PREVALENCE AND SPATIAL DISTRIBUTION OF CONTAMINANTS ASSOCIATED WITH A SUPERFUND SITE AMONG AQUATIC REPTILES IN SOUTHEAST GEORGIA

by

RICHARD G. BAUER

(Under the Direction of Laurie A. Fowler)

ABSTRACT

Brunswick, Georgia is the location of an Environmental Protection Agency Superfund site. Polychlorinated biphenyls (PCBs) and heavy metals associated with this site occur in upper-level predators at concentrations that could cause negative effects. The marsh around the Brunswick area is critical habitat to diamondback terrapins, loggerhead sea turtles, and American alligators. My research sought to quantify how much these three reptile species were being exposed to contaminants from the Superfund site. While terrapins closer to the site had higher levels of PCBs, we found no difference between PCB levels in terrapins found within a fishing advisory zone and terrapins found outside of the advisory zone. Mercury levels in loggerheads that nested primarily in the Brunswick area were similar to levels in loggerheads that nested elsewhere. Contaminant results in alligators varied, however many alligators contained lead levels that exceeded the lead limit placed on food by the Food and Drug Administration.

INDEX WORDS: Polychlorinated biphenyls, heavy metals, Superfund, diamondback terrapins, loggerhead sea turtles, American alligators, conservation, management, marsh

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B.S., Davidson College, 2012

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

2019

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May 2019

DEDICATION

My thesis is dedicated to all my friends and family that have kept me sane during these last few years. First, to my parents, Steven and Kristine. To my father, thank you for instilling the love of the natural world in me. From taking me to catch snakes, to tracking Ebola outbreaks in Africa, to setting up chemistry laboratories in the basement, thank you for encouraging me to follow my interests wherever they led. To my mother, thank you for always encouraging me to pursue my passion. Even if it meant putting up with me hiding snakes in the house or taking over the freezer with animal skulls to preserve. Additionally, I would like to thank my brother, Mike, for always encouraging me to believe in myself. For always pushing me to do my best but keeping me grounded during the turbulent times. I would also like to thank my girlfriend, Tori, for her unconditional assistance in my pursuits. Tori helped me with the practical parts of my projects, from wrestling alligators with me to running samples for heavy metals. And she was there for me during the later stages of my thesis, to emotionally support me during countless sleepless nights and episodes of self-doubt. I will forever be grateful for her help. And last but not least, to all my friends who were with me the entire way of this journey. From middle of the night alligator calls to stress relief breaks, you all helped in your own way to get me here.

ACKNOWLEDGEMENTS

First and foremost, I acknowledge the Jekyll Island Authority's Georgia Sea Turtle Center (GSTC), which is the whole reason why this project was possible. The Jekyll Island Foundation provided me with most of the funding for the chemical analyses of my samples, without which, none of this would have been possible. Individuals from the Jekyll Island Authority worthy of my gratitude are: Dr. Terry Norton, Breanna Ondich, Michelle Kaylor, Livvy Jones, Joseph Colbert, Yank Moore, Ben Carswell, and countless AmeriCorps members.

At the University of Georgia (UGA), I would like to first thank my advisor, Dr. Laurie Fowler, for being an unlimited source of positivity and understanding. I would also like to thank my committee members, Dr. Tracey Tuberville and Dr. Terry Norton, for their guidance and assistance over the course of this project. Additionally, I would like to thank the River Basin Center and the family of James E. Butler for supporting me through most of my master's education. Individuals at UGA that went above and beyond assisting me in my endeavors are: Gregory Skupien, Davide Zailo, Katie Mascovich, Darren Fraser, Lance Paden, Kristen Zemaitis, Dr. Kimberly Andrews, Dr. Brian Shamblin, and Lisa Gentit.

All methods described in this document have been approved by the University of Georgia Institutional Animal Care and Use Committee (Animal Use Protocols A2012 07-025-A2, A2015 10-044-Y3-A0, and A2016 10-011-Y1-A0).

TABLE OF CONTENTS

Page

ACKNOWLEDGMENTS……………………………………………………………..v

LIST OF TABLES………………………………………………………………….vii

LIST OF FIGURES…………………………………………………………………ix

CHAPTER

1 INTRODUCTION AND LITERATURE REVIEW……………………...1

2 SPATIAL DISTRIBUTION OF POLYCHLORINATED BIPEHNYLS AND MERCURY FROM A SUPERFUND SITE WITHIN A POPULATION OF DIAMONDBACK TERRAPINS IN BRUNSWICK, GEORIGA……………………………………………………………..33

3 MERCURY LEVELS OF LOGGERHEAD SEA TURTLES NESTING ON JEKYLL ISLAND, GEORIGA……………………………………..66

4 CONCENTRATIONS OF POLYCHLORINATED BIPHENYLS AND HEAVY METALS ASSOCIATED WITH A SUPERFUND SITE IN AMERICAN ALLIGATORS IN BRUNSWICK, GEORGIA…………104

5 SYNTHESIS AND RECOMMENDATIONS………………………….161

LIST OF TABLES

Page

Table 3.1: Summary of the linear mixed effects (LME) models used to test the effects of residency status and nesting in other states on mercury concentration in loggerhead sea turtle (*Caretta caretta*) skin samples from Jekyll Island, Georgia. Random effect variables are straight carapace length ("SCL"), populations status ("Status"; either Resident, Transient, or Unknown), and states nested in ("States"; either Georgia, Florida, South Carolina, or North Carolina). Individual genetic identification was included as a fixed effect ("ID"). Models are ranked according to Akaike's Information Criterion (AIC) values. *P* values were calculated by running an analysis of variance (ANOVA) for each experimental model against the null model for mercury concentration ("~ SCL + ID")……………………..99

Table 4.1: Summary of the results of the analysis of covariance (ANCOVA) of body condition index (Fulton's K) and sex (male, female) on Aroclor 1268, lead, and mercury levels in caudal tail scutes of American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA……………………………...144

Table 4.2: Summary of the analysis of covariance (ANCOVA) of combinations of Aroclor 1268, lead, and mercury concentrations in the caudal tail scutes of American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA

and their effects on blood values (packed cell volume, glucose, and total proteins). Significant correlations are highlighted in bold font…………………………...145

Table 4.3: Summary of studies that have measured lead levels in the caudal tail scutes of crocodilians. Lead concentrations are listed as the mean ± 1 SE. Studies that reported lead concentrations in dry weight were converted to wet weight (marked with *) by dividing the dry weight concentrations by 3.8 as calculated by Jeffree et al. (2001)………………………………………………………………..146

Table 4.4: Summary of studies that have measured mercury levels in the caudal tail scutes of crocodilians. Mercury concentrations are listed as the mean ± 1 SE. Studies that reported lead concentrations in dry weight were converted to wet weight (marked with *) by dividing the dry weight concentrations by 3.8 as calculated by Jeffree et al. (2001)………………………………………………147

LIST OF FIGURES

Page

Figure 2.1: Map of the study site, showing the LCP Chemicals Superfund site (crosshatched in red), the fishing advisory zone (hatched in yellow), the Downing Musgrove Causeway, and the GPS positions of the 30 diamondback terrapins (*Malaclemys terrapin*; blue dots) collected for this study along the Downing-Musgrove Causeway (DMC) leading to Jekyll Island…………………………...61

Figure 2.2: Comparison of Aroclor 1268 concentrations in diamondback terrapin (*Malaclemys terrapin*) livers from the North side of the Downing-Musgrove Causeway (DMC) and the South side of the DMC in Brunswick, Georgia (one-tailed t-test; t (11.133) = 1.218, p = 0.124)………………………………………62

Figure 2.3: Comparison of mercury concentrations in diamondback terrapin (*Malaclemys terrapin*) livers from the North side of the Downing-Musgrove Causeway (DMC) and the South side of the DMC in Brunswick, Georgia (one-tailed t-test; t (7.621) = 1.876, p = 0.049)………………………………………………………………63

Figure 2.4: Relationship between straight-line distance from the Linden Chemicals and Plastic (LCP) Superfund site and Aroclor 1268 concentrations in diamondback terrapin (*Malaclemys terrapin*) livers found along the Downing Musgrove Causeway in Brunswick, Georgia (linear regression; $F_{1,28}$ = 4.553, p = 0.042)…64

Figure 2.5: Relationship between straight-line distance from the Linden Chemicals and Plastic (LCP) Superfund site and log-transformed mercury concentrations in diamondback terrapin (*Malaclemys terrapin*) livers found along the Downing Musgrove Causeway in Brunswick, Georgia (linear regression; $F_{1,28} = 1.402$, $p = 0.246$)……………………………………………………………………….65

Figure 3.1: Map of the study site and the Turtle/Brunswick River Estuary (TBRE) Islands, showing the LCP Chemicals Superfund site (crosshatched in red), the fishing advisory zone (hatched in yellow), the TBRE, Jekyll Island, St. Simons Island, Sea Island, and Little St. Simons Island……………………………….....100

Figure 3.2: Relationship between log-transformed straight carapace length (SCL) measurements and mercury concentrations in loggerhead sea turtle (*Caretta caretta*) skin samples from Jekyll Island, Georgia (linear regression; $F_{1,52} = 8.122$, $p = 0.006$)…………………………………………………………………….101

Figure 3.3: Comparison of mercury concentrations in loggerhead sea turtle (*Caretta caretta*) skin samples from turtles that were sampled in both the 2015 and 2017 nesting seasons (paired t-test; t (7) = 1.611, $p = 0.151$)………………………..102

Figure 4.1: Map of the Turtle/Brunswick River Estuary, showing the LCP Chemicals Superfund site (outlined in red crosshatching), the fishing advisory zone (outline in yellow hatching) and the GPS positions of the 62 American alligators (*Alligator mississippiensis*; blue dots) collected for this study around Jekyll Island, Georgia, USA…………………………………………………………...148

Figure 4.2: Relationship between log-transformed body condition index (Fulton's K) and log-transformed Aroclor 1268 concentrations of skin samples in male and female American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA……………………………………………………………………………149

Figure 4.3: Relationship between log-transformed body condition index (Fulton's K) and log-transformed lead concentrations of skin samples in male and female American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA……………………………………………………………………………150

Figure 4.4: Relationship between log-transformed body condition index (Fulton's K) and log-transformed mercury concentrations of skin samples in male and female American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA……………………………………………………………………………151

Figure 4.5: Relationship between log-transformed distance from the LCP Chemical Superfund site and log-transformed Aroclor 1268 concentrations ($F_{1,62}$ = 0.144, p = 0.706) in caudal tail scutes of American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA……………………………………………...152

Figure 4.6: Relationship between log-transformed distance from the LCP Chemical Superfund site and log-transformed lead concentrations ($F_{1,62}$ = 0.122, p = 0.728) in caudal tail scutes of American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA………………………………………………………...153

Figure 4.7: Relationship between log-transformed distance from the LCP Chemical Superfund site and log-transformed mercury concentrations ($F_{1,61}$ = 0.044, p =

0.834) in caudal tail scutes of American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA……………………………………………...154

Figure 4.8: Comparison of Aroclor 1268 concentrations in American alligator (*Alligator mississppiensis*) caudal tail scutes from alligators that only use freshwater lagoons ("Island") and alligators that use marsh systems in Brunswick, Georgia (one-tailed t-test; t (3.047) = 1.478, p = 0.117)……………………………………155

Figure 4.9: Comparison of lead concentrations in American alligator (*Alligator mississppiensis*) caudal tail scutes from alligators that only use freshwater lagoons and alligators that use marsh systems in Brunswick, Georgia (one-tailed t-test; t (3.323) = 0.849, p = 0.226)…………………………………………………..156

Figure 4.10: Comparison of mercury concentrations in American alligator (*Alligator mississppiensis*) caudal tail scutes from alligators that only use freshwater lagoons and alligators that use marsh systems in Brunswick, Georgia (one-tailed t-test; t (7.945) = -1.039, p = 0.835)………………………………………………….157

Figure 4.11: Relationship between Aroclor 1268, lead, and mercury concentrations in caudal tail scutes and packed cell volume in the blood of American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA…………………..158

Figure 4.12: Relationship between Aroclor 1268, lead, and mercury concentrations in caudal tail scutes and glucose in the blood of American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA……………………………...159

Figure 4.13: Relationship between Aroclor 1268, lead, and mercury concentrations in caudal tail scutes and total proteins in the blood of American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA……………………………...160

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Site History

The Linden Chemicals and Plastic (LCP) site in Brunswick, Georgia has had a long history of industrial chemicals stored on its property. The site is approximately 550 acres in size, the majority being tidal marsh that borders the industrial facilities to the west and south (US EPA, 1996). The marsh system that is part of the LCP property is part of the Turtle/Brunswick River Estuary (TBRE). From 1919 to 1994, owners of the site performed operations that resulted in wastes being discharged into large holding pits and directly into the rivers of the tidal marsh (Kannan et al., 1997; Kannan et al., 1998). This resulted in the contamination of the dry upland soils, water, and aquatic sediments with heavy metals (mercury, lead, chromium, and zinc) and organic compounds (polychlorinated biphenyls [PCBs], polyaromatic hydrocarbons, and phenolic compounds; US EPA, 1996; Kannan et al., 1997). It is estimated that throughout the history of the site, over 37 tons of PCBs and 440 tons of mercury were released into the environment surrounding the industrial facilities (Kannan et al., 1998). The site was designated a Superfund site by the United States Environmental Protection Agency (EPA) and placed on the National Priorities List (NPL) in 1996. The state of Georgia designated the LCP Superfund site as the highest priority contaminated site in Georgia (US EPA, 1996). Initial testing in the marsh found that sediment samples collected adjacent to the Superfund site had PCB levels approximately 50 times higher than samples collected a relatively small distance away (500 meters; Kannan et al., 1997).

The LCP company was the only purchaser of Aroclor 1268 from the manufacturer in the southeastern United States, and only a limited amount of Aroclor 1268 was produced (Kannan et al., 1997; Maruya & Lee, 1998). The highly chlorinated and unique congener profile of Aroclor 1268 has allowed researchers to use it as an indicator of contamination associated with the LCP Superfund site (Kannan et al., 1997; Pulster et al., 2005). After the Superfund site was placed on the NPL, an initial study investigating the potential effects of PCBs on biota within the TBRE was conducted. The investigators examined a wide variety of organisms including invertebrates, fish, birds, and reptiles (Kannan et al., 1998). The researchers found that many species of animals within the TBRE had increased levels of PCBs, including Aroclor 1268, and these species served as good indicators for the exposure to contaminants associated with the LCP Superfund site. Further studies found that fish species inhabiting the TBRE had higher levels of Aroclor 1268 than fish captured from reference sites located 100 miles from Brunswick (Maruya & Lee, 1998). Researchers also found that while fish captured in the TBRE had significantly higher levels of Aroclor 1268, fish captured in the nearby cities of Savannah and Jacksonville still tested positive for Aroclor 1268 (Pulster et al., 2005). This may indicate that migratory fish species are transporting contaminants from the LCP Superfund site outside of the greater Brunswick area.

Recent studies have primarily focused on the effects of Aroclor 1268 on bottlenose dolphins (*Tursiops truncatus*). Sampling of dolphins in the TBRE found that dolphins residing in the TBRE had higher levels of PCBs than dolphins that were found stranded along the entire coast of Georgia (Pulster & Maruya, 2008; Pulster et al., 2009). Another study found that dolphins residing in the Brunswick area had higher PCBs and Aroclor 1268 than dolphins from a reference site (Sapelo Island National Estuarine Research Reserve) approximately 30 km northeast of the LCP site, however the Sapelo Island dolphins also had been exposed to Aroclor 1268 (Balmer et al., 2011). This study also found that dolphins sampled closer to the LCP Superfund site contained higher Aroclor 1268 levels than dolphins that were sampled further away from the site of contamination. Dolphins residing in the TBRE estuary were also found to have the highest total PCB levels of any cetacean tested (Balmer et al., 2011).

Some recent studies have also investigated the use of avian species as environmental indicators and analyzed the potential effects of contaminants from the LCP Superfund site on birds. Clapper rails (*Rallus longirostris*) that were year-round residents of the TRBE were found to bioaccumulate both mercury and Aroclor 1268 through the ingestion of marsh invertebrates at levels that could inhibit reproductive success (Cumbee et al., 2008). Additionally, a study analyzing the concentrations of mercury and Aroclor 1268 in least terns (*Sternula antillarum*) nesting along the Georgia coast found Aroclor 1268 profiles in birds at all sampling locations (Robinson et al., 2015). Robinson et al. (2015) found PCB level in least tern eggs greater than the concentration of PCBs associated with decreased hatch success (Becker et al., 1993; Harris et al., 1993; Hoffman et al., 1993). Additionally, mercury levels in the feathers and eggs of least terns that are associated with reproductive and kidney problems (Eisler, 1987; Robinson et al., 2015) The authors indicated that this suggests that Aroclor 1268 is being transported extensively along the Georgia coast via trophic transfer, and at levels that are potentially causing sub-lethal effects in least terns.

Past research has used the presence of Aroclor 1268 in wildlife species to indicate exposure to contaminants released from the LCP site (Kannan et al., 1997; Pulster et al., 2005). Initial studies indicated that due to the lipophilic properties of Aroclor 1268 and its inefficient transfer through the food web, the signature PCB of the LCP Superfund site may not be easily transported outside of the TBRE (Kannan et al., 1997; Kannan et al., 1998). However, more recent studies have suggested that Aroclor 1268 contamination from the LCP site is not limited to the TBRE and may be affecting wildlife at a much broader scale (Pulster et al., 2005; Pulster & Maruya, 2008; Pulster et al., 2009; Balmer et al., 2011; Robinson et al., 2015).

Contaminants of Concern

Polychlorinated biphenyls (PCBs) are synthetic organic chemicals that were used in industrial processes and incorporated into electrical and hydraulic equipment, plastics, paints, rubber, pigments and dyes (Fein et al., 1983). The United States banned the use of PCBs in 1977, however their resistance to biodegradation and ability to persist in the environment by bioaccumulating through the food chain have resulted in continued exposure risk to both humans and wildlife (Choi et al., 2006; Kjellerup et al., 2012). Polychlorinated biphenyls concentrate in aquatic environments from stormwater runoff of industrial areas where PCBs are deposited and can become sequestered in soils and sediments (Kannan et al., 1997; Cobb et al., 2002). Polychlorinated biphenyls have been shown to cause suppression of immune function, developmental abnormalities, liver damage, reproductive toxicity, endocrine disruption, neurological disruption, and population declines in upper trophic level organisms (Safe, 1993; Fox, 2001; Olenycz et al., 2015). These toxins have been linked to serious health effects in humans and wildlife even at low exposure levels (D'Ilio et al., 2011). In fish, PCBs have been linked to reproductive disfunction, endocrine disruption, and developmental abnormalities have been observed (Thomas, 1989; Elonen et al., 1998; Johnson et al., 1998). Similarly, decreased reproductive success and endocrine disruption has also been found in birds (Brunström et al., 1990; Bowerman et al., 1994). Mammals exposed to PCBs exhibit endocrine disruption, increased incidences of cancer, and decreased reproductive success (McNulty, 1985; Brouwer et al., 1989; Wiig et al., 1998) Polychlorinated biphenyls have been linked to reproductive disfunction, neurological problems, and increased incidences of cancer in humans (Morrisey & Schwetz, 1989; Jaacks & Staimez, 2015; Pérez-Maldonado et al., 2017).

While persistent organic pollutants can often be traced back to a point source of the pollution, heavy metals have many potential routes into the environment and can come from either point sources or non-point sources of pollution. Mercury is released into the environment by a variety of human causes including the burning of waste, peat, and fossil fuels, industrial activities (e.g., paper and pulp mills, mining, chloroalkali plants), and human crematoria (Reddy and Hayes, 1989; WHO, 1989; Hand and Friedman, 1990). Once released into the air, mercury can be transported to various ecosystems via atmospheric deposition and water currents (Yanochko et al., 1997). Direct release of mercury-containing effluents into aquatic systems, stormwater runoff to rivers that contains atmospheric mercury deposition that accumulates on terrestrial environments, and in situ microbial production of methylmercury are also ways that mercury can enter aquatic ecosystems (Obrist et al., 2018). The fact that mercury is so ubiquitous in the environment has led to the assertion that mercury contamination is the greatest environmental threat in the southeastern United States (Facemire et al., 1995). Lead also can be released into the environment through the use of lead bullets, smelting factories, or leaded gasoline (Camus et al., 1998). Heavy metals, such as lead and mercury, are known to bioaccumulate in organisms that occupy high levels on the food chain (Campbell et al., 2010).

Reptiles as Environmental Indicators

Long-lived vertebrates serve as ideal bioindicators of the effects of environmental contaminants such as PCBs and heavy metals (Rowe, 2008). Animals that have high site fidelity, long life spans, and delayed sexual maturity are optimal for studying the effects of contaminants on an ecosystem because they are resistant to periodic fluctuations in ecosystem conditions (Stearns, 1976). Reptiles have long lifespans and high site fidelity, making them ideally suited as bioindicators of contaminants (Meyers-Schöne & Walton, 1994; Crain & Guillette, 1998). Reptiles are also known to bioaccumulate persistent organic pollutants (POPs), such as PCBs, throughout their lifetime (Golet & Haines, 2001; Day et al., 2005). For this study, three species of reptiles that are long-lived animals were selected due to the fact that they exhibited the traits that are ideal for bioindicator species and varied in their dependence on the marsh ecosystem. Species that use the marsh ecosystem to varying extents should theoretically be exposed to contaminants within the TBRE at different rates. The three species selected for our study were diamondback terrapins (*Malaclemys terrpain*), loggerhead sea turtles (*Caretta caretta*), and American alligators (*Alligator mississippiensis*).

Study Species

The diamondback terrapin is a small emydid turtle that is found along the Atlantic coast from Massachusetts to Florida and along the Gulf coast from Florida to Texas. Diamondback terrapins are the only turtle in the United States to live in brackish water, inhabiting estuaries and salt marsh ecosystems. Carnivorous turtles, such as terrapins, are particularly useful in studying contaminants due to their upper trophic level status (Green et al., 2010). Studies comparing mercury contamination in diamondback terrapins and their prey (salt marsh periwinkle snails [*Littoraria irrorate*]) have found that terrapins are useful as a bioindicator species for mercury pollution in estuarine ecosystems, including the TBRE (Blanvillain et al., 2007). Additionally, other studies have shown the terrapins captured in close proximity to industrialized sites contained higher PCB exposure than terrapins from reference sites, showing that terrapins are good indicators for PCB contamination (Basile et al., 2011). Once abundant throughout their range, terrapin populations have declined from historic levels, mostly due to anthropogenic causes. Threats to terrapin populations include habitat degradation, mortality from human-subsidized predators (e.g., raccoons), mortality in crab pots, and road mortality (Wood & Herlands, 1997; Gibbons et al., 2001; Dorcas et al., 2007; Szerlag-Egger & McRobert, 2007; Grosse et al., 2011; Crawford et al., 2014). Although diamondback terrapins have no federal protection status, in Georgia they are listed as a species of "conservation concern" (GA DNR, 2009). Even though terrapin populations have been recovering in recent years, some populations around the Brunswick area may still be experiencing population decline (Crawford et al., 2014).

Loggerhead sea turtles occupy higher trophic levels than other sea turtle species and are therefore more likely to experience negative effects of bioaccumulative toxins such as PCBs and heavy metals due to their carnivorous nature (Maffucci et al., 2005; Perrault et al., 2017; Casini et al., 2018). Loggerheads feed primarily on large, benthic invertebrates such as blue crabs (*Callinectes sapidus*) and whelks (*Busycon* spp.; Wallace et al., 2009). Blue crabs are also commonly consumed by people, suggesting that if sea turtles are exposed to contaminants from prey species, humans that consume the same prey species may also be exposed. Additionally, like terrapins and alligators, loggerheads are long-lived animals that sometimes take up to 45 years to reach sexual maturity (Scott et al., 2012). These life history traits make them ideal bioindicator species of marine pollution and ecosystem health (Aguirre & Lutz, 2004; Keller et al., 2004; Andreani et al., 2008). Loggerheads are a protected species listed as federally threatened pursuant to the Endangered Species Act and endangered in the state of Georgia (GA DNR, 2011; US FWS, 2019*a*). Additionally, loggerhead sea turtles are listed as vulnerable on the International Union for Conservation of Nature (IUCN) Red List (Casale & Tucker, 2017). The main threats to the loggerhead identified by the IUCN are anthropogenic alterations to critical habitat, bycatch by the commercial fishing industry, invasive pathogens, marine pollution, and climate change. Evaluating the effects of pollution and contaminants on marine turtles such as loggerheads has recently become a global research priority (Hamann et al., 2010; Rees et al., 2016). Loggerhead sea turtles rely on nearshore areas during developmental life stages and as foraging grounds (Morreale & Standora, 2005; Scott, 2006). This proximity to shores increases the likelihood that loggerheads are exposed to pollutants from industrial areas (Day et al., 2005).

American alligators are model organisms for environmental studies due to their increased life spans and status as upper-level predators in ecosystems and have been used as indicator species of ecological health in previous species (Mazzotti et al., 2008; Eversole et al., 2015). Alligators also take an extended amount of time to reach sexual maturity compared to most vertebrates, making them particularly vulnerable to the negative effects of bioaccumulative toxins (Joanen & McNease, 1989; Dalrymple, 1996; Lance, 2003). Some alligators have taken over 15 years to reach sexual maturity (Wilkinson et al., 2016). Additionally, effects on alligator health attributed to pollutants have been correlated with similar effects on the health of humans that reside near contaminated areas (Guillette & Guillette, 1996). Alligators are federally listed as threatened due to similarity of appearance to other threatened species (US FWS, 2019*b*). Once an endangered species, alligator populations have recovered since being delisted and are a game species in the state of Georgia. Alligators are considered a keystone species, creating habitat for birds, reptiles, and amphibians that these animals depend on to survive (Kushlan & Kushlan, 1980; Kahn & Tansel, 2000; Campbell & Mazzotti, 2004; Palmer & Mazzotti; 2004). Additionally, alligators exert top-down control over prey species and have the ability to link ecosystems through trophic interactions (de Roos et al., 1998; McCann et al., 2005; Subalusky et al., 2009; Rosenblatt & Heithaus, 2011). Coastal alligators in Georgia that have been tracked using Global Positioning System telemetry have been found to make an average of 2.26 trips into marsh ecosystems each month (Nifong and Silliman, 2017). Nifong and Silliman (2017) found that these ventures into the marsh last from a period of a few hours to over a month at a time. Presumably, the reason alligators move from freshwater into brackish water ecosystems is for foraging opportunities. Coastal alligators forage on prey species that are commonly harvested commercially for human consumption, such as blue crabs (*Callinectes sapidus*), shrimp (*Palaeomonetes* sp.) mullet (*Mugil cephalus*), and silver perch (*Bairdiella chrysoura*) (Nifong and Silliman, 2013). If alligators are exposed to environmental contaminants by eating contaminated prey, humans that also eat these prey species may also be exposed to the same contaminants. Additionally, alligators are harvested throughout their range for human consumption in managed hunts, potentially exposing humans that consume them to bioaccumulated toxins.

