Distribution of Mercury in the American Alligator (*Alligator mississippiensis*), and Mercury Concentrations in the Species Across its Range

By

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(Under the Direction of I. Lehr Brisbin, Jr. and Steven B. Castleberry)

ABSTRACT

American alligators (*Alligator mississippiensis*) are top-level predators that can accumulate mercury in high concentrations. As human consumption of alligator continues to increase, there is an increased public health concern. I conducted two studies examining mercury concentrations in the American alligator. The first study was conducted on alligators from the Rockefeller Wildlife Refuge (RWR), Louisiana, to determine how mercury is distributed among body organ/tissue compartments. Samples from body organ/tissue compartments, including brain, gonad, heart, kidney, liver, and muscle were tested for mercury (Hg) and stable isotope ($\delta^{13}C$ and $\delta^{15}N$) signatures. Relationships between body organ/tissue compartments and non-invasive samples (blood, claws and dermal tail scutes) were examined to determine whether concentrations in non-invasive samples could be used to monitor populations non-lethally. Mercury concentrations in all organ/tissue compartments were correlated with each other, body size, and $\delta^{15}N$ signatures. The $\delta^{13}C$ signatures were not correlated with mercury concentrations or body size. Mercury was highest in the blood, followed by kidney and liver. Concentrations of mercury were lowest in gonad and brain tissue. Because mercury concentrations from blood, claws, and scutes were correlated with those of the internal organs/tissue compartments, non-lethal sampling methods may be a viable method of indexing

mercury burdens in body tissues. The second study involved examining tail muscle and liver samples from wild alligators in Alabama, Georgia, South Carolina, and the Rockefeller Wildlife Refuge in Louisiana, and an alligator farm in Mitchell County, Georgia to determine if mercury concentrations varied geographically in the species. The highest Hg concentrations were found in alligators from Glynn County, Georgia and southeast Alabama, while the lowest were found in the alligators from the RWR and the alligator farm. Differences among locations suggested that alligators could be used as biomonitors of mercury in the locations they inhabit.

INDEX WORDS: American alligator, mercury, stable isotopes, biomonitor

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B.S., The University of Georgia, 2001

A Thesis Submitted to the Graduate Faculty of the University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2004

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May 2004

ACKNOWLEDGEMENTS

I would like to thank my co-major professors, I. Lehr Brisbin, Jr. and Steven B. Castleberry, for their help. I also appreciate the effects of my committee members: Charles H. Jagoe and Micheal T. Mengak. Travis Glenn and Christopher Romanek were also a great help in this project. I am particularly grateful to I. Lehr Brisbin and Travis Glenn for allowing me to get out of the lab and catch alligators for other projects. I am fortunate to have worked at the Savannah River Ecology Laboratory (SREL), especially in the Ecotoxicology Laboratory under Heather Brant and in the Stable Isotope Laboratory under Lindy Paddock. A special thanks goes to Mandy Schable for allowing me to live in her home while working at SREL. I would also like to thank everyone who helped me in collecting samples: Travis Glenn, Ruth M. Elsey, Scooter Trosclair, and the rest of the Rockefeller Wildlife Refuge "Alligator Crew", Burks Laney (state trapper from Dothan, Alabama), Jackie Carter (state trapper from Folkston, Georgia), Johnny Williamson (state trapper from South Carolina), my uncle, Barry Moore (state trapper from Brunswick, Georgia), and his chief assistants, Eddie Brundage and Randy Lamb, and Shana Boyer and the crew from Alligator's Unlimited in Mitchell County, Georgia. My grandfather deserves special thanks for taking me on my first alligator adventure at the age of five. My dad and mom also deserve credit and my thanks for allowing me to be around animals all of my life, work with any animals I wanted, and for reminding me to watch my fingers and toes before going to look for alligators. Last, but definitely not least, I would like to thank my fiancé, Jason Haray, for always being there for me, whether it was to go check traps or to calm my nerves when this thesis came down to the wire. Thank you all; there is no way I could have done it without any of you.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Mercury is a naturally occurring element that is redistributed in the environment by geologic and biological cycles, as well as through human activities (Hord et al. 1990; Zillioux et al. 1993; Klaason 1996). Most naturally occurring mercury comes from degassing of the earth's crust, which releases an estimated 2700-6000 tons per year (Rudd 1995; Klaason 1996; World Health Organization 1990). Atmospheric transport and redistribution through rainwater may be the major route of naturally occurring mercury transport (Klaason 1996; Fitzgerald et al. 1998).

Anthropogenic activities, such as mining, shorten the ore phase of metals releasing them into the atmosphere (Chang and Cockerham 1994; Klaason 1996). Human use of mercury has resulted in direct and indirect releases into the environment, the major indirect source being the burning of fossil fuels, which releases approximately 5000 tons of mercury into the atmosphere annually (D'Itri 1972; Sorensen 1991). In the past, mercury was used in many industrial processes, and was released directly into the environment as a waste product. For example, mercury was used by the pulp and paper industry to create slimicides used in paper production and by the chlor-alkali industry as an amalgam and catalyst to lower the reactivity of the metal dissolved into the mercury (D'Itri 1972; Peters 1983; Eisler 1987). Additionally, mercury was used in many common practices and products, such as preserving wood and animal hides, photography, tattooing, felt manufacturing, household cleaners, dental fillings, paints, batteries, thermometers, and fluorescent lamps (Engel 1966; D'Itri 1972; Peters 1983). Mercury was also used in seed dressings to act as a bactericide and fungicide putting mercury directly into the food chain (D'Itri 1972; Peters 1983). Due to these anthropogenic and natural sources, mercury can be found in all plant and animal tissues. However, mercury levels generally depend on proximity to sources (D'Itri 1972).

Once mercury reaches the earth's surface, it either returns to the atmosphere to be recycled or is methylated by aquatic microorganisms (Klaason 1996). Methylation is the conversion of inorganic mercury (Hg^{2+}) to methyl mercury (MeHg) under anaerobic conditions, primarily through bacterial metabolism (D'Itri 1972; Sorensen 1991; Khan and Tansel 2000; Brant et al. 2002). Wetlands and reservoirs are important sites of mercury methylation (St. Louis et al. 1994; Porvari and Verta 1995; Rudd 1995). Methyl mercury is not readily excreted and is capable of accumulation over an organism's life (Watras and Bloom 1992). High mercury in living organisms from in and around contaminated sites may persist for more than 100 years, even after the source has been removed (Eisler 1987).

Methylated forms of mercury are highly toxic and more biologically mobile (D'Itri 1972; Eisler 1987; Zillioux et al. 1993). Once mercury is methylated, it is capable of biomagnifying in the food chain, reaching levels one up to one million times original levels (Fimreite and Karstad 1971; Jernlöv and Lann 1971; Mason et al. 1994; Klaason, 1996). Methyl mercury forms a complex cysteine group attached to an amino acid enabling it to cross cell membranes (Klaason 1996). This crossing of cell membranes may allow methyl mercury to affect cellular energy production and protein synthesis due to its binding to such structures as the endoplasmic reticulum, mitochondria, and nuclear envelope (Chang and Cockerham 1994). Alkylmercurials (methyl mercury) can easily pass the blood-brain barrier and destroy brain cells (D'Itri 1972; Zilliuox et al. 1993). High mercury concentrations have been shown to cause behavioral changes, as well as problems with growth, reproduction, and embryo/larval survival in aquatic species (Zillioux et al. 1993). Methyl mercury also is capable of passing through the placental barrier causing neurological damage to the fetus, without symptoms in the mother (D'Itri 1972; Sorensen 1991; Wolfe et al. 1998). Once in the body of an animal, methyl mercury binds with

protein and is found in muscle (Klaason 1996). Methyl mercury accounts for 99% of the mercury found in muscle tissue in fish and 95-99% of accumulated mercury in high trophic level organisms (Bloom et al. 1991; Bloom 1992; Huckabee et al. 1979).

Living organisms concentrate mercury, especially when it is in excess in the environment. The level of concentration depends on the type of organism and form of mercury (D'Itri 1972; Zillioux et al. 1993). The pathway is dependant on trophic level, duration and intensity of exposure, and environmental factors (Zillioux et al. 1993). Fluctuations may be observed in mercury levels among seasons, due to seasonal changes in an organism's diet (Wren 1986). Species are affected differently by mercury, depending on the dose-response relationship between the species in question and the type of mercury to which it has been exposed (Zillioux et al. 1993). D'Itri (1972) proposed that heat also affects mercury concentration through an increase in metabolism and uptake, a longer feeding period, an increase in bacterial growth, greater solubility of mercury, and increased methylation. All of these factors result in differential uptake of methyl mercury among organisms.

Mercury is considered to be the most serious environmental contaminant to fish and wildlife in the southeastern United States (Facemire 1995). Mercury contamination became of particular interest and concern in the Southeast after an endangered Florida panther (*Felis concolor coryi*) died in the Everglades National Park, Florida, USA from apparent mercury toxicosis (Roelke 1990). In 1992, the Florida Department of Health and Rehabilitative Services issued a mercury health advisory for 68 Florida waterways (Florida Department of Health and Rehabilitative Services 1992). The advisory was in response to statewide mercury studies conducted on several fish species by the Florida Game and Freshwater Fish Commission that found mercury concentrations above 0.5 mg Hg/Kg wet weight. This advisory urged people to

limit their consumption of largemouth bass (*Micropterus salmoides*), bowfin (*Amia calva*), and gar (*Lepisosteus* spp.) (Hord et al. 1990; Florida Department of Health and Rehabilitative Services 1992). Among birds, mercury has been found to be higher in those that eat primarily fish and other birds (Eisler 1987).

Environmental contaminants, particularly mercury, are a growing concern for several crocodilian species (Brisbin et al. 1998), including the American alligator. Studies examining mercury in American alligators have demonstrated varying concentrations among locations throughout the distribution. Alligator tail muscle from eight Florida lakes was tested for several different contaminants, including mercury (Delany et al. 1988). The average mercury concentration was 0.61 mg Hg/Kg wet weight and concentration varied among geographic locations. Hord et al. (1990) found that mercury levels in alligator muscle from Water Conservation Areas (WCA) in the Everglades National Park (ENP) in south Florida were high enough to warrant the cancellation of two consecutive alligator harvests. Heaton-Jones et al. (1997) confirmed that alligators from the Everglades National Park had higher mercury concentrations than those from surrounding areas. Ruckel (1993) tested alligator tail muscle from several locations in Georgia for mercury concentrations and found levels below the Food and Drug Administration (FDA) "action level" for fish, which is 1 mg Hg/Kg wet weight.

