The Lateral Extent and Spatial Variation of Mercury Exposure in Birds and their Prey Near a Polluted River

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College of William & Mary - Arts & Sciences

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The Lateral Extent and Spatial Variation of Mercury Exposure in Birds and their Prey near a Polluted River

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A Thesis presented to the Graduate Faculty of the College of William and Mary in Candidacy for the Degree of Master of Science

Department of Biology

The College of William and Mary
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This Thesis is submitted in partial fulfillment of the requirements for the degree of Master of Science

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Approved by the Committee, December, 2009

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Mercury contamination of waterways has become a global issue with the increase of industrial emissions during the last century. The South River of the Shenandoah Valley of Virginia is contaminated with mercury from an industrial plant that deposited mercury into the river from 1929-1950. The river and its associated biota have been monitored intensively since the 1990's and mercury levels have not decreased since then. The majority of previous studies have focused on the aquatic ecosystem; however, recent data demonstrated exposure in terrestrial songbird species sampled within 50 meters of the contaminated river. The present research investigated the distribution of mercury exposure in terrestrial songbirds and their prey across the floodplain.

The first objective was to describe the lateral extent of mercury exposure in songbirds nesting in the adjacent floodplain. By comparing mercury levels of four species of songbirds captured on nests at various distances from the river, the footprint of the mercury-impacted floodplain was described. The results using both adult and nestling Carolina wrens, Carolina chickadees, house wrens and eastern bluebirds, suggest that mercury is accumulated by terrestrial bird species foraging throughout the floodplain extending at least 400 meters from the river.

A second objective was to investigate the importance of flooding as a physical vector in transporting aquatic mercury to the terrestrial food chain. This was accomplished by recording various spatial variables that influence flooding at each bird nest sampling location. These spatial characteristics were then tested for their ability to predict avian mercury levels. The results suggest that flooding potential best predicts "hotspot" areas for mercury exposure. However, other variables, including distance from the proximate and ultimate sources of mercury also related to mercury exposure.

Previous work has established that mercury exposure in wildlife is largely through diet. In the floodplain of the South River, spider prey, in particular, has been shown to deliver the vast majority of mercury to terrestrial insectivorous songbirds. My study investigated whether mercury exposure of songbird prey, with particular focus on spiders, followed the same spatial trends as did the birds themselves.

The results concerning the lateral extent of mercury exposure in terrestrial spiders were inconclusive, most likely due to the lack of information regarding foraging territories of the spiders. However, flooding variables did relate to mercury for all types of prey collected, and especially in the case of spiders. This suggests that spiders inhabiting low-lying, flood prone areas are more likely to accumulate high amounts of mercury that they then deliver to the songbirds.

This study offers the first information about the spatial variability of floodplain mercury exposure on a biological scale. The compelling finding is that exposure risk extends for at least 400 meters from the mercury contaminated South River, which should be considered in all riverine mercury risk assessments as well as in future restoration efforts. Additionally, this study implicates flood waters as a vector of importance in transporting mercury from the aquatic ecosystem into the adjacent floodplain and terrestrial food chain.
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1. Chapter One: Background and Literature Review

1.1. Sources of Mercury Contamination

Mercury is a naturally occurring trace element in the earth’s crust that has no known benefit to biological organisms (Tchounwou et al., 2003). The sources of mercury to the global environment are both natural and anthropogenic. Natural sources include volcanic eruptions and weathering of rock that gradually exposes mercury trapped in the earth’s crust so that it is volatized into the atmosphere or incorporated into soils and biological organisms. Other natural sources of mercury to the atmosphere include emissions from soils and oceans and burning of biomass (Gustin et al., 2008). The majority of releases from biomass burning and soil emissions are re- emissions of mercury originally deposited from the air onto land (Gustin et al., 2008). The estimation of natural sources of mercury is difficult due to spatial and temporal variability and the lack of adequate analyses (Gustin et al., 2008). However, areas with ongoing or past geologic activity, such as volcanism or geothermal processes, contribute the majority of natural emissions and have been used to estimate overall emissions. A review of various studies has placed overall natural global emissions in the range of 800 to 3000 megagrams of mercury per year (Gustin et al., 2008; Nriagu, 1989). This is greater than the total estimated anthropogenic emissions of 2000 to 2400 megagrams of mercury per annum (Gustin et al., 2008). However, these figures do not include values for re- emissions, which may have greatly increased due to human activity.
1.1.1. Anthropogenic Sources and Increases

Total direct anthropogenic emissions have been estimated to be 33% of total emissions with the rest being attributed to natural emissions and re-emissions (Driscoll et al., 2007; Mason and Sheu, 2002). Mercury has been used throughout human history including for gold and non-ferrous metal production, industrial manufacturing, fungicides and medicines, and for use in everyday consumer products such as batteries and light bulbs. Although emissions from artisanal gold mining still contribute significantly to the global pool (248 tons in 2000), increased anthropogenic emissions began with large-scale industrialization (Pacyna et al., 2006; Boening, 2000). The large majority of anthropogenic emissions today stem from the burning of fossil fuels, mainly coal, for use in industrial and utility plants as well as residential furnaces (Boening, 2000; Burger and Gochfeld, 1997; Pacyna et al., 2006). Other major anthropogenic sources include non-ferrous metal production and waste disposal (Pacyna et al., 2006).

Although industrial use and disposal of mercury has become increasingly regulated, a substantial amount still enters into the global pool of mercury each year. Consumer waste contains many products with mercury, such as thermometers and other electronics that may volatilize mercury into the atmosphere or release it as leachate when they degrade in landfills. All of these sources have combined in recent decades to increase the global availability of mercury substantially. Using historical records in lake sediments, one study has estimated an increase by a factor of 2-5 from pre-industrial levels (Boening 2000). Because mercury is a naturally occurring element, human emissions have not increased the total amount of mercury in the world but have modified its form and local concentrations in ways that make it more available to biological organisms.
1.1.2. Point Source Contamination Sites

Mercury is emitted from both point sources and diffuse or non-point sources. Point source contamination comes from identifiable locations such as industrial discharges into the atmosphere or waterways. Point source emissions pose the largest threats to humans and wildlife living in the area of contamination. In the United States, identified point sources are subject to governmental regulation. Non-point sources are more difficult to control as they come from diffuse discharges and may span large areas. Diffuse point sources include leaching from contaminated industrial soils or landfills into waterways as well as re-emissions of mercury from contaminated soils and waterways into the atmosphere.

Point sources include localized atmospheric discharges of gaseous mercury into the atmosphere and direct discharges into water bodies or onto terrestrial soils. Atmospheric emissions in the United States are dominated by waste incineration and fossil fuel combustion in coal-fired plants. Aquatic discharges of mercury are dominated by direct discharges from water treatment plants and chlorine production plants in the United States.

Industrial discharge of inorganic mercury into waterways has been commonplace since the start of the industrial revolution (Tchounwou et al., 2003). Presumably, it was thought that the mercury would be diluted or buried in the sediments and therefore inflict no toxicity on biological organisms. However, historic emissions can become sources of non-point source emissions through the processes of local cycling and long-range transport. Through chemical transformation, mercury that is sequestered in sediments can become re-suspended and biologically available for uptake by organisms (USEPA, 3
1997; Wiener et al., 2003). For example, dredging, erosion, and/or flooding can cause re-suspension of particles. Once re-suspended, particles contaminated with mercury can be carried downstream, transported with dredge material or re-deposited on floodplain soils. Flooding is especially important because floodwaters can carry contaminants into floodplains where it could become available to terrestrial wildlife.

1.2. Mercury Speciation, Transport and Cycling

The global cycle of mercury is often described as having four interconnected compartments: atmospheric, aquatic, terrestrial and biotic (Wiener et al., 2003). Which compartment anthropogenic mercury becomes incorporated into depends on the type of mercury species released and the receiving environment (USEPA, 1997; Morel et al., 1998; Schroeder and Munthe, 1998). These compartments of mercury are not isolated from one another; rather complex interactions control the transport and cycling of mercury between compartments (Zillioux et al., 1993). For example, natural and anthropogenic mercury emitted to the atmosphere can be transported long distances before it is deposited and incorporated into a regional or local cycle (USEPA, 1997; Morel et al., 1998; Schroeder and Munthe, 1998). Mercury released to other media, including soil, vegetation and water is more likely to become incorporated into the local ecosystem cycle but it may be volatized and re-emitted to the atmosphere where it enters the global cycle (USEPA, 1997; Morel et al., 1998; Schroeder and Munthe, 1998). This characteristic makes estimates of newly released anthropogenic, natural, and re-emitted anthropogenic sources extremely difficult.

The transport and cycling of mercury is largely dependent on its chemical state at emission and whether it is subject to transformation into another mercury species. The
chemical speciation of mercury is complex and driven by local concentrations and environmental characteristics, such as the availability of water and binding molecules such as organic matter. Mercury speciation and cycling varies among different ecosystems and certain habitats may act as sinks while others may export mercury both to other habitats and to biological organisms (USEPA, 1997; Morel et al., 1998; Schroeder and Munthe 1998).

1.2.1. Chemical Forms of Mercury

Mercury can exist in three oxidation states in the environment including metallic or elemental mercury (Hg\(^0\)), mercurous mercury (Hg\(^{2+}\)), and mercuric mercury (Hg\(^{2+}\)) (USEPA, 1997). Elemental mercury occurs as a silver liquid at ambient temperatures and pressures (USEPA, 1997). However, liquid elemental mercury is not found in nature as it is quickly vaporized into a gaseous state or readily oxidized into Hg(II) species (total particulate mercury and reactive gaseous mercury) when in water (Morel et al., 1998). Mercurous and mercuric mercury are highly reactive and can form multiple inorganic mercuric salts and organomercuric chemical compounds (USEPA, 1997). Most mercury in the atmosphere is gaseous elemental mercury (Hg\(^0\)) while mercury encountered in all other media (aquatic, terrestrial, and biotic) is in the form of inorganic salts or organic compounds. The aquatic compartment is dominated by Hg(II) species that are either bound to ligand pairs in the water or in the sediments (Wiener et al., 2003). The terrestrial compartment mostly contains Hg(II) species that are bound to organic matter in the soils, while the biotic compartment is dominated by organomercurics, defined by the presence of a carbon-mercury (C-Hg) bond (Wiener et al., 2003; USEPA, 1997). Although both inorganic mercuric salts and organomercurics can be taken up by
organisms, such as aquatic invertebrates and fish, organomercurics are easily soluble across biological membranes and therefore are preferentially retained in animal tissues (USEPA, 1997).

1.2.2. Atmospheric Mercury and Long Range Transport

Atmospheric processes that control the exchange of gaseous elemental mercury (Hg\(^0\)) and various Hg(II) species drive the global cycle between the atmosphere and the other main compartments (aquatic, terrestrial, and biotic). Generally it is a two-way exchange with gaseous mercury emitted to the atmosphere, either naturally or due to anthropogenic actions, and some mercury removed from the atmosphere by oxidation to Hg(II) species and subsequent wet or dry deposition (Wiener et al., 2003). It is known that almost all of the mercury in the atmosphere is elemental gaseous mercury (Hg\(^0\)), which is water insoluble, and not subject to wet deposition during precipitation events (USEPA, 1997; Morel et al., 1998). Furthermore, the oxidation of Hg\(^0\) to Hg(II) species is a slow process; most atmospheric mercury is subject to long range transport, travelling long distances across the globe before being deposited on either land or water (Morel et al., 1998). According to one estimate by Mason et al. (1994) half of all anthropogenic mercury emitted to the atmosphere will enter the global cycle while the rest of it will be deposited locally or regionally. The average residence time of Hg\(^0\) in the atmosphere is estimated to be one year, which is ample time for it to be distributed across the globe via wind currents (Morel et al., 1998). Although the main sources of atmospheric mercury may be from point sources concentrated in industrial areas, mercury emissions may be deposited in remote and uninhabited parts of the world as well, making mercury a truly global pollutant (Morel et al., 1998).
Some amount of gaseous and particulate Hg(II) is emitted to the atmosphere from industrial sources. In contrast to Hg⁰, these species are likely to be deposited either locally or regionally during precipitation events because they are water soluble (USEPA, 1997). The United States Environmental Protection Agency (USEPA) estimated that 5-10 percent of Hg(II) emissions will be deposited within 100 kilometers of the emission point, with the rest being deposited regionally (USEPA, 1997). The average residence time of oxidized mercury (Hg(II)) is estimated to vary from hours to months depending on a host of environmental variables including precipitation and land use type (USEPA, 1997). Therefore, the species of mercury emitted to the air largely controls whether it will contaminate a remote system or the local or regional area.

1.2.3. Mercury in Freshwater Environments

Mercury enters freshwater ecosystems through atmospheric deposition, runoff from contaminated lands, or direct discharges from industrial sources. Inorganic liquid mercury that is released to freshwater systems as Hg⁰ is either oxidized by microorganisms to Hg(II) or released back to the atmosphere through evasion through transformation into a volatile form (Ullrich et al., 2001; Morel et al., 1998). At a given time, most mercury in freshwater environments is found as Hg(II) bound to sediments or to particulate organic matter in the water column (Ullrich et al., 2001).

Once mercury has been oxidized to Hg(II) species, its cycling becomes complex as it may be sequestered in the sediment, bound to particulate matter, taken up by biota or reduced again to Hg⁰ and re-emitted (Zillioux et al., 1993; USEPA, 1997; Morel et al., 1998; Ullrich et al., 2001). Therefore, it is difficult to track the ultimate fate of mercury in freshwater systems.
Of particular importance to my study is the fate of liquid inorganic mercury released into a river as direct discharge from an industrial source. Elemental mercury is not naturally present in its liquid state because it acts as a catalyst site for the oxidation to Hg(II) species (USEPA, 1997). Therefore, although some liquid mercury will be vaporized and evaded as Hg\(^0\), most of it will be oxidized to Hg(II) either as it sinks to the sediments or at the sediment-water interface (USEPA, 1997; Ullrich et al., 2001). Depending on whether it is bound to organic or inorganic molecules, these newly formed Hg(II) species are absorbed by aquatic organisms and fish directly from the water column, temporarily sequestered in the sediments or available for uptake by methylating bacteria (Morel et al., 1998; USEPA, 1997). If methylated by bacteria, the methyl mercury produced is available to enter the aquatic food chain where it can be accumulated to toxic amounts. Once in the food chain, methyl mercury may accumulate to increasing concentrations as it passes up through the trophic levels, according to the process of biomagnification described below (Wiener et al., 2003). However, some organisms are capable of excreting or de-methylating some amount of the methyl mercury they uptake (Wiener et al., 2003). Mercury that is excreted by organisms may once again enter the ecosystem cycle of mercury as Hg(II) species.

1.2.4. Mercury in Terrestrial Environments

Relative little is understood about the cycling of mercury in terrestrial environments in comparison to aquatic environments. Mercury can enter terrestrial ecosystems through atmospheric deposition (either wet or dry), direct industrial discharges onto soils, or by flooding from contaminated waterways (USEPA, 1997). Recent estimates indicate that the largest inventory of inorganic mercury is in terrestrial
soils; however little is known about the speciation and cycling of mercury within terrestrial soils (Wiener et al., 2003; Ullrich et al., 2001). Most of the mercury in terrestrial soils is thought to be Hg(II) species bound to organic matter or minerals and therefore generally not bioavailable (Wiener et al., 2003). However, there is some evidence for the presence of methyl mercury in forest tree leaves, leaf detritus on the forest floor, and in saturated or moist soils (Rimmer et al., 2005). This suggests that there is the potential for mercury uptake by terrestrial biota, which is supported by recent research on terrestrial organisms (Watras and Huckabee 1994; Wiener et al., 2003; Rimmer et al., 2005; Cristol et al., 2008). This recent research has elucidated the importance of understanding the cycling and speciation of terrestrial mercury, especially in environments subject to point source discharges.

Current research into the cycling of mercury in terrestrial ecosystems is focused on determining whether methyl mercury is produced within terrestrial soils or derived from external sources. There is evidence for atmospheric deposition of bioavailable methyl mercury, methylation within terrestrial soils, and additionally, methyl mercury from adjoining contaminated waterways being transported to terrestrial soils during flood events (Rimmer et al., 2005; Morel et al., 1998; Roulet et al., 2001). Some studies claim that acid rain increases the direct deposition of bioavailable methyl mercury; however the significance of this deposition is not agreed upon (Zilloux et al., 1993; Wiener et al., 2003). Other studies show support for the methylation potential of mercury in terrestrial soils by microorganisms, similar to the processes known in aquatic ecosystems (Roulet et al., 2001). However, the methylation potential is thought to be low in upland terrestrial soils (Wiener et al., 2003). Many studies have confirmed that the potential for
methylation is substantially increased in flooded soils under anoxic conditions (St. Louis et al., 2004). Anoxic conditions increase microbial activity, which is directly related to increased methylation (described in detail in section 1.2.5 Methylation and Methyl Mercury below). In addition to producing favorable conditions for methylation in terrestrial soils, frequent flooding has the potential to re-suspend inorganic mercury, organic Hg(II) species and methyl mercury that may be bound to organic river bottom sediments (USEPA, 1997; Gustin et al., 2006). Once re-suspended in the water column, bioavailable methyl mercury may be transported onto terrestrial soils through flooding. Most mercury in terrestrial soils is sequestered as inorganic and bound organic Hg(II) species. This sequestered mercury is largely unavailable to biota unless there is a high potential for methylation (see section 1.2.5 Methylation and Methyl Mercury).

Furthermore, recent evidence supports the claim that mercury can be transported from contaminated aquatic food chains into adjacent terrestrial food chains by way of biological subsidies, or organisms that provide a connection between the two separate food chains (see section 1.6 Subsidies and the Aquatic-Terrestrial Interface). These ideas suggest that the cycling of mercury in terrestrial environments is more active and complex than formerly described.

1.2.5. Methylation and Methyl Mercury

Methyl mercury is the mercury compound of most concern because it is highly toxic, readily accumulates in biological tissues and leads to exposure in humans and wildlife (Burger & Gochfeld 1997; Morel et al., 1998; Wiener et al., 2003; Celo et al., 2006). Methyl mercury is commonly formed in nature through the process of methylation. Mercury methylation is the conversion of particulate Hg(II) species of
mercury to methyl mercury ($\text{CH}_3\text{Hg}^+$) by a methyl-group donor while the reverse conversion of methyl mercury to inorganic mercury is termed de-methylation (Wiener et al., 2003). Methylation happens naturally in aquatic ecosystems via biotic processes (microbial metabolism) or abiotic processes (chemical methylation) (Celo et al., 2006; Morel et al., 1998). However, it is widely agreed that most methylation occurs via anaerobic bacteria, such as sulfate-reducing bacteria, in aquatic ecosystems (Burger & Gochfeld 1997; Wiener et al., 2003; Celo et al., 2006). The presence of methyl mercury in an ecosystem implies that there is a large amount of mercury available for methylation and that the rate of methylation is greater than that of de-methylation.

Biotic methylation and de-methylation is dependent on the rate of microbial activity, which in turn is determined by many environmental factors including nutrient availability, temperature, pH, redox potential, and the presence of binding organic and inorganic agents (Holmes and Lean, 2006; Celo et al., 2006). Many studies have found that moderately high temperatures and nutrient availability in organic matter increase bacterial activity and thereby increase the potential for methylation (Ullrich et al., 2001). Other environmental conditions determine the speciation of mercury and thereby control the dynamics of methylation. For example, in order for biotic methylation to occur, a significant amount of mercury must be available to be taken up by bacteria (Celo et al., 2006). In particular, neutral species, such as $\text{HgCl}_2$ or $\text{Hg(HS)}_2$, are more lipid soluble and easily cross cell membranes into bacteria where methylation occurs (Morel et al., 1998). Furthermore, methylation is thought to be more prominent in anaerobic environments at the interface between oxic and anoxic surfaces (Morel et al., 1998). However,
methylation can occur in the water column, albeit at slower rates (Ullrich et al., 2001; Morel et al., 1998; Tessier et al., 2007).

The influences of other environmental conditions are less well understood. For example, acidic environments have been found to favor methylation over the reduction of Hg(II) to Hg⁰ (which leads to evasion) whereas higher pH environments are correlated with higher Hg⁰ evasion (Ullrich et al., 2001). But some studies have shown that the addition of acid to lake sediments actually decreased the production of methyl mercury at the oxic-anoxic interface (Ullrich et al., 2001). The presence of sulfur is also particularly important because sulfate-reducing bacteria are known to be key methylators in anaerobic environments (Ullrich et al., 2001). In low sulfate concentrations, the addition of sulfates stimulates methylation (Ullrich et al., 2001). However, in high sulfate concentrations, methylation may be inhibited because of the production of sulfide that sequesters mercury in a biologically unavailable form (Ullrich et al., 2001). In a similar manner, the amount of organic matter present seems to determine its specific influence on methylation. Some studies have shown that the presence of organic matter actually enhances the rate of methylation by stimulating bacterial activity (Ullrich et al., 2001). However, when large amounts of organic matter are present, an increase in the complexation (or binding) of Hg(II) species occurs, reducing its availability for methylation (Ullrich et al., 2001). The influence of organic matter is further complicated by the pH of the environment. Some studies have shown that in low pH systems, organic matter is more likely to increase methylation while in neutral or high pH systems, the complexation of Hg(II) is more likely to occur (Ullrich et al., 2001).
The interaction among environmental factors increases the complexity of methylation/de-methylation processes and therefore, despite that large body of available research, we are still unable to accurately predict methylation rates in different habitats (Ullrich et al., 2001).

1.2.6. Bioaccumulation and Biomagnification

Bioaccumulation is the net accumulation of a contaminant in an organism. Methyl mercury readily crosses biological membranes, such as the blood-brain barrier, and is known to accumulate in aquatic organisms to levels much greater than that in the water (Wiener et al., 2003). Methyl mercury bioaccumulates in the tissues of organisms because it is lipid soluble (Kainz et al., 2006). Studies suggest that most accumulation is through ingestion of contaminated food rather than direct uptake from the water column (Morel et al., 1998). One study has shown that bioaccumulation increases with size in zooplankton, therefore fish that preferentially feed on larger zooplankton have higher methyl mercury concentrations via bioaccumulation (Kainz et al., 2006). This suggests that bioaccumulation in predators is dependent on their foraging habits.

Furthermore, mercury is known to biomagnify, or increase in concentration, up the food chain with each trophic level (Wiener et al., 2003). The number of trophic levels between predator and prey is crucial in predicting the mercury load in the predator (Morel et al., 1998, Cabana & Rasmussen, 1994). Studies have shown the usefulness of δ¹⁵N as an indicator of trophic level, suggesting that relative isotope concentrations can be used to predict contaminant biomagnification through the food web (Cabana & Rasmussen 1994). Biomagnification patterns are largely similar among food webs even
in systems that vary in ecosystem type, mercury source, and intensity of pollution (Weiner et al., 2003). However, the rate of bioaccumulation is dependent on foraging habits and habitats, as some habitats (e.g., wetlands) are more conducive to methylation processes than others (Morel et al., 1998; Wiener et al., 2003).

1.3. Human Health and Mercury Contamination

There have been multiple incidences of large-scale mercury contamination in humans, but the most infamous was in Minamata Bay, Japan, beginning in the 1950’s. A factory released large amounts of inorganic mercury that underwent a chemical conversion into the much more toxic organic form (Tchounwou et al., 2003). Many years later, residents of the area started to exhibit symptoms of what is now termed Minamata Disease (Tchounwou et al., 2003). There have also been instances of large-scale mercury poisoning from gold mining practices, one notable example taking place in the Amazon region of South America. Un-recycled mercury during gold mining practices often finds its way into waterways where it eventually contaminates the fish that many indigenous peoples in the Amazon are reliant upon in their diet (Gochfeld, 2003). Other instances of contamination are from the ingestion of seeds treated with fungicides containing mercury (Tchounwou et al., 2003; Gochfeld, 2003). These human casualties were caused by the ingestion of mercury through the diet. Recent studies have shown that the major current source of exposure to humans is fish and marine food items (Tchounwou et al., 2003; Gochfeld, 2003). However, other sources exist, including grain and meat products, skin lighteners, dental amalgams and the inhalation of inorganic mercury from the atmosphere (USEPA, 1997).
In recent years, there has been a global aim of defining a reference dose, or the dose that can be ingested daily over a lifetime with no significant adverse effect (Tchounwou et al., 2003). This is an enormous and difficult undertaking as the sources of exposure are diverse and the effects vary with form and route of exposure, age, health, genetic makeup and environment of the individual (Tchounwou et al., 2003). Mercury poisoning is known to cause an array of problems in humans including genotoxic, carcinogenic, and teratogenic effects (Tchounwou et al., 2003). However, the most well known effects are neurotoxic and include reduced motor skills, loss of memory, and slow mental response (Tchounwou et al., 2003). These effects vary greatly with the length and time of exposure in the individual. For example, high levels in a developing fetus can result in abortion, induced cerebral palsy, mental retardation, low birth weight or sensimotor dysfunctions (Gilbert and Grant-Webster, 1995).

1.3.1. Fish Consumption Advisories

In an effort to protect the public from the risk of consuming contaminated fish and water-dependent wildlife, such as ducks, turtles and muskrats, state, tribal and local governments monitor their waters for a variety of contaminants and issue fish consumption advisories when contaminant levels exceed established thresholds (USEPA, 2008; November 18, 2008 http://epa.gov/waterscience/fish/basic.htm). Although there are advisories issued for 44 different contaminants, 80% of the advisories in effect in 2006 involved five bio-accumulative contaminants: mercury, PCBs, chlordane, dioxins, and DDT (USEPA, March 20, 2009; http://www.epa.gov/fishadvisories). These contaminants accumulate in animal tissues at concentrations much higher than what is
found in their environment. In addition, concentrations of these contaminants are highest in organisms that feed higher on the food chain, such as humans.

Advisories may be issued for the general public or to more at risk subpopulations such as pregnant women, nursing mothers, or children (USEPA, 2009). Fish consumption advisories are usually recommendations, rather than regulations, to restrict consumption of certain locally caught fish from specific water bodies. There are five types of advisories issued ranging from consumption restrictions to legal bans on commercial fishing (USEPA, 2009).

As of 2006 there were a total of 3,852 fish consumption advisories on water bodies in 48 states, the District of Columbia, and territories or tribal areas (USEPA, 2009). Of these, 3,089 or 80% involved mercury. The number of mercury advisories has increased by 644 from 2004, attributable to new mercury advisories in 25 states (USEPA, 2009). Most new advisories are the direct result of increased monitoring rather than new sources (USEPA, 2009). Currently, 26% of the nation’s river miles are under a mercury fish consumption advisory (882,963 miles) (USEPA, 2009).

In the state of Virginia, there are 52 fish consumption advisories covering 4.7% of the state’s river miles. The South River has been placed under a no-consumption advisory for the general public for all fish, except stocked trout, since 1977 (USEPA, 2009). The advisory was issued because commonly caught species, except stocked trout, have an average mercury level that is consistently over the 0.5 ppm action level suggested by the USEPA. The high level of contamination in the fish suggests that other water-dependent wildlife or predators may also be at risk to mercury exposure.
1.4. Mercury and Wildlife

Exposure of wildlife to environmental contaminants occurs when receptors (species, populations, individuals) and contaminants co-exist in time and space and interact in some manner (Smith et al., 2007). However, knowledge of co-occurrence is insufficient to fully understand the risk of exposure to wildlife. Thorough assessments of exposure must take into account species-specific life history traits and the environment in which an organism resides to adequately define the intensity and length of exposure (Smith et al., 2007). For example, the potential of exposure to mercury differs among herbivores, omnivores, and carnivores due to the process of biomagnification. However, the bioavailability of a contaminant depends on physical processes in the environment such as methylation and the potential for transport between habitats. Therefore, the potential for wildlife exposure to mercury is dictated by both biotic and abiotic factors that increase or decrease the bioavailability of mercury and the subsequent likelihood of exposure (Smith et al., 2007). It is unrealistic to define the potential of exposure for all species and types of habitats and therefore exposure-risk groupings have been based on general habitat and diet similarities. This has led to a separation of mercury-related research along the physical boundary between aquatic and terrestrial ecosystems or between species considered to have aquatic diets and those considered to be terrestrial consumers. Although my research will provide reasons to move away from this thinking, I will examine aquatic and terrestrial organisms separately here.

1.4.1. Aquatic and Fish-eating Wildlife

Studies of mercury in wildlife have focused primarily on aquatic ecosystems and their associated biota, mainly because they are linked to human exposure. Wildlife in
aquatic ecosystems is at risk from both point source discharges of mercury and non-point sources from contaminated runoff and atmospheric deposition. Furthermore, there has been a general agreement that aquatic biota are more prone to toxicity because of the high methylation rates in aquatic ecosystems (Scheuhammer et al., 2007).

The literature is rich with studies on mercury exposure in aquatic invertebrates, fish and fish-eating predators (Chan et al., 2003; Scheuhammer, 2007; Wolfe et al., 1998; Frederick, 2002). From these studies, the movement of mercury through the food chain has been shown to occur from river sediments, aquatic vegetation, or directly from the water to aquatic invertebrates that are fed upon by small fish, thereby initiating the trophic cascade to higher order piscivorous species (Cremona et al., 2008; Cabana and Rasmussen, 1994; Hall et al., 1998). Aquatic invertebrates have been studied because of their importance as a food source, but relatively fewer studies have actually investigated the effects of mercury on aquatic invertebrates themselves. For example, many studies have investigated the accumulation patterns of different aquatic invertebrates; one study found that mussels (*Mytilus edulis*) accumulated methyl mercury in a predictable pattern (ex: mantle first, shell last) but that this pattern differed according to whether the mercury was inorganic or organic (Boening, 2000). Fewer studies have actually investigated the effects of mercury on aquatic invertebrates themselves. One laboratory study found that planarians or flatworms exhibited extreme tetratogenic effects, including the inability to regenerate normally, under various doses of mercury in their aquatic environment (Best et al., 1981). Multiple studies suggest that community parameters, such as species diversity and evenness, declined with increased sediment mercury (Suchanek et al., 1995). However, these studies did not attribute the decline of diversity solely to the
presence of mercury; rather, they suggested that multiple correlated environmental factors such as grain size, total organic carbon and water depth interact to produce effects at the community level (Suchanek et al., 1995). There are also studies that suggest that aquatic invertebrates exposed to mercury in their environment develop a tolerance that keeps them from exhibiting effects (Kraus et al., 1988; Vidal and Horne, 2002).

Fish have been the main target of mercury studies as there is the obvious human safety risk of eating contaminated fish. However, these studies have also provided much useful information on the accumulation of mercury by fish and associated effects at the individual and population levels. Fish assimilate methyl mercury much more readily than inorganic mercury and the vast majority of this methyl mercury is accumulated from their diet (USEPA, 1997). Many studies have corroborated the fact that within a given population, individual fish methyl mercury concentrations increase with age and size (McIntyre and Beauchamp, 2007; Storelli et al., 2007; USEPA, 1997). Laboratory dietary studies on fish have shown that methyl mercury is harmful to the central nervous system and fish exhibit such symptoms as incoordination, decreased responsiveness and swimming ability, reduced foraging, starvation and ultimately death (USEPA, 1997). Many of these symptoms impair the ability of individual fish to locate and capture prey as well as avoid predation affecting predator-prey interactions of the community. Additionally, studies have confirmed reduced spawning and gonadal development of juvenile fish fed ecologically realistic concentrations of methyl mercury until they reached maturity (Scheuhammer et al., 2007; USEPA, 1997). These effects of mercury can reduce the reproductive success of individuals possibly having a population effect over time (USEPA, 1997).
Piscivorous predators are of interest because they are long-lived, large-bodied, and feed within the aquatic food chain, all factors increasing risk of dietary methyl mercury exposure (Scheuhammer, 2007). These species include large predatory fish such as walleye (*Sander vitreus*), northern pike (*Esox lucius*), and lake trout (*Salvelinus namaycush*); mammals such as mink (*Mustela spp.*) and otter (*Lutra spp.*); and piscivorous birds such as common loons (*Gavia immer*), bald eagles (*Haliaeetus leucephalus*), osprey (*Pandion haliatus*), kingfishers (*Alcedo spp.*) and seabirds (Goodale et al., 2008; Cristol et al., 2008; Hink et al., 2007; Scheuhammer, 2007; Hopkins et al., 2007; Evers et al., 2005, Chan et al., 2003). Comparison between these species is difficult because of differences in physiology, diet, habitats, and geographic location that cause certain species to be more prone to exposure. However, there have been some comprehensive studies that have collected data points across United States in an attempt to identify the contaminants that pose the largest risk to wildlife (Scheuhammer et al., 2007; Hink et al., 2009; USEPA, 1997). In Scheuhammer et al. (2007) a number of piscivorous wildlife were reviewed for their risk of toxicity from environmental methyl mercury. This review stated that walleye, pike and lake trout are at risk for hormonal development effects at levels of 0.8 ppm or more (Scheuhammer et al., 2007). In the most recent of these studies, Hink et al. (2009) found that of a host of contaminants commonly found in freshwater fish, mercury (as well as PCB’s, total DDT and selenium) posed the greatest threat to bald eagle and mink populations nationwide. However, even though large mammalian predators such as otter and mink have been found to exhibit toxicity symptoms, research is lacking to determine if they are manifesting population level effects (Scheuhammer et al., 2007). Much more is known about piscivorous avian
predators such as the common loon, which has been the focus of a host of studies conducted in northeastern states. Evers et al. (2008) found that adult loon pairs with elevated methyl mercury body burdens produced fewer fledged chicks than pairs breeding on reference lakes. This same study also determined that common loon adults were more likely to exhibit adverse effects when their body burdens exceeded 3.0 ppm in their blood (Evers et al., 2008). Many mercury studies have also focused on seabirds because they are top predators that are highly dependent on fish in their diet. Leach’s storm petrels (*Oceandromo leucorhoa*) from the Pacific coast to the Atlantic have consistently been found to have elevated mercury levels. Most recently a study focusing on the Gulf of Maine found that petrels had levels high enough to cause adverse effects in both their blood and eggs (Goodale et al., 2008). This study also summarized findings that suggest mercury levels in seabirds are dependent on their feeding guild with piscivores having higher levels than invertivores or species that feed on both invertebrates and fish (Goodale et al., 2008). Furthermore, in comparisons of coastal feeders and species that feed on mesopelagic fish (deep sea fish), mesopelagic feeders consistently have higher mercury levels indicating mercury as a global pollutant rather than one associated with localized coastal sources (Goodale et al., 2008; Furness and Camphuysen, 1997).

### 1.4.2. Terrestrial Wildlife

Published reviews have pointed to the lack of information regarding exposure in terrestrial wildlife, but these understudied species have been the focus of some recent studies. Of particular interest are the characteristics that define the risk of exposure to terrestrial species. The routes of exposure for aquatic organisms are generally understood
and are similar among individual water bodies. This is due in part to the fact that contaminant distributions can be more homogenous within aquatic environments than terrestrial systems (Smith et al., 2007). Although there are complex exposure pathways in aquatic environments, the organisms are less capable of long distance movement if they are constrained to a single lake or wetland system (Smith et al., 2007). The poorly understood spatial and temporal distribution of contaminants in terrestrial systems combined with the mobility of terrestrial wildlife, such as birds or mammals, adds a great deal of uncertainty when estimating the exposure potential of terrestrial wildlife (Smith et al., 2007). Furthermore, terrestrial habitats are much less well-delineated than distinct water bodies and this allows considerable interaction and overlap of organisms between habitat types.