Effects of Contaminants of Concern on Reptiles

Environmental pollution poses a significant threat to human and wildlife health, particularly in marine environments (Naser, 2013). Persistent organic pollutants and heavy metals released from anthropogenic sources tend to accumulate in marine environments (Nriagu & Pacyna, 1988; Newman & Unger, 2003; Lamborg et al., 2014). Pollutants accumulate in marine sediments where they are exposed to benthic organisms. These contaminants can then bioaccumulate and biomagnify through the food chain as upper level predators consume benthic prey species (Caurant et al., 1999; Anan et al., 2001; Storelli et al., 2005; Camacho et al., 2013).

Pollutants such as PCBs and heavy metals have been found to negatively affect reptiles such as turtles and alligators. Polychlorinated biphenyls have been found to impair growth and survival rates of turtles, reverse temperature-dependent sex determination, and alter immune system function (Bergeron et al., 1994; Keller et al., 2004; Eisenrich et al., 2009). Exposure to PCBs has been linked to reduced growth, altered metabolic rate, and decreased bone density in diamondback terrapins (Holliday et al., 2009; Holliday & Holliday, 2012). Loggerheads that have increased levels of PCBs in blood have experienced decreased immune function and altered homeostasis of protein and carbohydrate levels in their blood (Keller et al., 2004; Keller et al., 2006; Camacho et al., 2013; Rousselet et al., 2017). Leatherback sea turtles (*Dermochelys coriacea*) have been shown to maternally transfer PCBs to offspring through the laying of eggs, and increased levels of PCBs in eggs have been linked to decreased hatch success (Stewart et al., 2011; Andrés et al., 2016). Polychlorinated biphenyls can cause endocrine disruption in alligators by acting as hormonal antagonists or altering the synthesis and degradation of hormones (Crain & Guillette, 1997). Additionally, juvenile male alligators exposed to PCBs have reduced testosterone levels when compared to juvenile male alligators that have no contaminant exposure (Guillette et al., 1999). Female alligators have been found to maternally transfer PCBs to their offspring via laying eggs, exposing the embryos to harmful levels of PCBs (Cobb et al., 2002). Additionally, embryonic alligators have exhibited development defects before hatching and can have decreased hatch success (Guillette et al., 1995; Cobb et al., 1997).

Heavy metal exposure can have similar negative effects on reptiles that are exposed to them. Mercury exposure has been linked to decreased hatch success in turtles and the alteration of gene expression in turtles (Hopkins et al., 2013; Meyers-Schöne et al., 2013). Sea turtles that have been exposed to mercury have also been shown to have decreased white blood cell counts and suppression of other immune system functions (Day et al., 2007; Komoroske et al., 2011). Additionally, sea turtles that have high levels of heavy metals have been linked with increased occurrence of fibropapillomatosis tumors (da Silva et al., 2016; Perrault et al., 2017). Heavy metals, including mercury and lead, have been suspected of causing sub-lethal effects in alligators, including altering sex steroid levels in exposed alligator populations (Guillette et al., 1999). Mercury has been associated with decreased health parameters, such as lower body condition indices (BMI) in American alligators (Nilsen et al., 2017). While heavy metal exposure typically does not directly result in mortality events, one alligator that was found dead at the Savannah River Site in South Carolina contained the highest levels of mercury recorded in any wildlife species, at levels above what would be needed to cause heavy metal toxicity (Brisbin et al., 1998; Campbell, 2003).

Thesis Objectives

The overall objective of my thesis is to assess the presence and spatial distribution of contaminant associated with the LCP Chemicals Superfund site among three species of aquatic reptiles found in Brunswick, Georgia. The majority of recent research surrounding the exposure of wildlife to contaminants from the LCP site has revolved around avian and marine mammal species (e.g., Cumbee et al., 2008; Pulster & Maruya, 2008; Pulster et al., 2009; Balmer et al., 2011; Robinson et al., 2015). However, there are indications that PCBs and heavy metals associated with the LCP site may be affecting reptilian species as well. Loggerhead sea turtles sampled along the Georgia coast have mercury levels high enough to potentially cause sublethal effects (Day et al., 2005; Day et al., 2007). Furthermore, loggerhead sea turtles sampled along the Georgia coast have tested positive for Aroclor 1268, even though they were captured outside of the Brunswick area (Day et al., 2005). This means that even though loggerheads are not year-round residents of the Brunswick area, they may be afflicted by the contaminants associated with the LCP Chemicals Superfund site. In addition to loggerheads, terrapins have also been found to have exposure to contaminants from the LCP site including Aroclor 1268 and mercury (Kannan et al., 1998; Blanvillain et al., 2007).

The three study species (diamondback terrapins, loggerhead sea turtles, and American alligators) are reptiles that are either protected species or have conservation concern regarding their population status. The three species are all aquatic in nature but represent three different aquatic systems. While they all use the marsh ecosystem to some extent, terrapins are a primarily estuarine species, loggerheads are primarily a marine species, and alligators are primarily a freshwater species. Additionally, they all have different levels of movement potential. Terrapins have high site fidelity with small home ranges compared to alligators and sea turtles, alligators have larger home ranges but still primarily stay within the greater Brunswick area, and loggerhead sea turtles are more regionally distributed. Additionally, loggerhead sea turtles and alligators feed on prey species in the marsh that are harvested and consumed by humans. If these animals are accumulating environmental contaminants as a result of ingesting these prey items, it is possible humans are as well. Furthermore, alligators in the Brunswick area are harvested for human consumption. If these animals have high levels of PCBs or heavy metal in their tissues, humans that consume them may be at risk. This project is an initial effort to assess the status of exposure of aquatic reptiles within the TBRE that are associated with the LCP Chemicals Superfund site. Depending on levels found within these species, further studies may be needed to assess the potential negative endpoints of these contaminants and the implications for conservation or further human health implications.

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CHAPTER 2

SPATIAL DISTRIBUTION OF POLYCHLORINATED BIPEHNYLS AND MERCURY FROM A SUPERFUND SITE WITHIN A POPULATION OF DIAMONDBACK TERRAPINS IN BRUNSWICK, GEORIGA[1]

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Abstract

Persistent pollutants such as heavy metals and polychlorinated biphenyls pose a large risk to both human and wildlife health. These contaminants resist natural degradation, are mobile in the environment through the food chain, and bioaccumulate in upper level predators. Brunswick, Georgia is home to a heavily contaminated EPA Superfund site with excessive levels of organic compounds and heavy metals, including mercury and Aroclor 1268, a rare, highly-chlorinated polychlorinated biphenyl only used at this specific location in the southeastern United States. Diamondback terrapins are an estuarine species of semi-aquatic turtle that has suffered decreases in numbers throughout its range, primarily to anthropogenic causes. Livers of fatally injured terrapins from nearby Jekyll Island were analyzed for Aroclor 1268 and mercury to determine the potential threat to terrapins within the Brunswick estuary. We also analyzed how concentrations of Aroclor 1268 and mercury differed between two populations separated by a man-made causeway and how concentrations differed with regards to distance from the Superfund site. Aroclor 1268 concentrations averaged 3.009 ± 0.246 mg/kg wet weight and mercury concentrations averaged 0.613 ± 0.092 mg/kg wet weight. Terrapins on the side of the causeway closer to the Superfund site had higher levels of mercury, but not Aroclor 1268. Additionally, we found that terrapins closer to the Superfund site had higher levels of Aroclor 1268 in their livers than terrapins captured further from the site. Aroclor 1268 concentrations in terrapin livers for this study were higher than Aroclor 1268 concentrations in terrapin livers tested directly adjacent to the site immediately after the Superfund designation was accorded by EPA in 1996. Increased concentrations at a site further from the original source of contamination could be indicative of contaminants being dispersed through the marsh ecosystem.

Introduction

Environmental pollution is a major concern for both human and wildlife health. Persistent pollutants such as polychlorinated biphenyls (PCBs) and heavy metals are a threat to ecosystems due to their hydrophobic nature and stable chemical structure, allowing them to persist and move throughout the environment without degradation (Kjellerup et al., 2012). PCBs have been linked to serious health effects in wildlife, even at low exposure levels (D'Ilio et al., 2011). Polychlorinated biphenyls can cause sublethal effects, such as altered hormone levels (Crain & Guillette, 1997). Reptiles exposed to PCBs have also been shown to maternally transfer PCBs to their offspring, causing developmental defects and decreased hatch success (Guillette et al., 1995; Cobb et al., 1997). In turtles, PCB exposure has been found to impair growth and survival rates (Eisenrich et al., 2009), reverse temperature-dependent sex determination (Bergeron et al., 1994), and alter immune function (Keller et al., 2004). Loggerheads that have increased levels of PCBs also display decreased immune function (Keller et al., 2004; Keller et al., 2006; Camacho et al., 2013; Rousselet et al., 2017).

Although less studied, there are also negative effects on turtles due to mercury exposure. Mercury has been hypothesized to cause sublethal effects in reptiles, such as altering sex steroid levels in exposed individuals (Guillette et al., 1999). Mercury exposure has been linked to decreased hatch success (Hopkins et al., 2013) and increased number of breaks in DNA strands of mercury-exposed turtles (Meyers-Schöne et al., 2013). Sea turtles that have increased mercury levels have suppressed immune function, such as decreased white blood cell counts (Day et al., 2007; Komoroske et al., 2011). The majority of studies investigating the effects of environmental contaminants have focused on clear negative effects, such as mortality events. There have been far fewer studies that have investigated the subtle negative effects of contaminants such as reduced reproduction and immune disfunction (Guillette et al., 1999).

Brunswick, Georgia is the location of a chloro-alkali plant that has operated since 1919 and was most recently owned by Linden Chemicals and Plastic (LCP Chemicals). This site was designated an Environmental Protection Agency (EPA) Superfund site in 1996 due to soil, groundwater, and sediment contaminated by heavy metals and organic contaminants (US EPA, 1996). Further investigation found high levels of mercury, lead, chromium, zinc, PCBs, and polycyclic aromatic hydrocarbons (PAHs) in the soil and waters adjacent to the Superfund site (Kannan et al., 1997; Maruya & Lee, 1998). Aroclor 1268, a rare, highly-chlorinated PCB was applied to electrical equipment used in chemical processing (Kannan et al., 1998). This specific PCB was only manufactured by one company that produced a limit amount of the chemical, and LCP Chemicals was the only user of this congener mixture in the southeastern U.S. (Kannan et al., 1997; Maruya & Lee, 1998). In addition to the 37 tons of PCBs that were released into the environment from the LCP site, an estimated 440 tons of mercury were also released (Kannan et al., 1998). However, while Aroclor 1268 is considered a point-source pollutant, mercury has other ways of entering the ecosystem. Other sources of mercury in aquatic environments include direct release of mercury-containing effluents, river runoff that contains atmospheric mercury deposition that accumulated on terrestrial environments, direct atmospheric mercury deposition into water bodies, and in situ microbial production of methylmercury (Obrist et al., 2018).

The amounts of heavy metals and PCBs found in the immediate vicinity of the LCP Chemicals site were high enough to potentially cause harm to biota inhabiting the Turtle/Brunswick River Estuary (TBRE) adjacent to the marsh surrounding the Superfund site (Figure 2.1). Initial studies mainly investigated the distribution of Aroclor 1268 and mercury among invertebrates, fish, and birds (Kannan et al., 1998; Maruya & Lee, 1998). However, more recent studies on the effects of these contaminants included bottlenose dolphins (*Tursiops truncatus*) (e.g., Pulster et al., 2009; Balmer et al., 2011; Wirth et al., 2014). The uniqueness of the Aroclor 1268 congener profile and the fact that LCP Chemicals is the only known source of the PCB in the region has allowed scientists to determine if organisms have been exposed to contamination from the LCP Chemicals site (e.g., Pulster et al., 2005; Pulster & Maruya, 2008; Cumbee et al., 2008; Pulster et al., 2009; Balmer et al., 2011; Robinson et al., 2015). Studies have been performed on organisms within the confines of the TBRE ecosystem (Cumbee et al., 2008) and on organisms on a broader regional scale (Balmer et al., 2011; Robinson et al., 2015).

Turtles are ideally suited as bioindicators of contaminants due to their long lifespans, high site fidelity, and status as upper-level consumers in the ecosystem (Meyers-Schöne & Walton, 1994; Crain & Guillette, 1998). Wild terrapins have been known to live 30 years or more (Brennessel, 2006). Turtles have also been shown to bioaccumulate POPs throughout their lifetime (Golet & Haines, 2001; Day et al., 2005). The diamondback terrapin (*Malaclemys terrapin*) is a small emydid turtle found along the Atlantic coast of the United States from Massachusetts to Florida and along the Gulf coast from Florida to Texas. Terrapins are the only turtle in North America that exclusively inhabit salt marshes, tidal rivers, and mangrove swamps throughout their range. Through capture-mark-recapture studies, scientists have found that individual terrapins have high site fidelity and are rarely found outside of the tidal creek where they were originally captured (Gibbons et al., 2001). Terrapins forage on invertebrates found in the salt marsh ecosystem including periwinkle snails (*Littoraria irrorata*), fiddler crabs (*Uca pugnax*), marsh crabs (*Sesarma reticulatum*), blue crabs (*Calinectes sapidus*), barnacles (*Balanus* spp.), and clams (*Polynesoda caroliniana*) (Tucker et al., 1995). Studies comparing mercury contamination in terrapins and their prey species have shown they can be used as a bioindicator species for mercury contamination in estuarine ecosystems (Blanvillain et al., 2007). In another study, terrapins closer to industrialized sites contained higher levels of PCBs than terrapins from reference sites, showing that terrapins are also good indicator species for PCB contamination (Basile et al., 2011).

Terrapin populations have declined across their range due to a variety of anthropogenic threats. Habitat fragmentation and degradation, overharvesting, drowning in abandoned crab pots, and mortalities from cars and boats have driven terrapins to near extinction in parts of their range (Conant 1964; Gibbons et al., 2001). Exposure to contaminants can potentially have negative effects on terrapin populations but is studied far less often than other threats (Basile et al., 2011). Kannan et al. (1998) examined the levels of Aroclor 1268 in terrapin livers as part of an initial investigation into what animal species were being exposed to contaminants from the LCP site. Terrapins found in creeks next to the Superfund site contained elevated levels of Aroclor 1268 (Kannan et al., 1998). An additional study compared mercury levels in blood and scutes from terrapins captured in the TBRE, however it did not look at the levels in the livers of the terrapins captured (Blanvillain et al., 2007). Scute samples collected from terrapins inhabiting the TBRE were found to have mercury concentrations 12 times higher than samples from terrapins in South Carolina (Blanvillain et al., 2007).

The objective of our study is to analyze diamondback terrapin livers for exposure to Aroclor 1268 and mercury in order to determine if terrapins in the TBRE are still accumulating toxins from the LCP Chemicals site and if there are any spatial differences of these contaminants within the population surrounding Jekyll Island, Georgia. While the LCP site contains a variety of contaminants of concern identified by the EPA, Aroclor 1268 and mercury were two of the contaminants that were released in the largest amounts. Additionally, Aroclor 1268 has been used to identify exposure to contaminants from the LCP site in previous studies (e.g., Pulster et al., 2005; Pulster & Maruya, 2008; Cumbee et al., 2008; Pulster et al., 2009; Balmer et al., 2011; Robinson et al., 2015). Terrapins around Jekyll Island are often hit by vehicles and killed on the causeway connecting the island to the mainland; by taking liver samples from these injured or dead animals, we did not have to sacrifice live, healthy individuals.

Methods

Study site

The study site for this project is Jekyll Island, Georgia, USA (Figure 2.1). Jekyll Island is a 2,238-ha barrier island located along the southeastern coast of Georgia. The island is located approximately 12 kilometers southeast of the city of Brunswick and is managed by the Jekyll Island Authority. Jekyll Island is connected to the mainland by the Downing-Musgrove Causeway (DMC), an 8.7-kilometer roadway that bisects an intertidal marsh ecosystem that is mainly comprised of cordgrass (*Spartina* spp.). This marsh ecosystem has a network of intertidal creeks that experience tides of 2-3 meters in amplitude, bringing the water to within 20 meters of the roadway (Grosse et al., 2011; Crawford et al., 2014*a*). The saltmarsh on the northern side of the DMC represents the southern boundary of the fish consumption advisory zone implemented by the Georgia Department of Natural Resources Environmental Protection Division due to PCB and mercury contamination from the LCP Chemicals Superfund site (GA DNR, 2018).

Roadways that fragment saltmarsh habitat containing terrapins attract female terrapins by providing elevated land to lay eggs (Butler et al., 2006; Szerlag-Egger & McRobert, 2007). Traffic along the DMC peaks from May to July, corresponding with terrapin nesting periods (Crawford et al., 2014*a*). This results in 100-400 terrapins being killed each summer along the DMC (Crawford et al., 2014*b*). The mortality rate for female terrapins is suggested to be severe enough to cause moderate to high population declines for the terrapin population around the DMC (Crawford et al., 2014*a*).

Jekyll Island is also home to the Jekyll Island Authority's Georgia Sea Turtle Center (GSTC), a rehabilitation facility specializing in the interconnection of wildlife rehabilitation, education, research, and mitigation strategies. Beginning in 2007 the GSTC began patrolling the DMC for terrapins between May and July in order to lessen roadway mortalities of terrapins and treat injured terrapins that had been struck by cars. Terrapins that are struck by cars on the causeway or otherwise killed are collected and brought back to the GSTC. Additionally, the GSTC participates in annual capture-mark-recapture surveys with researches from the University of Georgia (UGA). Surveys using seine nets demonstrate a higher number of terrapins captured in creeks south of the DMC compared to creeks north of the DMC.

Sample collection

Terrapins that had been hit by vehicles and injured or were already dead were collected and brought back to the GSTC. Terrapins that were too extensively injured for rehabilitation were humanely euthanized with sedatives. Individual turtles that died during rehabilitation or were euthanized were necropsied and liver samples were collected. Liver samples were placed into Whirl-Pak® sample bags (Nasco Fort Atkinson, WI, USA) and stored in an ultra-low freezer (-80°C) until contaminant analysis could be performed. Samples were frozen for no more than three years before all analyses were completed.

Contaminant analysis

Mercury analysis was performed using a Direct Mercury Analyzer (DMA-80: Milestone, Shelton, CT, USA). Liver samples were thawed completely and then ~0.01 grams wet weight of sample was measured. Weighed samples were then placed in individual quartz sample boats (Milestone, Shelton, CT, USA) and run through the DMA-80. The DMA-80 analyzes mercury by thermally decomposing the samples and using carrier gas to transport the mercury and combustion products into a catalyst chamber. A gold amalgamator captures the mercury species before releasing the mercury into the spectrophotometer's optical path. After every ten samples analyzed, both a system blank and system standard (DORM-3 Fish Protein; National Research Council Canada, Ottawa, Canada) were run to ensure proper calibration of the machine. Mercury concentrations are reported as means ± 1 standard error, and all concentrations are reported as wet weight.

For PCB analysis, liver samples were weighed to determine wet weight and then freeze dried. Dried liver samples were cut up to increase surface area and transferred into 50 mL polypropylene centrifuge tubes (Corning, Corning, NY, USA). Samples were treated with 10 mL of 1:1 solution of acetone and dichloromethane (Fisher Scientific, Waltham, MA, USA). The centrifuge tubes were placed in an ultrasonic bath and sonicated for 60 minutes. After sonication, tubes were centrifuged at 4,000 RPM for 20 minutes until the solution was clear. The clear supernatant layer was decanted into glass evaporation tubes. The liquid extraction process was repeated once more using another 10 mL of the acetone dichloromethane solution and added to the evaporation tubes. The combined extracts were carefully evaporated to near dryness at 60°C using a TurboVap® LV concentration workstation (Caliper Life Sciences, Hopkinton, MA, USA). After evaporation, 1 mL of methanol (VWR Chemicals, Radnor, PA, USA) was added to the evaporation tube, which was then vortexed for approximately 60 seconds. The methanol was carefully evaporated to near dryness under the same conditions. Afterwards, 0.8 mL of methanol was added to the evaporation tube and vortexed for approximately 60 seconds. The liquid was transferred into a 1 mL volumetric tube and brought to a final volume of one mL using methanol. This liquid was transferred into 2 mL glass crimp vials (Agilent Technologies, Santa Clara, CA, USA). The samples were analyzed on a Varian 4000 GC/MS (Varian Inc., Walnut Creek, CA, USA) and compared to an Aroclor 1268 standard (AccuStandard, New Haven, CT, USA). After every 10 samples were analyzed, an Aroclor 1268 standard and methanol blank were both run through the GC/MS to ensure quality control and assurance. The minimum detection limits for Aroclor 1268 concentrations by GC/MS was 0.005 mg/kg. Aroclor 1268 concentrations are reported as means ± 1 standard error, and all concentrations are reported as wet weight.

Statistics

When turtles were encountered on the DMC, they were classified as either being from the north side of the causeway or the south side of the causeway. If the turtle had not been observed on the road prior to being hit and no indications of the side it originated from were presented, it was classified as “unknown”. Distances of the terrapins from the LCP Chemicals site were calculated in ArcMap 10.5.1 (ESRI, Redlands, CA, USA). GPS points from the terrapin encounters were imported into ArcMap and the straight-line distance from the LCP Chemicals site (31.189440 N, 81.508330 S; US EPA, 2002) was calculated using the Measure tool.

Statistical tests were performed using program R 3.4.1 (R Core Team 2019). All data were tested for normality using the Shapiro-Wilk test. If data were not normally distributed, data were log-transformed prior to analysis. Contaminant levels of terrapins from the north population were tested against the south population using one-tailed Welch’s t-test. The t-test was one-tailed because one could assume terrapins from the north side should have higher contaminant levels than terrapins from the south side given that the northern terrapins are within the boundaries of the fishing advisory set by the Georgia DNR EPD. The relationships between distance from the LCP Chemicals site on Aroclor 1268 and mercury concentrations in terrapin livers were tested using a simple linear regression.

Results

Summary statistics

During May - July 2014, 31 female terrapins hit by automobiles were collected either dead on the DMC, or severely injured and euthanized (Figure 2.1). There were 7 terrapins originating from the north side of the DMC, 16 terrapins originating from the south, and 8 terrapins of unknown origin. GPS points were collected on 30 of the terrapins. The lone terrapin without an associated GPS point was brought to the GSTC by a citizen that could not provide information as to the location they found the terrapin. Terrapins were collected an average distance of 11.424 ± 0.347 km from the LCP Superfund site (Mean ± 1 SE; range of 9.318-15.313).

Population comparison

The average level of Aroclor 1268 in livers from terrapins originating from the north side (n=7) of the DMC was 3.565 ± 0.517 mg/kg wet weight (Mean ± 1 SE; range of 1.033-5.233 mg/kg). Average level of Aroclor 1268 in livers from terrapins from the south side (n=16) of the DMC was 2.818 ± 1.318 mg/kg wet weight (0.770-5.290 mg/kg). There was no significant difference in Aroclor 1268 concentrations between terrapins from north of the DMC and terrapins south of the DMC (t (11.133) = 1.218, p = 0.124; Figure 2.2), Mercury levels in livers from north of the DMC was 0.985 ± 0.285 mg/kg wet weight (0.286-2.311 mg/kg). Concentration of mercury in the livers of terrapins south of the DMC was 0.456 ± 0.049 mg/kg wet weight (0.142-1.028 mg/kg). There was a significant difference in mercury levels (t (7.621) = 1.876, p = 0.049; Figure 2.3).

Effect of distance on contaminant levels

The average level of Aroclor 1268 in liver tissue from the terrapins collected from the DMC was 3.009 ± 0.246 mg/kg wet weight (0.770-5.290 mg/kg). There was a significant negative correlation between distance from the Superfund site and Aroclor 1268 concentration ($F_{1,28} = 4.553$, $p = 0.042$; Figure 2.4). Mercury concentration in the livers of the terrapins was 0.613 ± 0.092 mg/kg wet weight (0.142-2.311 mg/kg). While there was a negative trend associated with the effects of distance from the LCP Chemicals site on mercury, this effect was not significant ($F_{1,28} = 1.402$, $p = 0.246$; Figure 2.5).

Discussion

There is still intense demand from Asian countries for turtle meat, resulting in the harvest of many species of turtles in the United States for exportation, including terrapins (Schaffer et al., 2008). The EPA and the Food and Drug Administration (FDA) are responsible for issuing recommendations regarding how high mercury levels can be in food to be considered safe for human consumption. However, there are no specific restrictions issued by the EPA or FDA regarding the limits of mercury that occur in turtle meat or turtle livers (Burger, 2002). The FDA and EPA recommend that fish meat be less than 0.46 mg/kg wet weight in mercury to be safe for human consumption (US EPA, 2018). From the terrapins sampled around DMC, 46.7% (14) of the livers exceeded the threshold recommended by the FDA and EPA. However, livers usually accumulate higher levels of mercury than corresponding muscle tissue. One study found mercury concentrations in terrapin livers to be over six times higher than mercury concentrations in muscle (Burger, 2002). It is therefore unlikely that terrapins in the TBRE contain mercury levels in muscle tissue that exceeded the threshold put forth by the EPA and

FDA. Furthermore, terrapin livers are not usually consumed by humans, reducing the potential threat to humans consuming terrapins from the TBRE (Burger, 2002).

Even if the terrapins in our study didn't exceed mercury levels set by the regulating agencies, the levels could still be high enough to negatively affect the health of the terrapins. A study analyzing mercury levels in liver and blood from three species of turtle captured in Brazil found liver concentrations to be as much as 20 to 100 times higher than blood concentrations (Eggins et al., 2015). The no observed effects concentration (NOEC) for mercury concentration in blood for loggerhead sea turtles (*Caretta caretta*) was found to be 0.05 mg/kg, above which individuals started exhibiting immunosuppression (Day et al., 2007). If we convert our liver mercury concentrations to hypothetical blood mercury levels using the results from the Eggins et al. (2015) study, between 0 and 16.7% (n=0-5) of the terrapins would exceed the NOEC for mercury concentration in blood. This rough translation could potentially mean that some terrapins in our study could have blood levels exceeding the NOEC in loggerhead sea turtle and may be suffering from decreased immune function. A study looking at mercury levels in the blood of terrapins captured in a tidal creek close to the LCP Chemicals site found increased levels compared to reference sites, with an average concentration of 0.746 ± 0.066 mg/kg (Blanvillain et al., 2007). This provides further support that terrapins around the DMC may have blood mercury levels higher than the NOEC.

The FDA recommends a limit of 0.2-3.0 mg/kg of total PCBs for all food types, with a 2.0 mg/kg threshold for fish, in order to be considered safe for human consumption (ATSDR, 2016). Based on the general threshold, 46.7-100% (14-30) of the terrapin livers sampled exceeded the FDA threshold depending on the conservative or the liberal estimate was used. Based on the FDA's PCB threshold in fish, 76.7% (23) of the terrapin livers had excessive amounts of PCBs. Livers typically accumulate PCBs at higher levels than muscle (Corsolini et al., 2000; Storelli et al., 2007; D'Ilio et al., 2011). Polychlorinated biphenyl concentrations in livers are approximately 11.5 times higher in turtles compared to muscle (D'Ilio et al., 2011). After converting our liver concentrations to rough muscle concentrations, 76.7% (n=23) of terrapins exceed the lowest limit of 0.2 mg/kg, with no limits exceeding the 2.0 mg/kg or 3.0 mg/kg limits.

