

MERCURY ACCUMULATION AND MOVEMENT ECOLOGY OF THE AMERICAN  
ALLIGATOR (*ALLIGATOR MISSISSIPPIENSIS*)

by

LAURA VANESSA KOJIMA

(Under the Direction of Benjamin B. Parrott)

ABSTRACT

Mercury (Hg) is a naturally occurring element but is also considered a widespread contaminant due to global anthropogenic activity. Even in moderate amounts, mercury (Hg) is an established neurotoxin and is associated with a range of adverse outcomes both in humans and wildlife. I investigated the accumulation of Hg in American alligators (*Alligator mississippiensis*) on the United States Department of Energy's Savannah River Site (SRS) and assessed the risk of Hg exposure from the consumption of wild caught alligator meat from alligators that originate from the SRS. I also examined the relationships between Hg body burdens and drivers of alligator movement behavior and home range. My results demonstrate the use of alligators as a bioindicator species and tied together the relationships between aspects of broader ecological health with that of human health through alligator ecology and toxicology.

INDEX WORDS: Bioindicator, Toxicology, Risk Assessment, GPS, Movement, Alligator

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LAURA VANESSA KOJIMA

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LAURA VANESSA KOJIMA

Major Professor:	Benjamin B. Parrott
Committee:	Tracey D. Tuberville
	Amanda Subalusky

Electronic Version Approved:

Ron Walcott  
Vice Provost for Graduate Education and Dean of the Graduate School  
The University of Georgia  
August 2023

## DEDICATION

This thesis is dedicated to those who paved the path before me to make science a more inclusive and accepting place.

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## TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS .....	v
LIST OF TABLES .....	x
LIST OF FIGURES.....	xii
CHAPTER	
1 INTRODUCTION .....	1
2 INTEGRATING MERCURY CONCENTRATIONS IN AMERICAN ALLIGATORS ( <i>ALLIGATOR MISSISSIPPIENSIS</i> ) WITH HUNTER CONSUMPTION SURVEYS TO ESTIMATE EXPOSURE RISK.....	5
Introduction.....	7
Methods.....	10
Results.....	15
Discussion .....	16
3 ENVIRONMENTAL CONTAMINANT BIOACCUMULATION IS ASSOCIATED WITH MOVEMENT BEHAVIOR IN A LONG-LIVED APEX PREDATOR .....	30
Introduction.....	31
Methods.....	33
Results.....	39
Discussion .....	43
Conclusion .....	49

4	CONCLUSION.....	59
	REFERENCES .....	64

## LIST OF TABLES

	Page
Table 2.1: Total mercury (THg) concentrations in tail muscle and whole blood samples from American alligators ( <i>Alligator mississippiensis</i> ) from two sites on the Savannah River Site (SRS), South Carolina, USA. Tail muscle was collected from individuals $\geq 180$ cm total length and whole blood was collected from all captured individuals. Predicted tail muscle (n = 9 predicted) THg concentrations were estimated using the linear equation (Eq. 3). Values are reported as mean $\pm 1$ SE (sample size; ranges).....	22
Table 2.2: Values for three scenarios (lower bound, average, and upper bound) of harvested American alligator ( <i>Alligator mississippiensis</i> ) meat consumption using South Carolina survey data from Tipton et al. (2017) to determine the frequency of consumption (times/year) and amount per meal (oz). THg concentration (mg/kg; ww) values from Savannah River Site, South Carolina alligator tail muscle, including predicted values (n = 40; $\geq 180$ cm total length).....	23
Table 2.3: Potential daily dietary exposure of mercury ( $\mu\text{g/day}$ ) from harvested alligator ( <i>Alligator mississippiensis</i> ) meat using South Carolina alligator harvest survey data from Tipton et al. 2017 and tail muscle mercury concentrations from alligators ( $\geq 180$ cm total length) sampled on the Savannah River Site.....	24
Table 3.1: Site of capture, identification, biological information (snout-vent-length (SVL) and mercury body burdens (Hg)), and GPS collection time frame of Savannah River Site, SC GPS tagged American alligators ( <i>Alligator mississippiensis</i> ) (n = 13).....	50

Table 3.2. AICc model output evaluating the most parsimonious model combinations for the relationship between explanatory variables and mean daily activity (s) of American alligators ( <i>Alligator mississippiensis</i> ) on the Savannah River Site, SC, in each season. Bolded values indicate top model combinations where $\Delta AICc \leq 2.00$ .....	51
Table 3.3. AICc model output evaluating the most parsimonious model combinations for the relationship between explanatory variables and mean daily distance (s) of American alligators ( <i>Alligator mississippiensis</i> ) on the Savannah River Site, SC, in each season. Bolded values indicate top model combinations where $\Delta AICc \leq 2.00$ .....	52
Table 3.4. AICc model output evaluating the most parsimonious model combinations for the relationship between home range size (ha) of Savannah River Site, SC American alligators ( <i>Alligator mississippiensis</i> ) and our explanatory variables per season. Bolded values indicate top model combinations where $\Delta AICc \leq 2.00$ .....	53

## LIST OF FIGURES

	Page
Figure 2.1: Map of South Carolina showing the location of alligator ( <i>Alligator mississippiensis</i> ) public hunt units and the Savannah River Site with respect to the Hunt Unit of concern (Unit 1- Southern Coastal). The map of the Savannah River Site denotes our sampling sites, Par Pond and L Lake, and their respective connections to the Savannah River.....	25
Figure 2.2: Pearson correlation ( $R$ ) between blood and tail muscle total mercury (THg) concentrations in American alligators ( <i>Alligator mississippiensis</i> ) captured in Par Pond and L Lake on the Savannah River Site, South Carolina, USA ( $n = 31$ ). All THg values are reported as wet weight.....	26
Figure 2.3: The relationship between THg, total length (TL), and site of capture in American alligators ( <i>Alligator mississippiensis</i> ) on the Savannah River Site (SRS). (a) Tail muscle THg was not significantly correlated to TL in either Par Pond or L Lake. Tail muscle values were obtained from alligators $\geq 180$ cm ( $n = 40$ ). (b) Whole blood THg was significantly correlated to TL in both Par Pond and L Lake ( $n = 53$ ). Significant differences between THg concentrations in Par Pond and L Lake were observed, with a more notable trend of bioaccumulation in alligators on Par Pond.....	27
Figure 2.4: The relationship between tail and blood THg and site of capture in American alligators ( <i>Alligator mississippiensis</i> ) on the Savannah River Site. Boxplots represent tail and blood THg including predicted values ( $n = 40$ ). For both tail muscle and blood,	

alligators on Par Pond had significantly higher levels of THg: (a)  $t = -6.27, p = 1.3 \times 10^{-6}$ ; (b)  $t = -6.60, p = 6.0 \times 10^{-7}$ . All THg values are reported as wet weight.....28

Figure 3.1. Daily activity and distance over time and per season. The mean daily (a) activity (s) and (b) distance (m) of Savannah River Site, South Carolina GPS tagged alligators (*Alligator mississippiensis*) (n = 13) during the data collection period (July 2020 – July 2022); trends in alligator daily activity and daily distance are denoted by the solid trend line and trends in the corresponding average daily temperature are denoted by the dashed trend line. The box plots show the range of daily movements [(c) activity (s) and (d) distance (m) traveled] of Savannah River Site, SC GPS tagged alligators (n = 13) per season. Colors denote season to account for variation in movement patterns among seasons.....54

Figure 3.2. Daily activity, distance, and snout-vent-length. The linear relationship between daily movement of Savannah River Site, SC (SRS) GPS tagged alligators (*Alligator mississippiensis*) (n = 13) and size (snout-vent-length) per season. Shaded areas between points represent the 95% confidence interval. (a) Mean daily activity (s) of SRS alligators and snout-vent-length. (b) Mean daily distance (m) of SRS alligators and snout-vent-length.....55

Figure 3.3. Daily activity, distance, and mercury. The linear relationship between daily movement of Savannah River Site, SC GPS tagged alligators (*Alligator mississippiensis*) (n = 13) and mercury body burdens (Hg; mg/kg ww) per season. Shaded areas between points represent the 95% confidence interval. (a) Mean daily activity (s) of SRS alligators and Hg. (b) Mean daily distance (m) of SRS alligators and Hg.....56

Figure 3.4. 95% Home Range Isopleth by Season. Home range isopleths of Savannah River Site, SC GPS tagged alligators ( <i>Alligator mississippiensis</i> ) on L Lake (n = 7) and Par Pond (n = 6) calculated using a Time Local Convex Hull approach. Home ranges of each individual tagged alligator are denoted by the shaded areas.....	57
--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	----

Figure 3.5. Home range and season. The relationship between home range size (ha) of Savannah River Site, SC GPS tagged alligators ( <i>Alligator mississippiensis</i> ) (n = 13) and season. Home ranges were calculated using a Time Local Convex Hull approach.....	58
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## CHAPTER 1

### INTRODUCTION

The production and release of contaminants due to anthropogenic activity results in continuous exposures for both humans and wildlife. The field of ecotoxicology has pioneered researcher addressing the adverse effects of contaminant exposure on wildlife, primarily focusing on fish, birds, and mammal. In general, reptiles are often neglected in ecotoxicological studies, despite many having a higher likelihood to be exposed to contaminants compared to other taxonomic groups. Furthermore, for animals whose life history makes them more susceptible to accumulating high amounts of contaminants and who are a game species, it is important to understand not only the effects of contaminant accumulation, but the risks surrounding consumption if hunted. My thesis aims to reduce this knowledge gap connecting these different facets involving ecological health with that of human health exploring the effects of a pervasive contaminant, mercury, on the ecology of the American alligator.

#### Mercury

Mercury is a naturally occurring element that has become an environmental pollutant as a result of being mobilized into aquatic and terrestrial ecosystems due to anthropogenic activity (e.g., gold mining, waste incineration, coal-burning power plants). Atmospheric transport is the foremost pathway of Hg emissions, nevertheless, land and ocean processes play an important role in the redistribution of Hg in terrestrial, freshwater, and marine ecosystems. Most notably, the production of methylmercury (e.g.,  $\text{CH}_3\text{Hg}^+$ ,  $(\text{CH}_3)_2\text{Hg}$ ; MeHg hereafter) in freshwater ecosystems (i.e., wetlands, watersheds, coastal zones) are particularly of concern. Following

deposition in aquatic sediments, sulfate-reducing bacterial can convert Hg to bioavailable methylmercury compounds which are potent neurotoxins that account for >95% of the Hg detected in biota (Bank et al., 2005, Compeau and Bartha, 1985, Wagemann et al., 1997). Hg is a contaminant of concern due to its ability to bioaccumulate over an individual's lifespan and biomagnify meaning it increases in concentration from lower to upper trophic levels (Chumchal et al., 2011, Snodgrass et al., 2000). An important distinction between Hg and most other atmospheric pollutants is that environmental and health impacts are only indirectly related to ambient atmospheric concentrations. The effects and levels of toxicity result from the uptake of Hg over an individual's lifespan via bioaccumulation and based on an individual's trophic position via biomagnification.

Hg is a well-known neurotoxin, documented to cause behavioral deficits. The neuron degeneration caused by Hg (Sakamoto et al. 1998) suggests that the metal could potentially disrupt the brain's ability to effectively control motor functions. The resulting behavioral effects may inhibit an organism's ability to capture prey, avoid predators, or successfully compete with others (Burke et al. 2010; Chin et al. 2013, Rice et al. 2014). Examining behavior is particularly crucial due to behavioral changes that result from environmental, chemical, and neurological variables between the organism and its surroundings can provide information in how anthropogenic influence is not only changing the environment but an organism's response to the change.

### *Mercury and Reptiles*

The life-history and physiological characteristics of reptilians make them ideal study candidates in the field of ecotoxicology. They are an excellent bioindicator group, useful for helping monitor the persistence of contaminants like mercury within an environment. Reptiles

are widespread globally inhabiting a variety of habitats, particularly those where mercury is most pervasive- aquatic habitats. Reptiles serve a crucial role as both predator and prey, with the few exceptions of some turtle and lizard species, most reptiles are strictly carnivorous, making them more susceptible to biomagnifying contaminants (Todd et al. 2010; van de Brink et al. 2016). In addition to their feeding preferences, reptilians are more at risk of high amounts of contaminant exposure and the subsequent bioaccumulation of contaminants due to their longer lifespans compared to that of other vertebrates (Hopkins, 2006; Bergeron et al. 2007, Rowe et al. 2008). Furthermore, reptiles exhibit high site fidelity leaving individuals susceptible to frequent interactions with contaminants within their environment (Hopkins, 2006; Bergeron et al. 2007).

### Objectives and Outline of Thesis

In this thesis, I report findings related to the bioaccumulation of Hg in the American alligator (*Alligator mississippiensis*) and also investigate the ecological influences of alligator movement behavior on the Savannah River Site (SRS) in South Carolina. The thesis research is aimed at addressing four objectives: 1) describing bioaccumulation trends in alligators on the SRS; 2) assessing the risk of mercury exposure from harvesting and consuming alligators that originate from the SRS; 3) determining environmental drivers and seasonal influence of alligator movement behavior using GPS data; and 4) investigating the relationship between mercury body burdens with behavior and other factors by evaluating fine scale movements and home ranges of alligators and comparing differences between alligators from a high-mercury lake with those from a nearby low-mercury lake. In Chapter 2 addresses the first two objectives to gain a better understanding of the risk of consuming game-species that originate in an area where contaminants persist. These data not only provide information on the trends of top predator mercury accumulation in our study area but provide a broader foundation for the assessment of

the consumption risk of aquatic game species. Chapter 3 addresses objectives 3 and 4 through identifying movement patterns of American alligators on the SRS and the relationship of alligator movement and mercury concentrations. Collectively, the work reported within this thesis advances our understanding of alligator movement ecology, and further provides information on the potential adverse effects of long-term mercury exposure as well as how movement behavior might influence mercury exposure dynamics.

CHAPTER 2

INTEGRATING MERCURY CONCENTRATIONS IN AMERICAN ALLIGATORS  
(*ALLIGATOR MISSISSIPPIENSIS*) WITH HUNTER CONSUMPTION SURVEYS TO  
ESTIMATE EXPOSURE RISK<sup>1</sup>

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<sup>1</sup>Kojima, L.V., Tuberville, T.D. and Parrott, B.B. (2023). Integrating Mercury Concentrations in American Alligators (*Alligator mississippiensis*) with Hunter Consumption Surveys to Estimate Exposure Risk. *Society of Environmental Toxicology and Chemistry*, 42: 525-534. Reprinted here with permission of publisher.

## ABSTRACT

Mercury is a naturally occurring element but is also considered a widespread contaminant due to global anthropogenic activity. Even in moderate amounts, mercury is an established neurotoxin and is associated with a range of adverse outcomes both in humans and wildlife. Humans in the U.S. are most commonly exposed to mercury through contaminated food or drinking water, and the consumption of game species, particularly those occupying higher trophic levels, has the potential to expose hunters to high concentrations of mercury. In this study, we determined mercury concentrations in tail muscle and blood from American alligators (*Alligator mississippiensis*) inhabiting a region (Savannah River Site, SC, USA) with known mercury contamination. We then integrated these data with alligator harvest records and previously published surveys of alligator meat consumption patterns to estimate potential exposure risk. We found that the average mercury concentrations in tail muscle (1.34 mg/kg, ww) from sampled alligators exceeded the recommended threshold for mercury exposure based on the World Health Organization's guidelines (0.5 mg/kg ww). In addition, based on regional consumption patterns reported for both adults and children, we estimated mercury exposures ( $\bar{x}_{Adult} = 0.419 \mu\text{g/kg/day}$ ;  $\bar{x}_{Child} = 2.24 \mu\text{g/kg/day}$ ) occurring well above the EPA methylmercury reference dose of  $0.1 \mu\text{g/kg/day}$ . Although the two reservoirs sampled in this study are not currently open to alligator hunting, they are connected to waters that are publicly accessible, and the extent of alligator mobility across these sites is not known. Together, the findings reported here further demonstrate the need for active monitoring of mercury concentrations in game species, which can convey substantial exposure risks to the public.