Studies also have demonstrated differences in mercury concentration among various body organ/tissue compartments in alligators. Yanochko et al. (1997) compared the mercury levels of several body organ/tissue compartments of alligators from Everglades National Park with those from a mercury-contaminated reactor cooling reservoir on the U.S. Department of Energy Savannah River Site (SRS) in South Carolina and found differences between these two locations, with significant correlations between mercury levels in the muscle versus dermal scutes, but no

differences between sexes. Jagoe et al. (1998) also compared mercury levels in alligators from the ENP, the Okefenokee National Wildlife Refuge in Georgia, the SRS, and several other sites in central Florida and again found differences among geographic locations and significant correlations between contaminant levels in various organ/tissue compartments. Burger et al. (2000) compared metals in alligators from three Florida lakes and between concentrations in non-lethally obtained samples such as skin and tail tips and found the non-invasive samples had significant positive correlations with mercury concentrations in internal organs. Elsey et al. (1999) examined mercury levels in alligators from Louisiana because of the increase in meat that was being processed from these animals for human consumption. She found muscle Hg concentrations ranging from 0.047 mg Hg/Kg wet weight to 0.386 mg Hg/Kg wet weight. Khan and Tansel (2000) determined mercury bioconcentration factors (BCFs), which is the ratio of the concentration of mercury in an organism or tissue to the concentration of mercury in the water the organism lives in, for alligators of differing ages inhabiting the Florida Everglades and found a relative increase in BCFs from juvenile to adult and high BCFs in liver, kidney, muscle, and tail scute, despite low mercury concentrations in surrounding water.

Because adult alligators feed at high trophic levels, are nonmigratory, and have a long life span (living 30+ years), the American alligator may be an extreme "limiting case" environmental monitor (Khan and Tansel 2000; Yanochko et al. 1997). As a biomonitor, the American alligator can show the presence or absence and quantify the relationship between damage and dose of mercury (Wren 1986). Adult alligators are considered the top predators in freshwater wetlands where they occur (Duvall and Barron, 2000). Higher trophic level feeders generally have higher mercury concentrations because of the ability of mercury to biomagnify (Jagoe et al. 1998;

Yanochko et al. 1997). Peters (1983) confirmed that alligators are capable of accumulating mercury in their tissues following dietary exposure.

A better understanding of the factors controlling the flow of contaminants, such as mercury, through the food web, can be obtained by studies of stable isotope ratios, particularly carbon and nitrogen (Romanek, pers comm). The trophic position of an organism within an ecological community can be estimated by determining its 15N/14N ratio (Hairston and Hairston 1993). Nitrogen naturally occurs as 15N/14N in a ratio of 99% 14N and 0.37% 15N. The stable isotope 15N, the heavier of the two isotopes, has been reported to increase 3-4 ‰ for each trophic level step (Schoeninger and DeNiro 1984; Minigawa and Wada 1984). The nitrogen stable isotope is expressed as $\delta^{15}N$ ({(15N/14N sample)/(15N/14N standard) –1}*1000) (Craig 1957). Mercury and $\delta^{15}N$ seem to be positively correlated in fish and raccoons (Kidd et al. 1995; Gaines et al. 2002). Additionally, the carbon stable isotopes 12C/13C can be used to determine whether an organism is feeding in a marine versus freshwater and terrestrial environment. These two stable isotopes occur in a ratio of 98.9% 12C to 1.1% 13C (Rounick and Winterbourn 1986). Carbon stable isotope ratios can distinguish between animals with diets made up of predominantly C3, C4 or CAM plants (Kelly 2000; Gannes et al. 1997). C4 plants, which include agricultural crops as well as marine plants, have an average delta value of -12.5 ‰, and C3 plants, which include plants that grow in freshwater and terrestrial areas, have an average delta value of –26.5 ‰ (Sage and Monson 1999; Ambrose and DeNiro 1986). Feeding location and mercury concentrations have been linked in a previous study conducted by Gariboldi et al. (1998) where wood storks feeding on freshwater prey items were consuming food with higher mercury concentrations than those feeding on primarily saltwater prey.

The primary objective of this thesis was to examine mercury concentrations in the American alligator. Chapter 2 examines differential distribution of mercury among various organ/tissue compartments. Chapter 3 examines mercury concentrations among geographic locations throughout the distribution. In both chapters, I use stable isotope signatures for $\delta^{13}C$ and $\delta^{15}N$ to examine relationships between mercury concentrations, trophic level, and feeding location (marine or freshwater and terrestrial environments) and their relationship to mercury concentrations. An additional objective was to address the potential of alligators to serve as biomonitors of environmental mercury.

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CHAPTER 2

DISTRIBUTION OF MERCURY IN ORGAN/TISSUE COMPARTMENTS OF THE AMERICAN ALLIGATOR (*ALLIGATOR MISSISSIPPIENSIS*)[1]

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[1]Moore, L. A., I. L. Brisbin, C. H. Jagoe, S. B. Castleberry, R. M. Elsey, T. C.. Glenn, S. B. Castleberry, and C. S. Romanek. To be submitted to *Journal of Wildlife Management*

Abstract

Mercury (Hg) concentration was examined in wild alligators inhabiting the Rockefeller Wildlife Refuge (RWR), Louisiana, to determine the extent of contamination and the distribution of mercury in various body organs and tissue compartments. Concentrations of mercury in claws and dermal tail scutes were compared to those in blood, brain, gonad, heart, kidney, liver, and muscle to determine if the former tissues, obtained by non-lethal sampling, could be used as measures of body burdens in internal organs. Stable isotope signatures for $\delta^{13}C$ and $\delta^{15}N$ were measured to examine trophic level, and feeding location (marine or freshwater) and their relationship to mercury concentrations. Mercury was found in all body organs and tissue compartments. Mercury concentrations were highest in blood (121.74±22.07 mg Hg/Kg wet weight), kidney (3.18±0.69 mg Hg/Kg dry weight), and liver (3.12±0.76 mg Hg/Kg dry weight). Non-invasive samples (blood, claws and dermal tail scutes) were positively correlated with all tissue Hg concentrations,(r^2 = 0.5129-0.9882 for blood, 0.3473-0.6370 for claws, and 0.3327-0.6485 for scutes). Mercury concentrations were positively correlated to trophic level ($\delta^{15}N$), but were not correlated with $\delta^{13}C$, suggesting the animals were feeding in the area where they were collected and not moving long distances to feed. Because mercury concentrations from blood, claws, and scutes were correlated with those of the internal organs/tissue compartments, non-lethal sampling methods may be a viable method of indexing mercury burdens in body tissues.

Introduction

Environmental contaminants are an ever-growing concern for several crocodilian species, with mercury (Hg) causing the most alarm (Brisbin et al. 1998). Mercury also is considered to be the most serious environmental contaminant, threatening fish and wildlife in the southeastern United States (Facemire et al. 1995). The effects of mercury on humans, fish, and many other wildlife species have been examined; however its effect on crocodilian species is not well studied (Eisler 1987; Heinz 1996; Hall, 1980). American alligators have been shown to be capable of accumulating mercury in their tissues following dietary exposure, with this accumulation exceeding U.S. Food & Drug Administration (FDA) limits for fish taken for human consumption (Peters 1983). Several studies have been conducted on contaminants, including mercury, in wild American alligators (*Alligator mississippiensis*). Some of these studies examined mercury in tail muscle to determine if there was a need for a public health concern (Delany et al. 1988; Hord et al. 1990; Ruckel 1993; Elsey et al., 1999). Concentrations in other tissues and at several geographic locations were examined in other studies (Heaton-Jones et al. 1997; Yanochko et al. 1997; Jagoe et al. 1998; Burger et al. 2000). Mercury is known to biomagnify in higher trophic level organisms (Eisler 1987; Wolfe et al. 1998). Because it is a top-predator, with adults feeding high on the food chain, is nonmigratory, and has a long life-span, the American alligator may represent an ideal environmental monitor for contaminant levels (Khan and Tansel 2000; Yanochko et al. 1997).

A better understanding of the factors controlling the flow of contaminants through the food web can be obtained by studies of stable isotope ratios, particularly those of carbon and nitrogen (Romanek pers comm). Trophic levels and energy flow within an ecological community can be estimated by testing for the 15N/14N ratio of the stable isotopes of nitrogen

(Hairston and Hairston. 1993). Mercury and $\delta^{15}N$ have a positively correlated relationship in fish and raccoons (Kidd et al. 1995; Gaines et al. 2002). The carbon stable isotopes 13C/12C can differentiate whether an animal is feeding in a marine or freshwater and terrestrial environment. Examining carbon stable isotope ratios can distinguish between animals with diets made up of predominantly C3, C4 or CAM plants (Kelly 2000; Gannes et al. 1997). C4 plants, which include agricultural crops as well as marine plants, have an average delta value of -12.5 ‰ and C3 plants, which include plants that grow in freshwater or freshwater and terrestrial areas, have an average delta value of –26.5 ‰ (Sage and Monson 1999; Ambrose and DeNiro 1986).

Alligator meat has restricted availability on the commercial market, therefore, the FDA has not set "action levels" for human consumption as it has for fish. In Louisiana alone, 350,000 kg of deboned meat is processed from wild alligators annually, with trends suggesting an increase in human consumption (Elsey et al. 1999). Mercury levels in alligators above FDA standards for other organisms, such as fish, could pose a significant human health threat.

The primary objective of this study was to examine mercury concentration in American alligator tissue from the Rockefeller Wildlife Refuge in Louisiana to determine the extent of contamination and the distribution of mercury in various body organs and tissue compartments. We also examined the relationship of mercury to carbon and nitrogen stable isotope signatures. Furthermore, we examined relationships of concentrations of mercury in blood, claws, and dermal tail scutes to body organs to determine if the former tissues, obtained by non-lethal sampling, could be used as measures of body burdens in internal organs.

Materials and Methods

Sample collection

Alligators were collected on 20 June 2002 in Unit 13 of the Rockefeller Wildlife Refuge (RWR), a 32,000-hectare coastal marsh located in eastern Cameron and western Vermilion Parishes in southwestern Louisiana. The refuge boundaries and predominant vegetation have been described previously by Joanen and McNease (1969). Alligators were collected by noosing from an airboat, placed into cages and transported to holding facilities. The following day alligators were sexed, measured to the nearest centimeter, euthanized by gunshot to the brain, and sampled. Samples taken included blood, brain, claws, gonad, heart, kidney, liver, muscle, and dermal tail scutes. Muscle tissue was taken from four locations: front leg (Anconeus), jaw (Pterygoides Internus), rear leg (Flexor Tibialis Internus), and tail (Longissimus). Muscles were tested separately because blood is shunted between the brain, heart, and tail excluding the other muscles during exertion (Pooley and Gans 1976).