Even if consuming contaminated terrapins does not pose a significant human health risk, these terrapins may still be being exposed to contaminants at levels high enough to cause sublethal effects. Most of the existing research on the effects of PCBs on semi-aquatic turtles revolves around egg development in snapping turtles (*Chelydra serpentina*) and slider turtles (*Trachemys scripta*; e.g., Gale et al., 2002; de Solla et al., 2008; Schnars et al., 2011; Matsumoto et al., 2014). None of these studies looked at other tissue concentrations in the affected turtles outside of the egg levels. The effects of PCB exposure on terrapins has mostly been limited to laboratory settings (e.g., Ford and Holliday, 2005; Holliday et al., 2009; Holliday and Holliday, 2012). These studies found intracoelomic injections of PCB 126 at a concentration of 20 mg/kg caused reduced growth, decreased hematocrit, decreased bone density, and altered metabolic rates in experimental terrapins.

Our study found a positive relationship between distance from the LCP site and Aroclor 1268 levels. Interestingly, our study found higher levels of PCBs in terrapin livers around the DMC than a previous study that analyzed terrapin livers for PCBs in Purvis Creek, a tidal creek closer to the LCP Chemicals site than the DMC. Terrapin livers sampled in Purvis Creek contained average PCB levels of 1.560 mg/kg (range 1.120-2.190 mg/kg; Kannan et al., 1998). Based on our spatial analyses, terrapins in Purvis Creek should have higher levels than terrapins around the DMC since they are closer to the source of the PCB contamination. However, PCBs do not biodegrade readily, and the area is still feeling the effects from Aroclor 1268 being released into the environment. There are even studies suggesting that Aroclor 1268 is moving through the food chain on a regional scale, showing up in dolphins, least terns (*Sternula antillarum*), and prey fish from Savannah to Jacksonville (Balmer et al., 2011; Wirth et al., 2014; Robinson et al., 2015).

Our study found no difference in Aroclor 1268 concentrations in terrapins from north of the DMC and those from south of the DMC. There is only one creek that joins the northern and southern sides of the marsh around the DMC, so the chance of terrapins moving back and forth via tidal creeks is highly unlikely. In addition to previous studies indicating high site fidelity (Gibbons et al., 2001), spatial studies of female terrapins surrounding the DMC reinforce the improbability that terrapins are moving freely across the causeway. Radio-telemetry data and GPS logger data have shown that female terrapins exclusively stay on either the north side of the causeway or the south side (Zailo et al., unpublished data). Additionally, no terrapins have even been captured on both sides of the causeway during mark-recapture efforts (Maerz & Crawford, unpublished data). Terrapins originally captured on the north side have only been recaptured on the north side, and vice versa for terrapins captured on the south side. The fact that there is little difference in Aroclor 1268 levels between the two populations may be indicative that Aroclor 1268 is becoming more pervasive in the environment through movement in the food chain as suggested in other studies (Balmer et al., 2011; Wirth et al., 2014; Robinson et al., 2015).

The southern population of terrapins did have significantly lower levels of mercury in their livers than terrapins from north of the DMC. While Aroclor 1268 has a very clear source, mercury is more ubiquitous in the aquatic environment. The northern population may have higher levels of mercury due to its proximity to the Turtle/Brunswick River. The Turtle/Brunswick River extends much further inland than Fancy Bluff Creek, the main tidal outflow for the southern marsh that flows into Jekyll Sound. All the waterways surrounding the city of Brunswick eventually drain into the Turtle/Brunswick River. Any chemical spills of mercury or mercury-containing effluents within the city limits should eventually make their way into the Turtle/Brunswick River. The EPA tracks the release of toxic compounds into the environment that could potentially harm humans or wildlife through the Toxic Release Inventory (TRI) Program. The only monitored source of mercury is an effluent from Brunswick Cellulose, LLC that is released directly into the Turtle/Brunswick River (US EPA, 2019). There are no major developed areas that drain into Fancy Bluff Creek, meaning that all anthropogenic sources of mercury associated with urbanized areas should be in the Turtle/Brunswick River. The linear regression analyzing the effect of distance from the LCP Chemicals site on mercury levels only found a slight decreasing trend, but no significant correlation between distance and mercury levels. Since our analyses of the effects of distance from the LCP Chemicals site on Aroclor 1268 levels did show a significant negative effect, it’s likely that the mercury levels are not due to a singular source of contamination, but rather multiple sources.

We found no difference in the Aroclor 1268 levels of terrapins from north of the DMC and terrapins south of the DMC. Since terrapins south of the causeway are outside of the fish advisory zone implemented by the Georgia EPD and are separated from the north by an impassable physical barrier, this suggests that animals outside of the advisory zone are being exposed at the same rates as animals inside the advisory zone. However, this result only pertains to one species of marsh wildlife. Additional information is needed to say for sure if the fishing advisory zone should be expanded. Future studies should investigate other species for Aroclor 1268 levels from inside and outside the fishing advisory zone. Specifically, these should include fish species that are commonly consumed by humans. Terrapins along the DMC are potentially experiencing moderate to high population declines as a result of roadway fatalities. If terrapins are experiencing sublethal effects as a result of contaminant exposure as well, this could be further impacting their population recovery. While this study was to quantify how much terrapins are being exposed, future studies are needed to determine the potential consequences this exposure is having on terrapins around the Brunswick area.

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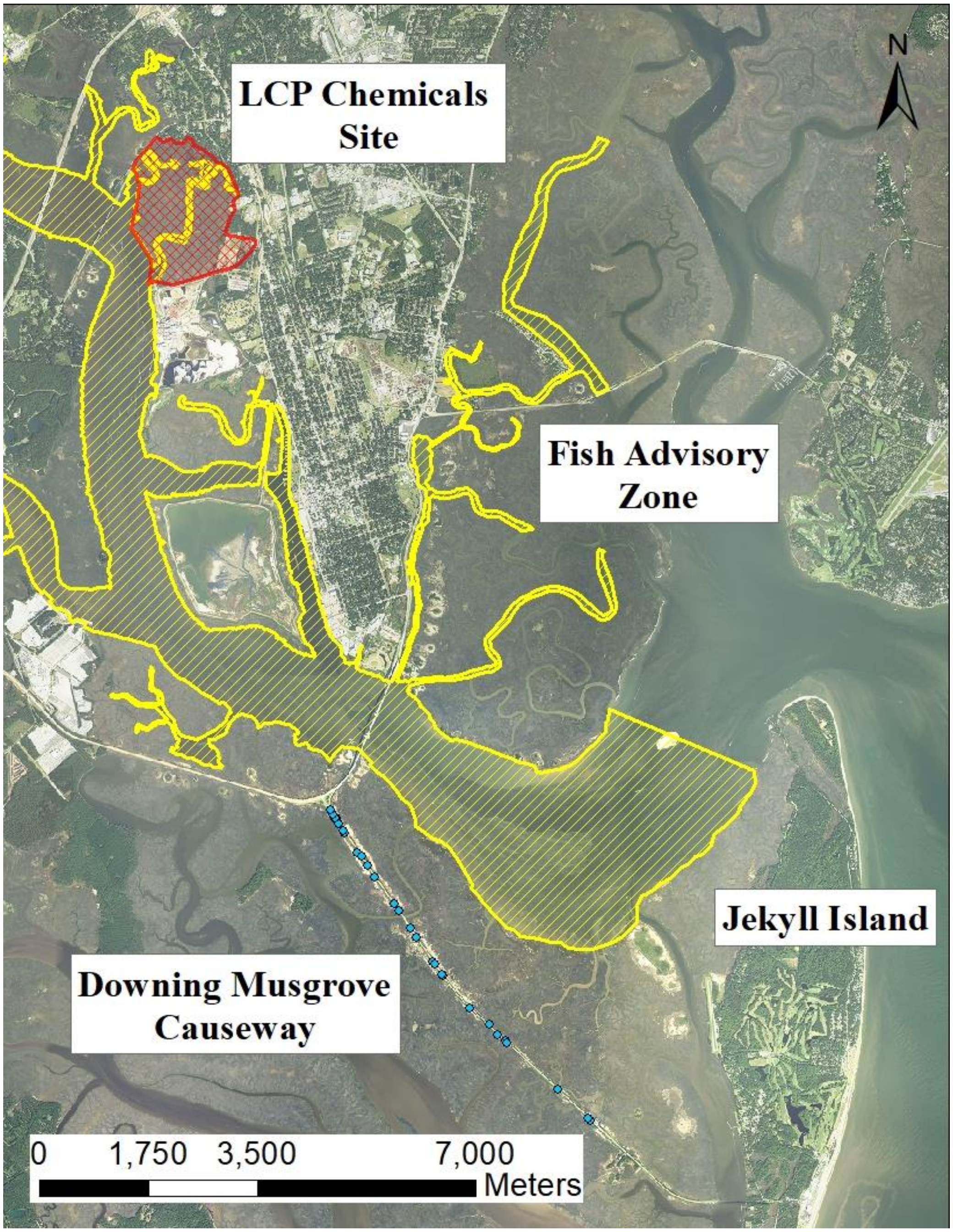

Figure 2.1: Map of the study site, showing the LCP Chemicals Superfund site (crosshatched in red), the fishing advisory zone (hatched in yellow), the Downing Musgrove Causeway, and the GPS positions of the 30 diamondback terrapins (*Malaclemys terrapin*; blue dots) collected for this study along the Downing-Musgrove Causeway (DMC) leading to Jekyll Island.

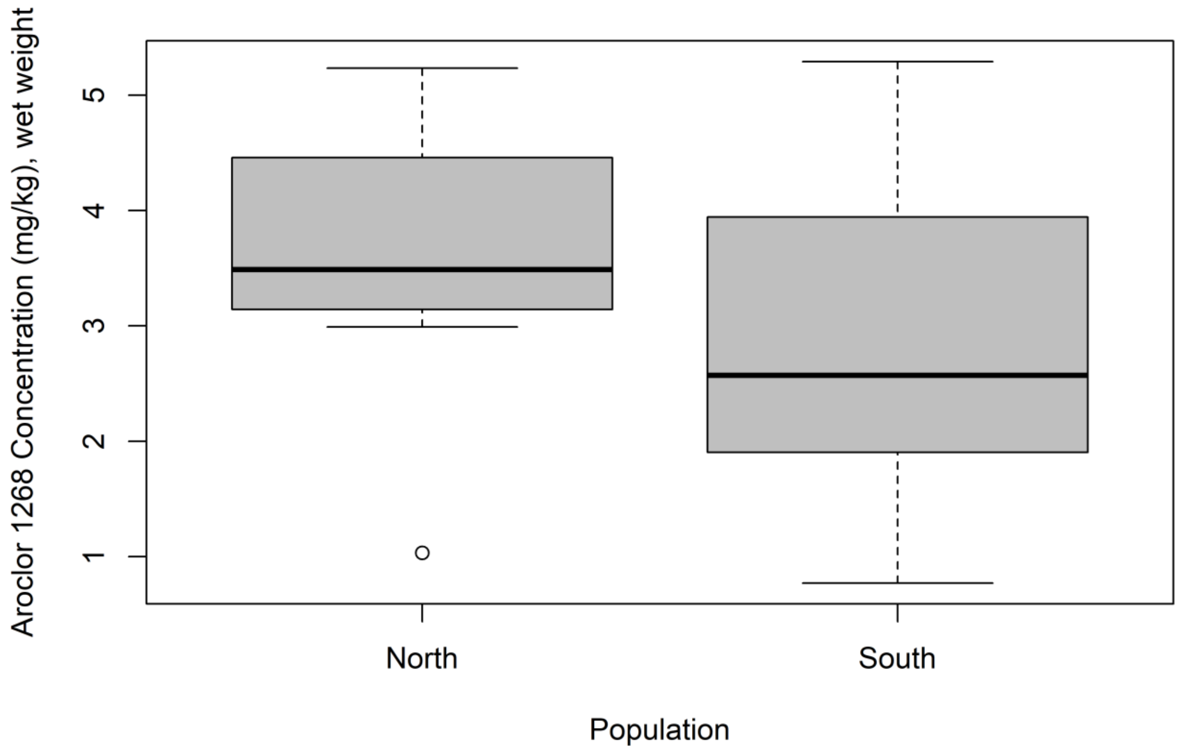

Figure 2.2: Comparison of Aroclor 1268 concentrations in diamondback terrapin (*Malaclemys terrapin*) livers from the North side of the Downing-Musgrove Causeway (DMC) and the South side of the DMC in Brunswick, Georgia (one-tailed t-test; t (11.133) = 1.218, p = 0.124).

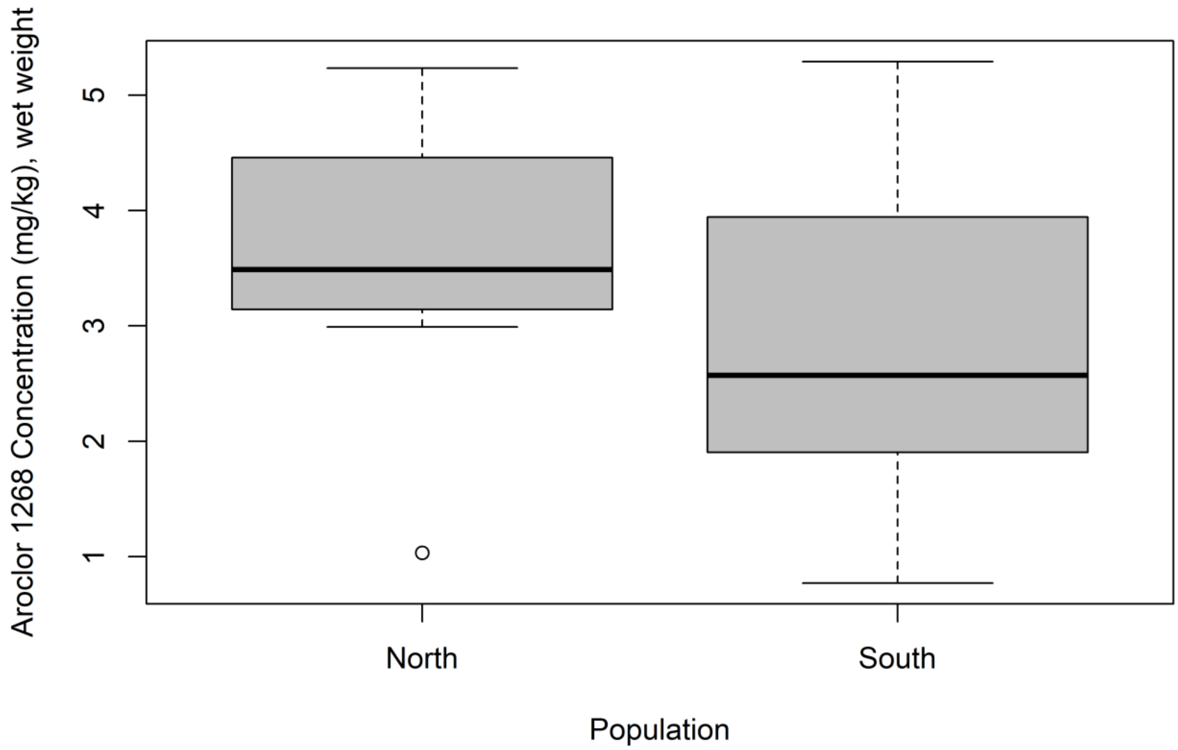

Figure 2.3: Comparison of mercury concentrations in diamondback terrapin (*Malaclemys terrapin*) livers from the North side of the Downing-Musgrove Causeway (DMC) and the South side of the DMC in Brunswick, Georgia (one-tailed t-test; t (7.621) = 1.876, p = 0.049).

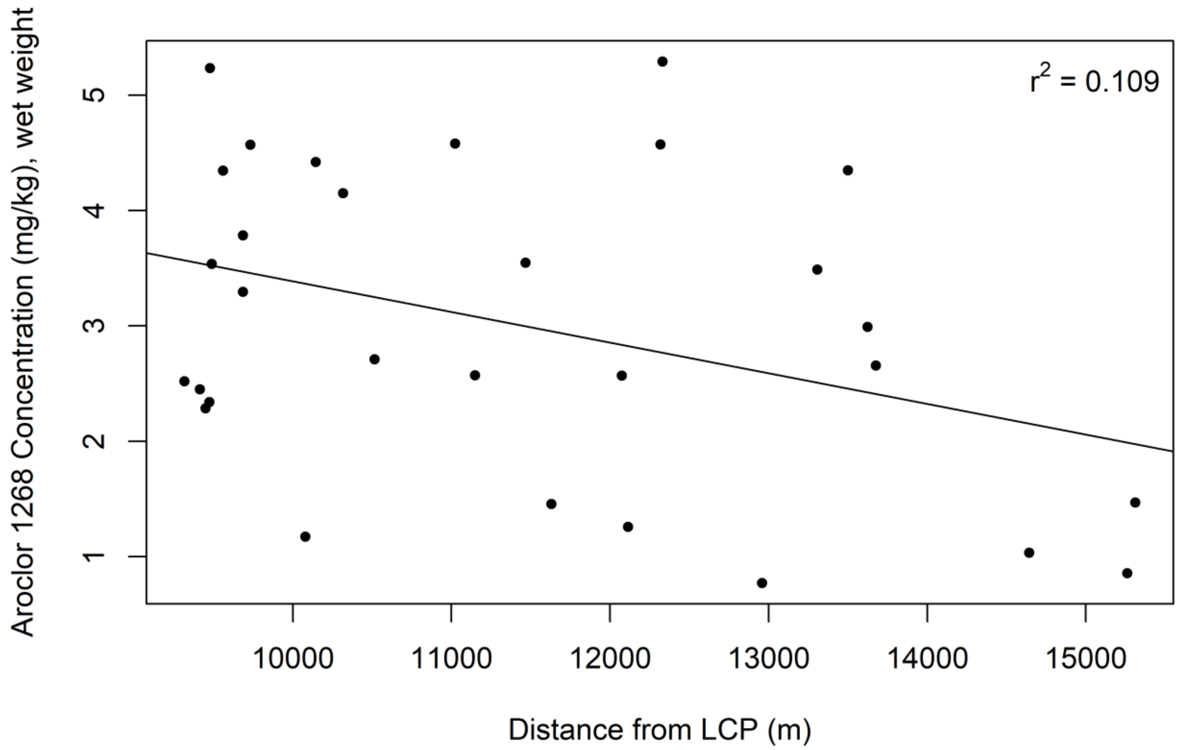

Figure 2.4: Relationship between straight-line distance from the Linden Chemicals and Plastic (LCP) Superfund site and Aroclor 1268 concentrations in diamondback terrapin (*Malaclemys terrapin*) livers found along the Downing Musgrove Causeway in Brunswick, Georgia (linear regression; $F_{1,28} = 4.553$, $p = 0.042$).

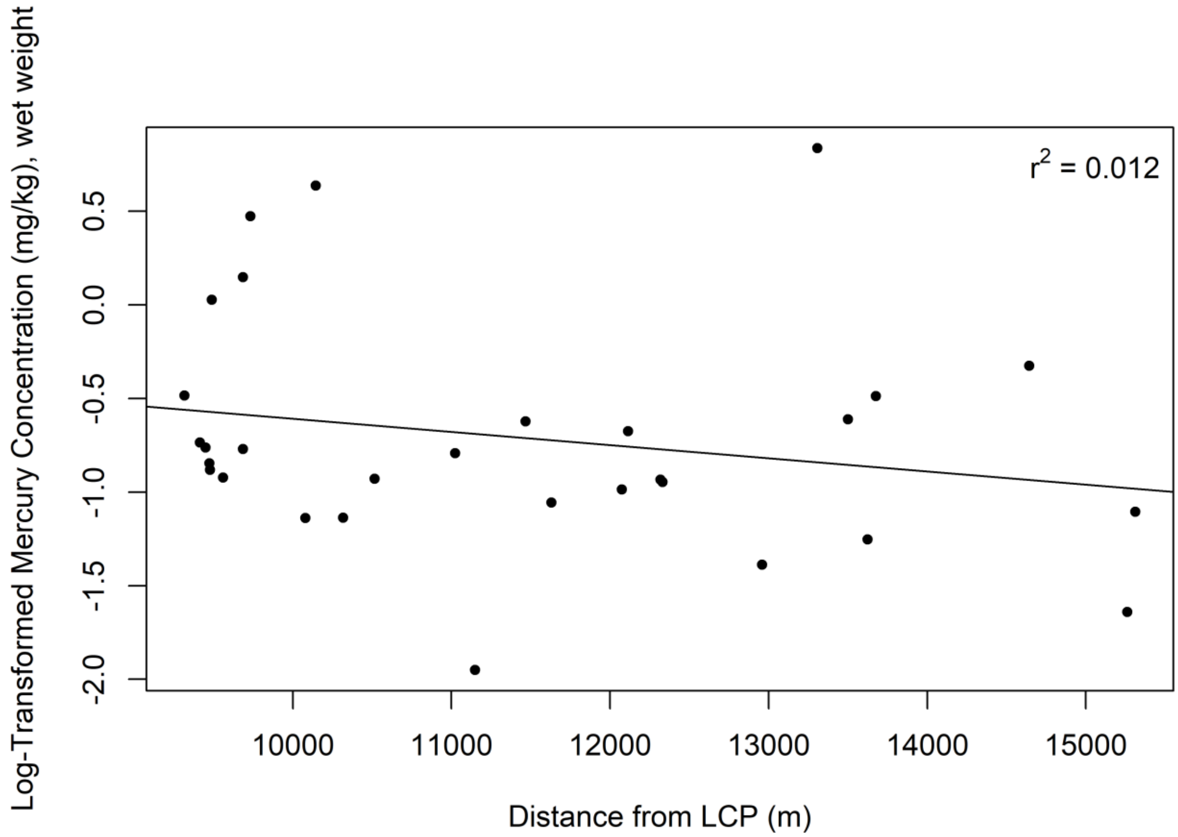

Figure 2.5: Relationship between straight-line distance from the Linden Chemicals and Plastic (LCP) Superfund site and log-transformed mercury concentrations in diamondback terrapin (*Malaclemys terrapin*) livers found along the Downing Musgrove Causeway in Brunswick, Georgia (linear regression; $F_{1,28} = 1.402$, $p = 0.246$).

CHAPTER 3

MERCURY LEVELS OF LOGGERHEAD SEA TURTLES NESTING ON JEKYLL ISLAND, GEORGIA[2]

[2] Bauer, R. G., K. M. Andrews, T. N. Norton, B. M. Shamblin, & L. A. Fowler. To be submitted to *Environmental Monitoring and Assessment*.

Abstract

Persistent pollutants such as heavy metals and polychlorinated biphenyls pose a large risk to both human and wildlife health. These contaminants resist natural degradation, are mobile in the environment through the food chain, and bioaccumulate in upper level predators. Brunswick, Georgia is home to a heavily contaminated EPA Superfund site with excessive levels of organic contaminants and heavy metals, including mercury. The effects of marine pollution such as heavy metals and other contaminants on sea turtles has become a research priority in recent years. We tested skin samples collected from nesting loggerhead sea turtle on Jekyll Island, Georgia for mercury levels to quantify the potential threat of contaminants from this Superfund site to sea turtles. Mercury concentrations averaged 0.028 ± 0.003 mg/kg wet weight. Mercury concentrations were negatively affected by straight carapace length but were not significantly affected by other morphometric measurements. Additionally, there were no differences in mercury concentration between turtles that laid nests mostly within the Brunswick area and turtles that laid nests mostly outside of the Brunswick area. Turtles that were sampled on Jekyll Island were sometimes documented to nest outside the state of Georgia, although this also did not have a significant effect on mercury levels of these loggerheads.

Introduction

Environmental pollution is a threat to both human and wildlife health, particularly in marine areas (Naser, 2013). Metals and other trace elements released from anthropogenic sources tend to accumulate in marine environments (Nriagu & Pacyna, 1988; Newman & Unger, 2003; Lamborg et al., 2014). Although the levels of heavy metals within the water column itself are relatively low, precipitation into marine sediments exposes benthic organisms to toxic levels of metals (Mas & Azcue, 1993). Metals are known to bioaccumulate and biomagnify throughout the food web as upper level predators feed on these benthic prey species (Caurant et al., 1999; Anan et al., 2001; Storelli et al., 2005; Camacho et al., 2013). Heavy metals are resistant to natural biodegradation and are potentially toxic to organisms even at low levels (Storelli et al., 2005; Day et al., 2007). Studies on fish, birds, and mammals have shown that heavy metal toxicity can cause neurotoxicity, liver and kidney damage, abnormal development, decreased reproductive output, and altered endocrine and immune function in afflicted individuals (Zelikoff et al., 1994; Hoffman et al., 2003).

Brunswick, Georgia is the location of a former chloroalkali facility that was most recently owned by Linden Chemicals and Plastic (LCP Chemicals). The LCP Chemicals site was designated an Environmental Protection Agency (EPA) Superfund site in 1996 due to soil, groundwater, and sediment contaminated by heavy metals and organic contaminants (US EPA, 1996). Further investigation found high levels of mercury, lead, chromium, zinc, PCBs, and polycyclic aromatic hydrocarbons (PAHs) in the soil and waters adjacent to the Superfund site (Kannan et al., 1997; Maruya & Lee, 1998). LCP Chemicals applied Aroclor 1268 to electrical equipment used in chemical processing (Kannan et al., 1998). This rare, highly-chlorinated PCB was only manufactured by one company, that produced a limited amount of the chemical, and LCP Chemicals was the only user of this congener mixture in the southeast U.S. (Kannan et al., 1997; Maruya & Lee, 1998). It is estimated that throughout the history of the site, over 37 tons of PCBs and 440 tons of mercury were released into the environment surrounding the industrial facilities (Kannan et al., 1998).

The amounts of heavy metals and PCBs found in the immediate vicinity of the LCP Chemicals site were high enough to potentially cause harm to biota inhabiting the Turtle/Brunswick River Estuary (TBRE) adjacent to the marsh surrounding the Superfund site (Figure 2.1). Initial studies mainly investigated the distribution of Aroclor 1268 and mercury among invertebrates, fish, and birds (Kannan et al., 1998; Maruya & Lee, 1998). However, more recent studies on the effects of these contaminants on organisms inhabiting the TBRE have been conducted on bottlenose dolphins (*Tursiops truncatus*) (e.g., Pulster & Maruya, 2008; Balmer et al., 2011; Wirth et al., 2014). PCB concentrations measured in upper level predators using the TBRE indicated that these animals were exposed to high enough concentrations to be exhibiting reproductive and immunosuppression (Pulster et al., 2009; Balmer et al., 2011). Additionally, Aroclor 1268 has been shown to be found in organisms not residing within the Brunswick area, suggesting that the contaminants from the LCP Superfund site may be moving through the food chain on a regional scale (Balmer et al., 2011; Wirth et al., 2014; Robinson et al., 2015).

Marine turtles rely on nearshore areas as critical habitat for foraging and developing (Morreale & Standora, 2005; Scott, 2006). This reliance on nearshore habitat increases the likelihood that sea turtles are exposed to heavy metals released from point sources such as coal burning power plants, steel mills, chloroalkali plants, and other industrial facilities (Day et al., 2005). Some species of sea turtles, such as loggerhead sea turtles (*Caretta caretta*), are long-lived upper level predators, making them susceptible to negative health effects of heavy metals (Maffucci et al., 2005). Due to these life history traits, sea turtles are thought to be reliable bioindicator species of marine pollution and ecosystem health (Aguirre & Lutz, 2004; Andreani et al., 2008). As a result, evaluating the effects of pollution and contaminants on marine turtles has recently become a global research priority (Hamann et al., 2010; Rees et al., 2016).