## INTRODUCTION

Although mercury is a naturally occurring element, anthropogenic activity has resulted in global hotspots with elevated concentrations capable of exerting adverse health effects on wildlife and humans. For example, coal combustion, chlor-alkali processing, and waste incineration introduce mercury into the environment through atmospheric transport and deposition (Jackson 1997; Driscoll et al. 2013). This dynamic is particularly consequential in aquatic ecosystems harboring sulfate reducing bacteria, which convert mercury to its more toxic and bioavailable form, methylmercury (MeHg) (Compeau and Bartha, 1985; Wagemann et al. 1997; Bank et al. 2005). Methylmercury bioaccumulates in organisms where it is readily absorbed into the bloodstream through the gastrointestinal tract and has a propensity to biomagnify across trophic levels (Wolfe et al. 1998; Chumchal et al. 2011; Bradley et al. 2017). Mercury has been documented to disrupt neuronal function in both humans and wildlife, negatively affecting coordination and movement, impairing both vision and speech, and weakening muscles (Sakamoto et al. 1998; EPA, 2022). Additionally, mercury has been found to negatively impact both the immune and endocrine systems, reproductive function, and in high amounts can result in mortality (Wolfe et al. 1998; Eisler, 2006; Scheuhammer et al. 2007; Tan et al. 2009; Wada et al. 2009; Todd et al. 2011).

In the United States, the main route of human mercury exposure is through direct ingestion from either contaminated food or water (Mahaffey, 2005; EPA, 2022). The consumption of game species holds value across cultural, economic, and conservation contexts, but for certain species, consumption can also serve as a direct source of contaminant exposure with attendant implications for public health (McCorquodale, 1997; Arnett and Southwick, 2015; Smith et al. 2018). In contrast to commercial agriculture, game species are often harvested from

spatially complex and heterogeneous landscapes and occupy a range of different habitats and trophic positions. In aquatic systems, contaminant concentrations in fish populations are often monitored by state and federal governments, but other game species (such as waterfowl, ungulates, alligators, and rabbits) are typically not given the same level of attention (Conder and Arblaster 2016; Smith et al. 2018). Globally, studies have investigated concentrations of contaminants in common game species, many of which have provided information on the risk of consuming game that has been killed with lead bullets and/or game meat that is sourced in close proximity to an area with known contamination (Swiergosz et al. 1993; Fachehoun et al. 2015; Oldenkamp et al. 2017; Morales et al. 2018; Arioli et al. 2019). While all game species can be exposed to contaminants, long-lived predatory species living in contaminated environments have potential to bioaccumulate high contaminant body burdens (Rowe, 2008), particularly for compounds such as mercury, which tend to biomagnify within an ecosystem.

The American alligator (*Alligator mississippiensis*) is an apex predator that inhabits a variety of freshwater and coastal habitats across ten states in the southern US, nine of which incorporate regulated alligator harvests into their wildlife management plans. Alligator harvests have served as an important mechanism for incentivizing the conservation of alligators and their habitat after these animals faced near extinction in the 1960s (Heykoop and Fechette, 2001). In addition to licensing fees that subsidize alligator management programs, direct and indirect economic benefits are realized for landowners, guides, and local communities (Powell, 2017; LDWF, 2021). Due to their long lifespans (> 60 years), high site fidelity, and high trophic status, alligators are an established bioindicator species for monitoring contaminants in aquatic environments (Rosenblatt and Heithaus, 2011; Nifong and Silliman, 2017; Wilkinson et al. 2016;

Lawson et al. 2020). These same attributes also convey substantial exposure risks to individuals that consume their meat.

The Savannah River forms the border between South Carolina and Georgia and is open to seasonal alligator harvests regulated by both states. Despite current consumption advisories for fish due to elevated mercury concentrations in the river, data on mercury concentrations in alligators are limited (SDHEC 2020). Flanking the Savannah River, the Department of Energy's Savannah River Site (SRS) is a former nuclear production plant which harbors reservoirs and wetlands with elevated concentrations of mercury (Jagoe et al. 1996; Fig.1). Although the SRS is not open to alligator hunting, it is possible that alligators move between the SRS and the Savannah River, making mercury-exposed alligators on the SRS potentially available to hunters. In this study, we quantified mercury concentrations in blood and tail muscle from alligators inhabiting the two former nuclear cooling reservoirs on the SRS that directly connect to the Savannah River through outfall streams. Our objectives were to (1) examine the relationship between body size and bioaccumulation of total mercury (THg) in harvest-sized alligators, (2) evaluate the relationship between THg concentrations in blood and tail muscle to determine if mercury concentrations in the former can serve as a proxy for concentrations in the latter, and (3) use published hunter consumption data to assess potential THg exposure risk associated with consuming these alligators. We predicted that THg concentrations would increase with size in alligators, THg concentrations in blood would reflect those measured in tail muscle, and the consumption of alligators occupying the SRS would present significant mercury exposure risks to humans.

## METHODS

### Study Area

The Department of Energy's Savannah River Site is a former nuclear production facility that houses two former nuclear reactor cooling reservoirs, Par Pond and L Lake. Elevated mercury concentrations in organisms inhabiting both reservoirs have been previously documented, likely originating from Hg-contaminated water pumped from the Savannah River below a now inactive off-site chlor-alkali plant (Kvartek et al. 1994; Jagoe et al. 1996). Par Pond, the larger of the two reservoirs (1100 ha), was constructed in 1958 to dissipate heat effluent from the P and R reactors on site. In 1991, a precautionary drawdown and repair of the Par Pond dam resulted in the resuspension of contaminated sediment in the reservoir, causing an increase in bioavailable mercury (DOE 1995). As a result, mercury concentrations in organisms inhabiting this reservoir have been well documented and monitored; however, mercury concentrations in alligators have not been assessed since the implementation of regulated harvests (Clay et al. 1978; Brisbin et al. 1992; Sugg et al. 1995; Yanochko et al. 1997; Jagoe et al. 1998; Peles et al. 2006; Brown et al. 2022). L Lake, the smaller (405 ha) and newer of the two reservoirs, was constructed in 1984 to dissipate heat effluent from the L Reactor. In contrast to Par Pond, the presence of mercury in L Lake biota has not received as much attention (Jagoe et al. 1996; Burger et al. 2003; Peles et al. 2006). Both reactors ceased operation in 1988 and their associated reservoirs currently serve as habitat for a variety of aquatic wildlife. Par Pond and L Lake drain into the Savannah River through two creeks, providing potential corridors for alligators to move between the reservoirs and the publicly accessible Savannah River (Fig. 2.1).

### *Alligator capture and sample collection*

From July-August 2020 and May-July 2021, we captured alligators using baited trip-snare traps (Murphy and Fendley, 1974), pole snares, or by hand. Immediately following capture, we collected blood samples from each alligator from the post-occipital venous sinus with a sterile 20-gauge needle and 30 mL syringe, which were transferred to either a 3 or 8 mL lithium heparin Vacutainer collection tube (BD, Franklin Lakes, NJ) and kept on wet ice for no longer than 5 hours before being frozen at  $-30^{\circ}\text{C}$  until analysis. We recorded morphological data including total length (cm) and sex and gave unmarked individuals unique scute clip marks (Bustard and Choudhury, 1981; Jennings et al. 1991; Rainwater et al. 2007). In addition, we injected individuals subcutaneously with a passive integrated transponder (PIT) tag (AVID, Norco, CA, USA) using a sterile 12-gauge needle (Wilkinson et al. 2016) at the right lateral area near the proximal base of the tail. For individuals with a total length  $\geq 180$  cm, we collected tail muscle at the left lateral area near the proximal base of the tail using a 10 mm Acuderm® biopsy punch (Acu-Punch® by Acuderm Inc., USA). The South Carolina Department of Natural Resources permits harvesting alligators with a total length of  $\geq 122$  cm; however at least 95% of alligators harvested in South Carolina are  $\geq 180$  cm, thus we considered only alligators  $\geq 180$  cm to be harvestable size (SCDNR, 2019; SCDNR, 2020; Butfiloski, 2021). To reduce potential discomfort, we administered 3 cc of 2% Lidocaine (Vet One®, USA) to the biopsy site using a 5-cc syringe and a 20-gauge 1.5 in needle prior to taking the biopsy. We released all alligators at their original capture location immediately following processing. We handled all alligators in accordance with approved protocols from the University of Georgia's Institutional Animal Care and Use Committee (AUP# A2020 02-026-Y2-A0). Scientific collection permits for capturing, handling, and collecting samples from alligators were issued by the South Carolina Department of Natural Resources (permits #SC-08-2020, #SC-08-2021).

### Quantifying total mercury in alligators

Prior to analysis, we thawed blood and tail muscle samples at room temperature. We homogenized blood samples using a vortex homogenizer for 30 s and placed a 50  $\mu$ L aliquot into a nickel weigh boat for analysis. We removed dermal tissue from the tail muscle biopsies using sterile shears and weighed each sample (wet weight to the nearest 0.001 g), freeze-dried it, then homogenized it using a Wig-L-Bug® grinder (Wig-L-Bug® Amalgamator, Crescent Dental Mfg Inc., USA). Once prepared, we weighed 0.005 g of each muscle sample and transferred the sample to a nickel weigh boat for analysis. We quantified total mercury (THg) concentrations in blood and tail muscle (mg/kg, wet weight) using a Direct Mercury Analyzer (DMA-80 EVO DUAL CELL, Milestone, Inc., Shelton, CT, USA; hereafter DMA) at the Savannah River Ecology Laboratory, University of Georgia (Aiken, SC, USA). The DMA uses a combination of thermal decomposition, gold amalgamation, catalytic conversion, and atomic absorption spectrometry to determine the mass fraction of THg in solid or liquid samples. For quality control, each run of 10 samples incorporated a blank, replicate, and a certified reference standard (TORT-3; National Research Council of Canada, Ottawa, Ontario). We originally obtained THg concentrations in tail muscle as dry weight (mg/kg) which we converted to wet weight (mg/kg) to account for sample preparation and phase differences (liquid vs. solid) between blood and tail muscle. We used the following formula to estimate the percent moisture content ( $M$ ) for each tail muscle sample (Eq. (1)) (Lusk et al. 2005; Lawson et al. 2020). The tail muscle sample's total mercury dry weight estimate ( $dw$ ) was then converted to wet weight ( $ww$ ) using the derived percent moisture content (Eq. (2)).

$$(1) \quad M = \frac{TM_w - TM_D}{TM_w} \times 100$$

$$(2) \quad ww = dw \times \left(1 - \frac{M}{100}\right)$$

$TM_w$  in Eq. (1) refers to the total mass of the wet sample that we transferred to the cryovial prior to freeze-drying, whereas  $TM_D$  is the mass of the sample after freeze-drying. All THg values are reported as mg/kg, ww.

### Statistical analysis

We performed all analyses with the statistical software RStudio v2021.09.1 (RStudio Team, 2021) and produced all figures using the package ggplot2 3.3.5 (Wickham, 2016). We tested data for normality and homogeneity of variance and log-transformed when necessary to fit basic assumptions of analysis. Preliminary analysis performing a Student's t-test found that THg in both tail muscle and whole blood did not differ significantly between sexes. Therefore, we did not consider sex in our models. We applied linear regression analyses to assess relationships between total length (TL) and THg concentrations in both tail and whole blood samples, in addition to evaluating the interaction between size and site on THg concentrations. A Student's t-test was used to assess the relationship between tail muscle THg concentrations and site of capture and whole blood THg concentrations and site of capture. Finally, we performed a Pearson correlation analysis to describe the relationship between blood THg and tail muscle THg values (both mg/kg, ww) based on individuals for which both sample types were available. The resulting regression constant and coefficient in Eq. (3) were then used to estimate muscle THg concentration from whole blood THg measurements for those alligators ( $\geq 180$  cm) for which a tail muscle sample was not collected.

$$(3) \quad y = a + bx$$

$$y = -.11 + 1.2x$$

In Eq. 3,  $a$  is the regression constant which is the mean response variable when the predictor (THg blood) values are set at zero whereas  $b$  is the regression coefficient. The  $x$  value in the equation is the predictor variable (THg blood concentrations) and  $y$  is the predicted tail muscle concentration (mg/kg, ww). We calculated descriptive statistics (mean, standard error, and range) of THg concentrations from tail muscle samples and their corresponding blood samples overall (SRS) and for each site separately (L Lake, Par Pond; Table 2.1).

#### Estimating consumption risk

We estimated consumption risk by combining our data on muscle THg concentrations in harvest-sized alligators ( $\geq 180$  cm TL) with published survey data of regional hunter consumption patterns of alligators (Tipton et al. 2017). Tipton et al. (2017) interviewed 23 recreational hunters who harvested alligators in South Carolina in 2015 and obtained information on planned consumption of the harvested meat, including the predicted meal size and predicted daily consumption frequency, to explore three consumption scenarios (lower bound, average, and upper bound scenarios). Additionally, Tipton et al. (2017) acquired site-specific information for two hunt units, including the Southern Coastal Unit (Fig. 2.1), where the SRS resides. We applied these same consumption scenarios to our muscle THg data to determine potential daily exposure associated with consuming alligators collected on the SRS (Table 2.2). In each scenario, adult (80 kg) and child (15 kg) body weights were used to account for exposure based on body weight. We examined dietary exposure scenarios for each reservoir and for both reservoirs combined to account for the risk of Hg exposure from alligators on one reservoir being

higher than the other and to estimate the average risk of eating alligators that originate from the Savannah River Site.

## RESULTS

### Mercury concentrations in alligator tissues

We sampled a total of 53 alligators across all size classes, 31 of which were harvestable alligators (total length  $\geq 180$  cm). THg concentrations in SRS alligators ranged from 0.077 – 4.33 mg/kg in tail muscle (n = 31) and 0.076 – 3.41 mg/kg in whole blood (n = 53; Table 2.1). We found that blood and tail THg were significantly and positively correlated ( $p = 1.1 \times 10^{-11}$ ,  $R^2 = 0.79$ , n = 31) (Fig. 2.2). Muscle THg increased with increasing TL; however, this relationship was not significant when considering data from both Par Pond and L Lake ( $p = 0.051$ ,  $R^2 = 0.30$ , n = 40) nor was the interaction of TL and site ( $p = 0.17$ ; Fig. 2.3a). Whole blood THg concentrations and TL were positively correlated ( $p = 1.3 \times 10^{-5}$ ,  $R^2 = 0.30$ , n = 53) and the interaction between TL and site was significant ( $p = 0.0004$ ,  $F = 32.76$ ) (Fig. 2.3b). In addition, harvest sized alligators (TL  $\geq 180$  cm) from Par Pond had significantly higher THg in tail muscle ( $\bar{x}_{Par\ Pond} = 1.97$  mg/kg, ww;  $\bar{x}_{L\ Lake} = 0.64$  mg/kg, ww;  $p = 1.3 \times 10^{-6}$ ) and blood ( $\bar{x}_{Par\ Pond} = 1.34$  mg/kg, ww;  $\bar{x}_{L\ Lake} = 0.60$  mg/kg, ww;  $p = 6.0 \times 10^{-7}$ ) when compared to harvest sized alligators from L Lake (Figs. 3.4a, b).