Some recent studies have examined mercury exposure in large-bodied mammals, bats, insectivorous birds, spiders, and amphibians. Life-history characteristics vary greatly among these different taxonomic groups, making interpretation of levels and prediction of effects difficult. However, the pathways of exposure are similar among the taxa and include ingestion, absorption through the skin, or inhalation (Smith et al., 2007). In addition, maternal transfer to eggs is known to be a significant source of mercury to developing young of mammals, birds, and amphibians (Hopkins et al., 2007; Hopkins et al, 2006).

Recent research has shown that terrestrial songbirds (order Passeriformes) and their prey accumulate mercury readily (Cristol et al., 2008). In 2008, Cristol et al. found that most of the mercury in the diet of affected songbirds was being delivered by spiders, which comprised greater than 25% of the biomass in the diets of several species and
contained greater than 75% of the ingested mercury. With such an obvious source of biologically available methyl mercury exposure, it is unlikely that inhalation, absorption or drinking are important contributors to overall exposure.

1.4.3. Sub-Lethal Effects of Mercury on Wildlife

Mercury toxicosis has been observed in wildlife individuals that ingest sufficient levels of methyl mercury in their prey or drinking water (Wolfe et al., 1998). In general, observed mercury toxicoses include damage to the nervous, excretory, and reproductive systems (Wolfe et al., 1998). For individuals, these effects can result in reduced survivorship, foraging success, mating success, and parental care as well as developmental and behavioral abnormalities (Wolfe et al., 1998; Scheuhammer, 2007; Kenow et al., 2003; Adams and Frederick, 2008; Brasso and Cristol, 2008; Evers et al., 2008). At the population level these effects can lead to declines in abundance, which is especially important for species at risk to other threats such as habitat loss. Due to the spatial variability in exposure, physiological differences between taxa, and foraging differences between individuals, it is difficult to determine levels at which wildlife will experience toxic effects. Currently accepted no-observed-adverse-effects-levels (NOAEL) for waterborne mercury have been established using piscivorous wildlife; however, this model is not easily generalized to other species that differ in size, diet, habitat, and sensitivity to mercury stress (Evers et al., 2008; Wolfe et al., 1998). Little is currently known about the levels of concern for omnivorous, insectivorous, or terrestrial species that have only recently become a focus of mercury studies. Therefore, although the negative impacts of mercury on wildlife have been widely documented, the levels that manifest these impacts across species are still uncertain.

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1.4.3.1. Sub-lethal Effects on Terrestrial Wildlife

There have been few field studies examining the effect of mercury contamination on terrestrial species. However, Brasso and Cristol (2008) found that tree swallows (*Tachycineta bicolor*), which feed primarily on flying aquatic invertebrates, produce about one less fledgling per nest on contaminated sites when compared to pairs breeding on reference sites without a history of mercury contamination. A similar study looked at reproductive success of eastern bluebirds (*Sialia sialis*), which have lower mercury levels than tree swallows, breeding on the same mercury-contaminated sites and found that there was no significant difference in clutch size, proportion hatched, or proportion fledged when compared to bluebirds breeding on reference sites (Condon, 2008).

Amphibians have also been a focus of mercury contamination studies because their permeable skin and dual habitats make them especially sensitive to both aquatic and terrestrial environments. However, research examining clutch size differences of American toads (*Bufo americanus*) breeding on contaminated and reference sites found no significant differences in their overall egg output (Bergeron, pers. comm., 2009). Ongoing research will examine the viability of these eggs to determine if maternal transfer of mercury is a threat for developing tadpoles as previous research has suggested for other contaminants (Hopkins et al., 2006). Sublethal effects of contaminants, including mercury, on amphibian populations are a focus of new research trying to decipher the causes of a worldwide decline of many amphibian species.

Very few studies have examined effects of mercury on terrestrial invertebrates. However, one study investigated the dose response effect of methyl mercury and selenium on house crickets (*Acheta domesticus L.*) and found that increased methyl
mercury doses resulted in decreased survival, growth and development (Ralston et al., 2006). Another study found that increasing the sediment mercury levels caused increased egg mortality and reduced nymphal hatching in two grasshopper species (Aiolopus thalassinus and Eyprepocnemis plorans) (Devkota and Schmidt, 1999). While Zhang et al. (2001) examined mercury contamination in German cockroaches (Blatella germanica) and found that accumulation in various tissues was associated with physical anomalies in the reproductive organs and fat bodies (Zhang et al., 2001).

Sub-lethal effects of mercury can be similar across species of wildlife; however, the levels needed to manifest these effects are highly variable between different taxa, species, and even individuals. Furthermore, effects are not always immediately apparent after exposure as there may be a latency period. Many of the effects mentioned above describe reproductive or survival metrics that are only observable after at least one breeding attempt or over an individual’s lifetime. Generalizing effects across species and/or taxa is very difficult and often inaccurate due to these factors as well as the variability in exposure (See sections 1.2 Mercury Transport and Cycling and 1.4 Mercury and Wildlife).

1.5. Birds as Biomonitor of Mercury Exposure

Although there exists some controversy over what makes a good biomonitor, there are a few agreed upon characteristics. These include such things as 1) well known life history traits, 2) consistent exposure to contaminants to allow the study of effects, 3) ease of monitoring, and 4) general interest by the public (Dauwe et al., 2003; Hilty and Merenlender, 2000). The use of birds as biomonitors satisfies many of these criteria as birds are a well-studied and ubiquitous taxon.
Birds have been used as biomonitors for a variety of human-induced stressors, including many contaminants (Cristol et al., 2008; Shriver et al., 2006; Rimmer et al., 2005; Dauwe et al., 2000, 2003, 2004; Eens et al., 1999; Furness and Camphuysen, 1997). Of particular importance to mercury studies is knowledge of foraging area and diet of a particular biomonitor and the nature of the tissue to be monitored (Hollamby et al., 2006). Similar to other wildlife, birds are mainly exposed to mercury through dietary uptake; therefore diet information is important in understanding the mechanism of exposure (See section 1.4 Mercury and Wildlife). Diet preferences have been documented for many species of birds but one needs to be wary of geographic differences in diet and use site specific information if available (Hollamby et al., 2006). For example, the diet of African fish eagle (Haliaeetus vocifer) has been documented to vary between sampling locations and individuals (Hollamby et al., 2006). At certain locations, carnivorous fish made up the majority of the diet while at other locations eagles were found to prey mostly on birds (Hollamby et al., 2006). Eagles that fed mostly on carnivorous fish were at the top of a longer food chain, which is correlated with increased mercury accumulation (See section 1.2.2.1 Bioaccumulation and Biomagnification).

Additionally, the foraging area and/or territory of the sampled individuals is also important when monitoring for mercury contamination in specific habitats or geographic locations (Hollamby et al., 2006). Even within a site, individuals may vary in microhabitats favored for foraging. Frederick et al., (2002) observed the separation on foraging grounds of white ibises (Eudocimus albus) from the same flock, with half of the adults foraging in coastal salt marshes and the other half foraging in freshwater estuaries, known to have higher prey mercury concentrations. Additionally, in a study of mercury
accumulation in seabirds of the Gulf of Maine, black guillemots (*Cepphus grylle*) were
documented foraging within 4 km of nesting sites on fish that were relatively immobile.
In contrast, common tern (*Sterna hirundo*) and double-crested cormorant (*Phalacrocorax
auritus*) adults fed on highly mobile schools of fish up to 40 km away from nesting
grounds (Goodale et al., 2008). This foraging difference was related to inter-island
mercury level differences between guillemot nesting sites that was not reflected in data
collected from terns and cormorants (Goodale et al., 2008). This example illustrates that
knowing the foraging territories of individuals is important since certain individuals may
increase their exposure with specific diet choices.

Characteristics such as sex, age, size and relative condition are easily assessed for
many species of birds, particularly songbirds. This allows one to make comparisons of
exposure between males and females, adults and nestlings, and large or small-bodied
species, increasing our understanding of which species and/or individuals are at a greater
risk of exposure. In addition, for territorial animals such as birds, the particular location
that an individual occupies will have specific characteristics that affect mercury
bioavailability.

The migratory status of a species is also important to document as many species
differ in their habitat and diet needs between their wintering and breeding grounds,
therefore varying their potential for exposure (Hollamby et al., 2006). Furthermore,
mercury availability may differ drastically between their breeding and wintering grounds
since these often lie in separate continents where regulations maybe more or less strict.
However, migratory species can still make valuable biomonitors if their migratory status
is taken into account when choosing which tissue to monitor (Hollamby et al., 2006).
1.5.1. Avian Tissue Comparison

Mercury is differentially distributed among tissues and therefore it is important to choose a particular tissue that suits monitoring needs. Past studies either collected dead specimens or sacrificed whole birds to look at the distribution of mercury among internal tissues. These studies provided information on the distribution of ingested mercury in internal tissues. One study that examined mercury distribution among tissues of common loon chicks found that mercury concentrations had a distribution as follows liver>kidney>muscle>carcass>brain (Kenow et al., 2007). However, another study that examined tissues in multiple water bird species found that kidney concentrations were greatest among internal tissues followed by liver>blood>muscle (Eagles-Smith et al., 2008). Both of these studies, and others, have reported that feathers have the highest concentrations followed by internal tissues (Kenow et al., 2007; Eagles-Smith et al., 2008). Eagles-Smith et al. (2008) investigated the relationship of tissue mercury levels in eight species of aquatic birds and found that blood was an excellent predictor of internal tissue concentrations while feathers were a weak predictor of internal tissue levels.

The two most commonly monitored tissues sampled non-lethally are blood and feathers. When mercury is ingested it first gets distributed among the red blood cells throughout the body before it is incorporated into any other body tissue (Morel et al., 1998). Therefore, blood mercury levels reflect short-term dietary uptake of mercury over approximately the previous two-week period (Evers et al., 2005). However, this is variable between species and dependent on the molting stage of the individual (Evers et al., 2005). For example, in young common loon chicks, growing hundreds or thousands of feathers, the half-life of mercury in the blood was estimated to be three days as
compared to 116 days in chicks that had completed feather growth (Fournier et al., 2002). Another study estimated a half-life of 84 days in non-molting adult mallards (*Anas platyrhynchos*) (Stickel et al., 1977). Recent studies have documented the fact that most of the mercury in blood is in the organic or methylated form of methyl mercury (Rimmer et al., 2005). This was corroborated at my study site where the total mercury in tree swallow blood samples was composed of 95% methyl mercury on average (Wada et al., 2009).

Feather is another non-lethal tissue that is easily monitored in nestling and adult birds. However, because of the toxicokinetics of feather mercury, accurate interpretation of levels is dependent on the age and migratory status of the individuals sampled (Eagles-Smith et al., 2008). Many studies have shown that feather growth provides an elimination route of body burden mercury for developing nestlings or molting adults (Condon and Cristol, 2009; Fournier et al., 2002). Methyl mercury has a high affinity for free thiol groups (-SH), which are abundant in feather keratin (Condon and Cristol, 2009). During feather growth, the feathers are connected to the body by a blood vessel that allows mercury circulating in the body to be transported to the feathers and become incorporated in the keratin structures. Developing nestlings are able to eliminate most of their mercury burden as they are growing hundreds of feathers at a given time. Adults undergoing their annual molt cycle or fledglings molting for the first time are also able to eliminate mercury into growing feathers (Condon and Cristol, 2009). However, since fewer feathers are growing at any one time, adults and fledglings are able to eliminate less of their body burden of mercury than developing nestlings. For all individuals, after feather growth is completed the blood vessel connecting each feather to the blood supply
atrophies and this route of mercury elimination is no longer available. In contrast to blood, feather mercury is stable once the blood vessel connection is lost; therefore it reflects only the mercury an individual was carrying at the time that the feather was grown (Eagles-Smith et al., 2008). However, we often do not know where an adult individual grew in a molting feather and then cannot be sure where they obtained the mercury burden reflected in their feathers. For this reason, it is important to use biomonitors for which the migratory behavior are well known. This complication is avoided when monitoring nestling feathers because nestlings are confined to one area and exposed only to dietary mercury or any maternal mercury received during egg-laying (Dauwe et al., 2004). Feathers sampled during this period of intense growth will reflect only mercury obtained from a small and defined area during the short nestling stage (Eagles Smith et al., 2008; Frederick et al., 2002).

1.5.2. Studies Using Birds as Biomonitor of Mercury Contamination

There have been numerous studies that have used birds to indicate mercury contamination. These studies have examined various tissues from an array of species varying from large birds of prey to small passerines. Here I will summarize a subset of these studies.

A large inclusive data set containing mercury blood levels from a range of birds, including piscivorous and insectivorous species, across northeastern North America was used to indicate areas of highest mercury contamination concern. They found that methyl mercury availability increased from marine, to estuarine and riverine systems, and was highest in lake habitats (Evers et al., 2005). The eggs of a commonly-studied piscivorous species, the common loon, were found to have decreasing mercury levels from east to

30
west, mimicking a geographic trend in mercury deposition rates and fish contamination (Evers et al., 2003).

Wading birds have also been used extensively as bioindicators of mercury contamination in aquatic habitats. One study used feathers sampled from great egret (Ardea alba) and white ibis nestlings to assess differences in mercury exposure between colonies located in coastal Florida and those located inland (Frederick et al., 2002). Coastal colonies consistently had lower feather mercury values than more inland colonies, suggesting that mercury accumulation in the prey base was more of a concern for inland populations (Frederick et al., 2002). Additionally, they detected a significant decline in mercury levels of all colonies over the long term, indicating a decrease in mercury availability in more recent years (Frederick et al., 2002).

Seabirds and other birds of prey have been historically used to assess mercury availability because they are large-bodied and exist high on the food chain making them ideal candidates to accumulate high levels of mercury (See sections 1.2.2.1 Bioaccumulation and Biomagnification and 1.4 Mercury and Wildlife). One of many studies used seabird feathers to assess changes in mercury availability of the northern Atlantic Ocean (Thompson et al., 1992). They found that, over the time period of study, there was an increase in mercury concentrations of birds sampled from the west and south of the British Isles (Thompson et al., 1992). Many studies have used birds of prey such as the bald eagle as biomonitor s of mercury as they were historically used to indicate pesticide exposure. One such study sampled feathers and blood from bald eagle nestlings in South Carolina to determine if they were accumulating mercury (Jagoe et al., 2002). Their results did indicate exposure of these nestlings; however, contrary to other studies,
they did not detect geographic differences between nests in coastal and inland regions (Jagoe et al., 2002).

In recent years there have been a growing number of studies using small-bodied passerines to monitor or indicate contamination. Passerines vary widely in habitat use, foraging strategies, diet, size and sensitivity to contaminants, so they have provided much-needed information on mercury contamination in a variety of habitats and ecosystems. One such study used two species of sharp-tailed sparrows to assess methyl mercury availability in coastal salt marshes of northeastern North America (Shriver et al., 2006). Using two similar species, Nelson’s sharp-tailed sparrow (*Ammodramus nelsoni*) and saltmarsh sharp-tailed sparrow (*Ammodramus caudacutus*) that breed solely in salt marsh habitat, they found that both species were accumulating elevated levels of mercury in their blood, although the larger-billed species (saltmarsh sharp-tailed sparrows) was accumulating higher levels (Shriver et al., 2006). From these results, they concluded that methylation in tidal salt marsh habitats is prominent and poses a risk for associated wildlife, especially in areas at risk of mercury deposition (Shriver et al., 2006).

Another study documented methyl mercury availability in a terrestrial montane system of northeast North America using a suite of insectivorous passerines (Rimmer et al., 2005). Although the methylation process occurring at these high elevation sites was not apparent, it was evident that exposure was a risk to terrestrial passerines. Of most importance was that the potentially endangered Bicknell’s thrush (*Catharus bicknelli*), a strictly terrestrial, insectivorous songbird, was susceptible to methyl mercury accumulation (Rimmer et al., 2005). From this they were able to conclude that even
species that reside in seemingly pristine montane forests are susceptible to mercury contamination.

Small passerines have also been used as biomonitors at sites of known point source contamination. Cristol et al. (2008) used terrestrial songbirds that breed in the floodplain of the South River in Virginia to determine that mercury in a river is mobile, either biologically or physically, and present in the terrestrial food chain within 50 m of the river. This study also showed that there was considerable variation between different species of birds according to their foraging strategy and specific diet choices, even though they were breeding in the same floodplain. Additionally, a series of studies has used great tits (Parus major) to indicate contamination by an array of heavy metals originating from a smelter site in southern Belgium (Janssens et al., 2001, 2003; Dauwe et al., 2004). These studies found significant heavy metal accumulation in the feathers of both adults and nestlings as well as in their caterpillar prey (Janssens et al., 2001; Dauwe et al., 2004).

Finally, a study investigated the occurrence of mercury in prothonotary warblers (Protonotaria citrea) breeding near chlor-alkali and DDT production facilities that released mercury in confined areas of the Tombigbee River floodplain (Adair et al., 2003). This study used nestling kidneys as the tissue of choice because it is the first internal organ to accumulate mercury to a high degree (Adair et al., 2003). They modeled the extent of exposure at two locations using mercury concentrations in soil samples taken close to each nest box, food items collected directly from nestlings, and nestling kidneys (Adair et al., 2003). Interestingly, they found the strongest correlations between soil and kidney as well as soil and food mercury concentrations (Adair et al., 2003).
2003). They did not find a significant correlation between the collected prey and nestling kidney samples and they concluded that the single food sample collected from broods of each nest box was inadequate to be representative of food mercury exposure (Adair et al., 2003). Furthermore, they were able to clearly demonstrate that nestlings reared by migratory adults that had no previous exposure were accumulating mercury within the short nestling period at a point source (Adair et al., 2003). Additionally, they correlated the extent of exposure in the nestlings with the availability of mercury in the soils surrounding their nest boxes and this provided a link between local availability and risk of exposure (Adair et al., 2003). Finally, during specific investigation of the collected prey items they established that spiders were accumulating significantly higher levels of mercury compared to the other prey types; however, they were unable to relate this to accumulation in the nestlings’ kidneys (Adair et al., 2003).

1.6. Studies Examining Spatial Variability & Extent of Contaminant Availability and Exposure

Few studies have investigated the spatial extent of contaminants originating from either point or non-point sources. Here, I will examine a few studies that attempted to determine the spatial extent and spatial variability of contaminant availability.

There have been studies that have attempted to quantify the amount of mercury deposition at increasing distances from point source atmospheric emissions. These studies have used a range of media to quantify deposition: air, soil, animal and plant. These studies have published different (sometimes competing) results; however, there is a general consensus that deposition is more likely to occur closer to the point source with lower concentrations as distance is increased. Many characteristics are likely to influence
the amount of deposition such as weather patterns, source and type of emissions, amount of emission and topographic features of the surrounding landscape.

One study looked at soil concentrations on a radial grid surrounding a large coal-fired plant in New Mexico (Crockett and Kinnison, 1979). This study had two major conclusions, one being that soil concentrations decreased with increasing distance from the plant suggesting that deposition was greater in the 1 km radius around the plant but dropped off rapidly, although soil concentrations were still elevated in the 30 km radius (Crockett and Kinnison, 1979). The also concluded that mercury concentrations throughout the study area were very low for the amount of mercury being emitted from the plant, suggesting that only a small portion was being locally deposited (Crockett and Kinnison, 1979).

Another study examined mercury concentrations in the air at increasing distances from a mercury mine in Guizhou, China (Tan et al., 2000). They found that air concentrations significantly decreased with increasing distance; however, they remained above background levels for 67 km (Tan et al., 2000).

A third study investigated the spatial variability of exposure to atmospheric point sources of mercury in northwest Spain. Specific study aims were to quantify the extent of mercury accumulation by grazing cattle at farms both upwind and downwind of industrial centers in Asturias and Galicia provinces (López Alonso et al., 2003). They found that cattle grazing on farms downwind accumulated significantly more mercury in their kidneys and this pattern was observed at distances as far as 200 km from the closest industrial center (López Alonso et al., 2003). Furthermore, although there was notable mercury accumulation in cattle upwind of industrial areas, there was no observed decline.
with increasing distance (Lopez Alonso et al., 2003). This study suggests that atmospheric mercury may be deposited more substantially within a close range downwind of the source, creating an exposure risk for downwind organisms.

The previously mentioned studies focused on atmospheric emissions of mercury. Very few studies, if any, have addressed a similar question with aquatic point sources of mercury. One pertinent study investigated the spatial variability of a variety of heavy metals including lead, copper, and zinc in floodplain soils flanking a river contaminated by non-point sources. They specifically aimed to classify variability of metal concentration in the soils according to depth and lateral extent from the river. Analysis of soil core samples taken at increasing distances from the stream bank channel showed that samples taken directly from the stream bank had the highest concentrations and a steep decline in concentrations of all metals was observed within 50 m extending laterally from the stream bank (Martin, 2000).

Due to the steep decline in metal concentrations within short distances of the stream bank channel, Martin (2000) suggested that metals were primarily deposited on the floodplain during flood events rather than by atmospheric deposition where one would expect no linear relationship. Furthermore, he reasoned that areas containing flood-borne metal deposits act as new non-point sources to the terrestrial environment and may also contribute high concentrations of metals back to the stream channel by erosion (Martin, 2000).

There are also some recent and current studies examining exposure to contaminants released from point sources. The majority of these studies are investigating the role of distance from atmospheric releases of contaminants (Lord, Society of 36
Ecotoxicology and Chemistry Annual Meeting 2009). Few studies have focused on the role of distance from a point source in explaining spatial patterns of exposure in free-living wildlife. However, there have been a few studies that have compared exposure in fish and other aquatic organisms from upstream and downstream of point sources. One recent example is a study that quantified exposure in free-living fish upstream and downstream of an effluent pipe that contained various pollutants including mercury (Vajda et al., 2008). They found, not surprisingly, that individuals directly downstream of the outflow pipe had elevated levels relative to individuals upstream of the pipe. This largely un-addressed question is important to ask when preparing sound risk assessments for wildlife. Without understanding the spatial extent of exposure from a point source it would be difficult to determine which species or habitats are at risk and should be included in an assessment.

1.6.1. Studies Assessing Spatial Variability & Extent of Mercury Exposure in Birds

Although a range of bird species (both large and small) have been used to indicate sites of mercury contamination, very few studies have investigated small scale differences in spatial variability of exposure. Some studies claim that the use of seabirds and birds of prey is advantageous because they feed at higher trophic levels and range over large areas (Furness and Camphuysen, 1997). Using species that range over a large area to indicate contamination offers information on the wide distribution of a contaminant but the heterogeneity detectable at smaller spatial scales, or within sites, is lost in the process (Burger et al., 1992; Janssens et al., 2001). A few studies have examined large scale regional differences, especially between industrial and more remote
areas (Dominguez and Montevecchi, 2003; Evers et al., 2003; Evers et al., 2005).

Additionally, a few studies have investigated differences in contaminant exposure between nest sites of the same study area (Goodale et al., 2008). However, none of these studies specifically investigated differences in mercury exposure between individuals inhabiting the same geographic location. In this section I will examine a few studies that have addressed this idea further.

Dominguez and Montevecchi (2003) found that levels of DDE (1,1-Dichloro-2,2-BIS(p-Chlorophenyl) Ethylene) and PCB (plychroniated biphenyl) in the blood of nestling bald eagles declined with nest distance (kilometers) from a former naval base. However, they did not find any relationship between distance and blood mercury concentrations. Another study found that nest site location was an important factor in explaining variation in contaminant levels of feathers taken from nestling Laysan and black-footed albatrosses (Diomedes immutabilis and Diomedes nigripes) (Burger and Gochfeld, 2000). Nest site location was most important in explaining lead levels because nestlings feeding near a building where lead paint had not been removed were observed feeding on lead chips (Burger and Gochfeld, 2000). However, no relationship was seen between distance from the dilapidated buildings and mercury levels in feathers (Burger and Gochfeld, 2000).

A study by Adair et al., (2003) mentioned in the previous section (1.5.2 Studies Using Birds as Biomonitors of Mercury Contamination), used nestling prothonotary warbler nestlings, their prey, and local soil samples to distinguish areas of increased mercury contamination at a known contamination site in Alabama. Using these three matrices they produced contour maps of mercury exposure that clearly defined areas of
high contamination (Adair et al., 2003). These areas were similar for soil and kidney matrices but differed for food mercury (Adair et al., 2003). This study demonstrates the usefulness of using biomonitors to determine areas of high risk within a single site. However, they were not able to investigate whether distance from the contaminated waterway was an important factor in defining these areas of high contamination because all nest boxes were placed at similar distances.

Finally, a series of studies investigated contaminant accumulation in adult and nestling great tits, a small insectivorous passerine, breeding along a pollution gradient. The most polluted sites were closest to a non-ferrous smelter that released heavy metals into the local atmosphere in Belgium (Janssens et al., 2001). These studies examined various contaminant concentrations in adult and nestling tail feathers, excreta, caterpillar prey, and vegetation at four sites ranging in distance from the pollution source: 0-350 m, 400-600 m, 2500 m and 4000 m from the source (Dauwe et al., 2004; Janssens et al., 2001). They found that for all four variables examined, mercury levels were higher at the site closest to the smelter (Dauwe et al., 2004; Janssens et al., 2001). However, the decrease in mercury concentrations was significant only for excreta, caterpillars and vegetation while it was non-significant for adult and nestling tail feathers (Dauwe et al., 2004; Janssens et al., 2001). The authors proposed that most of the metal contamination at their site was of inorganic forms and therefore was more likely to be excreted rather than incorporated into growing feathers (Dauwe et al., 2004). This would explain the stronger relationship seen with distance from the smelter and excreta, but does not explain how birds accumulated inorganic mercury, when their diet items presumably had incorporated organic mercury. It is also not clear why there was no relationship observed
between distance and nestling tail feathers, since there was significant mercury accumulation in the nestling tail feathers, which is known to be in the methylated form (Wada et al., 2009). One possibility may be that the use of adult tail feathers may not be indicative of local mercury exposure during the breeding season as the feathers would contain mercury accumulated during the previous year’s molt. However, this answer does not resolve why no relationship was seen between nestling tail feather mercury and distance from the smelter as the nestlings feather mercury should be indicative of their dietary mercury (See section 1.5.1 Avian Tissue Comparison).

I have found few studies on variability of exposure over a small spatial scale in any type of habitat (aquatic or terrestrial) or from either a point or non-point source. However, there are a few unpublished studies that investigated the extent of mercury exposure in aquatic biota downstream of a point source. A concurrent study on the South River study site in Virginia found that periphyton total mercury and methyl mercury concentrations steadily increased from the point source to approximately 7.5 km downstream (Tom, 2008). More recent sampling found a decrease in mercury around 32 km downstream (Tom, 2008). However, periphyton levels in the similarly contaminated Hollston River of Virginia were not found to decrease over a 136 km stretch downstream of the contamination source (Tom, 2008).

To the best of my knowledge, there are no studies that have investigated the spatial extent of mercury exposure of terrestrial biota due to mercury originating from an aquatic source. Only recently have researchers begun to understand the importance of material flux of biota, nutrients and/or contaminants from aquatic ecosystems to the adjacent terrestrial food chain. In the next section I will discuss the permeability of the
aquatic-terrestrial interface and summarize what we already know about material flux between systems and the mechanisms that drive it.

1.7. **Subsidies and the Aquatic-Terrestrial Interface**

Historically, ecological studies have focused on the cycling of nutrients and energy within habitats or ecosystems. In recent years, ecology at the landscape or ecosystem level has gained prominence and ecologists now regard systems as having permeable boundaries allowing the transfer of material between habitats (Ballinger and Lake, 2006). The permeability of a boundary, or ecotone, between two habitats is determined by the topography of the land as well as the structural complexity of the recipient habitat (Witman et al., 2004). Permeability is quantified as a combination of the concentration and velocity of the material being transported as well as the distance that the material travels into the recipient habitat (Witman et al., 2004). A boundary that is considered to be more permeable will lie between topographically similar habitats with few structural blockades. For example, in riparian systems, materials are generally deposited in floodplains where there is little change in relative elevation to the river, or on the inside of meanders where obstructions have slowed the flow (Malanson, 1993). Other studies have shown that the quantity and distance of seed dispersal into a forest is limited by densely vegetated edges relative to more gradual or sparse edges (Cadenasso and Pickett, 2001). These studies imply that topographic and vegetation factors along the aquatic-terrestrial interface determine the extent of material flux between ecosystems or habitats.
1.7.1. **Material Flux: Land to Water**

Material flux between habitats is often referred to as a subsidy, defined as a transfer of resources from a donor habitat to a recipient habitat or consumer that results in a benefit to the recipient (Polis et al., 1997). These resources include prey, detritus and nutrients that are transported across habitat borders by biological vectors, such as migrating animals, or physical vectors, such as water during flood events (Cadenasso et al., 2004; Polis et al., 1997). Much of the research at the land-water interface has been focused on the addition of nutrients and organic matter from surface water runoff to lakes, rivers and coastal waters (Ballinger and Lake, 2006). Many of these studies have focused on the importance of allochthonous or foreign inputs to the productivity of streams and have contributed to the development of the *River Continuum Concept*, which states that the role of terrestrial inputs decreases with stream size (Ballinger and Lake, 2006; Riley et al., 2004; Polis et al., 1997). Terrestrial inputs can have cascading effects on the food chain of recipient water bodies (Nakano et al., 1999; Polis et al., 1997). For example, in an experimentally manipulated study, the exclusion of terrestrial invertebrate inputs caused fish to shift their diet to aquatic invertebrates that feed on instream vegetation. This diet shift decreased instream herbivory and released the growth of algae within the stream (Baxter et al., 2005; Nakano et al., 1999).

1.7.2. **Material Flux: Water to Land**

Although the transport and consequence of terrestrial inputs to streams is well studied, there is a lack of research on the importance of aquatic subsidies to terrestrial food chains and ecosystems. This is in contrast to the idea that land-water ecotones are permeable. Recent research has switched from the unidirectional view of the land-water
interface where material flux is from the land to the water to a more cyclic view that incorporates the importance of allochthonous material exchange in both directions between aquatic and terrestrial ecosystems (Polis et al., 2004; Polis et al., 1997). The emergence of adult insects from streams has been a focal point of these studies, as they represent an important food source for riparian predators including birds, bats, and spiders (Burdon and Harding, 2008; Gray, 1993; Paetzold et al., 2006; Marczak and Richardson, 2007; Polis et al., 2004; Walters et al., 2008). Another mode of transport for subsidies to riparian lands is through flooding (Polis et al., 1997). For example, flooding transports nutrient rich sediments into floodplain areas that have been historically used as agricultural land (Polis et al., 1997). Both emergent insects and flood waters can be important vectors of nutrients, prey and other subsidies to riparian lands and biota. However, of more importance to this study is the importance of each of these mechanisms in transporting contaminants across the aquatic-terrestrial interface.

1.7.3. Movement of Contaminants across the Aquatic-Terrestrial Interface

Polis et al. (1997) defined a subsidy as a material that results in a benefit for the recipient, however superfluous nutrients or detrimental pollutants can also travel through the same mechanisms as beneficial subsidies (Walters et al., 2008). The transport of these contaminants can have negative impacts on the recipient consumer or ecosystem (Burdon and Harding, 2008). One very well known example is the runoff of surface waters containing excess nutrients and contaminants (Turner et al., 2008). This leads to eutrophication and contamination in small and large water bodies. The Gulf Coast near the mouth of the Mississippi River is a prime example of eutrophication because it
receives high levels of nutrients, primarily from the drainage of agricultural lands along the Mississippi River (Turner et al., 2008).

It is well known that mercury can be transported globally through weather patterns or locally within ecosystems, however its movement from aquatic to terrestrial habitats has been overlooked. The following sections will summarize studies that demonstrate how contaminants, such as mercury, cross the aquatic-terrestrial interface via the same mechanisms as the flow of nutrients, prey and detritus, namely through biological or physical vectors.

1.7.3.1. Emergent Insects as Biological Vectors

There have been multiple studies that point towards the transport or removal of contaminants from aquatic ecosystems through emerging insects. Aquatic insects undergo an ontogenic shift in their habitat needs as they hatch and develop in the water and emerge onto land as flying adults (Ballinger and Lake, 2006). Therefore, immature aquatic insects can accumulate contaminants from river sediments and potentially mobilize them back to the water column or to other aquatic organisms through predation (Currie et al., 1997; Menzie, 1980; Fairchild et al., 1992; Reynoldson, 1987). However, of more importance to this study, emerging adult aquatic insects can transfer body burdens of contaminants to the terrestrial ecosystem where their consumption has been linked to contamination of terrestrial predators, such as tree swallows (Currie et al., 1997, Fairchild et al., 1992; Walters et al., 2008). Times of emergence have been correlated to the higher densities of insectivorous birds and spiders in riparian zones relative to upland areas supporting the idea that emergent insects are an important food source to terrestrial predators (Ballinger and Lake, 2006; Gray, 1993).
Jackson and Fisher (1986) estimated that 97% of aquatic insect emergence (22.4 grams of carbon m$^{-2}$ yr$^{-1}$) is exported to the adjacent watershed where it feeds consumers such as ants, birds and bats. The patterns of emergence are still not well known, mostly because they vary greatly in space and time determined by a host of variables including weather patterns, ecotone structure, stream size, floodplain habitat etc. (Power et al., 2004). However, there have been some studies that have strived to quantify the abundance of emergent aquatic insects with distance from their aquatic source (Polis et al., 2004). These studies have shown that densities of emergent insects decline rapidly with distance from the river (Vander Zanden and Sanzone, 2004). One study in particular found that insect fluxes into the floodplain declined exponentially in numbers and biomass with a 50% reduction in just 10 m from the river’s edge (Power et al. 2004). Stream productivity and season were found to largely affect the lateral influx of emergent insects (Power et al., 2004). High density of emergent insects along shorelines has been correlated with abundance of riparian predators and therefore strongly supports the idea that these predators depend on aquatic subsidies (Polis et al., 2004).

These studies suggest that aquatic insect production and emergence may play a key role in the export of contaminants to riparian lands and associated fauna. Therefore, terrestrial organisms that rely more heavily on aquatic insects in their diet may be at a higher risk to exposure of some contaminants. A recent study investigated the reliance of web-building spiders (*Araneus* spp. and *Argiope aurantia*), ground-dwelling spiders (*Dolomedes* spp.) and herptiles on aquatic versus terrestrial food sources (Walters et al., 2008). They found that of the three predator types, web-building spiders relied most heavily on aquatic insects and therefore seemed to be the most at risk from pesticides.
exported out of the study stream (Walters et al., 2008). In addition, there is a recent study (Walters, unpublished) that addressed exposure of riparian spiders to PCB’s originating in Lake Hartwell of South Carolina. This study found that riparian spiders were relying heavily on emergent aquatic insects, which were credited for transporting the aquatic borne PCB’s to riparian spiders (Walters, unpublished). In addition, spiders that inhabited more upland areas were found to rely much less on aquatic insects in their diet which explained their lower levels of exposure (Walters, unpublished). This result suggests that distance from the river does play a role in understanding exposure to aquatic contaminants. However, even with the recent research focus on the transport of contaminants via subsidies, little attention is being paid to the spatial pattern of exposure in terrestrial organisms living near permeable ecotones.