Mercury Analyses

Samples were stored in separate, sterile Whirl-Pak® bags (NASCO) and frozen at –10ºC until testing. Samples were freeze dried on a Labconco freeze drier, then reweighed to determine moisture content, and homogenized before being tested using a DMA80 Direct Mercury Analyzer (Milestone, Inc, Monroe, CT) following EPA Method 7473, which tests for mercury in solids and solutions by thermal decomposition, gold amalgamation, thermal desorption and CVAA detection. Replicates and tissue standards certified for mercury concentration (DORM-2 (dogfish muscle), DOLT (dogfish liver), and TORT-2 (lobster hepatopancreas), purchased from the National Research Council of Canada (NRCC), Ottawa, Canada) were run with each set of

samples. Based on a 25 mg sample and an average blank of 0.5 ng Hg, the method detection limit (MDL) was 0.020 mg Hg/Kg.

Isotope Analyses

Lipids were extracted from freeze dried liver and tail muscle sample with a 2:1 ratio of chloroform:methanol mixture, rinsed with methanol, dried, and ~ 2mg of sample was analyzed for carbon and nitrogen using a continuous flow isotope ratio Delta+xls Mass Spectrometer (Finnigan-MAT, San Jose, Ca), with a Carlo Erba NC2500 Elemental Analyzer. At the beginning of each sample analysis an N2 working standard (Air) was introduced and the CO2 working standard (Pee Dee belemnite) was introduced at the end of each sample's conclusion (Mariotti 1983; Coplen 1996). External working standards of Dorm-2, dogfish muscle, were used and were found to be reproducible to $\pm$ 0.15 ‰ for both $\delta^{13}C$ and $\delta^{15}N$. The results of the stable isotope analyses are presented in per mil per volt units (‰) with a standard δ notation (Craig 1957).

Statistical analyses

Statistical analyses were conducted using SAS (SAS Institute, Cary, NC). All data were tested for normality and $\log_{10}$ transformed when necessary to meet the assumptions for parametric statistics. Differences in mercury concentration and stable isotope signatures between sexes were examined using analysis of covariance (ANCOVA). Body size was used as a covariate because alligators increase in size as they get older until an asymptotic limit is approached (Brisbin 1988). Paired t-tests were used to compare mercury concentration among muscle tissues examined (front leg, jaw, rear leg, and tail). Pearson's product moment correlations were used to determine relationships in mercury concentrations among tissues

examined and stable isotope signatures. Statistical results were considered significant when $P \leq 0.05$.

Results

Of the 27 alligators captured at RWR, 9 were female and 18 were male, ranging in total length from 90.2 cm to 268.6 cm (Figure 2.1). Mercury was detected in all tissues tested from the 27 alligators (Appendix 1). Mercury concentration in gonads ($F = 5.88$, $P = 0.0229$), and kidneys ($F = 4.63$, $P = 0.0412$), and $\delta^{15}N$ from tail tissue ($F = 6.52$, $P = 0.0171$), was higher in females. Other tissues examined did not differ between sexes.

Moisture content varied between tissues and may vary with alligator age and/or collection location (Yanochko *et al*. 1997); therefore, mercury concentrations were reported on a dry weight basis, for standardization. Blood samples were not dried before testing and mercury concentrations were therefore calculated as mg/kg wet weight.

Mercury concentration was highest in blood (121.74 ±22.07 mg Hg/Kg wet weight), followed by kidney (3.18 ±0.69 mg Hg/Kg dry weight) and liver (3.12 ±0.76 mg Hg/Kg dry weight), in all individuals (Table 2.1). Concentrations of mercury were lowest in gonad (0.25±0.06 mg Hg/Kg dry weight) and brain tissue (0.27±0.04 mg Hg/Kg dry weight).

Mercury concentration was positively correlated among all tissues, total length, and $\delta^{15}N$ (Table 2.2). However, $\delta^{13}C$ was not correlated with any tissue mercury concentrations, length, or $\delta^{15}N$. Notably, blood, claws and scutes were correlated with all mercury concentrations from other tissues and tail $\delta^{15}N$.

Among muscle tissue examined, mercury concentrations were highest in jaw muscle (0.55 ± 0.11 mg Hg/Kg dry weight), followed by tail muscle (0.48 ± 0.09 mg Hg/Kg dry weight),

rear leg muscle (0.42 ± 0.08 mg Hg/Kg dry weight), and front leg muscle (0.39 ± 0.07 mg Hg/Kg dry weight). All comparisons were significantly different ($P < 0.05$).

Discussion

Mercury was detected in all tissues from all alligators captured at Rockefeller Wildlife Refuge. Given the low method detection limit, this is not surprising. The blood mercury concentrations were high compared to other samples. For example, one adult female had a blood mercury concentration of 532.52 mg Hg/Kg wet weight.

Of all tissues/organs examined for mercury, only kidney and gonad differed between sexes, and were both higher in females. Delany et al. (1988) suggested that female alligators should have lower mercury concentrations than males, due to maternal transfer. However, in our study, many of the females had higher mercury concentrations than males. The differences in Hg concentrations observed could be due to the mobility patterns of the two sexes. Male alligators often will travel longer distances, up to a 256 ha, whereas females commonly stay in a smaller territory (Goodwin and Marion 1979). Therefore, males would possibly come into contact with a larger range of mercury concentrations in their diet and would be predicted to have higher concentrations. Females feeding in a small area take in a consistent amount of mercury, which may be small or large depending on the area. In areas with high mercury levels in lower trophic level organisms, female alligators may have high concentrations, which may have inflated values in our study.

There were large differences in mercury concentrations among tissues. Our results are consistent with Chang and Cockerham (1994) who stated that testing multiple tissues might allow differentiation between recent exposure and long-term exposure results because different tissues have different retention times. For example, liver is representative of dietary exposure,

while muscle represents long-term exposure (Yanochko et al. 1997; Khan and Tansel 2000). Liver tissues had one of the highest mercury concentrations observed, suggesting that the alligators were consuming high amounts of mercury in their diet. Blood represents levels of mercury during periods when mercury is moving between body compartments. In our study, blood samples had the highest mercury concentrations, suggesting high recent intakes of mercury.

Mercury concentrations in alligator muscle are of public health interest due to the increase in human consumption. In this study, the average muscle mercury concentration was 0.46 mg Hg/Kg dry weight. This concentration is approximately 0.10 mg Hg/Kg wet weight, which is just below the 0.13 mg Hg/Kg wet weight found in Louisiana alligators reported by Elsey et al. (1999). The concentrations reported in this study generally are lower than those reported in previous studies conducted in other locations. Yanochko et al. (1997) and Jagoe et al. (1998) found liver mercury concentrations and muscle mercury concentrations over ten times higher in Florida Everglades alligators than those from Louisiana reported in this study.

All tissue mercury concentrations were correlated with total length, other tissue mercury concentrations, and $\delta^{15}N$ from tail muscle. Because mercury concentrations from blood, claws and scutes were correlated with all of the other tissues, non-lethal mercury studies are possible and could be a valuable tool to monitor mercury concentrations over time. Similarly, Arnold (2000) found alligator claws correlated with several tissues such as muscle, kidney, liver, bone, spleen, and brain. Burger et al. (2000) found that mercury concentrations of non-invasive samples (skin and tail tip) were positively correlated to mercury concentrations of internal organs. Burger et al. (2000) suggests that skin would be the best bioindicator of internal mercury concentrations, particularly for the liver where metal concentrations are usually the highest. The

results of our study suggests that blood, claws, and dermal tail scutes samples are reliable for predicting mercury concentrations in internal organs and could prove useful in monitoring mercury in select locations.

As suspected, because larger alligators tend to eat at a higher trophic level, we found that larger animals had higher $\delta^{15}N$ values (Delany and Abercrombie 1986). Larger alligators may be exposed to higher mercury intake, as suggested by Rumbold et al (2002). However, Arnold (2000) found that mercury concentrations were not related to trophic level in alligators. Contrary to findings in Gariboldi et al. (1998) in a study of mercury concentration in wood storks (*Mycteria americana*), we found no significant relationships between $\delta^{13}C$ values and mercury concentration. This result is not surprising because all of the alligators were collected from one unit within the refuge. Observed relationships would have suggested that the individual(s) with differing $\delta^{13}C$ values had been feeding elsewhere. Similarly, Rumbold et al. (2002) found no correlation between $\delta^{13}C$ and mercury concentrations in alligators from a single water conservation area in the Florida Everglades.

In summary, we found mercury in all tissue/organ compartments and found positive correlations between all mercury concentrations and $\delta^{15}N$ signatures. However, $\delta^{13}C$ signatures were not correlated with mercury concentration. We also detected differences among the muscle tissues sampled. For future monitoring of alligator mercury concentrations, the results of this study suggest that tail muscle would be the best sample to test because it has an average mercury concentration and poses the most public health concern, because it is the muscle generally eaten. Nonetheless, our results suggest there is little public health concern in eating alligator meat from this location in Louisiana, because the concentrations are well below the 1.0 mg Hg/Kg wet

weight “action level” set by the FDA for edible fish. However, because mercury concentration is positively related to total length, larger individuals are likely to have higher concentrations.

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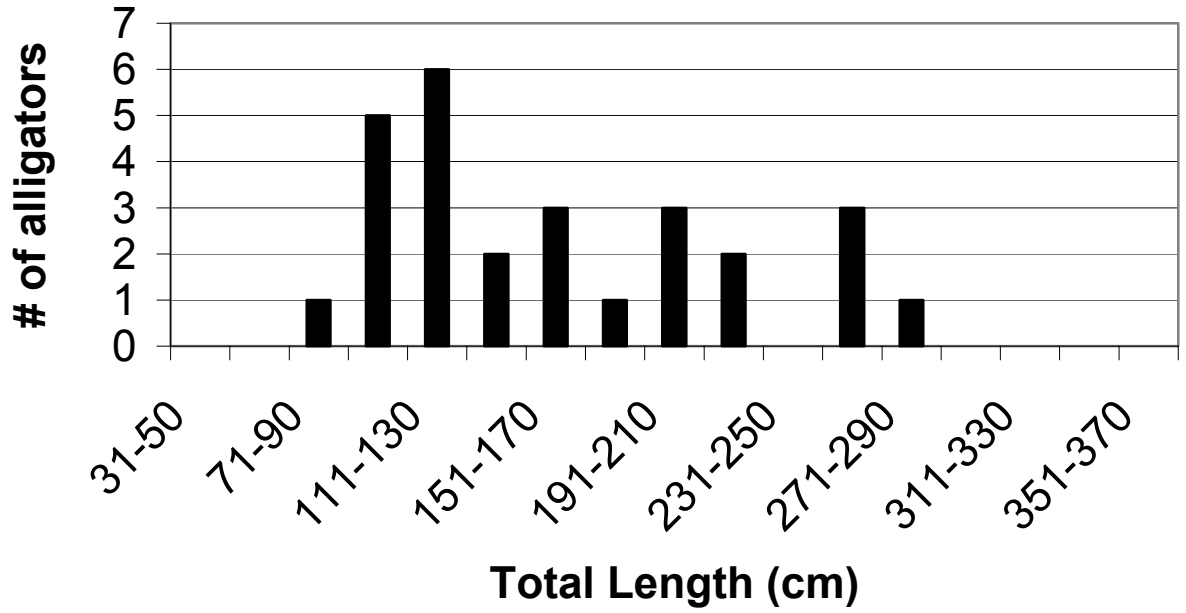

Figure 2.1. Total length distribution of American alligators collected from the Rockefeller Wildlife Refuge, Louisiana, June 2002.