### Potential consumption risk

Only alligators  $\geq 180$  cm were sampled for tail muscle and considered when analyzing consumption risk data. Additional tail muscle THg concentrations were estimated using the linear equation (Eq. 3) for 9 harvestable animals from which muscle samples were not collected (n = 40; 31 direct measurements, 9 predicted). Mean THg concentration in tail muscle of harvest sized alligators from the SRS ( $\bar{x} = 1.31 \pm .18$  mg/kg; ww) exceeded the World Health

Organization's recommended consumption concentration (0.5 mg/kg; ww) (Ikem and Egiebor 2005). When examining different exposure scenarios based on consumption rates and meal size survey data, there was a wide range of predicted exposures (summarized in Table 2.3). Potential dietary exposure across the SRS ranged from 0.036 – 363.92 µg/day with an average exposure of 33.54 µg/day. Estimated daily dietary exposure for adults ranged from 0.00045 – 4.55 µg/kg body weight/day, and using the average consumption scenario (average frequency x average serving size x average Hg concentration), we found the average dietary exposure for an adult was 0.419 µg/kg body weight per day. In the case of children, estimated daily dietary exposure ranged from 0.002 – 7.23 µg/kg body weight/day. This wide range in predicted THg exposure among scenarios can be attributed to the differences in THg concentrations in alligators between reservoirs, with the highest concentrations coming from Par Pond.

## DISCUSSION

### Total mercury concentrations in alligator whole blood and tail muscle

Our findings are consistent with mercury being prevalent in aquatic environments throughout the southeastern United States, where consumption of game species harvested through recreational hunting can lead to significant dietary exposures. Prior studies have used blood as a minimally invasive sample to evaluate mercury concentrations in crocodilians (Burger et al. 2007; Eggins et al. 2015; Nilsen et al. 2017a; Lawson et al. 2020; Lemaire et al. 2021), and our findings that whole blood THg concentrations can be used to infer tail muscle THg concentrations are consistent with those studies. For example, Nilsen et al. (2017) observed a similar relationship between concentrations of THg in blood and muscle of alligators in Florida, and reported that blood concentrations of selenium, rubidium, and zinc are also reflective of those in tail muscle. Strong correlations between THg in blood and other tissues have also been

documented in alligators from other study sites and in other reptiles, such as brown watersnakes (*Nerodia taxispilota*), collected on the Savannah River (Haskins et al. 2021a, b; Moore et al. 2022). Blood sampling protocols are usually less invasive than biopsy procedures and downstream analytical protocols are typically less time consuming. Taken together with findings from other studies, our results suggest that whole blood represents a minimally invasive and reliable proxy for THg concentrations in alligator tail muscle, which could be used in future monitoring applications.

We found that THg concentrations in blood were positively correlated with alligator length, which is in contrast to previous studies at the Savannah River Site reporting only scute Hg concentrations were correlated with alligator length (Yanochko et al. 1997; Jagoe et al. 1998). Studies at the Tom Yawkey Wildlife Center in South Carolina and Merritt Island National Wildlife Refuge in Florida have observed a nonlinear relationship between whole blood THg alligator length, suggesting accumulation occurs until growth cessation (Lawson et al. 2020). In black caimans (*Melanosuchus niger*) in French Guiana, a linear relationship between whole blood THg and total length was documented (Lemaire et al. 2021), and correlations between whole blood THg and total length have been observed in other reptiles, although the results are not always consistent (Burger et al. 2007; Schneider et al. 2010; Eggins et al. 2015). The relationship between tail muscle THg and total length in our study was not significant when considering combined data from the reservoirs. When examining this relationship separately at each reservoir, we found that the relationship was significant on Par Pond, but not on L Lake; however, analyses of tail muscle were restricted to samples from larger individuals ( $\geq 180$  cm) and thus lacked the variation in length included in our analyses of blood. Whereas there is broad support for the bioaccumulation of mercury across diverse taxa, increases in THg with

crocodilian length might not be solely attributable to bioaccumulation dynamics alone and can reflect ontogenetic shifts in diet (Platt et al. 2006; Wallace and Leslie 2008; Platt et al. 2013). Crocodilians, including alligators, typically feed at lower trophic levels during juvenile life stages compared to adults (Nifong et al. 2015). For example, in black caimans, THg in whole blood was positively correlated with both trophic position and total length (Lemaire et al. 2021). Similarly, THg liver and muscle concentrations in alligators in Texas were positively correlated with trophic position (Chumchal et al. 2011). Additional studies aimed at parsing the relative contributions of bioaccumulation and biomagnification via ontogenetic dietary shifts are promising avenues for better resolving the ecotoxicological dynamics that drive THg body burdens in crocodilians.

### Consumption Risk

Our results suggest the consumption of alligator tail muscle has the potential to convey substantial dietary exposure to Hg. The World Health Organization recommends not consuming food with mercury levels  $\geq 0.5$  mg/kg ww and the average THg concentrations in tail muscle from alligators on the Savannah River Site were well above this limit (Ikem and Egiebor 2005). Additionally, the Food and Drug Administration (FDA) recommends a limit of 0.46 mg/kg ww for weekly human consumption; however, frequent consumption of food products with Hg that have low concentrations (e.g., 3 servings per week that are  $\leq 0.15$  mg/kg; ww) are permissible (FDA, 2021). We derived daily dietary exposure of mercury from alligator tail muscle from lower and upper bound scenarios of consumption as well as the average consumption scenario. In addition, we considered how exposure varies between adults and children and calculated daily exposure for each group (Table 2.3). We showed that exposure can vary depending on the site from which the alligators originated, with consumption of alligators from Par Pond resulting in

greater exposure compared to L Lake. However, because reservoir of origin for alligators harvested off the Savannah River Site is likely to be unknown, we also estimated exposure for the SRS as a whole by combining values from the two reservoirs. Data from Tipton et al. (2017) showed that 45.5% of surveyed hunters reported consuming 3 oz of harvested alligator meat in a sitting with 63.6% reporting an expected consumption of alligator meat  $\geq$  once a month. Given the elevated Hg concentrations observed in alligators inhabiting the Savannah River Site, hunters that consume alligator meat from this area put themselves at a high risk of mercury exposure if they do not limit their consumption to at least one serving (3 oz) once a month. Other factors that influence THg exposure include sharing of harvested meat with children and vulnerable groups within a household, sharing with other households, and consumption of other game species (Smith et al. 2018). Tipton et al. (2017) reported that greater than 45% of successful alligator hunters in South Carolina planned to share alligator meat with children under 15 years old, with half of these individuals disclosing that their children were likely to consume alligator meat at the same frequency they did (Tipton et al 2017). Along with pregnant women, children are at greatest risk of the health effects of mercury exposure (FDA 2020). Children are more vulnerable to high Hg exposure due to the negative effects on their developing systems (WHO, 2006; Sly and Pronczuk 2007; Bose-O'Reilly et al. 2010). Thus, there are several factors to be considered when predicting risk of Hg exposure from alligator consumption including animal body burdens and site of origin, as well as consumption dynamics (e.g., frequency of consumption, body weight, portion size). In addition, overall Hg exposure risk will be influenced by the extent to which hunters consume other game species – an aspect that has received little attention (Smith et al. 2018).

Our analysis did not differentiate what proportion of THg is comprised in MeHg. However, muscle THg concentrations of brown watersnakes (*Nerodia taxispilota*) on the Savannah River are strongly correlated with MeHg, with the highest average percentage of MeHg in THg being found in muscle ( $79.4 \pm 1.7\%$ ; Haskins et al. 2021a). Similarly, MeHg comprises an average of 90% of the THg in fish muscle (Mason et al. 2000; Burger et al. 2014). In addition, Chumchal et al. (2011) documented that in alligators in Texas, MeHg in muscle was highly correlated with THg (81.6 %), and in a study considering two crocodile species (*Melanosuchus niger* and *Caiman crocodilus*), Gomes et al. (2020) found that MeHg in muscle tissues comprised between 84 – 94% of THg. Based on existing evidence, it is likely that MeHg is the primary contributor to THg measured in alligator tail muscle in this study and thus exceeds the reference dose (MeHg =  $0.1 \mu\text{g/kg/day}$ ) for most consumption scenarios (FDA, 2021).

Currently, there is no direct evidence of alligators moving between the Savannah River Site and the adjacent Savannah River. However, alligators have been documented to frequently move between water bodies, some more than 10 km (Subalusky et al. 2009; Rosenblatt and Heithaus, 2011; Fujisaki et al. 2014). During the course of our study, we captured two alligators on L Lake that were initially captured on Par Pond (approximately 7.7 km apart) in 2002 and 2007. The distance from L Lake to the Savannah River is approximately 8 km, suggesting that alligator movement from the Savannah River Site to the Savannah River is possible, if not likely. The Savannah River itself has a history of mercury contamination, probably related to a chlor-alkali plant upstream of the SRS that was active in the 1970s and nearby coal ash basins on the SRS (Kvartek et al. 1994). Due to elevated Hg concentrations in the Savannah River, the South Carolina Department of Health and Environmental Control has issued consumption advisories for common fish species in the river, such as largemouth bass (*Micropterus salmoides*), chain

pickerel (*Esox niger*), and spotted suckers (*Minytrema melanops*) (SDHEC 2020). Other fish species, such as bowfin (*Amia calva*) and largemouth bass downstream of the SRS, are prohibited from being fished due to elevated Hg concentrations (Burger et al. 2001, SDHEC 2020). As many of these fish species are likely to contribute to the alligator diet, consumption advisories for alligators from the Savannah River may also be warranted. Localized sampling of alligators (such as on the Savannah River) and an understanding of their landscape-scale movement patterns would help tailor consumption advisories to appropriately consider localized patterns of risk associated with alligator consumption.

**Table 2.1.** Total mercury (THg) concentrations in tail muscle and whole blood samples from American alligators (*Alligator mississippiensis*) from two sites on the Savannah River Site (SRS), South Carolina, USA. Tail muscle was collected from individuals  $\geq 180$  cm total length and whole blood was collected from all captured individuals. Predicted tail muscle (n = 9 predicted) THg concentrations were estimated using the linear equation (Eq. 3). Values are reported as mean  $\pm 1$  SE (sample size; ranges).

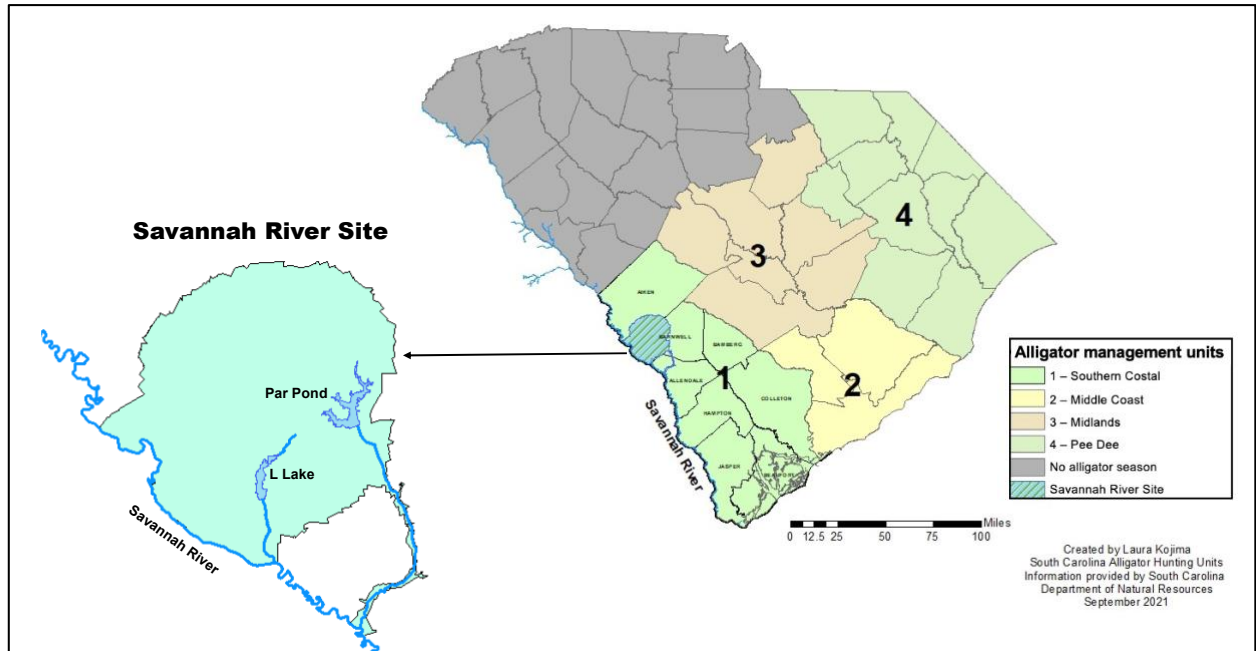
Site	Tail muscle (mg/kg ww)	Whole blood (mg/kg ww) <sup>1</sup>	Predicted tail muscle (mg/kg ww) <sup>2</sup>
SRS	1.31 $\pm$ .18 (n = 31; 0.08 – 4.33)	0.94 $\pm$ .10 (n = 53; 0.08 – 3.41)	1.34 $\pm$ .15 (n = 40; 0.08 – 4.33)
Par Pond	1.97 $\pm$ .22 (n = 17; 0.45 – 4.33)	1.34 $\pm$ .18 (n = 24; 0.08 – 3.41)	1.97 $\pm$ .20 (n = 21; 0.45 – 4.33)
L Lake	0.51 $\pm$ .05 (n = 14; 0.08 – 0.85)	0.60 $\pm$ .06 (n = 29; 0.10 – 1.21)	0.64 $\pm$ .08 (n = 19; 0.08 – 1.45)

**Table 2.2.** Values for three scenarios (lower bound, average, and upper bound) of harvested American alligator (*Alligator mississippiensis*) meat consumption using South Carolina survey data from Tipton et al. (2017) to determine the frequency of consumption (times/year) and amount per meal (oz). THg concentration (mg/kg; ww) values from Savannah River Site, South Carolina alligator tail muscle, including predicted values (n = 40;  $\geq 180$  cm total length).