1.7.3.2. Flooding as a Physical Vector

Flooding may also be responsible for the transport of aquatic mercury into riparian areas. Multiple studies have investigated the increased rate of methylation in recently flooded lands (St. Louis et al., 2004; Roulet et al., 2001). Flooding causes the organic carbon locked in the soils and plant material to be decomposed by bacterial respiration (St. Louis et al., 2004; Gilmour and Henry, 1991). Bacterial respiration unlocks much of the mercury stored in the soils and creates methyl mercury as a byproduct, which is easily accumulated by organisms (See sections 1.2.5 Methylation and Methyl Mercury and 1.4 Mercury and Wildlife). This process has been repeated in numerous flooded reservoirs worldwide. St. Louis et al. (2004) experimentally flooded a wetland in northwestern Ontario and recorded a 7-fold increase in the amount of methyl mercury as well as a simultaneous increase in the production of dissolved organic carbon.
Kelly et al. (1997) used an experimental boreal forest and found that there was a 39-fold increase in methyl mercury after flooding. The increase in bioavailable mercury after flooding was observed throughout the wetland food chain from vegetation to fish (Kelly et al., 1997). In both of these studies, areas that were previously carbon sinks were transformed into carbon exporters (St. Louis et al., 2004; Kelly et al., 1997). Additionally, St Louis et al. (2004) observed that, before flooding took place, the experimentally flooded wetland had been a store for mercury, mostly locked up in the peat moss, however, post flood sampling revealed that the newly created reservoir had become a mercury exporter as well. This has serious implications for reservoir creation worldwide, as they now total over 1 million acres (St. Louis et al., 2004). Areas naturally predisposed to flooding are subject to the same processes. Floodplain soils along Amazonian rivers are flooded annually. These inundated areas have been found to produce twice as much methyl mercury than during the dry season when the floodplain soils are not underwater (Roulet et al., 2001). Furthermore, soils that are rich in organic matter are also the areas with the highest rates of methylation owing to the availability of organic carbon (Roulet et al., 2001). This is especially important for areas receiving mercury loads, either through atmospheric deposition or from a local point source; flooding may unlock this mercury and make it biologically available to the local wildlife.

Additionally, there is some evidence that floodwaters can contaminate floodplain soils by remobilizing pollutants in river sediments and transporting them in alluvium (Adair et al., 2003). Pulkrabova et al. (2008) found that a major flooding of event of the River Elbe, Czech Republic, was responsible for increased DDT in floodplain soils; however the same trend was not seen for other persistent organic pollutants or polycyclic...
aromatic hydrocarbons (PAHs). A similar study on the River Elbe found that the flood of 2002 was responsible for the mercury contamination found in alluvial soils (Statchel et al., 2006). This study did not find a significant influence of landscape morphology (flood channels, plateaus, levees, and basins) on the concentration of mercury in the floodplain soils (Satchel, 2006). This may be explained by the fact that the flood of 2002 carried water over levees and broke holding chambers so that areas not normally subject to flooding were also inundated. In another study, Hilscherova et al. (2007) showed that flooding has the potential to act as a vector for PAHs from sediments into alluvial soils. They investigated the impact of floods on the redistribution of various organic pollutants found in river sediments of Europe and detected that over time, the PAHs increased in floodplain soils but decreased in river sediments (Hilscherova, 2007). In the case of mercury, both inorganic mercury and organic methyl mercury may be transported into floodplain soils where it either becomes sequestered or is taken up by lower trophic levels. Subsequent flood events producing anoxic conditions in these already contaminated soils can cause the in situ methylation of any inorganic mercury that was previously transported in the alluvium. Therefore, highly toxic methyl mercury can be produced in either the aquatic environment that acts as a point source for the adjacent areas, or methylation can occurs in the terrestrial soils themselves. Generally, large alluvial deposits are associated with larger rivers; however, the same processes may occur on smaller order streams.

1.8. Assessing Risk of Exposure for Wildlife

I have already described why mercury is a concern for wildlife, the ways in which species are exposed and how exposure has been measured in the past. I also reviewed
what is known about the spatial variability of mercury availability and exposure with a focus on studies that have used birds. Finally, I summarized recent ideas about how mercury travels between ecosystems, thereby increasing the range of biota at risk of exposure. In this section I will describe how this information is formally used to assess risk of exposure for wildlife by describing 1) the history and process of ecological risk assessments and 2) how risk assessments are used to design strategies to protect wildlife at contaminated sites.

1.8.1. The Process of Ecological Risk Assessment

Ecological risk assessments are used to analyze data, quantify uncertainties and evaluate the likelihood that adverse effects have or will occur as a result of exposure to stressors related to human activities (Hope, 2006; USEPA, 1997). Ecological risk assessments have multiple facets but their main utility is as a way to organize and present ecologically relevant information for risk managers or those making policy decisions (Hope, 2006).

Determining the species, or endpoints, to study when conducting a risk assessment is an important and first step in the process. Ideally, risk assessment endpoints should be a specific attribute of an ecologically important species for the site, for example disease or survival in wildlife (Hope, 2006). However, in ecotoxicology studies, determining whether certain species are indeed being exposed is a first step. This is encompassed in the first stage of an ecological assessment referred to as the “Problem Formulation Stage” (Hope, 2006).

The second stage is the characterization of exposure and effects in the chosen endpoint (Hope 2006; USEPA, 1997). Exposure characterization entails determining the
method of exposure to the endpoint as well as detailing the kinds of effects likely to occur (Hope, 2006). Within this framework it is important to understand the sources of the contaminant, the spatial and temporal distribution, and the co-occurrence of the individuals of interest with the contaminant (Hope, 2006). These last two points have not always been included in risk assessments and their exclusion causes less certainty of the actual risk to the endpoint in question (Hope, 2005). For example, if there is spatial variability in the presence of the contaminant, a free-living bird breeding may increase or decrease its contaminant load depending on where it chooses to move or forage. In order to accurately assess the probability of exposure for this individual (or species) it is necessary to understand the life history traits of the species and have a clear spatial profile of the contamination (Hope, 2005). If the species of interest feeds in the canopy but the contaminant is only available from the soil, this species may have less exposure than another endpoint. Furthermore, when assigning risk to a contaminant that is capable of biomagnification, the food web should be well understood and examined before pronouncing any endpoint as safe from exposure.

The next stage described in ecological risk assessment is interpretation of the data collected from the other two stages with the goal of estimating risk (Hope, 2006). Within this stage is the identification of data gaps and uncertainties that need to be included in the final risk assessment. In order to evaluate the consequences associated with any risk of exposure, the nature and intensity of observed adverse effects, spatial-temporal scales, and potential of recovery are taken into account for the final assessment (Hope, 2006). Once all the stages, interpretation, and uncertainties have been exposed and dealt with,
the most important step is to put the assessment in the hands of decision makers who can use the information to assign costs and inform restoration projects.

1.9. Information Gaps

Most risk assessments dealing with mercury have focused on the aquatic ecosystem as this is where the processes of bioaccumulation and biomagnification have been best documented. However, recent studies have pointed to the movement of mercury from aquatic sources into the adjacent terrestrial systems. Little research has focused on understanding mercury availability and exposure in terrestrial biota. Furthermore, although there have been a host of studies that have examined regional and site differences in mercury exposure, few have examined spatial variation on small scales. Understanding the factors that explain the variation in exposure on a small scale will better inform our understanding of mercury cycling. To my knowledge, no studies have investigated the maximum distance from an aquatic point source at which mercury exposure remains a risk for terrestrial biota. This information is imperative in performing accurate risk assessments as many more species and individuals may be at high risk of mercury exposure than previously believed. Future mercury risk assessments should take into consideration the connectivity between ecosystems and habitats to understand the scope of exposure more accurately. Additionally, by including information on the spatial variability and extent of mercury exposure, future risk assessments can increase their accuracy in predicting which habitats, species and individuals are at greatest risk.

1.10. South River Study Site

The South River is a fourth order stream with its headwaters originating on the western slope of the Blue Ridge Mountains near Waynesboro, Virginia. The river flows
in a northeasterly direction and joins the North River near Port Republic, VA to form the South Fork of the Shenandoah River. Eventually the South Fork joins the North Fork to form the Shenandoah River which ultimately finds its way to the Potomac River and Chesapeake Bay.

The floodplain of the South River is comprised mostly of agriculture and pasturelands with intermittent forest buffers of varying widths (Murphy, 2004). Although historically the South River was known for its sport fishing and other recreational activities, in recent times the river has been the recipient of urban, agricultural, and industrial pollutants.

Figure 1: Map of Study Area
1.11 History of Mercury Contamination

A factory owned by E.I. du Pont de Nemours (hereafter DuPont) located on the South River in Waynesboro, VA used mercuric sulfate as a catalyst in the production of acetate fibers between 1929 and 1950 (Carter, 1977). During this time, an unknown amount of mercury was leaked directly into the South River and adjacent soils at the factory site. Although the use of mercury as a catalyst was suspended in 1950, 22 years later globules of mercury were observed near the river and the river’s contamination was brought to public attention (Carter, 1977). To assess the safety risk to the local human population, sediment and fish tissue samples downstream of the Waynesboro factory were analyzed for mercury content. Sediment samples were found to have mercury concentrations up to 240 ppm, compared to 1 ppm upstream (Carter, 1977). Of more concern, the mercury levels of common game fish downstream were found to be well over the Food and Drug Administration “action” level of 0.5 ppm (Carter, 1977). In response, the Virginia Department of Environmental Quality issued a “no consumption” fish advisory in 1977 for all species taken on the South River extending from the Dupont factory in Waynesboro, VA to the South Fork Shenandoah River (approximately 77 river miles) (Carter, 1977).

1.12 Previous Research: Mercury Contamination of South River Biota

1.12.1. South River Science Team (SRST)

The result of a settlement between DuPont and the Virginia State Water Control Board was a trust fund created to fund research monitoring mercury contamination in the
South River for a 100-year period. In 2000, this trust fund was used to establish a team of researchers including academics, non-profit organizations, private industry scientists, and the United States Fish and Wildlife Service under the name of South River Science Team (SRST). The main objective of the SRST was to monitor mercury levels in the water, sediment and fish for the 77-mile stretch contaminated with mercury in an effort to understand the risk of exposure for the local human population. The majority of research conducted by the SRST has focused on the aquatic ecosystem with special attention to fish mercury levels, as they pose the greatest threat to humans. However, recent research has also focused specifically on the South River floodplain and its associated terrestrial biota.

1.12.1.1. Studies of Aquatic Biota

Monitoring of fish tissues downstream of the Waynesboro DuPont plant has been continuous since the fish consumption advisory was put into place in 1977. In contrast to expectations of the early SRST members, fish mercury levels have not decreased over time and remain elevated above the 0.5 ppm action level (Murphy 2004; VDEQ, 2007). The most recent survey of fish tissues occurred in the spring of 2007 and targeted multiple trophic levels; smallmouth bass (*Micropterus dolomieui*) (predators), redbreast sunfish (*Lepomis auritus*) (grazers), and white suckers (*Catostomus commersoni*) (bottom feeders) (VDEQ, 2007). The average total mercury levels for single fillets from 2007 were, respectively, 2.30 ppm, 1.70 ppm, and 1.50 ppm (VDEQ, 2007). Samples of stocked rainbow trout (*Oncorhynchus mykiss*) have also been continuously monitored but are below the 0.5 ppm action level and therefore deemed safe to consume (VDEQ, 2007). Studies have shown that, on average, 90% of the mercury in fish tissue is methyl mercury
and an action level of 1.0 ppm methyl mercury has been established by the U.S. Food and Drug Administration (VDEQ, 2007). All of the above sampled species (with the exception of the rainbow trout) have consistently had more than 1 ppm methyl mercury in their tissues and therefore the consumption advisory is still in place for fish taken from the South River.

In 2002, prey items of four species of fish (white suckers, channel catfish (*Ictalurus punctatus*), redbreast sunfish, and smallmouth bass) were collected and analyzed for total and methyl mercury concentrations (Murphy 2004). Collected prey items included aquatic invertebrate larval samples from six taxonomic orders including mayflies (*Ephemeroptera*), caddisflies (*Trichoptera*), damselflies (*Zygoptera*), dragonflies (*Anisoptera*), beetles (*Coleoptera*), and true flies (*Diptera*) (Murphy 2004). The mean total mercury concentrations of these orders ranged from 0.090 ± 0.61 ppm for beetles to 0.324 ± 0.76 ppm for true flies (Murphy 2004). The percent of the total mercury that was in the form of methyl mercury was also determined and averaged 5.2 ± 2.3% for beetles, 34.5 ± 5.0% for mayflies (detritivore-grazer), and 75.6% ± 13.3% for damselflies and dragonflies (predators) (Murphy 2004).

1.12.1.2. Studies of Terrestrial Biota

In general, few studies have assessed mercury accumulation in terrestrial biota. One study investigated mercury accumulation in various terrestrial compartments of the South River floodplain including soils, plants, terrestrial macro-invertebrates and a subset of terrestrial mammals (Cocking et al., 1991). Soil samples were taken from twenty-four
10x10 m² quadrats established at various sites along the South River. Total mercury concentrations in the soil samples were highly variable dependent on habitat type and other unmeasured variables. Soil samples taken from old field sites had a mean of 17.0 ± 1.3 ppm, while those from shrubland sites averaged 22.0 ± 3.7 ppm, and those from forested sites were more variable ranging from 11 to 84 ppm (Cocking et al., 1991). However, all soil samples from the South River floodplain were found to be elevated over samples taken from uncontaminated sites which averaged <0.1 ± 0.02 ppm (Cocking et al., 1991). Additionally, dry weight mercury concentrations were determined in the roots and leaves of both herbaceous and woody plants. High variability notwithstanding, mercury concentrations were higher in roots and stems and lower in leafy material (Cocking et al, 1991). Samples from contaminated sites (those above the detection limit) were found to be elevated over those from uncontaminated sites (Cocking et al., 1991).

Finally, this study also examined dry weight mercury concentrations in a subset of terrestrial macro-invertebrates and mammals. Earthworms (Lumbricus spp.), arachnids (order Araneae) and crickets (order Orthoptera family Gryllidae) were the only invertebrate taxa to consistently have elevated mercury concentrations at contaminated sites (Cocking et al., 1991). The subset of mammals collected were all found to have elevated mercury levels with the highest concentrations found in the liver (Cocking et al., 1991). However, it should be noted that this study had low sample sizes and collection was at only a maximum of three sites along the South River over different years and without spatial stratification.
During the spring of 2008 extensive soil sampling of the South River floodplain was undertaken by the Virginia Department of Environmental Quality in cooperation with the URS Corporation and DuPont. The main objectives of this study were to determine the distribution and extent of mercury concentrations in floodplain soils of the South River, determine if distribution of soil mercury follows that of land use, elevation, and distance from the river. The results of this study are intended for use in determining the potential of floodplain soils to act as a source to the terrestrial food chain and to the South River itself. Of the soil samples taken and analyzed for total mercury, 80% were elevated compared to regional background levels which range from 0.06-0.12 ppm (Jordan and Morrison, 2008). General trends suggest that soil total mercury levels decreased with increasing distance and elevation from the South River (Jordan and Morrison, 2008). Soil total mercury did vary significantly with land use type with wetlands and forested areas having higher mercury levels than pasture, open, and developed areas (Jordan and Morrison, 2008). These results show that mercury is present in terrestrial soils of the floodplain; however whether this mercury is abundantly methylated and made available to terrestrial biota is still unclear.

1.12.1.3. Avian Studies

Between 2005-2008, bird species breeding along the South River have been monitored to determine whether they are accumulating elevated levels of mercury. Species sampled included the piscivorous belted kingfisher (*Megaceryle alcyon*), aquatic aerial feeders such as the tree swallow and the northern rough-winged swallow (*Stelgidopteryx serripennis*), and a host of terrestrial insectivorous songbirds such as the
red-eyed vireo (*Vireo olivaceus*), Carolina wren (*Thryothorus ludovicianus*) and eastern bluebird. Of the suite of 13 species sampled, all but one were found to have elevated levels of mercury in their blood (Cristol et al., 2008). Of particular importance was that the majority of terrestrial-feeding species had similar if not higher levels to the aquatic-feeding species (Cristol et al., 2008).

This result sparked further research into exactly how these terrestrial species were obtaining such high levels. During 2006 and 2007, the prey delivered to developing nestlings by foraging adults was sampled from Carolina wrens, house wrens (*Troglodytes aedon*) and eastern bluebirds. Three prey orders were identified as making up the majority of the diet; Lepidopteran larvae and adults (caterpillars and moths), Orthoptera (grasshoppers and crickets), and Araneae (spiders) (Cristol et al., 2008). Of these prey types, spiders had the greatest total mercury burdens with an average of 1.24 ± 1.47 ppm dry weight (Cristol et al., 2008). This suggested that most of the mercury accumulating in the sampled terrestrial songbirds was being delivered through their spider prey. In support of this claim, spiders contained a higher proportion of their mercury as methyl mercury than other taxa (Cristol et al., 2008).

Presently, avian studies on the South River are focused on understanding the mechanisms by which terrestrial birds and their prey are exposed to mercury originating in the river. In addition to my current research examining factors that influence spatial variability of exposure in birds and their prey, a separate study is investigating the mechanisms by which mercury travels from the aquatic food chain into the terrestrial food chain.
1.13 Project Description

I investigated the spatial variability of mercury exposure in terrestrial insectivorous bird species that breed in the floodplain of a mercury-contaminated river. The main objective of this project was to describe the spatial extent of mercury exposure by comparing blood and feather mercury levels of adults and nestlings songbirds, respectively, along a distance gradient extending laterally from the polluted river (Chapter 2). The same questions were then addressed using insect prey and spiders and the same spatial factors and distance gradient used for birds to determine whether prey exposure mirrored predator exposure patterns (Chapter 3). Additionally, the flooding potential of foraging areas of both the songbirds and spiders was investigated as a potential mechanism in transporting mercury from the contaminated river onto floodplain soils.

1.14 Project Objectives and Predictions

1.14.1 Lateral Extent of Mercury Exposure in Terrestrial Songbirds

**Question:** What is the lateral extent of mercury exposure in terrestrial songbirds of the South River?

**Approach:** The lateral extent of mercury exposure for both adults and their nestling broods was investigated using the distance of their nests from the mercury-polluted river. Each occupied nest box was considered to be the central point of an adult pair’s foraging territory and therefore within the area of their potential mercury exposure. Nest boxes were placed along a distance gradient extending perpendicularly from the river’s shoreline. Regression analysis was used to determine if mercury exposure declined with
distance from the river. Additionally, mercury levels along the distance gradient were compared to reference levels in order to estimate a distance at which mercury exposure returned to reference levels. These analyses were conducted using both adult blood and nestling feathers to assess if the spatial extent of exposure differed among age groupings.

**Predictions:** I predicted that both adult blood and nestling feather mercury levels would decrease with distance from the river to a point at which it would be similar to reference levels. Furthermore, I predicted that blood mercury levels would no longer be elevated above reference levels for individuals nesting approximately 300 m from the river or outside of the floodplain.

1.14.2. Explaining Spatial Variation of Mercury Exposure in Terrestrial Songbirds

**Question:** What factors best explain the spatial variation of mercury exposure in terrestrial insectivorous songbirds of the mercury-polluted South River floodplain?

**Approach:** I created a general linear model to explain the variation in mercury exposure of terrestrial songbirds breeding in the floodplain of the South River. Species was included as a main factor because species differ in their foraging behavior and diet, which greatly influences mercury exposure. Additionally, I included five spatial factors that may relate with mercury availability and the potential for exposure within foraging territories. These factors include river kilometer downstream from the ultimate source of pollution, soil mercury content, lateral distance from the river, and flooding frequency (floodplain risk designation, i.e. “100 year floodplain”) and relative elevation of each nest box territory in comparison to the stream bank. These variables were used to predict
mercury exposure in both adult blood mercury levels and nestling feather mercury levels of four species of insectivorous songbirds that feed solely on terrestrial prey.

**Predictions:** Exposure to mercury requires that mercury is available in a bird’s environment, that there is potential for methylation, and that a bird feeds on contaminated prey. Therefore, I predicted that the flooding potential (flood risk and relative elevation) and distance from the river of a bird’s nest would best explain the variation in their exposure to mercury. Furthermore, because nestlings are confined to the nest box and have been exposed only to local conditions, I predicted that my model would better explain the variation in nestling feather mercury than adult blood.

1.14.3. **Lateral Extent of Mercury Exposure in Spiders of the Floodplain**

**Question:** Does mercury exposure of wolf spiders decline along a small-scale distance gradient.

**Approach:** Wolf spiders (family Lycosidae) were used to assess whether mercury accumulation declines along a small distance scale extending perpendicularly from the river. Lycosid spiders were chosen because they are the most abundant type of spider collected from my songbird study species. Spiders were collected from known distances away from the river by way of pitfall traps. Regression analysis was used to determine if spider whole body mercury levels declined with distance from the river. Finally, mercury levels along the small-scale distance gradient were compared to reference levels of spiders collected from reference sites in order to predict a distance at which mercury levels returned to average reference levels.

**Predictions:** I predicted that whole body mercury levels would decrease along the distance gradient.
1.14.4. Explaining Spatial Variation of Mercury Exposure in Terrestrial Prey

**Question:** What factors best explain the spatial variation of mercury exposure in the prey of terrestrial insectivorous songbirds of the mercury-polluted South River floodplain?

**Approach:** The main route of exposure for songbirds is known to be through dietary uptake, therefore their prey were sampled to determine which factors best describe mercury accumulation in songbird prey. Songbird prey was sampled directly from nestlings via the ligature method (see Section 3.3.2 Prey Sampling). A general linear model was created with invertebrate order as a main factor because previous research has shown that mercury accumulation differs significantly among invertebrate orders. The same suite of spatial factors were used to assess the spatial variability of mercury accumulation in the prey, namely, river kilometer downstream from the ultimate source of pollution, soil mercury availability, distance from the river, and flooding potential (flood risk and relative elevation) of each nest box territory from which the prey were collected. In addition, fresh weight was included as a predictor because body size varied substantially within prey types and body size is an important determinant of mercury accumulation.

Additionally, a second model was created to explain spatial variability in spider prey only because previous research and initial results indicated that spider prey was most influential in explaining bird mercury levels. This model included spider family, body size, and a measure of food chain length in addition to the spatial variables described above.
**Predictions:** I predicted that soil mercury availability and flooding potential would best explain mercury accumulation of invertebrate prey as they are present at the bottom of the food chain and thus more closely tied to overall mercury availability. However, in relation to the spider prey, I predicted that the same spatial factors that best explained the spatial variability in the birds would be most influential. Furthermore, as spiders are predatory, I predicted that food chain length would be an important factor in explaining mercury accumulation.
2. Chapter Two: Lateral Extent and Spatial Variation of Avian Mercury

Abstract

Four species of terrestrial insectivorous songbirds breeding in the floodplain of the mercury contaminated South River were used to investigate the spatial variability of mercury exposure. The use of adult blood mercury levels predicted that exposure would no longer be a risk for adults holding territories further than 400 meters from the contaminated river. However, nestling feather mercury levels suggested an even greater distance of 450 meters. In both dependent variables, flooding potential was found to best describe the spatial variability of mercury levels followed by the distance from the proximate and ultimate sources of mercury. Soil mercury availability was not found to be a significant predictor of mercury levels in either variable. These results should be included in future mercury risk assessments.

2.1. Introduction

Mercury released directly into waterways was originally thought to only pose a risk to aquatic-feeding species including many avian species such as the common loon (Gavia immer), great blue heron (Ardea herodias), and the tree swallow (Tachycineta bicolor) (Goodale et al., 2008; Cristol et al., 2008; Scheuhammer, 2007). However, recent studies have conclusively shown that terrestrial insectivorous avian species breeding along a mercury-contaminated river also accumulate high levels in their blood (Cristol et al., 2008). This revelation has sparked interest in understanding how terrestrial
species are exposed to aquatic mercury as well as understanding the mechanisms that deliver mercury to the terrestrial food chain.

The spatial extent of mercury contamination has not been adequately addressed in previous research and therefore is specifically addressed in this study. It has been shown that species with entirely terrestrial diets are still exposed to mercury arising from nearby aquatic sources (Cristol et al, 2008). But how far from these sources is exposure still a major risk? Many terrestrial wildlife species are very mobile and this has made addressing this question difficult. However, territorial breeding birds defend and forage in defined areas and therefore are well suited to explicitly answer this question.

A second previously unaddressed question regards the mechanisms by which terrestrial species are exposed to aquatic mercury. Two general hypotheses have been put forth, one being that aquatic mercury is transported from the aquatic food chain to the terrestrial food chain by way of biological vectors, such as emergent aquatic insects. The other non-mutually exclusive hypothesis suggests that aquatic mercury travels physically from aquatic systems to riparian areas by way of flood waters which deposit mercury on floodplain soils where it becomes available to the terrestrial food chain. Both of these hypotheses suggest that individuals that feed closer to the river are more likely to obtain mercury in their diet. If this is true than there should be an observable decline of mercury with distance from the river.

In the case that mercury is transported physically by way of flood waters, one would expect that individuals that forage in areas more likely to flood would obtain more
mercury in their diet. The flood potential of an area is directly related to its elevation relative to the closest waterway and in relation to the land around it as flood waters will take the path of least resistance. Flood waters can more easily flow into low-lying areas where there are few obstructions (Malanson, 1993). Furthermore, areas that are low-lying in relation to the surrounding land may produce stagnant pools of water, which create good habitat for methylation (St. Louis et al., 2004).

Relatively little is known about what causes variability of avian exposure to mercury within small areas. Within one habitat, such as a single meadow or a small forest tract, there is much variability among individuals with similar diet and foraging strategies resulting in certain individuals accumulating more mercury than others. My research addresses this problem by investigating whether there is a predictable pattern of mercury exposure according to a suite of spatial factors including flooding potential and distance from the proximate and ultimate sources of mercury of breeding territories.

2.2. Methods

2.2.1. Summary of Study Site

The South River has its headwaters in the Blue Ridge Mountains near Waynesboro, Virginia from which it flows North towards Port Republic, Virginia. Historically, the alluvial deposits of the South River have produced fertile agricultural lands along its banks and today the floodplain is composed mostly of privately owned cropland and pasturelands. In the 1940’s and 1950’s a factory owned by E.I. du Pont de Nemours (hereafter DuPont) used mercury as a catalyst in its production of acetate fibers.
During this time, an unknown amount of unrecycled mercury was leaked directly into the South River and adjacent soils at the factory site. Although the use of mercury was suspended in the 1950’s mercury still poses a threat to the wildlife of the South River. The Unites States and Fish and Wildlife Service has placed a “no consumption” fish advisory on the river warning fishermen that eating fish from the South River may be hazardous for the health.

2.2.2. Nest Box Trail

In 2005 a nest box trail was erected at mercury-contaminated sites spanning both sides of the South River from Waynesboro to its confluence with the North River at Port Republic, VA (See section 1.10.1 for map of study area). These sites consisted of either privately owned land or parkland managed by the cities of Waynesboro, Dooms, Crimora, or Grottoes. The number of nest boxes at each site depended on the size of area we had permission to use and therefore is not standard across all the sites. The boxes erected in 2005 were placed in habitat assumed suitable for nesting tree swallows and therefore were mostly in open fields and along edges. An effort was made to place the nest boxes approximately 50 m apart to reduce competition between nesting pairs, and all were within 50 m of the river. Nest boxes were also erected in reference areas that were known not to be contaminated with mercury beyond that of background levels for the Shenandoah Valley. Reference sites were chosen both upstream of Waynesboro, VA (location of the mercury source) and along the North and Middle Rivers, which join the South River to form the South Fork of the Shenandoah River at Port Republic, VA. Reference sites were also composed of privately owned land and public parkland. In
2006, additional nest boxes as well as plastic tube nesting structures were erected in woodland areas at both contaminated and reference sites to attract Carolina wrens and house wrens that prefer to breed in woody areas.

### 2.2.2.1. Nest Box Placement

Nest boxes were placed in suitable habitat according to species (See section 2.3.5 Study Species for detailed habitat preferences). For Carolina wrens and house wrens, nest boxes were erected in small clusters of 2-3 boxes in what was expected to be a single territory. Both of these woodland species build multiple “dummy” nests but only lay eggs in one nest (Friedman, 2007). Therefore, by placing nest boxes in clusters, it was more likely that one box would contain an active nest. Nest boxes for Carolina wrens were placed facing brush piles or fallen trees in woody areas. Boxes for house wrens were placed along the edges of woody and field areas or just inside the woody area. Nest boxes intended for eastern bluebirds were placed either in open areas such as field and yards or along edges, facing the open habitat. Boxes intended for Carolina chickadees were placed in small open areas within woody areas with mature trees. Although boxes were erected according to each species’ nesting preferences, birds sometimes chose boxes placed in uncharacteristic habitat for that species.

### 2.2.2.2. Nest Box Design

All nest boxes were built by Tom Meier of the W&M biology department staff according to the recommendations made by the North American Bluebird Society (http://www.nabluebirdsociety.org/nestboxspecs.htm). This consisted of a wooden nest
box attached to an aluminum pole of about 2 m length. Each nest box was also fitted with a predator baffle that prevented would be predators (domestic cats, black rat snakes, raccoons, etc.) from climbing the pole to access the nest. Figure 2 is a photograph of the standard nest box design used in this study.

Figure 2: Nest Box

2.2.2.3. Nest Box Distance Gradient

Additional nest boxes were again added in 2007 and then in 2008 to sites along the South River in order to create a distance gradient of available nest boxes stretching perpendicularly from the river. An effort was made to place boxes at 50 m intervals stretching laterally from the river for as far as the size of each research site allowed. A main thoroughfare (Rt. 340) runs parallel to the South River outside of designated floodplain area and therefore all sites were constrained by the highway if not lack of suitable habitat. Nest boxes were placed in open fields, along edges, and in wooded
habitat in order to attract all four of the study species (See section 2.3.6). Distances from river to nest box ranged from 0-608 m; however boxes were occupied out only to a distance of 460 m (See section 2.3.4 for additional information).

In 2007, there were a total of 172 nest boxes erected on contaminated sites in open fields or along forest edges (Table 1). A total of 190 nest boxes (boxes and tubes) were erected on contaminated sites in forested areas (See Table 1). Of all nest boxes in forested areas, 29% (47/189) were plastic tubes targeting Carolina wrens. Additional boxes were added in 2008 totaling 237 in open fields and along forest edges and 205 in forested areas (See Table 1). Of all nest boxes in forested areas in 2008, 23% (47) were plastic tubes.

Additionally, during the 2007 field season there were 163 nest boxes erected in open fields, edges, and wooded areas of reference sites, also placed to attract all four study species. In 2008 another 60 boxes were added to reference sites, making the total reference boxes 223. This was done in an effort to even out the number of boxes on contaminated and reference sites. The average distance from the nearest river (North and Middle Rivers) for nest boxes from which data was collected for this study was 40.41 meters, ranging from 0.002-195.95 meters.
Table 1: Contaminated Site Nest Box Totals by Year and Habitat Type

<table>
<thead>
<tr>
<th>Habitat Type</th>
<th>Number of Nest Boxes in each Year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2007</td>
</tr>
<tr>
<td>Open Field</td>
<td>172</td>
</tr>
<tr>
<td>Woods</td>
<td>190</td>
</tr>
<tr>
<td>Nest Boxes</td>
<td>143</td>
</tr>
<tr>
<td>Plastic Tubes</td>
<td>47</td>
</tr>
<tr>
<td>Total</td>
<td>362</td>
</tr>
</tbody>
</table>

2.2.3. Use of Geographic Information Systems (GIS)

During the summers of 2007 and 2008 Garmin eTrex Vista handheld Global Positioning Systems (GPS) navigator was used to record the exact coordinates (northing and easting) at which every box was located. Every box was given a unique alphanumeric code so that it could be distinguished by name. When boxes were moved they were given a new unique name and their new coordinates were recorded. Effort was made to only record GPS coordinates on days that were clear enough to get accuracy readings of within 5 meters. All GPS coordinates were then downloaded onto a computer using MapSource Software package 6.15.4. The downloaded coordinates were then used to create point shape files in ArcCatalog version 9.2 which were then plotted onto aerial photographs of the South River. The aerial photographs were taken in 2001 by the state of Virginia and are accessible through the GIS center at Radford University Virginia.
Geographic Information Network (VGIN). However, these aerial photographs were made available to the Cristol lab by the Virginia Department of Environmental Quality. These aerial photographs were used to digitize the shoreline of the South River from Waynesboro to Port Republic, as well as upstream portions where reference sites are located. The sections of the North and Middle Rivers that were bounded by nest boxes were also digitized. Both the east and west shorelines were digitized for all portions and these digitized portions of the river were then converted into line shape files. Using ArcMap version 9.2, a map of all study sites was created using the aerial photographs and then overlaid with the nest box point shape file and the river line files. Figure 3 shows the final result combining all the aerial photographs and shape files of the river. These files and maps were used in all further spatial analyses.
Figure 3: Map of South River
### 2.2.3.1 Determining Distance from the River

A spatial analysis tool of ArcGIS 9.2 called a spatial join was used to calculate the shortest distance between each box along contaminated portions of the South River and the closest shoreline point. This tool joins the attributes of two layers (point shape file and line shape file) to each other based on the location of the features in the layer, in this case the shortest distance between nest box and shoreline (ArcGIS 9.2 Desktop Help). The relationship between the two layers is then saved in an easily manipulated database. Table 2 gives totals of how many nest boxes (tubes included) fell into arbitrarily set distance categories. Figure 4 depicts the nest box locations according to distance category at one example site, the Wertman family private property at approximately river kilometer 14.5 (river mile 9) downstream of the contamination source in Waynesboro, Virginia. The color-coded buffers on either shoreline of the South River visually depict the distance category in which each nest box is erected.
Table 2: Distribution of Contaminated Boxes along Distance Gradient

<table>
<thead>
<tr>
<th>Distance Category (m)</th>
<th>Number of Boxes/Tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2007</td>
</tr>
<tr>
<td>0-10 m</td>
<td>87</td>
</tr>
<tr>
<td>10-50 m</td>
<td>156</td>
</tr>
<tr>
<td>50-100 m</td>
<td>65</td>
</tr>
<tr>
<td>100-200 m</td>
<td>48</td>
</tr>
<tr>
<td>200-300 m</td>
<td>3</td>
</tr>
<tr>
<td>300-400 m</td>
<td>2</td>
</tr>
<tr>
<td>400-500 m</td>
<td>51</td>
</tr>
<tr>
<td>500-600 m</td>
<td>0</td>
</tr>
<tr>
<td>&gt;600 m</td>
<td>0</td>
</tr>
</tbody>
</table>
2.2.3.2 Determining Relative Elevation

The relative elevation of every nest box was calculated as a method to assess the likelihood of flooding. Due to the existence of berms and other topographic features, nest boxes that were farther from the river are not always less likely to flood. Furthermore, this method offered a way to compare nest boxes that were the same distance from the river but in different floodplains, or had different flooding potentials.