Table 2.1. Mercury concentrations[1] and stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) in body organ/tissue compartments from American alligators collected at Rockefeller Wildlife Refuge, Louisiana, June 2002.

	MEAN	SE
Mercury		
Brain	0.27	0.04
Claw	0.76	0.13
Front Leg Muscle	0.39	0.07
Gonad	0.25	0.06
Heart	0.47	0.09
Jaw Muscle	0.56	0.11
Kidney	3.18	0.69
Rear Leg Muscle	0.42	0.08
Dermal Tail Scute	0.52	0.21
Tail Muscle	0.48	0.09
Blood	121.74	22.07
Average Muscle	0.46	0.09
$\delta^{15}N$		
Liver	5.24	0.18
Tail	5.45	0.12
$\delta^{13}C$		
Liver	-21.47	0.26
Tail	-20.41	0.27

[1]Mercury concentrations are expressed as mg/kg Hg dry weight, except for blood, which is expressed as mg/kg Hg wet weight.

Table. 2.2. Correlations among tissue mercury (Hg) concentrations, total length, and stable isotope signatures ($\delta^{15}N$ and $\delta^{13}C$) from American alligators sampled at Rockefeller Wildlife Refuge, Louisiana, June 2002.

	Length	Brain Hg	Claw Hg	Front Leg Hg	Gonad Hg	Heart Hg	Jaw Hg	Kidney Hg	Liver Hg	Rear Leg Hg	Scute Hg	Tail Hg	Blood Hg	Muscle Hg	Liver 15N	Liver 13C	Tail 15N	Tail 13C
Length		0.6731[2]	0.4117	0.6347	0.6460	0.6182	0.6456	0.7921	0.7238	0.6065	0.4445	0.6368	0.4793	0.6385	0.5652	-0.0652	0.4803	-0.0338
Brain Hg[1]	0.0002		0.7981	0.9240	0.8860	0.9601	0.9440	0.8789	0.8284	0.9356	0.8053	0.9485	0.7844	0.9476	0.4373	0.3234	0.5384	0.1584
Claw Hg	0.0328	<.0001		0.6159	0.6809	0.7410	0.7431	0.6890	0.6219	0.7271	0.5777	0.7498	0.5894	0.7162	0.2380	0.1667	0.4218	0.0982
Front Leg Hg	0.0004	<.0001	0.0006		0.8553	0.9585	0.9513	0.8383	0.7911	0.9602	0.8010	0.9507	0.7918	0.9747	0.4614	0.3967	0.4860	0.3018
Gonad Hg	0.0003	<.0001	<.0001	<.0001		0.8734	0.8865	0.8976	0.8188	0.8707	0.6558	0.9023	0.7602	0.8903	0.4503	0.3064	0.5424	0.1758
Heart Hg	0.0006	<.0001	<.0001	<.0001	<.0001		0.9828	0.8707	0.8327	0.9764	0.7728	0.9811	0.8303	0.9854	0.4420	0.4079	0.5194	0.3023
Jaw Hg	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001		0.8852	0.8232	0.9816	0.7394	0.9914	0.8421	0.9930	0.5050	0.3346	0.5581	0.2466
Kidney Hg	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.8770	0.8583	0.5875	0.8931	0.7594	0.8796	0.5254	0.1397	0.6033	0.1081
Liver Hg	<.0001	<.0001	0.0005	<.0001	<.0001	<.0001	<.0001	<.0001		0.7970	0.6137	0.8336	0.6504	0.8209	0.5230	0.0949	0.6021	0.0685
Rear Leg Hg	0.0008	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.7504	0.9870	0.3536	0.9933	0.4923	0.3742	0.5707	0.2623
Scute Hg	0.0202	<.0001	0.0016	<.0001	0.0002	<.0001	<.0001	0.0013	0.0007	<.0001		0.7484	0.5768	0.7659	0.3623	0.2390	0.3848	0.0663
Tail Hg	0.0004	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.8391	0.9941	0.4911	0.3460	0.5639	0.2607
Blood Hg	0.0132	<.0001	0.0015	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003	<.0001	0.002	<.0001		0.8391	0.6038	0.3721	0.5681	0.2727
Avg Muscle Hg	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.4947	0.3654	0.5526	0.2683
Liver 15N	0.0021	0.0255	0.2320	0.0154	0.0184	0.0210	0.0072	0.0049	0.0051	0.0091	0.0633	0.0093	0.0011	0.0087		-0.2008	0.8788	-0.3058
Liver 13C	0.7467	0.1071	0.4061	0.0405	0.1200	0.0347	0.0880	0.4870	0.6376	0.0545	0.2299	0.0771	0.0612	0.0609	0.3151		-0.2008	0.8399
Tail 15N	0.0112	0.0045	0.0284	0.0102	0.0035	0.0055	0.0025	0.0009	0.0009	0.0019	0.0475	0.0022	0.0025	0.0028	<.0001	0.3151		-0.3058
Tail 13C	0.8671	0.4396	0.6261	0.1261	0.3804	0.1254	0.2149	0.5916	0.7343	0.1863	0.7423	0.1891	0.1778	0.1761	0.1208	<.0001	0.1208	

[1]All Hg concentrations were log-transformed to meet the assumptions of parametric statistics

[2]Values above the diagonal are Pearson correlation coefficients (*r*) and those below the diagonal are the probabilities that these coefficients do not differ from zero.

CHAPTER 3

MERCURY CONCENTRATIONS IN THE AMERICAN ALLIGATOR (*ALLIGATOR MISSISSIPPIENSIS*)[1]

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[1]Moore, L. A., I. L. Brisbin, C. H. Jagoe, R. M. Elsey, T. C. Glenn, S. B. Castleberry, and C. S. Romanek. To be submitted to *Archives of Environmental Contamination and Toxicology.*

Abstract

Mercury (Hg) is a growing concern for many crocodilian species, including the American alligator (*Alligator mississippiensis*). Because alligators are top-level, long-lived predators, they are capable of accumulating considerable amounts of mercury in their tissues. Our objectives were to survey mercury concentrations in alligators from several locations within their geographic range to determine if alligators could be useful as biomonitors of mercury concentrations. Liver and tail muscle samples were taken from alligators collected by nuisance trappers in Alabama (n=10), Georgia (n=16), and South Carolina (n=3). Additionally, samples from 27 alligators were taken from Rockefeller Wildlife Refuge (RWR), Louisiana and 4 alligators from an alligator farm in Mitchell County, Georgia. Total mercury and stable isotope signatures ($\delta^{15}N$ and $\delta^{13}C$) were determined for all samples. Locations differed significantly in tissue mercury and stable isotope ratios. The highest mercury concentrations (19.41 ± 4.84 mg Hg/Kg dry weight for liver and 2.60 ± 0.65 mg Hg/Kg dry weight for tail) were found in Glynn County, Georgia. The lowest mercury concentrations (0.021 ± 0.006 mg Hg/Kg dry weight for liver and 0.023 ± 0.004 mg Hg/Kg dry weight for tail) were found in the farm alligators. The high mercury concentrations from Glynn County, Georgia are likely due the proximity to an EPA Superfund Site. Our results suggest that American alligators are capable of being used as biomonitors of mercury concentration in aquatic ecosystems.

Introduction

Environmental contaminants, especially mercury (Hg), are a growing concern in the conservation and management of several crocodilian species (Brisbin et al. 1998). Facemire et al. (1995) considered mercury to be the most serious environmental threat to fish and wildlife in the southeastern United States. Eisler (1987) reviewed the literature on the effects of mercury on humans, fish and other wildlife species. However, its effect on crocodilians is poorly understood. Crocodilians are capable of accumulating mercury in their tissues following dietary exposure at levels that exceed U. S. Food & Drug Administration (FDA) limits for fish taken for human consumption (Peters 1983). A small number of studies have been conducted on contaminants, including mercury, in wild American alligators (*Alligator mississippiensis*). Several studies examined mercury in tail muscle to determine if there was a need for a public health concern (Delany et al. 1988: Hord et al. 1990; Ruckel 1993; Elsey et al. 1999). Other studies examined concentrations in several tissues from animals collected at various locations across the range (Heaton-Jones et al. 1997; Yanochko et al. 1997; Jagoe et al. 1998; Burger et al. 2000).

The adult alligator is considered the top predator in freshwater wetlands in its range (Duvall and Barron 2000). Persistent contaminants, especially those that are not detoxified or excreted readily, such as methyl mercury, are generally more harmful to organisms at a higher trophic level (Hall 1980). The flow of contaminants through the food web can be traced by examining the 14N/15N ratio of the stable isotope nitrogen (Hairston and Hairston 1993). Nitrogen naturally occurs on earth as 14N/15N in a ratio of 99% of 14N and 0.37% 15N. The stable isotope 15N, the heavier of the two isotopes, has been reported to increase 3-4 ‰ for each trophic level step (Schoeninger and DeNiro 1984; Minigawa and Wada 1984). Mercury and

$\delta^{15}N$ have a positively correlated relationship in fish and raccoons (Kidd et al. 1995; Gaines et al. 2002).

Similarly, examining the carbon stable isotopes 12C/13C can differentiate whether an animal is feeding in marine versus freshwater and terrestrial environments. These two stable isotopes occur in a ratio of 98.9% of 12C to 1.1% of 13C (Rounick and Winterbourn 1986). Examining carbon stable isotope ratios can allow scientists to distinguish between animals with diets made up of predominantly C3, C4 or CAM plants (Kelly 2000; Gannes et al. 1997). C4 plants, which include agricultural crops, as well as marine plants, have an average delta value of -12.5 ‰ and C3 plants, which include plants that grow in freshwater areas, have an average delta value of -26.5 ‰ (Sage and Monson 1999; Ambrose and DeNiro 1986).