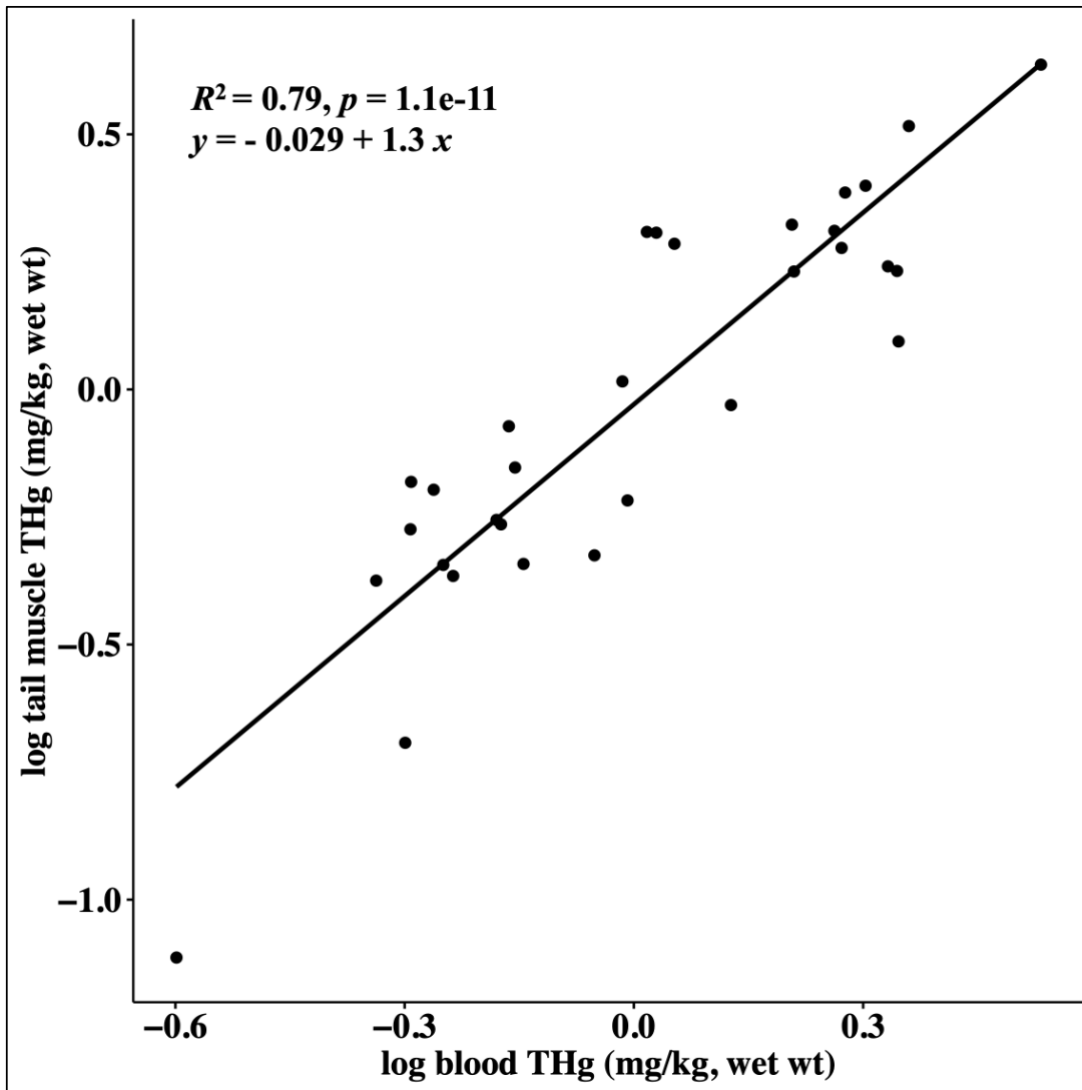
Scenario	Frequency of Consumption	Amount/meal	Tail THg concentration (mg/kg, ww)
Lower Bound	2 times/year	3 oz	0.08
Average	31 times/year	10.4 oz	1.34
Upper Bound	52 times/year	20.8 oz	4.33

**Table 2.3.** Potential daily dietary exposure of mercury ( $\mu\text{g/day}$ ) from harvested alligator (*Alligator mississippiensis*) meat using South Carolina alligator harvest survey data from Tipton et al. 2017 and tail muscle mercury concentrations from alligators ( $\geq 180$  cm total length) sampled on the Savannah River Site.

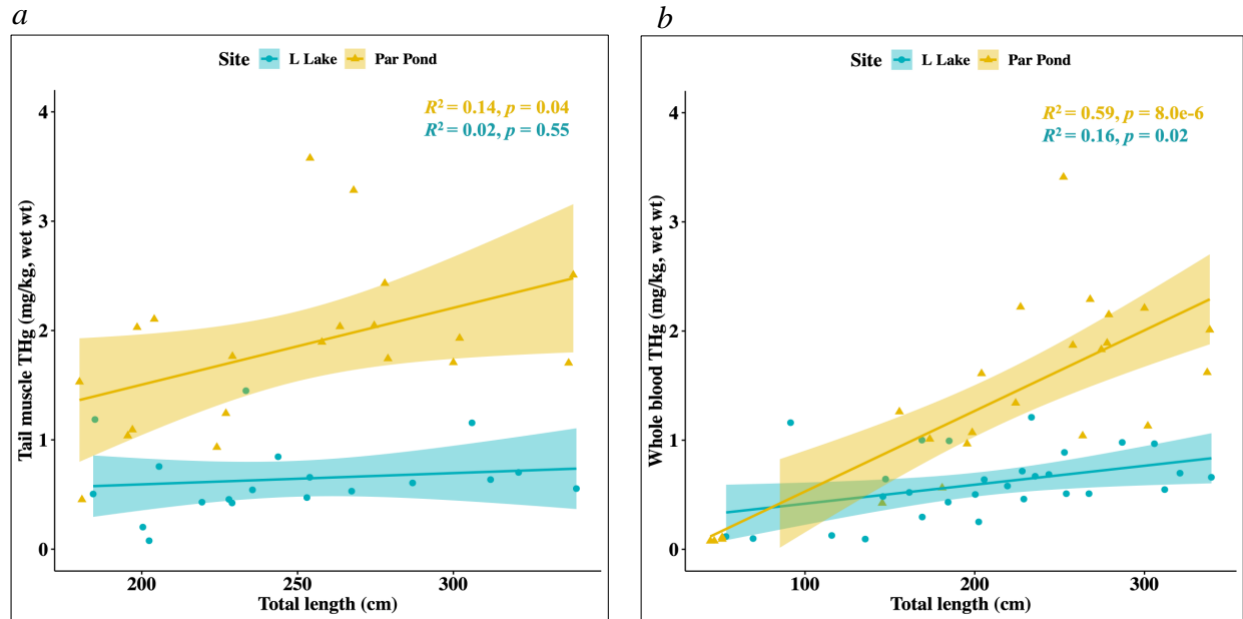
		Total ( $\mu\text{g/day}$ )	Adult ( $\mu\text{g/kg}$ body weight per day)	Child ( $\mu\text{g/kg}$ body weight per day)
<i>SRS (n = 40)</i>				
Lower Bound	Low frequency $\times$ Small serving $\times$ Low conc.	0.04	0.0005	0.002
Upper Bound	High frequency $\times$ Large serving $\times$ High conc.	363.92	4.55	24.26
Average	Avg. frequency $\times$ Avg. serving $\times$ Avg. conc.	33.54	0.42	2.24
<i>Par Pond (n = 21)</i>				
Lower Bound	Low frequency $\times$ Small serving $\times$ Low conc.	0.21	0.003	0.01
Upper Bound	High frequency $\times$ Large serving $\times$ High conc.	363.92	4.55	24.26
Average	Avg. frequency $\times$ Avg. serving $\times$ Avg. conc.	49.34	0.61	3.29
<i>L-Lake (n = 19)</i>				
Lower Bound	Low frequency $\times$ Small serving $\times$ Low conc.	0.04	0.0005	0.002
Upper Bound	High frequency $\times$ Large serving $\times$ High conc.	121.79	1.52	8.12
Average	Avg. frequency $\times$ Avg. serving $\times$ Avg. conc.	16.07	0.20	1.07



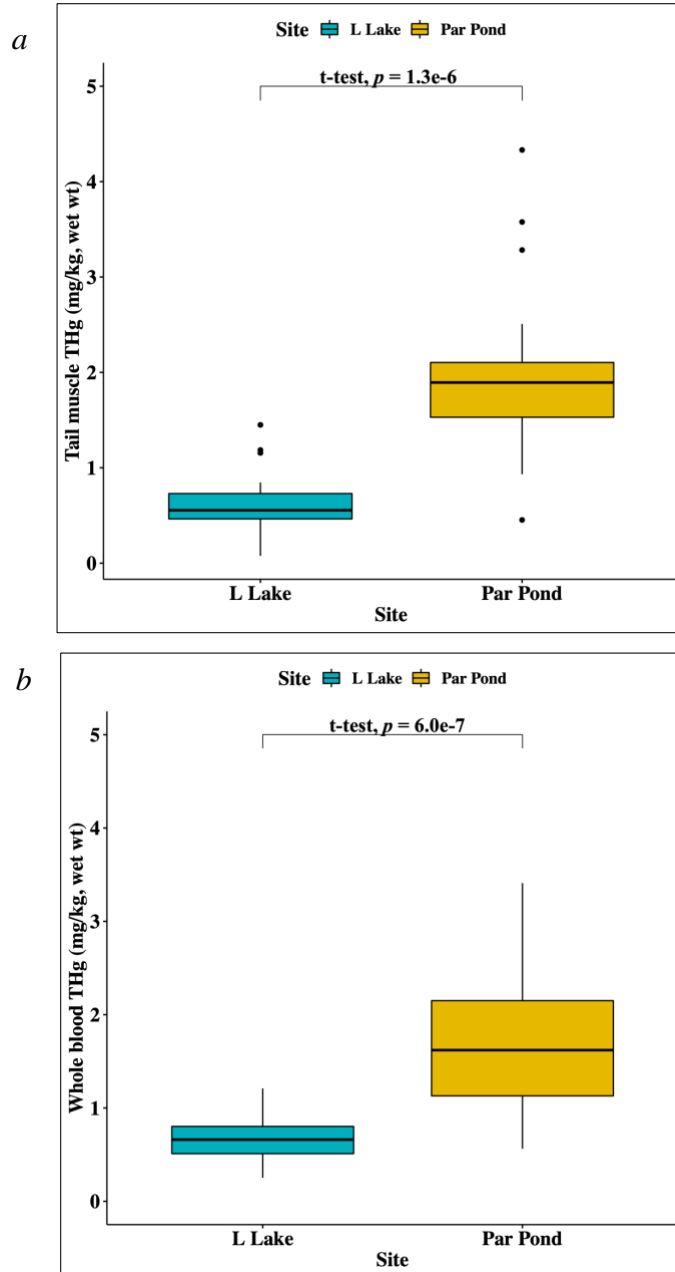
**Figure 2.1.** Map of South Carolina showing the location of alligator (*Alligator mississippiensis*) public hunt units and the Savannah River Site with respect to the Hunt Unit of concern (Unit 1- Southern Coastal). The map of the Savannah River Site denotes our sampling sites, Par Pond and L Lake, and their respective connections to the Savannah River.



**Figure 2.2.** Pearson correlation ( $R$ ) between blood and tail muscle total mercury (THg) concentrations in American alligators (*Alligator mississippiensis*) captured in Par Pond and L Lake on the Savannah River Site, South Carolina, USA ( $n = 31$ ). All THg values are reported as wet weight.



**Figure 2.3.** The relationship between THg, total length (TL), and site of capture in American alligators (*Alligator mississippiensis*) on the Savannah River Site (SRS). (a) Tail muscle THg was significantly correlated to TL in Par Pond, but not in L Lake. Tail muscle values were obtained from alligators  $\geq 180$  cm ( $n = 40$ ). (b) Whole blood THg was significantly correlated to TL in both Par Pond and L Lake ( $n = 53$ ). Significant differences between THg concentrations in Par Pond and L Lake were observed, with a more notable trend of bioaccumulation in alligators on Par Pond.



**Figure 2.4.** The relationship between tail and blood THg and site of capture in American alligators (*Alligator mississippiensis*) on the Savannah River Site. Boxplots represent tail and blood THg including predicted values ( $n = 40$ ). For both tail muscle and blood, alligators on Par Pond had significantly higher concentrations of THg: (a)  $t = -6.27$ ,  $p = 1.3 \times 10^{-6}$ ; (b)  $t = -6.60$ ,  $p = 6.0 \times 10^{-7}$ . All THg values are reported as wet weight.

## CHAPTER 3

# ENVIRONMENTAL CONTAMINANT BIOACCUMULATION IS ASSOCIATED WITH MOVEMENT BEHAVIOR IN A LONG-LIVED APEX PREDATOR<sup>2</sup>

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<sup>2</sup>Kojima, L.V., Kohl, M.T., Rainwater, T.R., Parrott, B.B., and Tuberville, T.D. To be submitted to *Journal of Animal Ecology*.

## ABSTRACT

Animal movement behavior provides insight into organismal and ecological function. Anthropogenic disturbances, such as urbanization and habitat fragmentation influence animal movement, but the effects of long-term exposures to environmental contaminants have yet to be examined. The long lifespans and predatory diets of crocodilians often lead to the bioaccumulation of persistent contaminants and confer a marked vulnerability to the consequent physiological effects resulting from elevated contaminant body burdens. In this study, we investigate the relationships between blood concentrations of mercury (Hg), a widespread contaminant with well characterized neurotoxicity, and movement patterns in free living, naturally exposed American alligators (*Alligator mississippiensis*). We sampled alligators from two former nuclear cooling reservoirs that vary with respect to historical Hg contamination and placed GPS transmitters on male alligators from each reservoir (13 total). Data collected across two years was analyzed using a linear mixed effects framework combined with AIC model selection to resolve the relationships linking seasonal alligator movement (daily activity (s) and daily distance (m)) and home range to climate conditions, individual traits, and Hg blood concentrations (mg/kg; wet weight). We found that climate conditions, alligator size (snout-vent-length), and Hg blood concentrations all contribute explanatory power towards alligator daily activity, but do not contribute to alligator daily distance. Furthermore, we found that Hg blood concentrations had a strong correlation with season home range size where individuals with elevated Hg had larger home ranges. These findings provide insight into how climate, anthropogenic contaminants, and individual traits relate to movement patterns resolved across seasonal scales.

## INTRODUCTION

Understanding how interactions between individuals and their environment are integrated over time to produce movement patterns over longer temporal scales is critical for assessing habitat requirements and functional roles within ecosystems. Movement behavior of large predators is especially informative for conservation and species management purposes given their top-down roles in shaping ecosystems (Wang et al. 2017) and can inform drivers of human-wildlife conflict (Baldwin and Bender, 2009). Environmental drivers like climate, habitat structure, resource availability, landscape configuration, and community structure can influence organismal physiology and elicit different behaviors depending on animal traits like size and sex (Nathan et al. 2008; Avgar et al. 2013; Kay et al. 2017; Parlin et al. 2018; Shaw, 2020; Lubitz et al. 2022). Thus, identifying how individual traits, together with climatic and landscape factors, affect movement patterns over spatially and temporally dynamic environments is fundamental to basic ecological science as well as conservation and management applications (Avgar et al. 2013).

Exposure to neurotoxic contaminants is known to negatively affect animal physiology and has the potential to influence movement behavior (Sakamoto et al. 1998; Vargas and Ponce-Canchihuamán, 2017; Bertram et al. 2022). Although mercury (Hg) is a naturally occurring heavy metal, it is also a widespread neurotoxin that can impair muscle strength, vision, and reaction response (Sakamoto et al. 1998; Wolfe et al. 1998; Eisler, 2006). Anthropogenic activities like mining, chlor-alkali processing, coal combustion, and waste incineration release mercury into the environment through atmospheric transport and deposition (Driscoll et al. 2013). Mercury persists in aquatic environments where it is often converted to a more bioavailable and toxic form, methylmercury (Compeau and Bartha, 1985). Studies examining the

effects of mercury exposure on movement and performance have primarily focused on embryonic and juvenile stages as well as smaller taxa (Sakamoto et al. 1998; Burke et al. 2010; Hassan et al. 2012; Chin et al. 2013; Johnson et al. 2023). For example, rats and mice exposed to mercury during gestational development are reported to have delayed reflexes, deficient movement capabilities, and impaired learning abilities (Inouye et al. 1985). Similarly, marsh periwinkle snails (*Littoraria irrorata*) dosed with mercury exhibit lower activity levels and reduced speed compared to an unexposed control group (Krull and Newman, 2022). Long-lived apex predators often serve as sentinel species for Hg biomonitoring and evaluating overall environmental health (Lawson et al. 2020), yet little research has investigated the relationship between Hg exposure and movement behavior in these organisms.

The American alligator (*Alligator mississippiensis*) inhabits a variety of freshwater and coastal habitats throughout the southeastern United States, and due to their high site fidelity, long lifespan, and high trophic status, alligators serve as bioindicator species for monitoring contaminants within these systems (Milnes and Guillette, Jr., 2008; Nifong and Silliman, 2017; Lawson et al. 2020; Kojima et al., 2023). Despite their high site fidelity, alligators have been observed to make long-range movements for short periods of time (Joanen and McNease, 1972), likely for hunting or when searching for mates. As ectotherms, their activity is driven by both weather and seasonality, particularly in late spring when mating occurs (Rosenblatt and Heithaus, 2011; Lawson et al. 2018; Vilet, 2020). Additionally, alligators occupy a wide range of freshwater habitats in which Hg has a propensity to settle and accumulate (Henke and Eversole 2019).