In order to accomplish this task, digital elevation models (DEMs) were downloaded from the United States Geological Survey (USGS) via the world wide web at http://edc2.usgs.gov/geodata/index.php. DEMS are raster files consisting of terrain elevations for ground positions at regularly spaced horizontal intervals (USGS; http://edc2.usgs.gov/geodata/index.php). The DEMs used for this analysis were 7.5 minutes, 1:250,000 scale with a horizontal grid of 10 meters. Elevations are recorded in meters relative to mean sea level. The DEMs for both Rockingham and Augusta counties were downloaded. A spatial analysis tool called Sample was used in ArcCatalog which creates a table that stores values of cells from a raster dataset (DEM) for a defined location, in this case the GPS coordinates of the nest boxes and of the South River shoreline digitized using a aerial photograph (ArcGIS 9.2 Desktop Help). For Sample to perform correctly, it is important that both the raster dataset and the shapefile are projected in the same coordinate system (in all cases the Universal Transverse Mercator (UTM) coordinate system was used). Once Sample has created a table, it needs to be joined to the shapefile according to a common attribute between both files. In this case, the DEM was used to store an elevation (above sea level) for every point along the South River shoreline (both eastern and western shoreline) as well as every nest box. These two
files were then joined using spatial join allowing the difference in elevation between every nest box and its closest shoreline point to be calculated and stored. This elevational difference is termed relative elevation throughout all further analyses. If the elevation at each nest box was above that of the closest shoreline point it received a positive relative elevation while if the elevation at a nest box was below that of the closest shoreline point, it received a negative relative elevation.

2.2.3.3. Determining Floodplain Designation

Every nest box was assigned to a particular floodplain designation as determined by the Hydrologic Engineering Center River Analysis System (HEC-RAS). HEC-RAS was first developed by the U.S. Department of Defense and models the hydraulics of water flow through natural rivers and other channels (U.S. Army Corps of Engineers). HEC-RAS was utilized by the URS Corporation to model inundation layers of the South River floodplain. Using the Hec-RAS application as well as GIS spatial analyst, URS developed floodplain analyses of 3 floodpains; 2-year, 5-year and 100-year. An example of their work is displayed in Figure 5.
Figure 5: Example Site Map Depicting South River Floodplain Designation
Produced by URS Corporation using HEC-RAS model for DuPont South River Project
URS Corporation forwarded me the GIS layers created using the HEC-RAS program and I added it to my GIS map of the South River. By combining my nest box layer and the floodplain layers, I was able to visually determine in which floodplain each nest box was located. Every nest box was assigned to one of the three floodplain zones. These floodplain designations can be interpreted according to the probability that each will be flooded. For example, a nest box in the 2-year floodplain has the probability of being flooded once out of every two years.

### 2.2.3.4. Determining Average Soil Mercury of Nest Box Territories

In 2008, the URS Corporation, in cooperation with the VDEQ, undertook a soils sampling project to determine the extent and distribution of mercury in floodplain soils of the South River. The results of this undertaking were used to determine if the presence and distribution of mercury in floodplain soils is a predictor of mercury exposure in terrestrial songbirds and their prey. Only surface soil samples taken from within 6 in. of the surface were used in this application as buried mercury is not likely available to foraging birds. Additionally, all soil concentrations used were analyzed for total mercury rather than methyl mercury solely due to the lack of adequate numbers of methyl mercury samples. Furthermore, since soil samples were taken independently of where birds were sampled, it was necessary to use ArcGIS to determine the average soil mercury in nest box territories. In order to accomplish this I created 100 m buffers around each nest box using the Buffer tool. I chose 100 m because this was typical maximal foraging area for my study species (See section 2.3.4. Study Species). Using the spatial analysis tool...
called spatial join, I was able to determine all the soil samples that were present inside each 100 m buffer. The number of soil samples taken within the buffer of each nest box area was variable and therefore, data were only used when at least two soil samples were available. After determining which buffers contained at least two soil samples or more, the soil sample concentrations were averaged resulting in one average soil concentration associated with each nest box foraging territory. These soil averages were used for all further analyses to determine the correlation of floodplain soil mercury and avian exposure to mercury.

2.2.4. Study Species

I studied four terrestrial, insectivorous avian species that commonly breed along the South River: Carolina wren, Carolina chickadee, house wren and eastern bluebird. These species were chosen because of their abundance, because they are all secondary cavity nesters and readily use artificial nest boxes, and because the variation in their foraging strategies suggests variation in their mercury exposure. To improve the generalizability of my results, I chose four species that varied in beak size, body size, and migratory status. Here I will detail what is known about the life history traits, breeding characteristics and foraging strategies of each study species.

2.2.4.1. Carolina Wren (*Thryothorus ludovicianus*)

The Carolina wren (CARW) is a common year-round resident throughout the southeastern United States and northern Mexico (Haggerty and Morton, 1995). Carolina wrens are non-migratory and maintain their territories year-round. Carolina wrens use a
wide range of woodland habitats that generally include moderate to dense shrub or brushy cover (Haggerty and Morton, 1995). Along the South River, Carolina wrens maintain territories in forests as well as in residential and urban areas with small wooded areas. Varying across their range, Carolina wrens can begin breeding as early as the last week in March and may continue through the end of August (Haggerty and Morton, 1995). Carolina wrens may have more than one clutch per season and each clutch typically contains 4-5 eggs, which are laid on successive days during the early morning (Haggerty and Morton, 1995). Incubation begins with the penultimate egg and is done almost exclusively by the female (Haggerty and Morton, 1995). Incubation is between 12-16 days but on average lasts 14 days (Haggerty and Morton, 1995). Hatching happens at any time of day but generally all eggs hatch within 10 hours of each other (Haggerty and Morton, 1995). Upon hatching, both parents feed; however, studies have reported that male feeds more often during first half of nestling period with female taking over during the second half (Haggerty and Morton, 1995). Nestlings generally fledge at about 12 days old but parents have been observed feeding fledglings up to 2 weeks after they left the nest (Haggerty and Morton, 1995). Fledglings generally leave natal territory by the time the second clutch has hatched (Haggerty and Morton, 1995).

Carolina wren males, and to a lesser extent females, maintain territories year-round and have been found to range between 1-4.1 hectares (Haggerty and Morton, 1995). However, territory size decreases as conspecific density increases (Haggerty and Morton, 1995). Nesting, foraging, and feeding of young generally occurs within territory boundaries (Haggerty and Morton, 1995).
Carolina wrens feed primarily on the ground, in leaf litter, around downed trees and near brush piles (Haggerty and Morton, 1995). They are considered gleaners from the ground and the lower portions of tree trunks (Haggerty and Morton, 1995). Carolina wrens are known to use their curved beaks to turn over leaf litter and other decaying vegetation (Haggerty and Morton, 1995). Carolina wrens feed mainly on insects (Lepidoptera and Orthoptera) and arachnids (Araneae and Opilione) (Haggerty and Morton, 1995; Friedman, 2007). Stomach contents have been found to consist of 94% animal matter and 6% vegetable matter (Haggerty and Morton, 1995). Animal matter consisted of a wide range including Lepidopterans (22%), Hemipterans (19%), Coleopterans (14%), Orthopterans (13%), Arachnids (11%), Hymenopterans (5%) and Dipterans (3%) (Haggerty and Morton, 1995). Recent studies reported that the nestling diet biomass consisted almost exclusively of Lepidopteran (mostly larvae) (>50%), Orthoptera (<10%) and arachnids, with Araneae making up more than 30% of the diet (Friedman, 2007).

2.2.4.2. Carolina Chickadees (*Poecile carolinensis*)

The Carolina chickadee (CACH) is a small (9-12 g) passerine that ranges from New Jersey to Texas and Pennsylvania to Kansas (Mostrom et al., 2002). The Carolina chickadee is also non-migratory and a year long resident in Virginia (Mostrom et al., 2002). Their habitat preferences are generally for mid to late successional hardwood forests (Mostrom et al., 2002). Selected nest sites are often chosen in cavities near forest edges, facing the clearing (Mostrom et al., 2002). Breeding pairs actively maintain territories that vary in size between 1.6-2.4 hectares (Mostrom et al., 2002). Territories
are first defended in late winter by the male and then by both adults during the breeding season. Carolina chickadees form wintering flocks during the winter that are then maintained by the dominant male and his mate (Mostrom et al., 2002). Breeding territories are used for nesting, foraging and nestling provisioning exclusively by the breeding pair, although there may be “floater” males on the periphery (Mostrom et al., 2002).

Carolina chickadees only produce one clutch per year, but may have a second clutch if the first clutch was failed (Mostrom et al., 2002). Nest building may begin 20 days prior to egg-laying which normally occurs in late March or early April (Mostrom et al., 2002). A typical clutch size is 6 eggs, laid one a day on successive days (Mostrom et al., 2002). Incubation normally begins when the last egg is laid but may occasionally begin with the penultimate egg (Mostrom et al., 2002). Female incubates exclusively for an average of 13 days (Mostrom et al., 2002). Hatching is often asynchronous and may happen over a 2-3 day period after which hatchlings are often brooded for up to a week by the female (Mostrom et al., 2002). Fledglings depart the nest approximately 16-19 days after hatching, sometimes asynchronously (Mostrom et al., 2002).

Carolina chickadees are considered arboreal gleaners and generally glean from small twigs and the underside of leaves of deciduous trees (Mostrom et al., 2002). Foraging height differs by tree type but typically occurs in the canopy or sub-canopy, and rarely on or near the ground (Mostrom et al., 2002). Diet during the summer consists primarily of animal food (insects and spiders) while during the winter, plant material is more often consumed (Mostrom et al., 2002). An investigation of stomach contents
found that Lepidopteran (larvae) were the most abundant food source during the summer followed by Hymenoptera and arachnids (Araneae and Opilione) (Mostrom et al., 2002). Small prey items are usually immediately consumed while larger items are cached for later consumption (Mostrom et al., 2002). Not much is known (prior to this study) of the Carolina chickadee nestling diet.

2.2.4.2. **House Wren (*Trogлогýtes aedon*)**

The house wren (HOWR) is a small (10-12 g) member of the wren family that prefers shrubby woodlands and very frequently uses artificial nest boxes (Johnson, 1998). The North American breeding range of the house wren extends from Southern Canada throughout the United States (Johnson, 1998). The house wren is migratory, spending winters in areas the southern United States and northern Mexico (Johnson, 1998). House wrens preferably select nest sites within 30 m of significant woody vegetation but avoid areas of dense vegetation (Johnson, 1998). One study showed that the probability of nest failure increased as vegetation density around the nest increased (Johnson, 1998).

The house wren is territorial and socially monogamous during the breeding season (Johnson, 1998). Active nests are usually greater than 30 m apart; however, in urban areas nests have been as close as 15 m apart (Johnson, 1998). The mean territory size has been estimated to be 0.5 ha (Johnson, 1998). Males will defend territories with multiple nest sites against neighboring resident males and unmated “floater” males; females will defend an active nest site against both male and female intruders (Johnson, 1998). However, there are a high number of intruder events (mainly by males) that result
in male intruders taking over an unused nest site or usurping the resident male altogether and adopting the female as a mate (Johnson, 1998).

Males and females arrive separately at breeding grounds in Virginia from late April to early May (Johnson, 1998). Clutch size is 6-7 eggs on average, but this varies with location within breeding range (Johnson, 1998). Eggs are laid in the early morning of successive days and incubation by the female begins the day after the last egg is laid (Johnson, 1998). Incubation is 12-13 days on average and generally hatch all in the same day; however the entire clutch may take 2-4 days to hatch (Johnson, 1998). Nestlings are fed by both parents and depart the nest between day 16 and day 18 (Johnson, 1998). All nestlings fledge within a few hours of each other (Johnson, 1998). House wrens may have second nesting attempts per season with the likelihood increasing at more southerly latitudes (Johnson, 1998).

House wren adults feed on terrestrial invertebrates gleaned from twigs and vegetation of the subcanopy, herbaceous ground cover, and the ground itself (Johnson, 1998). Stomach content examination of adults showed that the most abundant prey items were Araneae (mostly araneid spiders), Coleoptera, Hemiptera, and Lepidoptera (adult and larvae) (Johnson, 1998). However, more mobile taxa such as Diptera, Homoptera, and Collembola were also represented (Johnson, 1998). A study in Illinois observed nestling provisioning trips and found that nestlings were most often fed Orthoptera (37%), Lepidoptera (21%), araneid spiders 10%), and Opiliones (7%) (Johnson, 1998). In a recent study on the South River the nestling diet biomass was found to be mostly
Araneae (>25%), Lepidoptera (30%), Orthoptera (12%) and approximately 30% was comprised of other or unidentified taxa (Friedman, 2007).

2.2.4.4. **Eastern Bluebird (Sialia sialis)**

The eastern bluebird (EABL) is a member of the thrush family and is the largest of all four study species at about 28-32 g for adults (Gowaty and Plissner, 1998). Their breeding range extends through most of eastern North America from northern Nicaragua to southern Canada (Gowaty and Plissner, 1998). Eastern bluebirds are considered partial migrants meaning that within a population, certain individuals will migrate while others are residents on breeding grounds year-round (Gowaty and Plissner, 1998). Anecdotal evidence suggests that more northerly populations have a higher proportion of migrants than those with more mild winters (Gowaty and Plissner, 1998). The preferred natural breeding habitat consists of fire-maintained savanna, open stands of mature forests, and fields (Gowaty and Plissner, 1998). Eastern bluebirds will readily use nest boxes and natural cavities in residential yards, parks, pastureland and agricultural fields (Gowaty and Plissner, 1998).

Both males and females will defend the area around nest sites, which is where mating, nesting and foraging take place (Gowaty and Plissner, 1998). Studies have shown that defended areas during the breeding season can range from 1.1-8.4 hectares; however territory size is largely dependent on food availability, abundance of available nesting sites, and conspecific density (Gowaty and Plissner, 1998). Other estimations
suggest that all nesting, mating and foraging occurs within an area of 1 ha around the nest site (Ritchison, 2000).

Eastern bluebirds are socially monogamous and the duration of the pair bond is strongly associated with previous nesting success of the pair (Gowaty and Plissner, 1998). Mate-guarding does occur; however one study showed that 20% of nestlings within a population were produced by extra-pair copulations (Gowaty and Plissner, 1998).

Nest building by the female generally begins in late February or early March, with the onset of egg-laying approximately a week after nest completion (Gowaty and Plissner, 1998). An average clutch size is 5 eggs with eggs being laid in the early morning on successive days (Gowaty and Plissner, 1998). Incubation is solely by the female and begins on the day the last egg is laid (Gowaty and Plissner, 1998). Incubation is for 14 days on average and nestlings hatch synchronously, sometimes within minutes of each other (Gowaty and Plissner, 1998). Nestling provisioning is by both male and female and continues until approximately 3 weeks after fledgling (Gowaty and Plissner, 1998). Fledging occurs, on average, 18 days post hatching and may be asynchronous (Gowaty and Plissner, 1998). Second clutches are common and are more likely to occur in southerly parts of their breeding range (Ritchison, 2000).

Eastern bluebirds are considered ground foragers and use available perches to spot prey up to 40 m away, although most prey is within 20 m of perch (Gowaty and Plissner, 1998). Foraging areas are generally in open habitats with no overstory and
sparse ground cover (Gowaty and Plissner, 1998). Foraging generally takes place in close proximity to nest, especially during nestling provisioning (Gowaty and Plissner, 1998; Ritchison, 2000). During the breeding season the majority of prey eaten by adults consists of ground arthropods including Lepidoptera (adults and larvae) (32.4%), Orthoptera (25.6%), Araneae (11.3%), but fruits and other vegetable matter are also eaten (Gowaty and Plissner, 1998). A recent study determined that for eastern bluebirds nesting along the South River, approximately 25% of the biomass fed to nestlings consisted of Lepidoptera (larvae and pupae) and Orthoptera, while 20% was Aranea, and 15% Coleoptera (Friedman, 2007).

2.2.5. Nest Monitoring

Nest monitoring of all nest boxes along the trail (contaminated and reference) began in late February in both 2007 and 2008. Any old nests that were not removed at the end of the previous season were removed to make the box available for a new nest. During the early season (late February-early April) all nest boxes were checked for nest building once a week. Observations included the species, proportion of nest built and presence of adults in area. Once a nest was completed, the nest was visited a minimum of every three days to determine the nest fate including date of clutch initiation, clutch size and date of incubation. Egg-laying dates were then used to estimate hatching and fledgling dates. Nests were visited on estimated date of hatching to determine nest fate; if hatching had not occurred, the nest was re-visited over the following days until hatching was complete. Nests were also visited around estimated fledging date to determine success or failure of nest.
2.2.6. Methods of Adult Capture

Adults of all four species were captured with one of the following methods. The method of capture depended on the species, sex, number of previous capture attempts and nest box location. Attempts were made to capture both the male and female at all active contaminated nests. In addition, a minimum of 10 adults of each species were sampled from reference sites.

2.2.6.1. Pillow Case Technique

Previous work with Carolina wrens and Carolina chickadees on the South River determined that the females were not likely to abandon if captured during late incubation. A novel method of capture dubbed the “pillow case technique”, was invented by Scott Friedman of The College of William and Mary, and used solely for the capture of Carolina wren, Carolina chickadee and house wren females in order to increase the efficiency of capture for these difficult-to-handle species. This method entails approaching the nest during early morning hours when the female is still likely to be sitting on her eggs during the last few days of incubation. Once the nest is reached a bird bag was rapidly stuck in the entrance hole of the box to block the exit. A large pillow case was then thrown over the box, covering it entirely, and pinched at the bottom. The bird bag was then carefully removed from the entrance hole without opening an escape route. The female was then allowed to fly into the pillow case. The pillow case was then removed from the box and used as a large bird bag. Since females were still incubating during this capture attempt, an effort was made to quickly sample and free the individual.
In cases where the female was not found sitting on eggs during the first attempt of capture, they were captured using the target mist net technique after hatching was completed (See following section).

### 2.2.6.2. Target Mist Net Technique

Any females not captured on first attempt by the above method, as well as all non-incubating males, were captured using target mist netting. This entailed erecting a 6 or 12 m, 4-trammel, 36 millimeter mist net between two aluminum or titanium poles that were dug into the ground. Mist nets were erected in front of the nest box entrance hole in order to catch provisioning adults. All mist netting took place at least 2 days after the completion of hatching to reduce risk of abandonment after capture. Mist nets were erected and monitored from afar or visited every 10-20 minutes to determine if individuals had been captured. If both adults were observed to be in the area during a capture attempt, the nets were left open after the capture of the first adult to allow for capture of the second adult. However, whether or not one or both of the adults had been captured, nets were removed after a maximum of 60 minutes to allow the parents to return to provisioning their nestlings. For individuals that were increasingly wary, it often took more than one attempt throughout the nestling period to capture both adults. In some cases, I was not able to erect a mist net in such a way as to avoid detection by the adults and even after multiple attempts, these individuals could not be caught. In other cases, the nest was predated or abandoned before both adults could be captured.
2.2.6.3. Nest Box Traps

A simple nest box trap as described in Stutchbury and Robertson (1986) was used as the first attempt to catch either sex from all four species. This simple design involves making a trap door by cutting a credit card-sized piece of aluminum that is large enough to completely cover the entrance hole of the nest box (Stutchbury and Robertson, 1986). The trap door is attached to the inside wall of the nest box using duct tape and then propped up using a small stick, twig or piece of grass (Stutchbury and Robertson, 1986). The trap is activated when an adult enters the nest box hole and knocks the prop loose causing the trap door to fall (Stutchbury and Robertson, 1986).

After initial attempts to use this simple nest box trap design, it was apparent that this simple nest box trap design was not successful in the capture of Carolina wrens or house wrens. Carolina wrens were observed to prop the trap door open enough to escape after the trap had been closed (pers. observation). In addition, house wrens were observed escaping the nest box before the trap door was closed (pers. observation). Due to these observations, nearly all attempts to capture Carolina wrens or house wrens were made with either the “pillow case” or target mist net techniques.

I had some success using the simple nest box trap design to capture both eastern bluebirds and Carolina chickadees, especially on first capture attempts of unwary individuals. However, it became apparent that individuals that had previous experience with this trap design (either from previous years or from earlier in the same season) were wary and difficult to catch. Therefore, an improved design was used in which the
researcher activates the trap door (Friedman et al., 2007). This improved nest box trap design was used primarily for eastern bluebirds where one adult had been previously captured or where initial attempts with the more simple design had been unsuccessful.

2.2.6.4. Special Considerations

During my second data collection field season it was apparent that I was not going to have an adequate sample size of Carolina wrens because they had a very low nest box occupancy rate. Therefore, in order to increase my sample size, I located active territories without nest boxes. Because natural nests were hard to locate and not conducive to capture techniques, audio playback of a singing Carolina wren males was used to lure territorial males to the mist net. The tape player was placed and concealed just below an erected mist net so that when the desired male came to investigate the suspected intruder, he would become entangled in the mist net. An effort was made to observe the male initially and determine where his favorite singing spots were in order to erect the mist net within his territory. In these cases, the GPS location of the mist net was recorded as the center point of the individual’s territory and used in all further analyses.

2.2.7. Mercury Sampling of Adults

Upon capture, each adult was banded with a unique United States Geological Survey Bird Banding Laboratory (USGS BBL) metal band and subsequently sampled for mercury analysis. The focus of this study was on short-term mercury exposure and therefore blood was the chosen tissue for all adults because studies have shown that the half-life of mercury in the blood stream is on the order of 2 weeks (Evers et al., 2005).
Approximately 100 μL of blood for mercury analysis was sampled from the brachial vein using a 26 gauge, 0.5 inch needle. A detailed description of blood sampling can be found in Brasso (2007). The blood was collected in two 75μL tubes (one hepranized and one non-hepranized) and sealed with Critocaps ® and stored in a 10cc Becton Dickinson ® vacutainer to prevent breakage. Heparin is used as an anti-clotting agent and therefore samples stored in hepranized tubes were used for all mercury analysis (Friedman, 2007). However, duplicate blood samples were also collected in non-hepranized tubes when a Nitrogen isotope analyses was required, to avoid the nitrogen in heparin from contaminating the sample.

A minimum of 10 body feathers was collected as part of a larger project investigating mercury exposure in songbirds of the South River. Feather mercury of non-molting adults is indicative of long-term mercury exposure. All samples collected (blood and feathers) were stored in Ziploc bags labeled with the band number, species, date, sex, age and nest box location for each individual. The samples were then stored in a -25 degree Celsius freezer within 12 hours of collection and kept there until mercury analysis was performed.

2.2.8. Mercury Sampling of Nestlings

Short-term mercury exposure is best assessed using growing feathers in developing nestlings (See section 1.5.1. Avian Tissue Comparison). Therefore, a minimum of 10 body feathers was collected from every sampled nestling in both 2007 and 2008. In 2007, all nestlings of every brood were sampled and analyzed for mercury. However, after initial data analysis, it was apparent that there was little variation in
feather mercury exposure among nestlings of the same brood (See section 2.3.9. Sample Sizes and Independence). Therefore, during the 2008 season, a random 3 nestlings of every brood were sampled for mercury analysis. In broods of 3 or fewer nestlings, all nestlings were sampled.

When sampling broods, all nestlings were removed from nest and placed in bird bags. The first three randomly haphazardly removed nestlings were then fitted with a unique USGS BBL band, sampled for blood and feathers and measured for weight, wing and tail length. All unsampled nestlings were also fitted with a USGS BBL metal band but were not measured or sampled. Finally, all nestlings were returned to the nest in as little time as possible to minimize disturbance (approximately 20-30 minutes depending on size of the clutch).

An effort was made to sample body feathers from both the back/rump and chest of each nestling to avoid sampling only feathers that had grown simultaneously, this insured more representative estimate of mercury exposure. All broods were sampled one or more weeks after hatching to insure that suitable feather growth had occurred to warrant using feathers as a tissue for mercury analysis. Collected feathers were stored in small Ziploc bags labeled with band number, date, age, nest box and species for each sampled nestling. The ziploc bags and their contents were placed in a -25 Celsius freezer within 12 hours and were stored frozen until later mercury analysis.
2.2.9. Sample Preparation and Mercury Analysis

There was little necessary preparation of blood samples. They were simply removed from the freezer and allowed to thaw prior to analysis. However, feather samples were cleaned using deionized water to remove any residual mercury from their surface. The feather samples were then relocated to small manila envelopes with all necessary identification information, dried in a low-humidity chamber, and allowed to dry for approximately 48 hours prior to mercury analysis.

All mercury analysis took place in the Biology Department of the College of William & Mary campus using a Direct Mercury Analyzer (DMA-80 Milestone, Inc.). The DMA-80 uses cold vapor atomic absorbance spectroscopy to determine the nanograms (ng) of mercury in a sample. The sample weight is then used to determine the mercury concentration of the sample being analyzed. Detailed descriptions of the methodology by which the DMA-80 works can be found in the owners manual (DMA Manual, Milestone Inc.). In addition, all maintenance and calibrations were conducted according to suggestions found in the DMA Manual and from personal communication with DMA-80 troubleshooting staff.

The factory calculated instrument detection limit is 0.005 ng Hg (DMA Manual, Milestone Inc.). All avian blood and feather samples were above this detection limit. Before and after every tray of samples (approximately 40 samples) a set of standards (DORM-2 or DORM-3, and DOLT-3), a methods blank, and a sample blank to insure the quality of readings by the machine.
Duplicates of samples were run when possible approximately every 20 samples. Duplicates of blood were not true duplicates in that they were blood collected in two tubes from the same individual on the same date. But they were often taken from different wings and there may be variation in the amount of mercury actually present in each drop of blood. Duplicate feather samples were created by combining all collected body feathers from an individual nestling and splitting the combined sample into two equal portions. The relative percent difference (RPD) for all avian duplicate samples for this study was calculated using the equation $\text{RPD} = \frac{(x_1 - x_2)}{\frac{(x_1 + x_2)}{2}} \times 100$. The mean RPD for avian samples was $8.28 \pm 7.00\%$.

2.2.10. Sample Sizes, Independence and Normality

2.2.10.1. Adults

A total of 297 independent cases of sampled individuals were used in my analysis of adult mercury levels. Each case represents an individual adult bird that was captured and sampled for blood mercury regardless of year, species or sex. For most cases both the male and female were sampled from an active nest box, but each individual was considered an independent case. While most cases were individuals captured from a nest box with recorded geographic position coordinates, six Carolina wrens were caught opportunistically from known territories. The location of capture (mist net location) was recorded and used in estimating the territory location for these individuals.

Several nest boxes were occupied more than once throughout the season by different breeding pairs of the same species. I treated each capture (even when at the
same nest box) as an independent data point because blood mercury levels generally indicate exposure over the previous two weeks (Evers et al., 2005) and, in all cases except for one, more than three weeks had elapsed between sampling of different individuals from the same nest box. In addition, some nest boxes were occupied multiple times throughout the season but by breeding pairs of a different species. These samples are considered independent from each other because species differ in their foraging microhabitat and therefore should be exposed differentially to mercury. The 297 independent samples were from 159 different nest boxes (or territories).

An initial test for normality revealed that the adult blood mercury data were not normal, being right-skewed and having a positive kurtosis (skewness=2.347, kurtosis=6.257, Shapiro-Wilk Statistic=0.739, df=292, p<0.000). In order to satisfy assumptions of normality and homogeneity of variance all mercury values for adult blood and soil were log(10) transformed, resulting in data that did not differ significantly from a normal distribution (skewness=0.233, kurtosis=-0.258, Shapiro-Wilk Statistic=0.991, df=292, p=0.060).

2.2.10.2. Nestlings

Within clutch nestlings were not considered independent samples since they were reared by the same adult pair. Instead, the mean feather mercury of each brood was used to ensure independence. However, to justify the use of the brood mean I investigated the amount of variation within each brood. In 2007, body feathers were sampled from all nestlings within each of 85 broods (total of 319 nestlings) to determine the amount of within clutch variation of feather mercury levels. The average of the within clutch means
was 2.85 ppm while the average of the within-clutch standard deviations was 0.31 ppm. Using these values resulted in a coefficient of variation of 10% (CV=100(average StdDev/ average mean)). This suggests that there is little intraclutch variation of feather mercury values and justifies the use of mean brood feather mercury for all further analyses.

An initial test of normality found that the brood feather mercury data were not normal and had a postive kurtosis (skewness=1.838, kurtosis=5.285, Shapiro-Wilk Statistic=0.853, df=134, p<0.000). Simple log(10) transformation was used to normalize these data as described for blood mercury above, resulting data approximating a normal distribution (skewness=-0.051, kurtosis==0.391, Shapiro-Wilk Statistic=0.993, df=134, p=0.780).

2.2.11. Statistical Analysis of Adult Blood Matrix

The main objective of this study was to determine which of the measured spatial factors best explained mercury exposure in terrestrial songbirds. A general linear model was used to assess the fit of my data to the predictors. In this model, species was included as a main factor to account for variation in mercury exposure related to size, diet, and physiological differences between the four species.

2.2.11.1. Treatment of Year and Seasonal Effects

When adults of all species were combined, the year of capture did have a significant effect on log-transformed adult blood mercury levels (F=7.620 (1, 287), p=0.006). When each species was analyzed separately, only Carolina chickadees
(F=7.929 (1, 43), p=0.007) and eastern bluebirds (F=4.931 (1, 120), p=0.028) differed significantly between 2007 and 2008. Both of these species seemed to have lower blood mercury levels in 2008 (See Fig. 6).

There was also an effect of Julian day (ordinal date within season) when all species were combined (F=1.691 (7, 287), p=0.002). When Julian day was examined for each season (2007 and 2008) separately, it was highly significant in year 2007 (F=32.711 (1, 155), p<0.001) and nearly significant in 2008 (F=3.792 (1, 133), p=0.054). However, the trend was not consistent with Carolina chickadee mercury levels increasing over the season, Carolina wren and house wren levels decreasing and eastern bluebirds showing no change (See Fig. 7). Therefore, because no consistent trend was detected for either year or Julian day, these factors were not included as covariates in any additional models.

Figure 6: Comparison of Adult Blood Mercury by Year
2.2.11.2. Treatment of Sex Effects

Blood mercury levels did not differ significantly between female and male adult birds when all species were combined (F=0.150 (2, 284), p=0.861). No significant differences were found when each species was analyzed separately either (See Fig. 8). Therefore, sex was not used as a factor in further models.
2.2.11.3. Effect of Species on Adult Mercury Level

Species was found to be a significant factor in determining adult blood mercury levels ($F=87.79_{(3,292)}, p<0.0001$). When each species was looked at separately the result was (Carolina wren>Carolina chickadee>house wren>eastern bluebird (See Fig. 9). This relationship was the same for both 2007 and 2008 (See Table 3). Species was included in all models because it explained almost 50% of the variation in mercury levels.
Table 3: Mean Adult Blood Mercury Levels By Species and Year

<table>
<thead>
<tr>
<th>Species</th>
<th>Year</th>
<th>Mean ± Std. Dev. (ppm)</th>
<th>n</th>
<th>Species</th>
<th>Year</th>
<th>Mean ± Std. Dev. (ppm)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARW</td>
<td>2007</td>
<td>3.27 ± 1.83</td>
<td>27</td>
<td>HOWR</td>
<td>2007</td>
<td>1.15 ± 0.69</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>4.38 ± 2.60</td>
<td>10</td>
<td></td>
<td>2008</td>
<td>1.21 ± 0.74</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>3.83 ± 2.05</td>
<td>37</td>
<td>Mean</td>
<td>Mean</td>
<td>1.18 ± 0.72</td>
<td>92</td>
</tr>
<tr>
<td>CACH</td>
<td>2007</td>
<td>1.62 ± 0.83</td>
<td>19</td>
<td>EABL</td>
<td>2007</td>
<td>1.03 ± 0.67</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>0.97 ± 0.67</td>
<td>24</td>
<td></td>
<td>2008</td>
<td>0.76 ± 0.56</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>1.26 ± 0.80</td>
<td>43</td>
<td>Mean</td>
<td>Mean</td>
<td>0.92 ± 0.64</td>
<td>120</td>
</tr>
</tbody>
</table>

Figure 9: Comparison of Mean Adult Blood Mercury levels between species:
Presence of unique letter indicates significant difference at $\alpha=0.05$; note mercury values are log-transformed.
2.2.11.4. Principal Component Analysis for Adults

The spatial factors measured at each sampling location were used as covariates in the model. The individual variables included distance downstream from the ultimate source of mercury (river kilometer), flooding frequency (floodplain designation), relative elevation, distance from shoreline, and soil mercury availability within foraging territory. All spatial factors measured were significantly correlated with each other as is shown by their Pearson correlation coefficients and associated p-values in Table 4. To resolve this issue, principal component analysis was conducted using all the aforementioned spatial factors (soil mercury was converted to log scale). The number of principal components used in the final general linear model depended on the number of components needed to explain approximately 80% of the variance. These components did not necessarily all have Eigen values greater than the arbitrary cutoff of 1.0. No rotation was used in the principal component analysis.

Table 4: Correlation Coefficients of Spatial Variables included in Principle Component Analysis using Adult Blood Data (all cases significant at $\alpha=0.01$)

<table>
<thead>
<tr>
<th></th>
<th>Correlation Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flood Frequency</td>
</tr>
<tr>
<td>Flood Freq.</td>
<td>1.000</td>
</tr>
<tr>
<td>Rel. Elevation</td>
<td>-0.437</td>
</tr>
<tr>
<td>Distance (m)</td>
<td>-0.285</td>
</tr>
<tr>
<td>River Kilometer</td>
<td>-0.162</td>
</tr>
<tr>
<td>(log10) Soil Hg</td>
<td>0.256</td>
</tr>
</tbody>
</table>
2.2.12. Statistical Analysis of Nestling Feather Matrix

Analysis of nestling feather mercury levels was carried out in the same way as that for adult blood mercury levels. It should be noted that individual nestlings are not independent data points so all further analyses were done using brood means.

2.2.12.1. Treatment of Year and Seasonal Effects

In 2007 a total of 319 nestlings were sampled from 85 broods with a mean of 2.97 ± 1.93 ppm. In 2008 a total of 164 nestlings were sampled from 49 broods with a mean of 2.84 ± 2.11 ppm. When nestlings of all species were combined, year did not have a significant effect on log-transformed mean brood feather mercury ($F=0.165$ (1, 133); $p=0.685$). Additionally, when the species were analyzed separately there were no significant differences between years for any species (all $p>0.05$). However, Carolina chickadees did show a non-significant decrease in 2008 and Carolina wrens showed a non-significant increase in 2008 (See Fig. 10). Since the trends were not consistent across species, I decided that there was no significant difference in mercury availability between years and year was not included in any further models.
There was no significant effect of Julian day on mean brood feather mercury when all species were combined across the years ($F=0.967_{(55,133)}, p=0.547$). However, when year was analyzed separately, Julian date was not a significant factor in 2007 ($F=0.353_{(1,49)}, p=0.556$) but was in 2008 ($F=4.887_{(1,85)}, p=0.030$). In both years the trends mirrored those found with the adults; Carolina chickadee levels increasing over the season, Carolina wren and house wren nestlings showing a slight decrease, and eastern bluebird nestlings showing no relationship over the season (See Fig. 11). However, since this trend was not consistent among all species, Julian day was not included in any further models.
2.2.12.2. Effect of Species on Nestling Mercury Level

Nestling feather mercury did differ significantly between species; therefore species was used as an independent factor in all analyses (F=64.47(3,475), p<0.001). Carolina wrens had the highest mean at 6.80 ppm, followed by Carolina chickadees at 3.42 ppm, house wrens at 3.07 ppm, and eastern bluebirds at 2.17 ppm (See Table 5 and Fig. 12).
Table 5: Mean Nestling Feather Mercury Levels By Species and Year

<table>
<thead>
<tr>
<th>Species</th>
<th>Year</th>
<th>Mean ± Std. Dev. (ppm)</th>
<th>n</th>
<th>Species</th>
<th>Year</th>
<th>Mean ± Std. Dev. (ppm)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARW</td>
<td>2007</td>
<td>6.33 ± 1.71</td>
<td>22</td>
<td>HOWR</td>
<td>2007</td>
<td>2.90 ± 1.59</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>8.52 ± 5.48</td>
<td>6</td>
<td></td>
<td>2008</td>
<td>3.40 ± 1.57</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>6.80 ± 2.94</td>
<td>28</td>
<td></td>
<td>Mean</td>
<td>3.07 ± 1.60</td>
<td>123</td>
</tr>
<tr>
<td>CACH</td>
<td>2007</td>
<td>3.96 ± 2.24</td>
<td>61</td>
<td>EABL</td>
<td>2007</td>
<td>2.14 ± 1.15</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>2.55 ± 0.97</td>
<td>38</td>
<td></td>
<td>2008</td>
<td>2.24 ± 1.61</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>3.42 ± 1.98</td>
<td>99</td>
<td></td>
<td>Mean</td>
<td>2.17 ± 1.32</td>
<td>233</td>
</tr>
</tbody>
</table>

Figure 12: Comparison of Mean Brood Feather Mercury Levels between Species: Presence of unique letter indicates significant difference at α=0.05, note mercury values are log-transformed.