Exposure to heavy metals has been linked to multiple negative effects on the health of sea turtles. Fibropapillomatosis (FP) is a disease in sea turtles that causes afflicted individuals to grow tumors usually around the neck, eyes, tail, and fin regions (Foley et al., 2005; Duarte et al., 2012). Increased metal levels in sea turtles, including mercury, have been linked to increased FP tumor growth in green sea turtles (*Chelonia mydas*; da Silva et al., 2016; Perrault et al., 2017). Exposure to heavy metals has also been shown to decrease immune function in sea turtles. Mercury toxicity has been linked to decreased white blood cells and suppression of other immune functions in loggerhead and green sea turtles (Day et al., 2007; Komoroske et al., 2011). When compared to other species, sea turtles' immune systems start exhibiting decreases in function at significantly lower mercury levels than other animals (Day et al., 2007).

The loggerhead sea turtle is listed on the International Union for Conservation of Nature (IUCN) Red List (Casale & Tucker, 2017). The main threats to the loggerhead identified by the IUCN are anthropogenic alterations to critical habitat, bycatch by the commercial fishing industry, invasive pathogens, marine pollution, and climate change. Adult loggerheads occupy higher trophic levels than other sea turtle species and are therefore more likely to be negatively affected by biomagnified toxins such as heavy metals and polychlorinated biphenyls (Perrault et al., 2017; Casini et al., 2018). The

TBRE has been shown to provide critical habitat for female loggerheads during internesting periods (Scott, 2006). Additionally, loggerhead sea turtles sampled in Georgia have been shown to have mercury levels high enough to cause sublethal effects including suppression of the immune system (Day et al., 2005; Day et al., 2007). Additionally, one individual turtle captured along the Georgia coast had mercury levels 16 times higher than other individuals within the study and tested positive for Aroclor 1268 congeners, suggesting this turtle used the TBRE as a multi-season foraging site (Day et al., 2005). This indicates that loggerhead sea turtles are being exposed to contaminants linked to the LCP Superfund site at potentially dangerous levels. The objective of this study was to use a non-invasive way to test loggerhead sea turtles using the TBRE for mercury levels in a preliminary attempt to quantify the potential threat to loggerheads from contaminants associated with the LCP Superfund site.

Methods

Study site

The study site for this project is Jekyll Island, Georgia, USA. Jekyll Island is a 2,238-ha barrier island located along the southeastern coast of Georgia. The island is located approximately 12 kilometers southeast of the city of Brunswick and is managed by the Jekyll Island Authority. The Turtle/Brunswick River flows into the Atlantic Ocean at the northern tip of Jekyll Island. Jekyll Island is connected to the mainland via the Downing-Musgrove Causeway (DMC), an 8.7-kilometer roadway that bisects an intertidal marsh ecosystem that is mainly comprised of cordgrass (*Spartina* spp.). The saltmarsh on the northern side of the DMC represents the southern boundary of the fish consumption advisory zone for the Turtle/Brunswick River implemented in 1995 by the

Georgia Department of Natural Resources Environmental Protection Division due to PCB and heavy metal contamination from the LCP Chemicals Superfund site (GA EPD, 2018). Jekyll Island is also home to the Jekyll Island Authority's Georgia Sea Turtle Center (GSTC), a rehabilitation/education and research facility specializing in reptiles.

There are approximately 15 kilometers of beach on Jekyll Island that are used by sea turtles as nesting habitat. Most nests on Jekyll Island are laid by loggerhead sea turtles, however sporadic nesting of leatherback and green sea turtles have been observed (Ondich and Andrews, 2013). The beach has been surveyed for nesting sea turtles since 1972 by the Jekyll Island Sea Turtle Patrol, however the GSTC took over patrolling the nesting beach in 2007 (Ondich and Andrews, 2013). Satellite telemetry data from devices placed on female loggerheads nesting in Georgia have found that females will spend a majority of their inter-nesting periods (the time between laying nests) in near shore habitat, favoring estuaries and deep-channel habitats (Scott, 2006). Specifically, females nesting on Jekyll Island were shown to spend their internesting periods in and around the Turtle/Brunswick River Estuary (Scott, 2006).

Field sampling and sample collection

Field work for this project took place during peak nesting times during the 2015, 2016, and 2017 nesting seasons. Female loggerheads that were encountered emerging from the sea, nesting, or returning to the sea were intercepted. The shoulder area of the turtle was aseptically prepared with two alternating Betadine® Surgical Scrub (Purdue Pharma L.P., Stamford, CT, USA) and isopropyl alcohol applications. A skin sample was taken from each female the first time they were encountered that season using a sterile 6-millimeter biopsy punch (Miltex Inc., York, PA, USA). If time allowed, morphometric measurements of the turtle were taken. Curved carapace length (CCL) and curved carapace width (CCW) were taken using a soft measuring tape. Straight carapace length (SCL) and straight carapace width (SCW) were taken using calipers. Shell length measurements were taken using the straight-line distance (nuchal notch to most posterior notch) and width measurements were taken at the widest point of the shell. If a morphometric measurement was not successfully taken, that individual was excluded from the analysis corresponding to that measurement. Both shoulders of nesting females were scanned for the presence of Passive Integrated Transponder (PIT) tags and flippers were investigated for the presence of metal Inconel® flipper tags that are used to mark turtles both nationally and internationally (Ondich and Andrews, 2013). Skin samples were placed into Whirl-Pak® sample bags (Nasco Fort Atkinson, WI, USA) and kept at environmental temperature for no longer than 9 hours. After patrol ends around 6:00, tissue samples were logged and stored in an ultra-low freezer (-80°C) until mercury analysis could be performed.

Contaminant analysis

Mercury analysis was performed using a Direct Mercury Analyzer (DMA-80: Milestone, Shelton, CT, USA). Tissue biopsies were thawed completely and then weighed to the nearest 0.1 mg (wet weight). Weighed samples were then placed in individual quartz sample boats (Milestone, Shelton, CT, USA) and run through the DMA-80. The DMA-80 analyzes mercury by thermally decomposing the samples and using carrier gas to transport the mercury and combustion products into a catalyst chamber. A gold amalgamator captures the mercury species before releasing the mercury into the spectrophotometer’s optical path. After every ten samples analyzed, both a system blank and a system standard (DORM-3 Fish Protein; National Research Council Canada, Ottawa, Canada) were run to ensure proper calibration of the machine. Mercury levels are reported as means ± 1 standard error, and all concentrations are reported as wet weight.

Determining Nesting Histories

Researchers at the University of Georgia's Warnell School of Forestry and Natural Resources have been studying the genetics of nesting loggerhead sea turtles from Maryland to Florida (Shamblin et al., 2017). Genetic profiles are created for each loggerhead using a combination of skin and egg samples (Shamblin et al., 2007; Shamblin et al., 2009). Nesting histories were determined for all loggerhead sea turtles that we collected a mercury sample from to quantify how many nests they laid in the region and the location of those nests. Flipper tag and PIT tag identification for these turtles were cross-referenced with genetic IDs for turtles with corresponding physical tags to give us the individual genetic IDs for each individual. A complete annual nesting history for that turtle was then referenced using the genetic data gathered from egg and tissue samples from other research teams along the Atlantic coast. Although sea turtles generally nest in the same area each nesting season, sea turtles along the Georgia coast will often nest on multiple islands during a season. Therefore, even though we collected all our samples on turtles nesting on Jekyll Island, they would usually lay nests on other islands as well.

Sea turtles were broken into one of three categories depending on how many nests they laid within the BTRE area. For the purposes of this study, we defined the nesting beaches of the BTRE area as Jekyll Island, St. Simons Island, Sea Island, and Little St.

Simons Island (Figure 3.1). Although Jekyll Island and St. Simons Island are the two islands that border the Brunswick/Turtle River, St. Simons Island and Sea Island do not get many sea turtle nests due to the increased amount of beachfront development on those islands. Little St. Simons island is the first major nesting beach north of the mouth of the Turtle/Brunswick River. “Resident” turtles were defined as turtles that laid more than 50% of their nests on one of these four islands within the year the sample was collected. “Transient” turtles were defined as turtles that laid less than 25% of their nests on one of these four islands within a nesting season. Turtles that laid between 25% and 50% of their nests were classified as “unknown”. The estimated clutch frequency for loggerhead sea turtles nesting in Georgia is 4.70 (Shamblin et al., 2017), so only turtles that had at least 4 nests detected in the season the sample was collected were included in analysis.

Statistics

Statistical tests were performed using program R 3.4.1 (R Core Team 2019). All data were tested for normality using the Shapiro-Wilk test. If data were not normally distributed, data were log-transformed prior to analysis. The effects of size (SCL, CCL, SCW, and CCW) on mercury concentration were analyzed using simple linear regression. For turtles that were sampled in multiple years, the effects of time on mercury concentration were analyzed using a paired sample t-test. To test the effects of residency status (resident, transient, or unknown) and the effects of nesting in states outside of Georgia (Florida, South Carolina, or North Carolina), linear mixed effects (LME) models were used. This was accomplished by building a null LME model with SCL as a fixed effect and genetic ID as a random effect. Then LME models were constructed with either Status as a fixed effect, Other States as a fixed effect, or both Status and Other States as fixed effect. The results of these LME models were compared using Akaike Information Criterion (AIC) weights.

Results

Summary statistics

A total of 60 skin biopsies were collected from female loggerhead sea turtles nesting on Jekyll Island during the 2015 (n=19), 2016 (n=21), and 2017 (n= 20) nesting seasons. Ten turtles were sampled in multiple seasons. Nine turtles were sampled in both 2015 and 2017, and one turtle was sampled in both 2015 and 2016. Of the 60 samples collected, 31 were from loggerheads classified as residents of the TBRE area, 26 were from loggerheads classified as transients, and three turtles were unknown. From all the females sampled, 45 females only laid nests detected in Georgia during this study. In addition to these turtles that only nested in Georgia, nine females laid nests in Florida, four females laid nests in South Carolina, and two females laid nests in North Carolina.

Effect of body size on mercury concentration

The average concentration of mercury found in sampled loggerhead sea turtles was 0.028 ± 0.003 mg/kg wet weight (range of 0.003-0.095 mg/kg). One individual skin sample contained 0.264 mg/kg of mercury. This sample was excluded from analyses as an outlier. Since all the samples were measured by wet weight concentration, it was assumed this sample had dried out, causing the mercury concentration to be higher due to decreased weight.

Straight carapace lengths were collected from 54 females. The average straight carapace length was 91.135 ± 0.649 cm (81.3-100.7 cm). Curved carapace lengths were collected for 55 loggerheads that had tissues samples collected. The average curved carapace length was 98.305 ± 0.706 cm (87.8-108.5 cm). Straight carapace lengths were collected from 55 loggerheads. The average straight carapace length was 71.065 ± 0.524 cm (59.7-76.9 cm). Curved carapace widths were collected from 56 individuals. The average curved carapace width was 92.018 ± 0.738 cm (80.1-102.5 cm).

Linear regression of all four morphometric measurements (SCL, CCL, SCW, CCW) against mercury concentration revealed non-normally distributed residuals from the linear model. Therefore, mercury concentrations and morphometric measurements were log-transformed to produce normally distributed residuals. Straight carapace length had a significant negative effect on mercury concentrations in skin biopsies ($F_{1,52}$ = 8.122, p = 0.006; Figure 3.2). There was a negative relationship between curved carapace length on mercury concentrations, however this trend was not significant ($F_{1,53}$ = 3.056, p = 0.086). There was a negative relationship between straight carapace width and mercury concentrations in skin samples, however this trend was not significant ($F_{1,53}$ = 2.607, p = 0.112). Curved carapace width exhibited the weakest relationship with mercury concentrations in sampled loggerheads ($F_{1,54}$ = 0.194, p = 0.662).

Mercury concentrations of resampled individuals

Ten individual female loggerheads were sampled more than once during this study. Nine females were sampled in both 2015 and 2017. One female was sampled in both 2015 and 2016. The analysis of resampled individuals focused on the nine turtles that were sampled in 2015 and 2017. Using females that were all sampled at the same interval allowed us to use a paired t-test for analysis. One of the nine females was excluded from analysis as it was the individual with the abnormally high mercury concentration measured in the skin sample taken in 2017 (0.264 mg/kg). The average mercury concentration for females sampled in 2015 was 0.028 ± 0.006 mg/kg wet weight (0.008-0.064 mg/kg). The average mercury concentration for females sampled in 2017 was 0.025 ± 0.009 mg/kg wet weight (0.004-0.080 mg/kg). While the 2015 mercury concentrations passed the Shapiro-Wilk normality test, the 2017 mercury concentrations were not normally distributed. Therefore, mercury concentrations from both years were log-transformed, allowing them to pass the normality test before running the t-test. The paired t-test revealed that there was no significant difference between mercury concentrations in 2015 and 2017 (t (7) = 1.611, p = 0.151; Figure 3.3).

Linear mixed effect models

The results of the linear mixed-effects models are presented in Table 3.1. For the null linear mixed effects model, straight carapace length was used as a fixed effect and the DNA identification of the turtle was incorporated as a random effect. Straight carapace length was chosen because it was the only morphometric measurement to exhibit a significant effect on mercury concentrations of sampled females. We then designed three additional mixed effects models to compare with the null model. One model had residency status as a fixed effect, one model had additional states as a fixed effect, and one model had both residency status and other states as fixed effects. The linear mixed effects model that included states as a fixed effect was the strongest model in terms of Akaike Information Criterion (AIC). However, this model had a AIC weight of 0.464, while the model that only had SCL as a fixed effect had an AIC weight of 0.396. Both of these models had similar AIC weights, so it is most likely that neither accurately predicts mercury levels based on the effects tested.

Discussion

The effects of contaminants on sea turtles has become a research priority in recent years, however relatively few studies have focused on non-invasive methods of sampling sea turtles (Jerez et al., 2010; D'Ilio et al., 2011; Bucchia et al., 2015). There have only been a handful of studies analyzing the mercury concentrations in loggerhead sea turtles using skin biopsies (Day, 2003; Jerez et al., 2010; Casini et al., 2018). Unfortunately, these studies reported concentrations of mercury in dry weight, making direct comparisons difficult with our data, which are reported in wet weight. Jerez et al. (2010) found the average mercury concentration of skin samples collected from loggerheads in the Mediterranean Sea to be 0.08 ± 0.09 mg/kg dry weight (0.01-0.14 mg/kg; Jerez et al., 2010). Casini et al. (2018) found loggerheads in the Mediterranean Sea to have average mercury concentrations of 1.09 ± 0.72 mg/kg dry weight (0.22-3.22 mg/kg) in skin samples. (Casini et al., 2018). The average mean mercury concentration in this study was much lower than these studies (0.028 ± 0.003 mg/kg wet weight, range of 0.003-0.095 mg/kg), however the weight of the water in the wet samples may be diluting the mercury concentration. Skin samples from marine mammals have been found to be 73% water by weight (Yang & Mayazaki, 2018). If we used this number to convert our wet weight concentrations into dry weight concentrations, the average mercury level in this study would be 0.103 ± 0.011 mg/kg dry weight (0.011 – 0.351 mg/kg). This would be a little higher than the study by Jerez et al. (2010), but still lower than Casini et al. (2018). We also excluded one skin samples that had abnormally high mercury concentration (0.264 mg/kg wet weight), assuming it may have dried out during storage accounting for the unusually high level of mercury. However, if it was dried out, it would be within the range of mercury concentrations reported by previous studies (Jerez et al., 2010; Casini et al., 2018).

The EPA and the Food and Drug Administration (FDA) are responsible for issuing recommendations regarding how high mercury levels can be in food to be considered safe for human consumption. However, there are no specific restrictions issued by the EPA or FDA regarding the limits of mercury that occur in turtle meat (Burger, 2002). The FDA and EPA recommend that fish meat be less than 0.46 mg/kg wet weight in mercury to be safe for human consumption when eating one serving per week (US EPA, 2018). None of the skin samples from turtles in this study exceeded this level. Additionally, it is difficult to extrapolate potential levels of mercury in other organs of these turtles since studies that compared skin mercury concentrations to concentrations in other tissues used dry weight (Jerez et al., 2010). However, muscle samples in loggerheads contain 1.75 times more mercury than skin samples, liver samples contain 4.87 times more mercury than skin samples, and kidney samples contain 5.50 times more mercury than skin samples (Jerez et al., 2010). Muscle tissue is typically what is consumed by humans and is higher in mercury concentrations than skin. However, muscle tissue still does not accumulate as much mercury as other organs that have an affinity for mercury such as the liver and kidneys. Although consumption of sea turtles occurs in other parts of the world, the practice is almost none existent in North America presently (Witzell, 1994). Therefore, it is unlikely anyone in the region where these sea turtles' range could consume harmful amounts of mercury by eating them. Egg poaching in Georgia does still occur occasionally, however egg laying is not a major route for mercury elimination from the female (Sakai et al., 1995; Páez-Osuna et al., 2011).

Therefore, humans consuming eggs laid by these females are not likely to ingest harmful amounts of mercury.

The fact that mercury concentration decreased with straight carapace length is very peculiar. Contaminants that bioaccumulate and biomagnify, such as heavy metals, usually increase in concentration as animals grow and age (Lutcavage et al., 1997). However, studies investigating the effects of size on heavy metal concentrations in sea turtles have found mixed results (Kampalath et al., 2006; Páez-Osuna et al., 2011; Faust et al., 2014). Loggerhead sea turtles have mostly exhibited positive effects of body size on mercury concentrations (Kampalath et al., 2006). This positive effect of body size on metal concentrations has also been observed in Kemp's ridley sea turtles (*Lepidochelys kempii*; Wang, 2005). On the other hand, studies involving green sea turtles and olive ridley sea turtles (*Lepidochelys olivacea*) have typically shown negative effects of size on metal concentrations (Gordon et al., 1998; Sakai et al., 2000; Komoroske et al., 2011; Páez-Osuna et al., 2011; Faust et al., 2014). This negative effect has been theorized to be the result of a shift from an omnivorous diet to an herbivorous diet as juvenile green sea turtles mature (Bjorndal, 1997; Komoroske et al., 2011). Shifting from eating aquatic prey species to vegetation would limit the amount of mercury and other heavy metals green sea turtles could ingest. However, loggerhead sea turtles are more carnivorous than other sea turtles which would explain why most studies indicate size has positive effects on heavy metal accumulation (Bjorndal, 1997; Fanzellitti et al., 2004; Chen et al., 2008; Perrault et al., 2017; Donaton et al., 2019).

Even though our results seem counter intuitive, ours is not the first study to document a negative relationship between size and mercury concentrations in loggerhead sea turtles. Casini et al. (2018) also observed a decrease of mercury concentrations in skin samples as sea turtles aged. Although they did not record measurements of the turtles, they broke turtles into three age class; juveniles, subadults, and adults. The average mercury concentration was 0.99 ± 0.87 mg/kg dry weight (0.22-3.22 mg/kg) for juveniles, 1.26 ± 0.62 mg/kg dry weight (0.48-2.31 mg/kg) for subadults, and 0.67 ± 0.44 dry weight (0.36-0.98 mg/kg) for adults (Casini et al., 2018). Adult loggerhead sea turtles in our study exhibited the lowest concentrations of mercury in average concentration and range of concentration. Therefore, the results in our study that indicated larger loggerheads have lower concentrations of mercury are not without precedent. However, the exact cause for why larger loggerheads have lower levels of mercury needs to be determined in future studies that investigate specific diet and habitat use.

There are a few possible explanations for this effect. One way sea turtles offload contaminants is through the laying of eggs (Stewart et al., 2011; Andrés et al., 2016). However, multiple studies have shown that egg laying is not a major route for mercury elimination in loggerheads (Sakai et al., 1995; Páez-Osuna et al., 2011). Maternal transfer of mercury is therefore unlikely to be why there is a decrease in mercury as size increases. Juvenile loggerhead sea turtles in Georgia were found to be consuming higher proportions of debris associated with channel bottoms than adult loggerheads (Youngkin, 2001). Mercury has been found to be deposited in benthic organisms at higher rates than other organisms due to increased exposure to mercury-laced sediments (Jędruch et al., 2019). If juveniles are foraging on bottom-dwelling organisms at a higher rate than adults, this would cause them to be exposed to higher levels of contaminants associated with marine sediments, such as mercury.

There was no significant effect of residency status on mercury concentrations in loggerhead sea turtle samples that we sampled. The mean mercury concentrations between sea turtles that were resident nesters of the TBRE was not different from sea turtles that were sampled in the TBRE but laid most of their nests elsewhere. This is likely due to the pervasiveness of mercury contamination in the aquatic environment. Aroclor 1268 has been used as a "fingerprint" of contamination from the LCP Superfund in wildlife due to its rarity and unique congener signature (Pulster et al., 2009; Robinson et al., 2015). However, mercury is typically more ubiquitous in the environment. While Aroclor 1268 has a clear point source, mercury can be released into the environment from coal burning power plants, steel mills, chloroalkali plants, and atmospheric deposition (Day et al., 2005; Obrist et al., 2018). The sea turtles we sampled could be experiencing mercury contamination on a regional scale. Loggerhead sea turtles move great distances during their life stages, making it difficult to characterize their exposures to contaminants (Andreani et al., 2008). As loggerheads migrate along the Atlantic coast, they may be exposed to areas with higher mercury concentrations than Brunswick. For instance, based on data retrieved from the EPA's Toxic Release Inventory (TRI) Program, between 2015 and 2017, there was an average of 6.80 ± 0.06 pounds of mercury released into the air annually and an average of 0.43 ± 0.03 pounds of mercury released into the water annually in Brunswick (US EPA, 2019). On the other hand, other cities in the region released higher quantities of mercury. Savannah released an annual average of 11.33 ± 1.33 pounds of mercury and Jacksonville released an annual average of 243.72 ± 4.27 pounds of mercury. Both Savannah and Jacksonville have a higher amount of anthropogenic mercury being released into the environment than Brunswick.

Additionally, there may not be any noticeable differences in mercury levels due to the how contaminants are stored within skin samples. Skin samples have been shown to be effective indicators of heavy metal burden in internal organs (Jerez et al., 2010; Faust et al., 2014; Casini et al., 2018). Other non-invasive ways of sampling loggerhead sea turtles for contaminants have included drawing blood or collecting keratin samples from shell scutes (Day, 2003). Both scutes and skin samples have a longer retention time of contaminants, often reflecting a history of exposure on a scale of multiple years (Day et al., 2005). This means that skin samples collected from the turtles on Jekyll Island would not only include the contaminants they are exposed to during the nesting season around the TBRE, but also contaminants they are exposed to during the rest of the year and during years they do not nest. However, blood collected from loggerheads appears to be more indicative of what the sea turtle has been exposed to in the past few weeks or even days (Day et al., 2005). Future studies sampling the blood from turtles in the TBRE may be useful at clarifying the differences between turtles that spend a lot of times in the TBRE area and those that spend less time there. However, due to the rapid nature of mercury levels in blood, reference sites would likely be needed. Regardless, further research is warranted since sea turtles along the Georgia coast have been found to have high levels of Aroclor 1268 in the past (Day et al., 2005). This particular turtle that contained Aroclor 1268 congeners also had mercury concentrations 16 times higher than turtles sampled in the same sampling transects (Day et al., 2005). We discarded one abnormally high sample (10 times the average) thinking it may have dried out and become concentrated due to water loss. However, there is still a chance that this turtle had simply been exposed to extremely high levels of mercury compared to the others like the individual that Day et al. encountered.

The loggerhead sea turtle is listed as a threatened species under the Endangered Species Act and is listed as endangered in Georgia by the Georgia Department of Natural Resources (GA DNR, 2017; US FWS, 2019). Although the numbers of nests laid by loggerheads in Georgia has been increasing over recent years, this species is not considered recovered (Dodd, unpublished data). Prior studies have shown that loggerhead sea turtles have been exposed to Aroclor 1268, the fingerprint contaminant from the LCP Chemicals Superfund site. Future studies must be undertaken to determine if persistent pollutants from the LCP Chemicals site or other point sources of pollution are contributing to potential sublethal effects altering loggerheads' reproductive ability or long-term health. Blood mercury concentrations have been found to be approximately 25% of the mercury levels found in skin (Jerez et al., 2010). Using this estimate, the blood mercury levels in the loggerheads in this study hypothetically should be 0.026 ± 0.003 mg/kg dry weight (0.003 – 0.088). Blood mercury levels at 0.05 mg/kg dry weight have ben identified as the lowest mercury levels to start causing reduced immune function such as suppression of B-cell proliferation (Day et al., 2007). Therefore, at least a portion of the loggerhead sampled in this study could have sublethal effects like decreased immune function. Ideally, future studies would use heavy metal and PCB levels found in the blood of nesting or live-captured sea turtles to identify short term exposure levels to these contaminants of concern. Studies centering around short-term exposure would be more effective in management of these contaminants within a population of a protected species and would be more effective in comparing levels with prior studies investigating the effects of certain concentrations on sea turtle health parameters.

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Model	**df**	**AIC**	**ΔAIC**	***p***
~ SCL + States + ID	7	-256.35	0	0.097
~ SCL + ID	4	-256.03	0.32	-
~ SCL + Status + ID	6	-252.94	3.41	0.633
~ SCL + States + Status + ID	9	-252.68	4.25	0.248

Table 3.1: Summary of the linear mixed effects (LME) models used to test the effects of residency status and nesting in other states on mercury concentration in loggerhead sea turtle (*Caretta caretta*) skin samples from Jekyll Island, Georgia. Random effect variables are straight carapace length ("SCL"), populations status ("Status"; either Resident, Transient, or Unknown), and states nested in ("States"; either Georgia, Florida, South Carolina, or North Carolina). Individual genetic identification was included as a fixed effect ("ID"). Models are ranked according to Akaike's Information Criterion (AIC) values. *P* values were calculated by running an analysis of variance (ANOVA) for each experimental model against the null model for mercury concentration ("~ SCL + ID").