Alligators are territorial animals, with males shown to displace or even cannibalize smaller individuals, leading to an influence of individual size on home range size (Subalusky et

al. 2009; Rosenblatt and Heithaus, 2011; Lewis et al. 2014). Greater mobility in smaller individuals is thought to result from being driven out of territories by larger conspecifics (Garrick and Lang 1977; Vilet, 2020). Furthermore, alligators have been documented to be more abundant in natural habitats with little to no human impact or artificial structures (Gardner et al. 2016; Skupien and Andrews, 2017; Beal and Rosenblatt, 2020). Nevertheless, despite alligators' propensity to avoid human-impacted areas, it is unknown whether home range size is influenced by exposure to anthropogenically sourced contaminants.

Here we deploy GPS tracking devices onto adult male alligators to investigate how climatic drivers, blood Hg concentrations, and individual traits influence behavioral patterns within two freshwater reservoirs located on the Savannah River Site (SRS) in South Carolina that vary in contaminant history. In addition to estimating individual home ranges, we quantified movement and activity patterns across seasons to address two primary objectives: 1) resolve the relationships between climate conditions, individual size, and mercury blood concentrations on seasonal movement patterns, and 2) identify the contributions of individual traits and Hg blood concentrations to variation in home range size across different seasons.

## METHODS

### Study Area and Alligator Capture

The SRS is a former nuclear facility operated by the United States Department of Energy located in the Upper Coastal Plain of South Carolina. On the SRS are two inactive nuclear reactor cooling reservoirs, Par Pond and L Lake, that serve as habitat for wildlife. Par Pond (1,068 ha) was constructed in 1958 to dissipate heat effluent from the P and R reactors, and L Lake (405 ha) was constructed in 1984 to dissipate heat effluent from the L reactor; both ceased operation in 1988. While these reservoirs remain mostly undisturbed, elevated Hg concentrations

likely stemming from the initial filling of the reservoirs from the Savannah River, have been previously documented in organisms inhabiting them, including alligators (Clay et al. 1978; Brisbin et al. 1992; Yanochko et al. 1997; Jagoe et al. 1998; Kojima et al. 2023) and their potential prey (Sugg et al. 1995; Jagoe et al. 1996; Peles et al. 2006; Brown et al. 2022).

From July-August 2020 and May-July 2021, we targeted alligators having a total length (TL) of  $\geq 200$  cm using baited snare Murphy traps (Murphy and Fendley, 1974) and pole snares on both Par Pond and L Lake. Immediately following their capture, we collected blood samples from each alligator via the post-occipital venous sinus with a sterile 20-gauge needle and 30 mL syringe. We subsequently recorded morphological data including total length (cm), snout-vent-length (cm) and determined sex by cloacal examination of the genitalia. We transferred blood samples to either a 3 or 8 mL lithium heparin Vacutainer collection tube (BD, Franklin Lakes, NJ) and kept them on wet ice for no longer than 5 h before placing into a -30°C freezer until analysis. To individually mark each alligator, we injected passive integrated transponder tags (AVID, Norco, CA, USA) subcutaneously using a sterile 12-gauge needle in the right lateral area in the proximal base of the tail (Wilkinson et al. 2016). We then attached transmitters (methods described below), and then released individuals at their original capture location. We collected and handled alligators in accordance with approved protocols from the University of Georgia's Institutional Animal Care and Use Committee (AUP# A2020 02-026-Y2-A0). Scientific collection permits for capturing, handling, and collecting samples from alligators were issued by the South Carolina Department of Natural Resources (permits #SC-08-2020, #SC-08-2021).

#### GPS Data Collection

Global positioning system (GPS) technology provides fine-scale location data at a predetermined frequency, allowing researchers to examine movement patterns at a high

resolution; this is particularly useful for aquatic animals whose locations can be otherwise difficult to detect (Hebblewhite and Haydon 2010). From July 2020 to July 2022, we acquired alligator movement data using Telonics SeaTrkr-4370-4 GPS tags that collected location data through the ARGOS satellite system (Telonics Inc., Mesa, Arizona, USA). From July 2020 to August 2021, we fitted 7 adult male alligators (ranging from 219-321 cm TL) on L Lake with GPS units; from June 2021 to July 2021, we fitted 6 adult male alligators (ranging from 204-302 cm TL) on Par Pond (Table 3.1). Using methods from Brien et al. 2010, we attached GPS units surgically on the alligator's nuchal plate. We only included male alligators in the study due to their reported higher likelihood to move compared to female conspecifics (Fujisaki et al. 2014; Eversole and Henke, 2019).

We sought to balance the temporal resolution of data collection with battery life by programming units to collect location data every 4 hours from February through December and every 8 hours from December through February, corresponding to when alligators are most likely to move and most likely to be dormant, respectively (Henke and Eversole, 2019). We programmed the transmitters to collect data on activity (number of seconds active) via a three-axis accelerometer to provide additional data on alligator movement behavior. The activity sensor detected changes in acceleration or positioning in one or more of the axes transmissions (Telonics Gen4 GPS Systems, 2019).

We retrieved GPS data for all individuals as one of three types of point fix attempts: Succeeded, Resolved Quick Fix Pseudorange (QFP), and Unresolved QFP (Telonics, Inc., Arizona, USA). Succeeded fixes refer to location data calculated from at least four GPS satellites and require the unit to be above the water's surface for 30-90 seconds. For instances in which a 'succeeded fix' could not be acquired, we programed the transmitters to obtain resolved QFP

fixes, which require only short periods of surfacing time. Although QFP fixes have similar accuracy to succeeded fixes, their transmission is more data intensive and increases battery consumption. Unresolved QFP fixes have undetermined accuracy, but typically range from within a few hundred meters to several kilometers. In statistical software RStudio v4.0.5 (RStudio Team, 2021), we omitted all Unresolved QFP fixes from our data to maximize spatial accuracy and to remove spatial outliers. We removed location points from the first 48 hours after transmitter deployment to account for movement behaviors that alligators might exhibit following capture.

#### Quantifying total mercury in alligators

We quantified total mercury (THg) concentrations (mg/kg; wet weight) in alligator whole blood using a Direct Mercury Analyzer (DMA-80 EVO, Milestone, Inc., Shelton, CT, USA; hereafter DMA) at the Savannah River Ecology Laboratory, University of Georgia (Aiken, SC, USA). The DMA uses a combination of thermal decomposition, gold amalgamation, catalytic conversion, and atomic absorption spectrometry to determine the mass fraction of THg in solid or liquid samples. We thawed blood samples at room temperature and then placed them on a vortex homogenizer for 30 s. We then transferred 50  $\mu$ L to a nickel weigh boat for analysis in the DMA. For quality control, each run of 10 samples incorporated a blank, replicate, and a certified reference standard (TORT-3; National Research Council of Canada, Ottawa, Ontario).

#### Weather Data

We obtained weather data for the period of July 2020 – July 2022 from the Savannah River National Laboratory's N-Area meteorological tower located on the Savannah River Site approximately 8 kilometers from L Lake and 12 kilometers from Par Pond. The original data was

in 15-min intervals, which we aggregated to obtain daily-scale weather values. Daily variables included mean air temperature (°C), mean rainfall (mm), and mean wind speed (m/s).

#### Statistical analysis: Movement and Home Range

We aggregated 4 h timestamp data to 8 h intervals for consistency when analyzing the GPS data across seasons and to reduce missing data points for individual 4 h data collection periods. We quantified alligator movement using both activity (seconds) and distance (meters), generating three values of each metric per day. We obtained activity values from the transmitter accelerometers and calculated distance from GPS location data using the *adehabitatLT* package in R (Calenge 2006). We obtained the daily averages for activity and distance by calculating the means of the three values within the 24 h collection period (8 h timestamp intervals) for the corresponding movement metric using the *dplyr* package in R (Wickham et al. 2022). For all individuals we had > 11 months of data, although during the period July 2020 through June 2021 we only had transmitters deployed on alligators on L Lake (i.e., none on Par Pond); we included all available data for all analyses.

To calculate home ranges, we used the Time Local Convex Hull (T-LoCoH) approach in RStudio using the T-LoCoH package (Baker et al. 2015; Schweiger et al. 2015), which is one of the few methods that can handle both spatially and temporally autocorrelated GPS data. In particular, T-LoCoH has been shown to outperform other traditional home range calculation techniques in excluding areas known to not be used and is more adept at identifying geographical features like barriers, lakes, and inhospitable terrain (Baker et al. 2015; Lyons et al. 2013; Getz et al. 2007), making it particularly suited for extracting home range size in our study system.

To consider seasonal variation in alligator movement, we divided home ranges into seasons and calculated the core area 95% density isopleth for each season. We classified seasons

based on the calendar year for the Northern Hemisphere and used each equinox and solstice as the official start of the four seasons (spring, summer, fall, and winter).

#### Model selection: Daily Movement and Activity Behavior

To assess the relationship between our explanatory and response variables, we used a full-subsets information theoretic approach to construct a complete model set that evaluated movement in response to different combinations of our explanatory variables and compared all the models in this set using Akaike Information Criterion (AIC) model selection. We constructed a global model using a linear mixed effects framework (SVL + Hg + average daily temperature + average daily wind speed + average daily rainfall) with ID and Site as random effects using the lme4 package in R (Bates et al. 2015) and used the dredge function in the package MuMIn (Barton, 2014) to construct a complete model set based on the fit of the global model. We did this for each response variable (daily activity, daily movement) per season using the same global model combination. We used SVL instead of TL because one of our tagged alligators was missing part of its tail. We expanded our analysis to identify the best fit model of movement behavior within each season rather than including season as an explanatory variable due to strong seasonal patterns in movement behavior. Across all model sets, we selected the model(s) with the lowest AICc score ( $\Delta AICc \leq 2.0$ ), using the AICcmodavg package in R (Mazerolle, 2020) to identify our best fitting model. In R, we standardized SVL, Hg, and our climatic variables and extracted beta coefficient values to assess the strength of the predictor variables in the models.

#### Model selection: Home Range

We used the full-subsets information theoretic approach to investigate the relationships of alligator size and Hg body burdens between seasonal home range size (ha) where Site, ID, and Year were random effects. We developed a global model including all our explanatory variables

(SVL and Hg) for the construction of our model sets using the MuMIn package and dredge function. Across each season, model(s) with the lowest AICc model(s) ( $\Delta AICc \leq 2.0$ ) were considered to have equivocal support. For the home range model outputs, we standardized SVL and Hg and extracted beta coefficient values to assess the strength of the predictor variables in the models.

## RESULTS

### Alligator Daily Movement and Activity Behavior

We analyzed data from 8,507 GPS points (Succeeded and Resolved QFP) obtained from a total of 13 adult alligators during a two-year collection period ( $n = 7$  L Lake;  $n = 6$  Par Pond). Alligators ranged from 104–163 cm SVL and their blood mercury ranged from 0.77–2.21 mg/kg ww (Table 3.1). We evaluated the relationship between our response variables, mean daily distance and mean daily activity, by performing a Spearman correlation and found a significant yet weak relationship between the two variables ( $p < 0.001$ ;  $\rho = 0.19$ ; Supplemental). As these metrics were not strongly correlated, we modeled activity and distance separately. We examined the relationships between our response variables and season using a linear mixed effects model where ID and Site were random effects, which revealed a significant relationship between activity and season ( $p < 0.001$ ;  $R^2 = 0.26$ ) (Figs. 3.1a, c), and a significant yet weak relationship between distance and season ( $p < 0.001$ ;  $R^2 = 0.02$ ; Figs. 3.1b, d).

### Alligator Daily Movement and Activity Behavior: Spring

Average daily activity was highest in spring compared to the other three seasons at 161 s/day. Distance averaged 433 m/day, but was not highest in the spring. During the spring, activity appeared to peak on days with average temperatures reaching 25°C and distance peaked at 20°C, whereas both response variables appeared lowest at 10°C (Figs. 3.1a, b). We tested all

combinations of predictor variables to assess a best fit model for both mean daily activity and mean daily distance using AICc model selection. The global model explaining mean daily activity during the spring season ( $R^2 = 0.27$ ) was best supported and included SVL, Hg, average daily temperature, average daily wind speed, and average daily rainfall (Table 3.2). We determined the relative effect of each explanatory variable based on the beta value output and found a moderate negative association between SVL and mean daily activity ( $\beta = -43.53$ ) and a positive but weak relationship with Hg and mean daily activity ( $\beta = 5.18$ ; Figs. 3.2a, 3.3a). Climate explanatory variables all had moderate positive effects on activity in the spring (average daily temperature ( $\beta = 38.52$ ), average daily wind speed ( $\beta = 11.65$ ), and average daily rainfall ( $\beta = 4.88$ )).

We identified two supported models ( $\Delta AICc \leq 2.0$ ) when examining daily distance during Spring (Table 3.3), with the highest ranked model including Hg and climate variables ( $R^2 = 0.02$ ), but not SVL, whereas the second-best supported model included all variables ( $R^2 = 0.02$ ;  $\Delta AICc = 0.76$ ). In our highest ranked model, temperature ( $\beta = 37.30$ ) and wind speed ( $\beta = -26.61$ ) had the highest beta values, with average daily rainfall ( $\beta = 2.47$ ) and Hg having weaker relationships with mean daily distance ( $\beta = 1.26$ ). Similarly, in the second-most supported model, the climate variables had a stronger association with mean daily distance compared to SVL and Hg (Table 3.3; Figs. 3.2b, 3.3b).

#### Alligator Daily Movement and Activity Behavior: Summer

Daily mean activity of SRS alligators during the summer was 141 s/day and daily distance averaged 358 m/day. Alligators appeared most active at 25°C during this season and less active at <22°C. The best supported model for examining predictors of activity during the summer season was the global model (Table 3.2), which included all explanatory variables ( $R^2 =$

0.32). Based on the beta coefficient values, we determined that SVL ( $\beta = -47.73$ ) and average daily temperature ( $\beta = 20.07$ ) had the strongest associations with mean daily activity in the spring compared to Hg ( $\beta = -11.60$ ), average daily wind speed ( $\beta = 10.92$ ), and average daily rainfall ( $\beta = 0.98$ ; Figs. 3.2a, 3.3a). When evaluating mean daily distance, two models were supported (Table 3.3), with the highest ranked model including Hg and climate variables ( $R^2 = 0.01$ ), but not SVL, and the global model being our second ranked model ( $R^2 = 0.01$ ;  $\Delta AICc = 1.13$ )

#### Alligator Daily Movement and Activity Behavior: Fall

Mean daily activity averaged 91 s/day during fall, whereas distance was the highest compared to other seasons averaging 453 m/day. During fall, alligator activity peaked at approximately 22.5°C and decreased under 15°C. Two models explaining mean daily activity during the fall had equivocal support (Table 3.2), with the highest ranked model ( $R^2 = 0.12$ ) including Hg and the climate variables and the second ranked model being the global model ( $R^2 = 0.12$ ;  $\Delta AICc = 1.18$ ). The climate variables had the strongest associations with mean daily activity in the fall where average daily rainfall ( $\beta = -15.21$ ) had negative relationship with mean daily activity, and average daily temperature ( $\beta = 19.40$ ) and average daily wind speed ( $\beta = 9.43$ ) had positive effects. Hg ( $\beta = -3.56$ ) had a weak negative association with mean daily activity. In the second ranked model, SVL ( $\beta = -7.43$ ), in addition to Hg ( $\beta = -2.59$ ) and average daily rainfall ( $\beta = -15.20$ ), had negative relationships with mean daily activity, whereas average daily temperature ( $\beta = 19.33$ ) and average daily wind speed ( $\beta = 9.41$ ) had positive effects and again we found strong associations with our climate variables and mean daily activity (Figs. 3.2a, b).

