by humans, even the smallest immature alligator sampled from Lake Waccamaw (total length 111.5 cm, SVL 58.5 cm) had a blood tHg level (360 ng/g) sufficiently high to result in an estimated muscle concentration of 0.332 mg/kg (Table S2).

**Blood biochemistry**

Our analysis of blood chemistry values and multiple regression analysis found few changes across parameters that were correlated with tHg concentrations (Table 4). For the entire sampled study population, only uric acid and phosphorous were positively correlated with log$_{10}$ transformed [tHg], Spearman’s $r$(57) = 0.375, $p = 0.004$ and $r$(64) = 0.363, $p = 0.003$. Using Kruskal–Wallis tests (Chi square = 12.51, $p = 0.002$, df = 2) followed with Dunn’s multiple comparison test we also found a significant increase in uric acid in the samples from Lake Waccamaw compared to samples from the St Johns River ($p = 0.001$). Those findings suggest that a small but detectable impact on renal function is associated with elevated Hg exposure at Lake Waccamaw. Elevated phosphorous concentrations are also predictive of kidney disease in humans, and an established indicator of renal dysfunction in experimental animal studies (O’Seaghdha et al. 2011; Raikou 2021). However, the importance of the correlation between increasing Hg and serum phosphorous concentrations that we observed is less clear; complex interactions between agricultural phosphorus run-off, eutrophication, and bioavailable Hg in aquatic and estuarine environments cannot be ruled out as contributing to the changes in plasma serum phosphorous that we observed (Pakhomova et al. 2018; Soerensen et al. 2016). While AST in St Johns River samples was significantly elevated compared to each of the NC sites, a concomitant increase in creatine kinase that was significantly correlated with SVL (Spearman’s $r$(68) = 0.333, $p = 0.008$) was also observed in this population of larger alligators from the St Johns River. We interpret those increases in AST and CK as unrelated to exposure, but rather due to factors related to sampling and muscle release of these enzymes. The significant increase in blood glucose found in the Cape Fear River population ($p = 0.03$) was not correlated with blood concentrations of tHg, and therefore does not appear related to Hg exposure (Table 4).

### Conclusions

Methyl mercury bioaccumulation in aquatic environments is driven in part by atmospheric deposition of mercury from anthropogenic sources, however numerous local factors influence the degree to which organic Hg bioaccumulates in biota. The results of our analysis show that American alligators in coastal North Carolina are subject to widespread exposure to mercury. The elevated concentrations of blood Hg found in alligators at the Lake Waccamaw site suggest that bioavailable organic Hg are higher in the Lake Waccamaw ecosystem, and that factors related to the extensive wetlands surrounding this site are likely increasing the
bioavailability and bioaccumulation of Hg. Similar to previous studies, we found that alligator populations from sites in close geographical proximity may have very different blood tHg concentrations (Nilsen et al. 2017a). We recommend that ongoing biomonitoring studies be expanded across additional sites that include various ecological and hydrological conditions found throughout the range of American alligators. Further, expanded biomonitoring studies are needed to better understand the impacts of contaminants on alligator populations, and possible risks to humans from consuming contaminated alligator meat. Only deaths are needed to better understand the impacts of American alligators. Further, expanded biomonitoring studies are needed across additional sites that include various ecological and hydrological conditions found throughout the range of American alligators. Further, expanded biomonitoring studies are needed across additional sites that include various ecological and hydrological conditions found throughout the range of American alligators.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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