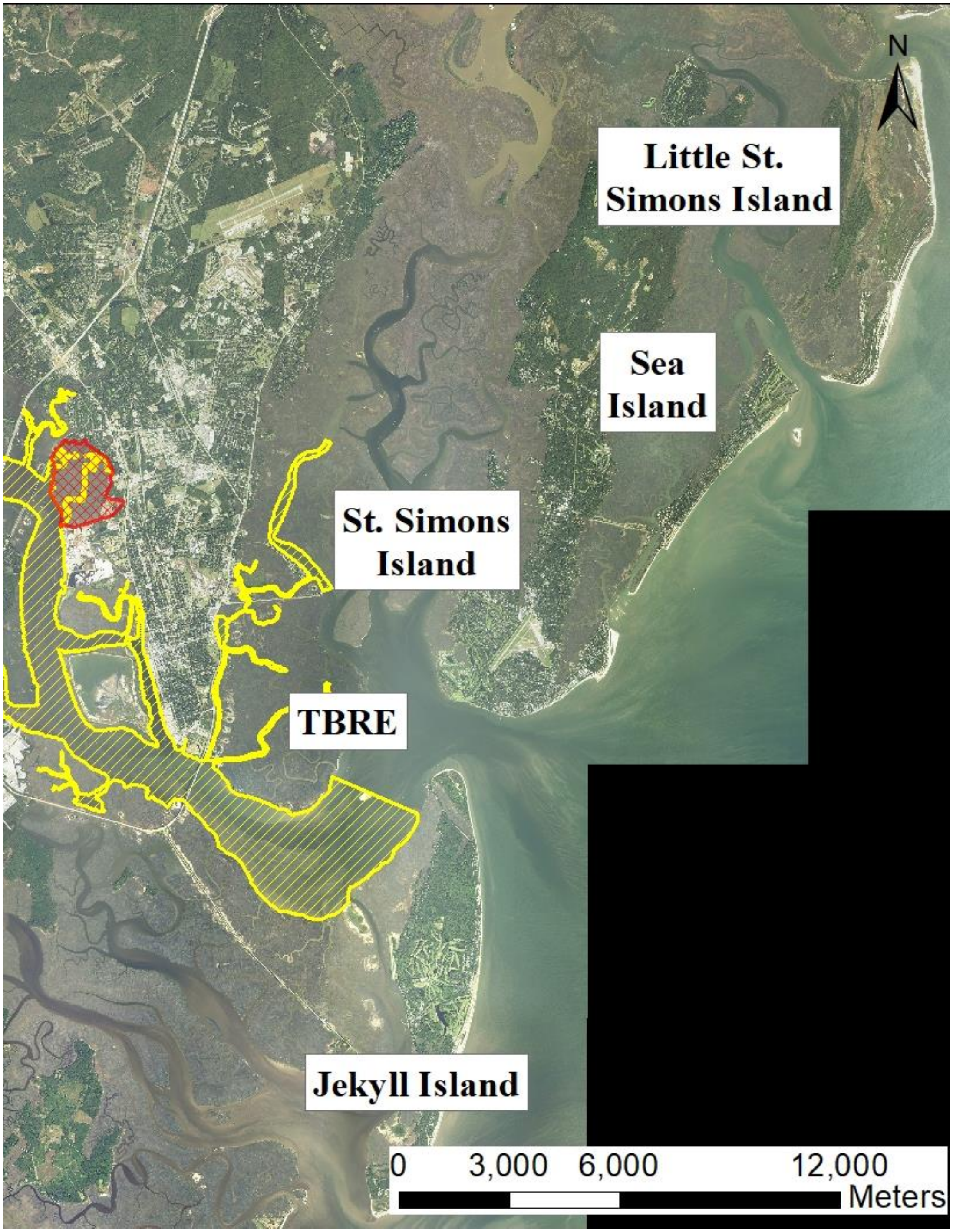

Figure 3.1: Map of the study site and the Turtle/Brunswick River Estuary (TBRE) Islands, showing the LCP Chemicals Superfund site (crosshatched in red), the fishing advisory zone (hatched in yellow), the TBRE, Jekyll Island, St. Simons Island, Sea Island, and Little St. Simons Island.

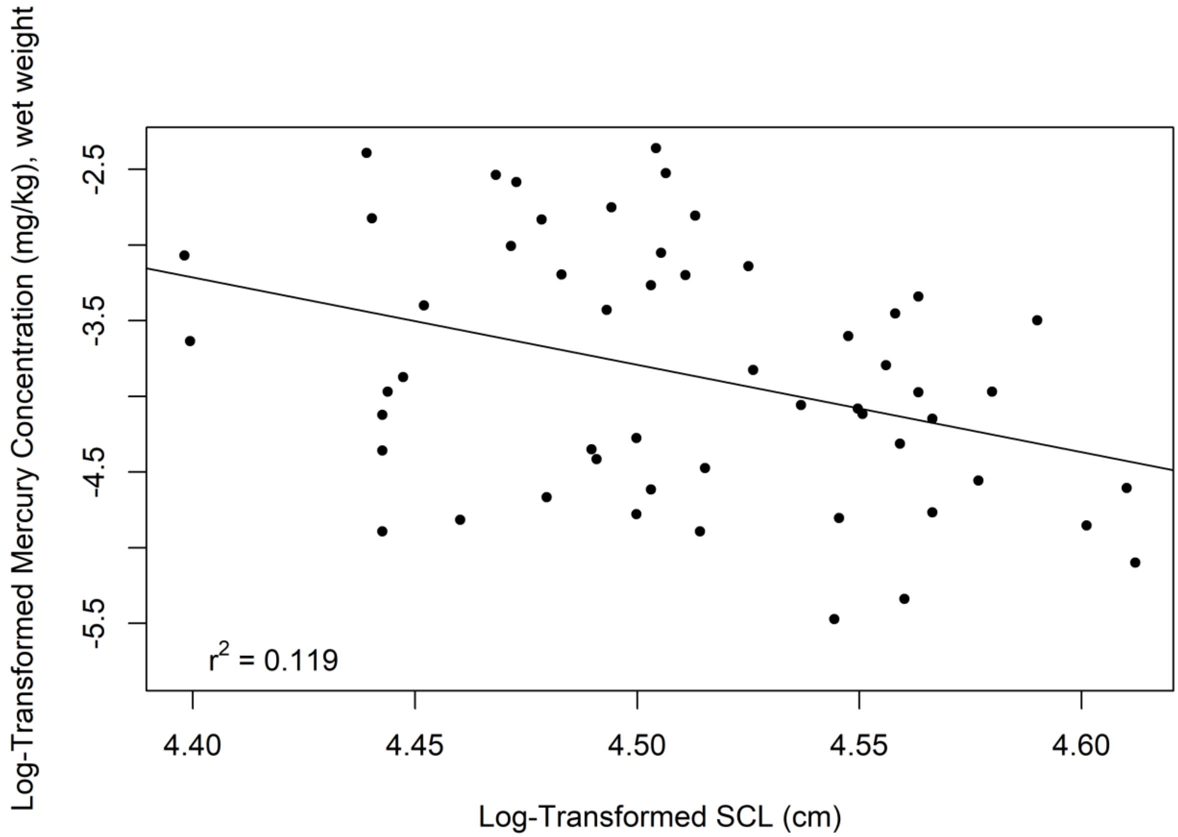

Figure 3.2: Relationship between log-transformed straight carapace length (SCL) measurements and mercury concentrations in loggerhead sea turtle (*Caretta caretta*) skin samples from Jekyll Island, Georgia (linear regression; $F_{1,52} = 8.122$, $p = 0.006$).

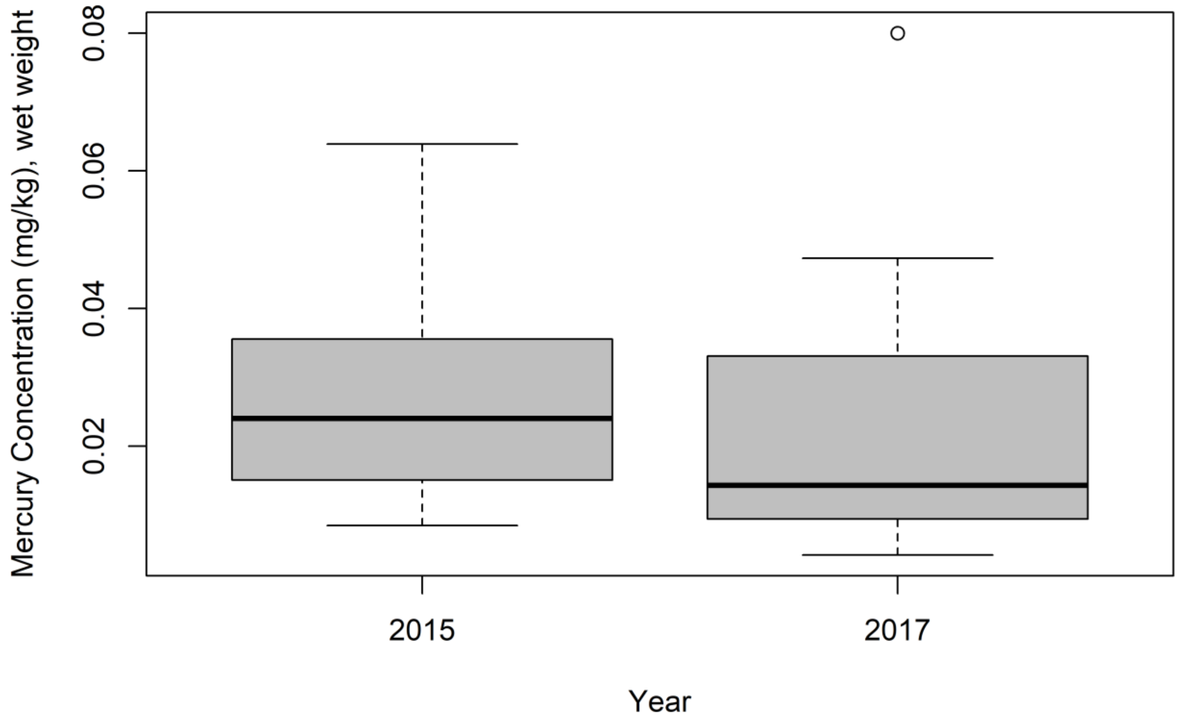

Figure 3.3: Comparison of mercury concentrations in loggerhead sea turtle (*Caretta caretta*) skin samples from turtles that were sampled in both the 2015 and 2017 nesting seasons (paired t-test; t (7) = 1.611, p = 0.151).

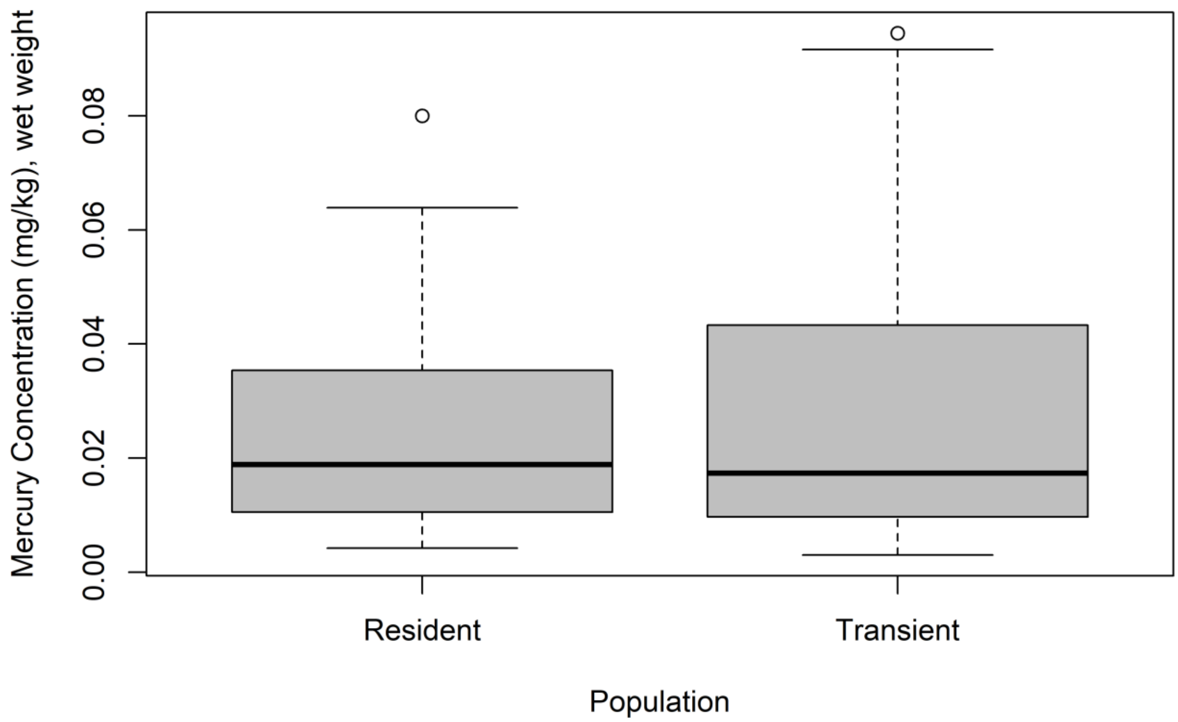

Figure 3.4: Comparison of mercury concentrations in loggerhead sea turtle (*Caretta caretta*) skin samples from turtles that laid more than 50% of their nests in the Turtle/Brunswick River Estuary area ("Resident") and turtles that laid less than 25% of their nests in the Turtle/Brunswick River Estuary area ("Transient"; one-tailed t-test; t (42.861) = -0.665, p = 0.745).

CHAPTER 4

CONCENTRATIONS OF POLYCHLORINATED BIPHENYLS AND HEAVY METALS ASSOCIATED WITH A SUPERFUND SITE IN AMERICAN ALLIGATORS IN BRUSNWICK, GEORGIA, USA[3]

[3] Bauer, R. G., K. M. Andrews, T. N. Norton, & L. A. Fowler. To be submitted to *Environmental Health Perspectives*.

Abstract

Environmental pollutants pose a threat to both human and wildlife health. Contaminants such as heavy metals and persistent organic pollutants are particularly dangerous due to their resistance to natural degradation and ability to bioaccumulate through the food chain. Brunswick in coastal southeastern Georgia is home to a heavily contaminated Environmental Protection Agency Superfund site with excessive levels of organic contaminants and heavy metals, including mercury, lead, and Aroclor 1268, a rare, highly-chlorinated biphenyl only used at this specific location. Prior studies on the distribution of these contaminants in the Brunswick estuary have primarily focused on marine mammals, birds, and fish species. However, American alligators in the Brunswick area also use the marsh ecosystem for movement between barrier islands and for foraging opportunities, potentially exposing them to these contaminants of concern. We quantified lead, mercury, and Aroclor 1268 in caudal tail scutes and examined how these contaminants affected health parameters of alligators through diagnostic blood values. Scute lead concentrations averaged 7.113 ± 1.980 mg/kg wet weight, mercury concentrations averaged 0.157 ± 0.023 mg/kg wet weight, and Aroclor 1268 concentrations averaged 1.532 ± 0.251 mg/kg wet weight. There was no significant effect of body size or sex on any of the contaminant concentrations examined. Additionally, there was no significant effects of distance of the capture location from the Superfund site or habitat use on concentrations of any of the contaminants we measured. There were positive effects of Aroclor 1268, lead, and mercury concentrations on packed cell volume values in blood from alligators. There was a significant negative relationship between Aroclor 1268 and glucose levels, yet there was a positive relationship between mercury

and glucose values. However, it is difficult to determine if the contaminants themselves are the cause of altered blood values or if there are other confounding variables. Lead levels of tail scutes from alligators were much higher than reported in previous studies using crocodilians, and mercury levels were comparable to other studies on crocodilians in Georgia and Florida. Additionally, a large number of alligators sampled in this study exceeded limits of PCBs and lead set for human consumption of food.

Introduction

Persistent environmental pollutants are a threat to both human and wildlife health. Contaminants such as persistent organic pollutants (POPs) and heavy metals tend to accumulate in aquatic environments as a result of runoff from terrestrial sources of contamination (Nriagu & Pacyna, 1988; Newman & Unger, 2003; Lamborg et al., 2014). Polychlorinated biphenyls are a specific type of POP that gained popularity in the early 1900s and were incorporated into multiple industrial processes (Fein et al., 1983). Although the use of PCBs was banned in 1977, their resistance to biodegradation and ability to bioaccumulate in organisms have resulted in a long-term threat to wildlife health (Choi et al., 2006). Mercury is released into the environment through coal burning power plants, steel mills, chloroalkali plants, and atmospheric deposition (Day et al., 2005; Obrist et al., 2018). Lead can enter the ecosystem through multiple point sources such as lead bullets and smelting sites, or non-point sources, like the use of leaded gasoline (Camus et al., 1998). Once heavy metals enter aquatic ecosystems, levels within the water column itself are relatively low. However, metals precipitate into marine sediments, exposing benthic organisms to potentially toxic levels of metals. Similar to PCBs, the heavy metals will then bioaccumulate through the food web as predators feed on benthic prey species (Caurant et al., 1999; Anan et al., 2001; Storelli et al., 2005; Camacho et al., 2013). Heavy metals and PCBs are d;angerous to wildlife because both can cause serious health effects even at relatively low exposure levels (Storelli et al., 2005; Day et al., 2007, D'Ilio et al., 2011). PCBs have been linked to numerous toxic effects on wildlife, including endocrine system disfunction, immunotoxicity, developmental abnormalities, and alterations in metabolism (Hoffman et al., 2003). Studies on fish, birds, mammals, and reptiles have shown that heavy metal toxicity can cause neurotoxicity, liver and kidney damage, abnormal development, decreased reproductive output, and altered endocrine and immune function in afflicted individuals (Zelikoff et al., 1994; Hoffman et al., 2003, Perrault et al., 2017*b*).

Brunswick, Georgia is the location of a former chloroalkali facility that was most recently owned by Linden Chemicals and Plastic (LCP Chemicals). The LCP Chemicals site was designated an Environmental Protection Agency (EPA) Superfund site in 1996 due to soil, groundwater, and sediment contamination by heavy metals and organic contaminants (US EPA, 1996). Further investigation found high levels of mercury, lead, chromium, zinc, PCBs, and polycyclic aromatic hydrocarbons (PAHs) in the soil and waters adjacent to the Superfund site (Kannan et al., 1997; Maruya & Lee, 1998). LCP Chemicals applied Aroclor 1268 to electrical equipment used in chemical processing (Kannan et al., 1998). This rare, highly-chlorinated PCB was only manufactured by one company that produced a limited amount of the chemical, and LCP Chemicals was the only user of this congener mixture in the southeast U.S. (Kannan et al., 1997; Maruya & Lee, 1998). Industrial wastes were discharged into holding pits on-site and directly into the marsh, resulting in the contamination of the marsh sediments with heavy metals and persistent organic pollutants (Kannan et al., 1997). It is estimated that throughout the history of the site, over 37 tons of PCBs and 440 tons of mercury were released into the environment surrounding the industrial facilities (Kannan et al., 1998).

The amounts of heavy metals and PCBs found in the immediate vicinity of the LCP Chemicals site were high enough to potentially cause harm to biota inhabiting the Turtle/Brunswick River Estuary (TBRE) adjacent to the marsh surrounding the Superfund site (Figure 4.1). Initial studies mainly investigated the distribution of Aroclor 1268 and mercury among invertebrates, fish, and birds (Kannan et al., 1998; Maruya & Lee, 1998, Blanvillain et al., 2007). However, more recent studies on the effects of these contaminants on organisms inhabiting the TBRE have included bottlenose dolphins (*Tursiops truncatus*) (e.g., Pulster & Maruya, 2008; Balmer et al., 2011; Wirth et al., 2014). PCB concentrations measured in upper level predators using the TBRE are high enough to affect reproductive and immune system function (Pulster et al., 2009; Balmer et al., 2011). Additionally, detection of Aroclor 1268 in some biota has not been limited to the Brunswick area, suggesting that the contaminants from the LCP Superfund site may be moving through the food chain on a regional scale through the movements of highly mobile species (Balmer et al., 2011; Wirth et al., 2014; Robinson et al., 2015).

There are no current records implicating PCB exposure with mortality events in alligators. However, understanding the sub-lethal effects of PCB exposure on alligators can provide insight into broader effects of contaminant exposure outside of noticeable mortality events (Guillette et al., 1999). PCBs have been shown to cause endocrine disruption in alligators by acting as hormonal antagonists or altering the synthesis and degradation of hormones (Crain & Guillette, 1997). Juvenile male alligators exposed to

PCBs have reduced testosterone levels in plasma when compared to alligators from reference locations (Guillette et al., 1999). Alligators with increased levels of PCBs have been linked to differentiated gene expression in juveniles from contaminated and reference locations (Hale et al., 2017). Alligator embryos are also susceptible to PCB exposure, primarily from maternal transfer (Cobb et al., 2002). Differences in reproductive hormone levels and gonadal modifications in neonatal alligators at the time of hatching have shown PCBs can cause organizational modifications in developing alligators (Guillette et al., 1995). Alligators exposed to elevated levels of POPs have produced offspring with polyovular follicles, poorly organized testes, and smaller phalluses (Guillette et al., 1995; Guillette et al., 1999). PCBs have also been theorized to cause reduced hatching success in alligators (Cobb et al., 1997).

Heavy metal exposure, like PCB exposure, is not typically attributed to mortality events in alligators. One case study involving a deceased alligator at the Savannah River Site in South Carolina, however, documented the highest levels of mercury recorded in any wildlife species, at levels well above what would be needed to cause heavy metal toxicity (Brisbin et al., 1998; Campbell, 2003). However, heavy metals have been suspected of causing sub-lethal effects, including altering sex steroid levels in alligators (Guillette et al., 1999). However, most of the research conducted on heavy metal exposure in alligators has been at locations with other pollutants such as PCBs and pesticides, making it difficult to attribute sublethal effects to heavy metal exposure (Burger et al., 2000).

Monitoring blood values in wildlife can be useful in evaluating the health of wildlife populations and diagnosing potential threats (Johnson et al., 2014). Basic blood diagnostics such as hematocrit or packed cell volume (PCV) can aid in determining the general health of an individual, including anemia or dehydration (Yu et al., 2011). Hematology can be used to detect conditions such as anemia, inflammatory diseases, parasitism, and other pathological disorders (Campbell, 2006). Both persistent organic pollutants and heavy metals have been shown to alter hematology in wildlife (McConnell, 1985; Grasman et al., 2000). Low hematocrit can be a sign of anemia, potentially caused by exposure to heavy metals or other contaminants (Peterson, 2002). Rats and primates exposed to PCBs have exhibited decreased hematocrit (PCV), red blood cell counts, and hemoglobin (Bruckner et al., 1973; Arnold et al., 1993; Chu et al., 1994). Exposure to organochlorine contaminants such as PCBs has been suggested to cause anemia in loggerhead sea turtles based on the correlation of lower PCV with increased PCB levels (*Caretta caretta*; Keller et al., 2004). Lead levels have been linked to increased glucose levels and total solids, while mercury has been shown to negatively affect glucose and total proteins (Kolesarova et al., 2008). Mercury has been linked to increased total proteins in the blood of Kemp's ridley (*Lepidochelys kempii*) and green sea turtles (*Chelonia mydas*; Perrault et al., 2017*a*). On the other hand, lead decreases total solids, iron, albumin, and alpha globulins in loggerhead sea turtles (Perrault et al., 2017*b*).

Long-lived vertebrates serve as ideal bioindicators of the effects of persistent pollutants due to their increased life spans, delayed sexual maturity, and upper-trophic level status as predators (Rowe, 2008). In this sense, American alligators (*Alligator mississippiensis*) are model organisms due to their life history traits (e.g., upper-level predator, long life span, delayed sexual maturity) and have been used as indicators of ecological health in previous studies (Mazzotti et al., 2008; Eversole et al., 2015). Alligators also take an extended amount of time to reach sexual maturity compared to most vertebrates, making them particularly vulnerable to the effects of bioaccumulative toxins as they have more time before reproducing to accumulate toxins that have negative effects on reproduction (Joanen & McNease, 1989; Dalrymple, 1996; Lance, 2003). Additionally, effects on alligator health attributed to pollutants have been correlated with similar effects in human populations, such as abnormal hormonal levels (Guillette & Guillette, 1996).

Alligators are considered a keystone species, meaning other wildlife species depend on them to survive (Khan & Tansel, 2000). Alligators create vital habitat for birds, reptiles, and amphibians through the creation of wallows and dens (Kushlan & Kushlan, 1980; Campbell & Mazzotti, 2004; Palmer & Mazzotti, 2004). Additionally, alligators serve critical roles in the food webs they occupy. They exhibit top-down control over prey species and can link discrete ecosystems through trophic interactions and movement between habitats? (de Roos et al., 1998; McCann et al., 2005; Subalusky et al., 2009; Rosenblatt & Heithaus, 2011). Coastal alligators forage on prey species that are commonly harvested commercially for human consumption (Nifong & Silliman, 2013). Additionally, wild alligators are harvested throughout their range in managed hunts for human consumption, potentially exposing humans to organic and inorganic pollutants the alligator has accumulated. The objective of this study was to assess the levels and potential effects of three contaminants of concern (Aroclor 1268, lead, and mercury) associated with the LCP Chemical Superfund site on a population of American alligators in the Brunswick area to determine the threat level to alligators and identify potential human health impacts.

Methods

Study site

The study site for this project was Jekyll Island, Georgia, USA. Jekyll Island is a 2,238-hectare barrier island along the southeastern coast of Georgia, located approximately 12 kilometers southeast of the LCP Superfund site. There are 34 freshwater lagoons on Jekyll with the majority (23) being located on the golf courses in the central part of the island (Skupien et al., 2016; Skupien & Andrews, 2017). The island is connected to the mainland via the Downing-Musgrove Causeway (DMC), an 8.7-kilometer roadway that bisects an intertidal marsh ecosystem that is mainly comprised of cordgrass (*Spartina* spp.). The saltmarsh on the northern side of the DMC represents the southern boundary of the fish consumption advisory zone for the Turtle/Brunswick River implemented by the Georgia Department of Natural Resources Environmental Protection Division due to PCB and heavy metal contamination from the LCP Chemicals Superfund site (GA EPD, 2018).

Jekyll Island is also home to the Jekyll Island Authority's Georgia Sea Turtle Center (GSTC), a rehabilitation facility specializing in the interconnection of wildlife rehabilitation, education, research, and mitigation strategies. Researchers from the GSTC and University of Georgia (UGA) have performed capture-mark-recapture on alligators since 2011. Additionally, spatial ecology studies have been performed to analyze movements and home ranges of adult alligators. While most alligators use freshwater habitats, some individuals use the marsh ecosystem (Skupien et al., 2016). Additionally, both adults and sub-adult alligators have been observed moving significant distances from Jekyll Island. One sub-adult alligator moved over 12 kilometers from its original capture location to the parking lot of the Brunswick Port Authority, and another adult alligator moved 9 kilometers from its original location into the outskirts of the city of Brunswick.

Sample collection

Alligator caudal tail scutes were collected during regular mark-recapture surveys or through opportunistic wildlife response calls. The area around the collection site was disinfected by applying two coats of alternating Betadine® Surgical Scrub (Purdue Pharma L.P., Stamford, CT, USA) and isopropyl alcohol. A sterile scalpel blade was used to remove the tail scute, and the tissue was placed in a Whirl-Pak® sample bag (Nasco Fort Atkinson, WI, USA) and placed on ice. Tissue samples were stored in an ultra-low freezer (-80°C) until contaminant analysis could be performed. Alligator sex was determined by visual inspection on smaller individuals. Male alligators have penes that are relatively large, round, and reddish whereas females have clitores that are smaller and white or pale red in comparison. For large individuals, sex was determined by manual palpation of the cloaca to feel for the presence or absence of a phallus. Additionally, a visual health exam was conducted on all alligators sampled in order to identify any physical abnormalities, injuries, or other symptoms of underlying conditions.

Morphometric measurements were collected on all captured individuals. Total length, snout-vent length, body girth, and tail girth were collected using a soft measuring tape to the nearest 0.1 cm. Head measurements including head length and head width were collected using calipers to the nearest 0.1 cm. Body condition indices can be useful in exploring the relationships between individual health and bioaccumulated contaminants. One such index is the Fulton's K index, which compares one skeletal size measurement with a measurement of volume into a ratio. Historically, total length or snout-vent length have been compared with mass (Zweig, 2003). However, mass can be difficult to obtain in the field, especially for larger individuals. Therefore, tail girth has been shown to be a suitable substitute for mass in Fulton's K calculations (Zweig et al., 2014). We calculated Fulton's K for captured alligators by comparing head length with tail girth (HL/TG), which has been shown to be the most accurate representation of body condition in alligators due to the decreased amount of error in these measurements between researchers (Zweig et al., 2003). The average Fulton's K ratios based on HL/TG measurement of alligators in south Florida was 1.447 ± 0.005 (0.994 – 1.725).