For mean daily distance, two model combinations were closely ranked (Table 3.3), with the combination of Hg and climate ( $R^2 = 0.01$ ) ranking highest and the global model ranking

second ( $R^2 = 0.02$ ;  $\Delta AICc = 0.02$ ). Despite the model's weak relationship with mean daily distance, we found that Hg ( $\beta = -13.95$ ), average daily temperature ( $\beta = -22.52$ ), and average daily rainfall ( $\beta = -107.78$ ) were all negatively related to mean daily distance and that average daily wind speed had a weak positive association with mean daily distance ( $\beta = 0.86$ ; Figs. 3.2b, 3.3b).

#### Alligator Daily Movement and Activity Behavior: Winter

Both mean daily activity and distance were lowest during the winter season with activity averaging 62 s/day and distance averaging 287 m/day. For both distance and activity, alligators movement was greatest during days in which average temperature exceeded 15°C, but otherwise appeared limited by the cold. When testing all model combinations for the best fit model predictor of mean daily activity during the winter season, the global model was best supported ( $R^2 = 0.21$ ; Table 3.2). We found that most beta coefficients were lower for the winter season compared to the other seasons and that mean daily activity had the strongest associations with SVL ( $\beta = -14.91$ ; Fig. 3.2a) and average daily temperature ( $\beta = 11.81$ ). Hg ( $\beta = 0.51$ ; Fig. 3.3a), average daily wind speed ( $\beta = 1.16$ ), and average daily rainfall ( $\beta = -4.63$ ) all had weak associations with mean daily activity in the winter.

Similar to other seasons, two models were supported when evaluating daily distance, with Hg and the climate variables ranked highest ( $R^2 = 0.03$ ) and the global model ranked second ( $R^2 = 0.04$ ;  $\Delta AICc = 0.001$ ; Table 3.3). Hg ( $\beta = 31.49$ ) was positively related to both mean daily distance and average daily temperature ( $\beta = 68.04$ ). Average daily rainfall ( $\beta = -5.49$ ) and average daily wind speed ( $\beta = -37.71$ ) had negative associations with mean daily distance in the winter (Figs. 3.2b, 3.3b).

### Home range

We assessed individual home ranges for each season and year (2020–2022), resulting in 83 home range isopleths (Fig. 3.4). Home range sizes varied per season, with alligators having the largest average home range in the fall ( $\bar{x} = 69.11$  ha) and the smallest in the winter ( $\bar{x} = 17.92$ ; Fig. 3.5a). Average home range size was 54.50 ha in the spring and 34.96 ha in the summer. We evaluated potential influences on home range by testing for the best combinations of our explanatory variables—size (SVL) and mercury body burdens (Hg)—across each season. Given the differences in Hg concentrations in alligators between Par Pond and L Lake (Kojima et al. 2023) we considered Site as a random effect.

In spring, the top supported model ( $R^2 = 0.88$ ; Table 3.4) only included Hg ( $\beta = 14.02$ ) and not SVL (Fig. 3.5b). We found that the best fit explaining summer home range ( $R^2 = 0.84$ ; Table 3.4) included only Hg ( $\beta = -18.76$ ) as well; however, Hg had a negative association with home range size and was the only season in which we observed this trend (Fig. 3.5b). In fall, two models were supported, with the top model ( $R^2 = 0.93$ ) including the combination of SVL ( $\beta = 21.67$ ) and Hg ( $\beta = 54.29$ ) and the second ranked model ( $\Delta AICc \leq 0.05$ ;  $R^2 = 0.95$ ; Table 3.4) including only Hg ( $\beta = 108.66$ ; Fig. 3.5b). The best supported model for winter included only Hg ( $\beta = 33.19$ ) ( $R^2 = 0.93$ ; Table 3.4; Fig. 3.5b).

## DISCUSSION

Here, we focused on seasonal differences in alligator movement patterns and how the role that morphological and environmental factors play in influencing movement behavior may differ. Further, we wanted to assess the relationship of mercury body burdens with explanatory factors to identify potential movement patterns that could provide information on the possible neurological effects that Hg accumulation could have on alligators. Consistent with other studies,

alligator movement patterns appeared to be heavily affected by seasonality, with the greatest mean daily distance observed during the fall season and mean daily activity occurring during the spring season, and the lowest for both occurring during winter (Rosenblatt and Heithaus 2011; Beal and Rosenblatt 2020). Furthermore, we found that mean daily activity was most correlated to average daily temperature, with the alligators peaking in activity when temperatures were approaching a max of 25°C, and size, with larger alligators being less active across all seasons. We did not detect robust relationships between our explanatory variables and average daily distance (m). These findings are not completely unexpected given that we did not find a strong correlation between daily activity and daily distance. Additionally, alligators are reported to exhibit site fidelity (Fujisaki et al. 2014; Lawson et al. 2020) and based on our results, we suspect that our tagged alligators did not move great distances but were instead active within their respective home ranges. Activity data is likely to reflect a range of different behaviors like moving between basking spots, searching for food, and mating. The only notable distances traveled by individuals were two alligators, one from Par Pond and one from L Lake, that left their respective reservoirs temporarily during the 2022 Spring season, which coincided with the breeding season.

### *Climatic Influences*

For many species of herpetofauna, climatic conditions, in addition to season, are primary drivers of movement (Todd and Winne, 2006; Todd et al. 2007; Drabik-Hamshare and Downs, 2017; Eskew and Todd, 2017; Parlin et al. 2018). We report a positive relationship between temperature and movement, with a decline in mean daily activity and mean daily distance traveled during colder temperatures, consistent with movement behavior of other ectotherms. Daily wind speed was also correlated with alligator activity, having a positive association with

mean daily activity during all seasons. However, we found the opposite relationship with wind speed and mean daily distance, in which wind speed had a negative association with daily distance during the spring, summer, and winter and a positive association in the fall. Little information on how reptiles react to wind is reported, despite wind speed being documented to disrupt the ability to sense threats and navigate surroundings in other taxonomic groups (Cherry and Barton, 2017). Due to convective cooling effects associated with wind exposure, it may be more difficult for ectotherms to thermoregulate on windy days, and thus a negative relationship between wind speed and daily distance might be expected. We found the positive relationship between activity and wind speed interesting; however, it may reflect GPS tagged alligators moving to avoid the windy conditions (Sun et al. 2001; Steelman and Dorcas, 2010).

#### Associations with size

Our findings demonstrate that during seasons in which alligators were highly mobile (spring and summer), smaller alligators were more active compared to their larger counterparts. This is consistent with other studies in which sub-adult male alligators were more mobile compared to other size classes and to females (Subalusky et al. 2009; Rosenblatt and Heithaus, 2011). Despite the constrained size classes in this study, smaller adult alligators would be presumed to move to avoid predation risk posed by larger conspecifics. Large adult alligators have been reported to exhibit aggression, particularly during the breeding season, which may result in cannibalization (Hutton, 1989; Lawson et al. 2018). Additionally, smaller ectotherms will heat and cool faster than larger individuals (Stevenson, 1985), which could be the case for our tagged alligators resulting in an increase in activity as smaller individuals move to find basking and shaded areas. However, despite smaller alligators being more active than their larger conspecifics, we found a weak association between size and mean daily distance, suggesting that

additional studies incorporating a broader range of size classes may be needed to more fully resolve these dynamics.

#### Associations with mercury body burdens

Mercury appeared in the best fit models for both mean daily distance and mean daily activity outputs. However, given the weak association of our explanatory variables with mean daily distance, it is difficult to determine the biological significance of explanatory variables with respect to mean daily distance. Nevertheless, we found that Hg had a negative association with mean daily activity and mean daily distance during the summer and fall seasons and a weak positive association with our response variables during spring and winter (Figs. 3.3a, b). Greater movement in winter by alligators with higher Hg levels could have detrimental effects as alligators normally undergo a state of brumation to withstand low temperatures (Brisbin et al. 1982; Brandt and Mazzotti, 1990). During brumation, alligators have been reported to reduce foraging behaviors and this has been suggested to result in increased mercury concentrations due to the metabolizing of fat reserves and muscle (Keller et al. 2014; Nilsen et al. 2019). Thus, elevated winter movement might enhance mobilization of mercury stores, leading to exhaustion and a weakened immune system (Wright and Cooper, 1981; Zimmerman et al. 2017).

In previous studies, organisms from Hg-contaminated sites were observed to exhibit behavioral alterations that can influence survival. For example, northern two-lined salamanders (*Eurycea bislineata*) from a Hg-contaminated site displayed delayed reaction responses when being prodded during a set of locomotion trials in comparison to those from a reference site (Burke et al. 2010). Yet, given the observational nature of our study, we cannot establish a clear cause-and-effect relationship between Hg body burdens and alligator movement and activity. Further, while models derived from our AIC model selection approach often incorporated Hg,

Hg bioaccumulates in alligators (Nilsen et al. 2017a; Nilsen et al. 2017b; Lawson et al. 2020; Kojima et al. 2023), and is thus confounded with individual size. In alligators with snout-vent-lengths  $\geq 182$  cm, the size which growth cessation begins in males, it has been reported that Hg concentrations begin to decline and there is no longer a correlation between Hg concentrations and SVL (Lawson et al. 2020). All our tagged alligators were large, adult males but were under this range. We did not find a correlation between alligator size and Hg concentrations in our tagged alligators which may be due to this cessation in Hg concentrations in large adults. In a previous study at the same study sites, we did find a correlation between blood Hg concentrations and alligator body size when considering alligators of all life stages (total length: 44.2 – 339.5 cm; Kojima et al. 2023). Thus, blood Hg can vary with alligator body size in our study system but the relationship was not detected when only considering adult individuals. Additional studies seeking to disentangle the influences of animal size and blood Hg concentrations are needed to fully resolve the relationship between Hg and variation in alligator movement patterns.

#### *Drivers of alligator home range*

Mercury was included in the best fit model for home range size across all seasons. We found a positive relationship between Hg and home range size across all seasons except summer, during which Hg was negatively associated with home range size. The positive relationship between Hg and alligator home range size may be due to the correlation between alligator size and Hg, however we did not find that home range size was strongly associated with size of our tagged alligators. One possibility is that, given the neurotoxic effects of Hg accumulation, alligators with high Hg levels are less capable of finding prey, resulting in the need to forage over a larger area, hence resulting in a larger home range. Difficulties with prey capture have

been recorded in the Burke et al. (2010) study in which northern two-lined salamanders (*Eurycea bislineata*) from an uncontaminated reference site were more efficient at prey capture and were documented to eat twice as much prey compared to individuals from a Hg-contaminated site. Similarly, neonatal northern watersnakes (*Nerodia sipedon*) born from mothers collected at a Hg-contaminated site exhibited decreased motivation to feed and displayed reduced strike efficiency compared to snakes born from mothers from a reference site (Chin et al. 2013). Despite these reported consequences of Hg on behavior and function, herpetofauna have largely been documented to circumvent the detrimental effects of contamination (Wada et al. 2011; Nilsen et al. 2017a; Haskins et al. 2019). Furthermore, despite there not being a strong association of SVL with home range size, elevated Hg body burdens are more likely to occur in larger individuals due to bioaccumulation, and the relationship between size and Hg could confound further interpretation.

In other species, home range size has been shown to scale with body size due to the positive relationship between body mass and metabolic costs, leading to the conclusion that home range size should match organismal energetic needs (McNab, 1963; Kleiber, 1975; Hendriks, 2007; Todd and Nowakowski, 2020). For example, snakes, lizards, and turtles have all exhibited an increase in home range size with an increase in body mass (Perry and Garland, 2002; Slavenko et al. 2016; Todd and Nowakowski, 2020). However, factors like sex, social structure, and habitat use and availability also contribute to this relationship. The average home range size of tagged alligators in the current study was largest during the season (fall), where a positive association with SVL was observed. While spring coincides with alligator breeding season when two primary plasma androgens, testosterone and dehydroepiandrosterone (DHEA), peak in large adults (Hamlin et al. 2011), it has been reported that in the fall, alligators > 135 cm

SVL experience another peak in DHEA, but not testosterone (Hamlin et al. 2011). It is possible that increases in these androgens heighten territorial behavior, which can contribute to larger home ranges, particularly for larger individuals which have the highest levels of androgens. As previously discussed, however, almost half of our tagged alligators are > 135 cm, limiting the variation that would be exhibited if we had a wider range of size classes, which is likely why we do not see the effect of alligator size on home range size in the spring. Future research should include a resource selection analyses given that evaluating habitat is likely to provide insight into the habitat characteristics that might drive home range size variation. Ultimately, season strongly influenced home range size, which was expected due to alligators being documented to expand their home ranges during the spring season and reduce their home ranges during the winter season when they brumate (Goodwin and Marion, 1979; Fujisaki et al. 2014; Lewis et al. 2014).

## CONCLUSION

Considering the relationships between alligator movement and size, climate, and contaminant body burdens can inform researchers on important ecological dynamics and identify potential threats regarding persistent contaminants in the environment. The American alligator is the most studied crocodilian species, and yet there is limited information on the adverse effects of contaminants like mercury on adults (but see Milnes et al. 2008; Nilsen et al. 2017a).

Understanding alligator movement and the ecological threats they might face can provide critical insight into other crocodilian species, over half of which are imperiled and difficult to study due to their diminishing numbers (IUCN, 2022; Mascarenhas-Junior et al. 2023). A comprehensive understanding of alligator movement patterns, ecology, and anthropogenic disturbances, such as environmental contamination, is crucial for conservation efforts aimed at protecting this iconic species and their environment.

**Table 3.1.** Site of capture, identification, biological information (snout-vent-length (SVL) and mercury body burdens (Hg)), and GPS collection time frame of Savannah River Site, SC GPS tagged American alligators (*Alligator mississippiensis*) (n = 13).

Site	ID	SVL (cm)	Hg (mg/kg; ww)	Tag Deployment	Data Collection End Date	Length of Data Collection
L Lake	718358	110	0.58	7/16/20	4/20/22	640 days
L Lake	718361	163	0.55	7/15/20	7/20/22	728 days
L Lake	718362	158	0.70	7/22/20	7/20/22	725 days
L Lake	718363	132	0.89	8/18/20	7/20/22	698 days
L Lake	718364	116	0.72	8/18/20	7/20/22	698 days
L Lake	718365	142	0.51	8/18/20	6/6/22	653 days
L Lake	718368	116	0.46	7/22/20	6/21/22	696 days
Par Pond	718355	136	1.87	6/14/21	7/20/22	399 days
Par Pond	718356	105	1.34	6/9/21	7/20/22	398 days
Par Pond	718357	143	2.15	7/7/21	7/20/22	375 days
Par Pond	718359	163	2.21	6/3/21	7/20/22	409 days
Par Pond	718366	104	1.61	6/9/21	5/16/22	338 days
Par Pond	718367	156	1.13	6/29/21	7/20/22	383 days

**Table 3.2.** AICc model output evaluating the most parsimonious model combinations for the relationship between explanatory variables and mean daily activity (s) of American alligators (*Alligator mississippiensis*) on the Savannah River Site, SC, in each season. Bolded values indicate top model combinations where  $\Delta AICc \leq 2.00$ .