2.2.12.3 Effect of Days Since Hatching on Nestling Feather Mercury

Nestlings were not always of the same age when sampled; therefore I investigated whether there was a significant effect of age (days since hatching) on feather mercury
values. Because species were sampled at different ages, due to length of nestling period, each species was investigated separately. Age did not have a significant effect on nestling feather mercury values for any species (all p>0.5; See Table 6). This relationship is shown graphically in Figure 13 which suggests that there is a slight trend for nestling feather mercury levels to increase with age. However, for all species, the $r^2$ was less than 0.07, suggesting that this relationship is very weak.

**Table 6: The Effect of Days Post Hatch on Nestling Feather Mercury Levels (ppm):**
The effect was not significant for any individual species ($\alpha=0.05$). The range of days post hatch differed for each species along with the length of the nestling stage.

<table>
<thead>
<tr>
<th>Species</th>
<th>Age Range</th>
<th>Degrees of Freedom</th>
<th>F-statistic</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARW</td>
<td>8-14</td>
<td>1, 26</td>
<td>1.708</td>
<td>0.203</td>
</tr>
<tr>
<td>CACH</td>
<td>7-15</td>
<td>1, 98</td>
<td>2.484</td>
<td>0.118</td>
</tr>
<tr>
<td>HOWR</td>
<td>9-15</td>
<td>1, 93</td>
<td>3.388</td>
<td>0.069</td>
</tr>
<tr>
<td>EABL</td>
<td>6-18</td>
<td>1, 198</td>
<td>1.808</td>
<td>0.180</td>
</tr>
</tbody>
</table>
2.2.12.4 Principal Component Analysis for Nestlings

All nestlings were sampled from active nest boxes and were therefore associated with a distance from the river, relative elevation, soil mercury value and river kilometer from the source of contamination. However, because there was considerable correlation between these spatial factors (See Table 7), principal component analysis was used to produce new uncorrelated variables for further analysis. A total of 133 cases were used in the principal component analysis with each case representing a different brood of nestlings. No rotation was used in the principal component analysis and five components were produced to explain 100% of the total variance in my data. However, the first three components explained a total of 79.3% of the variance and these were extracted and interpreted for further analyses.
Table 7: Correlation Coefficients of Spatial Variables included in Principle Component Analysis (All cases significant at $\alpha=0.05$)

<table>
<thead>
<tr>
<th></th>
<th>Flooding Frequency</th>
<th>Relative Elevation (m)</th>
<th>Distance from River (m)</th>
<th>River Kilometer</th>
<th>Log(10) Soil Mercury (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flooding Frequency</td>
<td>1.000</td>
<td>-0.416</td>
<td>-0.297</td>
<td>-0.229</td>
<td>0.169</td>
</tr>
<tr>
<td>Rel. Elevation (m)</td>
<td>-0.416</td>
<td>1.000</td>
<td>0.283</td>
<td>0.354</td>
<td>-0.207</td>
</tr>
<tr>
<td>Distance from River (m)</td>
<td>-0.297</td>
<td>0.283</td>
<td>1.000</td>
<td>-0.160</td>
<td>-0.201</td>
</tr>
<tr>
<td>River Kilometer (km)</td>
<td>-0.229</td>
<td>0.354</td>
<td>-0.160</td>
<td>1.000</td>
<td>-0.167</td>
</tr>
<tr>
<td>Log(10) Soil Hg (ppm)</td>
<td>0.169</td>
<td>-0.207</td>
<td>-0.201</td>
<td>-0.167</td>
<td>1.000</td>
</tr>
</tbody>
</table>

2.3. Results Using Adult Blood Matrix

2.3.1. Results of Lateral Extent of Adult Exposure to Mercury

The original objective of this study was to investigate how far out into the floodplain of the South River songbirds are exposed to mercury. Therefore, I will first address the lateral extent of mercury exposure in adult blood. Adult birds were sampled from nest boxes placed along a distance gradient extending from 1-460 meters perpendicularly from the shore of the river.

In the investigation of adult blood mercury, a total of 285 adults were used (30 Carolina wrens (CARW), 43 Carolina chickadees (CACH), 92 house wrens (HOWR), and 120 eastern bluebirds (EABL). This includes both males and females sampled from 159 different nest box locations spread throughout 20 different sites along the contaminated portion of the South River.
Initial results with all species combined show that adult blood mercury levels did decline significantly with distance from the river’s shoreline (using log(10) blood mercury values and log(10) distance; F=46.523 (1, 284), p=0.000, adjusted $r^2=0.137$). This is best visualized in Figure 14, where all species show a marked decline in blood mercury levels along a lateral distance gradient.

**Figure 14: Decline of Adult Blood Mercury Level with Distance**

Blood mercury levels (ppm) for adults from all species declines with nest box distance from the shoreline (m)

I wanted to determine whether the decline in blood mercury levels was similar among the four species. In order to assess this I looked at the interaction between species and distance, which was not significant (F=1.715 (3, 284), p=0.164). However, visual representations (See Fig. 15 a-b showing both un-transformed and log-transformed data) did clearly show that that there were differing patterns among the species, although this
difference was not found to be significant in the combined species model. To further examine this I performed post hoc tests for each species separately (See Table 8). The results showed that Carolina wrens had the steepest decline to zero (260.5 m) with a correlation coefficient of 0.634. Carolina chickadees had the next steepest decline (317.8 m) with a correlation coefficient of 0.471. House wrens had a similar slope to Carolina chickadees (279.8 m) with a correlation coefficient of 0.461. Finally, eastern bluebirds had the shallowest decline (459.6 m) with a correlation coefficient of 0.373.

Table 8: Regression Statistics for relationship between log-transformed Adult Blood Mercury (ppm) and Distance from the River (m) for each species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Distance Gradient (m)/ Range (m)</th>
<th># of Adults Sampled</th>
<th>R</th>
<th>F-Statistic</th>
<th>df</th>
<th>P-value</th>
<th>Adjusted r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARW</td>
<td>(1.893-262.3410)/260.448</td>
<td>30</td>
<td>0.634</td>
<td>18.819</td>
<td>1, 29</td>
<td>&lt;0.001</td>
<td>0.390</td>
</tr>
<tr>
<td>CACH</td>
<td>(1.087-318.590)/317.772</td>
<td>43</td>
<td>0.471</td>
<td>11.662</td>
<td>1, 42</td>
<td>0.001</td>
<td>0.194</td>
</tr>
<tr>
<td>HOWR</td>
<td>(1.087-280.875)/279.788</td>
<td>92</td>
<td>0.461</td>
<td>24.262</td>
<td>1, 91</td>
<td>&lt;0.001</td>
<td>0.152</td>
</tr>
<tr>
<td>EABL</td>
<td>(1.161-460.755)/459.594</td>
<td>120</td>
<td>0.373</td>
<td>19.120</td>
<td>1, 119</td>
<td>&lt;0.001</td>
<td>0.085</td>
</tr>
</tbody>
</table>
Figure 15: (a) Un-transformed and (b) Log-transformed data showing the difference in slopes between species blood mercury levels as distance from the river increases. All species and sexes combined from 2007 and 2008.
In order to test whether these differences in slopes were significant, I conducted an analysis of covariance (ANCOVA) along the distance range shared by all species (1-262 m). This resulted in the exclusion of 17 cases (2 CACH, 2 HOWR and 13 EABL). The ANCOVA was performed using species as a main independent factor, blood mercury level as the dependent factor and log-transformed distance as the covariate. The regressions line of mercury level vs. distance from the river were not significantly different from each other ($F=2.584_{(3, 267)}$, $p=0.054$), meaning that the mercury levels of all four species declined with distance in the same general way (See Fig. 16 a-b). Since the regressions were not significantly different, the second step of the ANCOVA was performed.
Figure 16: Regression of blood mercury values for all four species along standardized distance range of 1-262 meters. (a) Using Un-transformed data (b) Using log-transformed data
The second step in an ANCOVA tests whether the y-intercepts of each species are significantly different from each other. This step proved to be significant ($F=39.451(3, 260)$, $p<0.001$) showing that the y-intercepts of all four species were not equal to each other (See Table 9). Therefore, it can be said that species had a significant effect on mercury values, independent of distance from the river. To reveal which species had significantly different y-intercepts, I performed pair-wise comparisons between species using Bonferroni adjusted means of log-transformed blood mercury values (See Table 10). This analysis revealed that the y-intercept of Carolina wrens was significantly different from the other three species. In addition, the y-intercept of eastern bluebirds was significantly different from the other three species. Finally, there was no difference in slope between Carolina chickadees and house wrens.

Table 9: Results for Test of Equality of Means for All Species shows that species do differ in their mean log-transformed blood mercury values (ppm), covariate=log-transformed distance (range 1-262 meters)

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean (ppm)</th>
<th>Std. Error</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>CACH</td>
<td>0.018</td>
<td>0.040</td>
<td>-0.060</td>
</tr>
<tr>
<td>CARW</td>
<td>0.465</td>
<td>0.046</td>
<td>0.373</td>
</tr>
<tr>
<td>EABL</td>
<td>-0.108</td>
<td>0.025</td>
<td>-0.157</td>
</tr>
<tr>
<td>HOWR</td>
<td>0.031</td>
<td>0.027</td>
<td>-0.023</td>
</tr>
</tbody>
</table>
Table 10: Pairwise Comparisons between Bonferroni adjusted means for all species. Comparison of log(10) blood mercury levels as explained by log(10) distance from the river. (* Delineates significance at the α=0.05 level)

<table>
<thead>
<tr>
<th>(I) Species</th>
<th>(J) Species</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>CACH</td>
<td>CARW</td>
<td>-0.446*</td>
<td>0.061</td>
<td>0.000</td>
<td>-0.609</td>
<td>-0.284</td>
</tr>
<tr>
<td>EABL</td>
<td></td>
<td>0.127*</td>
<td>0.047</td>
<td>0.045</td>
<td>0.002</td>
<td>0.251</td>
</tr>
<tr>
<td>HOWR</td>
<td></td>
<td>-0.013</td>
<td>0.048</td>
<td>1.000</td>
<td>-0.141</td>
<td>0.115</td>
</tr>
<tr>
<td>CARW</td>
<td>CACH</td>
<td>0.446*</td>
<td>0.061</td>
<td>0.000</td>
<td>0.284</td>
<td>0.609</td>
</tr>
<tr>
<td>EABL</td>
<td></td>
<td>0.573*</td>
<td>0.053</td>
<td>0.000</td>
<td>0.433</td>
<td>0.713</td>
</tr>
<tr>
<td>HOWR</td>
<td></td>
<td>0.433*</td>
<td>0.054</td>
<td>0.000</td>
<td>0.290</td>
<td>0.577</td>
</tr>
<tr>
<td>EABL</td>
<td>CACH</td>
<td>-0.127*</td>
<td>0.047</td>
<td>0.045</td>
<td>-0.251</td>
<td>-0.002</td>
</tr>
<tr>
<td>CARW</td>
<td></td>
<td>-0.573*</td>
<td>0.053</td>
<td>0.000</td>
<td>-0.713</td>
<td>-0.433</td>
</tr>
<tr>
<td>HOWR</td>
<td></td>
<td>-0.140*</td>
<td>0.037</td>
<td>0.001</td>
<td>-0.238</td>
<td>-0.041</td>
</tr>
<tr>
<td>HOWR</td>
<td>CACH</td>
<td>0.013</td>
<td>0.048</td>
<td>1.000</td>
<td>-0.115</td>
<td>0.141</td>
</tr>
<tr>
<td>CARW</td>
<td></td>
<td>-0.433*</td>
<td>0.054</td>
<td>0.000</td>
<td>-0.577</td>
<td>-0.290</td>
</tr>
<tr>
<td>EABL</td>
<td></td>
<td>0.140*</td>
<td>0.037</td>
<td>0.001</td>
<td>0.041</td>
<td>0.238</td>
</tr>
</tbody>
</table>

2.3.2. At what Distance Do Mercury Levels Decline to Mean Reference Level?

A second objective of this distance study was to identify a key distance from the river at which adult blood mercury levels declined to reference levels. In order to accomplish this a minimum of 10 adult individuals of each species were sampled from reference sites in 2008. Adequate data were not available for 2007. Mean reference mercury levels differed significantly among species ($F=11.503_{(1,44)}, p<0.001$) with Carolina wrens having the highest mean value (0.20 ppm), followed by house wrens 118
(0.12 ppm), eastern bluebirds (0.10 ppm) and Carolina chickadees (0.09 ppm) (See Table 12 and Fig. 17).

Table 11: Mean blood Mercury Levels for Reference Adults sampled in 2008. CARW had highest reference values followed by HOWR, EABL and CACH.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean ± Std. Dev. (ppm)</th>
<th>Sample Size (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARW</td>
<td>30.20 ± 0.055</td>
<td>10</td>
</tr>
<tr>
<td>CACH</td>
<td>0.09 ± 0.04</td>
<td>12</td>
</tr>
<tr>
<td>HOWR</td>
<td>0.12 ± 0.54</td>
<td>10</td>
</tr>
<tr>
<td>EABL</td>
<td>0.10 ± 0.04</td>
<td>13</td>
</tr>
<tr>
<td>TOTAL</td>
<td>0.12 ± 0.06</td>
<td>45</td>
</tr>
</tbody>
</table>

Figure 17: Box plot depicting significant difference between mean blood mercury levels of contaminated versus reference adult individuals by species. All four species were significantly different at $\alpha=0.05$. Error bars depict 95% confidence intervals.
Visual extrapolation was first used to identify the distance at which mercury levels declined to mean reference levels. All four species were initially graphed individually with nest site distance from the river predicting individual blood mercury levels. The mean reference blood mercury level of each individual species was added as a reference line to each graph. A linear regression line was fit to each graph and the point at which the fit line intersected with the reference line was estimated using extrapolation. Log-transformed mercury values were not used so as to maximize ease of interpretation. The predicted distance varied by species but was always greater than 250 m from the river (See Fig. 18 a-d). In an effort to generalize findings, all species were combined in a single graph and the extrapolation procedure repeated. This method predicted that blood mercury levels for adult songbirds would decline to reference at approximately 400 meters from the river (Fig. 19).
Figure 18 (a-d): Decline of Adult Blood Mercury Level (ppm) with Distance from the River for each Species Individually. Black line depicts linear regression fit with 95% confidence intervals (dashed black lines) and green line is mean 2008 reference levels for species. Intersection of the fit line and reference line predicts distance at which blood mercury levels decline to reference levels.

(a) Predicted Distance for Carolina wrens is between 250-275 meters

Species: CARW
(b) Predicted Distance for Carolina chickadees is between 325-350 meters

Species: CACH

(c) Predicted Distance for house wrens is 350 meters

Species: HOWR
(d) Predicted Distance for eastern bluebirds is between 600-650 meters

Figure 19: Generalized Songbird Predicted Distance at which Adult Blood Mercury Levels Decline to Mean Reference Level (all species combined) with 95% Confidence Intervals.

Predicted Distance = 400 meters
2.3.3. Results of Spatial Variation in Adult Blood Mercury Level

2.3.3.1. Results of Principal Component Analysis

The results of the principal component analysis yielded three components that explained, cumulatively, 79.26% of the variance in the included variables (See Table 13). The first two extracted components had Eigen values greater than 1.0 (2.0 and 1.15 respectively). The third extracted component had an Eigen value was 0.82 but explained more than 16.35% of the variance in the data so is included here (See Table 12).

Due to the large amount of variance and correlation in my data, some of my variables had high loading scores on multiple components. This made biologically interpreting the last three components difficult and therefore they are not discussed further. The loadings of each extracted component are shown in Table 13.

The first component accounts for 39.90% of the variance in the data and loaded most heavily for relative elevation (0.782) and flood frequency (0.729), but also for distance from the river (0.594) and soil mercury (-0.591). Taking into account the signs of each loading, the first component can be interpreted as the flooding potential because an adult nesting at a nest box territory with a high PC1 score would be at a high elevation and far from the river, with infrequent flooding and low soil mercury. The second component (PC2) explained 23.00% of the variance and loaded most heavily for the two distance variables, river kilometer (0.839) and distance from the river (-0.644) and is thus interpreted as the distance component. A bird with a high PC2 score would be far downstream and close to the river shoreline. Finally, the third component (PC3) explained the least of my variance (16.35%) but still had an Eigen value close to the rule-
of-thumb 1.0 cutoff (0.818). PC3 loaded heavily only for soil mercury (0.782) and is interpreted as the soil mercury component. Soil mercury seems to be a poor predictor of blood mercury, although there is some evidence of positive correlation (See Fig. 20).

**Table 12: Total Variance Explained by Initial and Extracted Principal Components with Associated Eigenvalues**

<table>
<thead>
<tr>
<th>Total Variance Explained by Components</th>
<th>Eigenvalue</th>
<th>% of Variance</th>
<th>Cumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0</td>
<td>39.90</td>
<td>39.90</td>
</tr>
<tr>
<td>2</td>
<td>1.15</td>
<td>23.00</td>
<td>62.91</td>
</tr>
<tr>
<td>3</td>
<td>0.82</td>
<td>16.35</td>
<td>79.26</td>
</tr>
<tr>
<td>4</td>
<td>0.63</td>
<td>12.51</td>
<td>91.77</td>
</tr>
<tr>
<td>5</td>
<td>0.41</td>
<td>8.23</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Table 13: Loadings of Extracted Principal Components** (Explained 79.257% of Total Variance)

<table>
<thead>
<tr>
<th>Principal Component Loadings</th>
<th>Principal Component Number and Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC1: Flooding Potential</td>
</tr>
<tr>
<td>Floodplain Designation</td>
<td>0.729</td>
</tr>
<tr>
<td>Relative Elevation (m)</td>
<td>0.782</td>
</tr>
<tr>
<td>Distance from River (m)</td>
<td>0.594</td>
</tr>
<tr>
<td>River Kilometer (km)</td>
<td>0.387</td>
</tr>
<tr>
<td>Log(10) Soil Hg (ppm)</td>
<td>-0.591</td>
</tr>
</tbody>
</table>
2.3.3.2. Results of General Linear Model Using Adults

A general linear model was used to identify the significance of each component in explaining mercury levels in adult birds. Species was included as a main factor in the model as mercury levels vary significantly between species. In addition, all interactions between species and each component were initially included in the model. There were a total of 285 individual cases used in the general linear model, composed of 30 Carolina wrens, 43 Carolina chickadees, 92 house wrens and 120 eastern bluebirds.

The initial model included species, PC1, PC2, PC3 and all interactions between species and each component. Species was a significant predictor of adult mercury level (F-statistic of $23.175_{(3,284)}$ and a $p= 0.000$). Flooding potential (PC1) and distance (PC2)
were also both significant factors in predicting adult blood mercury levels (PC1; F=70.756 (1, 284), p=0.000) and (PC2; F=28.463 (1, 284), p=0.000) (See Fig. 21 and 22). Soil mercury availability (PC3) was not a significant factor (F=0.581 (1, 284), p=0.447) (See Fig. 23).

The final model explained approximately 51.7% of the variation in adult blood mercury values and included species as a main factor, PC1 and PC2 as covariates, and the interaction of species and PC2 (F=36.923 (8, 284), p<0.000, adjusted r²=0.517). PC3 was removed from the model because it did not improve the predictive power of the model. When the effect sizes of each factor were examined, PC1 had the largest effect size with a partial Eta squared value of 0.285, followed closely by species (0.226), the interaction of species and PC2 had the next largest effect size (0.103) but PC2 on its own had a small effect size of only 0.097.
Figure 21: Flooding Potential (PC1) as a Predictor of Log-transformed Adult Blood Mercury

- More Frequent Flooding, Lower Relative Elevation
- Less Frequent Flooding, Higher Relative Elevation

- CARW: $R^2$ Unear = 0.198
- CACH: $R^2$ Linear = 0.225
- HOWR: $R^2$ Linear = 0.122
- EABL: $R^2$ Linear = 0.294
Figure 22: Distance Measures (PC2) as a Predictor of Log-transformed Adult Blood Mercury

![Graph showing distance measures (PC2) as a predictor of log-transformed adult blood mercury levels.](image)

- **Species**
  - CARW
  - CACH
  - HOWR
  - EABL

- **Slopes and R-squared values**
  - CARW: $R^2$ Linear = 0.095
  - CACH: $R^2$ Linear = 0.194
  - HOWR: $R^2$ Linear = 0.098
  - EABL: $R^2$ Linear = 0.001

- **Legend**
  - Less Downstream, Farther from the River
  - Farther Downstream, Closer to the River
Figure 23: Soil Mercury Availability (PC3) as a Predictor of Log-transformed Adult Blood Mercury

Species
- CARW
- CACH
- HOWR
- EABL

**PC3**

- CARW: R² Linear = 0.014
- CACH: R² Linear = 0.04
- HOWR: R² Linear = 2.407
- EABL: R² Linear = 0.041

Log-transformed Adult Blood Mercury (ppm)

Low Soil Mercury | High Soil Mercury

Soil Mercury (PC3)
2.4. Results Using Nestling Feather Matrix

2.4.1. Results of Lateral Extent of Nestling Exposure to Mercury

In the investigation of nestling feather mercury, a total of 483 nestlings from 134 broods were used (10 CARW, 22 CACH, 36 HOWR, and 66 EABL). Nestling broods were sampled from 82 different nest box locations spread throughout 13 different sites along the contaminated portion of the South River.

Initial results with all species combined show that mean brood feather mercury levels declined significantly with distance from the shoreline (using log(10) mean brood feather mercury values and log(10) distance values; $F=12.096 (1, 133), p=0.001$, adjusted $r^2=0.077$). This is best visualized in Figure 24, where all species show a marked decline in blood mercury levels along a lateral distance gradient.

Figure 24: Brood Feather Mercury Levels (ppm) for Nestlings from all Species Declines with Nest Box Distance from the Shoreline (m)
I wanted to know whether the decline in feather mercury levels was similar among the four species, so each species was analyzed separately. In order to assess this I looked at the interaction between species and distance, which was not significant (F=1.069(3, 133), p=0.365). However, visual representations (See Fig. 25 a-b) showing both untransformed and log-transformed data) clearly show that that there were different slopes among the species, although this difference was not significant in the combined species model. To further examine this I performed post hoc tests for each species separately (See Table 14). The results show that Carolina chickadees had the steepest decline (317.5 m) with a correlation coefficient of 0.713. Carolina wrens had the next steepest decline (90.8 m) with a correlation coefficient of 0.453. House wrens and eastern bluebirds had the flattest slopes: House wrens had the steeper slope (256.35 m) with a correlation coefficient of 0.335, while eastern bluebirds (459.6 m) had a correlation coefficient of 0.288.

Table 14: Regression Statistics for Relationship between Log-transformed Brood Feather Mercury (ppm) and Distance from the River (m) for each species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Distance Gradient (m)/ Range (m)</th>
<th># of Broods Sampled</th>
<th>R</th>
<th>F-Statistic</th>
<th>df</th>
<th>P-value</th>
<th>Adjusted r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARW</td>
<td>(9.276-100.085)/ 90.808</td>
<td>10</td>
<td>0.453</td>
<td>2.061</td>
<td>1,8</td>
<td>0.189</td>
<td>0.107</td>
</tr>
<tr>
<td>CACH</td>
<td>(1.087-318.590)/ 317.502</td>
<td>22</td>
<td>0.713</td>
<td>20.707</td>
<td>1,20</td>
<td>&lt;0.001</td>
<td>0.282</td>
</tr>
<tr>
<td>HOWR</td>
<td>(1.087-257.435)/ 256.348</td>
<td>36</td>
<td>0.335</td>
<td>4.294</td>
<td>1,34</td>
<td>0.046</td>
<td>0.051</td>
</tr>
<tr>
<td>EABL</td>
<td>(1.161-460.755)/ 459.594</td>
<td>66</td>
<td>0.288</td>
<td>5.797</td>
<td>1,64</td>
<td>0.019</td>
<td>0.089</td>
</tr>
</tbody>
</table>
Figure 25: Un-transformed (a) and log-transformed (b) data showing the difference in slopes between species brood feather mercury levels as distance from the river increases. All species and sexes combined from 2007 and 2008.
In order to test whether the differences in slopes between species were significant I conducted an analysis of covariance (ANCOVA). However this could not be done over a shared distance range without excluding all HOWR broods; therefore the entire distance range of 1-459 m was used. The ANCOVA was performed using species as the independent factor, feather mercury value as the dependent factor and log-transformed distance as the covariate. The interaction between species and distance was not significant (F=20.605(3, 77), p=0.614). Therefore there was not a significant difference in the regressions of each species to each other and the second part of the ANCOVA was performed.

The second step in an ANCOVA tests whether the y-intercepts of each species are significantly different from each other. This step proved to be significant (F=8.414(3, 77), p<0.001) showing that the y-intercepts of all four species were not equal to each other (See Table 15). Therefore, it can be said that species had a significant effect on mercury values, independent of distance from the river. To reveal which species had significantly different y-intercepts, I performed pair-wise comparisons between species using Bonferroni adjusted means of log-transformed blood mercury values (See Table 16). This analysis revealed that the y-intercept of Carolina wrens was significantly different from the other three species with all p-values<0.001. In addition, the y-intercept of eastern bluebirds was significantly different from the other three species (all p<0.001). Finally, there was no difference in slope between Carolina chickadees and house wrens (p=0.690). These differences mirror the slope differences in adult blood mercury values.
Table 15: Results for Test of Equality of Means for Species shows that species differ in their mean log-transformed brood feather mercury values (ppm), covariate = log(10)transformed distance (entire distance range included)

<table>
<thead>
<tr>
<th>Test for Equality of Means Between Species</th>
<th>Mean (ppm)</th>
<th>Std. Error</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARW</td>
<td>0.451</td>
<td>0.049</td>
<td>0.353 - 0.548</td>
</tr>
<tr>
<td>CACH</td>
<td>0.764</td>
<td>0.073</td>
<td>0.619 - 0.908</td>
</tr>
<tr>
<td>HOWR</td>
<td>0.248</td>
<td>0.028</td>
<td>0.192 - 0.304</td>
</tr>
<tr>
<td>EABL</td>
<td>0.476</td>
<td>0.039</td>
<td>0.399 - 0.552</td>
</tr>
</tbody>
</table>

Table 16: Pairwise comparisons between Bonferroni adjusted means for all species. Comparison of log(10) brood feather mercury levels as explained by log(10) distance from the river. (*Delineates significance at the α=0.05 level)

<table>
<thead>
<tr>
<th>Bonferroni Adjusted Pairwise Comparisons between Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) Species</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>CARW</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>CACH</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>HOWR</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>EABL</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
2.4.2. At what Distance Do Feather Mercury Levels Decline to Mean Reference Level?

Using the same procedure as with adult blood mercury I identified a key distance from the river at which nestling feather mercury levels declined to reference levels. In addition to 134 broods sampled from contaminated sites, nestling broods were also sampled from reference sites. However, I did not have Carolina wren nests on reference sites in 2008 and only one house wren reference nest had nestlings survive to sampling age in 2008. Therefore, all reference broods sampled throughout the duration of bird studies on the South River were combined. These samples spanned the years 2006-08, but due to the extremely small sample sizes, the effect of year on reference nestling feather mercury could not be reliably ascertained. Sample sizes were small in general, therefore this comparison between contaminated and reference feather mercury has limited interpretability.

Mean reference mercury levels differed significantly among species (F=4.058 (3,30), p=0.032) with Carolina chickadees having the highest mean value (0.30 ± 0.11 ppm), followed by Carolina wrens (0.29 ± 0.01 ppm), house wrens (0.22 ± 0.01 ppm) and eastern bluebirds (0.21 ± 0.01 ppm) (See Table 17). Reference feather mercury values did differ significantly from contaminated values (F=28.860(2,506), p<0.001; See Fig. 26).
Table 17: Mean Reference Nestling Feather Mercury by Species

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean ± Std. Dev. (ppm)</th>
<th>Sample Size (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CACH</td>
<td>0.30 ± 0.11</td>
<td>12</td>
</tr>
<tr>
<td>CARW</td>
<td>0.29 ± 0.01</td>
<td>2</td>
</tr>
<tr>
<td>HOWR</td>
<td>0.22 ± 0.01</td>
<td>2</td>
</tr>
<tr>
<td>EABL</td>
<td>0.21 ± 0.01</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>0.25 ± 0.03</td>
<td>22</td>
</tr>
</tbody>
</table>

Figure 26: Box plot depicting significant difference between mean brood feather mercury levels of contaminated versus mean reference broods by species. All four species were significantly different at $\alpha=0.05$. Error bars depict 95% confidence intervals.
Visual extrapolation was used to identify a key distance at which mercury levels declined to mean reference levels. All four species were initially graphed individually with nest site distance from the river predicting mean brood feather mercury levels. The mean reference feather mercury level of each individual species was added as a reference line to each graph. Finally a linear regression line was fit to each graph and the point at which the fit line intersected with the reference line estimated using extrapolation. Log-transformed mercury values were not used so as to maximize ease of interpretation. The predicted distance varied by species but was always greater than 250 m from the river (See Fig. 27 a-d). In an effort to generalize findings, all terrestrial avian species are combined in a single graph, which predicted that feather mercury levels would decline to reference at 450 meters from the river (Fig. 28).
Figure 27 (a-d): Decline of Brood Feather Mercury Level with Distance from the River for each Species individually. Black line depicts linear regression fit with 95% confidence intervals and green line is mean reference levels for species. Intersection of the fit line and reference line predicts distance at which blood mercury levels decline to reference levels.

(a) Predicted Distance for Carolina wrens is between 250-275 m

(b) Predicted Distance for Carolina chickadees is 275 meters
(c) Predicted Distance for house wrens is 600 meters

![Graph for House Wrens (HOWR)]

Species: HOWR

\[ R^2 \text{ Linear } = 0.051 \]

(d) Predicted Distance for eastern bluebirds is 575 meters

![Graph for Eastern Bluebirds (EABL)]

Species: EABL

\[ R^2 \text{ Linear } = 0.089 \]
Figure 28: Generalized Predicted Distance at which Brood Feather Mercury Levels Decline to Mean Reference Level for all species combined. Solid black line depicts mean of all species combined with 95% confidence intervals (black dashed lines). The dashed green line depicts mean reference level for all species combined in 2008.

**Predicted Distance for All Species Combined** = 450 meters

**Species**
- CARW
- CACH
- HOWR
- EABL

**Fit line for Total**

**R² Linear** = 0.087
2.4.3. Results of Spatial Variation in Nestling Feather Mercury Levels

2.4.3.1. Results of Principal Component Analysis

The results of the principal component analysis yielded three components that explained, cumulatively, 79.30% of the variance (See Table 18). The first two extracted components had Eigen values greater than 1.0 (1.92 and 1.17 respectively). The third extracted component had an Eigen value was 0.87 but explained 17.56% of the variance in my data so is included.

The first component (PC 1) explained almost 40% of the variance in my data and loaded heavily for relative elevation (0.790) and flooding frequency (0.735). PCI loaded less heavily for distance from the river (0.506), river mile (0.491) and log-transformed soil mercury (-0.511). Since PCI loaded most strongly for both variables that determine flooding potential, it is interpreted as the flooding potential component. The second component (PC2) explained 23.39% of the variance and loaded most heavily for river kilometer (0.769) and distance from the river (-0.746). Both of these variables describe the distance of each case from the ultimate and proximate sources of mercury; therefore PC2 is interpreted as the distance measures component. Finally, the third component (PC3) loaded heavily for log-transformed soil mercury (0.844) and weakly for all other variables, therefore PC3 is interpreted as the soil mercury availability component. The component scores and interpretations can be seen in Table 19.
Table 18: Total Variance Explained by Extracted Principal Components

<table>
<thead>
<tr>
<th>Component Number</th>
<th>Eigenvalue</th>
<th>% of Variance</th>
<th>Cumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.92</td>
<td>38.44</td>
<td>38.44</td>
</tr>
<tr>
<td>2</td>
<td>1.17</td>
<td>23.39</td>
<td>61.84</td>
</tr>
<tr>
<td>3</td>
<td>0.87</td>
<td>17.45</td>
<td>79.28</td>
</tr>
<tr>
<td>4</td>
<td>0.59</td>
<td>11.73</td>
<td>91.01</td>
</tr>
<tr>
<td>5</td>
<td>0.45</td>
<td>8.99</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Table 19: Loadings for all Extracted Principal Components

<table>
<thead>
<tr>
<th>Component Number and Interpretation</th>
<th>PC1: Flooding Potential</th>
<th>PC2: Distance Measures</th>
<th>PC3: Soil Mercury Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floodplain Designation</td>
<td>0.735</td>
<td>0.070</td>
<td>0.335</td>
</tr>
<tr>
<td>Relative Elevation (m)</td>
<td>0.790</td>
<td>0.108</td>
<td>0.212</td>
</tr>
<tr>
<td>Distance from River (m)</td>
<td>0.506</td>
<td>-0.746</td>
<td>0.054</td>
</tr>
<tr>
<td>River Kilometer (km)</td>
<td>0.491</td>
<td>0.769</td>
<td>-0.021</td>
</tr>
<tr>
<td>Log(10) Soil Hg (ppm)</td>
<td>-0.511</td>
<td>0.068</td>
<td>0.844</td>
</tr>
</tbody>
</table>

2.4.3.2. Results of General Linear Model using Nestlings

The predictive power of each component was investigated in a general linear model. The initial model included log-transformed mean brood mercury as the dependent variable and species as a main factor. Species was included because nestling feather mercury levels vary significantly by species due to differences in nestling provisioning diet as well as physiological differences. The effect of each component was investigated by including them as covariates. The interaction between species and each component was also included in the initial model. All 134 broods were included in the
model (10 Carolina wrens (CARW), 22 Carolina chickadees (CACH), 36 house wrens (HOWR) and 66 eastern bluebirds (EABL)). Broods from 2007 and 2008 were combined because no effect of year was detected.