The primary objective of this study was to examine mercury levels in American alligator tail muscle and liver tissue across the southeastern United States. Additionally, we examined the $\delta^{15}N$ and $\delta^{13}C$ signatures to determine potential links between trophic level and mercury concentration and to differentiate differences in mercury concentration between marine and freshwater alligator populations. Our results provide data regarding the applicability of the American alligator as a biomonitor for contaminants in aquatic ecosystems.

Study Sites

Liver and tail muscle (Longissimus) samples were taken from wild alligators collected by nuisance trappers in Alabama (n=10), Georgia (n=16), and South Carolina (n=3) and by refuge personnel from Rockefeller Wildlife Refuge (RWR), Louisiana (n=27) (Figure 3.1). An additional four alligators were sampled from an alligator farm in Mitchell County, Georgia. Alligators from Alabama were collected from Tallapoosa, Covington, Geneva, and Houston Counties. The South Carolina samples were taken from Beaufort County. Samples from wild alligators in Georgia were taken from Brantley, Camden, Glynn, and Pierce Counties (Figure

3.1). In Georgia, Brantley, Camden, and Pierce were considered a single collection locality (Camden) separate from Glynn County collections (Glynn).

Materials and Methods

Mercury Analysis

Alligators were sexed, weighed, and total length (TL) in centimeters was measured at time of capture, before processing. Samples taken from each alligator were stored in separate, sterile Whirl-Pak bags (Nasco) and frozen at -10^{o} C until testing. Samples were freeze dried in a Labconco freeze drier at -44^{o} C under a pressure of 12 x 10^{3}Mbar, reweighed for moisture content, and homogenized before being analyzed for total Hg using a DMA80 Direct Mercury Analyzer (Milestone, Inc, Monroe, CT) following EPA Method 7473, which tests for total mercury in solids and solutions by thermal decomposition, gold amalgamation, thermal desorption and CVAA detection. Replicates and tissue standards of certified mercury concentration (DORM-2 (dogfish muscle), DOLT (dogfish liver), and TORT-2 (lobster hepatopancreas), purchased from the National Research Council of Canada (NRCC), Ottawa, Canada) were run with each set of samples. Based on a 25 mg sample and an average blank of 0.5 ng Hg, the method detection limit (MDL) was 0.020 mg Hg/Kg.

Isotope Analysis

The freeze dried liver and tail muscle samples were lipid extracted with a 2:1 ratio of chloroform:methanol mixture, rinsed with methanol, dried, and ~ 2 mg of sample was placed in a pressed tin capsule. A continuous flow isotope ratio Delta+xls Mass Spectrometer (Finnigan-MAT, San Jose, Ca), with a Carlo Erba NC2500 Elemental Analyzer was used to analyze tissue samples. All liver and tail muscle samples were analyzed for stable isotope signatures of carbon and nitrogen. In the testing process they were converted to CO_2 and N_2 in the oxidation and reduction furnaces of the elemental analyzer. The mass spectrometer separated the CO_2 and N_2

and measured for 12C/13C and 13N/14N ratios. At the beginning of each sample analysis an N_2 working standard (AIR) was introduced and the CO_2 working standard (Pee Dee belemnite) was introduced at the end of each sample's conclusion (Mariotti 1983; Coplen 1996). The results of the stable isotope analyses are presented in per mil per volt units (‰) with a standard δ notation (Craig 1957). External working standards of Dorm-2, dogfish muscle and liver, were used and reproducible to $\pm$ 0.15 ‰ for $\delta^{13}C$ and $\delta^{15}N$.

Statistical Analysis

Statistical analyses were conducted using SAS (SAS Institute, Cary, NC). All data were tested for normality and $\log_{10}$ transformed when necessary to meet the assumptions for parametric statistics. Differences in mercury concentration and stable isotope signatures between sexes and among locations were examined using analysis of covariance (ANCOVA). Body size was used as a covariate because alligators increase in size as they get older until an asymptotic limit is approached (Brisbin 1988). Tukey's multiple comparison procedure was used when significant differences were detected with ANCOVA. Statistical results were considered significant when $P \leq 0.05$.

Results

Alligators ranged between 90 cm to 353 cm in total length, with the largest alligator captured in Glynn County, Georgia and the smallest at RWR, Louisiana (Appendix 2). South Carolina alligators had the highest average total length (276.9 cm) (Figure 3.2).

Initial analysis demonstrated that liver ($F = 0.55$, $P = 0.46$) and tail muscle ($F = 2.20$, $P = 0.14$) mercury concentrations did not differ between sexes, thus, males and females were pooled for subsequent analyses. Locations exhibited similar trends for liver and tail muscle. Glynn County and Camden County, Georgia had the highest mean liver mercury concentrations of all locations sampled (Table 3.1). South Carolina and Alabama had intermediate levels, while RWR

and the alligator farm had the lowest mean liver mercury concentrations. For tail muscle, Glynn County and Camden County had high mean mercury concentrations. However, mean tail muscle mercury concentration was higher in Alabama than in Camden County. As with liver concentrations, the lowest values were from RWR and the alligator farm.

Liver ($F = 11.03$, $P < 0.0001$) and tail muscle ($F = 14.56$, $P < 0.0001$) $\delta^{15}N$ values were higher in Glynn County and Camden County than all other locations (Figure 3.3), indicating that those alligators were feeding at a higher trophic level than those from the other locations (Minigawa and Wada 1984). The Alabama site had lower $\delta^{13}C$ values than all other sites except the South Carolina and Glynn County, Georgia (Figure 3.4). The highest $\delta^{13}C$ value was from the farm alligators.

Discussion

We found mercury in all tissues of all alligators sampled. Concentrations in some individuals were quite high (Appendix 2). For example, one alligator from Camden County, Georgia had a liver concentration over 90 mg Hg/Kg dry weight, which is over five times the mean concentration for that population. This individual was a male captured near the St. Mary's River, where fish consumption advisories have been issued (Facemire et al. 1995). This individual may have inflated values for the Camden County sample and may partially explain the high variance observed. Arnold (2000) examined mercury in alligators from the Okefenokee Swamp, which is in the same general area as Camden County, and found mercury concentrations less than half of what we found in our Camden County samples. Why this individual had an excessively high mercury concentration is unknown.

Consumption advisories also have been issued for rivers in the southern section of South Carolina, as well as the Intercoastal Waterway (Facemire et al. 1995). However, the largest alligator from the South Carolina location had a tail muscle tissue concentration of only 0.996

mg Hg/Kg dry weight, which is over five times lower than concentrations found in Florida Everglades alligators by Jagoe et al. (1998). The sample size for South Carolina was small (n = 3), which may account for the discrepancy. Additionally, these alligators were collected in area where the water source (fresh or tidal) is controlled by a rice field trunk system, which also may have contributed to the differences observed between our study and previous studies.

Overall the alligators from Glynn County, Georgia had the highest mercury concentrations. A major source of mercury in Glynn County is the LCP Chemicals Superfund Site. This site covers 222.6 hectares, with the majority of the acreage in tidal marsh along the Turtle River and Purvis Creek. In the past 70 years an oil refinery, paint manufacturing company, power plant, and chlor-alkali plant were located on the site. Polychlorinated biphenyls (PCBs), semi-volatile contamination, and mercury are all prevalent in the site's soils, groundwater, flora, and fauna. The Environmental Protection Agency estimates that over 172 metric tons of mercury were unaccounted for in the area during the years of 1955 through 1979 when Allied Signal and LCP controlled the site. Seafood consumption advisories have been in place for part of the Turtle River and the entire Purvis Creek as a result of studies that found excessive mercury and PCBs in aquatic organisms [www.epa.gov/region4/waste/npl/nplga/lcpincga.htm].

The farm alligators had consistently low mercury concentrations in liver and tail muscle tissue. This is likely a result of strictly controlled diet consisting of commercial alligator food and ground chicken. The primary ingredient of the commercial alligator food is grain products followed by animal protein. The farm alligators have a diet low in mercury, unlike the wild alligators that are eating organisms that have mercury in their tissues.

We found no difference in mercury concentrations between sexes, which is consistent with previous studies (Elsey et al. 1999; Ruckel 1995; Jagoe et al. 1998). However, our finding

could be due to the small number of females tested in this study. Most alligators harvested by nuisance trappers are male (73% in our sample), because females generally stay in a small home territory, while larger males will travel longer distances (Goodwin and Marion 1979). Previous studies (Yanochko et al. 1997; Jagoe et al. 1998) showed that larger, older alligators had higher mercury concentrations. Lodge (1994) explained that young alligators feed on invertebrates and as they grow they begin feeding on larger prey items. We demonstrated a correlation between $\delta^{15}N$ measurements and total length, suggesting that larger alligators feed higher on the food chain.

Mercury levels were higher in liver tissue than in tail muscle. The liver acts as a storage and redistribution center of ingested mercury, while muscle tissue acts as a sink for accumulated mercury (Sorensen 1991). Total mercury was examined in this study, which may explain the high liver mercury values. Jernlov and Lann (1971) stated that examining total mercury may not be useful when looking at bioaccumulation rates, because total mercury contains both methyl mercury (MeHg) and inorganic mercury. They also stated that the proportion of MeHg to inorganic mercury is generally larger in muscle tissue and lower in liver tissue. Fish muscle tissue contains 99% MeHg and MeHg accounts for 95-99% of accumulated mercury in muscle tissue of high trophic level organisms (Huckabee et al. 1979; Bloom et al. 1991; Bloom 1992). Methyl mercury in alligator muscle appears to be accumulated in a similar manner (Moore et al., unpublished data). Therefore, with liver containing higher amounts of inorganic mercury the total mercury level would be higher.

The Alabama location had the lowest levels of $\delta^{13}C$, suggesting the individuals were feeding in a freshwater environment. The Alabama location was inland, and thus, alligators did not have access to saltwater habitats. In a study conducted by Gariboldi et al. (1998), wood storks feeding on freshwater prey items were consuming food with higher mercury

concentrations than those feeding on primarily saltwater prey. However, the mercury levels in Alabama were not the highest in our study. The highest mercury concentrations in our study were from Glynn County, Georgia, which is not surprising due to the close proximity to the EPA Superfund Site. The South Carolina alligators were harvested from an area with a dike system so the amount of fresh versus salt water can be controlled, possibly explaining the relatively low $\delta^{13}C$ values. All of the other sites were in close proximity to the coast, which is consistent with the higher $\delta^{13}C$ values observed. The farm alligators had the highest $\delta^{13}C$ values, which is likely due to their strictly controlled diet. The diet is primarily grain products, which are C4 plants, resulting in high $\delta^{13}C$ values.