Blood collection was attempted on all individuals larger than one-meter total length. The area directly behind the skull was disinfected using two applications of alternating Betadine® Surgical Scrub (Purdue Pharma L.P., Stamford, CT, USA) and isopropyl alcohol. Blood was drawn from the supraoccipital sinus using a 20-gauge, 1.5-inch needle and a 3 mL heparinized syringe. Blood samples were wrapped in a towel and placed in a cooler with ice until they could be analyzed at the GSTC after sampling was complete, between 30 minutes and 3 hours after sampling. Glucose was measured using a FreeStyle Freedom Lite blood glucometer (Abbott Laboratories, Chicago, IL, USA) once the blood was transported back to the GSTC. A small amount of heparinized whole blood was transferred to a microhematocrit tube and centrifuged to measure packed cell volume

(PCV). Total solids in the plasma were measured by using a refractometer. All blood values were analyzed in duplicate.

Contaminant analysis

Mercury analysis was performed using a Direct Mercury Analyzer (DMA-80: Milestone, Shelton, CT, USA). Tail scute samples were thawed completely and then ~0.01 grams wet weight of sample was measured. Weighed samples were then placed in individual quartz sample boats (Milestone, Shelton, CT, USA) and run through the DMA-80. The DMA-80 analyzes mercury by thermally decomposing the samples and using carrier gas to transport the mercury and combustion products into a catalyst chamber. A gold amalgamator captures the mercury species before releasing the mercury into the spectrophotometer's optical path. After every ten samples analyzed, both a system blank and system standard (DORM-3 Fish Protein; National Research Council Canada, Ottawa, Canada) were run to ensure proper calibration of the machine. Mercury levels are reported as means ± 1 standard error, and all concentrations are based on wet weight.

For lead analysis, 0.2 grams of tissue were taken and weighed wet. Samples were placed in 5 mL of trace metal grade nitric acid (70% HNO_3; JT Baker Inc., Phillipsburg, NJ, USA) and then digested using microwave-assisted acid digestion. The remaining solution was diluted with 20 mL of double deionized water and transferred into 50 mL polypropylene centrifuge tubes (Corning, Corning, NY, USA). One mL of this solution was further diluted in 9 mL of 1% nitric acid in preparation for chemical analysis. Inductively coupled plasma-mass spectrometry (ICP-MS; Thermo Scientific, Waltham, MA, USA) was used to analyze for lead concentrations. Lead standards diluted in 1%

nitric acid were analyzed to quantify lead concentrations (SPEX CertiPrep, Metcuhen, NJ, USA). Standards and 1% nitric acid blanks were analyzed after every 10 samples run to ensure quality control and assurance. The minimum detection limit for lead concentrations by ICP-MS was 0.001 mg/kg. Lead levels are reported as means ± 1 standard error, and all concentrations are based on wet weight.

For PCB analysis, scute tissue samples were weighed to the nearest 0.1 mg to determine wet weight and placed into 50 mLpolypropylene centrifuge tubes (Corning, Corning, NY, USA). Samples were treated with 10 mL of 1:1 solution of acetone and dichloromethane (Fisher Scientific, Waltham, MA, USA). The centrifuge tubes were placed in an ultrasonic bath and sonicated for 60 minutes. After sonication, tubes were centrifuged at 4,000 RPM for 20 minutes until the solution was clear. The clear supernatant layer was decanted into glass evaporation tubes. The liquid extraction process was repeated once more using another 10 mL of the acetone dichloromethane solution added to the evaporation tubes. The combined extracts were carefully evaporated to near dryness at 60°C using a TurboVap® LV concentration workstation (Caliper Life Sciences, Hopkinton, MA, USA). After evaporation, 1 mL of methanol (VWR Chemicals, Radnor, PA, USA) was added to the evaporation tube, which was then vortexed for approximately 60 seconds. The methanol was carefully evaporated to near dryness under the same conditions. Afterwards, 0.8 mL of methanol was added to the evaporation tube and vortexed for approximately 60 seconds. The liquid was transferred into a 1 mL volumetric tube and brought to a final volume of 1 mL using methanol. This liquid was transferred into 2 mL glass crimp vials (Agilent Technologies, Santa Clara, CA, USA). The samples were analyzed using gas chromatography-mass spectrometry

(GC/MS; Varian Inc., Walnut Creek, CA, USA) and compared to an Aroclor 1268 standard (AccuStandard, New Haven, CT, USA). After every 10 samples analyzed, an Aroclor 1268 standard and methanol blank were both run through the GC/MS to ensure quality control and assurance. The minimum detection limits for Aroclor 1268 concentrations by GC/MS was 0.005 mg/kg. Aroclor 1268 levels are reported as means ± 1 standard error, and all concentrations are based on wet weight.

Habitat Use

Caudal tail samples were collected from alligators that were included in previous radiotelemetry studies using very high frequency (VHF) radio transmitters and global positioning system (GPS) loggers (Skupien et al., 2016). These alligators were tracked using a combination of VHF telemetry, GPS loggers, or both for at least a full year. Animals with VHF transmitters were manually tracked at least twice per week. The GPS loggers were programmed to record a position at least 10 times per day and the data was downloaded remotely once per month. Alligators that used the marsh at least once during the time they were monitored were considered "marsh habitat" alligators. If the animal only used freshwater lagoons on the island during the monitoring period, they were considered "freshwater habitat" alligators. If multiple samples were collected from the same tracked alligator, the contaminant values were averaged between the two samples so that each individual only had one set of concentrations associated with it.

Statistics

Distances of the alligators from the LCP Chemicals site were calculated in ArcMap 10.5.1 (ESRI, Redlands, CA, USA). GPS points from alligator capture locations were imported into ArcMap and the straight-line distance from the LCP Chemicals site (31.189440 N, 81.508330 S; US EPA, 2002) was calculated using the Measure tool.

Statistical tests were performed using program R 3.4.1 (R Core Team 2019). All data were tested for normality using the Shapiro-Wilk test. If data were not normally distributed, they were log-transformed prior to analysis. The effects of sex and Fulton's K on contaminant concentrations were analyzed using analysis of covariance (ANCOVA). The relationship between distance from the LCP Chemicals site and contaminant concentrations was tested using simple linear regressions. The differences between contaminant concentrations of alligators that used marsh habitat and alligators that only used freshwater habitats was tested using one-tailed Welch's t-test. Effects of Aroclor 1268, lead, and mercury on packed-cell volume, glucose, and total solids were each tested using ANCOVA. The effects of each contaminant were tested individually, along with interactions between Aroclor 1268 and lead, Aroclor 1268 and mercury, lead and mercury, and all three contaminants together.

Results

Summary statistics

Alligators were sampled between May 2012 and May 2018, resulting in the collection of 64 tissue samples (Figure 4.1). Twenty-five samples were from females, 37 samples were from males, and 2 samples were from alligators of unknown sex. Total length was collected from all 64 alligators sampled, with an average length of 198.55 ± 8.50 cm (range: 54.6 – 339.1 cm). Head length measurements were collected from 60 individuals, with a mean length of 26.878 ± 1.249 cm (13.1-48.3 cm). Tail girth measurements were collected from 61 individuals with a mean circumference of 43.334 ± 2.302 cm (19.7-99.1 cm). Two samples tested for Aroclor 1268 were below the detection limit (0.005 mg/kg) and half the detection limit (0.0025 mg/kg) was substituted for their concentration. The average Aroclor 1268 concentration for the 64 samples was 1.532 ± 0.251 mg/kg wet weight (0.0025-8.211 mg/kg). Two samples analyzed for lead were below the detection limit (0.001 mg/kg) and half the detection limit (0.0005 mg/kg) was substituted for their concentration. The 64 samples' lead concentration averaged 7.113 ± 1.980 mg/kg wet weight (0.0005-80.052 mg/kg). Sixty-three samples were analyzed for mercury, with an average concentration of 0.157 ± 0.023 mg/kg wet weight (0.005-0.893 mg/kg).

Relationship between contaminant levels and sex and body condition

Of the 37 male alligators sampled, head length and tail girth measurements were collected from 34. Head length and tail girth measurements were collected from all 25 females sampled. The average Fulton's K score for male alligators was 0.626 ± 0.010 (0.462-0.739). The average Fulton's K score for female alligators was 0.653 ± 0.008 (0.541-0.760). Aroclor 1268 concentrations averaged 1.532 ± 0.251 mg/kg wet weight (0.0025-6.310 mg/kg) in males and averaged 1.950 ± 0.432 mg/kg wet weight (0.013-8.211 mg/kg) in females (Figure 4.2). Lead concentrations averaged 7.113 ± 1.980 mg/kg wet weight (0.0005-80.052 mg/kg) in males and averaged 7.812 ± 2.407 mg/kg wet weight (0.009-55.064 mg/kg) in females (Figure 4.3). Mercury concentrations averaged 0.190 ± 0.036 (0.009-0.893 mg/kg) in males and averaged 0.115 ± 0.018 mg/kg wet weight (0.005-0.373 mg/kg) in females (Figure 4.4). There was no significant relationship between any of the independent variables (sex, Fulton's K score, or the interaction of sex and Fulton's K score on Aroclor 1268, lead, or mercury concentrations (Table 4.1).

Relationship between contaminant levels and distance from LCP Chemicals site

The average distance of the alligator capture locations from the LCP Chemicals site was 15.975 ± 0.220 kilometers (11.143-19.999 km; Figure 4.1). There was a positive relationship between distance and Aroclor 1268 concentrations, however this relationship was not significant ($F_{1,62} = 0.144$, $p = 0.706$; Figure 4.5). There was no relationship between distance from LCP Chemicals on lead concentration in alligators ($F_{1,62} = 0.122$, $p = 0.728$; Figure 4.6). or mercury concentrations in alligators ($F_{1,61} = 0.044$, $p = 0.834$; Figure 4.7).

Habitat Use

Ten samples were collected from alligators that had been tracked using VHF telemetry or GPS loggers. Three alligators were tracked using VHF telemetry, two were tracked using GPS loggers, and five were tracked using a combination of VHF telemetry and GPS loggers. The mean number of relocations obtained for alligators tracked using VHF telemetry was 118.88 ± 13.86 (65 – 192). The mean number of relocations collected by GPS loggers on alligators was 2308.29 ± 602.99 (725 – 4544). Four of these alligators had been documented using the marsh habitat and the other six individuals had been observed only using freshwater ponds on the island. Aroclor 1268 and lead concentrations were higher in alligators that used the marsh environment compared to animals that remained in freshwater ecosystems, however neither of these were significant effects. The mean Aroclor 1268 concentration for individuals using marsh habitat was 2.626 ± 1.471 mg/kg wet weight (0.189-6.310 mg/kg). The mean Aroclor 1268 concentration for alligators that only used island ponds was 0.616 ± 0.205 mg/kg wet weight (0.048-1.655 mg/kg). There was no significant difference in Aroclor 1268 concentrations in alligators that had different habitat usages (t (3.047) = 1.478, p = 0.117; Figure 4.8). The mean lead concentration for alligators that used marsh habitats was 24.119 ± 18.879 mg/kg wet weight (0.020-80.052 mg/kg). The average lead concentration for alligators that only used island ponds was 6.685 ± 3.817 mg/kg wet weight (0.395-26.454 mg/kg). There was no significant difference in lead concentrations in alligators that had different habitat usages (t (3.323) = 0.849, p = 0.226; Figure 4.9). The average mercury concentration for alligators that used marsh habitats was 0.166 ± 0.084 mg/kg wet weight (0.046-0.404 mg/kg). The mean mercury concentration for alligators that only used freshwater habitats was 0.286 ± 0.104 mg/kg wet weight (0.065-0.849 mg/kg). There was no significant difference in mercury concentrations in alligators that had different habitat usages (t (7.945) = -1.039, p = 0.835; Figure 4.10).

Relationship of contaminants on blood values

Blood was drawn from 21 alligators that were sampled for contaminants. The average packed cell volume (PCV) was 25.714 ± 1.666% (16-45%; n=21). Aroclor 1268, lead, and mercury all exhibited positive relationships with PCV, meaning PCV increased as concentrations of contaminants also increased (Figure 4.11). However, the only significant relationship was between PCV and the interaction between Aroclor 1268, lead, and mercury combined ($F_{1,13}$ = 5.082, p = 0.042; Table 4.2).

The average glucose level was 84.050 ± 3.927 mg/dL (56-131 mg/dL; n=20). Aroclor 1268 exhibited a negative relationship on glucose levels, meaning glucose levels decreased as contaminant concentrations increased (Figure 4.12). This is in contrast to lead and mercury, which both showed positive relationships with glucose levels. However, only the interaction between Aroclor 1268 and mercury showed a significant relationship with glucose levels ($F_{1,12} = 9.156$, $p = 0.011$; Table 4.2)

The average total solids level was 4.995 ± 0.228 g/dL (2.0-7.2 g/dL; n=20). Mercury concentrations had a positive relationship on total protein level, lead concentrations had a slightly positive relationship, and Aroclor 1268 concentrations had negative relationships on total solids levels in blood (Figure 4.13). However, none of these were significant, and no interactions among the contaminants were significant (Table 4.2).

Discussion

Tail scutes from alligators sampled in this study contained a wide range of Aroclor 1268, lead, and mercury concentrations. Caudal tail scutes have frequently been used for studying heavy metal concentrations in crocodilian populations (Odierna, 1995; Heaton-Jones et al., 1997; Yanochko et al., 1997; Jagoe et al., 1998; Burger et al., 2000; Rainwater et al., 2007; Trillanes et al., 2014; Buenfil-Rojas et al., 2015; Nilsen et al., 2017). A summary of prior studies that analyzed caudal tail scutes from American alligators, American crocodiles (*Crocodylus acutus*), and Morelet's crocodiles (*Crocodylus moreletii*) for lead is shown in Table 4.3. The average lead concentration in alligators sampled in this study (7.342 ± 2.038 mg/kg wet weight) was much higher than the average lead concentrations found in alligators in Florida (Burger et al., 2000; Nilsen et al., 2017). Additionally, the lead levels found in this study was much higher than American and Morelet's crocodiles from Belize and Mexico (Rainwater et al., 2007;

Trillanes et al., 2014). In a separate study not listed in Table 4.3, multiple caiman species in Brazil were found to have lead levels ranging from less than 0.5 mg/kg to 84 mg/kg (Odierna, 1995). Most of these individuals had less than 0.5 mg/kg of lead, but 34.4% had levels between 0.5 mg/kg and 2.0 mg/kg, and 14.5% had levels greater than 2.0 mg/kg of lead (Odierna, 1995; Brazaitis et al., 1996). These values are much lower than the values we observed in alligators in this study, with 45.2% (n=28) animals having lead concentrations greater than 2.0 mg/kg wet weight, the highest category of lead concentration defined by Odierna (1995) and Brazaitis et al. (1996).

Mercury levels in caudal tail scutes of alligators have been investigated in many studies across the southeastern United States. A summary of the mercury concentrations found in caudal tail scutes is shown in Table 4.4. Studies that tested alligators on the Savannah River Site in South Carolina and the Florida Everglades found average mercury concentrations of 1.03 ± 0.42 mg/kg wet weight (Heaton-Jones et al., 1997), 1.205 ± 0.166 mg/kg wet weight (Yanochko et al., 1997), 1.534 ± 0.274 mg/kg wet weight Jagoe et al., 1998), and 0.319 mg/kg wet weight (Nilsen et al., 2017). All of these studies found mercury concentrations in alligators higher than the average mercury concentrations in this study (0.157 ± 0.023 mg/kg wet weight). However, the levels in this study are comparable to alligators from central Florida, and are higher than alligators from the Okefenokee Swamp, located approximately 70 kilometers west of Jekyll Island (Burger et al., 2000; Jagoe et al., 1998). This suggests that mercury levels in alligators are highly variable at a regional level. The mercury concentrations found in alligators in this study area are also comparable to studies investigating mercury in crocodilians in Central America. The individuals in our study had higher mercury concentrations than American and Morelet's crocodiles in Belize and Mexico (Rainwater et al., 2007; Trillanes et al., 2014). However, another study investigating different populations of Morelet's crocodiles in Mexico found concentrations of mercury higher than our study (Buenfil-Rojas et al., 2015).

Although caudal tail scutes are commonly used to monitor heavy metals in alligators, only one study previous to this one has used scutes for PCB analysis (Gonzalez-Jauregui et al., 2012). Unfortunately, the total PCB levels in the scutes were not reported for this study, making us unable to compare PCB burdens. However, the authors did mention that only 50% of the Morelet's crocodiles in their study showed PCB contamination. This is much lower than the 96.9% (n=62) of individuals with measurable PCB levels in our study.

The average PCV for alligators ranges between 16.3 and 20.5%, glucose averages 74 mg/dL, and total solids levels average between 4.3 and 5.1 g/dL (Dessauer, 1970; Johnson et al., 2014). Blood parameters measured in this study were fairly close to these reference ranges (mean PCV 25.7%, mean glucose 84.1 mg/dL, mean total proteins 5.0 g/dL). Packed cell volume was higher in alligators that had higher levels of Aroclor 1268, lead, and mercury in this study. Additionally, the interaction between all three contaminants was significantly positively correlated with PCV values. This is contradictory to past research on the effects of contaminants on blood diagnostics. Most studies have found that PCBs typically cause lower PCV values, leading to anemia in exposed wildlife (Bruckner et al., 1973; Arnold et al., 1993; Chu et al., 1994; Keller et al., 2004). The PCV values may also be indicative of other health effects on the alligators. The PCV may be higher due to dehydration from the stress of capture.

However, the mean PCV in our study was not unusually higher than what is found in healthy animals (Dessauer, 1970).

The interaction between Aroclor 1268 and mercury had a significant relationship on glucose levels in alligators, however the two contaminants exhibited different effects. Aroclor 1268 had a negative relationship on glucose levels, whereas mercury had a positive relationship on glucose levels. Previous research on mammals has found mercury to have negative relationships on glucose levels (Kolesarova et al., 2008). However, glucose levels in alligators can vary drastically between individuals and time of year. Additionally, there are many other factors that may affect glucose levels in sampled alligators including stress to the animal during capture, the specific type of capture method used, stress due to disease, and others. Furthermore, glucose levels are negatively correlated with warmer temperatures in alligators (Coulson & Hernandez, 1953). Therefore, variation in glucose levels in our study may be attributed to the weather during capture. Additionally, variation may be explained by the time since the individuals' last meal. Alligators do not need to eat regular meals and can fast for long periods of time due to being ectothermic. If an alligator has fasted for a period of time, glucose levels may be lower than normal. The time between blood collection and sampling could also be a factor in glucose levels. Glucose will drop even after being collected from an animal if it is in contact with red blood cells. Blood that took longer to transport back to the GSTC (approximately 3 hours) would be lower than blood that was processed quicker (approximately 30 minutes). However, the time between blood collection and processing was not recorded.

Furthermore, our study revealed no relationships of contaminant concentrations on total solids levels. This is also contrary to other studies showing significant effects of lead and mercury on plasma total solids (Kolesarova et al., 2008; Perrault et al., 2017*a*; Perrault et al., 2017*b*). Ultimately, concentrations of all three contaminants of concern did not account for the variation between blood values in the alligators we tested based on the low r^2 values, even in models that had significant interactions. Although the effects of PCBs and heavy metals have been examined in other wildlife species, only a few studies have been conducted on reptiles (Keller et al, 2004; Yu et al., 2011; Perrault et al., 2017*a*, Perrault et al., 2017*b*). It is therefore difficult to conclude that PCB and heavy metal exposure is causing physiological effects in the alligator in the Brunswick area. Further study is required in order to determine the potential negative effects on alligators caused by exposure to contaminants associated with the LCP Chemicals Superfund site.

Analysis between concentrations of contaminants and distance from the LCP site did not show any significant correlations. This is not very surprising given the habits of alligator movement in the Brunswick area. Alligators from Jekyll Island that were tracked using VHF telemetry and GPS loggers have expansive home ranges (Skupien et al., 2016). Additionally, alligators from Jekyll Island have variation in their habitat use. Some individuals have been found to use the marsh ecosystem extensively, presumably for feeding, whereas other individuals prefer to remain on Jekyll Island exclusively. The fact that alligators are highly mobile makes geographic analysis of contaminant concentrations on this scale difficult. Both alligators that were captured on the island directly to the North of Jekyll Island (St. Simons Island; Figure 4.1) were originally captured and tagged on Jekyll Island. If these animals had not been marked prior to appearing on St. Simons Island, it would be impossible to tell that they were actually from Jekyll Island, much further from the LCP Chemicals site. Additionally, one of these animals had an original capture location on Jekyll Island 14.5 kilometers from the LCP Chemical site but had also been documented in downtown Brunswick as close as 7.0 kilometers to the Superfund site. Without finer scale spatial data on each individual sampled, it is difficult to model the effects of distance from the LCP Chemicals site on contaminant concentrations. Other studies investigating the geographic distribution of contaminants associated with the LCP Chemicals site among wildlife were on a broader, regional scale (Balmer et al., 2011; Wirth et al., 2014; Robinson et al., 2015).

Heavy metal concentrations in caudal tail scutes have been shown to correlate strongly with heavy metal concentrations in other tissues, including muscle tissue (Burger et al., 2000; Nilsen et al., 2017). This is important since alligators can be legally harvested and alligators are, in fact, regularly harvested from the Brunswick area for consumption. The state of Georgia has 10 hunting zones for alligator harvest throughout the state. The zone that includes Brunswick had the second greatest number of harvests during the 2017 hunting season (GA DNR, 2017). Additionally, the number of harvests in this zone has been increasing, with the 2014-2016 harvests being three of the four largest in the past 15 years. The 2017 harvest had the same number of permits issued to hunters as 2016 but had a sharp decline in the number of hunters that successfully harvested an alligator (GA DNR, 2017).

People that are consuming meat from alligators exposed to PCBs or heavy metals from the LCP site in Brunswick may be putting themselves at risk. The EPA and the Food and Drug Administration (FDA) are responsible for issuing recommendations regarding how high mercury levels can be in food to be considered safe for human consumption. The FDA and EPA recommend that fish meat have < 0.46 mg/kg mercury (wet weight) to be safe for human consumption when eating one serving per week (US EPA, 2018). From the animals included in our study, 4.7% (n=3) exceeded this level of mercury. The FDA has set a limit of 0.1 mg/kg of lead in food for human consumption (US FDA, 2018*a*). From the alligators we sampled, 73.4% (n=47) exceeded this threshold. However, our study analyzed the caudal tail scutes, not the muscle itself, which is the tissue humans are most likely to consume. Typically, alligator muscle has higher levels of heavy metals than the scutes, so a larger percentage of our animals are potentially above the recommended safe level of mercury concentration (Burger et al., 2000; Nilsen et al., 2017). Using the results of Burger et al. (2000) and Nilsen et al. (2017), we can extrapolate the hypothetical muscle concentrations of mercury and lead from the alligators in this study. Based on these conversions 60.3% (n=38) of the alligators exceeded the 0.1 mg/kg limit for lead in muscle tissue and 1.6% (n=1) of the alligators exceeded the 0.46 mg/kg limit for mercury in muscle tissue. Additionally, the FDA has implemented a limit of 3 mg/kg of PCBs in food for human consumption as an action level (US FDA, 2018*b*). From the individuals we sampled, 20.3% (n=13) exceeded the FDA threshold for PCB concentrations. There are no studies investigating the correlation between PCBs in scute tissues and PCB concentrations in muscle tissue, however it can reasonably be assumed that muscle concentrations will be higher, as is the case with heavy metals. Therefore, potentially a greater number of individuals sampled exceeded the FDA threshold.

Previous research has also found heavy metal concentrations above the limit that is deemed safe for human consumption (Yanochko et al., 1997; Jagoe et al., 1998). These results led to the temporary suspension of the alligator harvest in Florida in 1989 and 1990 (Campbell, 2003). Continued monitoring of harvested wild alligators intended for human consumption has been recommended for both South Carolina and Georgia, however no consistent testing for contaminants has been implemented (Ruckel, 1993; Bowles, 1996).

Although we cannot draw any conclusions regarding the potential effects these contaminants are having on the alligator population within the Brunswick area, our results reinforce the need for persistent monitoring of alligator meat for contaminants. Lead and PCB concentrations in the tissue samples we collected are much higher than previously reported for alligators and other species of crocodilians. We also had individuals that exceeded levels set by the EPA and FDA as safe for human consumption in PCBs, lead, and mercury. Care needs to be taken by hunters in their consumption of alligators captured in proximity to Superfund sites in the Brunswick area. Furthermore, alligators in coastal areas often eat the same prey species that humans consume (Nifong & Silliman, 2013). Assuming alligators in this study mainly accumulated PCBs and heavy metals through their prey, seafood harvested in Brunswick may contain contaminants that high enough to cause high levels of bioaccumulation in humans as well. Further research is needed to investigate the potential human health risk of eating seafood from the Brunswick area.

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Contaminant	Response	df	Mean Squares	F value	*p*
Aroclor 1268	Fulton's K	1	1.550	0.359	0.551
	Sex	1	5.765	1.336	0.253
	Fulton's K * Sex	1	0.181	0.062	0.804
	Residuals	55	4.315	-	-
Lead	Fulton's K	1	1.182	0.155	0.695
	Sex	1	25.694	3.370	0.072
	Fulton's K * Sex	1	3.364	0.441	0.509
	Residuals	55	7.624	-	-
Mercury	Fulton's K	1	0.107	0.104	0.749
	Sex	1	0.711	0.688	0.411
	Fulton's K * Sex	1	0.141	0.137	0.713
	Residuals	54	1.033	-	-

Table 4.1: Summary of the results of the analysis of covariance (ANCOVA) of body condition index (Fulton's K) and sex (male, female) on Aroclor 1268, lead, and mercury levels in caudal tail scutes of American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA.

Blood	Response	df	Mean Squares	F value	*p*
Packed Cell Volume	Aroclor 1268	1	73.01	1.334	0.269
	Lead	1	0.01	0.0002	0.988
	Mercury	1	42.13	0.770	0.396
	Aroclor 1268 * Lead	1	23.61	0.431	0.523
	Aroclor 1268 * Mercury	1	35.46	0.648	0.435
	Lead * Mercury	1	2.28	0.042	0.841
	Aroclor 1268 * Lead *Mercury	1	278.18	5.082	**0.042**
	Residuals	13	54.74	-	-
Glucose	Aroclor 1268	1	11.59	0.058	0.813
	Lead	1	93.11	0.468	0.507
	Mercury	1	430.39	2.162	0.167
	Aroclor 1268 * Lead	1	544.07	2.734	0.124
	Aroclor 1268 * Mercury	1	1822.49	9.156	**0.011**
	Lead * Mercury	1	308.75	1.551	0.237
	Aroclor 1268 * Lead *Mercury	1	260.05	1.307	0.275
	Residuals	13	199.04	-	-
Total Proteins	Aroclor 1268	1	2.399	2.076	0.175
	Lead	1	1.332	1.153	0.304
	Mercury	1	0.042	0.037	0.851
	Aroclor 1268 * Lead	1	0.178	0.154	0.701
	Aroclor 1268 * Mercury	1	1.766	1.528	0.240
	Lead * Mercury	1	0.030	0.026	0.874
	Aroclor 1268 * Lead *Mercury	1	0.135	0.117	0.738
	Residuals	12	1.156	-	-

Table 4.2: Summary of the analysis of covariance (ANCOVA) of combinations of Aroclor 1268, lead, and mercury concentrations in the caudal tail scutes of American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA and their effects on blood values (packed cell volume, glucose, and total proteins). Significant correlations are highlighted in bold font.