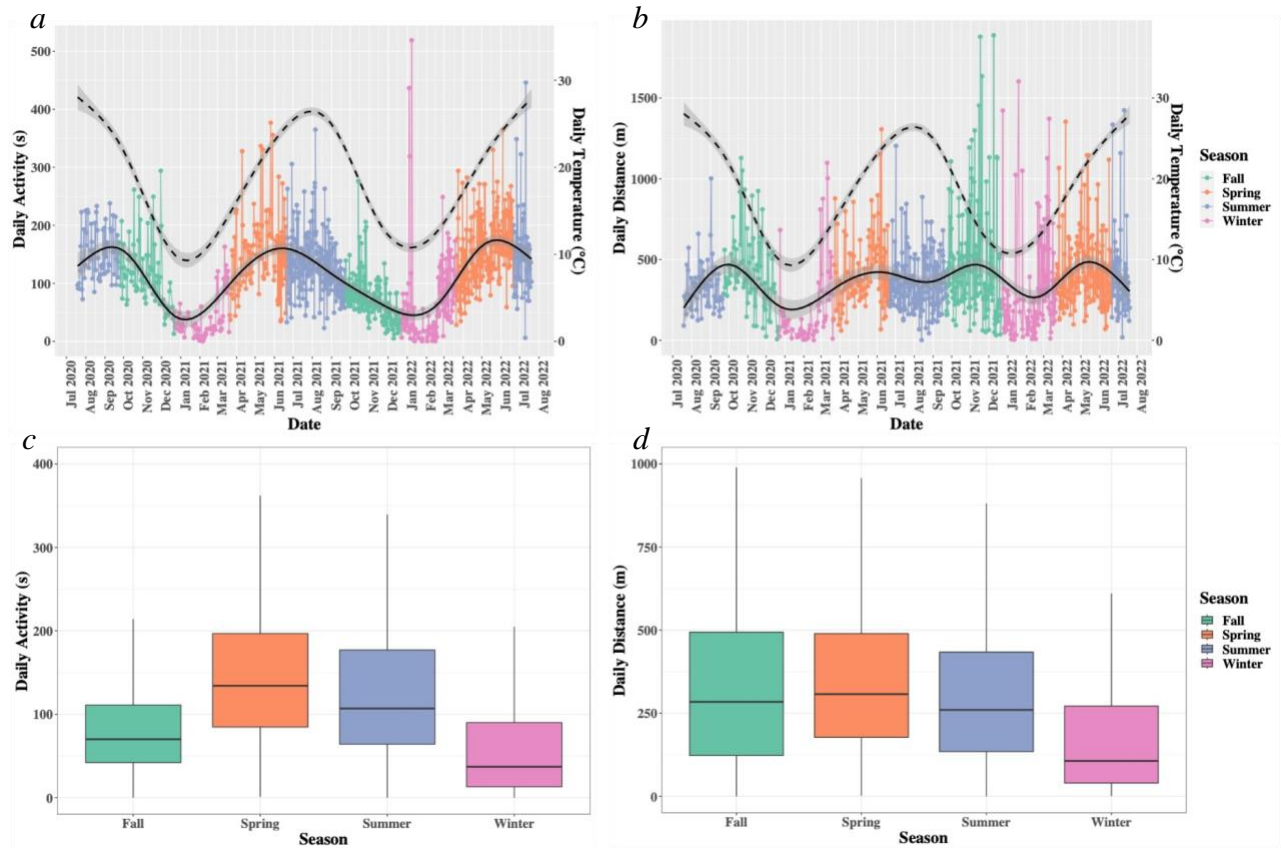
Model Sets: Mean Daily Activity		R <sup>2</sup>	df	AICc	$\Delta AICc$	AICcWt	Log like
<b>Spring</b>							
	<b>SVL + Temperature + Wind speed + Rainfall + Hg</b>	<b>0.27</b>	<b>9</b>	<b>16047.70</b>	<b>0.00</b>	<b>0.93</b>	<b>-8014.78</b>
	SVL + Temperature + Wind speed + Rainfall	0.26	8	16053.16	5.46	0.06	-8018.53
	SVL + Temperature + Wind speed + Hg	0.26	8	16057.43	9.73	0.01	-8020.66
<b>Summer</b>							
	<b>SVL + Temperature + Wind speed + Rainfall + Hg</b>	<b>0.32</b>	<b>9</b>	<b>16334.08</b>	<b>0.00</b>	<b>0.89</b>	<b>-8157.98</b>
	SVL + Temperature + Wind speed + Hg	0.32	8	16340.36	6.28	0.04	-8162.13
	SVL + Wind speed + Rainfall + Hg	0.32	8	16340.79	6.71	0.03	-8162.34
<b>Fall</b>							
	<b>Temperature + Wind speed + Rainfall + Hg</b>	<b>0.12</b>	<b>8</b>	<b>12848.19</b>	<b>0.00</b>	<b>0.59</b>	<b>-6416.03</b>
	<b>SVL + Temperature + Wind speed + Rainfall + Hg</b>	<b>0.12</b>	<b>9</b>	<b>12849.37</b>	<b>1.18</b>	<b>0.33</b>	<b>-6415.60</b>
	Temperature + Wind speed + Rainfall	0.11	7	12852.99	4.80	0.05	-6419.44
<b>Winter</b>							
	<b>SVL + Temperature + Wind speed + Rainfall + Hg</b>	<b>0.21</b>	<b>9</b>	<b>8949.94</b>	<b>0.00</b>	<b>0.72</b>	<b>-4465.86</b>
	SVL + Temperature + Rainfall + Hg	0.22	8	8952.86	2.92	0.17	-4468.34
	SVL + Temperature + Wind speed + Rainfall	0.20	8	8954.76	4.82	0.07	-4469.29
Where ID and Site are random effects							

**Table 3.3.** AICc model output evaluating the most parsimonious model combinations for the relationship between explanatory variables and mean daily distance (s) of American alligators (*Alligator mississippiensis*) on the Savannah River Site, SC, in each season. Bolded values indicate top model combinations where  $\Delta AICc \leq 2.00$ .

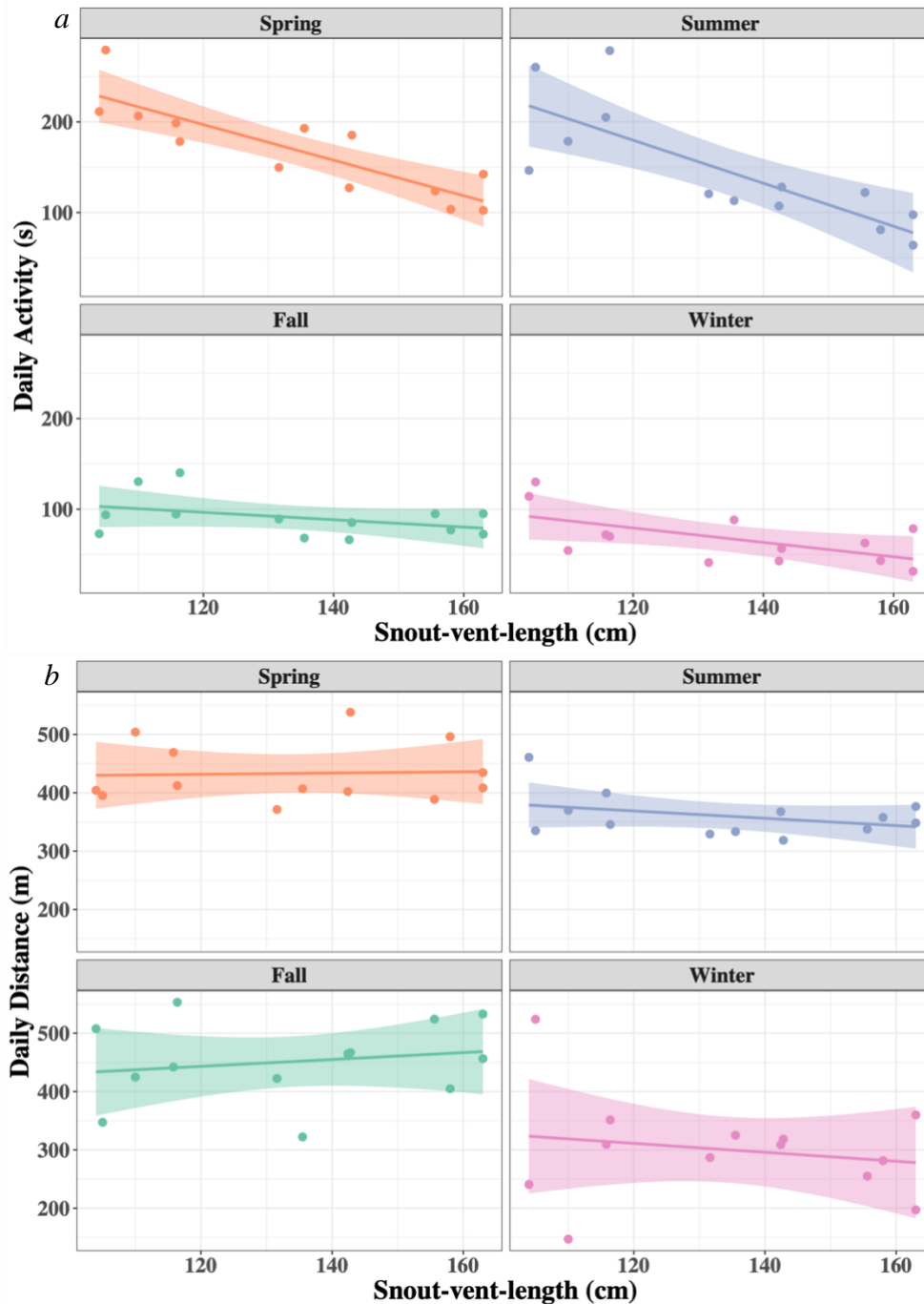
Model Sets: Mean Daily Distance		R <sup>2</sup>	df	AICc	$\Delta AICc$	AICcWt	Log like
<b>Spring</b>							
	Temperature + Wind speed + Rainfall + Hg	<b>0.02</b>	<b>8</b>	<b>19934.73</b>	<b>0.00</b>	<b>0.55</b>	<b>-9959.31</b>
	SVL + Temperature + Wind speed + Rainfall + Hg	<b>0.02</b>	<b>9</b>	<b>19935.50</b>	<b>0.76</b>	<b>0.38</b>	<b>-9958.68</b>
	Temperature + Wind speed + Rainfall	0.02	7	19940.99	6.25	0.02	-9963.45
<b>Summer</b>							
	Temperature + Wind speed + Rainfall + Hg	<b>0.01</b>	<b>8</b>	<b>19664.85</b>	<b>0.00</b>	<b>0.56</b>	<b>-9824.37</b>
	SVL + Temperature + Wind speed + Rainfall + Hg	<b>0.01</b>	<b>9</b>	<b>19665.97</b>	<b>1.13</b>	<b>0.32</b>	<b>-9823.92</b>
	Wind speed + Rainfall + Hg	0.01	7	19668.68	4.83	0.05	-9827.80
<b>Fall</b>							
	Temperature + Wind speed + Rainfall + Hg	<b>0.01</b>	<b>8</b>	<b>16893.74</b>	<b>0.00</b>	<b>0.41</b>	<b>-8438.80</b>
	SVL + Temperature + Wind speed + Rainfall + Hg	<b>0.02</b>	<b>9</b>	<b>16893.76</b>	<b>0.02</b>	<b>0.40</b>	<b>-8437.80</b>
	SVL + Wind speed + Rainfall + Hg	0.01	8	16897.33	3.59	0.07	-8440.60
<b>Winter</b>							
	Temperature + Wind speed + Rainfall + Hg	<b>0.03</b>	<b>8</b>	<b>12107.10</b>	<b>0.00</b>	<b>0.49</b>	<b>-6045.46</b>
	SVL + Temperature + Wind speed + Rainfall + Hg	<b>0.04</b>	<b>9</b>	<b>12107.11</b>	<b>0.00</b>	<b>0.49</b>	<b>-6044.44</b>
	Temperature + Wind speed + Rainfall	0.03	7	12115.80	8.70	0.01	-6050.83
Where ID and Site are random effects							

**Table 3.4.** AICc model output evaluating the most parsimonious model combinations for the relationship between home range size (ha) of Savannah River Site, SC American alligators (*Alligator mississippiensis*) and our explanatory variables per season. Bolded values indicate top model combinations where  $\Delta AICc \leq 2.00$ .

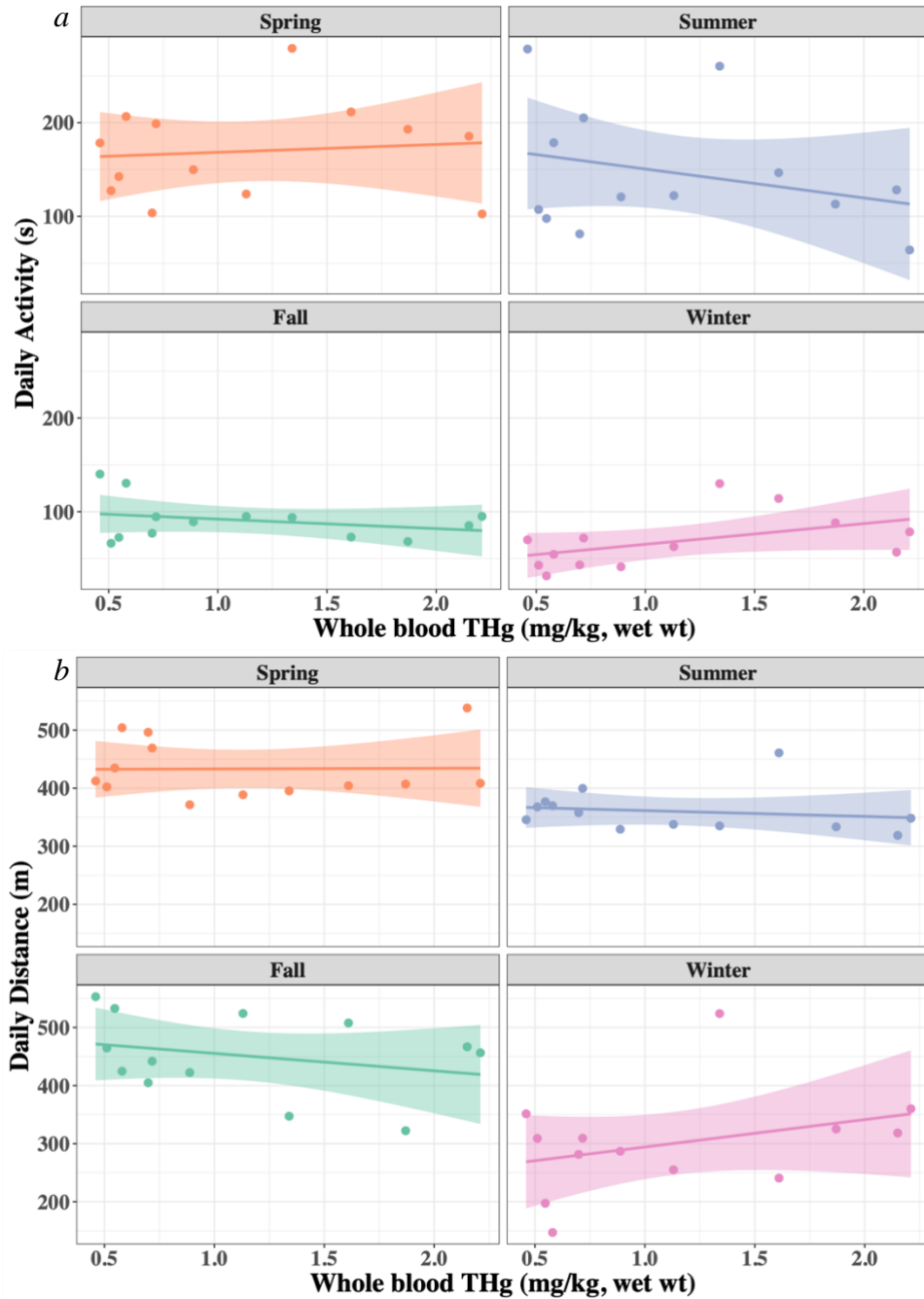
Model Sets: Home Range Size		R <sup>2</sup>	df	AICc	$\Delta AICc$	AICcWt	Log like
<b>Spring</b>							
	<b>Hg</b>	<b>0.88</b>	<b>6</b>	<b>188.30</b>	<b>0.00</b>	<b>0.68</b>	<b>-84.33</b>
	Hg + SVL	0.83	7	191.34	3.03	0.15	-83.07
<b>Summer</b>							
	<b>Hg</b>	<b>0.84</b>	<b>6</b>	<b>246.50</b>	<b>0.00</b>	<b>0.81</b>	<b>-114.92</b>
	Hg + SVL	0.85	7	250.22	3.72	0.13	-114.82
<b>Fall</b>							
	<b>Hg + SVL</b>	<b>0.93</b>	<b>7</b>	<b>217.87</b>	<b>0.00</b>	<b>0.50</b>	<b>-97.63</b>
	<b>Hg</b>	<b>0.95</b>	<b>6</b>	<b>217.92</b>	<b>0.05</b>	<b>0.48</b>	<b>-99.96</b>
<b>Winter</b>							
	<b>Hg</b>	<b>0.93</b>	<b>6</b>	<b>188.84</b>	<b>0.00</b>	<b>0.73</b>	<b>-84.92</b>
	Hg + SVL	0.90	7	191.19	2.35	0.23	-83.50
Where ID, Site, and Year are random effects							