As expected, species had a significant effect ($F=12.498_{(3, 132)}, p<0.001$), as did PC1 (flooding potential, $F=5.357_{(1, 132)}, p=0.022$) and PC2 (distance measures, $F=7.794_{(1, 132)}, p=0.006$) (See Fig. 29 and 30). However, PC3 (soil mercury availability) did not have a significant effect on brood feather mercury levels ($F=0.508_{(1, 132)}, p=0.477$) and was not included in subsequent models (See Fig. 31). Neither the interaction of species and PC1 ($F=2.020_{(3, 132)}, p=0.115$) nor species and PC3 ($F=0.461_{(3, 132)}, p=0.710$), were significant in the initial model. However, the interaction between species and PC2 (distance measures) was significant ($F=5.170_{(3, 132)}, p=0.002$) suggesting that there was an unequal number of each species measured at both distances from the river and distance downstream.

The final model included species as a main factor, PC1 and PC2 as covariates and the interaction of species and PC2. This model explained 51.6% of the variability in mean brood feather mercury ($r^2=0.516$). Species was highly significant ($F=13.645_{(3, 132)}, p<0.001$). Both flooding potential (PC1) ($F=51.753_{(3, 132)}, p<0.001$) and the distance measures (PC2) ($F=6.901_{(3, 132)}, p=0.010$) had significant effects. Flooding potential is an important predictor for Carolina chickadees and eastern bluebirds but has a weak relationship for Carolina wrens and house wrens with a general trend of decreasing brood feather mercury as flooding frequency decreases and relative elevation above the river increases (Fig. 29). The interaction between species and the distance measures was once again significant ($F=4.774_{(3, 132)}, p=0.003$). This interaction is graphically shown in
Figure 30, which suggests that both Carolina wren and Carolina chickadee brood feather mercury increased with river mile downstream and decreased as lateral distance from the river increases. The relationship was very weak for both house wrens and eastern bluebirds, suggesting that river mile and lateral distance from the river are not important predictors for these species.

When the effect sizes of each factor were compared, PC1 accounted for the highest amount of variation with a partial Eta squared value of 0.294, followed closely by species (0.248), the interaction of species and PC2, which had modest effect size of 0.104. PC2 on its own had the smallest effect size of just 0.053.
Figure 29: Flooding Potential (PC1) as a Predictor of Log-transformed Brood Feather Mercury
Figure 30: Distance Measures (PC2) as a Predictor of Log-transformed Brood Feather Mercury
2.5. Discussion

2.5.1. Seasonal Variation in Mercury Levels

2.5.1.1. Adults

There was a significant difference in adult mercury levels between the 2007 and 2008 seasons when all species were combined. However, significant differences were detected individually only for Carolina chickadees and eastern bluebirds, with both having lower levels in 2008. In 2007, only 29% of the bluebirds sampled were from
nests more than 100 meters from the river shore, while in 2008 this number grew to 38%, suggesting that lower levels between years may have been an artifact of sampling inconsistencies for this species. Concerning Carolina chickadees, approximately equal numbers were sampled from >100 m away from the river in each year (26% in 2007 versus 30% in 2008). However, the distance range for chickadees was extended from 188 m in 2007 to 318 meters in 2008. Given that mercury levels did decrease with distance from the river, it seems likely that the lower mercury levels in 2008 were a result of more individuals being sampled from territories farther from the river with less mercury available.

When the effect of date within season (Julian day) was examined for both years combined, it was significant. However, there seemed to be no distinguishable trend of adult blood mercury levels with Julian day within either the 2007 or 2008 seasons when comparing each species separately. Carolina chickadee mercury levels increased over the season, while Carolina wren, house wren and eastern bluebirds all decreased with Julina day for both years. Since there was no consistent pattern among the species, there is no predictable change in mercury availability over the season. The differences seen within each species may be due to changes in diet or possibly may be an artifact of one of the other many variables.

For example, the length of breeding season varies among the species and may be important in explaining the discrepancies in mercury levels with increasing Julian day. For instance, the Carolina chickadee only lays one clutch per season and therefore has a shortened but early breeding season with the vast majority of nest initiation dates being in late April and early May. However, the house wren is migratory and does not begin
nesting on the South River until late May or early June. Furthermore, both the Carolina wren and eastern bluebird have long seasons with some nests as early as late April and some as late as the end of August.

In addition, the analysis of Julian day does not take into account the effect of distance from the river. Adults may have been sampled at increasingly far or increasingly closer distances from the river over the duration of the season and the effect of Julian day may be an artifact of distance not being included as a factor. However, in a subsequent analysis I included only those individuals that were captured at nest box territories less than or equal to 50 m from the river in an effort to control for the effect of distance from the river. The results of this additional analysis found that Julian day was non-significant when distance from the river was controlled for in both the 2007 season when \(F=3.227_{(1,77)}, p=0.076\) and the 2008 season \(F=0.373_{(1,52)}, p=0.544\). Therefore, Julian day does not seem to be a significant factor in explaining mercury levels since no consistent pattern can be found among the species. In order to accurately determine whether there is seasonal variation in mercury exposure one would need to focus on one species or individual that is sampled at similar distances and habitat throughout the season.

2.5.1.2. Nestlings

There was no significant effect of year on brood feather levels when all species were combined or when each species was analyzed separately. Therefore, year was not used as a factor in any of the final models predicting mercury exposure of nestlings.
Julian day was also found not to be a significant factor in either the 2007 or 2008 season when all species were combined. Additionally, when each species was analyzed separately for the effect of Julian day in each season, no significant results were detected. However, though Julian day did not have any effect of brood feather mercury levels, to be consistent with the analysis of adults, I performed another analysis using only broods that were sampled from within 50 m of the river for each species and across both seasons. This analysis found only one significant effect of Julian day on house wrens broods sampled in 2008 ($F=25.074$, $p=0.038$). For all other species in both seasons, there was no effect of Julian day. Since no consistent trend could be detected, Julian day seemed to be an unimportant factor in predicting brood feather mercury. However, these results should be interpreted with caution because sample sizes were extremely low for Carolina wrens in both years ($n < 5$) and for house wrens in 2008 ($n = 5$).

### 2.5.2. Effect of Sex on Adult Mercury Levels

There was no significant difference between mercury levels of females and males of any study species. This is contrary to some studies that suggest females have an additional mercury excretion route when laying eggs (Dauwe et al., 2004). Males do not have this route of excretion and therefore might be expected to have higher mercury loads than females. However, in this study all females were caught either during the last week of incubation or after hatching. The half-life of mercury in the blood has been established to be about 2 weeks (Evers et al., 2005) and the incubation period for all four species was, on average, about 2 weeks long. Therefore mercury deposition into eggs
may have had no effect on many of the samples taken for this study, as they were collected 1-3 weeks after the last egg was laid.

Additionally, some cases of sex differences in mercury levels have been from sexually dimorphic species such as common loons (Evers et al., 2005). This difference is normally attributed to the differences in resource utilization among the species (Aulen and Lundberg, 1991). For example, in common loons where the male is heavier than the female, it was found that males generally eat larger fish than females, thereby increasing their mercury accumulation (Evers et al., 2005). Although Carolina wrens and eastern bluebirds are somewhat sexually dimorphic species, differences in diet have not been confirmed and it is likely that both sexes have similar diets.

2.5.3. Effect of Age on Nestling Feather Mercury

Age (days post hatch) did have a significant effect on feather mercury values of nestlings with mercury levels slightly increasing with age (See section 2.2.12.3. Effect of Days Post Hatch on Nestling Feather Mercury). One possible explanation is that feathers were sampled from nestlings while they were still growing. Therefore, when older nestlings were sampled, their growing feathers had more time to accumulate mercury from the blood system than feathers sampled from younger nestlings. However, since age was not a significant factor when each species investigated individually, the variation in feather mercury levels attributed to age is minimal. In addition, feather growth is such that mercury level should not change in a given part of the feather after it emerges from the sheath. Thus, higher levels in more developed feathers indicate higher mercury
concentration in the last-grown portions rather than further infusion into earlier-grown portions.

2.5.4. Effect of Species on Mercury Levels

Species was a significant factor in predicting mercury levels for both adult and nestlings. The four study species varied in their size (both as nestlings and adults), their habitat types, diet and foraging strategies (See Section 2.2.4. Study Species). Carolina wrens consistently had the highest mercury levels and eastern bluebirds consistently had the lowest levels. These two species were also the largest in terms of weight, with eastern bluebirds having a mean weight of 29.75 ± 3.32 grams and Carolina wrens weighing 19.63 ± 1.87 grams. House wrens were slightly heavier than Carolina chickadees with mean weights of 11.09 ± 1.02 grams and 9.34 ± 0.09 grams, respectively. Many studies have correlated size with mercury level (Morel et al., 1998) (See Section 1.2.5. Methylation and Methyl Mercury); however, the majority of these studies have examined aquatic invertebrates and fish species with indeterminate growth. This same relationship does not seem to hold true for songbirds species sampled in this study.

Habitat type has also been found to a play a role in dictating mercury exposure. Of the four study species, eastern bluebirds were the only species to nest almost exclusively in open areas such as fields, agricultural areas and parks. The other three species used more dense woody areas to various extents. Carolina wrens nested in areas of thick vegetation while house wrens and Carolina chickadees nested in both mature woodlands and along edges along open areas. According to mean mercury levels, my
results suggest that open areas may contain less available mercury than woodland areas. However, this is an imperfect analysis because it does not take into account actual diet preferences among the species.

Previous work on the South River, as well as other studies, has confirmed that the majority of avian mercury exposure is through the diet. Furthermore, previous studies (Friedman, 2007; Cristol et al., 2008) have shown that prey items commonly eaten by songbirds on the South River differ in their mercury accumulation with spiders accumulating the most. Therefore, it is logical that the diet differences among my four study species are the driving force behind the differences in their mercury exposure.

Finally, species that feed on similar prey items may increase or decrease their mercury loads according to where they forage for these items, in terms of microhabitat. Foraging strategies of my four study species vary from Carolina wrens which forage in leaf litter, to house wrens and Carolina chickadees which glean prey from vegetation both in the sub-canopy and the canopy, to eastern bluebirds which are ground foragers in open areas where they spot prey from perches (See Section 2.2.4. Study Species). Prey types taken from these different microhabitats may vary in species, size, and potential of mercury accumulation. Habitats and microhabitats vary in their methylation potentials, thereby influencing the amount of mercury available to species in the area (See section 1.2.5. Methylation and Methyl Mercury).

### 2.5.5. Lateral Extent of Exposure in Terrestrial Songbirds

The primary objective of this work was to determine how far from the contaminated South River terrestrial songbirds were still exposed to mercury. This is an
important and previously unaddressed question that has implications for how future mercury risk assessments are conducted.

### 2.5.5.1. Adults

This study concluded that terrestrial insectivorous songbirds were being exposed to mercury arising from the contaminated river even when they maintained breeding territories as far as 400 meters from the river. This is a compelling result because it suggests that species throughout the floodplain, and even those breeding outside of the traditional floodplain, are still exposed to mercury originating in the river itself.

All species displayed a similar decline with distance from the river; however, the extent of contamination and the predicted distance at which exposure would decline to reference levels was variable. Average territory size and degree of territoriality may offer one explanation for the differences seen between species. Territory size may be important because species or individuals with larger territories may feed over a wider area and therefore may not be as indicative of the mercury contamination in the immediate nest box area. In this case, the distance of each nest box from the river may not be an accurate measure of the typical distance from the river at which an individual foraged. The literature suggests that eastern bluebirds maintain the largest territories (1.1-8.4 ha) followed by Carolina wren (1-4.1 ha), the Carolina chickadee (1.6-2.4 ha) and finally the house wren (0.2-0.5 ha) (See section 2.3.4. Study Species). However, territory size is largely dependent on con-specific density, resource availability (e.g., nest sites and food), and resource patchiness (Elchuk and Wiebe, 2002). These three characteristics were largely site dependent with some sites having more natural woodland
areas that supported multiple Carolina wren, Carolina chickadee and house wren pairs but few eastern bluebird pairs. Other sites had a few small woodland patches that supported few woodland species but multiple eastern bluebird pairs. Because I did not measure territory sizes of individuals, the use of territory size to predict the decline of mercury values with distance from the river is limited. Additional information on foraging sites and territoriality would only strengthen the conclusion that the decline of mercury exposure is more pronounced in species with smaller foraging territories.

2.5.5.1.1. Difference Between Males and Females

Due to sex role differentiation in all four of my study species, I hypothesized that females may be better indicators of mercury availability in the immediate nest site location than males. This hypothesis was based on two ideas, one being that the in order to maximize energy gain for amount of energy consumed during foraging events, breeding adults should conform to the principles of the central place foraging. However, the principles may be disparately applicable to males and females according to other duties. For example, studies have shown that passerine males are typically more aggressive in territory defense (Fedy and Stutchbury, 2005). It has also been suggested that the female is more active in defending the actual nest site from con-specific female intruders and predators while the male is generally more active in patrolling the entire territory area in defense of his territorial position as well as potential predators (Pons et al., 2008; Fedy and Stutchbury, 2005). This suggests that the male may forage over a wider area for both himself and the nestlings while the female forages in a smaller range around the nest.
Secondly, throughout this study all females were either caught during the last days of incubation or in the first week after hatching was completed. The Birds of North America (BNA) species accounts for Carolina wrens, Carolina chickadees, and eastern bluebirds state that the female clutch attentiveness comprises 60-75% of her daily activity (Gowaty and Plissner, 1998; Mostrom et al., 2002; Haggerty and Morton, 1995). During this time, she is generally confined to the nest box and takes only very short foraging forays lasting, on average, 5 minutes (Gowaty and Plissner, 1998; Mostrom et al., 2002; Haggerty and Morton, 1995). It is also mentioned that for all three of these species (CARW, CACH, EABL) the male is likely to deliver prey items to the incubating female (Gowaty and Plissner, 1998; Mostrom et al., 2002; Haggerty and Morton, 1995). According to the principles of the central foraging theory, as described above, the male would exert less energy by delivering prey items found at close distance to the incubating female. However, when feeding himself, it would be more advantageous to forage throughout the territory in order to defend his position and detect predators at the same time. With these differences in mind, one would expect females caught during or just after incubation to be better indicators of mercury in the immediate nest location.

2.5.5.2. Nestlings

Using brood feather mercury as the endpoint provided a slightly different result from that with adult blood, with the lateral extent of mercury contamination extending for 450 meters into the floodplain. I originally predicted that the brood feather matrix would offer a more accurate indication of mercury availability in the immediate nest site area since the nestlings are confined to the nest box and any mercury in their system would
have been accumulated during their nestling stage on the South River or from female deposition into their egg (Eagles Smith et al., 2008; Frederick et al., 2002). This hypothesis was strengthened by the principles of the central foraging theory that assumes that a central place forager (in this case a breeding adult) maximizes their net rate of energy to the central place (nest site) (Elchuk and Wiebe, 2002).

The selection of an optimal foraging territory should be based on risk of predation, food abundance, food availability and competition from both con-specifics and other species (Elchuk and Wiebe, 2002). However, this does not take into account the time and energy expenditure associated with the distance traveled from the central place, the energy exertion of carrying a heavier load of prey items, or the energy nutrition of prey items (Elchuk and Wiebe, 2002). Foraging distance and nutrition value of delivered prey items become extremely important factors when provisioning for young and protecting against would-be nestling predators. To reduce the risk of predation to the nestlings, one would expect parents to provision their nestlings with the most nutritious prey items found near the nest site. Under this circumstance, nestlings should be better indicators of mercury availability near the nest site. However, the results of the feather mercury versus distance from the river regressions were much weaker for the nestlings than the adults overall (See Table 20). Therefore my original hypothesis is not supported.

Alternative explanations for the unexpected weaker relationship of nestlings with nest site distance may be explained by possible differences in diet between adults and nestlings. Some studies and observations have suggested that adults provision their nestlings with higher quality or nutritional food than they forage for themselves (Arnold et al., 2007). Spiders are nutritionally similar to other common prey (Lepidoptera);
however, they contain much higher portions of taurine, which has been related to proper stress responsiveness and cognitive function in nestling birds (Arnold et al., 2007). Therefore, parents may be feeding their nestlings a higher proportion (numbers or biomass) of high quality spiders than is reflected in prey abundance. In order to seek out these high quality spiders, parents may have to search over larger areas or farther from the nest than they would forage for themselves.

This idea is further supported by a possible explanation that as the season progresses, the areas immediately surrounding the central place (nest site) will have been exploited and will no longer be optimal foraging habitats. To best provide for their nestlings, the adults will have to take longer trips to farther patches within their territories. Additionally, it has been suggested that nestling provisioning changes according to the age and begging of the chicks (Grieco, 2002). Some studies have suggested that as nestlings, age, the size of prey delivered increases (Grieco, 2002). It has also been suggested that in order to obtain larger prey items, parents take longer foraging trips to farther distances within their territories (Grieco, 2002). Since all nestlings in this study were sampled between day 6 and fledging, it is likely that their feather mercury reflects dietary mercury obtained from larger prey items located on the periphery of each territory rather than in the immediate area. Therefore, their feather mercury levels would reflect mercury availability over a larger area while blood mercury levels of the parents may reflect mercury laden prey found more opportunistically near the nest site. These last two hypotheses would better explain the stronger correlation of adult mercury than nestling mercury with nest site distance from the river.
Table 20: Comparison of Predicted Distances at which Mercury Levels Return to Reference Levels and Regression Fits of Mercury Values (ppm) versus Distance (m) from the River for both Adult Blood and Brood Feathers.

<table>
<thead>
<tr>
<th>Species</th>
<th>Predicted Distances</th>
<th>Regression Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult Blood</td>
<td>Nestling Feather</td>
</tr>
<tr>
<td>CARW</td>
<td>250-275 m</td>
<td>250-275 m</td>
</tr>
<tr>
<td>CACH</td>
<td>325-350 m</td>
<td>275 m</td>
</tr>
<tr>
<td>HOWR</td>
<td>350 m</td>
<td>600 m</td>
</tr>
<tr>
<td>EABL</td>
<td>600-650 m</td>
<td>575 m</td>
</tr>
<tr>
<td>Combined</td>
<td>400 m</td>
<td>450 m</td>
</tr>
</tbody>
</table>

2.5.5.3. Small and Unequal Sample Sizes

The primary objectives of this work were to first decipher whether terrestrial birds sampled throughout the South River floodplain were being exposed to mercury originating in the river and then indicate a distance at which mercury levels were no longer above those of reference levels, on average. The first objective was clearly met with the conclusion that all individuals sampled on the South River had mercury levels well above the average reference level. The second objective was met by using visual extrapolation of results to determine a distance, for each species and for all species combined, at which mercury levels would not be elevated. However, for many of the species, the predicted distance was outside of the distance range from which samples were actually taken. Additionally, for all species, both adults and nestlings, more samples were from closer distances (< 100 m) than from farther distances (> 100 m). This was a result of the lack of nesting pairs using available boxes at farther distances and
reduces confidence in the regressions. In a subsequent analysis when regressions were recalculated separately for males and females, all cases resulted in better fit regressions predicting closer distances for females, with the exception of house wrens (See Appendix A). However, when the predicted distance is averaged across females and males of all adult species, the end result still predicts a distance of 405 m. Therefore, without further study I believe it is valid to suggest that terrestrial insectivorous birds can be exposed to mercury through their diet even when foraging in territories up to 400-450 m from the aquatic source of mercury.

Future work would greatly enhance this study if sample sizes of each species were kept consistent across the distance gradient; however, when working with free-living organisms, this is not feasible. Despite this flaw, using a field-based study to address these important questions provides much needed data about how mercury exposure varies in a natural floodplain food web rather than one constructed in a laboratory setting.

2.5.6. Spatial Variation of Exposure in Terrestrial Songbirds

A secondary objective of this project was to investigate the spatial variation of mercury exposure in insectivorous songbirds of the South River floodplain. This questions needs to be addressed to better understand how mercury moves through ecosystems. It is especially important in the case of the mercury-polluted South River because previous research has found that terrestrial species are exposed to mercury from the river (Cristol et al., 2008). However, to date, no research has investigated how mercury is transported from the aquatic system into the terrestrial food chain. My work initiates this long-term investigation and offers some basic information about areas where
mercury exposure is more prevalent and the processes that control the spatial variation of mercury.

2.5.6.1. Adults

A set of spatial variables was used to predict the mercury exposure of adult songbirds breeding along the South River. The initial principal component analysis reduced these variables to three components; the first describing the flooding potential of a territory, the second describing the distance from both the proximate (river shoreline) and ultimate (DuPont site) sources of mercury, and the third describing the amount of mercury available in the soil surrounding each nest site. The predicting power of each of these components was then assessed to determine which of them are most important in assessing risk of exposure.

The final model explained about 50% of the variation of mercury levels and included species, flooding potential (PC1) and distance measures (PC2) as factors. After examining the predicting strength of each factor, it was determined that species is extremely important in understanding exposure. Species encompasses the size, habitat, diet, foraging strategies and physiology of each individual. Since we know that the vast majority of exposure to mercury is through the diet (See section 1.2.6. Bioaccumulation & Biomagnification), it follows that blood mercury levels should differ significantly among species with different foraging behaviors (See section 2.2.4. Study Species).

The most compelling result was that the flooding potential of each bird’s territory was found to be significant in predicting mercury exposure. The trend of decreased blood mercury levels with decreased flooding potential was similar among all four species.
Carolina chickadees and eastern bluebirds had the strongest regression coefficients with PC1 while house wrens and Carolina wrens had slightly weaker regressions. However the interaction of PC1 and species was not significant so the differences between the species is likely due to the fact that not all species were sampled from the same locations and there may be a greater range of flooding potentials in certain species than others.

The flooding potential component (PC1) loaded positively for floodplain, relative elevation, distance and river kilometer while it loaded negatively for soil mercury. The results of the general linear model suggest that this component was the best predictor of adult blood mercury levels with mercury levels declining with increasing PC1 component scores. This strongly suggests that these variables are important in determining the risk of exposure of songbirds in the South River floodplain.

Additionally, the flooding potential component loaded most strongly for flood frequency of each nest site, based on floodplain delineations, and the relative elevation of the nest site area to the shoreline. Relative elevation was deemed an important variable because it distinguishes between points lying in the same floodplain. For example, two Carolina wrens may choose nest boxes within the 2-year floodplain zone, however, one territory may be a meter less in relative elevation than the other. Therefore, although the likelihood that each territory will flood is once every 2 years, the site with lower elevation will retain floodwaters for a longer time period and may also serve as a drainage point within the 2-year floodplain. Anoxic conditions are likely to increase methylation rates as described in Section 2.2.3.2. Determining Relative Elevation. Therefore, although both Carolina wren territories are equally likely to receive mercury transported through floodwaters, bioavailable methyl mercury is more likely to be
produced in the territory that retains water for a longer time period. Therefore, as was predicted in my original hypothesis, the frequency of flooding is negatively related to the relative elevation of each territory and these two variables combined are important predictors of hot spot areas for mercury exposure.

The distance measures component was also found to be an important predictor of blood mercury levels, although its effect size was substantially lower than that of the flooding potential component. All four species had increasing mercury levels with distance downstream from the factory and as the distance from the shoreline decreased. The regression coefficients for all species were generally weak but strongest for Carolina chickadees. This may once again be indicative of an unbalanced design with birds being sampled closer to the river at some sites, but only for some species. The overall result suggests that individuals of all species are more likely to be exposed if they chose territories farther down the contaminated section of the South River (river kilometer) and if they chose territories closer to the river (distance from the river). However, the loadings of this component also suggest that there is a strong interaction between river kilometer and distance from the river with more samples taken closer to the river at farther downstream sites (due to habitat constraints). Furthermore, the interaction of PC2 and species was significant, suggesting that the species differed in the distance gradients (either distance downstream or lateral distance) at which they were sampled. The added complexities of these two interactions make it difficult to disentangle the effect of river kilometer. Despite this, the effect of distance from the river is validated when each species is looked at separately as in the previous analysis determining the lateral extent of
mercury exposure (See section 2.5.5. *Lateral Extent of Mercury Exposure in Terrestrial Songbirds*).

In an effort to determine if river kilometer had an effect on the likelihood of mercury exposure, I looked at each species separately and found that there was not a significant effect on either Carolina wrens or Carolina chickadees; however, there was a significant effect on house wrens and eastern bluebirds. The trend was not consistent among the species with house wren and Carolina wren levels increasing with river kilometer and eastern bluebird levels decreasing at the farthest sites. In an effort to control for the effect of distance from the river, I re-analyzed each species separately but used only individuals sampled from territories within 50 meters of the river. This analysis resulted in river kilometer having a significant effect only on house wrens (See Appendix B for graphs). In order to truly understand the effect of river kilometer it would be necessary to control for other factors such as species and distance from the river. My results clearly show that mercury exposure is determined by distance from the proximate source of mercury, in this case the river, but the importance of distance from the ultimate source (DuPont factory) is ambiguous.

The third principal component, deemed the soil mercury availability component (PC3), was driven mostly by soil mercury availability in the territory of each breeding pair and was found to have a marginal effect size in the final general linear model. When the importance of this component was investigated it was found not to be a good indicator of adult mercury level. The regression coefficients for all species were weak, underscoring the lack of importance of PC3 as a predictor of adult mercury level. The trend was highly inconsistent with Carolina wrens and eastern bluebirds having lower
blood mercury levels as the availability of soil mercury in their territories increased. However, Carolina chickadees seemed to show an increase of blood mercury levels with the increase of soil mercury availability. House wren blood mercury levels had no relationship with soil total mercury availability. These results contradict the hypothesis that mercury exposure would increase with the increase of soil total mercury.

The soil mercury availability component loaded positively for soil mercury, relative elevation and distance from the river while it loaded negatively for flood frequency and river kilometer. This suggests that soil mercury increases as the distance from the river increases and the flooding potential decreases. Since flooding and distance should correlate with mercury in soil, I suggest the third component is composed of leftover variation not explained by the first two components but in fact, is not biologically interpretable or important.

In order to determine the true importance of soil mercury availability in predicting mercury levels I analyzed the relationship of blood mercury levels with the basic variable of soil mercury as it was produced using the methods described in 2.2.3.4. Determining Average Soil Mercury of Nest Box Territories. This analysis showed that there was a trend of increasing blood mercury levels with increasing soil mercury levels, however for no species was this trend significant. The soil mercury variable was composed of total mercury (THg) levels of surface soil samples. Future work attempting to understand the relationship between soil mercury availability and mercury exposure should look at the methyl mercury (meHg) content of the soil samples rather than total mercury as methyl mercury is the bioavailable form. Methyl mercury is most likely to enter into the terrestrial food web and be passed from prey to predator up the food chain.
2.5.6.2. Nestlings

The results of the general linear model investigating the spatial variation of
nestling exposure were very similar to those described for adults. The final model
explained 51.6% of the variation in brood feather mercury levels. Both the flooding
potential (PC1) and distance measures (PC2) components were significant in predicting
brood feather mercury levels. There was once again a significant interaction of PC2 with
species. However, akin to the adults, the soil mercury availability component (PC3) was
not significant and therefore not used in the final model.

The first component, deemed the flooding potential component, had the largest
effect size and explained much more of the variation in brood feather mercury than the
second component (distance measures). PC1 loaded positively for floodplain, relative
elevation, distance from the river and river kilometer while it loaded negatively for soil
mercury. The results suggest that brood mercury decreases as the flooding potential
decreases, distance from the river increases, distance downstream increases and soil
mercury decreases. However, like the adults, flooding potential loaded heavily for the
frequency of flooding and the relative elevation of each nest site, suggesting that these
two variables are most important in predicting which areas will accumulate mercury
originating from the South River. These variables are also important determinants of the
potential for methylation, which is directly related to exposure in biological organisms
(See Section 1.2.5. Methylation and Methyl Mercury).

When investigating the prediction power of flooding potential (PC1), I found that
eastern bluebird brood feather mercury had the strongest regression coefficient and
declined as flooding potential of the nest box area decreased. Carolina chickadees had
the next strongest coefficient followed by a weak house wren coefficient. Carolina wren broods had almost no linear relationship with flooding potential, which is likely due to the very small sample size of Carolina wren nestlings. Sample size is a problem throughout my nestling analysis.

The distance measures component (PC2) was also a good predictor of brood feather mercury levels, albeit a slightly weaker predictor accounting for 23% of the variation with a weak effect size. This relationship is likely mostly driven by the lateral distance variable rather than river kilometer. Once again there was a significant interaction of PC2 and species for Carolina wrens and Carolina chickadees increasing as the distance downstream increased and lateral distance from the river decreased. House wrens followed the same trend but had a much weaker relationship. Finally, eastern bluebirds seemed to have no relationship at all with a very weak regression coefficient. When river kilometer was investigated singly for each species, it was a significant factor only for eastern bluebirds. In the case of eastern bluebirds, river kilometer may be driving the relationship with PC2 rather than lateral distance from the river.

As discussed with the adults, the loadings of river kilometer and lateral distance on PC2 suggest that as river kilometer increases the lateral distance decreases. Therefore, it is likely that more nestlings were sampled closer to the river at farther downstream sites than at other closer downstream sites.

Finally, similar to the adult results, brood feather mercury was not well predicted by soil mercury availability in each territory (all $r^2 < 0.04$). However, unlike the adults, there was a similar trend among all four species when the data was visualized in Figure 31. For all four species, there seemed to be an increase of feather mercury with increased
soil mercury availability. The reason why this trend was more consistent for the nestling feathers than adult blood is not clear. It may be a spurious result related to the fact that fewer broods than individual adults were sampled over the course of the study.

2.5.6.3. Note on Use of Soil Mercury

Contradictory to my original hypotheses, the amount of total mercury in the soils of each pair’s territory was not a good predictor of whether or not individuals would be exposed to elevated levels of mercury. However, this result may be due to improper study design and the fact that soil sampling was not done as part of the bird study. Rather, soil sampling was part of a separate study and the application of the soil data to this study was opportunistic. Therefore the locations of soil samples were not always consistent with nest box locations. Additionally, in order to get an accurate measurement of soil mercury within the each territory, multiple soil samples would need to be taken from many points within the territory. I believe the abundance of soil samples used for this study were not adequate in generating accurate averages of soil mercury availability. Finally, since methyl mercury is the type likely to accumulate in the terrestrial food chain, the use of soil methyl mercury levels, rather than total mercury values, would have greatly improved the model.

2.6 General Conclusions

The main conclusions that this work offers regarding avian mercury exposure is that mercury is not sedentary but is capable of being transported hundreds of meters from the aquatic source into the adjacent terrestrial food chain. Contrary to previous beliefs, species directly or indirectly connected to the river because they are aquatic feeders or
forage along the shoreline are not the only species susceptible to aquatic mercury. Furthermore, exposure to aquatic mercury reaches far beyond the riparian corridor to individuals breeding throughout the floodplain. In future mercury risk assessments to determine which species and individuals are at risk to exposure, I would recommend including all avian species within a 400 meter stretch on either side of the contaminated water body. I believe that this still may be a conservative estimate as the potential for mercury exposure may be spread over a wider area, but including this 400 meter swath would protect many more species and to a higher degree than previous risk assessments without being infeasible for restoration efforts.

This study also offers some of the first information regarding the spatial variation of mercury. Specifically, my results offer evidence that flooding is the main vector by which mercury is transported from an aquatic source onto the floodplain where it becomes available for uptake by biological organisms. The fact that distance from the proximate and ultimate sources of mercury were significant factors in the final model predicting mercury exposure suggests that aquatic insect subsidies to the floodplain are also an important vector in transporting mercury across the aquatic-terrestrial interface. However, further study is needed to quantify the true importance of biological vectors in the transport of mercury. My results suggest that both flood regimes and biological vectors are important in the transport of mercury and are not mutually exclusive. Future studies should investigate the importance of biological vectors (i.e. emergent aquatic insects) to the food chain of terrestrial songbirds. Additionally, more study is needed to understand the spatial and temporal variation in peak emergences. Emergent insects may deliver mercury to the terrestrial food chain with immediate increases seen in riparian...
predators while floodwaters may transport mercury to floodplain soils where it is then incorporated into the terrestrial food chain over time, rather than in observable pulses. In conclusion I propose that both types of vectors are integral in understanding how terrestrial organisms can be exposed to aquatic mercury.
3. Chapter Three: Lateral Extent and Spatial Variation of Prey Mercury

Abstract

Prey items collected from four species of terrestrial insectivorous songbirds breeding in the floodplain of the mercury contaminated South River were used to investigate the spatial variability of mercury exposure. Mercury levels of individual prey orders differed significantly, although there were large amounts of variation within prey types. Principal component analysis suggests that flooding potential, and soil mercury availability to a lesser extent, best predict prey mercury levels. Spiders were analyzed separately because of their importance in understanding avian mercury. Wolf spiders (Araneae, Lycosidae) were collected from pitfall traps to determine the lateral extent of spider mercury. The wolf spiders did not exhibit a clear decline of mercury with distance from the contaminated river, suggesting that wolf spiders may range over larger territories than previously thought. Flooding potential was found to best describe the spatial variability of spider prey mercury, highlighting the importance of floodwaters as a physical vector of contaminants such as mercury.

3.1. Introduction

Previous work has shown that diet, specifically spiders, are the pathway by which terrestrial insectivorous songbirds obtain their mercury loads (Cristol et al., 2008). Therefore, in addressing the questions of spatial extent and variability of mercury exposure it is imperative to focus on spider prey items. Spiders are riparian predators and will feed opportunistically on invertebrates that are in abundance (Ahrens and Kraus, 2006). In particular, spiders are known to prey on adult aquatic insects that have
emerged from aquatic sources (Ahrens and Kraus, 2006). It has been suggested that the vast majority of aquatic insects will not travel far from the aquatic source from which they emerged and are more abundant closer to the water (Wittman, Ellis and Anderson, 2003). Spiders that feed closer to the river may incorporate more aquatic insects in their diet by directly feeding on them and therefore may increase their mercury load which they deliver to their avian insectivorous predators. In the previous chapter, we observed that songbird mercury levels do decline with distance from the contaminated river. We hypothesized that this decline of avian mercury with distance may be due to either a change in diet in which they fed on spiders with less mercury (because the spiders eat fewer aquatic insects) or that mercury was less likely to be deposited by flood waters with distance from the river. To better understand the mobility of mercury, the lateral extent of spider mercury was investigated. This was addressed by comparing mercury levels of spiders collected from pitfalls placed along a distance gradient extending laterally from the river.

Additionally, in order to examine the relative importance of flooding potential and selected spatial variables were examined for their importance in predicting mercury levels of prey items collected from four insectivorous bird species breeding along the South River. The avian portion of these research study found that flooding potential was the most important spatial factor in predicting avian mercury levels. Therefore, it was investigated in a model built to predict prey mercury and subsequently spider mercury.

By addressing the roles of flooding potential and distance (proximal and ultimate) in multiple matrices (birds, prey and spiders) I am able to better understand the route that
mercury takes to reach avian riparian predators as well as the mechanisms that work to transport mercury across the water-lane interface.

3.2. Methods

3.2.1. Spider Sampling with Pitfall Traps

In 2007, spiders were sampled from three sites (Wertman’s Private Property, Desportes’ Private Property and Grand Caverns Regional Park) using pitfall traps (See Fig. 1 for a Map of all Study Sites). Pitfall traps were placed in accessible areas along a distance gradient stretching 135 meters perpendicularly from the river at each site. The number of pitfall traps placed at each site was determined by the size of the site and whether permission was granted from the various landowners.