Species	Location	n	Dry weight (mg/kg)	Wet weight (mg/kg)	Study
A. mississippiensis	Central Florida, USA	22		0.101 ± 0.041	Burger et al., 2000
A. mississippiensis	Florida, USA	30		0.030	Nilsen et al., 2017
A. mississippiensis	Brunswick, Georgia, USA	63		7.113 ± 1.980	This study
C. acutus	Río Grande de Tárcoles, Costa Rica	6		0.492 ± 0.420	Rainwater et al., 2007
C. moreletii	New River Watershed, Belize	10		0.110 ± 0.078	Rainwater et al., 2007
C. moreletii	Champotón River, Mexico	7	0.064 ± 0.045	0.017 ± 0.012*	Trillanes et al., 2014
C. moreletii	Biosphere Reserve Los Petenes-Celestún, Mexico	10	0.065 ± 0.058	0.017 ± 0.015*	Trillanes et al., 2014
C. moreletii	Mocú and Silvituc lagoons, Mexico	6	0.017 ± 0.010	0.004 ± 0.003*	Trillanes et al., 2014

Table 4.3: Summary of studies that have measured lead levels in the caudal tail scutes of crocodilians. Lead concentrations are listed as the mean ± 1 SE. Studies that reported lead concentrations in dry weight were converted to wet weight (marked with *) by dividing the dry weight concentrations by 3.8 as calculated by Jeffree et al. (2001).

Species	Location	n	Dry weight (mg/kg)	Wet weight (mg/kg)	Study
A. mississippiensis	Everglades, Florida, USA	12		1.03 ± 0.42	Heaton-Jones et al., 1997
A. mississippiensis	Florida, USA	12		0.340 ± 0.330	Heaton-Jones et al., 1997
A. mississippiensis	Holiday Park, Florida, USA	7	5.120 ± 1.010	1.347 ± 0.266*	Yanochko et al., 1997
A. mississippiensis	WCA-3A, Florida, USA	10	6.330 ± 1.040	1.666 ± 0.274*	Yanochko et al., 1997
A. mississippiensis	Savannah River Site, South Carolina, USA	39	4.580 ± 0.630	1.205 ± 0.166*	Yanochko et al., 1997
A. mississippiensis	Everglades, Florida, USA	17	5.830 ± 1.040	1.534 ± 0.274*	Jagoe et al., 1998
A. mississippiensis	Central Florida, USA	20	0.520 ± 0.090	0.137 ± 0.024*	Jagoe et al., 1998
A. mississippiensis	Okefenokee Swamp, Georgia, USA	9	0.290 ± 0.030	0.076 ± 0.008*	Jagoe et al., 1998
A. mississippiensis	Savannah River Site, South Carolina, USA	39	5.140 ± 0.640	1.353 ± 0.168*	Jagoe et al., 1998
A. mississippiensis	Central Florida, USA	22		0.051 ± 0.009	Burger et al., 2000
A. mississippiensis	Florida, USA	30		0.319	Nilsen et al., 2017
A. mississippiensis	Brunswick, Georgia, USA	62		0.157 ± 0.023	This study
C. acutus	Río Grande de Tárcoles, Costa Rica	6		0.094 ± 0.027	Rainwater et al., 2007
C. moreletii	New River Watershed, Belize	10		0.073 ± 0.020	Rainwater et al., 2007
C. moreletii	Gold Button Lagoon, Belize	9		0.099 ± 0.022	Rainwater et al., 2007
C. moreletii	Champotón River, Mexico	7	0.064 ± 0.045	0.017 ± 0.012*	Trillanes et al., 2014
C. moreletii	Biosphere Reserve Los Petenes-Celestún, Mexico	10	0.065 ± 0.058	0.017 ± 0.015*	Trillanes et al., 2014
C. moreletii	Mocú and Silvituc lagoons, Mexico	6	0.017 ± 0.010	0.004 ± 0.003*	Trillanes et al., 2014
C. moreletii	Pucte, Rio Hondo, Mexico	9		0.261 ± 0.165	Buenfil-Rojas et al., 2015
C. moreletii	Cocoyol, Rio Hondo, Mexico	7		0.233 ± 0.101	Buenfil-Rojas et al., 2015
C. moreletii	La Union, Rio Hondo, Mexico	8		0.562 ± 0.636	Buenfil-Rojas et al., 2015

Table 4.4: Summary of studies that have measured mercury levels in the caudal tail scutes of crocodilians. Mercury concentrations are listed as the mean ± 1 SE. Studies that reported lead concentrations in dry weight were converted to wet weight (marked with *) by dividing the dry weight concentrations by 3.8 as calculated by Jeffree et al. (2001).

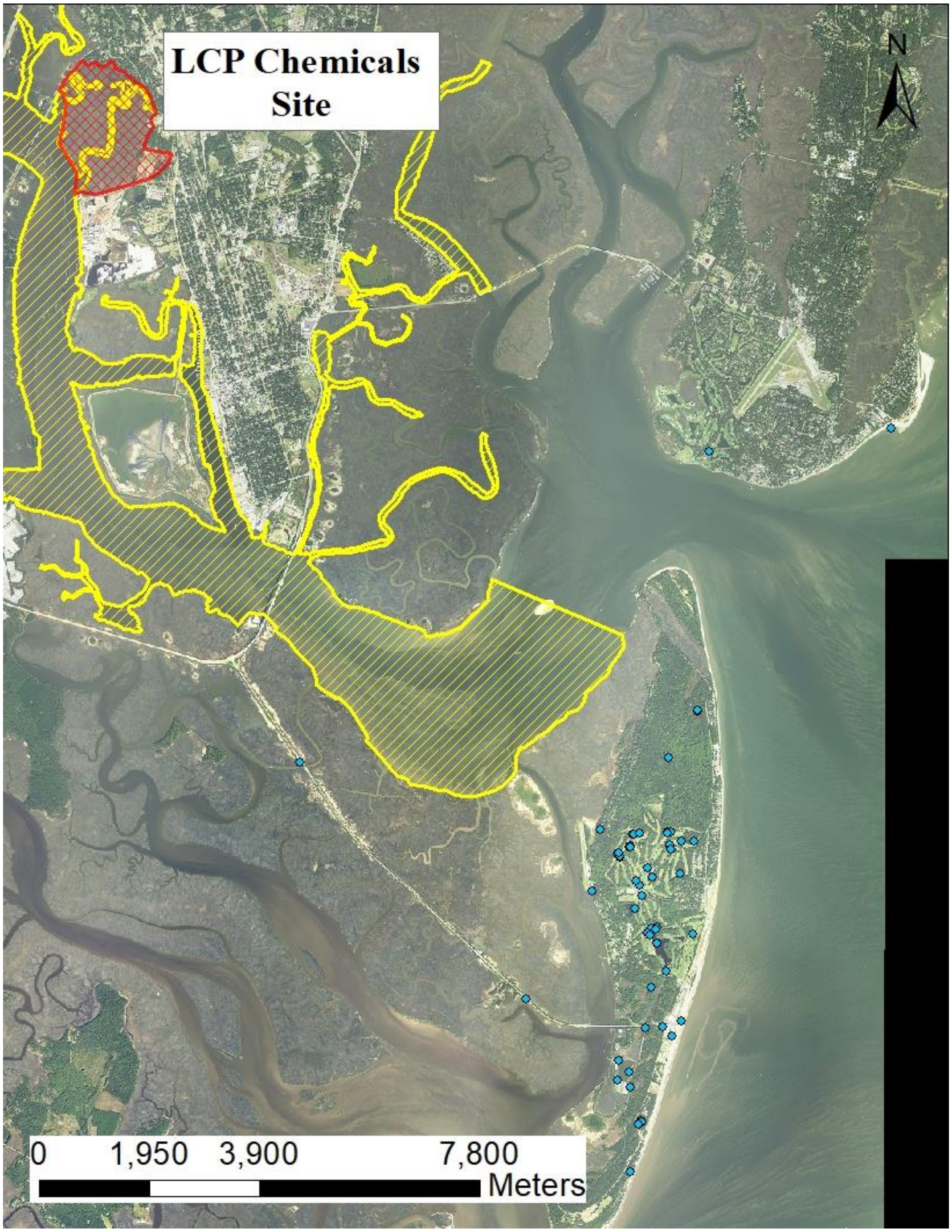

Figure 4.1: Map of the Turtle/Brunswick River Estuary, showing the LCP Chemicals Superfund site (outlined in red crosshatching), the fishing advisory zone (outline in yellow hatching) and the GPS positions of the 62 American alligators (*Alligator mississippiensis*; blue dots) collected for this study around Jekyll Island, Georgia, USA.

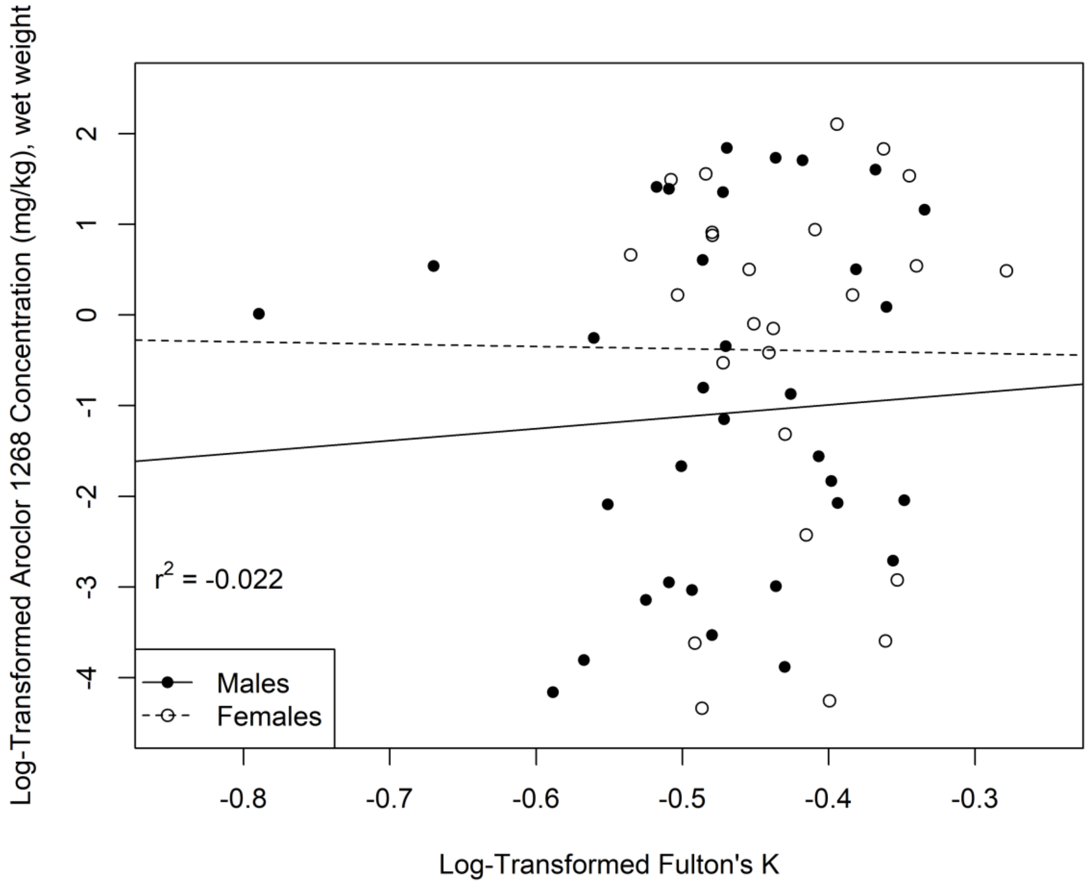

Figure 4.2: Relationship between log-transformed body condition index (Fulton's K) and log-transformed Aroclor 1268 concentrations of skin samples in male and female American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA.

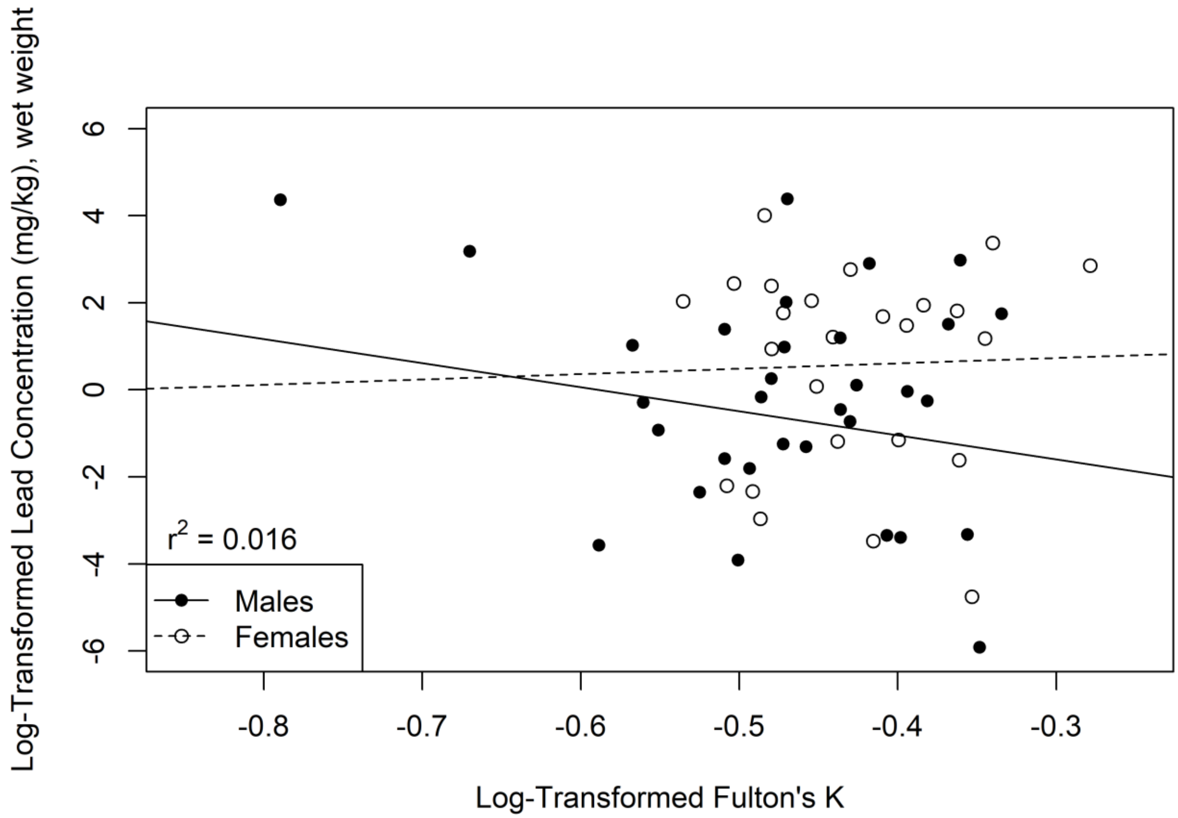

Figure 4.3: Relationship between log-transformed body condition index (Fulton's K) and log-transformed lead concentrations of skin samples in male and female American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA.

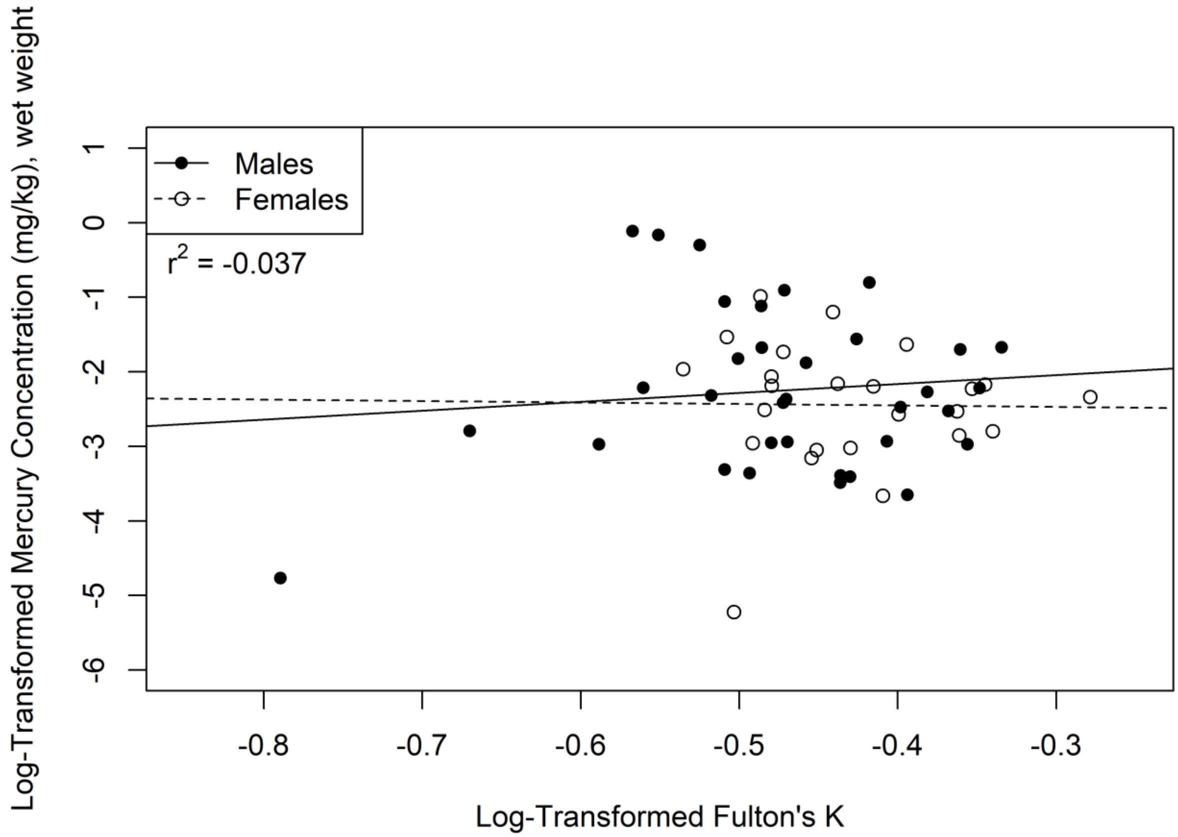

Figure 4.4: Relationship between log-transformed body condition index (Fulton's K) and log-transformed mercury concentrations of skin samples in male and female American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA.

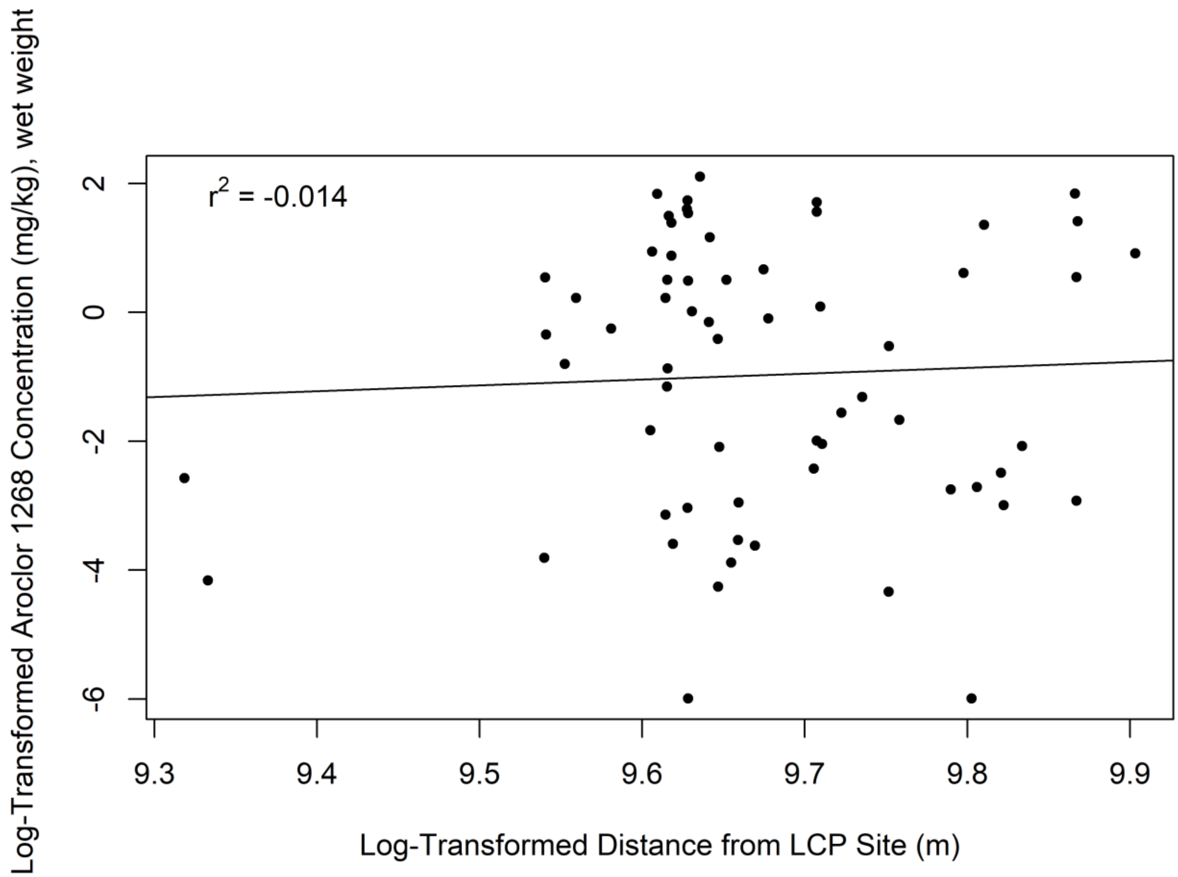

Figure 4.5: Relationship between log-transformed distance from the LCP Chemical Superfund site and log-transformed Aroclor 1268 concentrations ($F_{1,62}$ = 0.144, p = 0.706) in caudal tail scutes of American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA.

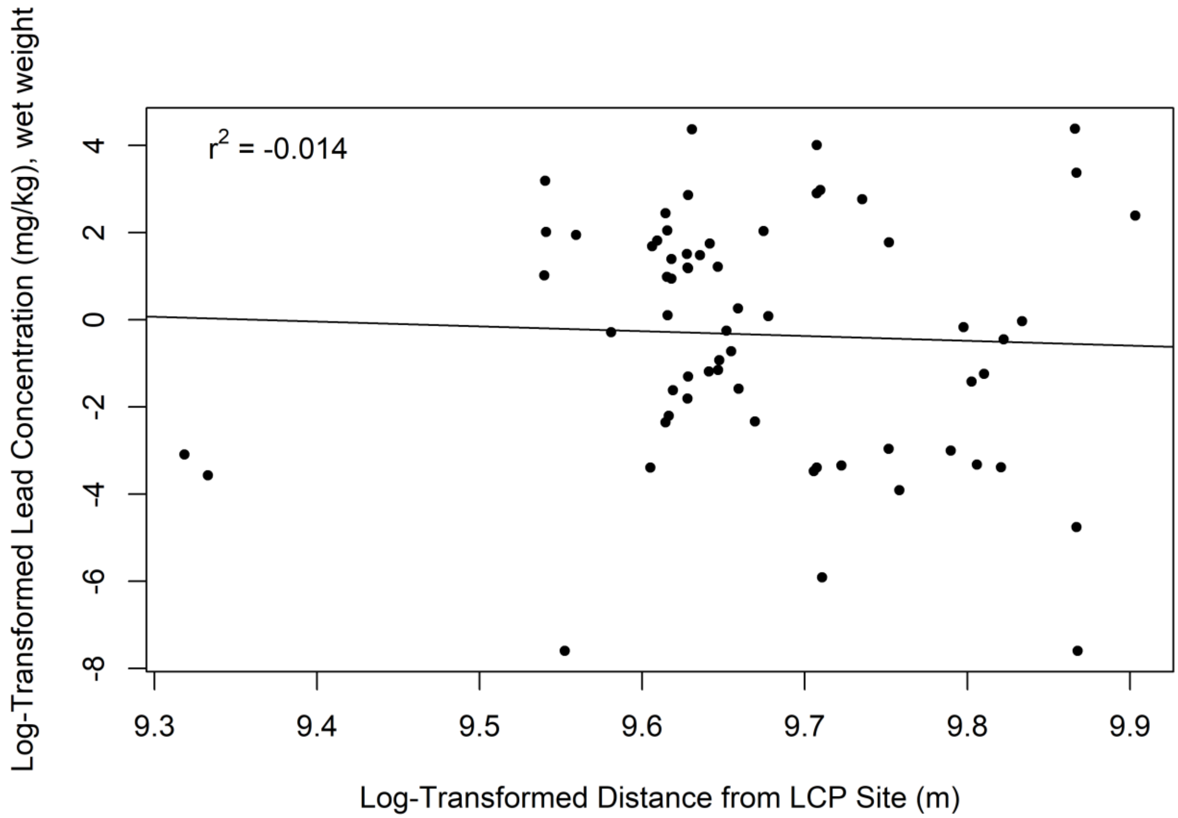

Figure 4.6: Relationship between log-transformed distance from the LCP Chemical Superfund site and log-transformed lead concentrations ($F_{1,62}$ = 0.122, p = 0.728) in caudal tail scutes of American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA.

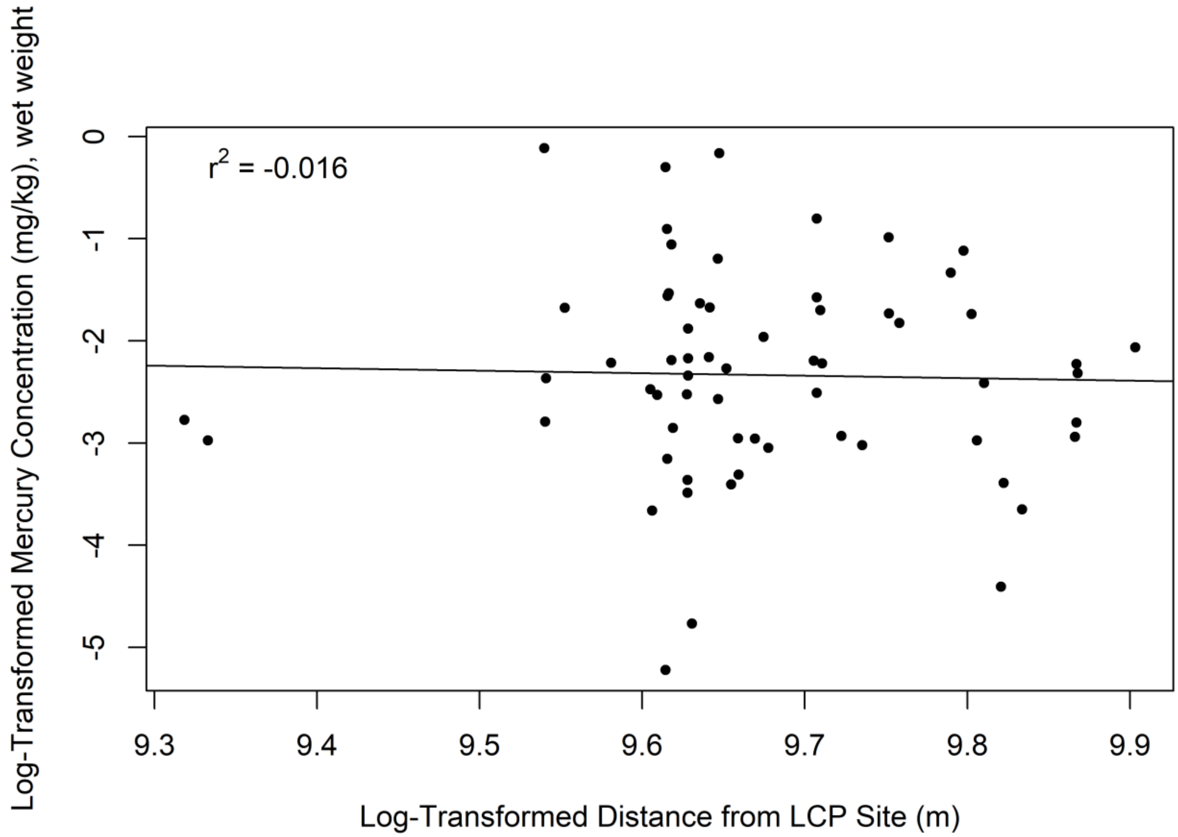

Figure 4.7: Relationship between log-transformed distance from the LCP Chemical Superfund site and log-transformed mercury concentrations ($F_{1,61} = 0.044$, $p = 0.834$) in caudal tail scutes of American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA.