We found that mercury concentrations differed among geographic locations. These differences in geographic location in this study and previous studies may result from differing mercury inputs from both local and atmospheric sources. It could also be due to differences in methylation or bioavailability among locations. Locations with known point sources, such as Glynn County, should be a concern when monitoring mercury concentrations in the biota.

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Table 3.1. Mean (±SE) total mercury concentrations (ppm) in liver and tail tissues of American alligators from six sites. Sample size (n) is denoted in parentheses

	Liver	Tail Muscle
	Mean ± SE	Mean ± SE
Alabama	10.78±4.26^{ab} (4)	2.00±0.49^{ab} (10)
Glynn County, GA	19.41±4.84^{a} (9)	2.60±0.65^{a} (9)
Camden County, GA	17.49±14.88^{bc} (6)	1.89±1.12^{bc} (7)
Farm	0.021±0.006^{d} (4)	0.023±0.004^{d} (4)
Louisiana	3.12±0.76^{c} (27)	0.48±0.09^{c} (27)
South Carolina	11.92±4.87^{ab} (3)	0.55±0.23^{abc} (3)

Within tissues, locations with the same superscript letter are not significantly different at $\alpha = 0.05$.

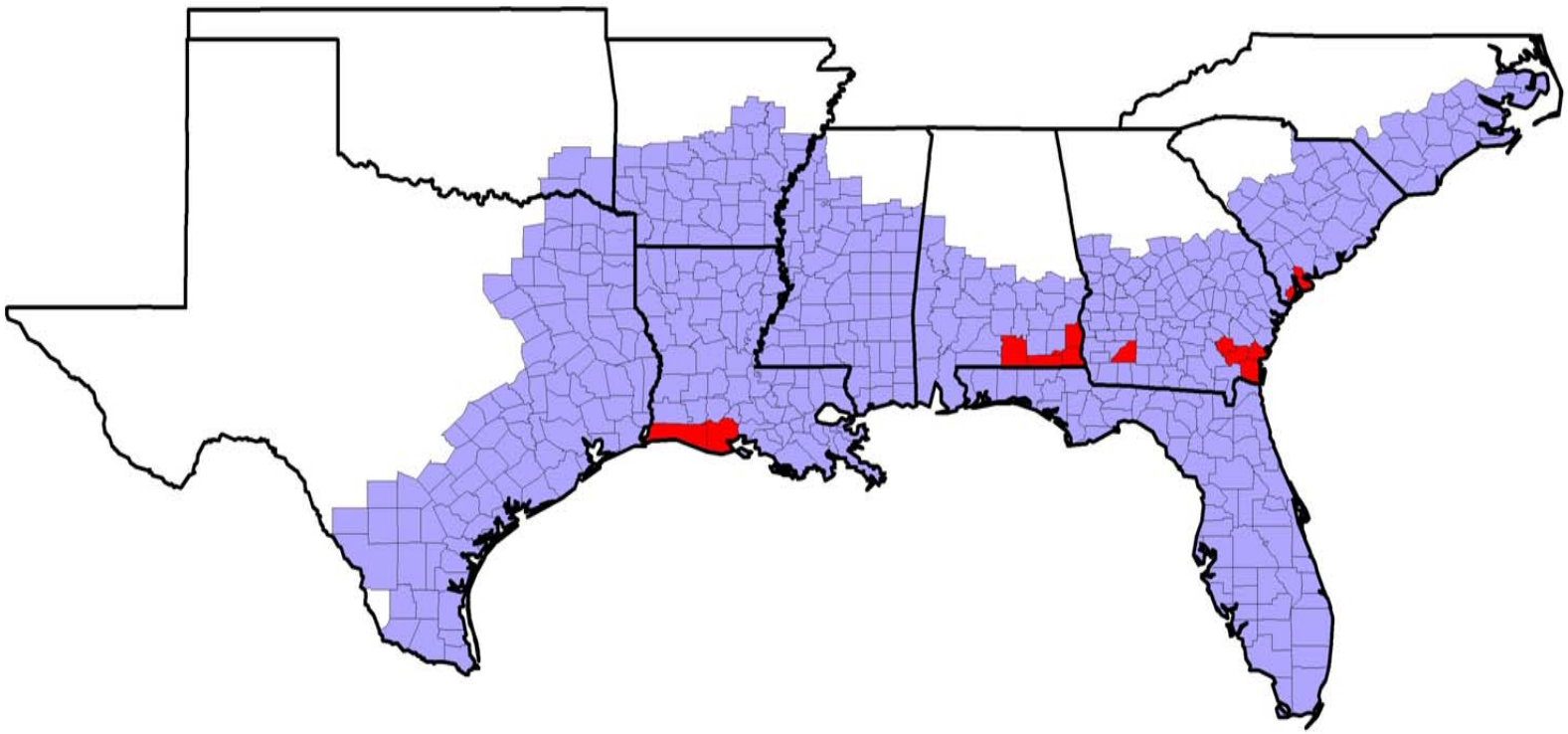

Figure 3.1. Map of the Southeast, including the range of the American alligator, with the sample locations highlighted.

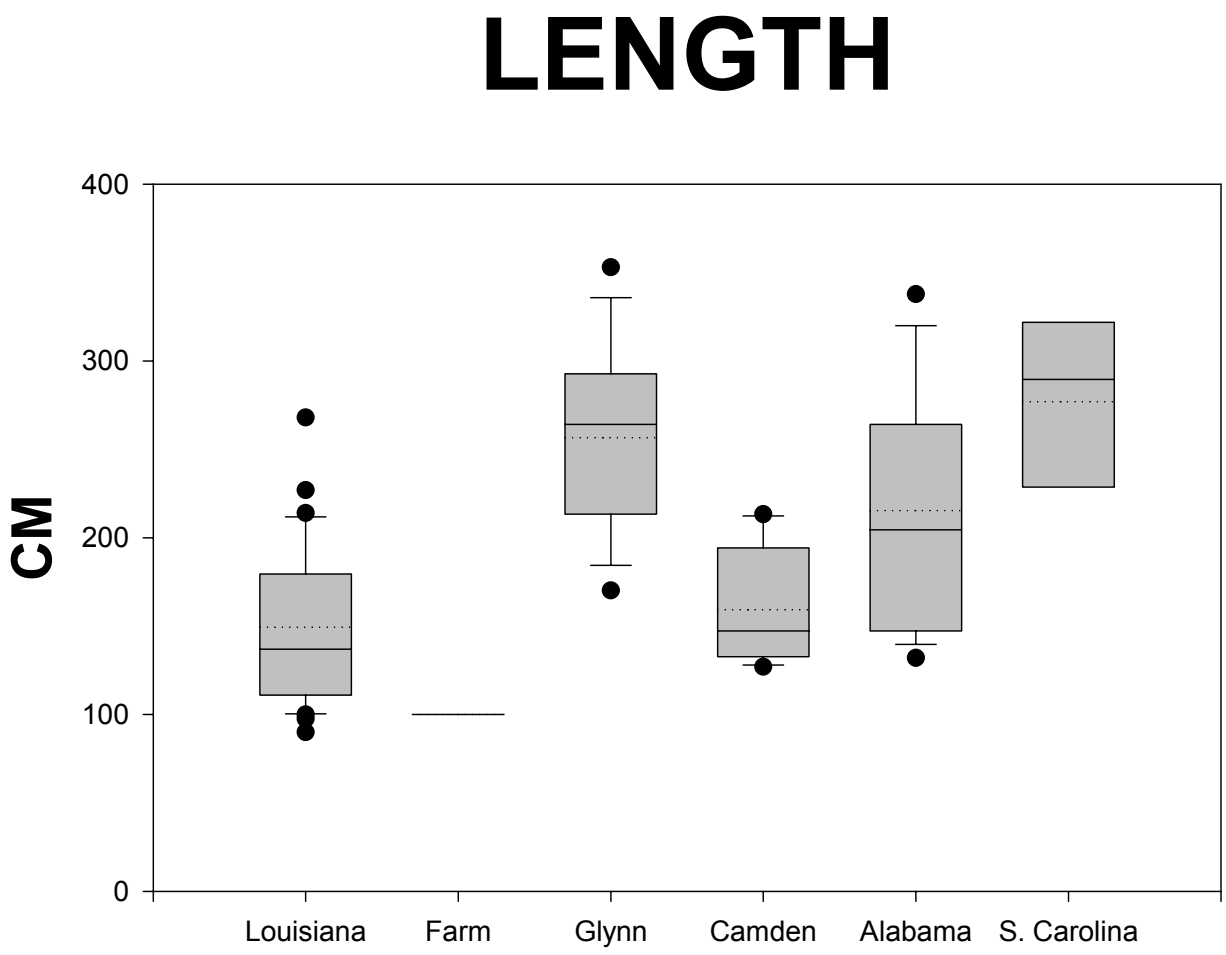

Figure 3.2. Box plots of total lengths of alligators sampled at six locations in the southeastern U.S. Dashed lines represent mean length and solid lines indicate (from bottom to top) the 5, 25, 50, 75 and 95 percentiles, respectively. Values outside the 5th and 95th percentiles are plotted as dots.