3.2.1.1. Pitfall Design and Placement

Each pitfall consisted of a 1 quart plastic Mix n’ Measure paint mixing container with a volume of 28 ounces (http://www.e-encore.com) purchased at Lowe’s Home Improvement. Pitfalls were placed 50 meters apart, on average; however the distance separating each pitfall was determined by microtopography (i.e. if a tree or rock was present). Small holes were dug in the ground and excess dirt removed so that each pitfall could be placed in the ground with its rim flush. Excess dirt removed from the hole was used to make the area around each pitfall flat. Care was used in not creating mounds around each pitfall that would deter spiders from approaching the trap.

Various studies have used different techniques to capture abundant numbers of specific invertebrates in pitfall traps. These techniques range from adding some kind of
killing solution to the bottom of each pitfall, using mesh netting and placing funnels in pitfalls to deter predators, attaching fences to guide certain invertebrates to the traps and an assortment of covers to prevent the collection of rainwater (Brennan et al., 2005; Schmidt et al., 2006; Baker and Barmuta, 2006). However, many of these studies were concerned with measuring abundance and eventually preservation of items. My use of pitfall traps was simply as a way to collect spiders at varying distances from the river for destructive mercury analysis. Therefore, I chose the simplest pitfall design with no added killing solution or predator deterrence as shown in Figure 32. I chose not to add any sort of killing solution because I was unsure of the effect any solution would have on the mercury content of each spider. Finally, trap covers were not used because pitfall traps were left open for a maximum of 24 hours and were used on days when rain was not in the forecast.

**Figure 32: Pitfall Trap**
3.2.1.2. Pitfall Distance Gradient

At the Wertman’s private property, a total of 12 pitfalls were placed approximately 10 meters apart allowing for a total distance range of 4-139 meters (135 m). Of these pitfalls, seven were placed in open field habitat while five were placed in an adjacent woodland area. There were a total of 8 pitfalls placed approximately 10 meters apart on the Desportes’ private property, placed in woodland habitat and stretching 23-103 meters (80 m) from the river. Finally, a total of 12 pitfalls were placed approximately 5 meters apart in woodland habitat of the Grand Caverns Regional Park, ranging from 37-102 meters (65 m) from the river.

3.2.1.3. Pitfall Sample Collection

Pitfall trapping took place during the summer season of 2007 with the first collection occurring on 5/23/07 and the final collection on 9/02/07. Pitfall traps were placed at each site once a week and left open for 24 hours. Collections took place the following day and all pitfalls were removed. Because the holes dug for each pitfall were maintained, each pitfall was placed in the same location throughout the season.

All spiders captured were collected from each location regardless of family or size. Additionally, a representative sample of all other items captured from each pitfall during each collection period was kept. All items were given a unique identification code using the site, date of collection, pitfall and item number and then stored in small glass vials (1-2 dram shell vials) on ice while in the field (Fig. 33). All glass vials from the same pitfall collection were additionally stored in a small Ziploc® bag with identification
information recorded on the outside to prevent loss of the sample if the vial was to break during transport. Each night after collection, each item was identified, weighed and stored in a -30°C freezer until future analysis.

Figure 33: Prey Collection

3.2.1.4. Spider Identification

An attempt was made to identify all spiders collected to family. Identification was made possible with a dissecting scope, insect and spider field guides and the use of online taxonomic keys and pictures (Nearctic Spider Database: http://www.canadianarachnology.org/data/spiders/, University of Kentucky Critter Files: http://www.uky.edu/Ag/CritterFiles/casefile/spiders/spiderfile.htm, http://bugguide.net/). A family designation was given only for samples where certainty of identification was
100%; all other items were only identified to order. Photographs were taken of at least one specimen of each type of spider to document correct spider identification. The eye pattern is the most distinguishable characteristic between spider families.

Spiders of the family Lycosidae were used in determining the lateral extent of mercury exposure because they are abundant along the South River and were found to make up a large percentage of spiders collected from insectivorous songbirds during a previous study (Cristol, unpublished data). The eye pattern of spiders in the family Lycosidae (wolf spiders) consists of 4 small frontal facing eyes in a horizontal line just above the chelicerae, two large frontal facing eyes above the group of 4 eyes, and 2 large eyes (same size as large frontal facing eyes) on the upside of the head that form a symmetrical square with the two large frontal facing eyes. It is this square arrangement of the up-facing and frontal facing pairs that sets wolf spiders apart from fishing spiders of the family Pisauridae which exhibit a “u” pattern rather than a square pattern. An example of the eye pattern associated with spiders of the family Lycosidae (wolf spiders) is shown in Figure 34.

**Figure 34: Rabid Wolf Spider Eye Pattern (Lycosidae rabidae)**
3.2.2. Prey Sampling

Prey items were collected from nestlings of all four of the study species (CARW, CAHC, HOWR and EABL) used in the avian part of this project (See section 2.3.4. Study Species). Essentially, provisioning adults were used as “insect collectors”. By using prey items delivered by the adults themselves, it is possible to make a comparison between the spatial variation of mercury exposure in adult and nestling birds and that of their prey items. An effort was made to collect prey items from nest boxes where both the adults and nestlings had been sampled for mercury. However, this was not always the case as there were instances where the nestlings of sampled adults did not survive long enough to be sampled for prey.

3.2.2.1. Ligature Method

The ligature method entails placing a constricting device around the neck of each nestling, which prevents them from swallowing food items while not inhibiting breathing (Johnson et al., 1980). Studies have shown that when ligatures are placed correctly, loose enough to allow breathing but tight enough to restrict swallowing, strangulation can be reduced to less than one percent (Mellot and Woods, 1993). The ligature method is advantageous for prey collection because many items can be collected at one time and the food items are usually intact, allowing for proper identification and size determination (Johnson et al., 1980). Generally, the ligature method is used as a way to assess diet. In these studies, the assumption that parent and nestling diets are similar is made (Friedman, 2007). There have been studies that corroborate this assumption (Gowaty and Plissner, 1988; Johnson, 1998; Cristol et al., 2008). However, my study was solely concerned
with the collection of prey items for mercury analysis and not in the accuracy of diet analysis.

### 3.2.2.2. Ligature Application and Collection

Small plastic 4-inch long cable ties were used as ligatures in this study because of their low cost and ease of application and removal. Ligatures were applied to all nestlings of a brood after their wing feathers had erupted but before their tail feathers were unsheathed. This time period corresponded to different ages among the four species but did standardize the developmental period. Using this time period reduced the risk of mortality to young nestlings (Johnson et al. 1980). Additionally, some studies have shown that smaller food items are more likely to slip past the ligatures in older nestlings (Johnson et al., 1980). By applying ligatures before the tail feathers became unsheathed, the risk of premature fledging of nestlings with ligatures attached was reduced. However, despite these precautions, I did have one brood of Carolina chickadee nestlings prematurely fledge during a ligature session, leading to their certain death. No cases of strangulation occurred during the study.

In order to apply ligatures, all nestlings of the brood were removed and placed temporarily in a bird bag. Nestlings were removed one at a time and fitted with a ligature and then replaced back in the nest. Ligatures were left in place for up to one hour at a time to ensure that parental feedings took place. During the first few ligature trials, I observed parental feedings from afar for the sampling hour and then removed all non-swallowed prey items and removed the ligatures. Ligatures were removed by carefully using a sharp edge to cut them. After I was confident of my abilities, I ran multiple
ligature trials at once, which did not allow for observation of all nests. For all ligature trials, un-swallowed food items were removed after the sampling hour rather than after each parental feeding. This minimized disturbance to feeding adults, ensuring that each trial resulted in the successful collection of prey items.

Prey items collected via the ligature method were removed from the nestlings’ mouth with the help of small forceps and placed in small, clean glass jars (1-2 dram shell vials). Additionally, as a precautionary measure, all vials from one ligature were placed in the same Ziploc® bag with a ligature trial number, date, species, and nest box written on the outside. These vials were then kept on ice for the duration of the day. Each night all prey items were removed, identified to order, weighed and placed in a -30°Celsius freezer until future analysis.

3.2.3. Sample Preparation

All invertebrate samples, collected via the pitfall and ligature methods, were kept frozen during all times except during final mercury analysis. Every sample was given a unique identifier to avoid loss of data. Pitfall samples were coded with the date, pitfall and item collected.

In preparation for mercury analysis, all samples were removed from the freezer, and freeze-dried using a Labconco © Benchtop Freeze Dry System. Samples were generally left on the freeze drier for 24-48 hours; however due to differing water contents, some samples were removed earlier or left longer. Following freeze-drying, all samples were re-weighed to obtain a dry weight. This allowed for calculation of the solid
fraction and fresh weight mercury content (See Appendix C for calculation). After dry weight was recorded, all samples were homogenized to a powder using a clean glass stirring rod. Finally, every sample was assigned another unique identifier referred to as the Direct Mercury Analyzer (DMA) number for ease of recording during mercury analysis.

### 3.2.4. Mercury Analysis

All total mercury values were obtained using a Direct Mercury Analyzer (DMA) at the College of William & Mary. For detailed information on total mercury analysis please refer to Section 2.3.8, *Sample Preparation and Mercury Analysis* in Chapter two.

#### 3.2.4.1. Duplicate Samples

As described in Section 2.3.8, *Sample Preparation and Mercury Analysis*, one duplicate sample was analyzed for every 20 samples. Duplicate samples of insects were true duplicates because they were taken from the same whole body homogenized powder. However, not all invertebrate samples were large enough to provide duplicates. The relative percent difference (RPD) between duplicates of all invertebrate samples (pitfalls and ligatures) above the minimum detection limit was calculated to be $20.63 \pm 29.80\%$ (calculated with 87 duplicates).

#### 3.2.4.2. Minimum Detection Limit

The factory-calculated minimum detection limit (MDL) of the William & Mary DMA was 0.0055 nanograms of mercury (ng Hg) (See Friedman, 2007 for more details on this calculation). Many methods have been put forth for dealing with samples that fall
below the minimum detection limit as described in Friedman (2007). Almost all methods have their associated pros and cons. However, for the purposes of this study, the actual readings were used for all samples that fell below the detection limit. The method of using the actual machine readings is described in Helsel (2005). The main rationale for this decision is that this study is primarily concerned with the variation of mercury levels between samples rather than the accuracy of mercury content. Although there is the potential for bias when using this method, the variation between samples is retained.

Of the 135 spiders collected via the pitfall method, none of those sampled for mercury (87 samples) fell below the MDL. However, 55 out of the 899 ligature samples (of all orders) analyzed for mercury fell below the MDL of 0.0055 ng Hg. Since this accounted for a small portion of all ligature samples (6.12%) the actual machine readings were used in all cases. Of the 231 ligature spiders analyzed for mercury, no samples fell below the minimum detection limit.

3.2.4.3. Methyl Mercury Analysis

A subsample of all ligature samples run for total mercury were additionally sent to Quicksilver Scientific (www.quicksilverscientific.com) for mercury speciation analysis using Liquid Chromatographic Mercury Speciation. This process separates and independently measures different forms of mercury (inorganic, methyl) in a sample in one simultaneous step (Quicksilver Scientific, 2009). Since this process reports both the inorganic Hg(II) and methyl mercury content, the total mercury content of each sample can be calculated as Hg(II) + meHg = Total Hg. Therefore, a check was run using
samples sent to both the William & Mary DMA and Quicksilver Scientific; the results of which can be seen in Appendix D.

3.2.5. Isotope Analysis

A subsample of 165 spiders was sent to two different labs for carbon and nitrogen isotope analysis. Eighty-eight samples were sent to the University of California Stable Isotope Laboratory (http://stableisotopelab.ucdavis.edu/) while seventy-seven samples were sent to the Colorado Plateau Stable Isotope Laboratory (http://www.mpcer.nau.edu/isotopelab/). Both labs use the same analytical process involving an elemental analyzer interfaced with a continuous flow Isotope Ratio Mass Spectrometer (IRMS). The machinery used by each laboratory is different but unfortunately, comparisons between the laboratories cannot be made because duplicates were not sent. Sample preparation for isotope analysis consists of freeze-drying and weighing out exact amounts of each type of sample as specified by each laboratory (Consult websites above for detailed information on sample preparation). All samples are then packaged in tin capsules and sent to the appropriate laboratory. The results provided by each lab include the delta (δ) values of the heavier to lighter isotope for both carbon (δ^{13}C) and nitrogen (δ^{15}N). These delta values are expressed as relative to PeeDee Belemnite for Carbon and air for Nitrogen.

3.2.6. Geographic Information Systems (GIS)

Every spider collected from a pitfall trap was assigned a distance from the river according to the lateral distance of each pitfall from the shoreline of the river. The locations of each pitfall and the subsequent determination of distance from the river was
calculated using the same methods described in Section 2.3.3.1 *Determining Distance from the River*. Site maps displaying the pitfall locations are shown in Figure 35.

Prey samples were collected from active nests along the same nest box trail used for the avian study. For more specific information regarding the nest box trail, refer to Section 2.3.2. *Nest Box Trail*. Additionally, GIS was used to determine the spatial variables used in the analysis (Distance from the River, Relative Elevation, Floodplain Designation, Average Soil Mercury). See Section 2.3.3. *Use of Geographic Information Systems* for detailed information on how each of these variables was calculated.
Figure 35: Site Maps Depicting Pitfall Locations

Wertman's Property

Desportes' Property

Grand Caverns Regional Park
3.3. Statistical Analysis

All statistical analyses were conducted using PASW Statistics 17.0 from SPSS Incorporation. All data was examined for deviance from normality and transformed as needed.

3.3.1. Tests of Normality and Data Transformations

Prior to the assessment of data with principal component analysis and general linear models, tests of normality were run to determine deviance from a normal distribution.

3.3.1.1. Pitfall Spider Data

The initial test of normality including dry weight mercury values only from Lycosid spiders revealed that the data were not normally distributed (Shapiro-Wilk Statistic=0.950, df=43, p=0.011). Additionally, the data had a positive kurtosis (2.941). Simple Log(10) transformation was performed to normalize these data before it was used in the general linear model assessing the variables of distance from the river and size (kurtosis 0.607, Shapiro-Wilk Statistic=0.956, df=43, p=0.099).

3.3.1.2. Ligature Prey Data

When the normality of mercury levels for ligature prey items (orders with sample sizes less than 5 and unknowns removed) was assessed, the data were found to be non-normal, right-skewed and have positive kurtosis (skewness=7.127, kurtosis=71.022, Shapiro-Wilk Statistic=0.352, df=898, p<0.000). The outliers were determined for each
prey order separately and removed. An outlier was determined to be a value that deviated from the mean by three or more standard deviations. After removal of the outliers, all mercury levels were log(10) transformed and normality was reassessed \( (\text{skewness}=-1.740, \text{kurtosis}=3.516, \text{Shapiro-Wilk Statistic}=0.826, \text{df} = 805, p<0.000) \). True normality was not obtained but the data fit the assumptions of normality significantly better and parametric tests were used.

### 3.3.1.3. Spider Prey Data

Spiders collected via the ligature method were analyzed separately and were therefore tested for normality separately. Initial tests of non-transformed data revealed that the data were right-skewed and with positive kurtosis and did not conform to the expectations of normality \( (\text{skewness}=2.425, \text{kurtosis}=7.725, \text{Shapiro-Wilk Statistic}=0.748, \text{df}=228, p<0.000) \). Log(10) transformation was applied to the spider data and this normalized the data \( (\text{skewness}=0.308, \text{kurtosis}=0.177, \text{Shapiro-Wilk Statistic}=0.989, \text{df}=228, p = 0.091) \).

### 3.3.2. Statistical Analysis of Pitfall Spiders

A total of 135 spiders were collected from pitfalls in order to assess the lateral extent of mercury exposure in spiders of the South River floodplain. Every spider was considered an independent sample in this case, even if they were sampled from the same pitfall, because each spider forages independently. In an effort to standardize the type of spider, only Lycosid spiders (wolf spiders) were used, reducing the sample size to 65. Linear regression analysis was used to assess whether there is a significant decline of
spider mercury level (dependent variable) with distance from the river (independent variable).

Additionally, an attempt was made to predict the distance at which spider levels would decline to those collected from reference sites. In order to accomplish this, the average mercury level of 21 reference spiders was calculated as 0.07 ppm dry weight and used as a reference line in the regression graphs and visual extrapolation was used to predict the distance at which samples returned to baseline level.

3.3.2.1. Treatment of Year and Seasonal Effects

All pitfall spiders were collected during the 2007 season from 5/23/07-9/02/07. To determine whether there were seasonal effects on Lycosid spider mercury level I used regression analysis. The results suggest that there was no effect of date collected (F=1.332 (1, 63), p=0.253, r²=0.021). This is depicted in Figure 36 below.
3.3.2.2. Treatment of Size Effects

Because size has been shown to affect mercury level in various species (Storelli et al., 2007; McIntyre and Beauchamp, 2007), I looked at the effect of size on Lycosid spiders collected from pitfalls. I found that fresh weight (weight at time of collection prior to freezing) did not have a significant effect on mercury levels ($F=0.934_{(1,653)}$, $p=0.38$, $r^2=0.015$). However, there is a trend of increasing mercury level with increasing size of spider as depicted in Figure 37.
Figure 37: Effect of Fresh Weight on Spider Mercury Level

3.3.2.3. Treatment of Site and Habitat Effects on Mercury Level

Since pitfalls were placed in both open fields and woodland areas at the Wertman’s private property site, it was possible to assess the effect of habitat. A total of 21 spiders were collected from the Wertman site from pitfalls placed in open fields and 16 were collected from pitfalls in woodland habitat. The effect of habitat was significant (F=6.487, p=0.016, r^2=0.173) with spiders collected from woodlands having a higher average (1.93 ± 1.21 ppm) than spiders collected from open fields (1.15 ± 0.76 ppm). This trend is shown in Figure 38. Since there was a significant difference in mean spider
mercury from each habitat type, spiders collected from woods were excluded in final statistical analyses. This further reduced the sample size of pitfall spiders to $n=43$.

**Figure 38: Effect of Habitat on Spider Mercury Level**

Using only spiders collected from woodland areas, the effect of site was assessed. This included spiders collected from three different sites which vary by river kilometer downstream from the contamination source (DESP, km=22.5, $n=15$; GRCA, km=31.4, $n=16$; WERT, km=14.5, $n=12$). There was not a significant difference in mercury level between the sites ($F=1.319_{(2, 43)}$, $p=0.215$, $r^2=0.062$) as depicted in Figure 39 and spiders from all sites were combined in statistical analyses.
3.3.3. Statistical Analysis of Ligature Samples

A total of 899 ligature samples were collected over the 2007 and 2008 seasons and analyzed for total mercury. After the exclusion of outliers, orders with sample sizes less than five items, and unidentified items, the total number of samples was reduced to 854. Every invertebrate collected was considered an independent sample in all further analyses. In order to understand the variation in mercury levels the following effects were examined.

3.3.3.1. Treatment of Year and Seasonal Effects

Ligature samples were collected from all four avian study species throughout both the 2007 and 2008 season. When all prey orders were combined, there was not a
significant difference between the years (F=0.05(1, 803), p=0.944, r²=0.0) (see Fig. 40). Additionally, when each prey order was examined individually, there were no significant effects of year (all p>0.05).

Figure 40: Mercury Level of all Ligature Samples by Year

The date of collection (Julian day) was also examined for each season individually. For samples collected in 2007, Julian day was significant (F=9.105(1, 508), p=0.003, r²=0.13). In 2008, Julian day was nearly significant (F=0.403(1, 291), p=0.526, r²=0.037). However, when each prey order was examined separately for each season, Julian day only proved to be significant for Coleoptera in 2007 (n=30, F=10.081(1, 30),
p=0.004, \( r^2 = 0.508; 2008 \), Lepidoptera adults in 2008 (\( n=21 \), \( F=4.496_{(1, 21)} \), \( p=0.046 \), 
\( r^2 = 0.420 \)) and Lepidoptera larvae collected in both seasons (2007; \( n=161 \), \( F=15.636_{(1, 161)} \), 
\( p<0.000 \), \( r^2 = 0.298 \); 2008, \( n=105 \), \( F=11.143_{(1, 105)} \), \( p=0.001 \), \( r^2 = 0.310 \)). Julian day did not
have a significant effect on any other prey order, however there are some trends within
prey orders evident in Figure 41(a-b). Both Araneae and Opiliones samples show a slight
increase of mercury levels later in the season while both Coleoptera and Opisthophora
(earthworms) and Lepidoptera seem to have highest mercury levels in the middle of each
season. Despite these possible trends, there is no consistency between prey types;
therefore it cannot be assumed that mercury availability varies in a predictable manner
throughout the summer seasons.
Figure 41: Trends of Prey Mercury with Date of Collection (Julian day)

(a)

(b)
It is important for later analyses to note that year and Julian day were not significant factors in predicting spider mercury level (Year, \(F=1.043_{(1, 229)}, p=0.612\); Julian day, 2007, \(F=0.259_{(1, 129)}, p=0.612, r^2=0.043\); 2008 \(F=0.002_{(1, 87)}, p=0.962, r^2=0.005\)).

### 3.3.3.2. Treatment of Size Effects on Mercury Level

The effect of size on all prey orders of ligature samples was difficult to detect as many other variables are involved in predicting mercury level, including location and prey type. Univariate analyses revealed that when all prey orders were combined, there was no significant effect of size or fresh weight (\(F=01.007_{(1, 784)}, p=0.316, r^2=0.036\)). These results are depicted in Figure 42. However, when each prey order was analyzed separately, size did prove to have a significant effect on samples of Coleoptera (\(F=5.689_{(1, 37)}, p=0.022, r^2=0.365\)), Lepidopteran adults (\(F=10.079_{(1, 52)}, p=0.003, r^2=0.403\)), and Orthoptera (\(F=11.777_{(1, 127)}, p=0.001, r^2=0.292\)). When just these three prey orders were examined, the trends were not consistent with mercury level rising with size in Coleoptera and decreasing with size in both Lepidopteran adults and Orthoptera (See Fig. 43). Therefore, the effect of size could not be disentangled from other variables and is not included in the final model, although this may result in unaccounted for variation.
Figure 42: Effect of Fresh Weight on Ligature Mercury Level
Figure 43: Effect of Fresh Weight on Mercury Level of (a) Coleoptera, (b) Lepidopteran adults and (c) Orthoptera

(a) Coleoptera

(b) Lepidoptera

(c) Orthoptera
3.3.3.3. **Relationship of Delta Nitrogen-15 and Mercury and Size**

Almost all spiders collected via the ligature method were analyzed for the ratio of heavy to light isotope of both carbon and nitrogen as a way to distinguish between foraging strategies of spiders. For the purposes of this study, delta nitrogen-15 ($\delta^{15}N$) was examined because literature suggests that mercury biomagnifies in organisms of higher trophic levels which are distinguished by $\delta^{15}N$ (See section 1.2.6.). Additionally, some studies have suggested that $\delta^{15}N$ and size may co-vary in that $\delta^{15}N$ increases with size (See section 1.2.6.). In the final general linear model built to explain variation in mercury levels of spiders a main factor, such as size or delta nitrogen-15, was needed to better capture variation due to spider trophic position. To choose the better main factor between size and delta nitrogen-15 I investigated whether they are correlated to each other and how well they explained mercury levels independently.

Size or fresh weight did not predictably explain spider mercury levels ($F=0.070_{(1, 218)}$, $p=0.792$, $r^2=0.18$). However, delta nitrogen-15 ($\delta^{15}N$) does predictable correlate with total mercury levels ($F=10.327_{(1, 161)}$, $p=0.002$, $r^2=0.246$) (See Fig. 44). Mercury levels increase with increasing values of $\delta^{15}N$ as would be expected from the literature. This relationship is even stronger when only the methyl mercury fraction of total mercury values is used ($F=8.773_{(1, 52)}$, $p=0.005$, $r^2=0.380$).

Additionally, the correlation of size and delta nitrogen-15 was examined to determine if they were independent variables. The Pearson’s correlation was determined to be 0.203 and the covariance 0.052. This suggests that these two variables are indeed
independent of each other. These results provided the basis of my decision to include delta nitrogen-15 but not size in the final general linear model for spider prey.

**Figure 44: Relationship of Delta Nitrogen-15 on Spider Mercury Level**

3.3.3.4. **Treatment of Habitat Effects on Ligature Mercury Level**

The habitat type associated with each nest box was also assigned to each prey item collected from that nest box. This assumes that adults are foraging in similar habitat of that from which the nest box is located, which may not always be an accurate assumption. However, this assumption was useful in determining if habitat type was having a significant effect on prey mercury level. When each prey order was analyzed separately, habitat type did have a significant effect on Lepidiopteran larvae (fields n=143, woods n=127; F=15.587(1,268), p<0.000, r²=0.056), Opiliones (fields n=4, woods
n=11; F=5.158(1, 13), p=0.041, r^2=0.284) and Orthoptera (fields n=86, woods n=46; 
F=9.553(1, 132), p=0.002, r^2=0.068). However, habitat type did not have a significant 
effect on any of the other prey orders, though in general samples collected from woody 
areas had higher mercury levels than those collected from open fields (See Fig. 45). 
Despite this apparent trend, there were only ample sample sizes in each habitat type to 
adequately test for differences in Lepidopteran larvae and Orthoptera, which appeared to 
have opposite trends. For Lepidopterans, those collected from fields had a lower mean 
(n=127, 0.04 ± 0.11 ppm) than those collected from woodland habitat (n=143, 0.08 ± 
0.19 ppm). Orthopteran samples had the opposite trend with those collected from open 
fields having a higher mean mercury level (n=86, 0.07 ± 0.12 ppm) than those collected 
from woodland areas (n=45, 0.19 ± 0.30 ppm).

It should be noted that even with adequate sample sizes for all spiders collected 
via ligature, habitat type was still not found to be a significant factor in predicting 
mercury level, however similar to the pitfall results, spiders collected from woody areas 
had a higher average mercury level than those collected from open fields (Field n=151 
mean=0.96 ± 1.71 ppm, Wood n=79 mean=1.29 ± 1.29 ppm; F=2.243(1, 230), p=0.136).
3.3.3.5. Differences in Mercury Level Among Prey Orders

Prey order was a significant factor and therefore was included in all analyses 
\(F=56.963 (1, 803), p<0.000, r^2=0.393\). Table 21 lists the sample sizes and mean dry weight mercury levels for all prey orders collected via the ligature method. Figure 46 depicts the large variation in mercury level between prey orders as well as within prey orders. The prey orders are organized in order of decreasing mean mercury level.
Table 21: Sample Sizes of Mean Dry Weight Total Mercury for Prey Orders

<table>
<thead>
<tr>
<th>Prey Order/Class</th>
<th>Mean Total Mercury Level (Dry Weight ppm)</th>
<th>Sample Size (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARANEAE</td>
<td>1.07 ± 1.58</td>
<td>230</td>
</tr>
<tr>
<td>BLATTARIA</td>
<td>0.31</td>
<td>1</td>
</tr>
<tr>
<td>COLEOPTERA</td>
<td>3.88 ± 6.77</td>
<td>42</td>
</tr>
<tr>
<td>COLEOPTERA LARVAE</td>
<td>2.20 ± 2.45</td>
<td>4</td>
</tr>
<tr>
<td>CLASS DIPLOPODA</td>
<td>0.17 ± 0.11</td>
<td>5</td>
</tr>
<tr>
<td>DIPTERA</td>
<td>1.69 ± 2.77</td>
<td>3</td>
</tr>
<tr>
<td>EPHEMEROPTERA</td>
<td>1.10</td>
<td>1</td>
</tr>
<tr>
<td>HEMIPTERA</td>
<td>0.87 ± 0.40</td>
<td>2</td>
</tr>
<tr>
<td>HOMOPTERA</td>
<td>0.22</td>
<td>1</td>
</tr>
<tr>
<td>HYMENOPTERA</td>
<td>0.10 ± 0.13</td>
<td>21</td>
</tr>
<tr>
<td>ISOPODA</td>
<td>0.99 ± 0.99</td>
<td>8</td>
</tr>
<tr>
<td>LEPIDOPTERA ADULT</td>
<td>0.07 ± 0.20</td>
<td>56</td>
</tr>
<tr>
<td>LEPIDOPTERA LARVAE</td>
<td>0.14 ± 0.74</td>
<td>274</td>
</tr>
<tr>
<td>LEPIDOPTERA PUPAE</td>
<td>0.04 ± 0.03</td>
<td>4</td>
</tr>
<tr>
<td>MOLLUSK</td>
<td>0.68 ± 0.95</td>
<td>2</td>
</tr>
<tr>
<td>Odonata</td>
<td>1.47 ± 1.32</td>
<td>2</td>
</tr>
<tr>
<td>Opilione</td>
<td>1.04 ± 2.09</td>
<td>16</td>
</tr>
<tr>
<td>OPISTHOPORA</td>
<td>1.90 ± 1.90</td>
<td>32</td>
</tr>
<tr>
<td>ORTHOPTERA</td>
<td>0.18 ± 0.57</td>
<td>134</td>
</tr>
<tr>
<td>UNKNOWN PUPAE</td>
<td>0.46 ± 1.11</td>
<td>6</td>
</tr>
<tr>
<td>Seed</td>
<td>0.01</td>
<td>1</td>
</tr>
<tr>
<td>UNIDENTIFIED ITEMS</td>
<td>1.60 ± 3.00</td>
<td>51</td>
</tr>
<tr>
<td>Total</td>
<td>0.75 ± 2.12</td>
<td>898</td>
</tr>
</tbody>
</table>
3.3.3.5.1. Difference in Methyl Mercury Content of Prey Orders

A subsample of the most highly represented prey orders were also analyzed for methyl mercury (meHg) content. Figure 47 depicts the variation in dry weight methyl mercury (ppm) among the prey orders analyzed ($F=3.879_{(1, 129)}$, $p=0.001$, $r^2=0.153$). Additionally, Figure 48 shows the mean percent methyl mercury content of each prey order. Percent methyl mercury was calculated as dry weight meHg ppm/dry weight total...
mercury (THg) ppm for each prey item and the mean was calculated for the entire prey order.

Table 22: Sample Sizes of each Prey Order Analyzed for Methyl Mercury Content

<table>
<thead>
<tr>
<th>Prey Order</th>
<th>Mean Methyl Mercury Level (Dry Weight ppm)</th>
<th>Mean Percent Methyl Mercury</th>
<th>Sample Size (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARAN</td>
<td>0.59 ± 0.80</td>
<td>0.45 ± 0.23</td>
<td>57</td>
</tr>
<tr>
<td>OPIL</td>
<td>0.36 ± 0.34</td>
<td>0.53 ± 0.34</td>
<td>5</td>
</tr>
<tr>
<td>COLE</td>
<td>0.08 ± 0.10</td>
<td>0.05 ± 0.07</td>
<td>17</td>
</tr>
<tr>
<td>OPIS</td>
<td>0.06 ± 0.08</td>
<td>0.04 ± 0.04</td>
<td>11</td>
</tr>
<tr>
<td>ORTH</td>
<td>0.06 ± 0.18</td>
<td>0.29 ± 0.28</td>
<td>26</td>
</tr>
<tr>
<td>LEPL</td>
<td>0.01 ± 0.01</td>
<td>0.24 ± 0.22</td>
<td>13</td>
</tr>
<tr>
<td>LEPA</td>
<td>0.004 ± 0.002</td>
<td>0.49 ± 0.40</td>
<td>11</td>
</tr>
</tbody>
</table>

Figure 47: Methyl Mercury Content of Prey Orders
3.3.4. Principal Component Analysis Using All Ligature Prey Items

A suite of spatial factors was used as covariates in a general linear model in order to determine their importance in predicting mercury levels. The individual variables used in the model for all ligature items included distance downstream from the ultimate source of mercury (river kilometer), flooding frequency (floodplain), relative elevation, distance from shoreline, and soil mercury availability within nest box territory. All spatial factors measured were significantly correlated with each other as is shown by their Pearson correlation coefficients and associated p-values in Table 23. To resolve this issue, principal component analysis was conducted using all the aforementioned spatial factors.
(soil mercury was converted to log(10) scale). The number of components used in the final general linear model depended on the number of components needed to explain approximately 80% of the variance. These components did not necessarily all have Eigen values greater than the arbitrary cutoff of 1.0. No rotation was used in the principal component analysis.

Table 23: Correlation Coefficients and Significance of Spatial Variables included in PC Analysis of Ligature Prey Samples (significance at $\alpha=0.05$)

<table>
<thead>
<tr>
<th>Correlation Coefficient Matrix</th>
<th>Flood Frequency</th>
<th>Rel. Elevation (m)</th>
<th>Distance (m)</th>
<th>River Kilometer (km)</th>
<th>Log(10) Soil Hg (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flood Frequency</td>
<td>NA</td>
<td>0.551</td>
<td>0.203</td>
<td>0.292</td>
<td>-0.146</td>
</tr>
<tr>
<td>Rel. Elevation (m)</td>
<td>0.551</td>
<td>NA</td>
<td>0.372</td>
<td>0.242</td>
<td>-0.381</td>
</tr>
<tr>
<td>Distance from River (m)</td>
<td>0.203</td>
<td>0.372</td>
<td>NA</td>
<td>-0.204</td>
<td>-0.248</td>
</tr>
<tr>
<td>River Kilometer (km)</td>
<td>0.292</td>
<td>0.242</td>
<td>-0.204</td>
<td>NA</td>
<td>-0.043</td>
</tr>
<tr>
<td>(log10) Soil Hg (ppm)</td>
<td>-0.146</td>
<td>-0.381</td>
<td>-0.248</td>
<td>-0.043</td>
<td>NA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Significance (1-tailed) $\alpha = 0.05$</th>
<th>Flood Frequency</th>
<th>Rel. Elevation (m)</th>
<th>Distance (m)</th>
<th>River Kilometer (km)</th>
<th>Log(10) Soil Hg (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flood Frequency</td>
<td>NA</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Rel. Elevation (m)</td>
<td>0.000</td>
<td>NA</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Distance from River (m)</td>
<td>0.000</td>
<td>0.000</td>
<td>NA</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>River Kilometer (km)</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>NA</td>
<td>0.106</td>
</tr>
<tr>
<td>(log10) Soil Hg (ppm)</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.106</td>
<td>NA</td>
</tr>
</tbody>
</table>

3.3.5. General Linear Model Analysis Using All Ligature Prey Items

A general linear model was built using the principal components described above as well as prey order. Prey order was used as a main factor in the model because it was significantly important in predicting mercury levels of collected prey items (See section 3.4.3.5. Differences in Mercury Among Prey Orders).
3.3.6. Principal Component Analysis Using Ligature Spiders

The suite of spatial factors used in the factor analysis of all ligature prey items was again used in the analysis of spider prey. Spiders were analyzed separately because of their importance in delivering mercury to avian insectivores (Cristol et al., 2008). Due to the correlation of many of these spatial variables (See Table 24), principal component analysis was again used to produce uncorrelated components that explain the most amount of variation in the data.