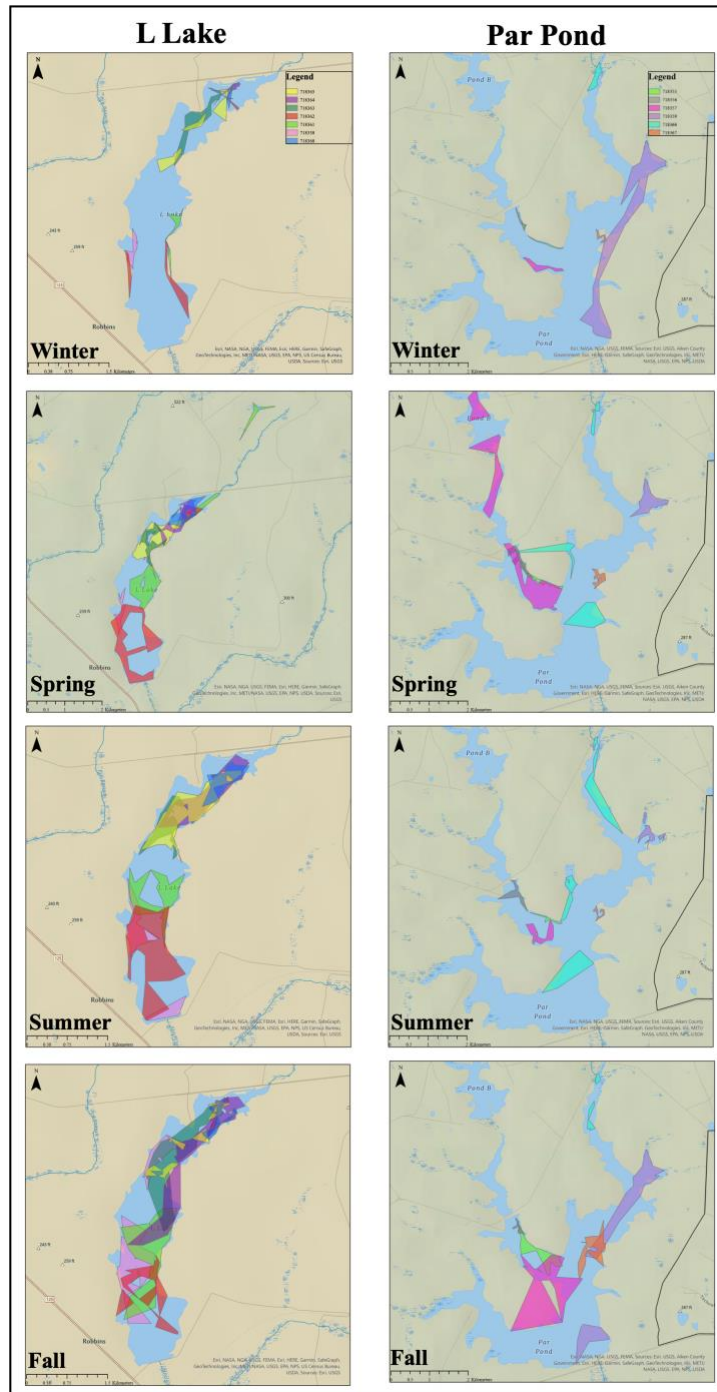
**Figure 3.1. Daily activity and distance over time and per season.** The mean daily (a) activity (s) and (b) distance (m) of Savannah River Site, South Carolina GPS tagged alligators (*Alligator mississippiensis*) (n = 13) during the data collection period (July 2020 – July 2022); trends in alligator daily activity and daily distance are denoted by the solid trend line and trends in the corresponding average daily temperature are denoted by the dashed trend line. The box plots show the range of daily movements [(c) activity (s) and (d) distance (m) traveled] of Savannah River Site, SC GPS tagged alligators (n = 13) per season. Colors denote season to account for variation in movement patterns among seasons.



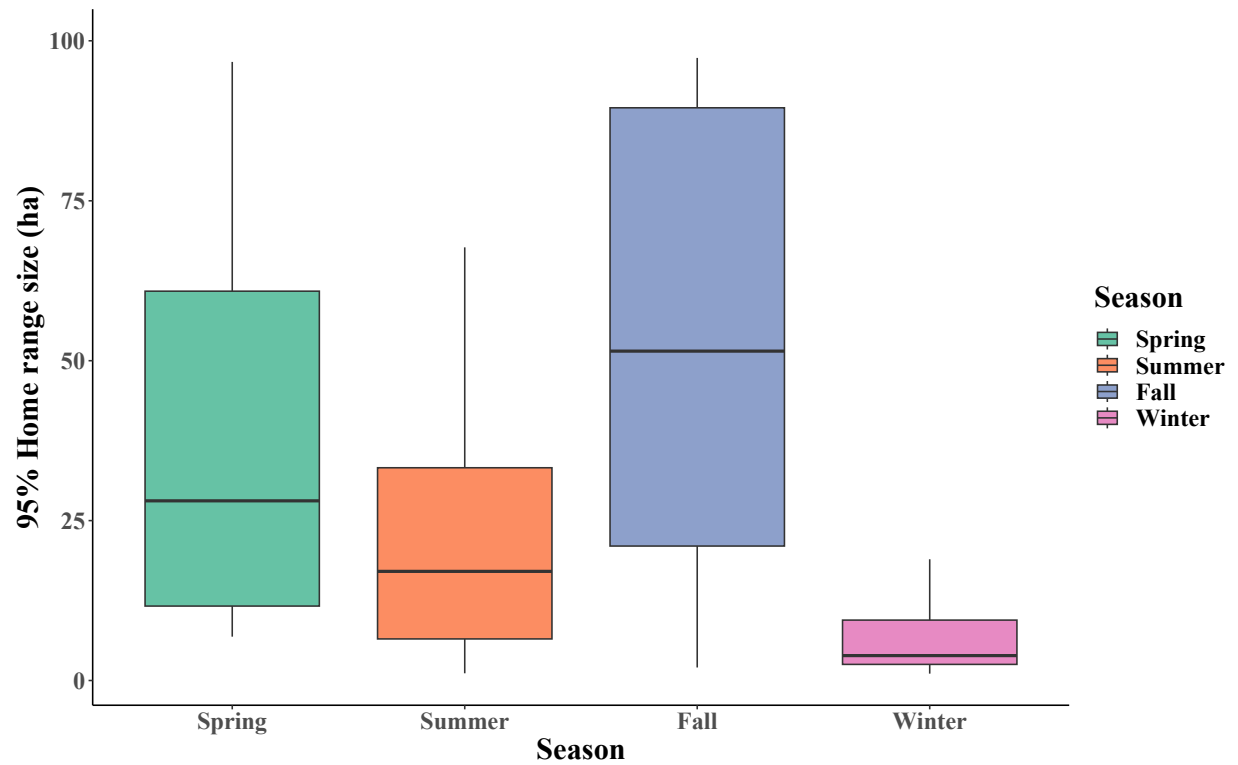
**Figure 3.2. Daily activity, distance, and snout-vent-length.** The linear relationship between daily movement of Savannah River Site, SC (SRS) GPS tagged alligators (*Alligator mississippiensis*) ( $n = 13$ ) and size (snout-vent-length) per season. Shaded areas between points represent the 95% confidence interval. (a) Mean daily activity (s) of SRS alligators and snout-vent-length. (b) Mean daily distance (m) of SRS alligators and snout-vent-length.



**Figure 3.3. Daily activity, distance, and mercury.** The linear relationship between daily movement of Savannah River Site (SRS), SC GPS tagged alligators (*Alligator mississippiensis*) ( $n = 13$ ) and mercury body burdens (Hg; mg/kg ww) per season. Shaded areas between points represent the 95% confidence interval. (a) Mean daily activity (s) of SRS alligators and Hg. (b) Mean daily distance (m) of SRS alligators and Hg.



**Figure 3.4. 95% Home Range Isopleth by Season.** Home range isopleths of Savannah River Site, SC GPS tagged alligators (*Alligator mississippiensis*) on L Lake (n = 7) and Par Pond (n = 6) calculated using a Time Local Convex Hull approach. Home ranges of each individual tagged alligator are denoted by the shaded areas.



**Figure 3.5. Home range and season.** The relationships between home range size (ha) of Savannah River Site, SC GPS tagged alligators (*Alligator mississippiensis*) (n = 13) and season. Home ranges were calculated using a Time Local Convex Hull approach.

## CONCLUSION

As a game species with a wide geographic range, the American alligator has proved to be a useful species for monitoring environmental health and potentially a sentinel for human health. Their life history as a top predator as adults and long-life spans make them more likely to biomagnify and bioaccumulate contaminants, respectively, in their environments providing valuable information on the status of ecosystems that are vulnerable to environmental pollution. Additionally, as aquatic reptiles, alligators occupy a variety of freshwater habitats where pervasive contaminants such as mercury (Hg) can persist and methylate, being converted to its more toxic and bioavailable form, methylmercury. And while the adverse effects of Hg have been well studied in both humans and wildlife, there is a lack of knowledge resolving the link between Hg exposure in wildlife with that of human health. Particularly, this gap ignores the effects of Hg exposure in long-lived top predators such as the alligator that can also be consumed by humans. My thesis aimed to tie together this disconnect, by addressing the risks of Hg exposure by consuming harvested Hg-contaminated alligator meat and the effects of elevated Hg body burdens on alligator movement behavior.

A few important caveats should be considered when interpreting our findings. In Chapter 2, we discuss the risk associated with consuming harvested alligator meat from alligators that occupy the Savannah River Site (SRS). While Hg levels in alligators from the SRS could be reflective of those on the Savannah River given the water source of the reservoirs on the SRS is from the Savannah River, the processes of Hg methylation and accumulation do differ between habitats. Therefore, we cannot conclusively infer the Hg concentrations in alligators on the

Savannah River. However, given the elevated Hg concentrations in fish that are top piscivores on the Savannah River, we do suspect that the average levels of alligators would be in between that of the alligators on L Lake and Par Pond and thus, pose a potential risk to humans who may consume them. Furthermore, another caveat to consider is the trends in hunting and sharing of harvested meat after the COVID-19 pandemic. Our research began in 2020 and when considering the demography of harvested alligators on the Savannah River, we found a decrease in hunting activity on the Savannah River between 2019 and 2020 (SCDNR, 2019; SCDNR, 2020). Additionally, the harvest data used in our study was collected in 2015, so trends of harvest and consumption patterns have likely changed over time. Nonetheless, our study showed that aquatic game species that are likely to accumulate contaminants and put consumers at risk do warrant warnings and advisories in areas where there is a known history of mercury contamination. We recommend additional sampling of alligators on the Savannah River and in areas like Superfund Sites across the Southeastern U.S. where alligator hunting is permitted. These data will provide valuable information not only on the persistence of contaminants within the environment, but also the potential exposure risks to hunters. It is also important to note that on the Savannah River Site there has been documentation of other contaminants aside from Hg such as radiocesium (Burger et al. 2001). Therefore, other historical contaminants and emerging contaminants should be considered within these assessments.

By understanding both the human and wildlife health aspects of alligator ecology and Hg exposure, we have better insight on the broader consequences of Hg exposure in the environment. In Chapter 3, we assessed the relationship of environmental drivers, individual traits, and blood Hg concentrations with seasonal alligator movement, which we quantified using average daily distance (m) and average daily activity (s). We further investigated the

relationships of blood Hg concentrations and alligator size with seasonal home range size (ha). As Chapter 3 revealed, the combination of our climate variables, snout-vent-length, and Hg blood concentrations had the most explanatory power for all seasons when assessing the relationship with average daily activity. We initially were concerned about the potential relationship between snout-vent-length and Hg blood concentrations given that in Chapter 2 there was a significant correlation between size and Hg. However, we did not find the same correlation in our tagged alligators, which may be due to the constrained size class compared to Chapter 2 where we had samples across all life stages. Hg blood concentrations and snout-vent-length both appearing in the top models further validates their individual non-confounding relationships with alligator activity. As expected, climate conditions and alligator size had explanatory power over alligator activity with average daily temperature having the strongest association with alligator activity and larger alligators being less likely to be active across all seasons. We did not find such strong associations with activity and blood Hg concentrations; however, we did report those with elevated Hg were less active during the fall, a season which alligators would be more likely to move large distances. This ties into our Chapter 2 given that the annual alligator hunt occurs in the fall and there is a potential for alligators with elevated blood Hg concentrations being easier targets to hunt due to elevated blood Hg concentrations resulting in delayed reaction response.

In contrast, when assessing the relationship with seasonal average daily distance, we found that the combination of our explanatory variables had weak explanatory power over average daily distance. Given these results, the data likely needs to be reduced to a shorter temporal scale. Rather than assessing average daily distance, it may be best to assess the data in intervals of four hours for the spring, summer, and fall seasons and eight-hour intervals for the

winter season based on the respective data collection timestamps. Additionally, we can also compare distances between day and night and even factor in the lunar cycle as that has been suspected to influence behavior in herpetofauna (Perry and Fisher, 2006). Nonetheless, the lack of robust explanatory power by our covariates was surprising given trends in reptile movement, primarily by that of climate conditions.

Lastly, in Chapter 3 we wanted some basic insight in seasonal alligator home range and were interested in investigating the relationships with alligator size and Hg blood concentrations on alligator home range size. We found that for almost all seasons, Hg alone had the home range size, except for fall which had two top models with the highest ranked including Hg and snout-vent-length. We found these relationships particularly interesting given that the trends between blood Hg concentrations and home range and snout-vent-length and home range were very similar. Yet based on the models and the lack of correlation between alligator size and blood Hg concentrations, blood Hg concentrations contributed the most explanatory power. Going forward, to better understand SRS alligator home range and their overall ecology it would be useful to conduct a resource selection function analysis and collect habitat data including basking habitat, covered water, open water, and collecting water depth, water temperature, and potentially even sampling the sediment and water for Hg concentrations. This information could be useful to not only understand how the alligator is using their environment, but also if alligator movements are increasing their likelihood of Hg exposure.

My research demonstrates the potential routes and consequential effects of Hg exposure in both humans and wildlife. As mentioned, there is still limited research on alligators or other long-lived top predator species at risk of elevated Hg contamination. My results suggest that we need to bridge the disconnect between human health and wildlife health in the field of

toxicology. In areas where there is evidence of contamination it is pertinent to prioritize sampling of wildlife that are game species and have life histories that make them ideal bioindicators for the environment. My research provides insight in the persistence of Hg in an alligator population that occupies an area with historical documentation of Hg in addition to the risk associated with the harvest and consumption of alligators that originate from this area. Furthermore, we were able to describe the limited role elevated Hg concentrations might be playing in influencing alligator movement behavior and overall survival. Moving forward, a comprehensive understanding of alligator movement patterns, ecology, and anthropogenic disturbances, such as environmental contamination, is crucial for efforts aimed at protecting this iconic species and their environment.

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