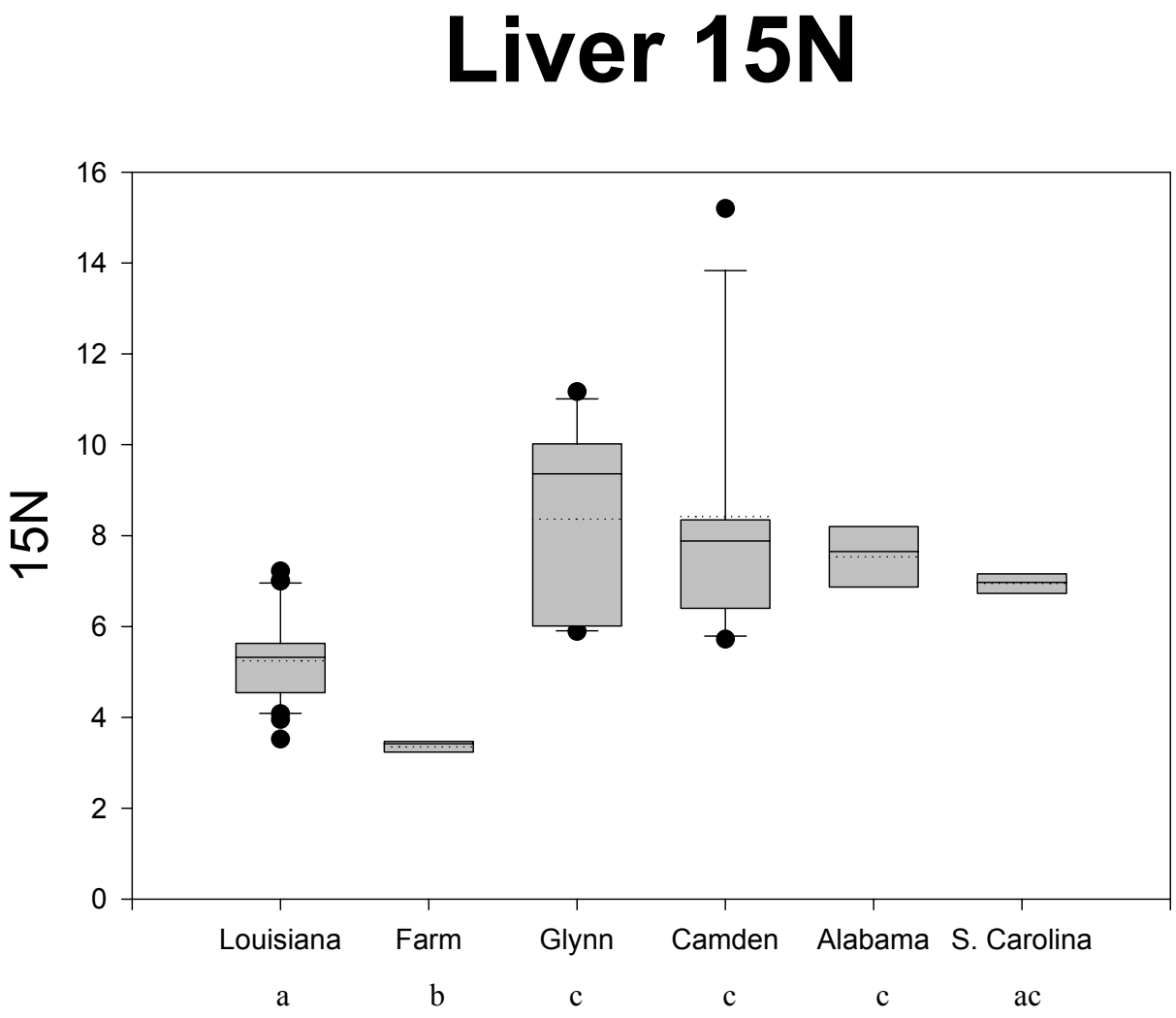

Figure 3.3. Box plots of $\delta^{15}N$ of alligators sampled at six locations in the southeastern U.S. Dashed lines represent mean length and solid lines indicate (from bottom to top) the 5, 25, 50, 75 and 95 percentiles, respectively. Values outside the 5th and 95th percentiles are plotted as dots. Locations with the same letter are not significantly different by ANCOVA using Tukey's multiple comparison procedure at $P < 0.05$.

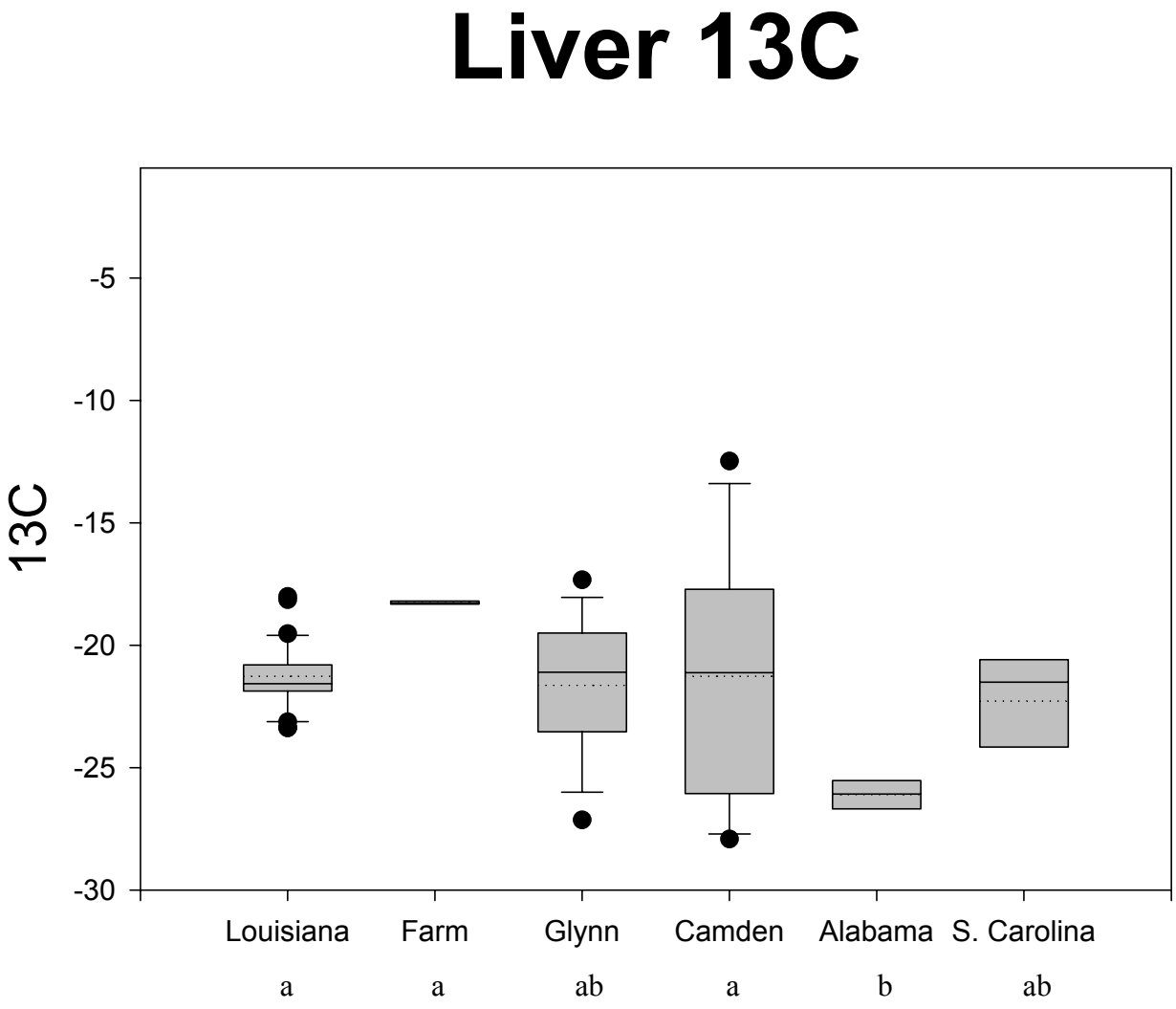

Figure 3.4. Box plots of $\delta^{13}C$ of alligators sampled at six locations in the southeastern U.S. Dashed lines represent mean length and solid lines indicate (from bottom to top) the 5, 25, 50, 75 and 95 percentiles, respectively. Values outside the 5th and 95th percentiles are plotted as dots. Locations with the same letter are not significantly different by ANCOVA using Tukey's multiple comparison procedure at $P < 0.05$.

CHAPTER 4

CONCLUSIONS

The human health hazards from alligator meat consumption could be a serious health problem. Unlike fish, which have been shown to pose a health hazard due to accumulated contaminants, alligator meat is not eaten as commonly by most people. However, certain groups of people, such as processors and trappers, may be at more risk from mercury contaminated alligator meat due to a higher consumption level. My results suggest that high-risk groups may need to monitor their consumption rates, depending on geographic location, particularly in respect to size of the alligators they consume. The results of this study and others, suggest a monitoring program should be developed, especially on alligators taken for human consumption. The differences in mercury concentrations between geographic locations in this study and in previous studies may result from differing mercury inputs from both local and atmospheric sources. It could also be due to differences in methylation or bioavailability among locations.

This study, along with the other studies on this species, have shown that alligators are potential bioindicators of mercury contamination in aquatic systems, because they inhabit aquatic systems where mercury often accumulates, they are long-lived and they are top-level predators (Brisbin et al., 1998). As biomonitors, alligators could help scientists track changes in mercury concentrations in different locations. My results also suggest that non-invasive samples can be used to examine changes in mercury concentrations over time through repeated testing of the same populations.

More studies on the types of mercury in alligator tissues need to be completed to achieve a clearer picture into the bioaccumulation process of mercury in the species. Jernlov and Lann (1971) stated that examining total mercury may not be very useful when looking at bioaccumulation rates, because total mercury contains MeHg and inorganic mercury. They also stated that the proportion of MeHg to inorganic Hg is generally larger in muscle tissue and lower in liver tissue. Fish muscle tissue contains 99% MeHg and it is said that MeHg accounts for 95-

99% of accumulated mercury in muscle tissue of high trophic level organisms (Huckabee et al. 1979; Bloom et al. 1991; Bloom 1992). It is currently unknown if alligators are capable of excreting mercury or at what rate excretion occurs, warranting further study.

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APPENDICES

Appendix 1. Total Hg from all tissues and stable isotope data from Louisiana alligators