Table 24: Correlation Coefficients of Spatial Variables included in PC Analysis of Spider Prey Samples (all cases significant at $\alpha=0.05$)

<table>
<thead>
<tr>
<th>Correlation Coefficients</th>
<th>Flood Frequency</th>
<th>Relative Elevation</th>
<th>Distance from River</th>
<th>River Kilometer</th>
<th>(log10) Soil Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flood Frequency</td>
<td>NA</td>
<td>0.470</td>
<td>0.130</td>
<td>.292</td>
<td>-0.219</td>
</tr>
<tr>
<td>Relative Elevation (m)</td>
<td>0.470</td>
<td>NA</td>
<td>0.259</td>
<td>.242</td>
<td>-0.376</td>
</tr>
<tr>
<td>Distance from River (m)</td>
<td>0.130</td>
<td>0.259</td>
<td>NA</td>
<td>-.204</td>
<td>-0.136</td>
</tr>
<tr>
<td>River Kilometer (km)</td>
<td>0.255</td>
<td>0.302</td>
<td>-0.262</td>
<td>NA</td>
<td>-0.164</td>
</tr>
<tr>
<td>(log10) Soil Hg (ppm)</td>
<td>-0.219</td>
<td>-0.376</td>
<td>-0.136</td>
<td>-.043</td>
<td>NA</td>
</tr>
</tbody>
</table>

3.3.7. General Linear Model Analysis Using Ligature Spiders

The general linear model used to predict mercury levels in ligature spiders contained delta nitrogen-15 ($\delta^{15}$N) as a main factor because it is a proxy for trophic position or length of food chain and therefore can distinguish between spiders with different foraging strategies. Additionally, $\delta^{15}$N has been found to be correlated with size in other studies (Cabana and Rasmussen, 1994), although I did not find a high degree of
correlation in my data; therefore, $\delta^{15}N$ may be useful in distinguishing between spiders of different size as well as various foraging strategies.

3.4. Results

3.4.1. Lateral Extent of Mercury Exposure in Wolf Spiders

In order to assess whether spider mercury levels declined with distance from the contaminated river, I used simple regression analysis to examine the predictive power of distance from the river. Log-transformed mercury levels of Lycosid spiders collected only from pitfalls placed in open field habitat were used in this analysis. The results of this model suggest that distance was not important in predicting spider mercury ($F=0.032, (1, 33), p=0.858, r^2=0.001$). The lack of decline of mercury values with distance is clearly depicted in Figure 49 which provides the regression results of spider mercury level versus pitfall distance from the river ($r^2=0.001$). This result was only slightly improved when all Lycosid spiders were combined regardless of habitat type ($n=65, r^2=0.0004$). Since no decline with distance was observed, it was not possible to determine a distance at which spider mercury level decline to reference levels using this method.
3.4.2. Spatial Variation of Mercury Exposure in Terrestrial Prey

3.4.2.1. Results of Principal Component Analysis

The results of the principal component analysis yielded three components that explained, cumulatively, 82.15% of the variance (See Table 25). The first two extracted components had Eigen values greater than 1.0 (2.04 and 1.26 respectively). The third extracted component had an Eigen value of 0.81 but explained more than 16.25% of the variance in my data so is included here (See Table 26).

The first component (PC1) accounts for 40.69% of the variance in my data and loaded most heavily for relative elevation (0.874) and flood frequency or floodplain zone (0.744), but also for distance from the river (0.531) and soil mercury (-0.568). Taking
into account the signs of each loading, the first component can be interpreted as the flooding potential because a case with a high PC1 score would be at a high elevation and far from the river, with infrequent flooding and low soil mercury. The second component (PC2) explained 25.20% of the variance and loaded most heavily for each of my distance variables, river kilometer (0.815) and distance from the river (-0.653). PC2 can be interpreted as the distance component. Finally, the third component (PC3) explained the least of my variance (16.25%) but still had an Eigen value close to the rule-of-thumb 1.0 cutoff (0.81). PC3 loaded heavily only for soil mercury (0.745) and is interpreted as the soil mercury component.

Table 25: Total Variance Explained by Extracted Principal Components with Associated Eigenvalues

<table>
<thead>
<tr>
<th>Component</th>
<th>Total Variance Explained by Components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eigenvalue</td>
</tr>
<tr>
<td>1</td>
<td>2.035</td>
</tr>
<tr>
<td>2</td>
<td>1.260</td>
</tr>
<tr>
<td>3</td>
<td>0.813</td>
</tr>
<tr>
<td>4</td>
<td>0.519</td>
</tr>
<tr>
<td>5</td>
<td>0.373</td>
</tr>
</tbody>
</table>

* Bold Components Extracted
Table 26: Loadings of Extracted Principal Components

<table>
<thead>
<tr>
<th>Component Number and Interpretation</th>
<th>PC1: Flooding Potential</th>
<th>PC2: Distance Measures</th>
<th>PC3: Soil Mercury Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floodplain Designation</td>
<td>0.744</td>
<td>0.293</td>
<td>0.377</td>
</tr>
<tr>
<td>Relative Elevation (m)</td>
<td>0.874</td>
<td>0.024</td>
<td>0.053</td>
</tr>
<tr>
<td>Distance from River (m)</td>
<td>0.531</td>
<td>-0.653</td>
<td>0.289</td>
</tr>
<tr>
<td>River Kilometer (km)</td>
<td>0.334</td>
<td>0.815</td>
<td>-0.170</td>
</tr>
<tr>
<td>(Log10) Soil Hg (ppm)</td>
<td>-0.568</td>
<td>0.289</td>
<td>0.745</td>
</tr>
</tbody>
</table>

3.4.2.2. Results of General Linear Model

A general linear model was used to identify the significance of each principal component in explaining prey mercury levels. Prey order was included as a main factor in the model as mercury levels vary significantly between prey order (See Section 3.4.3.5. Differences in Mercury Level Among Prey Orders). A total of 788 individual cases containing mercury values and component scores for all three components were used in the model after outliers were deleted from the dataset.

The initial model included prey order as a fixed factor and PC1, PC2, PC3 as covariates. Prey order was a significant predictor of prey mercury level ($F=53.541_{(9,788)}$ and $p<0.000$). Flooding potential (PC1) was also a significant factor in predicting prey mercury levels ($F=14.826_{(1,788)}$, $p<0.000$). However, neither PC2 nor PC3 were significant predictors and were removed from the final model ($PC2 F=0.212_{(1,788)}$, $p=0.646$; $PC3 F=1.626_{(1,788)}$, $p=0.203$) (See Fig. 51 and 52).

The final model included species as a main factor and PC1 as a covariate and accounted for approximately 40% of the variation in prey mercury levels ($r^2=0.403$). In
this model, prey order had the largest partial Eta effect size of 0.385 while that of PC1 was 0.019. Although the flooding potential component proved to be significant in the overall model when its predictive power was examined in regression analysis, it only explained approximately 1.4% of the variation in prey mercury ($r^2=0.014$; See Fig. 50).

**Figure 50: Flooding Potential (PC1) as a Predictor of Prey Mercury**
Figure 51: Distance Measures (PC2) as a Predictor of Prey Mercury
Figure 52: Soil Mercury Availability (PC3) as a Predictor of Prey Mercury
3.4.3. Spatial Variation of Mercury Exposure in Terrestrial Spider Prey

3.4.3.1. Results of Principal Component Analysis

The results of the principal component analysis yielded three components that explained, cumulatively, 80.02% of the variance (See Table 27). The first two extracted components had Eigen values greater than 1.0 (1.96 and 1.26 respectively). The third extracted component had an Eigen value of 0.789 but explained 15.79% of the variance in my data so is included here (See Table 28).

The first component (PCI) accounts for 39.04% of the variance in my data and loaded most heavily for relative elevation (0.840) and flood frequency or floodplain zone (0.729), but also for distance from the river (0.282) and soil mercury (-0.625). Taking into account the signs of each loading, the first component can be interpreted as the flooding potential because a case with a high PC1 score would be at a high elevation and far from the river, with infrequent flooding and low soil mercury. The second component (PC2) explained 25.20% of the variance and loaded most heavily for each of my distance variables, river kilometer (-0.719) and distance from the river (0.850). PC2 can be interpreted as the distance component. Finally, the third component (PC3) explained the least of my variance (15.79%) but still had an Eigen value close to the rule-of-thumb 1.0 cutoff (0.789). PC3 loaded heavily only for soil mercury (0.738) and is interpreted as the soil mercury availability component. However, it also loaded for floodplain zone (0.472) suggesting that soil mercury increases as the floodplain designation increases; however, this is not biologically interpretable.
Table 27: Total Variance Explained by Extracted Principal Components with Associated Eigenvalues

<table>
<thead>
<tr>
<th>Component</th>
<th>Total Variance Explained by Components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eigenvalue</td>
</tr>
<tr>
<td>1</td>
<td>1.95</td>
</tr>
<tr>
<td>2</td>
<td>1.26</td>
</tr>
<tr>
<td>3</td>
<td>0.79</td>
</tr>
<tr>
<td>4</td>
<td>0.57</td>
</tr>
<tr>
<td>5</td>
<td>0.43</td>
</tr>
</tbody>
</table>

* Bold Components Extracted

Table 28: Loadings of Extracted Components

<table>
<thead>
<tr>
<th>Component Number and Interpretation</th>
<th>PC1: Flooding Potential</th>
<th>PC2: Distance Measures</th>
<th>PC3: Soil Mercury Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floodplain Designation</td>
<td>0.729</td>
<td>-0.032</td>
<td>0.472</td>
</tr>
<tr>
<td>Relative Elevation (m)</td>
<td>0.840</td>
<td>0.092</td>
<td>0.085</td>
</tr>
<tr>
<td>Distance from River (m)</td>
<td>0.282</td>
<td>0.850</td>
<td>0.117</td>
</tr>
<tr>
<td>River Kilometer (km)</td>
<td>0.493</td>
<td>-0.719</td>
<td>0.025</td>
</tr>
<tr>
<td>(Log10) Soil Hg (ppm)</td>
<td>-0.625</td>
<td>0.097</td>
<td>0.738</td>
</tr>
</tbody>
</table>

3.4.3.2. Results of General Linear Model

A general linear model was used to identify the significance of each principal component in explaining mercury levels in spiders. Delta nitrogen-15 (δ^{15}N) of each spider was included as a main factor in the model as δ^{15}N is related to trophic position of individuals as explained in Section 1.2.5. Methylation and Methyl Mercury. Each principal component was included as a covariate resulting in the use of 161 individual...
cases containing mercury values, $\delta^{15}$N and scores for all three principal components, after outliers were deleted from the dataset.

The initial model included $\delta^{15}$N as a fixed factor and PC1, PC2, PC3 as covariates. Delta nitrogen-15 was a significant predictor of spider mercury level ($F=5.359_{(1,161)}$ and a $p=0.022$). Flooding potential (PC1) was also a significant factor in predicting spider mercury levels ($F=27.818_{(1,161)}$, $p<0000$) (See Fig. 53). The distance measures component (PC2) was also a significant predictor ($F=4.134_{(1,161)}$, $p=0.044$) but soil mercury availability (PC3) did not prove to be a significant factor and was removed from the final model ($F=0.261_{(1,161)}$, $p=0.610$) (See Fig. 54 and 55). The final model included $\delta^{15}$N and PC1 and PC2 as covariates and accounted for approximately 22% of the variation in prey mercury levels ($r^2=0.221$). In the final model, PC1 explained by far the most variation having a partial Eta effect size of 0.151 while that of $\delta^{15}$N was just 0.033 and PC2 0.026. The predictive power of PC1 was found to be 11.6% using regression analysis (See Fig. 53).
Figure 53: Flooding Potential (PC1) as a Predictor of Spider Prey Mercury

Log-Transformed Mercury Level (Dry Weight ppm)

Flooding Potential (PC1)

$R^2$ Linear $= 0.116$
Figure 54: Distance Measures (PC2) as a Predictor of Spider Prey Mercury

Distance Measures (PC2)
Figure 55: Soil Mercury Availability (PC3) as a Predictor of Spider Prey Mercury

R\textsuperscript{2} Linear = 4.287E-4

Soil Mercury Availability (PC3)
3.5. Discussion of Prey Mercury Results

3.5.1. Seasonal Variation

Seasonal changes in mercury levels were investigated in spiders collected from pitfalls, spiders collected as prey from songbirds, and all prey types combined. The results show that mercury levels did not differ between the two years of collection but that Julian day did have an effect on some prey types. This is likely due to differences in development and diet or species change over the season within prey types. Predatory invertebrates such as spiders, Opiliones and Coleoptera may change their diet according to what types of prey are most available at different times during the season. Certain prey types hatch or emerge synchronously in large numbers (Polis et al., 2004). One example is emergent aquatic invertebrates, such as mayflies and caddisflies, which serve as prey for a large number of riparian predators including some types of spiders (Walters et al., 2008; Power et al., 2004). Emergent aquatic invertebrates generally emerge when the environmental conditions are suitable, which means that they may be available as prey in large quantities but for short time spans (Polis et al., 2004). However, other prey types such as Lepidoptera and Orthoptera are more likely timed with climatic changes that spur on vegetative growth. Caterpillars, in particular, rely solely on vegetative matter in their diet and their mercury values may be indicative of changes in mercury accumulation in vegetation over the season. Alternately, different species of caterpillars may be available at different times during the summer. Therefore, mercury levels are not likely to show a consistent trend among different prey groups throughout the season and this is reflected in my results.
3.5.2. How does Size Relate to Mercury Level in Invertebrates?

I also investigated the importance of invertebrate size or fresh weight in understanding the variation in mercury levels. For all invertebrate types, size was not found to be an important factor but there was a general trend of increasing mercury levels with increasing size of invertebrate. When the mercury levels of prey types were considered individually, a few did have a significant relationship with size; however, the trends were inconsistent with mercury levels increasing with size of beetle and decreasing with size of moth and grasshopper. This suggests that the relationship of size and mercury accumulation cannot be generalized over a broad array of invertebrate types.

It was surprising that even when wolf spiders were examined alone, size still did not have a significant effect. Despite this result, there was a trend of increasing mercury with increasing size of spider suggesting that if this question was investigated more thoroughly with more samples of a larger size range, it is likely that size would have a significant effect. Despite these results, it is imperative to keep in mind that invertebrate mercury levels vary in accordance with multiple variables and not just size.

3.5.3. How does Habitat Type Affect Mercury Levels?

The design of this study allowed for some preliminary investigations into how mercury accumulation varies according to habitat. Spiders were collected via pitfalls placed both in open field and woodland habitats. The habitat type from which spiders were collected did have a significant effect on mercury levels with spiders from woody areas having higher mercury levels. This result was somewhat supported by prey mercury levels, although habitat designation was less certain because birds do not
necessarily forage in the same habitat as their nest box. When the mean mercury level of prey collected from nest boxes placed in open fields was compared to the mean mercury level of prey collected from woody areas, habitat type was non-significant. Despite this, for all prey types collected from both habitat types, those collected from woody areas had higher mean mercury levels, with the exception of grasshoppers.

I hypothesize that differences in methylation potential are the cause of suspected higher mercury levels in spiders and insects collected from woody habitats over those collected from field habitats. Woody areas along the South river generally had a thick cover of detritus, which is broken down by bacteria that are also capable of methylation. I decided to investigate whether the same trend was seen when methyl mercury content rather than total mercury was investigated. This information is summarized in Table 29 and visually shown in Figure 56. Although, I had a low sample size of insects analyzed for methyl mercury, there does seem to be a general trend of greater percent methyl mercury in insects and spiders collected from woody areas over those from open fields. Statistically speaking, habitat does have a significant effect on methyl mercury content when all ligature items are combined ($F=19.339$ (1, 139), $p<0.000$). The suspected increase of methylation potential in woodland areas may be a result of less controlled flooding and water retention in these areas. The majority of land along the South River is used for agriculture or grazing land; however areas that are frequently inundated are not exploited for these purposes. Therefore, the more natural and possibly frequent flooding behavior in woodland areas may play a part in providing environments for methylation to occur. Much of this is speculative but the data do lend further support to the hypothesis that
woody areas are more likely to have higher methylation rates. However, additional studies are needed to corroborate this finding.

Table 29: Mean Percent Methyl Mercury Content of Prey Orders by Habitat

<table>
<thead>
<tr>
<th>Prey Order/Class</th>
<th>Habitat (F/W)</th>
<th>Mean ± Std. Dev.</th>
<th>Sample Size (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARAN</td>
<td>F</td>
<td>41.35 ± 20.88</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>50.90 ± 25.15</td>
<td>20</td>
</tr>
<tr>
<td>COLE</td>
<td>F</td>
<td>5.02 ± 7.45</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>1.73</td>
<td>1</td>
</tr>
<tr>
<td>LEPA</td>
<td>F</td>
<td>46.82 ± 37.89</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>53.46 ± 51.81</td>
<td>3</td>
</tr>
<tr>
<td>OPIL</td>
<td>F</td>
<td>29.72 ± 18.34</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>87.84 ± 0.17</td>
<td>2</td>
</tr>
<tr>
<td>ORTH</td>
<td>F</td>
<td>22.40 ± 26.53</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>41.61 ± 27.34</td>
<td>9</td>
</tr>
</tbody>
</table>

Figure 56: Percent Methyl Mercury Content of Prey Orders by Habitat
3.5.4. Mercury Level Varies Significantly Among Prey Orders

This study further confirms that mercury levels do vary significantly among prey orders with predatory invertebrates having higher mean mercury levels than omnivores and herbivores. This finding follows in the footsteps of previous work on the South River that concluded that of the three most abundant prey types, spiders had significantly higher mercury (Cristol et al., 2008, Friedman, 2007). Additionally, my work shows that the mercury in predatory spiders is comprised of a substantially higher percent of methyl mercury than in other omnivorous and herbivorous invertebrates. This is not a surprising result as the literature has shown that mercury biomagnifies with every trophic level (See section 1.2.5.).

3.5.5. Discussion of the Lateral Extent of Spider Mercury

The lateral extent of spider mercury was specifically investigated in this study because it was hypothesized to be the mechanism behind the significant decline of avian mercury levels with distance from the South River. Riparian spiders may obtain some of their diet from emergent aquatic insects, which accumulate mercury during their larval stage in the South River. Therefore, spiders could act as an intermediary through which aquatic mercury is biomagnified before accumulating in avian insectivores. However, no decline of spider mercury was observed along pitfall transects, and distance from the river proved to be a non-significant factor in predicting mercury levels in spiders collected from birds. These results were not expected and here I offer a few possible explanations.
The distance gradient used to determine the lateral extent of spider mercury extended only to 150 meters from the river, which may not have been far enough to distinguish a trend. Additionally, the home range size of wolf spiders is not well known and some studies have suggested that they have small home ranges and are not likely to move out of a 1 m² area over several sequential days (Ahrens and Kraus, 2006). However, other studies have suggested that they have substantially larger territories and will completely exit an area of 900 m² within several days (Kiss and Samu, 2000). These studies offer very different conclusions. If the latter is true, then the distance between pitfalls may not have been adequate to distinguish between spiders using two different foraging territories. Since wolf spiders are known as hunting or roaming spiders, they may not forage within predetermined territories but rather forage where the prey are more abundant. This idea is supported by studies that have discovered an abundance of spiders at aquatic-land interfaces versus in more inland areas (Ahrens and Kraus, 2006).

Furthermore, the abundance of spiders along aquatic-land interfaces has been reported to increase during times of emergence of aquatic invertebrates (Henschel, 2001). This seems a likely explanation as to why spider mercury levels exhibited no relationship with distance. The distance of each pitfall from the river may not have been indicative of the actual foraging location of the spider, and spiders may have congregated in “hot spot” areas to feed rather than maintaining foraging territories.

My results suggest that spiders foraging throughout the floodplain are susceptible to mercury originating in the river. Earlier in this discussion I alluded to the idea that these results suggest that spiders feed on aquatic subsidies as far as 150 meters from the South River. This conclusion is contrary to that of Briers et al. (2005), who concluded
that riparian wolf spiders of desert streams obtain a large portion of their diet (40%) from emergent insects but that the importance of aquatic insects to their diet dropped off dramatically with distance from the stream comprising 1% of the diet at just 20 m from the shoreline (Briers et al., 2005). This suggests that aquatic subsidies do not extend far into the riparian corridor of desert streams. However, the South River is dramatically different than a desert stream, having a wider floodplain that extends to 400 m at its widest section. Aquatic insects emerging from the South River may travel further inland than those emerging from desert streams, suggesting that aquatic mercury transported out of the South River may be accumulated in riparian spiders throughout the floodplain rather than within a few meters of the shoreline.

Finally, as an additional analysis to further support or contradict the lack of relationship of pitfall spiders with distance from the river, I looked at the importance of distance from the river in predicting mercury levels in prey spiders collected directly from terrestrial songbirds via the ligature method. In this analysis, each spider was associated with the nest box distance from the river; not the spiders exact foraging position but an estimate of where the bird foraged the spider. In this statistical analysis, distance was again not an important factor in predicting mercury exposure ($F=2.389_{(1, 229)}$, $p=0.124$). However, ligature spiders did show a slight trend of decreasing mercury levels with distance from the river as shown in Figure 57. However, the distance at which spider mercury levels decline to reference could not be predicted with confidence because spiders were not collected far enough from the river. Figure 57 depicts the average reference spider mercury level at 0.07 ppm; however, contaminated spiders did not decline to this level within the 400 m stretch from the river at which they were in...
collected. There is a lot of variation in spider mercury levels that is not taken into account in this analysis. However, the varying foraging strategies may offer an explanation as to why spiders collected via ligature do exhibit some decline with distance while wolf spiders collected via pitfall trapping did not exhibit any trend. In addition, it may have been the greater distance over which the prey spiders were collected.

Spiders collected via the ligature method were of all types (wolf (*Lycosidae*), fishing (*Pisauridae*), orb weavers (*Araneidae*), long-jawed orb weavers (*Tetragnathidae*), jumping spiders (*Salticidae*), crab spiders (*Thomisidae*), funnel-web weavers (*Agelenidae*), cobweb spiders (*Theriidae*), sac spiders (*Clubionidae*), lynx spiders (*Oxypidae*), running crab spiders (*Philodromidae*) rather than only wolf spiders as collected via pitfall trapping (See Appendix E for mercury levels of spiders by taxonomic family). Some of these spider families have been shown to primarily feed on emergent aquatic insects (long-jawed orb weavers) while others are thought to obtain a large portion of their diet from emergent aquatic insects but may also feed on terrestrial insects (orb weavers) (Walters et al., 2008; Polis et al., 2004). Those families that rely more heavily on aquatic insects are likely to exhibit a stronger trend with distance from the river while those that rely more heavily on terrestrial insects are more likely to exhibit a weaker trend. Since there are representatives of spiders from a host of families in the samples collected via the ligature method, one would expect a stronger trend with distance than shown by wolf spiders collected via pitfall trapping. Therefore, the trend visualized in Figure 57 is most likely driven by spiders from families that rely more heavily on aquatic insects in their diet.
3.5.6. Discussion of Principal Component and General Linear Model Results Using All Prey Types and Spider Prey Only

The results using data from all prey types, collected via the ligature method, indicate that flooding potential is the most important factor (of those investigated) in predicting mercury levels. This result is further strengthened when spiders are examined singularly. The flooding potential component loaded most heavily for floodplain and relative elevation but also loaded positively for distance from the river and river kilometer downstream, and negatively for soil mercury. Therefore, all of these variables
are somewhat important in understanding the spatial variability in invertebrate mercury levels.

Also notable was that the flooding potential component was actually more important in predicting spider mercury levels than spider delta nitrogen-15 value ($\delta^{15}\text{N}$). The delta nitrogen-15 variable was used to distinguish between types of spiders that may differ in their foraging strategies, diet, size and other physiological aspects. The fact the flooding potential resulted as a stronger predictor suggests that the foraging location, rather than foraging strategy, is more important in determining mercury exposure in spiders.

The distance measures component, which loaded most strongly for distance from the river and river kilometer in both the model predicting mercury in all prey types and the model predicting only spider prey mercury, proved to be significant and was included in the final models. However, the distance measures had a relatively small effect on mercury levels when effect sizes were compared among all factors in each model. When the relationship of mercury levels in all prey types and the distance measures component was investigated, I found that there was a weak trend of decreasing mercury levels as both distance from the river and distance downstream decreased.

The third principal component, which loaded exclusively for soil mercury, was also not significant using either all prey types or just spiders. Despite these results, there were still some apparent but weak trends. When the soil mercury component was further examined, prey mercury levels suggested a trend of increasing mercury levels with increasing soil mercury availability. However, this trend disappears when only spiders
are included in the analyses, suggesting that the trend is driven by other more sedentary prey types, including Lepidoptera and Orthoptera. I suggest that if soil samples had been taken in conjunction with foraging areas of the four avian study species (from which all insect and spider prey were collected), soil mercury would have been a better indicator of mercury exposure in both insect and spider prey. Additionally, the use of methyl mercury content of soil samples rather than total mercury content would have better predicted mercury levels of the insect and spider endpoints.

4. Overall Conclusions of Study

The major objective of this work was to decipher the spatial patterns governing mercury exposure in terrestrial songbirds and their prey. There are two noteworthy conclusions from this study, the first being that terrestrial songbirds and their prey are at risk to mercury exposure throughout the floodplain of a mercury-contaminated river. This question has not been previously addressed and suggests that many more species and individuals are at risk than have traditionally been included in risk assessments. Secondly, my study supports the hypothesis that flooding potential predicts mercury exposure, suggesting that flood waters are an important vector in transporting mercury across the aquatic-terrestrial interface. Therefore, flooding potential is important in understanding how aquatic mercury makes it way into the terrestrial food chain of the adjacent floodplain. The major take home message is that species and individuals throughout the floodplain should be taken into consideration in all future risk assessments of mercury-contaminated rivers. Finally, the invertebrate portion of this study focused on spiders because they were deemed important transporters of mercury to higher order
predators in previous research. However, we still have little understanding of how spiders obtain their high doses of mercury. Future work should focus on the importance of emergent insects to the spiders most commonly eaten by terrestrial songbirds on the South River floodplain (wolf, orb weavers, fishing, and crab spiders). My study offers some additional pieces to this puzzle by supporting the hypothesis that wolf spiders venture and forage over much larger areas than some previous studies have suggested, or for some other reason have mercury levels that do not closely correlate to their locations of capture. Furthermore, spiders that are collected from woody habitats and those areas more likely to flood consistently had higher mercury levels. Although much more work is needed until we can fully understand this story, especially investigating the importance of biological vectors in transporting mercury, this study offers some of the first empirical data describing the mechanisms that control how aquatic mercury finds its way up the terrestrial food chain.
4. References


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Appendix A: Adult Blood Mercury Decline with Distance according to Species and Sex

a) Blue refers to adults with unknown sex:
   Females ($r^2=0.32$) predicted distance = 160 m;
   Males ($r^2=0.43$) predicted distance = 225
b) Females \( (r^2=0.24) \) predicted distance = 280 m;  
Males \( (r^2=0.15) \) predicted distance = 450 m

Species: CACH

![Graph showing blood mercury levels versus distance from river for CACH species.]

F: \( R^2 \text{ Linear} = 0.244 \)  
M: \( R^2 \text{ Linear} = 0.15 \)

c) Females \( (r^2=0.32) \) predicted distance = 480 m;  
Males \( (r^2=0.43) \) predicted distance = 290 m

Species: HOWR

![Graph showing blood mercury levels versus distance from river for HOWR species.]

F: \( R^2 \text{ Linear} = 0.13 \)  
M: \( R^2 \text{ Linear} = 0.204 \)
d) Females ($r^2=0.11$) predicted distance = 625 m; 
Males ($r^2=0.07$) predicted distance = 700 m
Appendix B: Importance of River Mile in Predicting Adult Mercury Levels

1) Effect of River Mile on Mean Adult Mercury Levels By Species

![Graph showing the effect of river mile on mean adult mercury levels by species.]

2) Effect of River Mile on Mean Adult Mercury Level Sampled within 50 meters of the South River by Species
(a) CARW

![Graph showing the effect of river mile on mean adult mercury levels for CARW species.]
(b) CACH

Species: CACH

Mean Blood Mercury (ppm)

River Mile

(c) HDWR

Species: HDWR

Mean Blood Mercury (ppm)

River Mile

269
(d) EABL

![Graph showing Mean Blood Mercury (ppm) for Species EABL across River Mile values from 1.7 to 22.0. Peaks and troughs indicate fluctuations in mercury levels.](image)

River Mile: 1.7, 2.9, 5.1, 9.2, 9.5, 9.9, 10.9, 11.3, 13.9, 14.2, 16.4, 17.4, 19.6, 19.9, 22.0

Mean Blood Mercury (ppm): 0.0, 0.5, 0.7, 1.2, 1.5, 2.0
Appendix C: Calculation of Invertebrate Fresh Weight Mercury Level

Dry weight mercury concentrations are used for all invertebrates reported throughout this study, however, fresh weigh concentrations are often more useful in the application of results to the field. Therefore, here I present the calculations for fresh weight mercury and report the mean fresh weight mercury levels for all prey orders collected. Since not all collected prey items have associated fresh or dry weights, the sample sizes reported for fresh weight levels will differ from that of dry weight seen in Table 22.

\[
\text{Fresh Weight Mercury (ppm)} = \text{Dry Weight Mercury (ppm)} \times \left( \frac{\text{Dry Weight(g)}}{\text{Fresh weight(g)}} \right)
\]
Table 30: Sample Sizes and Mean Mercury Level by Prey Group in Fresh Weight Parts per Million (ppm)

<table>
<thead>
<tr>
<th>Prey Order/Class</th>
<th>Mean Mercury Level ± Std. Dev. (Fresh Weight ppm)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARANEAE</td>
<td>0.36 ± 1.18</td>
<td>222</td>
</tr>
<tr>
<td>BLATTARIA</td>
<td>0.09</td>
<td>1</td>
</tr>
<tr>
<td>COLEOPTERA</td>
<td>1.07 ± 2.00</td>
<td>42</td>
</tr>
<tr>
<td>COLEOPTERA LARVAE</td>
<td>0.47 ± 0.52</td>
<td>4</td>
</tr>
<tr>
<td>CLASS DIPLOPODA</td>
<td>0.03 ± 0.04</td>
<td>5</td>
</tr>
<tr>
<td>DIPTERA</td>
<td>0.41 ± 0.68</td>
<td>3</td>
</tr>
<tr>
<td>EPHEMEROPTERA</td>
<td>0.46</td>
<td>1</td>
</tr>
<tr>
<td>HEMIPTERA</td>
<td>0.17</td>
<td>1</td>
</tr>
<tr>
<td>HOMOPTERA</td>
<td>0.05</td>
<td>1</td>
</tr>
<tr>
<td>HYMENOPTERA</td>
<td>0.03 ± 0.03</td>
<td>20</td>
</tr>
<tr>
<td>ISOPODA</td>
<td>0.31 ± 0.32</td>
<td>8</td>
</tr>
<tr>
<td>LEPIDOPTERA ADULT</td>
<td>0.02 ± 0.05</td>
<td>55</td>
</tr>
<tr>
<td>LEPIDOPTERA LARVAE</td>
<td>0.04 ± 0.23</td>
<td>269</td>
</tr>
<tr>
<td>LEPIDOPTERA PUPAE</td>
<td>0.02 ± 0.02</td>
<td>4</td>
</tr>
<tr>
<td>MOLLUSK</td>
<td>0.0002</td>
<td>1</td>
</tr>
<tr>
<td>ODONATA</td>
<td>0.26 ± 0.23</td>
<td>2</td>
</tr>
<tr>
<td>OPILIONE</td>
<td>0.28 ± 0.59</td>
<td>15</td>
</tr>
<tr>
<td>OPISTHOPORA</td>
<td>0.56 ± 0.59</td>
<td>32</td>
</tr>
<tr>
<td>ORTHOPTERA</td>
<td>0.05 ± 0.22</td>
<td>132</td>
</tr>
<tr>
<td>UNKNOWN PUPAE</td>
<td>0.31 ± 0.76</td>
<td>6</td>
</tr>
<tr>
<td>SEED</td>
<td>0.009</td>
<td>1</td>
</tr>
<tr>
<td>UNIDENTIFIED ITEMS</td>
<td>0.39 ± 0.76</td>
<td>49</td>
</tr>
<tr>
<td>Total</td>
<td>0.22 ± 0.83</td>
<td>874</td>
</tr>
</tbody>
</table>
Appendix D: Correlation of College of William & Mary Mercury Analysis Results with Results from Quicksilver Analytical

Correlation and Descriptives

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quicksilver Total Mercury (ppm)</td>
<td>2.75</td>
<td>8.95</td>
<td>32</td>
</tr>
<tr>
<td>W&amp;M Total Mercury (ppm)</td>
<td>3.05</td>
<td>10.03</td>
<td>32</td>
</tr>
</tbody>
</table>

Pearson Correlation = 1.00, p<0.000

$R^2_{\text{Linear}} = 0.995$
Appendix E: Mean Mercury Levels, Delta Nitrogen-15 Values and Sample Sizes of Spider Families Collected as Prey from Terrestrial Songbirds (CARW, CACH, HOWR and EABL) (Fresh Weight ppm)

<table>
<thead>
<tr>
<th>Spider Family</th>
<th>Mean ± Std. Deviation (ppm)</th>
<th>Sample Size (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aglenidae</td>
<td>0.50 ± 0.53</td>
<td>10</td>
</tr>
<tr>
<td>Araneidae</td>
<td>0.36 ± 0.32</td>
<td>9</td>
</tr>
<tr>
<td>Clubionidae</td>
<td>0.27 ± 0.08</td>
<td>3</td>
</tr>
<tr>
<td>Lycosidae</td>
<td>0.21 ± 0.25</td>
<td>47</td>
</tr>
<tr>
<td>Oxypodiodes</td>
<td>0.02</td>
<td>1</td>
</tr>
<tr>
<td>Pisauridae</td>
<td>0.77 ± 0.83</td>
<td>3</td>
</tr>
<tr>
<td>Salticidae</td>
<td>0.30 ± 0.14</td>
<td>17</td>
</tr>
<tr>
<td>Theridiidae</td>
<td>0.23 ± 0.16</td>
<td>8</td>
</tr>
<tr>
<td>Thomisidae</td>
<td>0.21 ± 0.24</td>
<td>24</td>
</tr>
<tr>
<td>Unknown</td>
<td>0.27 ± 0.38</td>
<td>98</td>
</tr>
<tr>
<td>Total</td>
<td>0.27 ± 0.35</td>
<td>220</td>
</tr>
</tbody>
</table>

* Unknowns include both those samples for which taxonomic family could not be identified or those that were prepared for mercury analysis (freeze-dried and homogenized) without identification to the level family.

Mean Mercury Levels of Spider Families Collected as Prey from Terrestrial Songbirds (fresh weight ppm)
Mean Delta Nitrogen-15 ($^{15}N/_{\text{oo}}$) Value of Spider Families Collected as Prey from Terrestrial Songbirds (fresh weight ppm)
Mikaela Gioia Selene Howie was born in Morgantown, West Virginia on October 24, 1978 to two newly arrived immigrants from Dublin, Ireland. Her father (Dr. Michael Britchford Howie) is originally from Blackpool, England while her mother (Maria Olga Llata del Rio Howie) is originally from Santander, Spain. Her two siblings (sister Cliona Anne Howie and brother Morgan Andre Howie) were born in Dublin, Ireland making Mikaela the only member of her family to be born in the United States of America. After receiving a B.S. in Ecology and Evolutionary Biology, with Anthropology as a second major, from Tulane University in 2001, Mikaela took several ornithological field jobs in North America including Tucson, Arizona, Central Mexico and Columbus, Ohio. During this time she also worked at the Tulane Museum of Natural History as a curatorial assistant of the extensive fish collection. In 2006, she joined the Department of Biology at the College of William and Mary where she successfully defended her Master’s thesis December 2009.