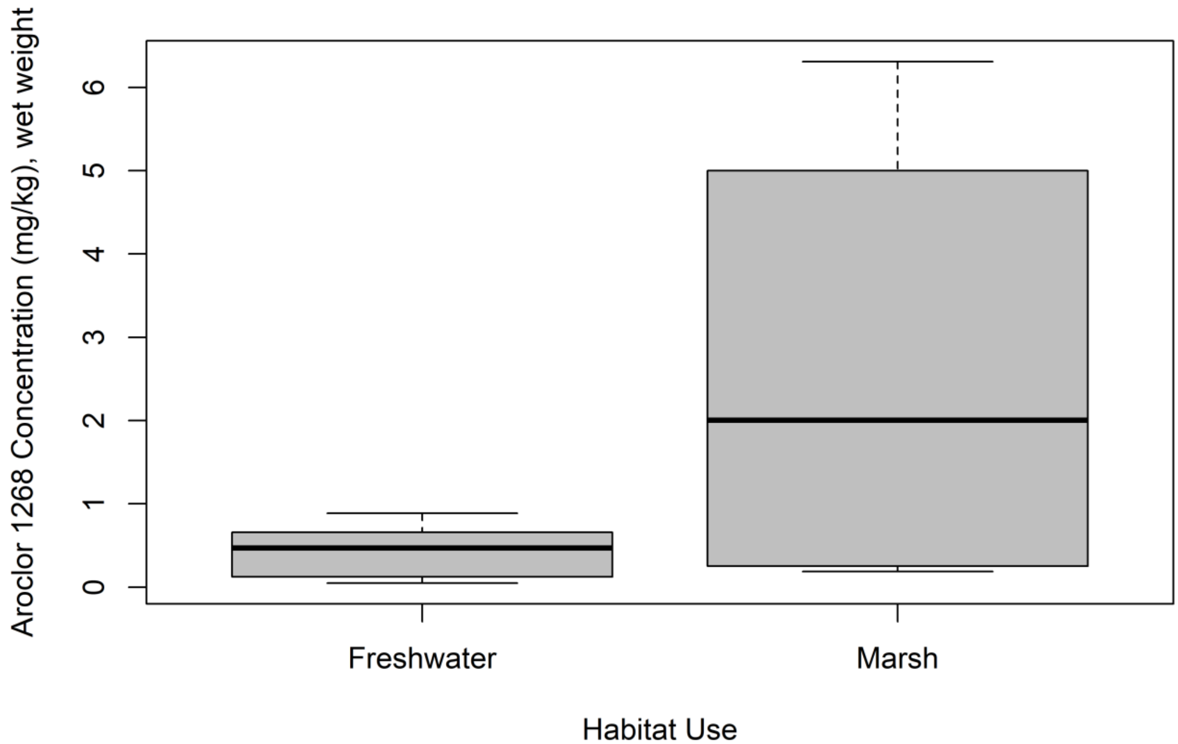

Figure 4.8: Comparison of Aroclor 1268 concentrations in American alligator (*Alligator mississppiensis*) caudal tail scutes from alligators that only use freshwater lagoons ("Island") and alligators that use marsh systems in Brunswick, Georgia (one-tailed t-test; t (3.047) = 1.478, p = 0.117).

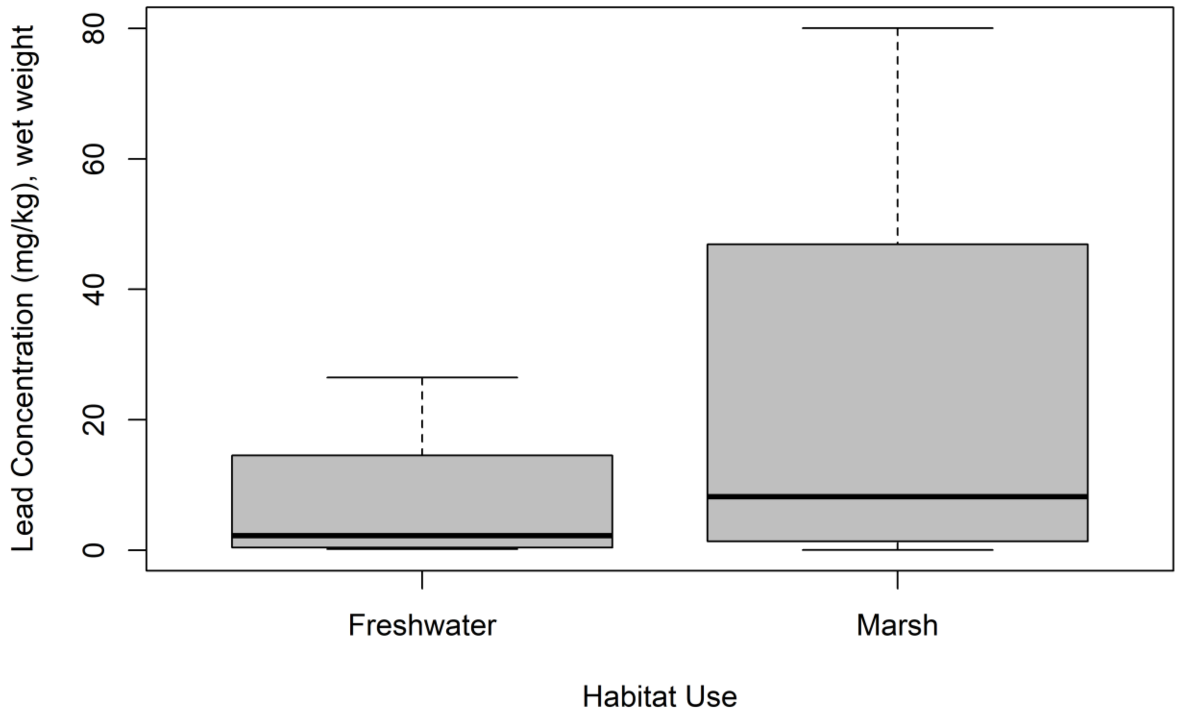

Figure 4.9: Comparison of lead concentrations in American alligator (*Alligator mississppiensis*) caudal tail scutes from alligators that only use freshwater lagoons and alligators that use marsh systems in Brunswick, Georgia (one-tailed t-test; t (3.323) = 0.849, p = 0.226).

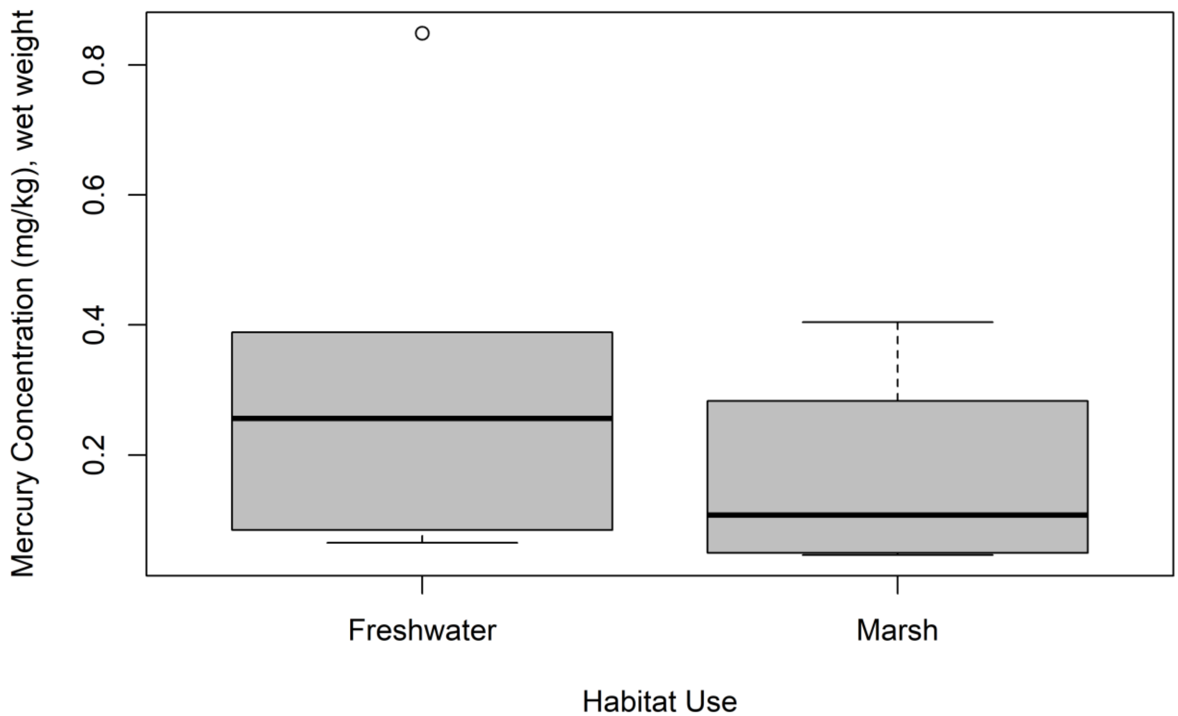

Figure 4.10: Comparison of mercury concentrations in American alligator (*Alligator mississppiensis*) caudal tail scutes from alligators that only use freshwater lagoons and alligators that use marsh systems in Brunswick, Georgia (one-tailed t-test; t (7.945) = -1.039, p = 0.835).

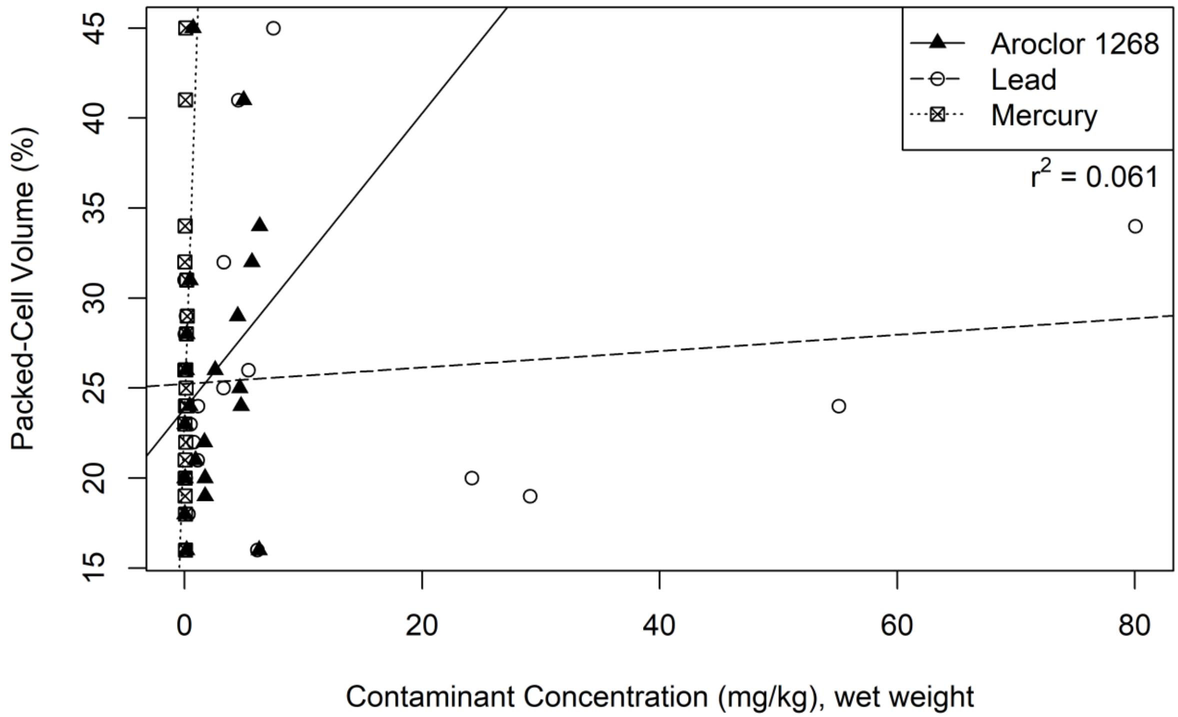

Figure 4.11: Relationship between Aroclor 1268, lead, and mercury concentrations in caudal tail scutes and packed cell volume in the blood of American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA.

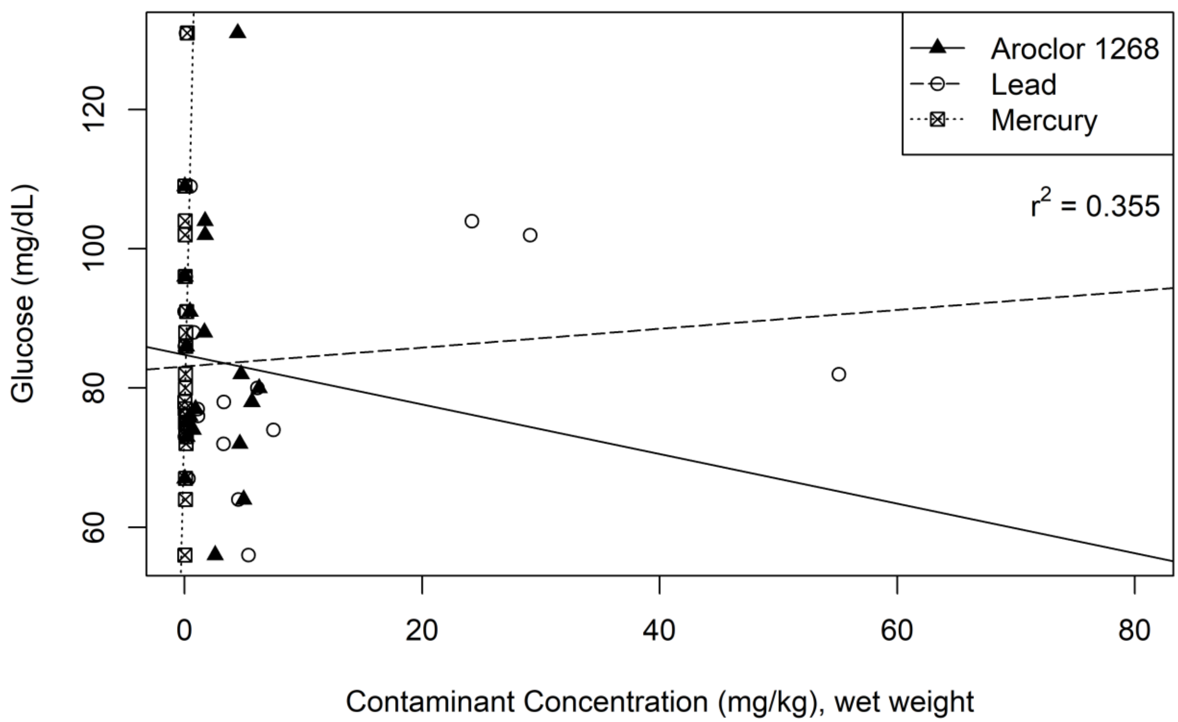

Figure 4.12: Relationship between Aroclor 1268, lead, and mercury concentrations in caudal tail scutes and glucose in the blood of American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA.

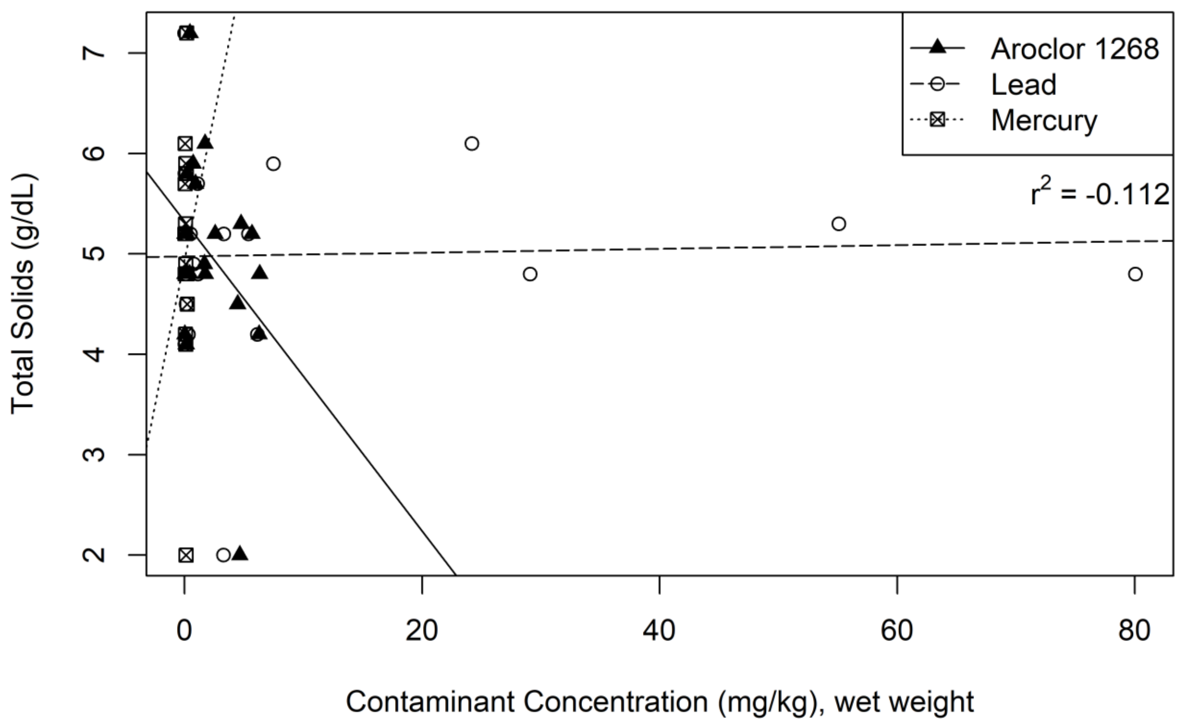

Figure 4.13: Relationship between Aroclor 1268, lead, and mercury concentrations in caudal tail scutes and total proteins in the blood of American alligators (*Alligator mississippiensis*) from Brunswick, Georgia, USA.

CHAPTER 5

SYNTHESIS AND RECCOMENDATIONS

The contaminants that were released into the environment as a result of the industrial activities at the LCP Superfund site pose a credible threat to wildlife health. Polychlorinated biphenyls (PCBs) and heavy metals have been identified in a variety of organisms inhabiting the marsh around the LCP site (e.g., Kannan et al., 1998; Blanvillain et al., 2007; Cumbee et al., 2008; Balmer et al., 2011). Aroclor 1268, the unique PCB associated with the site, has been hypothesized to cause decreases in reproductive success in clapper rails around the Brunswick area (Cumbee et al., 2008). Additionally, Aroclor 1268 has been identified as a potential cause of sublethal effects in bottlenose dolphins inhabiting the marshes around Brunswick (Balmer et al., 2011). Recent studies indicate that Aroclor 1268 may be spread to other parts of the region by highly mobile species that have been exposed to and bioaccumulated Aroclor 1268 traveling outside the Brunswick area (Pulster et al., 2005; Pulster & Maruya, 2008; Pulster et al., 2009; Balmer et al., 2011; Robinson et al., 2015). Contaminants from this site may be a threat to both wildlife and human health due to persistence of PCBs and heavy metals in the ecosystem combined with the human consumption of seafood from the area.

In chapter 2 we found high levels of Aroclor 1268 in diamondback terrapins caught outside as well as inside the fishing advisory zone implemented by the Georgia Environmental Protection Division (EPD). This finding suggests that the fishing advisory zone might not be conservative enough to protect people from the effects of PCBs from the LCP Superfund site. Capture-mark-recapture and spatial ecology studies of diamondback terrapins by other researchers in the area have found no documented movement of individuals from one side of the Downing Musgrove Causeway to the other side (Maerz & Crawford, unpublished data; Zailo, unpublished data). Therefore, we assume that the similar levels Aroclor 1268 observed in terrapins captured inside and outside the fishing advisory zones were a result of the spread of Aroclor 1268 outside the advisory area rather than due to movement of terrapins into and out of the advisory area. However, these findings alone do not mandate changing the fishing advisory zone. More research is needed before an informed decision can be made. Future research should investigate if prey species caught by anglers outside the fishing advisory zone differ from those caught within the advisory zone. In addition to determining whether Aroclor 1268 has pervaded the food chain in areas outside the advisory area and will also more explicitly quantify the potential harmful effects to humans by directly measuring levels in species humans are likely to consume.

In chapter 4, we demonstrated that caudal tail scutes collected from alligators in the Brunswick area have high levels of PCB and lead contamination. A high percentage (73.4%) of alligators sampled in our study showed lead concentrations above the limit of 0.1 mg/kg recommended by the FDA for human consumption (US FDA, 2018*a*). Additionally, nearly all (96.9%) of the alligators contained a measurable level of PCBs in their caudal tail tissue, with 20.3% of alligators containing higher than the 3.0 mg/kg limit of PCBs recommended by the FDA (US FDA, 2018*b*). These findings could indicate potential human health impacts to people that consume alligator meat since contaminants in alligator scutes are also present in alligator muscle from the same individuals (Burger et al., 2000; Nilsen et al., 2017). However, while contaminant loads in caudal tail scutes correlate highly with contaminants in muscle tissue, muscle tissue tends to have higher concentrations than tail scutes (Burger et al., 2000; Nielsen et al., 2017). Therefore, it is possible that the number of animals that exceed FDA recommendations for lead and PCBs in our study may be conservative, as most people consume the muscle tissue of alligators. Historically, high levels of heavy metals have led to the suspension of managed alligator harvests in other states (Campbell, 2003). Additionally, monitoring of contaminant levels in harvested alligators has been suggested for South Carolina and Georgia, but no formal monitoring has been implemented (Ruckel, 1993; Bowles, 1996). The alligator hunting zone that Brunswick is in is the second largest in the state of Georgia and has been increasing in popularity since 2014 (GA DNR, 2017). These high levels of contaminants combined with the popularity of harvesting alligators within the area indicates a credible risk to humans, justifying the need for a regular monitoring protocol of wild harvested alligators for contaminants of concern.

In chapter 3 we demonstrated that there was no difference in mercury levels from skin samples collected from loggerheads that nest frequently in the Brunswick area and skin samples collected from loggerheads that nest outside the Brunswick area. The objective of this chapter was to quantify exposure of loggerheads to contaminants associated with the LCP Superfund site. While the results of the loggerhead sampling were unable to differentiate between mercury bioaccumulation of loggerheads that laid most of their nests in the Brunswick area with those turtles that laid nests outside of the Brunswick area, the tissues we were able to use may not have been conducive to detecting such differences. Skin and scute samples from sea turtles have long retention times of contaminants and tend to reflect exposure over multiple years (Day et al., 2005). Loggerheads in the southeastern United States typically lay nests every 2.5 to 3 years (Richardson et al., 1978; Bjorndal et al., 1983). Therefore, loggerhead turtles nesting on the TBRE may only spend time near Brunswick during the nesting season, approximately three months every two or three years. This represents a very small subset of their lifetime. Testing blood from these animals may offer a more precise picture of exposure within recent days or weeks (Day et al., 2005). Additionally, individual turtles may nest in different areas during different nesting seasons. For instance, one turtle that nested in both 2015 and 2017 was classified as a transient in 2015 but classified as a resident in 2017. This female none of her nests in the Brunswick region in 2015 but laid all of her nests on TBRE islands in 2017. This would further complicate the issue of comparing tissues that account for multiple years of bioaccumulating contaminants. Previously, Day et al. (2005) demonstrated that blood from loggerheads along the coast of Georgia contained detectible levels of mercury and Aroclor 1268. These loggerheads tested positive for contaminants associated with the LCP site even though they were sampled outside of the advisory area implemented by the Georgia DNR. Blood samples would provide enough material to analyze for PCBs (more than one gram) without being too invasive. Further sampling using a different tissue, such as blood, may be more useful at monitoring exposure to mercury and Aroclor 1268 originating from the LCP in this highly mobile, migratory species. Additionally, necropsying dead, stranded sea turtles would provide an opportunity to sample other types of tissues in loggerheads. This would also allow researchers to collect substantial quantities of different tissues to test for PCBs that require more than one gram to analyze. Sampling multiple types of tissue could give a clear picture of the lifetime exposure limits of sea turltes.

The findings from our study indicate that further investigation into the potential effects of contaminants from the LCP site on the Brunswick area are warranted. Future studies in coastal Georgia investing contaminants associated with the LCP site should consider health effects in conjunction with contaminant levels. One adult female loggerhead sea turtle in our study contained mercury at a much higher concentration than others (nearly 10 times the mean mercury concentration). This sample was discarded as an outlier and was assumed to have dried out prior to analysis, as all samples were analyzed on a wet weight basis. However, a loggerhead turtle in a previous study was also found to have mercury levels 16 times higher than the average mercury concentration of other loggerheads in the same study (Day et al., 2005). This individual also tested positive for Arolcor 1268, indicating exposure to contaminants from the LCP site. Thus, the contaminant levels measured in the outlier we excluded from our study could potentially be an accurate reading, corresponding to an individual turtle that had bioaccumulated much higher levels than observed in other sampled turtles. Additionally, the terrapin livers sampled in our study contained almost double the amount of Aroclor 1268 compared to terrapins tested during 1995 (Kannan et al., 1998). However, sex and size were not reported by Kannan et al. (1998). If the terrapins tested by Kannan et al.

(1998) were also adult females, it could indicate that terrapins are continuing to bioaccumulate PCBs from the site, even 18 years after the LCP site was placed on the National Priorities List (NPL) by the Environmental Protection Agency (EPA). Terrapins around the Brunswick area are already suffering moderate population declines (Crawford et al., 2014). Further influences on their population from contaminants at levels that could potentially cause sublethal effects and inhibit their reproductive output may exacerbate this decline.

The overall objective for our study was to quantify the level of exposure of aquatic reptiles to contaminants associated with the LCP Superfund site and investigate how spatial variations affected their exposure levels. We found that reptiles that use the marsh habitat around Brunswick are still bioaccumulating contaminants associated with the LCP site 23 years after the EPA listed the Superfund site on the NPL. Some of the levels in these animals are high enough that they could be causing sublethal effects that are detrimental to the population's health and recovery. Further studies are necessary to assess the negative endpoints in these species that may be happening due to exposure to PCBs and heavy metals. Additionally, our study indicates that humans consuming animals from the Brunswick estuary may still be at risk for exposure to these contaminants. Further investigations are needed to determine the threat level to people in order to take necessary steps to protect humans from the effects of these contaminants.

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