ID	Sex	Length CM	Brain Dry	Claw Dry	Front Dry	Gonad Dry	Heart Dry	Jaw Dry	Kidney Dry	Liver Dry	Rear Dry	Scute Dry	Tail Dry	Whole Blood	Muscle Dry	Liver 15N	Tail 15N	Liver 13C	Tail 13C
53	F	227	0.60	1.30	0.64	0.65	0.76	1.04	16.10	16.87	0.70	0.42	0.91	252.59	0.82	7.22	6.68	-23.08	-21.49
55	F	192	0.25	0.51	0.41	0.35	0.47	0.73	5.91	3.81	0.47	0.14	0.53	193.26	0.53	6.78	6.56	-23.37	-22.73
100-Y	F	192	0.30	0.52	0.41	0.31	0.42	0.54	3.73	2.91	0.38	1.06	0.51	66.15	0.46	7.00	6.28	-21.76	-20.22
101-A	F	167	1.14	3.79	1.83	1.53	2.46	3.03	10.97	8.41	2.21	5.83	2.46	532.52	2.38	5.64	5.91	-18.14	-18.12
102-B	F	111	0.10	0.40	0.13	0.13	0.22	0.25	1.16	0.75	0.23	0.07	0.26	75.82	0.22	5.35	5.76	-21.44	-20.91
103-C	M	123	0.11	0.06	0.34	0.11	0.22	0.25	0.75	0.63	0.23	0.21	0.22	72.06	0.26	4.97	4.88	-20.00	-19.94
104-D	M	117	0.19	0.36	0.25	0.08	0.28	0.33	0.80	0.70	0.33	0.27	0.28	98.01	0.30	5.66	5.78	-21.99	-22.07
105-E	F	164	0.52	1.19	1.06	0.45	1.25	1.41	7.33	7.56	1.23	0.38	1.34	270.09	1.26	5.32	5.70	-18.01	-16.36
106-F	M	129	0.21	0.54	0.31	0.13	0.44	0.45	1.63	12.18	0.33	0.47	0.37	90.94	0.36	5.70	5.87	-21.73	-20.96
107-G	M	113	0.11	0.60	0.25	0.13	0.26	0.31	0.89	0.66	0.24	0.15	0.24	63.89	0.26	5.47	5.48	-21.68	-19.93
108-H	M	172	0.29	0.83	0.39	0.31	0.49	0.57	4.80	2.72	0.40	0.32	0.48	98.96	0.46	5.07	5.65	-22.24	-22.07
109-I	M	159	0.38	0.85	0.41	0.19	0.53	0.54	3.11	1.53	0.41	0.88	0.45	.	0.45	3.95	4.93	-21.11	-20.80
110-J	M	102	0.15	0.36	0.19	0.09	0.22	0.24	0.59	0.46	0.18	0.21	0.20	61.61	0.20	4.80	4.93	-21.02	-19.97
111-K	M	106	0.12	0.36	0.17	0.07	0.23	0.24	0.97	0.52	0.18	0.06	0.21	75.97	0.20	5.16	5.46	-19.85	-18.95
112-L	F	90	.	0.93	0.20	0.22	0.23	0.31	1.22	0.84	0.25	0.26	0.29	112.56	0.26	4.41	5.09	-19.89	-20.42
113-M	M	105	0.22	0.87	0.27	0.09	0.32	0.35	1.21	0.84	0.30	0.31	0.29	92.83	0.30	5.59	6.01	-21.57	-21.63
114-N	F	137	0.16	0.63	0.20	0.09	0.26	0.32	1.47	0.98	0.25	0.08	0.27	87.44	0.26	5.42	5.52	-21.28	-20.49
115-O	M	268	0.29	0.81	0.53	0.19	0.50	0.71	4.16	3.04	0.58	0.46	0.57	176.70	0.60	7.00	5.99	-21.87	-20.47
116-P	M	168	0.29	0.76	0.44	0.27	0.54	0.58	3.80	2.95	0.44	0.49	0.50	319.79	0.49	5.59	4.93	-20.75	-18.88
117-Q	M	156	0.16	0.46	0.25	0.10	0.29	0.40	1.24	1.14	0.24	0.35	0.30	52.07	0.30	5.09	4.92	-21.59	-21.29
118-R	M	214	0.35	0.98	0.39	0.29	0.54	0.56	5.54	4.25	0.42	0.31	0.51	84.55	0.47	4.54	5.37	-20.95	-20.95
119-S	M	182	0.20	0.47	0.30	0.22	0.32	0.31	2.57	2.66	0.25	0.14	0.27	49.01	0.28	4.08	4.79	-19.53	-18.52
120-T	M	203	0.41	1.11	0.41	0.31	0.54	0.61	2.20	4.68	0.45	0.75	0.56	76.54	0.51	5.33	5.64	-21.87	-21.33
121-U	M	98	0.14	0.59	0.25	0.07	0.23	0.26	1.03	0.96	0.24	0.40	0.27	29.38	0.26	4.12	5.00	-23.37	-21.97
122-V	M	100	0.07	0.25	0.11	0.03	0.15	0.20	0.49	0.29	0.13	0.03	0.15	33.13	0.15	3.52	3.97	-21.67	-18.83
123-W	F	111	0.08	0.42	0.12	0.09	0.13	0.17	0.96	0.70	0.15	0.06	0.17	44.23	0.15	4.24	4.99	-23.13	-21.26
124-X	M	131	0.18	0.57	0.23	0.19	0.28	0.37	1.33	1.24	0.27	0.04	0.32	55.07	0.30	4.55	5.07	-21.29	-20.58

Appendix 2. Liver and Tail muscle total Hg and stable isotope data from all six sites.

ID	Sex	Location	Length	Liver dry	Tail dry	Liver 15N	Tail 15N	Liver 13C	Tail13C
905053	F	LOUISIANA	227	16.871	0.907	7.22	6.68	-23.08	-21.49
905055	F	LOUISIANA	192	3.806	0.534	6.78	6.56	-23.37	-22.73
905100-Y	F	LOUISIANA	192.2	2.905	0.51	7	6.28	-21.76	-20.22
905101-A	F	LOUISIANA	167.4	8.412	2.455	5.64	5.91	-18.14	-18.12
905102-B	F	LOUISIANA	111	0.752	0.256	5.35	5.76	-21.44	-20.91
905103-C	M	LOUISIANA	123.2	0.628	0.224	4.97	4.88	-20	-19.94
905104-D	M	LOUISIANA	116.8	0.698	0.277	5.66	5.78	-21.99	-22.07
905105-E	F	LOUISIANA	164	7.555	1.337	5.32	5.7	-18.01	-16.36
905106-F	M	LOUISIANA	129	12.182	0.37	5.7	5.87	-21.73	-20.96
905107-G	M	LOUISIANA	113	0.663	0.244	5.47	5.48	-21.68	-19.93
905108-H	M	LOUISIANA	171.8	2.717	0.476	5.07	5.65	-22.24	-22.07
905109-I	M	LOUISIANA	159	1.53	0.447	3.95	4.93	-21.11	-20.8
905110-J	M	LOUISIANA	102	0.463	0.202	4.8	4.93	-21.02	-19.97
905111-K	M	LOUISIANA	106	0.516	0.208	5.16	5.46	-19.85	-18.95
905112-L	F	LOUISIANA	90	0.84	0.285	4.41	5.09	-19.89	-20.42
905113-M	M	LOUISIANA	105	0.835	0.289	5.59	6.01	-21.57	-21.63
905114-N	F	LOUISIANA	137	0.977	0.273	5.42	5.52	-21.28	-20.49
905115-O	M	LOUISIANA	268	3.038	0.571	7	5.99	-21.87	-20.47
905116-P	M	LOUISIANA	168	2.948	0.503	5.59	4.93	-20.75	-18.88
905117-Q	M	LOUISIANA	156	1.135	0.301	5.09	4.92	-21.59	-21.29
905118-R	M	LOUISIANA	214	4.245	0.509	4.54	5.37	-20.95	-20.95
905119-S	M	LOUISIANA	182	2.664	0.267	4.08	4.79	-19.53	-18.52
905120-T	M	LOUISIANA	203	4.681	0.562	5.33	5.64	-21.87	-21.33
905121-U	M	LOUISIANA	97.6	0.961	0.269	4.12	5	-23.37	-21.97
905122-V	M	LOUISIANA	100	0.292	0.154	3.52	3.97	-21.67	-18.83
905123-W	F	LOUISIANA	111	0.696	0.166	4.24	4.99	-23.13	-21.26
905124-X	M	LOUISIANA	131	1.243	0.318	4.55	5.07	-21.29	-20.58
AU1	.	FARM	.	0.017	0.029	3.38	3.46	-18.32	-18.54
AU2	.	FARM	.	0.012	0.021	3.48	3.61	-18.23	-18.37
AU3	.	FARM	.	0.016	0.033	3.46	3.65	-18.15	-18.45
AU4	.	FARM	.	0.038	0.015	3.09	3.6	-18.32	-18.21
BG1	M	GLYNN COUNTY	309.88	19.842	2.913	11.17	10.78	-17.33	-16.79
BG2	M	GLYNN COUNTY	264.16	13.17	2.588	9.77	9.7	-24.3	-17.55
BG3	M	GLYNN COUNTY	220.98	10.646	2.409	6.04	6.19	-23.27	-22.04
BG4	M	GLYNN COUNTY	281.94	14.583	0.857	9.36	8.69	-19.11	-23.7
BG5	M	GLYNN COUNTY	353.06	51.819	4.924	10.77	10.72	-21.1	-20.07
BG6	F	GLYNN COUNTY	215.9	34.375	6.474	5.89	6.2	-22.82	-21.99
BG7	M	GLYNN COUNTY	287.02	11.239	1.328	9.75	10.11	-20.07	-18.51
BG8	M	GLYNN COUNTY	205.74	6.937	0.748	6.62	6.77	-19.63	-18.85
BG9	F	GLYNN COUNTY	170.18	12.091	1.127	5.93	6.48	-27.14	-27.04
SC1	M	SOUTH CAROLINA	332.74	21.649	0.996	7.22	7.62	-25.04	-24.76
SC2	M	SOUTH CAROLINA	289.56	7.323	0.325	6.65	8.02	-21.51	-20.55
SC3	M	SOUTH CAROLINA	208.28	6.776	0.316	6.97	7.35	-20.28	-20.58
AL1	M	ALABAMA	302.26	.	0.272	.	5.6	.	-19.42
AL2	F	ALABAMA	264.16	.	5.044	.	8.78	.	-25.07
AL3	M	ALABAMA	337.82	.	1.217	.	8.06	.	-23.72
AL4	M	ALABAMA	208.28	.	2.966	.	8.33	.	-26.94
AL5	M	ALABAMA	226.06	.	2.615	.	10.67	.	-25.46
AL6	M	ALABAMA	132.08	.	0.427	.	6.59	.	-20.43
AL7	M	ALABAMA	147.32	8.687	1.353	6.19	6.54	-26.45	-26.11
AL8	F	ALABAMA	200.66	5.295	1.201	7.75	7.57	-25.36	-23.3
AL9	M	ALABAMA	147.32	5.779	1.093	7.55	8.17	-26.91	-26.64
AL10	F	ALABAMA	187.96	23.366	3.769	8.65	9.21	-25.7	-25.78
C1*	F	CAMDEN COUNTY	147.32	11.194	4.368	7.4	8.13	-27.92	-26.51
C2	M	CAMDEN COUNTY	152.4	0.794	0.175	6.07	6.48	-23.68	-21.86
C3	F	CAMDEN COUNTY	132.08	0.649	0.18	8.28	8.03	-19.76	-20.4
C4	M	CAMDEN COUNTY	127	0.684	0.189	7.88	8.4	-21.12	-17.56
C5	F	CAMDEN COUNTY	134.62	0.214	0.059	5.72	6.07	-26.85	-25.9
C6	M	CAMDEN COUNTY	208.28	91.393	7.642	15.2	14.97	-12.48	-12.26
C7	M	CAMDEN COUNTY	213.36	.	0.6	8.37	8.62	-17.03	-16.02

Each (.) denotes a sample that was not collected from that animal.
*This alligator had a stub-tail.